Genetic and epigenetic mechanisms regulating the G1-S transcriptional wave in fission yeast

Alberto González Medina

PhD Thesis UPF

Barcelona, 2018

Treball dirigit pel Dr. José Ayté del Olmo

Oxidative Stress and Cell Cycle Group

Departament de Ciències Experimentals i de la Salut (DCEXS)

Programa de Doctorat en Biomedicina de la Universitat Pompeu Fabra

MOVING ON !!

ACKNOWLEDGMENTS

Esta tesis va dedica a mis padres, sois una inspiración continúa, sin vosotros nada de esto hubiera sido posible, gracias por apoyarme en todo y por tratar de entenderme, gracias por estar siempre al teléfono cuando os necesito, si soy alguien en la vida será siempre gracias a vosotros, y a los valores que me habéis transmitido. Esta tesis también te la dedico a ti hermano, porque siempre has estado ahí cuando te he necesitado; os llevaré a los tres siempre en mi corazón.

Gracias a José y a Elena por haber hecho de mi un CIENTÍFICO, por haberme dado la oportunidad de disfrutar durante estos cuatro años en este estupendo laboratorio, y por haberme escuchado. Gracias especialmente a José por la paciencia que has tenido conmigo, sobre todo corrigiéndome la tesis. Espero saber transmitir y poner en práctica todo el conocimiento que me habéis dado.

Quiero agradecer especialmente a toda la gente que ha pasado por el laboratorio y que me han apoyado durante estos 4 años. En primera lugar, daros las gracias a los que guiaron mis primeros pasos en el 383, mi Isita, Patri y Javi. Gracias por acogerme desde el primer momento, y por enseñarme tanto. Nunca se me olvidarán nuestras meriendas ni nuestros viernes de chocolate. Iva gracias por el paper. Laura, gracias por todas las compras del Amazon y por el colchón hinchable; Alba, mi ibérica favorita, te debo un viaje a la Feria; Stefan, siempre riéndome con tus tonterías; Luis, si hay alguien que me ha sabido entender durante todo este tiempo ese has sido tú, nuestras horas de gimnasio, de fiesta, de laboratorio…; Claudia, mi #Instagramer, #itgirl, #influencer, #fotógrafa favorita, siempre serás mi Lady ChIP. Rodrigo, me llevo nuestros bailes de fiesta y el haberme enseñado el trap, sin ti no sería el mismo influencer; Marga, muchísimas gracias por todo lo que me has enseñado, eres un ejemplo a seguir, y siempre serás mi fulanita; Esther, mi niña, estoy muy orgulloso de ti y de lo que has aprendido, seguro que llegas muy lejos; Cris, que nos gusta a nosotras un relio, muchas gracias por dejarme tu casa, siempre estaré ahí

para una cervecita; Monste, drag queen, que diga drama queen, gracias por toda la ayuda que me has prestado con la tesis, y fuera en el piso, ojalá Amancio te regale algún día un escaparate para ti sola; Sonia ¿pero qué la pasa? gracias por traer el Yoga a mi vida, ahora yo traeré a Manolo García a la tuya. Gracias Sue porque siempre has estado dispuesta a resolverme cualquier duda y por último a Mercé, por todo el coñazo que te he dado.

También agradecer a los compañeros de los Posas, Inmuno y Viro, por haberme dejado material siempre que lo he necesitado, y por todas las Beer Sessions que hemos levantado.

A mis chicas de cocina: Montse, Mari, Chelo y Enri. Sois una alegría para todos, me lo he pasado genial con vosotras, nunca os olvidaré y seguro que os echo en falta allá donde vaya.

Mi familia Calabria, gracias porque mi vida en Barcelona no hubiera sido lo mismo sin vosotros: Ana, René, Paula, Lidia, Sara, Yus e Ibon.

Para terminar, me gustaría agradecer todas las personas que han pasado durante estos 4 años por Barcelona para verme y algunos de los cuales se han convertido en hijos predilectos de Barcelona: Josun, siempre seremos embajadoras del SONAR, gracias por el #summervibes; Tomy contigo empezó mi tesis y contigo ha acabado, dejamos atrás Dublín, Niza y Los Ángeles pero muchos otros destinos nos están esperando; Nathan, mi cari, siempre recordaré nuestras noches en Arena y mi paso por Mallorca; Tam, nena, gracias por haber venido a visitarme y haberme dado fuerzas cuando más lo necesitaba; María, La puerros, mi señora favorita, sabes que de mayor quiero ser como tú; Mariana, mi hermana argentina y mi mentora de la vida; Sheyla, Tomás y Marta, 25 años de amistad y los que nos quedan; Nuria, Noelia, Raquel, Silvia y Elena, mis gitanas favoritas.

No me puedo dejar fuera a mis dos mejores amigas catalanas: Silvia, tienes ese espíritu que tanto me gusta que siempre nos lleva a hacer travesuras. Laura, cari, te admiro un montón, gracias por haber estado pendiente de mí en mis primeros momentos en Barcelona.

SUMMARY

SUMMARY

When cells start their life cycle have to face the decisive decision whether to remain in quiescence state (G0) or to pass a point of no return which leads to cellular proliferation. This point, which is known as “Start” in yeast and “Restriction Point” in mammalians, takes place at the end of G1 phase of cell cycle and depends on the activation of specific transcription factors. These transcription factors regulate a wide variety of genes involved in the DNA replication, DNA repair and the progression of the cell cycle. The transcription of genes required for S phase has to be finely regulated. Alteration in some components of this pathway can trigger uncontrolled cell cycle progression and tumorigenic processes in higher eukaryotes. In this work, we use the fission yeast *Schizosaccharomyces pombe* to study the regulation of the transcription factor MBF (functional homolog of E2F-RB in metazoans). MBF regulates the expression of genes required for the G1-Stransition and its alteration leads to genetic instability in yeast. In previous work, we have described how the co-repressors Nrm1 and Yox1 bind to MBF at the end of S phase, inhibiting the MBF-dependent transcription. But the molecular mechanisms that regulate the activation of MBF at the onset of each cell cycle it is still an unresolved question. We have developed a reporter system to measure the changes in the activity of MBF in live cells using flow cytometry. Thanks to this reporter system, we have been able to find new regulators of MBF such as tRNA methyltransferases and COP9/Signalosome which can alter positively and negatively the MBF-dependent expression respectively. Furthermore, we have also studied which proteins are necessary to modify the chromatin structure when the MBF-dependent genes are active. In this sense, we have found that the

remodeler complex INO80 (which participates in the exchange of histone variant H2A.Z with H2A) and the histone acetyltransferase Gcn5 (through the acetylation of histone H3 at lysine 9 and lysine 18) help to active the MBF-regulated genes.

RESUMEN

Cuando las células comienzan su ciclo vital tienen que enfrentarse a la decisión de mantenerse en un estado de quiescencia (G0) o por el contrario pasar un punto de no retorno que conduce a la proliferación celular. Este punto conocido como “Start” en levaduras y “Restriction Point” en metazoos tiene lugar al final de la fase G1 del ciclo celular, y depende de la activación de una serie de factores de transcripción, entre otros reguladores. Estos factores de transcripción regulan una amplia variedad de genes involucrados en la replicación del ADN, en la respuesta al daño al ADN y en la progresión del ciclo celular. Esta transcripción de genes necesarios para la fase S del ciclo celular tiene que ser finamente regulada ya que la alteración en alguno de los componentes de esta vía puede desencadenar procesos tumorales en eucariotas superiores. En este trabajo nosotros usamos la levadura de fisión *Schizosaccharomyces pombe* para estudiar la regulación del factor de transcripción MBF (homólogo funcional de E2F-RB en metazoos), el cual está encargado de regular la expresión de genes necesarios para el paso de la transición G1-S y cuya alteración conduce a la inestabilidad génica en levaduras. Anteriormente en el laboratorio hemos descrito que los co-represores Nrm1 y Yox1 se unen a MBF al final de la fase S inhibiendo la transcripción de genes dependientes de MBF. Pero determinar molecularmente cómo MBF se activa al inicio de cada ciclo es una cuestión hoy en día sin resolver. Durante este trabajo hemos desarrollado un sistema reportero que mide las variaciones en la actividad de MBF en las células vivas usando citometría de flujo. Gracias a esta metodología hemos sido capaces de encontrar nuevos reguladores de MBF como tRNA metiltransferasas y COP9/Signalosome que pueden

alterar positiva y negativamente la expresión de genes MBF respectivamente. Por otra parte, hemos estudiado qué proteínas son necesarias para modificar la estructura de la cromatina cuando los genes dependientes de MBF se encuentran activos. En este sentido, hemos encontrado que el complejo remodelador de cromatina INO80 que remplaza la variante de la histona H2A.Z por H2A y la histona acetiltransferasa Gcn5, a través de la acetilación de la histona 3 lisina 9 (H3K9) y lisina 18 (H3K18), promueven directamente la transcripción de genes necesarios para la transición G1-S del ciclo celular.

PREFACE

The proper transition from G1 to S phase of the cell cycle is vitally important for the control of eukaryotic cell proliferation, and its deregulation may trigger oncogenic processes. The pass through G1 to S phase is regulated by a point of no return known as “Restriction Point” in mammalian cells and “Start” in yeast, and also by the checkpoints, which ensure the accurate completion of cell cycle processes. The important role of the Restriction Point and the checkpoints for maintaining the appropriate cell division cycle control is confirmed by the high number of mutations that are detected in the components of these pathways during oncogenesis.

The G1-S transcriptional network codifies many proteins required in downstream processes of S phase and related to DNA replication, repair and cell cycle progression. Therefore, missregulation of this transcriptional program leads to inefficient DNA replication (or replicative stress) originating what ultimately can produce DNA damage. Mechanisms that regulate replicative stress checkpoint, DNA damage checkpoint and G1-S transcriptional program converge in the same family of transcription factors (E2F in mammalian cells, and MBF in fission yeast). Thus, cells cope with the different stresses managing checkpoints that modulate the expression of G1-S genes.

As G1-S transcriptional regulation is largely conserved in eukaryotic organisms, we have used the fission yeast *S. pombe* to show new regulators of G1-S transcriptional wave. In this thesis, we have investigated the biological relevance of both genetic and epigenetic factors that provide new insights into the mechanisms that control G1-S transcriptional program.

INDEX

Page

INTRODUCTION

1. *Schizosaccharomyces pombe*

The fission yeast *Schizosaccharomyces pombe* is an eukaryotic unicellular organism extensively used a model organism due to its simple growth conditions in the laboratory, and its easy genetic manipulation. Since the 1950s, studies in *S pombe* have led to the discovery of genes involved in fundamental mechanisms of molecular and cellular biology. Additionally, the growth of *S pombe* occurs by length extension and divides by bipartition, forming a septum at a central position of the wall. This feature allows the identification of the phase of cell cycle in which cells are by simple microscope observation.

The fission yeast genome is divided between just three relative large chromosomes with 5036 genes. Moreover, *S. pombe* presents several characteristics that make it attractive for genetic studies: the laboratory strains can be either haploid or diploid. The haploids strains are specially interesting to find characteristic phenotypes of mutant alleles which are frequently recessive. Yeast can maintain autonomous plasmids that reversibly alter the behaviour of the stains. And also, yeasts have active homologous recombination mechanisms which allow to introduce easily mutations or deletions in a specific allele.

A major reason for using *S. pombe* to study the basics molecular processes is that this yeast has evolved less rapidly than its distant cousin *Saccharomyces cerevisae*, sharing more features with metazoan cells. Specially *S. pombe* share a great molecular similarity to higher eukaryotes regarding its mechanism of cell cycle control. Moreover, *S. cerevisiae* has lost some genes (338) which are conserved between *S. pombe* and mammalian cells, which

makes fission yeast a complementary experimental system to budding yeast. But *S. pombe* has not historically had many practical applications as *S. cerevisiae*. That is one of the main reasons why the scientific research has been more limited in fission yeast.

2. Mitotic Cell Cycle

One of the main objectives of the cell cycle is to maintain the proper timing of events destined to replicate the genetic material and segregate it into two daughter cells. The cell cycle is divided into four phases according to the chronological order of events: Gap1 (G1), DNA Synthesis (S), Gap 2 (G2), and Mitosis (M). During G1, cells sense extracellular signals (e.g availability of nutrients or growth factors) that determine whether to commit cell division, in a process called START (Restriction Point in mammalian cells). Following this point, cells duplicate DNA and centrosomes (the microtubule-organizing structure) during S phase. In G2, cells sense intracellular signals from checkpoint pathways that ensure that replication has been completed properly and the mitotic spindle machinery is functional. During M phase, duplicated chromosomes are separated and each daughter cell receives one of the sister chromatids from each homologue pair. Alternatively, cells can exit G1 to enter a resting phase of the cycle called G0, in which they can remain for long periods without proliferating. At this point, yeast cells can execute conjugation or sexual differentiation, reducing from one diploid cell (2N) to four haploid cells (N); this process is known as meiosis. An important peculiarity of meiosis is the recombination that takes place between DNA replication and meiosis I. Through recombination there is a genetic exchange between the homologous chromosomes.

2.1. Cell Cycle in fission yeast

The fission yeast (*Schizosaccharomyces pombe*) cell cycle has some peculiarities, which differ from other eukaryotes (Fig. 1):

- A short G1 phase takes place between mitosis and cell septation, because the DNA replicates immediately after mitosis. That is the reason why the cells have two nuclei during G1 phase.

- During S phase, DNA replication is initiated before completion of the cytokinesis, so newborn daughter cells are already in G2. This makes that asynchronous growing cultures have a peculiar flow cytometry profile compared to other eukaryotes, showing a single peak of 2C DNA content.

- G2 phase occupies around 70% of the cell cycle, thereupon the most of cells will be in G2 phase in an asynchronous culture.

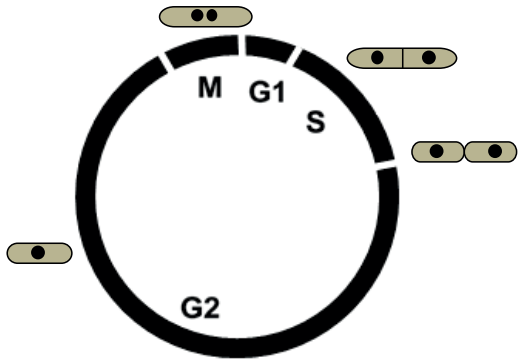

Figure 1. The fission yeast cell cycle.

S. pombe grows exponentially by length extension during G2 phase until the onset of mitosis, and completion of cellular division with the formation of septum. This characteristic growth makes possible to place a cell in a specific phase of cell cycle by simple microscopy. This feature also allowed the isolation of *S. pombe* cell cycle mutants by Paul Nurse in the mid-1970, and the identification of *cdc* genes (cell division cycle) (Nurse et al., 1976). This finding was pioneering in the investigation of cell cycle control. Some of the strains with cell cycle defects are easily recognized, because they show characteristic changes in cell length at restrictive temperature (36ºC), but they grow normally at permissive temperature (25ºC) (Fig.2).

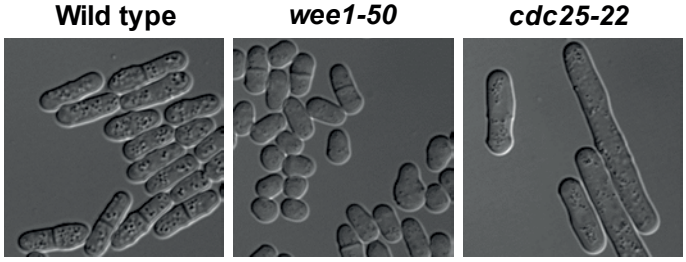

Figure 2. *S. pombe* cells. Wild-type cells have a size of 9-10 µm, but *cdc25* mutant grows much longer, and *wee1* mutant divides at a much smaller size when they grow at restrictive temperature.

As most of these genes are essential, the strains carrying this type of mutations were isolated as temperature-sensitive (ts) mutants. Many of these mutants have been divided into two groups. In the first group are *cdc* mutants, which are unable to progress through the cell cycle at nonpermissive temperature; they are represented as very long cells because they continue to grow in length but fail to divide. One of these strains is the Cdc25 phosphatase mutant, *cdc25-22*. On the other hand, *wee* mutants form smaller cells because they enter rapidly in M phase, shortening

G2 and bypassing cell size checkpoint, which normally prevents small cells from entering mitosis. Mutant strains that are smaller when they reach mitosis extend their G1 phase until they achieve the threshold of size required to progress through cell cycle (Fig. 2).

2.2. CDK/Cyclin complexes

The eukaryotic cell cycle is a highly regulated process that depends on mechanisms, which ensure faithful replication and segregation of the genetic material. Cyclin-dependent kinases (CDKs) are the central regulatory enzymes in the progression of cell cycle (Morgan, 1997). They regulate the transition through different phases of the cell cycle through their binding to the activating subunits, the cyclins.

In mammalian cells, multiple CDKs can associate with different cyclins to drive distinct events in the cell cycle. In simpler eukaryotes such as yeasts, a single CDK enzyme associates with several cyclins. For example, in budding yeast, a single CDK protein (Cdc28) can associate with as many as nine different cyclins: three G1 cyclin (Cln1-3), two S phase cyclins (Clb5 and Clb6) and four mitotic cyclins (Clb1-Clb4) (Enserink and Kolodner, 2010). The concentration of the cyclins can be regulated transcriptionally, post-transcriptionally or by controlling protein synthesis and degradation rates (Bloom and Cross, 2007).

In fission yeast, the oscillation activity of the cyclins is controlled by the master CDK, Cdc2, which binds to different cyclins, such as Cdc13, Cig2, Cig1 and Puc1, depending on the phase of the cell cycle (Hayles et al., 1994).

Cdc13 is the single essential B type cyclin required for entry into mitosis (Fisher and Nurse 1996; Mondesert et al., 1996). The association of Cdc2/Cdc13 kinase with replication origins and the phosphorylation of pre-replication components avoid chromosomal reduplication, ensuring the maintenance of ploidy (Wuarin et al., 2002). Cdc13 levels fluctuate during the cell cycle, raising progressively during S phase and G2, and reaching a maximum peak when cells are in mitosis (at metaphase). Finally, Cdc13 drops to minimum levels in anaphase due to its proteolytic degradation by the APC complex (Creanor and Mitchison 1996; Yamano et al., 2000). This degradation results in Cdk1 inactivation and reactivation of phosphatases such as PP1, PP2A, and Cdc14 (Grallert et al., 2015). New synthesis of Cdc13 is initiated in the next S phase.

Cig2 is a B-type cyclin, which promotes S phase initiation (Connolly and Beach 1994; Mondesert et al., 1996). Cig2 has a role in the regulation of the S phase, associating with specific regions of chromatin, like promoters of genes regulated by the Cdc10 transcription factor. Cig2/Cdc2 may prevent endoreduplication through the phosphorylation and degradation of Cdc18, a protein necessary to recognize the replication origins (Wuarin et al., 2002); or by suppressing transcription through the Cdc10/Res1/Res2 complex (Ayté et al., 2001). Cig2 is degraded during G2 and M phase by the SCF complex, while the APC complex degrades Cig2 during anaphase and G1 (Yamano et al., 2000).

Cig1 is a B type cyclin, with a minor contribution to the onset of S phase (Fisher and Nurse, 1996; Mondesert et al., 1996). Cdc2/Cig1 is implicated in the phosphorylation of Rum1 (a Cdk1 inhibitor) inducing its degradation (Benito et al., 1998). It was shown that Cig1,

as Cdc13, is completely degraded during mitosis via the APC complex (Blanco et al., 2000).

Puc1 is closely related to *S. cerevisiae* Cln cyclins. It was described that Puc1 promotes progression through G1, but its role remains unclear. Additionally, Puc1 plays an active role in regulating the length of G1 (Martin-Castellanos et al., 2000).

2.3 G1-S transition, Start

One of the most important points of cell cycle control takes place at the end of the G1 phase in a process called Start in yeast and Restriction Point in mammalian cells. At this moment of the cell cycle, cells decide to continue a new vegetative cycle or to enter in a sexual cycle or remain in a quiescent state. This is a point of no return and it triggers a new round of cell division.

External factors are determinant to promote or not the progression through Start. In the case of yeast, external nutrient levels are crucial to determine if cells commit a new cell cycle. In mammalian cells, proliferation and passage through the Restriction Point depends on the appropriate extracellular signals (mitogens) and in many tissues cells may stay permanently in the G0 quiescent state (Pardee, 1989). Cell cycle events are largely independent of extracellular factors once cells enter into S phase.

Passing through Start requires the activation of specific CDKs and the activation of a transcriptional program, which involves from tens (in yeast) to hundreds of genes (in metazoans) required for the early events of the cell cycle (Bertoli et al., 2013). In *S. pombe*, this transition depends on the CDK Cdc2 and the protein complex Cdc10/Res1/Res2, which is part of the transcription factor MBF (see

below) (Simanis et al., 1987). In *S. cerevisiae*, the key regulators of the G1-S transition are the CDK Cdc28 and two related transcription factors: SBF and MBF (Epstein and Cross, 1992).

In higher eukaryotes, a failure to proper regulate cell cycle entry can result in abnormal division and is frequently associated with cancer (Massague, 2004).

2.4 DNA replication and S phase

The duplication of genetic material takes place during S phase, when DNA synthesis starts at multiple replication origins located along the chromosomes. The accurate control of DNA replication is of vital importance to avoid failures in duplication of genome, which lead to aneuploidy of the daughter cells or to increased gene dosage. Assembly and disassembly of protein complexes at the origin of DNA replication is crucial in the control of the S phase.

The DNA replication process starts early in the cell cycle. The origin recognition complex (ORC) composed of six proteins (ORC1–6) binds to the origins of replication (ORI) during M phase, peaking at the M/G1 transition (Wu and Nurse, 2009). In *S. pombe* the homologue of the Orc4 subunit, Orp4 recognizes DNA through its AT-hook motif (Chuang and Kelly, 1999) and binds AT-rich islands located preferentially in intergenic regions (Segurado et al., 2003). This recognition differs from *S. cerevisiae*, where origins of DNA replication are much simpler and contain autonomously replicating sequences (ARS) (Newlon and Theis, 1993). Next, in G1, Cdc18 (CDC6 in mammalian cells and *S. cerevisiae*) and Cdt1 are recruited in a process called origin licensing and form the pre-replicative complexes (pre-RC) together with the six subunits of the replicative

MCM helicase that unwinds the DNA (Takeda and Dutta, 2005). The MCM complex is loaded at origins in an inactive form and is activated in a manner that requires CDK and Hsk1 DDK (Dbf4-dependent kinase) phosphorylation, which allows recruitment of additional factors, including Cdc45. When CDK becomes active in late G1 promotes complex assembly and replisome loading, coordinating the timing of replication initiation at hundreds of origins. In addition, CDK inhibits any further licensing (Siddiqui et al., 2013).

Finally, the active origin is unwound, and the replication forks are established. The process of starting replication is called origin firing. Origins fire from hundreds of different chromosomal sites in a stochastic way, in which the number of AT tracts in a sequence is the major determinant (Dai et al., 2005). Not all the origins fire at the same time: euchromatic regions have more origins that fire early in S-phase, and heterochromatic regions are enriched in origins that fire late (Bell and Dutta, 2002). The number of active replicons increases gradually, peaking at mid S-phase, and then decrease toward the end of S-phase. In this way, the accumulation of active replicons on unreplicated regions promote end of DNA replication (Kaykov and Nurse, 2015).

The increasing CDK activity trough S phase is essential to inhibit the assembly of a new pre-RCs and therefore, to ensure a single round of DNA synthesis per cycle. An example of this regulation is the phosphorylation of Cdc18 by Cdc2, which marks Cdc18 for ubiquitin-mediated proteolysis (Kominami and Toda, 1997).

It is remarkable to mention that in *S. pombe* due to the extremely short G1 phase, the G1 events necessary for the onset of S phase begins during the previous cycle, at the same time that mitotic events take place (Nurse and Thuriaux, 1977).

2.5 G2/M regulation

The onset of mitosis is regulated principally by the phosphorylation of the CDK, Cdc2. Wee1 is the kinase responsible for the phosphorylation of Tyr15 and the subsequent inhibition of Cdc2 (Russell and Nurse 1987; Parker et al., 1992). The phosphatase Cdc25 dephosphorylates this conserved residue, activating Cdc2 and triggering mitosis (Fig. 3) (Russell and Nurse, 1986; Gould and Nurse, 1989; Moreno et al., 1989). Two pathways regulate Tyr15 phosphorylation upstream of Wee1 and Cdc25: the mitogen-activated protein kinases of the nutritional stress response (SR) and the cell geometry sensing (CGS) pathways. The SR pathway links the MAP kinase Sty1 with the recruitment of Polo kinase (Plo1) to the spindle pole body and the subsequent CDK activation (Petersen and Hagan 2005; Petersen and Nurse, 2007), or the kinase complex TORC1 with the activation of greatwall-endosulfine (Ppk18-Igo1) pathway that inhibits protein phosphatase PP2A promoting entry into mitosis (Chica et al., 2016); therefore, this pathway controls the nutritional modulation of the mitotic entry. The CGS pathway is composed by the Cdr1 and Cdr2 kinases, which phosphorylate Wee1 in response to cell geometry, and involves polar gradients of the DYRK-family kinase Pom1 along the axis of the cell (Martin and Berthelot-Grosjean 2009; Moseley et al., 2009) (Fig. 3).

More recently, other proteins have been implicated in the regulation of the G2/M transition independently of Tyr15 phosphorylation. These proteins might be involved in cellular localization or promote posttranslational modification of Cdc2 (Navarro and Nurse, 2012).

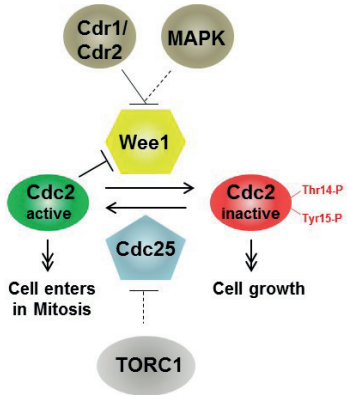

Figure 3. Regulation of Cdc2 phosphorylation. Wee1 mediate inactivation of Cdk1 through the phosphorylation of Tyr14 and Tyr15. In other way, phosphatase Cdc25 dephosphorylates Cdc2, promoting entry in Mitosis. The Stress-nutritional response (SR) pathway through MAPK and TORC1 and the cell geometry-sensing pathway (CGS) through Cdr1/Cdr2 regulate both Wee1 and Cdc25.

2.6 Temporal ordering of the cell cycle

The proper timing of events destined to DNA replication and segregation of sister chromatids is an essential characteristic of the cell cycle. The fluctuating activity of different CDKs is crucial for cell cycle progression. With the discovery in yeast that the same CDK promotes both the entry in S phase and mitosis (Nurse and Bissett 1981; Piggott et al., 1982) brought the question of how a CDK can act at different phases of the cell cycle and still maintain the right sequence of events. Three ideas, not mutually exclusive, might help to solve the question about the cell cycle ordering.

- Checkpoints that control late events do not occur until earlier events have been completed (Hartwell and Weinert, 1989).

- Specific cyclins that associate with the Cdk1 at different times establish the order of the different phases of the cell cycle by phosphorylating specific substrates (Surana et al., 1991).

- Quantitative levels of Cdk1 activity triggers first S phase and later Mitosis (Stern and Nurse, 1996; Coudreuse and Nurse, 2010).

2.6.1 Checkpoints mechanisms

Checkpoints are mechanisms, which ensure that certain conditions are fulfilled to let continue with the cell cycle. Hartwell and Weinert proposed the idea that the ordering of cell cycle depends on a checkpoint that prevents the onset of mitosis until DNA replication has been completed (Hartwell and Weinert 1989). The idea that there is a control mechanism ordering the cell cycle comes from the observation that mutations in DNA polymerases, or the block of ribonucleotide reductase by hydroxyurea prevent cell cycle progression into mitosis (Hartwell, 1978). According with this idea, elimination of the checkpoint may have catastrophic consequences for the cell. But current understanding places the checkpoint mechanisms in the recognition of damage rather than the ordering of the cell cycle (Bartek et al., 2004). Some evidences question this theory: for example, it has been proved that when there is an inefficient replication origin licensing, the DNA replication progress more slowly during S phase and mitosis starts before DNA replication is completed (Lengronne and Schwob, 2002). This fact points that there should exist other mechanisms that order the different phases of the cell cycle. Moreover, a clear example that checkpoints are not responsible for ordering of cell cycle is the

embryonic cell cycle of *Xenopus laevis*, which proceed without functional checkpoint. In this organism, cell divisions are simplified cell cycles that alternate between DNA replication and mitosis without cell growth or gap phases and in a highly synchronous manner (Blow and Laskey 1986; Hutchison et al., 1987).

2.6.2 Cyclin specificity

Cyclins are essential regulators of CDKs that activate the kinase activity and target it to its substrates. The discovery of G1 cyclins in budding yeast Cln1-Cln3, and cyclins required for mitosis Clb1-Clb4, suggested that a network of cyclins control the passage through cell cycle, with one cyclin specific for each phase of the cycle; therefore, the sequential expression of the cyclins could be responsible for ordering the different phases of cell cycle. However, various researches have shown by deletion or replacement of individual or a combination of S phase cyclins that some cyclins can be dispensable. For example, the ordering of cell cycle remains unaltered if S phase cyclins are replaced by mitotic cyclins in both budding yeast and vertebrates (Hu and Aparicio, 2005; Moore et al., 2003). One of the best examples of cyclin replacement is the “minimal CDK control network” generated in *S. pombe*, where a single cyclin, Cdc13, is sufficient to ensure the proper progression of cell cycle (Coudreuse and Nurse 2010). In addition to cyclins, CDKs can also be eliminated in higher eukaryotes, where CDK1 can substitute for all the other interphase CDKs (CDK2, CDK3, CDK4 and CDK6) in murine cell lines (Santamaria et al., 2007). In contrast, mitotic cyclins are essential. Therefore, most cyclins appear dispensable for the correct cell cycle progression.

2.6.3 Quantitative model

This model is based on quantitative changes in the level of CDK activity, which are crucial for initiation of S phase and mitosis (Stern and Nurse, 1996). To support this model, a single cyclin-CDK chimera between cyclin Cdc13 and the CDK1 Cdc2 (Cdc13-L-Cdc2) was generated in *S. pombe*, where it could substitute all the mitotic cyclins *(Δcig1*, ∆*puc1*, ∆*cig2*, ∆*cdc13*). This Cdc2 was mutated to make it sensitive to ATP analogues (Cdc2as) (Dischinger et al., 2008). Applying different concentrations of ATP analogue (NM-PP1), the passage through S phase and mitosis could be regulated and it was possible to demonstrate that the threshold of active CDK is lower for S phase than for Mitosis (Coudreuse and Nurse, 2010).

Subsequent studies identified three types of CDK substrates: early substrates which are related with DNA replication and are phosphorylated within G1-S transition and across the cell cycle; mid substrates, which show an increased phosphorylation at both transitions, peaking at G2/M; and late substrates, that are related with mitotic process and are phosphorylated at G2/M. All these substrates are simultaneously dephosphorylated at mitotic exit, when Cdc13 is targeted for degradation by APC. In these studies, it was shown that some mid substrates respond to cyclin specificity, therefore cyclin-substrate specificity provides a further layer of regulation in the timing of some CDK substrates (Swaffer et al., 2016).

Finally, the fact that there are substrates than can be phosphorylated along the complete cell cycle raises the question of how a CDK phosphorylates one substrate earlier than another. It is known that CDK substrate phosphorylation is quickly lost when CDK activity is chemically inhibited (Holt et al., 2009) suggesting that phosphatases counteract CDK activity.

In budding yeast, the phosphatase Cdc55 counteracts CDK phosphatase Cdc55 counteracrts CDK phosphorylation, dephosphorylating specifically threonines from early substrates (but not serines). Therefore, late substrates are more sensitive to Cdc55, establishing an increased CDK threshold for their phosphorylation (Godfrey et al., 2017).

With all this information, it seems to be that a quantitative mechanism, where the central player CDK1 and phosphatases drive multiple reactions of phosphorylation/dephosphorylation, promotes the progression of cell cycle. This progression is produced when specific thresholds of the phosphorylation events, marked by selectivity of phosphatases, are passed. Together with this quantitative model, cyclin specificity and checkpoint contribute to orchestrate the proper timing of the cell cycle.

3. Cell-cycle dependent transcription in fission yeast

In fission yeast, DNA microarrays originally identified around 500 genes that showed a cell cycle-associated periodic transcription (Rustici et al., 2004; Peng et al., 2005; Oliva et al., 2005; Marguerat et al., 2006). These genes were expressed at different times, so they could be grouped in different transcriptional waves; therefore, the genes of each wave peak at specific phase of the cell cycle.

3.1 Gene expression

Phase-specific regulation of gene expression through transcription plays a central role in the regulation of cell cycle. The molecular mechanism that controls the synthesis of messenger RNA (mRNA) by RNA polymerase II (Pol II) has been described in detail (Fig.4). Upstream of the start codon of the ORF (Open Reading Frame) is located the promoter region. Within this region, there are short conserved DNA motifs, which are recognized by diverse transcription factors. The first of these motifs is the TATA box, localized 25-40 bp upstream of the Start of transcription, and where the TATA binding protein (TBP) binds to TBP associated factors (TAFs). These general transcription factors (GTFs) recruit the RNA poll II to form the transcription preinitiation complex (PIC), then mRNA synthesis starts (Shandilya and Roberts, 2012; Thomas and Chiang, 2006). The Mediator complex is a multiprotein co-regulator, which functions as a link between the general transcription machinery and gene-specific transcription regulators (Biddick and

Young 2005; Bjorklund and Gustafsson 2005). Some genes, known as housekeeping genes, only need RNA pol II, GTFs and the Mediator complex to be transcribed at relatively constant rates. But for inducible genes, the promoter region structure is more complex. Additional DNA motifs upstream of the TATA box are present. These motifs are known as upstream activating sequences (UASs) or upstream repression sequences (URSs), where further transcription factors are able to bind regulating RNA pol II activity and promoting gene expression at specific time or under specific conditions (Guarente, 1988). In the context of cell cycle, an extensive number of UASs has been described, in addition to specific transcription factor complexes, which bind to these UASs regions. Regulation of transcription can be affected not only by the activity of transcription factors; there are mechanisms that regulate the expression of these transcription factors like their nuclear location, posttranslational modifications and degradation.

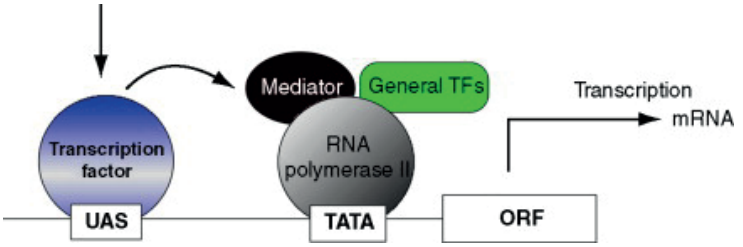

Figure 4. Molecular mechanism of gene transcription controlled by RNA polymerase II. The opening reading frame (ORF) of a gene is transcribed into mRNA by the joint action of RNA polymerase II, general transcription factors (TFs), Mediator complex, and the action of specific transcription factors, which bind to upstream activating sequences (UAS) of the promoter (adapted from McInerny 2011).

3.2 Cell cycle-specific transcription

Transcriptome analyses have identified four major clusters of gene expression whose periodicity coincides with the principal stages of the cell cycle. Each cluster is under the control of a specific transcription factor, which binds to specific motif within the promoter:

- The MBF (MCB-Binding Factor) complex is responsible for activating M/G1 and early G1 genes that encode for proteins involved in both the G1-S transition and the onset of DNA synthesis during S phase. MBF upregulates 80 genes containing the MCB (MluI Cell Cycle Box) sequence in their promoters (Lowndes et al., 1992).

- In late G1, a set of periodic genes involved in cytokinesis is regulated by the transcription factor Ace2. Some of these genes code for the main hydrolytic enzymes responsible for septum dissolution (Alonso-Nunez et al., 2005).

- The expression of histone genes in S phase depends on the transcription factor Ams2, which binds to GATA motif at promoters (Takayama and Takahashi, 2007). It is interesting to note that although *ams2* expression relies on MBF, there is no evidence for a direct connection between MBF-dependent transcription and the expression of histone genes.

- A small group of weakly induced genes have been identified during G2 phase; among them we can find *spd1*, *psu1*, and *rds1*. All these genes have a putative common sequence UAS localized at promoters (Rustici et al., 2004), but not transcription factor has been identified yet.

- Finally, a number of genes induced at mitosis are regulated by the PBF (PCB-Binding Factor) complex. Most genes encode products required for processes at the end of the cell cycle, such as chromosome separation, cytokinesis, and septation. The promoters of these genes are under the control of PBF contain forkhead-binding sites and/or the DNA-binding sites named PCBs (Pombe Cell cycle Boxes) (Anderson et al., 2002). PBF is formed mainly by two forkhead-like transcription factors, Fkh2 and Sep1; the MADS box-like protein, Mbx1; the kinase Plo1, the phosphatase Clp1, and the anillin-like protein Mid1 (Buck et al., 2004; Bulmer et al., 2004; Papadopoulou et al., 2008; Agarwal et al., 2010; Papadopoulou et al., 2010). Nevertheless, only the two forkhead transcription factors are necessary for the periodicity of the M phase genes. Both transcription factors have complementary and opposing roles in the regulation of mitosis: Sep1 has an activator role, triggering maximum transcription levels when it binds to mitotic gene promoters; for this reason, *sep1* mutants cause reduced transcription. In contrast, Fkh2 is only bound to PCB promoters when gene expression is reduced, which supports its negative role (Suarez et al., 2015). Recently it was discovered the transcription factor, Sak1, an essential gene and a member of the highly-conserved RFX family that activates most of the mitotic genes; which means that Sep1 mainly has an active role in genes involved in septation (Garg et al., 2015).

3.3 Transcriptional program during G1-S transition

During the G1 phase, CDK activity promotes the pass through G1-S transition and the onset of S phase. In G1-S transition proteins that regulate downstream cell cycle events are produced. Therefore, the G1-S transcriptional program is implicated in two fundamental aspects of cell cycle regulation: cell division cycle control and maintenance of genome stability.

The mechanisms of G1 cell cycle control are highly conserved from yeast to metazoans. In general terms, CDK1 phosphorylates transcriptional inhibitors and releases them from transcription factors, activating G1-S genes as G1 cyclins. The expression of these cyclins promotes a positive feedback loop. Among the expressed genes, negative regulatory proteins are expressed as well. These regulatory repressors are part of a negative autoregulatory feedback loop. They bind to G1-S gene promoters to turn off transcription when cells progress to S phase. Moreover, these transcriptional repressors are targeted by the checkpoint kinases to regulate G1-S transcription during cell arrest.

3.3.1. *S. pombe:* MBF

MBF (MluI cell cycle box (MCB) Binding Factor) belongs to a family of transcription factors that regulate cell cycle progression; specifically, MBF contributes to the timely expression of early genes, which regulates the G1 to S phase transition.

MBF is a multisubunit transcription factor composed by the core subunits Cdc10, Res1 and Res2; and few other regulatory components (Fig. 5). MBF mediates G1-S specific transcription of

around 80 genes with roles in DNA replication and repair, cell cycle control and other related functions (Baum et al., 1997; Peng et al., 2005; Rusciti et al., 2004; Aligianni et al., 2009). Well-established MBF targets are *cdc22* (ribonucleotide reductase) (Fernandez Sarabia et al., 1993), *cig2* (S phase cyclin) (Connolly and Beach, 1994), *cdc18* and *cdt1* (both are part of the DNA replication machinery) (Kelly et al., 1993; Hofmann and Beach 1994).

All these genes share a DNA motif in the promoters, which are called MCB elements (ACGCGTNA), due to the presence of an *Mlu*I restriction site in the consensus sequence. MCB motifs are present in several copies in the promoter, being the number, orientation and spacing of this motif essential for the activation of transcription (Maqbool et al., 2003). Furthermore, isolated MCB elements can provide cell cycle-regulated transcription of a heterologous gene (Lowndes et al., 1992).

MBF is a high molecular weight complex (about 0.7 MDa) identified by its binding activity to DNA motifs using gel retardation assay. Despite this, only few components of the complex have been described so far. The best-characterized MBF components (Cdc10, Res1 and Res2) have constant protein levels over the cell cycle (Simanis and Nurse 1989; Whitehall et al., 1999). Moreover, it is well known that the core subunit Cdc10 binds to its target promoters throughout the cell cycle (Wuarin et al., 2002), but MBF is only active at the end of Mitosis, during G1 and S phase, pointing that regulation of MBF-dependent transcription is not achieved merely by modulating the DNA binding activity of the complex. It is still unclear how the complex is activated at M phase and inactivated during S phase, remaining inactive during G2; but strong evidences indicate that MBF is regulated by posttranslational modifications of the core

components, additional proteins or proteins that modify chromatin structure.

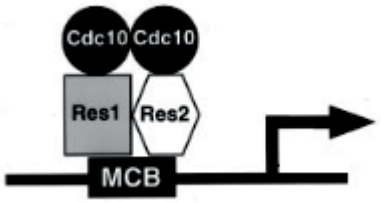

Figure 5. Model of MBF complex. MBF complex contains the heterodimers Res1/Cdc10 and Res2/Cdc10, which bind to MCB elements located in the promoters (adapted from Whitehall et al 1999).

Cdc10

Cdc10 is considered as the active component of the complex, since when it is functionally inactivated the transcription is reduced. Moreover, cells harbouring temperature-sensitive *cdc10* alleles are arrested at Start when they are grown at the restrictive temperature (Simanis and Nurse, 1989). Cdc10 binds DNA through its partners Res1 and Res2, which are the DNA binding subunits of the complex.

The C-terminal part of the protein has an essential role for the function of MBF, since it participates in the formation of the complex (Reymond et al., 1993). A truncated form of the protein (Cdc10-C4), lacking 61 amino acids of its C terminus, leads to a highly induced transcription of MBF genes throughout cell cycle when is grown at low temperature (McInerny et al., 1995). Cdc10 contains ankyrin motifs in its sequence (Aves et al., 1985) (Breeden and Nasmyth, 1987). Ankyrin is a 33-residue sequence motif, which is present in many proteins spanning a wide range of functions and is considered as a scaffold for protein-protein interaction. The number of repeats per protein varies from one to 33, for example Cdc10 contains 24 copies of this repeat (Chakrabarty and Parekh, 2014). In the case of

MBF complex, ankyrin repeats seem to have a role in stabilizing the complex through interactions with other proteins rather than direct interactions Cdc10/Res1/Res2 (Ayte et al., 1995; Whitehall et al., 1999).

Overexpression of Cdc10 under a strong inducible promoter (*nmt1*) does not affect gene expression of MBF dependent genes during the cell cycle (White et al., 2001). The fact that its regulation is maintained despite this overexpression reinforces the idea that other regulators, rather than the amount of protein, control the activity of MBF complex.

Res1 and Res2

Res1 and Res2 represent the DNA binding subunit of the complex. Both proteins have similar size and overlapping functions. Res1 and Res2 have a strong homology in their N-terminal region, through which bind to DNA, and also in the two-central located ankyrin motifs, which do not play a role in the formation of MBF (Ayte et al., 1995) but are essential for mediating cell cycle-regulated transcription (Whitehall et al., 1999). Res1 and Res2 are different in the C-terminal portion, which have several domains required for their functions (Fig. 6). Therefore, the functional specificity of both proteins is solely determined by the C-terminal portion (Sturm and Okayama, 1996). The C-terminal serves as a platform to interact with Cdc10 as well (Ayte et al., 1995; Zhu et al., 1997).

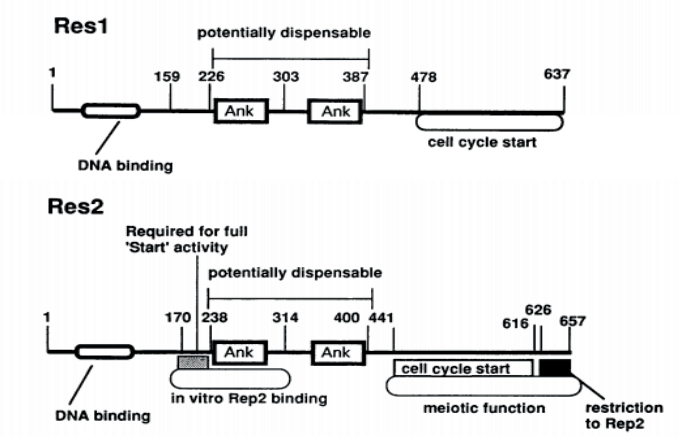

Figure 6. Scheme of functional domains in Res1 and Res2. Res1 and Res2 proteins are represented indicating the domains required for their different functions. The numbers indicate the position of amino acids (adapted from Sturm and Okayama 1996).

Res1 was identified as a suppressor of *cdc10* (Tanaka et al., 1992). Overexpression of Res1 or only its N-terminal portion can rescue the lethal phenotype of strains bearing a temperature sensitive allele of *cdc10*. For its part, the overexpression of Res1 in a wild type context induces growth arrest in G1 (Ayte et al., 1995). While *cdc10* is an essential gene, *res1* can be deleted and viability is maintained, although disruption of *res1* causes growth defect at high and low temperature. Moreover, *res1* mutant cells are unable to normally induce transcription of MBF-dependent genes, indicating that Res1 plays a role in the activation of transcription (Tanaka et al., 1992).

Res2 has its main role in meiosis, where is required for premeiotic DNA synthesis and spore formation (Miyamoto et al., 1994). Although Res2 also forms part of the mitotic MBF complex (Miyamoto et al., 1994; Ayte et al., 1997; Whitehall et al., 1999). It has been described that overexpression of Res2 can suppress

Δ*res1* defects (Miyamoto et al., 1994). *Δres2* cells show derepression of some MBF-dependent genes, which represents the opposite effect that *res1* mutant (Baum et al., 1997). This observation may be explained because the binding of the co-activator Rep2 is decreased (see below).

These opposite effects of Res1 and Res2 led to think that changes in the stoichiometry of both proteins in the complex could alter the activity of MBF, inactivating the complex during G2 (Baum et al., 1997). But later studies demonstrated that Res proteins are associated with Cdc10 throughout the cell cycle, arguing against the previous model (Whitehall et al., 1999). More recent microarray data have shown that the regulation is more complicated, and each Res1 and Res2 protein is required for the activation and repression of a different subset of genes (Dutta et al., 2008). For instance, *Δres1* cells have defects to induce transcription of *cdc18*, *cdt1* and *cig2* genes, whilst *Δres2* cells show an increase of expression of most MBF-dependent genes except *yox1*, *cig2* and *mik1*. Furthermore, in *res1* mutants, *cdc22* is upregulated, while *cdc18* is downregulated (Baum et al., 1997; Dutta et al., 2008). Thus, deeper studies about these proteins are necessary to know more about the regulation of MBF.

Co-repressors Nrm1 and Yox1

MBF binds to MCB motifs throughout the cell cycle but is maintained in an inactive state by two co-repressors, Nrm1 (Negative Regulator of MBF targets) and Yox1. Both *nrm1* and *yox1* genes have been described as MBF targets, and therefore participate in a negative feedback loop (de Bruin et al., 2006; Aligianni et al., 2009). The deletion of either of the two genes leads to an increase and constitutive MBF transcription. Moreover, both

co-repressors accumulate during S phase, and their binding to MBF promoters peaks when the transcription is repressed. Nrm1 binds MBF through Cdc10, and more specifically through its C terminal (de Bruin et al., 2008). Yox1 homeodomain protein physically associates with the MBF complex through Nrm1 (Gómez-Escoda et al., 2011). Yox1 has three phosphorylation sites: Ser6, which is a target of CDK when MBF dependent transcription is activated; and Ser114 and Thr115 which are phosphorylation sites for the Cds1 checkpoint (Caetano et al., 2011; Gomez-Escoda et al., 2011). Therefore, Yox1 and Nrm1 are both co-repressors of MBF transcription, but Yox1 needs Nrm1 to be loaded on MBF promoters.

Co-activator Rep2

Rep2 is one of the less characterized subunits of the MBF complex. *rep2* encodes for a zinc finger protein which was isolated as a suppressor of a temperature-sensitive *cdc10* mutant (Nakashima et al., 1995). The C-terminal domain of Rep2 contains a Res2 binding and a transcriptional activation domain, therefore, Rep2 interacts directly with Res2 and works as a co-activator of the MBF complex during mitotic cycle. However, it does not seem to contribute to the periodicity of the MBF genes (Tahara et al., 1998). Deletion of *rep2* shows a downregulation of MBF-dependent transcription (Baum et al., 1997) and cells deleted for *rep2* are unable to start a new cell mitotic cycle at low temperature (Nakashima et al., 1995). It has been proposed that levels of Rep2 oscillate along with cell cycle, and its degradation depends on APC (Anaphase-Promoting-Complex)/ Ste9 (Chu et al., 2009).

Other components of MBF

The mitotic cyclin Cig2 is the product of one of the MBF regulated genes. It has been described that Cig2 binds MBF via Res2 at the end of S phase and phosphorylates Res1 at residue S130. This phosphorylation inactivates the complex upon exit from S phase. Therefore, Cig2 forms an inhibitory feedback loop with MBF (Ayté et al., 2001). This finding was the first evidence of a direct regulation of MBF transcription by CDKs in *S. pombe.* Thus, there are two negative regulatory circuits to ensure the timing of MBF-dependent transcription (co-represors Nrm1/Yox1 and the CDK-cyclin Cdc2/Cig2), indicating the robustness of the regulation of this complex.

Rep1, a homolog of Rep2 (Nakashima et al., 1995), was first described as a component of the meiotic MBF, without a function in the control of mitotic transcription (Tanaka et al., 1992). Deletion of *rep1* does not influence growth properties (Sugiyama et al., 1994). However, overexpression of Rep1 in mitotic cycle results in deregulation of MBF genes, which become constitutively transcribed throughout the cell cycle (White et al., 2001). This evidence indicates that Rep1 could be considered a possible activator of the complex, at least during meiosis.

3.3.2 *S. cerevisiae*: SBF/MBF

In budding yeast, SBF and MBF are the two complexes involved in the regulation of the G1-S transcription program, which includes over 200 genes (Amon et al., 1993; Breeden, 1996; Bean et al., 2005). The MBF complex contains the Swi6 protein (a common subunit), and Mbp1 (DNA binding protein). MBF binds to promoter sequences containing the conserved MCB recognition sequence ACGCGTNA. MBF actives the expression of genes predominantly

involved in DNA replication (*POL1, POL2*), S phase initiation (*CLB5, CLB6*) and DNA repair (Bean et al. 2005; Ferrezuelo et al. 2010; Wittenberg and Reed 2005).

The SBF (Swi4 Cell-cycle Box) complex consists of Swi6 and the Swi4 (DNA binding protein). SBF recognizes the SCB (Swi4 Cell-cycle Box) sequence CRCGAA, but it can bind MCB elements as well (Partridge et al., 1997). SBF targets include genes involved in cell cycle progression (*CLN1, CLN2*), cell morphogenesis (*PCL1, PCL2*), spindle pole body duplication (*FKS1, FKS2*), and other growth-related functions (*GIN4*).

The distribution of the genes in different categories, depending on MCB or SCB sequences is not strict; and genome wide analyses have shown that actually there is a functional overlap between both complexes (Iyer et al., 2001). Otherwise, the inactivation of a single complex has a moderate phenotypic effect, but deletion of both Mbp1 and Swi4 leads to non-viability (Koch et al., 1993), suggesting that one of both transcription complexes is enough to ensure the transition G1 to S.

Despite the high functional overlap between SBF and MBF, there are considerably different mechanisms of regulation between the two complexes. SBF binds to target promoters at the beginning of G1 phase but is inhibited by the repressor Whi5 (Costanzo et al., 2004; de Bruin et al., 2004). The activation of G1-S genes is promoted by the CDK-cyclin Cln3-Cdc28, which is attached to the endoplasmic reticulum (ER) membrane (Wang et al., 2004b). In late G1, the chaperone Ydj1 releases Cln3-Cdc28 from the ER, allowing the phosphorylation of Whi5, MBF and SBF, and triggering the transcription of genes necessary for G1-S transition (Verges et al., 2007). Among these genes, two additional G1 cyclins, *CLN1* and *CLN2* are expressed. Both cyclins contribute to the nuclear exclusion

of Whi5 and complete a positive feedback loop (Skotheim et al., 2008). Recently it was described that Whi7, a homolog of Whi5, helps to retain Cln3 in the ER and collaborates with Whi5 in the repression of SBF-dependent transcription (Gomar-Alba et al., 2017). Upon S phase entry, SBF-dependent transcription is inactivated via mitotic cyclins phosphorylation of Swi4, which disrupts the binding of SBF to the promoters (Amon et al., 1993). For its part, MBF-dependent transcription is inactivated by Nrm1 via a negative feedback loop similar to *S. pombe* (de Bruin et al., 2006).

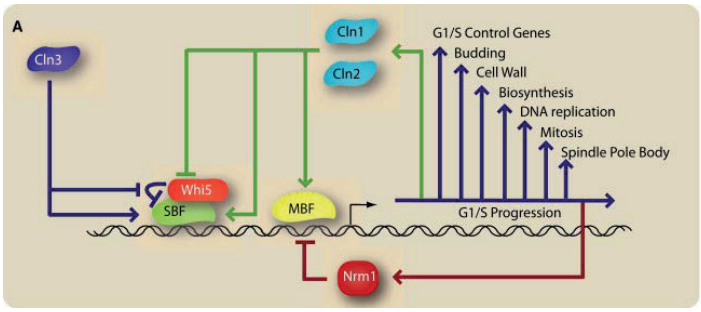

Figure 7. Diagram of the G1-S transition regulon. At the beginning of S phase SBF is inhibited by the repressor Whi5. When Cln3 is released from endoplasmic reticulum, phosphorylates and initiates Whi5 inactivation, allowing some G1-S transcription. Among the first genes induces are the cyclins Cln1 and Cln2, which complete the positive feedback loop through the inactivation and nuclear exclusion of Whi5 (adapted from Eser et al., 2011).

3.3.3 Mammalians: E2F/DP

In mammalian cells, the family of E2F transcription factors associates with its heterodimerization partner DP (Differentiation-related Polypeptide) to regulate the transcription of genes necessary for DNA synthesis and cell cycle progression through S phase. Although E2F is a large family of transcription factors, only E2F1-3 have a function as transcriptional activators, while E2F4-8 have a

function as co-repressors (Takahashi et al., 2000; Giangrande et al., 2004; Rowland and Bernards 2006; Lammens et al., 2009). E2F is inhibited by RB (Retinoblastoma tumor suppressor) protein family, which is composed of RB, and the RB-like proteins p107 and p130 (Dick and Rubin, 2013). RB associates with E2F1-3, while p107 and p130 are associated with the co-repressors E2F4-8 (DeGregori and Johnson, 2006). This association between RB and E2F/DP occurs via two domains: the pocket domain and the C-terminal domain (Chow and Dean, 1996).

RB is subjected to a fine tune regulation. The phosphorylation carried out by different kinases, such as Cyclin D-CDK4/6 and Cyclin E-CDK2, induces allosteric changes that block the function of the pocket domain. As well as being phosphorylated, RB is acetylated, methylated, ubiquitylated, and SUMOylated (Macdonald and Dick, 2012). These conformational changes in domains determine the association with E2F/DP and with co-regulatory subunit, like histone deacetylases (HDACs), histone methyltransferases or chromatin remodeling factors (Talluri and Dick, 2012; Uchida, 2016).

During the last 20 years it was accepted that cyclin D-CDK4/CDK6 inactivates RB during early G1 phase by progressive multi-phosphorylation, called hypo-phosphorylation releasing E2F transcription factors that specifically active the expression of the cyclin E, which then activates CDK2 and completes RB inactivation by hyper-phosphorylation at the Restriction Point. But recent biochemical studies have shown that RB has three different phosphorylated states: un-phosphorylated, mono-phosphorylated or hyper-phosphorylated (Narasimha et al., 2014). The first two forms of phosphorylation promote the binding of RB to E2F and also promote the recruitment of inhibitor proteins like HDAC1, which inhibits gene transcription by blocking access of transcription factors

to the promoters (Brehm et al., 1998). RB remains un-phophorylated in quiescent cells, but in early G1, RB is mono-phosphorylated by cyclin D–CDK4/6 complex in response to growth stimuli (Narasimha et al., 2014). At the restriction point, RB is multi-phosphorylated by cyclin E-CDK2, preventing its binding to E2F, which is critical for cells to promote cell cycle progression (Yao et al., 2008). The activated E2F transcription factors can recruit major histone acetyl transferases, the GCN5 complex (Lang et al., 2001) and the Tip60 complex (Taubert et al., 2004); allowing the recruitment of Pol II and promoting E2F target gene expression. The G1-S genes encode many of the factors required for DNA replication (PCNA, ORC1, CDT1), DNA repair (BRCA1/2), transcription (E2F1, E2F2, E2F7, E2F8,) or even Cyclin/CDK (cyclin A1, cyclin E1/E2, CDK2). G1-S cell cycle genes will be also expressed promoting a positive autoregulatory feedback loop that amplifies E2F activation function (Fig.8). During late S phase when DNA synthesis is completed, the decrease in CDK activity and the activity of PP1 (Protein Phosphatase 1) dephosphorylate RB, forming again a complex with E2F proteins and repressing the transcription of cell cycle progression genes (Kolupaeva and Janssens, 2013).

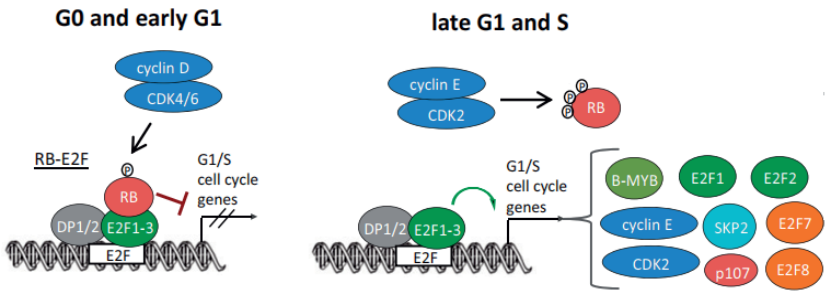

Figure 8. In G0 and early G1 phase, when RB is un-phosphorylated or mono-phosphorylated by cyclin D-CDK4/6, remains attached to E2F/DP complexes, blocking the expression of cell cycle genes. When cells are encouraged to start a new mitotic cell cycle, cyclin E-CDK2 becomes activated and phosphorylates RB,

allowing the activation of expression of G1-S cell cycle genes. Many of these genes codify for cell cycle regulators, which form a positive feedback loop that enables passage through the restriction point and entry into S phase (adapted from Fischer and Müller 2017).

E2Fs also have a significant role in the activation of apoptotic genes in response to DNA damage: E2F1 is phosphorylated by two kinases activated by double-strand DNA breaks: ATM (Ataxia-Telangiectasia Mutated) and CHK2 (Checkpoint Kinase 2). Also, RB is phosphorylated by CHK2 (Bartek and Lukas, 2003; Zannini et al., 2014). All these changes in posttranslational modifications result in repression of the E2F-dependent transcription and the activation of pro-apoptotic genes (Wang et al., 2004a). In addition to the function in cell cycle control, the RB–E2F has a role as suppressor of several genes involved in pluripotency, cellular metabolism, innate immunity, and cytokine signalling (Kitajima and Takahashi, 2017).

3.4 Missregulation of the G1-S transition

RB1 was the first gene identified as a potential tumour suppressor, because its mutation and inactivation coincide with the formation of retinoblastoma tumours (Knudson, 1971). Later, it was observed that almost all human cancer cells carry mutations in components of the RB pathway, thus the inactivation of the RB pathway seems to be indispensable for uncontrolled proliferation in cancer cells (Malumbres and Barbacid, 2001; Santamaria et al., 2007) RB pathway can be disrupted through differential mechanisms:

- Amplification and overexpression of D-type cyclins, which enhances RB inactivation and promotes cell proliferation. For example, Cyclin D1 is overexpressed in adenomas and in many breast cancers (Arnold and Papanikolaou, 2005; Diehl, 2002)

- Loss of p16 tumor suppressor. p16 is a direct inhibitor of CDK4/6 and is implicated in the activation of the senescence program, which limits aberrant proliferation and tumorigenesis. p16 is deleted or epigenetically silenced in a large number of cancers (Malumbres and Barbacid, 2001). Also, mutations of CDK4, which bypass p16 action are found in human melanomas (Wolfel et al., 1995).

- Direct perturbations of RB function. Loss of RB results in chromosomal instability inducing defects during DNA replication and abnormal chromosome segregation (Kennedy et al., 2000; Eguchi et al., 2007); prevents induction of cellular senescence, which represents a mechanism to response to oncogenic stress (Collado et al., 2007); promotes angiogenesis to overcome the growth-limiting effects of hypoxia (Gabellini et al., 2006) and is associated with increased metastatic potential through the increase of expression of genes like cyclooxygenase 2 (COX2), which increase cell motility in some cancers (Hui et al., 1999).

Therefore, understanding the modulation of p16/cyclinD-Cdk4,6/RB pathway and the RB status is an important goal of the medicine today for the development of tailored therapy.

3.5 S-phase checkpoints

Cells are continuously exposed to DNA damage putting at risk the genetic material of the cell and compromising its viability (Ciccia and Elledge, 2010). S phase represents a special vulnerable time for cells to counteract DNA damage, since lesions act as a physical impediment to the replicative machinery. DNA damage can be induced by exogenous sources or generated during DNA metabolism. Normal cellular processes may produce the alteration of DNA as a side effect. For example, it is possible to find DNA alterations caused by misincorporation of dNTPs during DNA replication (Lindahl and Barnes, 2000). Additionally, reactive oxygen species (ROS), generated from normal cellular metabolism (respiration in the mitochondria), oxidize DNA bases and produce DNA breaks (Hegde et al., 2008).

The exogenous sources are produced by physical or chemical agents from environmental, for example the ionizing radiation (IR) or ultraviolet (UV) light from sunlight (Hoeijmakers, 2009). IR can induce oxidation of DNA bases and generate single strand breaks (SSBs) and double strand breaks (DSBs) of the DNA. UV light produces DNA damage by covalent binding of pyrimidines, causing SSBs of the DNA, and replication fork pausing as well. Example of chemical agents are methyl methanesulfonate (MMS) that introduces mutations by methylation of bases in the DNA; or topoisomerase inhibitors camptothecin (CPT), which inhibits topoisomerase I, inducing the formation of SSBs or DSBs.

Checkpoints are responsible for maintaining cell viability by ensuring accurate completion of cell cycle processes and coordinating the DNA damage response (DDR). DDR is a signal transduction pathway that senses DNA damage and replicative

stress. In fission yeast the DDR is constrained mainly to the central sensor kinase Rad3 (the analogue of ATM/ATR in metazoan). Rad3 can activate two different checkpoints depending on the nature of the damage, driving a cascade of phosphorylation events, which recruits and activates DNA repair proteins at the damaged sites. Rad3 also activates the effector kinases Cds1 or Chk1 (CHK2 and CHK1 in metazoan). Both kinases regulate the transcriptional activity of MBF (Dutta et al., 2008; Dutta and Rhind 2009) and block the cell cycle progression through the phosphorylation and inhibition of the phosphatase Cdc25 preventing the activation of CDK1 (Walworth et al., 1993; Furnai et al., 1997).

The replicative stress checkpoint has as main objectives the arrest of the cell cycle, the stabilization of replication forks, and the regulation of MBF dependent-transcription. Hydroxyurea (HU) treatment is frequently used to induce the replicative checkpoint because it is a competitive inhibitor of ribonucleotide reductase, leading to deoxyribonucleotides depletion, fork stalling and the activation of the checkpoint (Kelly and Brown, 2000). When cells are treated with HU, and DNA synthesis checkpoint is activated, the sensor kinase Rad3 phosphorylates the effector kinase Cds1, which phosphorylates the co-repressor Yox1 at Ser-114 and Thr-115, releasing Yox1 from MBF, and activating the MBF-dependent transcription (Fig. 9) (Caetano et al., 2011; Gómez-Escoda et al., 2011). Therefore, cells treated with HU have all MBF transcripts upregulated (Dutta et al., 2008). The up-regulation of MBF-dependent genes is important for cells to survive during prolonged replication arrest. For example: *cdc22* encodes the large subunit of ribonucleotide reductase and it is necessary to synthetize extra dNTPs to complete DNA synthesis; *mrc1* encodes a replication fork protein that stabilize stalled forks and *mik1* produce a tyrosine kinase

that inhibit Cdc2 via phosphorylation (Dutta and Rhind 2009). More recently, it has been described that the catalytic subunit of TORC2 (Tor1) and its major substrate Gad8 bind to MBF and are required for its fully activation in response to replicative stress (Cohen et al. 2016).

The DNA damage checkpoint has an objective to block the cell cycle and the MBF transcription program until the damage has been solved. Chemical o physical agents as gamma-IR induce predominantly double-strand breaks, which cannot be efficiently repaired during G1 by a haploid cell, such as *S. pombe*, because their repair requires a sister-chromatid recombination template. As consequence of this effect, commonly the DNA damage checkpoint is induced. In this case, Rad3 phosphorylates the effector kinase Chk1, which phosphorylates directly the core subunit of the MBF complex Cdc10, at two different sites of its carboxy-terminal (Dutta and Rhind 2009). This phosphorylation leads to the release of the MBF complex from the promoters and thus, the inactivation of some genes from S-phase transcriptional program (Fig. 9) (Ivanova et al., 2013).

Therefore, in *S. pombe* the two main checkpoints are ensuring the accurate progression of the cell cycle through the modulation of MBF transcription with outcomes that go in opposite direction: whereas DNA replication checkpoint targets Yox1 causing de-repression of transcription, DNA damage checkpoint targets Cdc10 promoting the downregulation of MBF-dependent transcription (Fig. 9).

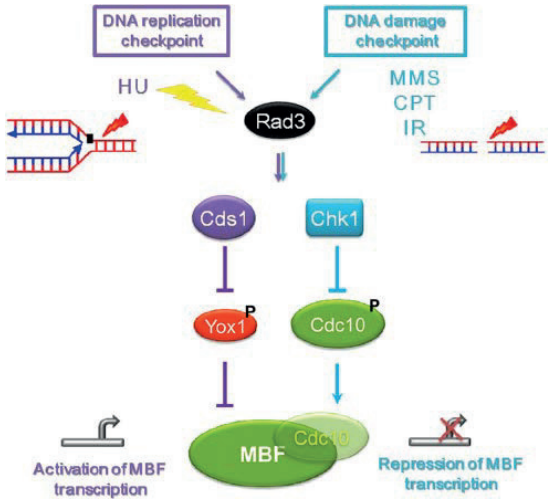

Figure 9. Integrative scheme of the DNA damage and the DNA replication checkpoints on the MBF complex. When fission yeast cells suffer replicative stress, Cds1 is activated, and phosphorylates Yox1, which no longer can bind the MBF complex, alleviating the transcriptional repression of genes required for DNA synthesis. But when cells are exposed to DNA damage, the effector kinase Chk1 directly phosphorylates the core component of the MBF Cdc10. The consequence of this phosphorylation is contrary to what happens under replicative stress, the release of Cdc10 from its target promoters and the repression of MBF-dependent transcription (adapted from Ivanova et al., 2013).

4. Chromatin remodeling and histone modifications

Chromatin is the association between DNA and nucleosomes that allows the compaction of the DNA in the nucleus. Chromatin can be classified into euchromatin, which is gene rich, less condensed and actively transcribed, and heterochromatin, which is gene poor, highly condensed and found at the pericentromeric DNA repeats, telomeres, and the mating-type locus in *S. pombe* (Grewal and Jia, 2007). Nucleosomes consist of histone octamers containing two copies of each of the four histone proteins H2A, H2B, H3 and H4, and 146 bp of DNA which are wrapped around the octamer (Richmond and Davey, 2003). Compacted DNA in nucleosomes generally is less accessible for DNA binding factors than free DNA (Bell et al., 2011). Indeed, the structure of chromatin can be regulated by several mechanisms like histone modifications, disruption of histone-DNA interaction, DNA methylation and the replacement of canonical histones by histone variants. Therefore, understanding the nucleosome positioning mechanisms is essential to better understand genome regulation. It is known that the major part of nucleosomes has well-defined positions, chiefly in regions that have important roles for regulation, like promoters or replication origins (Jiang and Pugh, 2009; Iyer 2012). Specifically, genes start with a characteristic wide (150-200 bp) nucleosome depleted region (NDR) just upstream of the transcription start site (TSS) that is flanked by the +1 nucleosome (the first nucleosome downstream of the TSS) and the -1 nucleosome (the first nucleosome upstream of the TSS), configuring the canonical promoter chromatin structure (−1 nucleosome/NDR/+1 nucleosome). +1 nucleosome is the first of a

set of nucleosomes that are extended into the gene body. Most functional UASs appear to be located in the nucleosome-depleted region of yeast promoters directly upstream of the TSS, because this depleted region presents unimpeded access to the binding of the transcription factors (Fig.10).

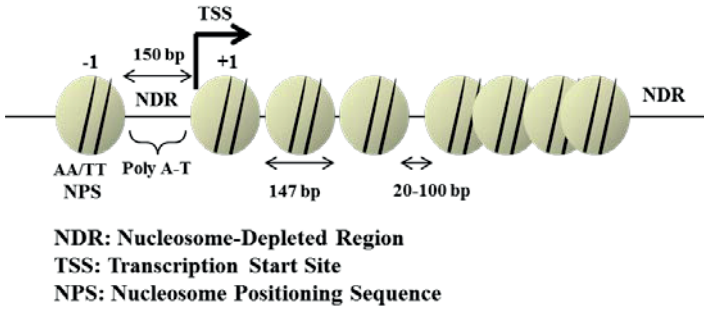

Figure 10. Scheme of nucleosome positioning around gene promoter. Gene promoter architecture typically have a large NDR (≈150bp) upstream of the TSS. The NDR contains poly (dA-dT) tracts, DNA sequences that resist bending. But, AA/TT or CC/GG dinucleotides repeating every 10 bp provide a curvature favorable to nucleosome formation and stability. When these repetitions are extender across ≈150bp can work as NPS.

4.1 Chromatin remodelers

Chromatin remodelers are ATPase proteins, which use the energy of ATP hydrolysis to relocate DNA around nucleosomes, disrupting histone-DNA interaction. There are at least five families of chromatin-remodeling complexes based on structural or functional domains: SWI/SNF, ISWI, INO80, CHD and Mi-2 (Mohrmann and Verrijzer, 2005). Fission yeast lacks ISWI-type remodelers. Figure 11 summarizes the different actions of the chromatin remodelers.

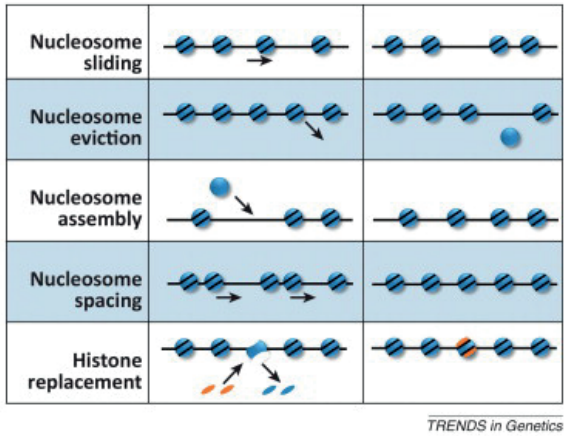

Figure 11. Modification activities carried out by chromatin remodelers and their end results on nucleosome placement or composition (adapted from Petty and Pillus 2013).

4.1.1 SWI/SNF

There are two subfamilies of remodeling complexes inside of this group: the SWI/SNF-class and the RSC. Both complexes function in the sliding and eviction of nucleosomes. Although both complexes have ATP-dependent nucleosome-remodeling activity, they have distinct compositions. In *S. pombe* SWI/SNF complex contains 12 different proteins (Snf22, Sol1, Snf5, Ssr1, Arp9, Snf59, Ssr2, Arp42, Ssr3, Ssr4, Snf30 and Tfg3), whereas RSC contains 13 different subunits (Snf21, Rsc1, Rsc9, Rsc4, Ssr1, Arp9, Ssr2, Arp42, Ssr3, Sfh1, Rsc58, Ssr4, Rsc7), some of them are shared with SWI/SNF (Monahan et al., 2008).

SWI/SNF controls the mRNA levels of 2-5% of all genes in budding yeast (Rando and Winston, 2012), being required for both activation and repression of transcription of a subset of genes; for example, it is necessary for the activation of transcription of ribosomal protein genes and heat shock factor genes (Shivaswamy and Iyer, 2008); SWI/SNF also represses the transcription of hexose

transport and iron uptake genes (Monahan et al., 2008). SWI/SNF mediates telomeric and rDNA silencing (Dror and Winston, 2004), DNA repair (Chai et al., 2005a) and regulates alternative splicing (Batsche et al., 2006; Waldholm et al., 2011; Patrick et al., 2015). Several studies suggest that SWI/SNF collaborates with other transcriptional regulators like SAGA complex (Sudarsanam et al., 1999; Govind et al., 2005; Mitra et al., 2006).

RSC regulates transcription of genes transcribed by RNA polymerases II and III, is necessary to maintain nucleosome free regions (NFRs) (Badis et al., 2008) and appears to have roles in cell cycle progression (Cao et al., 1997), as well as in kinetochore function (Hsu et al., 2003), sister chromatid cohesion (Huang et al., 2004), DNA repair (Shim et al., 2007) and maintenance of ploidy (Campsteijn et al., 2007).

4.1.2 CHD1

In fission yeast, this group contains two CHD1 (chromo-helicase/ATPase DNA binding remodelers) proteins, Hrp1 and Hrp3, which are required for chromosome segregation (Yoo et al., 2000), the maintenance of silencing at the centromeric region (Walfridsson et al., 2005; Shim et al., 2012) and the repression of cryptic promoter activity (Wang et al., 2005). CHD1 factors are localized in promoter regions where they help in the nucleosome disassembly near the transcription start site and regulate also nucleosome density in coding regions (Walfridsson et al., 2007). Therefore, CDH1 remodeler complex controls global chromatin structures regulating both euchromatin and heterochromatin.

4.1.3 INO80

This family includes the INO80 and the SWR1 remodeling complexes, which share common subunits like the AAA+ ATPase subunits Rvb1 and Rvb2 and the Arp4-actin dimer. Both complexes affect the distribution of the histone variant H2A.Z (Pht1 in fission yeast). Histones can be classified in canonical histones, which are expressed and deposited into chromatin just after DNA replication; and histone variants, which are expressed throughout the cell cycle, and its incorporation is replication-independent (Verreault et al., 1996 Ahmad and Henikoff 2001, Shelby et al., 2000). Histone variants confer different structures and functionalities to specific genomic regions (Chakravarthy et al., 2004). At the level of transcription, the ORF is characterized by high levels of H2A.Z at the nucleosome +1 at the TSS, with variable H2A.Z at -1 nucleosome, and low levels of H2A.Z downstream of the +1 nucleosome (Albert et al., 2007; Raisner et al., 2005; Zhang et al., 2005).

INO80 was identified in a genetic screen in *S. cerevisi*ae for defective mutants in activating expression to inositol depletion response (Ebbert et al., 1999). It affects promoter of genes by nucleosome remodeling, nucleosome eviction, H2A.Z removal, and the establishment of nucleosome spacing (Shen et al., 2000; Attikum et al., 2007; Udugama et al., 2011; Papamichos-Chronakis et al., 2011). In this way, INO80 regulates several biological functions: activates transcription by helping to position the -1 and +1 nucleosomes establishing the nucleosome free region (Klopf et al., 2016; Krietenstein et al., 2016), represses transcription of non-coding RNAs by exchanging the H2A.Z variant (Marquardt et al., 2014) or evicts directly the transcription machinery (Poli et al., 2016). INO80 also works in the maintenance of genome stability, promoting replication fork re-start after stalling of replication forks (Shimada et

al., 2008) and prevents transcription collisions by removing RNA polymerase II during replicative stress (Lafon et al., 2015). INO80 intervenes in the double-strand breaks response, promoting eviction of nucleosomes from DNA, which leads to chromatin decompaction and enhances the accessibility of the repair machinery (Neumann et al., 2012; Seeber et al., 2013). INO80 has been implicated in the activation of genes related to metabolic and osmotic stresses (Barbaric at al., 2007; Ford et al., 2008, Hogan et al., 2010). In *S. pombe,* INO80 complex contains 15 subunits, 14 of them are conserved and a novel factor Iec1, which is important for the checkpoint response (Hogan et al., 2010). Recently, it has been shown that INO80 works together with Hrp1 to mediate replacement of histone H3 with Cnp1 at regional centromeres (Choi et al., 2017).

The SWR complex is specialized in the exchange of histone H2A for the histone variant H2A.Z of chromatin at promoters, centromeres and subtelomeric regions (Krogan et al., 2003; Kobor et al., 2004; Raisner et al., 2005). It has been described that the SWR complex is also recruited to broken DNA, promoting the association of proteins required for efficient double strands break (DSB) repair (van Attikum et al., 2007). Thus, SWR1 has a positive role in the activation of damage checkpoint (Kalocsay et al., 2009).

4.1.4 Mi-2

The Mi-2 homolog in *S. pombe,* Mit1, belongs to the multienzyme complex called SHREC (Snf2/HDAC-containing Repressor Complex). SHREC complex mediates the repression of heterochromatin transcription through two enzymatic functions: Mit1, which is a chromatin remodeler and the histone deacetylase Clr3, which targets lysine 14 of histone H3 (Sugiyama et al., 2007). Mit1 is located around heterochromatin regions at centromeres,

subtelomeres and the mating-type region, and functions synergistically with the histone methyltransferase Set2 antagonizing RNA Pol II access around these regions (Creamer et al., 2014). Mit1 was also described to function in the nucleosome organization of euchromatic gene coding regions (Lantermann et al., 2010), but genome-wide nucleosome position mapping indicates that there are no significant changes in the chromatin structure of these regions in *mit1* mutant strains (Hennig et al., 2012).

4.2 Histone modifications

Chromatin modifiers catalyze the addition and removal of post-translational modifications from histones and other chromatin-related proteins. The histone N-terminal tails are subjected to various posttranslational modifications, including chemical groups such as acetylation, methylation, phosphorylation, and the binding of proteins as ubiquitin and small ubiquitin-like modifier (SUMO) (Grant, 2001) (Fig. 12). These modifications can impact in cellular processes such as transcription regulation, chromatin assembly, DNA repair and cell cycle progression (Sterner and Berger, 2000). Two hypotheses explain the effect of histone modification on transcription; firstly, it is thought that chromatin structure can be modulated via alteration of DNA-nucleosome interaction, changing the histone charge. In this sense, histone acetylation unfolds chromatin structure by neutralizing the positive charge of lysines, decreasing their affinity for the negatively charge of DNA and allowing the entry of the transcription machinery to the promoters (Kouzarides, 2007). Secondly, histone modification serves as anchor points for binding proteins which have specialized domains

such as bromodomains or chromodomains, and which are able to affect positively or negatively transcription (Bottomley, 2004).

Histone modifications are not mutually exclusive, and it has been reported a large number of cases in which there is a crosstalk between different modifications. For example, HDAC activity is especially important to prepare the histones for methyltransferases by removing acetyl groups that block methylation. More specifically, it has been described that the depletion of HDACs like Clr3 and Sir2 correlates with a global reduction in the heterochromatin marks H3K9me2 and H3K9me3 (Shankaranarayana et al., 2003; Yamada et al., 2005).

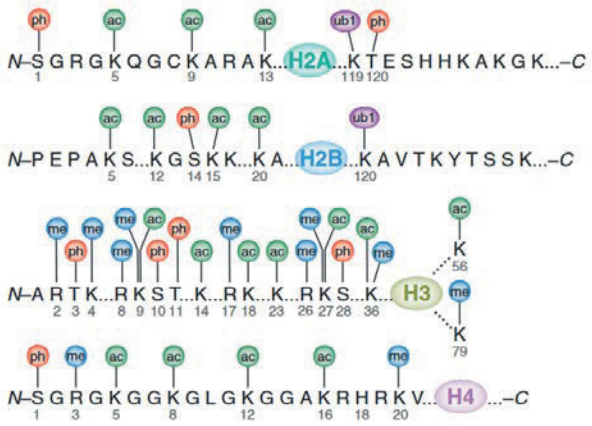

Figure 12. Post-translational modifications of human histone tails. The modifications shown are acetylation (ac), methylation (me), phosphorylation (ph) and ubiquitination (ub1) (Bhaumik et al., 2007).

4.2.1 Histone Acetylases (HATs)

HATs catalyze the addition of an acetyl group ($COCH_3$) from the pseudo-substrate acetyl coenzyme A (acetyl-CoA) to the ε-amino group of lysines on the N-terminus of histones. Many HATs can act not only on histones, but also on non-histone proteins (cytoplasmic

as well as nuclear) and they are also called lysine acetyltransferases (KATs) (Talbert et al., 2012).

HAT enzymes can be classified into several groups based on their conserved protein domains. The two main families of HATs are the GNAT (GCN5-related N-acetyltransferase) family and MYST (MOZ, Ybf2/Sas3, Sas2 and Tip60) family. GNAT family is characterized by having a C-terminal bromodomain (Neuwald and Landsman, 1997); whereas MYST family is characterized by containing zinc fingers as well as chromo-domains (Avvakumov and Cote, 2007).

Acetylation is one of the most characterized modifications in *S. pombe*. Lysine acetylation promotes molecular processes, which can lead to two biochemical consequences: the recruitment of coactivator complexes through conserved domains, such as bromodomains; or the participation of co-repressor complexes through HDAC (Fig 13). Therefore, HATs have been widely linked to transcriptional activation and homeostasis.

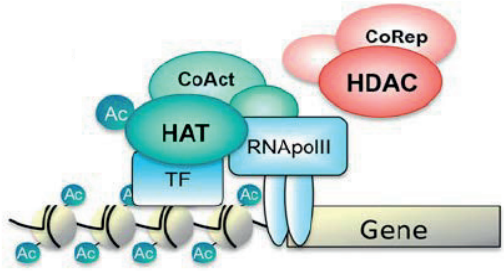

Figure 13. Balance between acetylation and deacetylation at gene promoters. During transcription, the HAT acetylates nucleosomes at specific promoter sites, promoting the recruitment of coactivators (CoAct). Histone acetylation can create a more open chromatin structure, allowing the binding of transcriptional factors (TF) and the initiation of transcription by RNA polymerase II (RNApolII) as well. After that, the HDAC re-establish the deacetylation state of chromatin leading to proper transcriptional homeostasis (Adapted from Schneider et al., 2013).

Hat1

The Hat1 histone acetyltransferase is responsible for the acetylation of newly synthesized histone H4 at lysines 5 and 12 (H4K5 and H4K12) (Benson et al., 2007). Hat1 has a role in damage repair being recruited to the sites of DNA double-strand breaks (Qin and Parthun 2006; Benson et al., 2007). Hat1 also participates in the establishment and organization of telomeric silencing .

Gcn5

Gcn5 (General Control Non-derepressible 5), one of the members of the GNAT family, is the best-characterized HAT and serves as a prototype for histone acetyltransferase studies. Gcn5 was identified in a screening for factors involved in amino acid biogenesis (Penn et al., 1983) but later studies identified Gcn5 as a subunit of several transcriptional co-activator complexes (Georgakopoulos and Thireos, 1992). Co-activators are defined by their direct interaction with the basal transcription machinery and/or open chromatin structure at gene promoter (Thomas and Chiang 2006; Hahn and Young 2011).

In fission yeast, Gcn5 belongs to the conserved SAGA complex, a multifunctional co-activator that comprises nineteen subunits. SAGA subunits are conserved and organized into five separate modules with diverse activities: structural core, binding module, histone acetyltransferase (HAT), histone deubiquitinase (DUB) and TATA-box binding protein (TBP) module (Fig.14).

- The **structural core** is composed by Spt7, Spt20, Ada1, Taf5, Taf6, Taf9, Taf10 and Taf12. The TBP-associated factors (TAFs) are shared with the co-activator TFIID (see below). That is the reason why the deletion of these TAFs is not viable

(Yamamoto et al., 1997; Mitsuzawa and Ishihama, 2002; Helmlinger et al., 2011).

- The **transcription activator binding protein** Tra1 which is the largest component of SAGA, although it does not contribute significantly to the integrity of SAGA (Helmlinger et al. 2011). It has been suggested that Tra1 may serve as a scaffold for the assembly and recruitment of other co-activator complexes to target genes, but it is not absolutely required for the recruitment of SAGA to promoters (Brown et al., 2001; Fishburn et al., 2005; Reeves and Hahn, 2005; Helmlinger et al., 2011).

- The **histone acetyltranferase module** formed by Gcn5, Ada2, Ada3 and Sgf29 acetylates histone H3 mainly on residues K9 and K14, but also in K18 and K36 (Nugent et al., 2010; Pai et al., 2014). Ada2 and Ada3 increase the activity of Gcn5 and broadens the lysine specificity of Gcn5 to acetylate multiple lysine residues (Grant et al., 1999; Balasubramanian et al., 2002). Sgf29 contains a tandem Tudor domain, which recognizes H3K4me3 and enhances processivity by the HAT module on methylated substrates (Bian et al., 2011; Ringel et al., 2015)

- The **histone deubiquitinase (DUB) module** contains the catalytic subunit Ubp8 as well as Sgf11, Sgf73 and Sus1. Ubp8 mediates deubiquitylation of histone H2B around coding regions, and its deletion reduces transcription of SAGA-regulated genes. The disruption of Ubp8-mediated deubiquitylation of H2B also alters levels of gene-associated H3K4 methylation and H3K36 methylation (Henry et al.,

2003). In other hand, Sgf73 binds to upstream activating sequences, facilitating assembly of the transcription preinitiation complex (Shukla et al., 2006).

- The **TATA-box binding protein module (TBP)** comprises both Spt3 and Spt8, which recruit general factor TATA-binding protein (TBP) to specific promoters (Sermwittayawong and Tan 2006; Baptista et al. 2017).

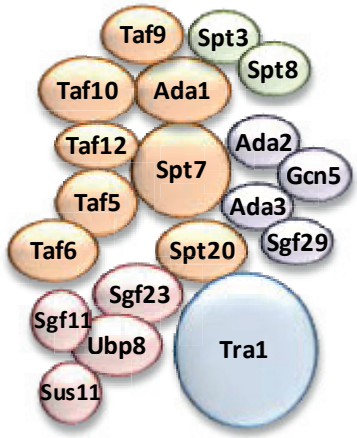

Figure 14. Schematic organization of *S. pombe* SAGA complex. Structural subunits are coloured orange; the transcription activator binding protein Tra1, blue; HAT module, lavender; TBP module, green; and DUB module, pink.

As we have described above, the recruitment of SAGA to gene promoters can be mediated by the interaction of Tra1 with specific transcriptional activators, but also Gcn5 has a bromodomain, which can bind acetylated H3 and H4 N-terminal tails, potentiating cooperative nucleosome acetylation of histone H3 (Owen et al., 2000; Li et al., 2009). This acetylation carried out by the HAT module allows to open up the chromatin landscape for binding of additional transcription factors and the pre-initiation complex (PIC) (Balasubramanian et al., 2002). The Spt3 subunit also collaborates

in the recruitment of TBP to facilitate PIC formation and transcriptional activation (Mohibullah and Hahn, 2008). Gcn5, together with SAGA, has been shown not only to be present at promoters but also to localize to coding regions where they accompany Pol II during elongation and function to acetylate and subsequently evict nucleosomes from gene coding regions (Govind et al., 2007; Sanso et al., 2011). The DUB module also promotes transcription elongation through deubiquitination of H2B, which allows the recruitment of the Ctk1 kinase and subsequent Ser2 phosphorylation of the Pol II C-terminal domain (Henry et al., 2003). It is remarkable that the inactivation of specific subunits affects the stability of the corresponding module but not the overall integrity of the SAGA complex (Lee et al., 2011).

Of the nineteen SAGA subunits, five are shared with TFIID (Transcription Factor II D). TFIID is a general transcriptional factor comprised of TBP and 14 TAFs, and acts as a platform for assembly of transcription PIC (Berger et al., 2011). Therefore, SAGA and TFIID share this set of subunits and also both bind activators and TBP; but SAGA has no known DNA binding activity or interactions with other components of the basal transcription machinery (Warfield et al., 2017). Genes can be classified in two types: housekeeping genes, whose promoters lack a consensus TATA box and have the +1 nucleosome adjacent to the place of PIC formation; and regulated genes, whose promoters contain TATA box and are modulated by chromatin remodelers or histone modifications (Basehoar et al., 2004; Huisinga and Pugh, 2004). Housekeeping genes are typically TFIID dominant and inducible genes are SAGA dominant (Basehoar et al., 2004; Huisinga and Pugh, 2004; Rhee and Pugh, 2011). At present, there is controversy because is not totally clear which of these two transcription factors are recruited to a specific set of genes

(Bonnet et al., 2014; Grunberg et al., 2016). The two last studies carried out in budding yeast concluded that each complex independently contributes to the expression of all genes transcribed by RNA pol II: SAGA complex is located in the UAS elements, overlapping with Mediator, and TFIID is mapped around the transcription start site, but the growth conditions may change the magnitude of TFIID dependence (Fig.15). Thus, these emerging models do not support the idea that each coactivator is preferably located in a particular promoter, and open new questions about how key regulators as sequence-specific or DNA-binding TFs affect the recruitment and the biological function of these two co-activators (Baptista et al., 2017; Warfield et al., 2017; Taatjes et al., 2017).

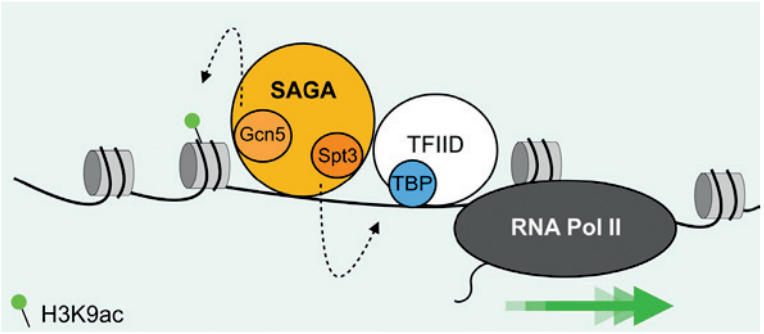

Figure 15. SAGA and TFIID work together in the transcription of almost all yeast genes. Gcn5 through the acetylation of histone, and Sp3 through the binding of TBP, synergizes to promote transcription (adapted from Baptista et al., 2017).

Gcn5 and SAGA complex contribute to other aspects of gene expression as well:

- SAGA collaborates with chromatin remodelers in the regulation of gene transcription. For example, it has been described that SAGA is required for SNF/SWI recruitment to *inv1* gene, which is required for hydrolysis of sucrose and raffinose (Ahn et al., 2012).

- Microarray analysis have shown a role of Gcn5 in the regulation genes involved in salt stress response (Johnsson et al., 2006).

- Gcn5 also regulates negatively gene expression, repressing the transcription of sexual differentiation genes (Helmlinger et al., 2008).

- During DSB repair, Gcn5 acetylates H3K36, increasing chromatin accessibility and promoting the homologous recombination pathway (Pai et al., 2014).

- Gcn5 plays a significant role in the recruitment of condensin, evicting nucleosomes and promoting the efficient binding of condensin components at the nucleosome depleted regions (Toselli-Mollereau et al., 2016).

- Evidence indicate a possible role of Gcn5 in the initiation of DNA replication in *S. cerevisiae.* For instance, Gcn5 physically associates with replication origins (Espinosa et al., 2010) and stimulates origin activity (Vogelauer et al., 2002), probably through the decondensation of chromatin structure to allow the entry of DNA replication machinery.

In metazoans, GCN5 was identified in another co-activator complex in addition to SAGA, called ATAC (Ada Two A Containing) (Nagy and Tora, 2007). In addition to the regulation of transcription, GCN5 participates in several cellular processes such as DNA repair (Brand et al. 2001; Tamburini and Tyler, 2005), telomere maintenance (Atanassov et al., 2009), DNA replication (Paolinelli et al., 2009), cell cycle progression (Orpinell et al., 2010) and nucleosome assembly (Burgess et al., 2010). GCN5 acetylates

lysines 9, 14, 18, 27 and 56 of histone H3, and lysines 8 and 16 of histone H4 (Brownell et al., 1996; Kuo et al., 1996; Tjeertes et al., 2009; Burgess et al., 2010). GCN5 has a potential role during different stages of development and tumorigenesis (Armas-Pineda et al., 2007; Wang and Dent, 2014). In fact, accumulated evidences suggest that GCN5 is involved in the regulation of oncogenic genes. For example, it has been shown that GCN5 expression is significantly upregulated in human colon adenocarcinoma tissues (Chen et al., 2013; Yin et al., 2015). In addition, GCN5 interacts with both Myc and E2F transcription factors, regulating their target genes (Lang et al., 2001; Liu et al., 2003b).

Mst1

Mst1 belongs to MYST family and forms a complex similar to the NuA4 complex of budding yeast (Shevchenko et al., 2008), catalyzing the acetylation of histone H3 at lysine 4 (H3K4), histone H4 at lysines 5, 8, 12 and 16 (H4K5, H4K8, H4K12 and H4K16) and the histone variant H2A.Z (Kim et al., 2009; Xhemalce and Kouzarides 2010; Ruan et al., 2015). Mst1 is essential for viability and mutants are sensitive to a large number of genotoxic agents including HU, UV irradiation, MMS and bleomycin, pointing that Mst1 has an active role in the maintenance of genome stability. Mst1 was found interacting with Res2, a core subunit of MBF (Gomez et al., 2005). Moreover, Mst1 has an active role in the prevention of further heterochromatin spreading through the stabilization of Bdf2, which inhibits the action of the HDAC Sir2 (Wang et al., 2013).

Mst2

Mst2 is part of a complex similar to the NuA3 complex of budding yeast, which functions redundantly with Gcn5 to regulate the acetylation of histone H3 lysine 14 (H3K14) (Nugent et al., 2010; Wang et al., 2012). It has been suggested that H3K14ac mediates the DNA damage response through the chromatin compaction as well as the recruitment of the chromatin remodeler RSC, similar to what happens in *S. cerevisiae*, where RSC promotes the recruitment of ATM/ATR sensor kinase to DSB sites (Shim et al., 2007; Wang et al., 2012). Mst2 counteracts heterochromatin spreading through the regulation of histone turnover (Reddy et al., 2011; Wang et al., 2015). Furthermore, it has been described how Mst2 binds to all transcriptionally active regions, protecting them from the formation of heterochromatin. In this way, Mst2 complex is recruited through Pdp3 to H3K36me3. H3K36me3 is a hallmark of transcription, established by Set2 that interacts with RNA polymerase II. Mst2 also acetylates Brl1, which is part of HULC complex, involved in ubiquitination of histone H2B and universally linked to active gene transcription (Flury et al., 2017).

Rtt109

The acetylation of histone H3 lysine 56 (H3K56) is under the control of the HAT Rtt109. H3K56ac is limited to S phase, disappearing during G2/M phases of the cell cycle (Masumoto et al., 2005; Xhemalce et al., 2007). H3K56ac is involved in transcription, replication and DNA damage response:

- H3K56ac allows the chromatin to be disassembled during transcriptional activation of specific genes and enables the recruitment of the SWI/SNF remodelling complex regulating

positively the gene activity (Xu et al., 2005; Williams et al., 2008).

- H3K56ac is deposited on newly synthesized histones before incorporation onto DNA, enhancing the affinity of the histone chaperones for newly synthesized histone H3 (Li et al., 2008; Su et al., 2012). Moreover, H3K56ac suppresses the transcription of newly replicated DNA along the S phase, maintaining expression homeostasis during this time when the DNA dosage of different genes transiently differs. Thanks to this buffering effect, the expression of genes that replicate early and late remains constant, and the DNA dosage has a limited influence on mRNA synthesis rates (Voichek et al., 2016).

- Several evidences point to a role of H3K56ac as a guardian genome stability. H3K56ac is increased after exposure of genotoxic effect and subset activation of checkpoint kinases (Tanaka et al., 2012). Defects in the acetylation of H3K56 produce genome instability and sensitivity to DNA damage agents (Masumoto et al., 2005; Xhemalce et al., 2007). These defects can be produced by a failure to complete the repair of DNA lesions or by defects in the completion of DNA replication (Wurtele et al., 2012). In this way, acetylation of H3K56 by Rtt109 establishes a favorable chromatin structure for DNA damage repair machinery to access damaged DNA (Masumoto et al., 2005). And at the end of DNA repair, deacetylation of H3K56ac leads to restoring the original chromatin structure being a signal for the completion of repair (Chen et al., 2008a).

Therefore, H3K56ac can influence on chromatin structure in opposite ways, while in DNA replication and DNA damage repair, H3K56ac promotes nucleosome assembly, during transcription activation promotes nucleosome disassembly.

Studies carried out in *S. cerevisiae* have also shown a close relationship between Rtt109 and Gcn5. Genetic evidences indicate that Rtt109 and Gcn5 work in parallel in response to DNA damage (Fillingham et al. 2008; Li et al. 2008; Burgess et al. 2010). Furthermore, it could be a crosstalk between both HATs during replication coupled assembly (Li et al., 2008). This can be explained, at least in part, because Rtt109 also catalyse the acetylation of H3K9 and H3K27, two residues that are mostly acetylated by Gcn5 (Berndsen et al., 2008). It is remarkable to note that although Gcn5 can acetylate H3K56 in mammalian cells, in budding yeast *gcn5* mutant does not affect the global levels of H3K56ac, but when cells are synchronized shows deregulation of H3K56ac along the cell cycle (Burgess et al. 2010).

4.2.2 Histone Deacetylases (HDACs)

HDACs are conserved enzymes that deacetylates groups from lysines on histone tails. These enzymes counteract the action of HATs, and therefore, HDACs generally act as co-repressors in gene expression (with some exceptions). The deacetylation of histones may repress transcriptional processes by blocking the access to promoters of several chromatin-remodeling complexes such as SWI/SNF or basal transcriptional machinery, surprising the PIC formation at promoter regions (Deckert and Struhl, 2002). In addition to this role, HDACs are important in other chromatin-dependent processes such as DNA repair and replication (Kurdistani and Grunstein, 2003). HDACs are classified according to the structure of

their active sites into three classes: the Class I Rpd3-like proteins, the Class II Hda1-like proteins and the Class III Sir2-like proteins (also called sirtuins). The Class I and Class II are related enzymes and they share a conserved central enzymatic domain. The Class III enzymes need NAD^+ to deacetylate acetyl groups. Like the HATs, HDACs are able to interact with a large number of non-histone proteins, promoting the removal of its acetylation. In mammalian cells, the best characterized example is the tumor suppressor protein p53, which is deacetylated by HDAC1, promoting its ubiquitination and degradation (Luo et al., 2000).

Fission yeast possesses members of all three classes: Clr6 and Hos2 (class I), Clr3 (class II) and Sir2, Hst2 and Hst4 (Sir2 family or sirtuins) (Ekwall, 2005). Through the removal of acetyl groups from lysines, HDACs not only alter transcription, but also promote the establishment of alternative posttranslational lysine modifications such as methylation, ubiquitination, and sumoylation. For example, it has been described that Clr3 and Sir2 depletion correlates with a reduction in H3K9me2 and H3K9me3 (Abshiru et al., 2016).

Clr6

Clr6, the homolog of Rpd3 in *S.cerevisiae*, is an essential HDAC that removes the acetyl group of histone H3 at lysines 9, 14, 18, 23 and 27 (H3K9, H3K14, H3K18, H3K23 and H3K27); and histone H4 at lysines 5, 8, 12 and 16 (H4K5, H4K8, H4K12 and H4K16) (Wiren et al. 2005; Abshiru et al. 2016). Clr6 exists in two physically and functionally distinct complexes: complex I and complex II (Nicolas et al. 2007). Later, it was defined a variant of complex I, which was called complex I´´ (Zilio et al., 2014). Complex I regulates the silencing of mating locus and centromeres, and also the acetylation of coding regions (Nicolas et al., 2007). Complex I'' controls the

expression of Tf2 elements, noncoding RNA (ncRNA) and stress related genes (Zilio et al., 2014). Complex II regulates histones acetylation at coding regions, suppressing aberrant transcription (antisense) and is important for protecting DNA from genotoxic agents (Nicolas et al., 2007). Moreover, RNA pol II promotes the recruitment of Clr6 complex II to deacetylate histones behind the elongating RNAPII and restores the chromatin (Lee et al., 2017) state. Finally, Clr6 has been implicated in the repression of some meiosis-induced genes (Wiren et al., 2005).

Hos2

Hos2, also known as Hda1, is a Class I HDAC which has a major role in the activation of gene expression targeting H4K16ac around 5' region of highly active genes (Sinha et al., 2006; Wiren et al., 2005). This role is contrary to the typical function of HDACs; an elevated proportion of highly expressed genes with low H4K16ac are involved in major metabolic pathways; therefore, Hos2 can be particularly important in order to boost their expression for rapid growth (Wiren et al., 2005). More recently it has been described that Hos2 promotes faithful cytokinesis (Grewal et al., 2012).

Clr3

The HDAC Clr3 deacetylates primarily H3K14Ac, which are previously K9me2 and K9me3. Clr3 exerts its function around the mating locus, centromere and telomere regions, limiting the access of RNA Pol II to these regions (Sugiyama et al. 2007; Buscaino et al., 2013; Abshiru et al., 2016). Furthermore, Clr3 also has an active role in transcription during stress conditions (Wiren et al., 2005).

Sir2

Sir2 is a nuclear protein that preferably deacetylates H3K4ac, H4K16ac and to a less extent H3K9 and H3K14 (Xhemalce and Kouzarides 2010; Alper et al., 2013; Abshiru et al., 2016). Sir2 works together with Clr3, promoting the recruitment of gene silencing complexes to heterochromatin regions (Shankaranarayana et al., 2003; Freeman-Cook et al., 2005; Yamada et al. 2005; Buscaino et al., 2013) (See below).

Hst2

Hst2 is the less characterized HDAC. Hst2 deacetylates H3K9ac located within ORFs (Durand-Dubief et al., 2007). Hst2 is required for rDNA and centromeric silencing and represses genes involved in transport and membrane function (Durand-Dubief et al., 2007).

Hst4

Hst4 is the last member of sirtuins, which have redundant roles in heterochromatin formation (Durand-Dubief et al., 2007). Hst4 carries out the deacetylation of H3K56ac. As we have mentioned above, the acetylation of H3K56 occurs during S phase and disappear during G2/M (Xhemalce et al., 2007). Several evidences point to a role of Hst4 in the DNA damage response: *hst4* mutant cells exhibit fragmented DNA, have the DNA damage checkpoint activated, and are synthetic lethal with several components of the DNA damage checkpoint, like Rad3, Chk1 and Cds1 (Haldar and Kamakaka, 2008). Levels of Hst4 are downregulated in response to DNA damage (Haldar and Kamakaka et al. 2008). More specifically, the sensor kinase Rad3 mediates the degradation of Hst4, being targeted by ubiquitin-mediated proteolysis upon DNA damage (Thaminy et al., 2007; Tanaka et al., 2012). Hst4 has also been

found interacting with the checkpoint sensors Rad9, Rad1 and Hus1 (Chang et al., 2011). And interestingly, in Δ*hst4* strains the homologous recombination repair protein Rad52 appears mostly located in the origins of replication (Zhou et al., 2013).

Hst4 also represses genes that are involved in aminoacid biosynthesis, oxidoreductase activity and Tf2 retrotransposons (Durand-Dubief et al., 2007). Hst4 is associated with the silencing of centromeric and telomeric regions (Chang et al., 2010), being the acetylation of H3K56 required for telomere tethering to nuclear periphery (Tanaka et al., 2012).

4.2.3 Histone Methyltransferases (HMTs)

Histone methylation is defined as the transfer of one, two, or three methyl groups from S-adenosyl-L-methionine to lysine or arginine residues of histone proteins by histone methyltransferases (HMTs). Enzymes that contain a conserved SET domain are implicated in the lysine methylation of histones (Rea et al., 2000). In *S. pombe* there are at least nine SET domain proteins (Set1-3, Clr4, Set5-9) being Set1, Set2 and Clr4 the better characterized methyltransferases, for its homology with SET1, SET2 and SUV39H in mammalian cells. Although histone acetylation is generally associated with positive transcriptional activity, histone methylations are correlated with both activation and silencing of transcription.

Set1

Set1 is required for histone H3 lysine 4 methylation (H3K4me) and is associated with active genes within euchromatic regions (Noma et al., 2001; Noma and Grewal, 2002). Set1 belongs to Set1C/COMPASS complex, which contains eight subunits (Set1,

Ash2, Spf1, Swd3, Swd1, Swd2.1, Shg1 and Sdc1) (Roguev et al., 2003; Roguev et al., 2004). The subunits have various roles on the stability of the complex, on the states of different H3K4me and also, they have diverse effects in the transcriptome (Lorenz et al., 2014; Mikheyeva et al., 2014).

Set1 is recruited by the Pol II elongation machinery to 5′ region of active ORFs, interacting with Pol II C-terminal domain (CTD) at serine 5 but not serine 2. Thus, Set1 acts during transcription elongation being a universal marker of active genes in eukaryotes. The methylation within the coding region persists after transcription, indicating that H3K4me provides a molecular memory of recent transcriptional events (Ng et al., 2003). Set1 also has repressive functions on repetitive elements and participates in the silencing and nuclear organization of Tf2 retrotransposons by antagonizing the function of the H3K4 histone acetyltransferase Mst1 (Lorenz et al., 2012), where maintains the repression in *mat* locus and subtelomeric regions (Mikheyeva et al., 2014) . It is also recruited to promoters of stress-response genes in a Atf1-dependent manner (Lorenz et al., 2014). Thereby, depending on the genomic context, H3K4me can act as a marker of transcriptional activation or as a marker of repression of heterochromatic loci (Figure 16).

H3K4me can exist as monomethylation (H3K4me1), dimethylation (H3K4me2), or trimethylation (H3K4me3) (Santos-Rosa et al., 2002). Every pattern of methylation is specific of an ORF region. In this way, studies in *S. cerevisiae* have shown that while H3K4me3 occurs at the beginning of actively transcribed genes, dimethylated H3K4me2 is located in the middle of genes, and H3K4me1 is especially found within the end of genes (Pokholok et al., 2005).

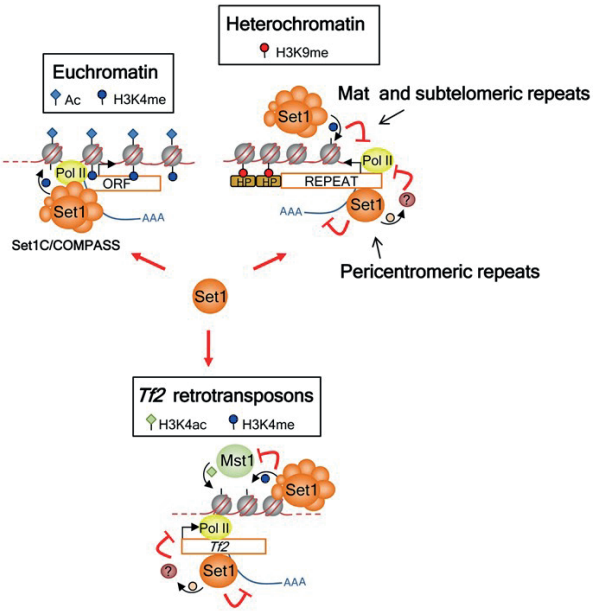

Figure 16. Roles of Set1C in genome processes in *S. pombe* (adapted from Mikheyeva et al., 2014).

An essential aspect of H3K4me regulation is its relationship to monoubiquitination of histone H2B (H2Bub1), which is another conserved modification linked to highly expressed genes (Xiao et al. 2005). The monoubiquitination of histone H2B (H2Bub1) must precede H4K3me (Dover et al. 2002). The monoubiquinitation of H2B at a conserved C-terminal lysine (K119 in fission yeast and K120 in mammals) regulates transcription participating in two different pathways: one that stimulates transcription elongation through the establishment of positive feedback loop between Cdk9 and H2Bub1 and which is H3K4me-independent (Sanso et al., 2012) and another that enhances interaction of Set1 with chromatin promoting H3K4me and downstream effects (Tanny et al., 2007; Racine et al., 2012).

Set2

Histone H3 lysine 36 methylation (H3K36me) is under the control of histone acetyltransferase Set2 (Noma and Grewal, 2002). In mammalian cells, SETD2 has been described as a tumor suppressor gene, playing an important role in maintaining genome stability (Kanu et al., 2015). Set2 methylation of H3K36 has been associated with RNA Pol II elongation in both budding and fission yeasts (Hampsey and Reinberg 2003; Morris et al., 2005; Kizer et al., 2005). Studies in fission yeast have proved that H3K36 methylation is cell cycle regulated, with a peak of H3K36me3 around the G1-S transition (Pai et al., 2014). In fact, MBF promoter genes are H3K36 di and trimethylated, facilitating DNA replication and the transcriptional response to genotoxic stress (Pai et al., 2017). Set2-dependent H3K36 methylation also has an active role in DNA repair pathway, reducing chromatin accessibility, promoting non-homologous end joining (NHEJ) at DSB and regulating the activation of Chk1 (Pai et al. 2014; Lim et al. 2018). Set2 is also responsible for repressing cryptic promoter activity (Hennig et al., 2012) and suppressing a subset of RNAs including heterochromatic and subtelomeric RNAs, in part via recruitment of HDAC Clr6 complex II or in conjunction with Mit1 and SHREC complex (Chen et al., 2008b; Creamer et al., 2014; Suzuki et al., 2016).

Clr4

Clr4 is the single enzyme responsible for introducing methylation at lysine 9 of histone H3 (H3K9me), specially di- and trimethylation (H3K9me2, H3K9me3) (Cheng et al., 2005). Clr4 forms a complex called Clr4 multiprotein complex (CLRC), which is associated with the RITS iRNA effector complex and both are responsible for the heterochromatin initiation and spreading (Motamedi et al., 2004;

Djupedal and Ekwall 2009). Clr4 behaves like a reader and writer of H3K9 methylation through the action of different domains (Horita et al., 2001). In this way, Clr4 sets the H3K9 methylation pattern, which is recognized by the chromodomains of Clr4 and the heterochromatic protein Swi6 (HP1 in mammalian cells). H3K9me is also recognized by proteins involved in the recruitment of chromatin modifiers, like the HDACs Clr3 and Sir2, promoting the silencing of chromatin and restricting the access of RNA polymerase II to heterochromatin (Bannister et al., 2001; Nakayama et al., 2001; Sugiyama et al., 2007). Then, the further recruitment of Clr4 leads to the methylation of neighbouring nucleosomes (Haldar et al., 2011) .

The H3K9me acts not only as a hallmark of heterochromatin. More recently it has been shown that Clr4 promotes the generation of siRNA through the methylation of Mlo3, an essential factor in the production of centromeric siRNA (Zhang et al., 2011).

4.2.4 Histone Demethylases (HDMs)

The enzymes responsible for removing methyl groups from methyl-lysine or arginine residues are named histone demethylases. The best characterized HDMs are those that have the highly conserved JmjC domain. This domain has a histone demethylase catalytic activity capable of demethylating histones, generating formaldehyde and succinate (Tsukada et al., 2006). *S. pombe* contains seven JmjC proteins, but it is not clear if all of them have histone demethylase activity (Fig. 17) (Huarte et al., 2007). Epe1 is the JmjC containing protein most widely studied in *S. pombe*. Epe1 catalyses the removal of H3K9 methylation counteracting the heterochromatin stability. In fact, deletion of *epe1* promotes continuous spreading of heterochromatin-associated histone

modifications (Ayoub et al., 2003; Trewick et al. 2007; Yu et al., 2018). Epe1 may be also regulated in response to environmental signals to preserve or eliminate H3K9 methylation at specific loci (Zofall et al., 2012).

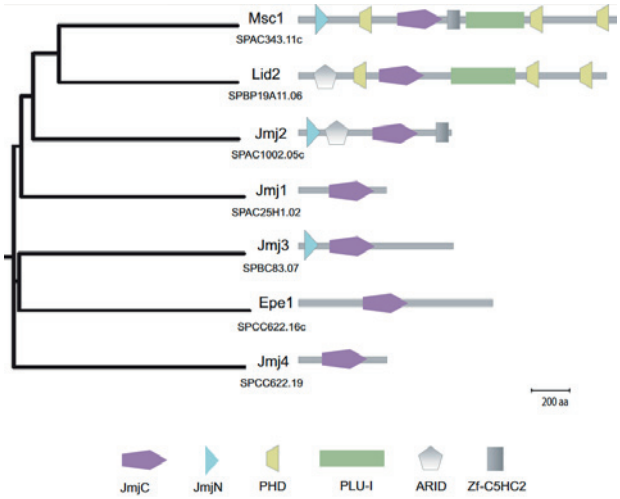

Figure 17. Phylogenic tree of JmjC domain-containing proteins in *S.pombe*. Schematic representation of the JmjC domain proteins with various domains predicted (adaptated from Huarte et al., 2007).

4.3 Chromatin and Cell Cycle

Chromatin structure and cell cycle regulation are deeply related. A good evidence for this point comes from the observation that many mutants of chromatin modifiers exhibit problems in the control of the cell cycle; and other mutants with altered cell cycle regulation show abnormalities in the silencing of some genes.

As previously described, DNA replication begins when ORC recognizes and binds to the origins of replication. The replication origins are usually situated in accessible genomic regions; indeed,

origins are located generally near of gene promoters (Cadoret et al., 2008; Cayrou et al., 2011). Epigenetics features are involved in the location of replication origins: these regions are enriched in the histone variant H2A.Z (Mechali et al., 2013) and in the histone modification H3K4me3. Some studies in *Drosophila* suggest that SAGA complex is necessary to open chromatin and lead to ORC binding (Vorobyeva et al., 2013). Besides, some chromatin modifiers are necessary for the assembly of the pre-RC: H3K4 methylation promotes the assembly of the pre-RC (Rizzardi et al., 2012).

The timing of replication is also regulated by chromatin organization. The accessibility of initiation factors to different origins is regulated by histone acetylation. For example, in *Drosophila* H3K18ac and H3K27ac are associated with early replication (Eaton et al., 2011) and in *S. cerevisiae*, deletion of HDAC Rpd3 provokes early activation of late-firing origins (Aparicio et al., 2004).

For the progression of replication fork is necessary the disruption of nucleosomes, which is dependent on the chromatin remodeler SWI/SNF family (Groth et al., 2007) and histone chaperones, which help to transfer nucleosomes to the daughter strands (Gambus et al., 2006).

Another important aspect in the replication is the inheritance of epigenetic marks. It seems that the proliferating cell nuclear antigen (PCNA) has a major role in this process, coupling chromatin restoration to replication. In mammalian cells, PCNA works with some chromatin modifiers like SNF2H, Dnmt1 and HDACs, promoting repressive marks after new DNA synthesis (Groth et al., 2007).

It is essential that replication is not initiated more than once per replication origin in each cell cycle to avoid re-replication and

endoreduplication processes. Some mechanisms targeting various pre-RC subunits involve histone modifications. A good example of this aspect is H4K29me2 in animal cells, which inhibits pre-RC formation until it is removed during the next G1 phase (Dorn and Cook, 2011).

During mitosis, chromatin condensation is associated with various histone modifications such as phosphorylation of H3S10, H2B ubiquitination, and phosphorylation of histone H1 (Xu et al., 2009).

All these evidences show how epigenetics plays a crucial role in the location of replication origins, the timing of replication, the progression of the replication forks and tin the segregation of the chromosomes in eukaryotic cells.

4.4 Chromatin in health and cancer

As described above, the organization of the genome into a specific structure can influence the ability of genes to be active or silenced, and this organization of genome is under the control of histone modifiers and chromatin remodelers. Therefore, the changes introduced by these chromatin modifiers determine the epigenome of an individual. Recent advances have shown that mutations in genes that affect global epigenetic profiles work together with genetic alterations to give rise to diseases such as cancer (Jones and Baylin 2002; Egger and Liang 2004). Cancer epigenome is characterized by changes in DNA methylation, histone modifications patterns and expression of chromatin remodeler enzymes that lead to aberrant gene expression or silencing. These epigenetic changes, also known as epimutations, can lead to silencing of tumor

promoting tumorigenesis processes (Sharma et al., 2010). In this sense, HDACs play a key role in transcriptional inactivation of tumor suppressor genes. For example, mutations in HDAC2, HDAC4 and HDAC9 have been involved in colorectal cancer, breast and prostate adenocarcinoma respectively (Ropero et al., 2006; Sjoblom et al., 2006; Hanigan et al., 2008; Berger et al., 2011). In recent years, the pharmaceutical industry has concentrated its effort on the discovery of drugs that selectively inhibit HDACs or HMTs and are now used as therapeutic compounds; for example, the small-molecule inhibitor JQ-1 that inhibits the binding of transcription factor BRD4 to acetylated lysine residues (Matzuk et al., 2012; Muhar et al., 2018) or ORY1001, which is an inhibitor of the lysine demethylase LSD1 (Maes et al., 2018). The principle that guides this type of epigenetic therapy is that the reversal of epigenetic silencing will restore the cell state, and therefore, the cancer defense mechanism such as the expression of the cycling-dependent kinase inhibitors p16 and p21 (Egger et al., 2004).

The chromatin remodelers can also lead to aberrant gene silencing of tumor suppressors preventing the access to the promoters of these genes. Alterations of SWI/SNF function through modifications of some of its subunits have been widely implicated in cancer (Roberts and Orkin, 2004). For example, loss of SNF5, which is a *bona fide* tumor suppressor, is observed in pediatric cancers (Versteege et al., 1998; Chai et al., 2005). Moreover, the catalytic subunits BRM and BRG1 are silenced in a variety of cancer cell lines and primary tumors, such as lung cancers (Reisman et al., 2003; Marquez-Vilendrer et al., 2016). Besides the chromatin remodelers, the histone variant H2A.Z is also overexpressed in several types of cancers (Svotelis et al., 2009).

In summary, histone modifiers play pivotal roles in the development of numerous diseases. Therefore, the exploration of specific functions of each histone modifier and chromatin remodeler and the identification of their targets are necessary to provide new and promising epigenetic therapies in the future.

OBJETIVES

Our group is interested in characterizing how the transcription factor MBF is regulated in parallel to cell cycle progression. In this thesis, we aimed to discover new regulator proteins capable of modulating MBF activity, and to characterize how these regulators influence the G1-S transcriptional program.

Specifically, the main objectives of this thesis were:

1. Identification of new MBF regulators using fluorescence-based system which can identify changes in the activity of the MBF complex *in vivo*.

2. Study the regulation of chromatin features at MBF-regulated promoters.

RESULTS

CHAPTER I

A functional genome-wide genetic screening identifies new pathways controlling the G1-S transcriptional wave

MBF is the sole transcriptional factor complex that drives the G1-S transcriptional program in fission yeast. This transcriptional network include genes involved in DNA replication (*cdc18* and *cdt1*), DNA repair (*ssb1* and *rad21*) and cell cycle progression (*cig2* and *yox1*). Previously, we have characterized how MBF is one of the final targets of replicative and DNA damage checkpoints. But the regulation of the MBF complex in an unperturbed cell cycle remains largely unknown. With this work we intend to develop a tool based on flow cytometry for the screening of non-essential mutants that interfere with the activity of MBF. To do that we generated a reporter strain containing the fluorescence protein YFP fused to the MBF-dependent gene *cdc22* (Cdc22-YFP) and we crossed it with a haploid gene deletion collection. Using flow cytometry, we were able to quantify the fluorescence of each mutant strain, and after normalization with its cellular size we generated a representative ratio of MBF activity per each knockout strain. After a careful analysis we could classified the different mutants basing on their ontologies. Thereby, we could confirm the already known regulator of MBF like the co-repressors Yox1 and Nrm1 and the co-activator Rep2. Among the stains with lower ratios, we could find mutants with the translational processes compromised (proteins of Elongator complex, and tRNA modification pathway). In other way, mutants

with higher ratios were involved with cell cycle regulation and DNA repair.

To completely validate the genetic screening, we decided to characterize some mutants of the tRNA methylation pathway, in which the ratio of MBF activity was reduced, and mutants of COP9/Signalosome complex, which have the MBF activity induced. The analysis of some tRNA methyltransferases mutants showed that the MBF-dependent expression was downregulated in many of them. Among these mutant strains defective in translation, we can find the Δ*trm112*, Δ*mtq2* and Δ*trm9*, which form a complex that modifies nucleotides in tRNAs. In regard to the mutants of COP9/Signalosome complex, we confirmed that the activity of MBF was increased. These mutants *(*Δ*ddb1,* Δ*csn1,* Δ*csn2*) showed to be sensitive to HU, pointing a genetic interaction with replication checkpoint, which was confirmed by tetrad dissection. Therefore, thanks to fluorescence-based reporter, we have been able to identify several regulators that in an indirect way, can modulate the MBF-dependent transcription.

Gaspa L, González-Medina A, Hidalgo E, Ayté J. A functional genome-wide genetic screening identifies new pathways controlling the G1/S transcriptional wave. Cell Cycle. 2016 Mar 3;15(5):720–9. DOI: 10.1080/15384101.2016.1148839

CHAPTER II

The INO80 complex activates the transcription of S phase genes in a cell cycle regulated manner

To further characterize proteins that directly regulate MBF-dependent transcription, we decided to use a proteomic approach. After the immunoprecipitation of the MBF core component Cdc10 tagged with HA (Cdc10-HA), we identified potential partners of MBF using iTRAQ followed by LC/MS/MS/ (combination of liquid chromatography (LC) with mass spectrometry). Among the identified proteins, we noticed that several members of INO80 complex were present (Ies4, Iec5 Alp5 and Rvb1). INO80 is an ATP-dependent chromatin remodeling complex, that regulates the structure of the chromatin trough the exchange of the histone variant H2A.Z (Pht1 in *S. pombe*) with H2B, promoting the establishment of the nucleosome free region around the proximal promoter region. In this work, we have characterized how INO80 complex regulates the transcription of MBF-regulated genes. In this sense, we confirmed the interaction of several subunits of INO80 (Ies4 and Ino80) with Cdc10 by Co-IP. We have also shown that the binding of INO80 to MBF promoters, which was cell cycle regulated, correlated with the activation of MBF-dependent expression. Additionally, we confirmed that INO80 was involved in the activation of G1/S genes. The histone variant H2A.Z is frequently accumulated at -1 and +1 nucleosomes at the TSS. Here, we observed that chromatin accessibility is dynamically altered when we eliminated H2A.Z (Δ*pht1)*, and the nucleosome depleted region was restrained at the MBF-dependent promoters. Finally, we characterized the acetylation of the histone H2A.Z (H2A.Zac) around these promoters, showing that the acetylation was

cell cycle regulated. Moreover, the synchronization of a mutant that mimics the hypoacetylated (pht1-4KR) state demonstrated that the histone variant H2A.Z was necessary to maintain the gene expression homeostasis of G1/S phase.

Knezevic I, González-Medina A, Gaspa L, Hidalgo E, Ayté J. The INO80 complex activates the transcription of S-phase genes in a cell cycle-regulated manner. FEBS J. 2018 Oct 1;285(20):3870–81. DOI: 10.1111/febs.14640

CHAPTER III

Gcn5-mediated acetylation of H3K9 and H3K18 promotes the G1/S transcriptional wave

Passage through START requires the induction of G1/S transcriptional program, which is under the control of the MBF complex in fission yeast. MBF-dependent transcription is induced at the onset of metaphase and inactivated at the end of S phase through a negative feedback loop, where co-repressors Yox1 and Nrm1 bind to MBF until Nrm1 is phosphorylated by CDK1 and released from chromatin. Although mechanisms of MBF-transcription repression have been widely described, the precise molecular mechanism involved in the activation of MBF at the onset of each cell cycle remains still unknown. In this context, chromatin represents an impediment to the access of transcription factors to DNA and the regulation of the state of chromatin by altering chromatin structure or recruiting histone modifiers could play a key role in the regulation of MBF-dependent transcription. Histone acetyltranferases (HATs) catalyze the addition of an acetyl group to lysine residues at the N-terminus of histones and non-histone proteins. When MBF transcription is fully active, we observed that the HAT Gcn5 together with the SAGA complex bind to the MBF promoters through the co-activator Rep2. Moreover, Gcn5 is recruited to MBF promoters in a cell cycle dependent manner, peaking in G1 phase and dropping during G2, inversely to the co-repressors. These results suggest that the co-repressors Yox1 and Nrm1 act as physical barriers preventing the binding of Gcn5 to MBF promoters. We have also shown that Gcn5 acetylates specifically lysine residues 9 and 18 on histone H3 of MBF promoters at the

onset of the cell cycle. When we synchronized a Δ*gcn5* mutant we found that cell cycle was delayed compared to the wild type strain, and that MBF-dependent transcription was delayed and downregulated due to the lower levels of acetylation of the MBF promoters of this mutant. Therefore, Gcn5 is necessary for the full activation and proper timing of MBF genes expression.

González-Medina A, Hidalgo E, Ayté J. Gcn5-mediated acetylation at MBF-regulated promoters induces the G1/S transcriptional wave. Nucleic Acids Res. 2019 Sep 19;47(16):8439–51. DOI: 10.1093/nar/gkz561

DISCUSSION

At the beginning of the cell cycle, cells have to decide whether to switch between quiescence and proliferation states. Start marks irreversibly the commitment to the completion of the cell cycle. This point takes place during G1 and is under the control of the transcription factor MBF. The mechanisms that regulate MBF remain poorly understood. The main studies about the regulation of MBF activity have been focused on the inactivation of the complex. In this sense, the co-repressors Nrm1 and Yox1 are responsible for the inactivation of the complex at the end of S phase and during G2 phase. Both proteins are MBF target genes, therefore, the MBF-dependent transcription creates a negative feedback loop that maintains the homeostasis of the network. Regarding the activation of the complex, only Rep2 is known to work as co-activator, since its deletion leads to a reduced transcription of MBF-dependent genes. However, these co-activator and co-repressors have not any enzymatic activity that could directly alter the function of MBF complex; therefore, the search of new MBF regulators is necessary to further understand how is regulated the transcriptional network of the G1-S transition.

Fluorescence-based reporter of MBF activity

With the aim of discovering new proteins that regulate the function of MBF, we developed a flow cytometry–based approach to analyse the MBF activity. We used this reporter to perform a genome-wide screening with the aim to isolated non-essential proteins that may regulate the MBF activity. Since flow cytometry is a laser-based technology mainly used to quantify fluorescence, we decided to fuse the MBF regulated gene cdc22 with YFP as a reporter of MBF activity. Among the different conditions that we tested, we showed that fluorescence chimera Cdc22-YFP was the best option to monitor

the variations of MBF activity because it shows changes in the ratio of fluorescence when the MBF activity is altered and the steady-state levels are maintained; therefore, the variations in the fluorescence of Cdc22-YFP that we measured were a true reflection of the transcription changes caused by MBF complex. We were able to assay more than 2700 different mutants, and we reported several novel factors affecting the MBF-dependent transcription. We identified some enzymes involved in the maturation of tRNAs that support the transcription of MBF genes, and components COP9/Signalosome and E3 ligase complex Ddb1-Cul4Cdt2 that promote the downregulation of MBF-dependent transcription.

As we have shown, the fluorescence-based method that we have described in this work is a highly quantitative method of measuring expression of MBF-dependent transcription. However, this method has the limitation that can only can be applied for the search of non-essential genial genes. Since Cdc22 and YFP are long-lived proteins, we cannot detect fast changes in the transcriptional activity of MBF, especially those that decrease transcription. Therefore, future screenings using a short-lived reporter system which is rapidly degraded will be necessary to detect fast changes in MBF activity, especially those that downregulate the transcription. For example, a short-lived reported system could be the GFP protein with a degron sequence which can be recognized to target for degradation by the ubiquitin-proteasome system, allowing the detection transient changes in MBF activity.

tRNA methyltransferases and the Elongator complex promote an accurate MBF activity

Among the strains with the MBF-dependent transcription downregulated, we found several mutants of the tRNA methylation

pathway, the Elongator complex and some ribosomal proteins. The impaired MBF activity in these mutants points that an efficient and faithful translation is necessary to maintain stable levels of MBF-dependent transcription. Transfer RNAs (tRNAs) are the molecular adapters expressed by RNA polymerase III that connect the different codons of mRNA with the aminoacids encoded by these codons. As the genetic code is degenerated, many aminoacids are encoded by more than one triplet. The tRNAs must recognize more than one codon, because there are far fewer tRNA genes than codons. For this reason, tRNAs are highly modified (up to 100 different modifications have been described) (Agris et al., 2007), influencing the stability and the structure of the tRNA when recognize different codons. These modifications have two main consequences: the increase of the efficiency of the tRNA when recognizes the codons, which maintain the levels of newly synthetized proteins (Kuger et al., 1998); and the increase of the fidelity of the translation processes in order to avoid mutations in the newly synthetized proteins (Patil et al., 2012).

In our genetic screening we identified some enzymes responsible for catalysing the modifications at specific sites of tRNAs. Among these enzymes, we found Elongator complex proteins Elp1, Elp4 and Elp6, and the tRNAs methyltransferase protein Trm112 which is an auxiliary protein that dimerizes with Trm9 and Trm11 to form an active enzyme, or with Mtq2 to form the translation release factor eRF1. Both group of enzymes work together in the modification at wobble position (U_{34}) of tRNALysUUU, tRNAGlnUUG and tRNAGluUUC; while Elongator introduces an acetyl group at position 5 of U_{34} (cm^5U_{34}), tRNAs methyltransferases introduce a methyl group at U_{34} to yield mcm5U_{34}. Elongator also works with Ctu1 to generate the thiolated form $mcm^5s^2U_{34}$. Both modifications allow the

generation of an anticodon 5′-UUN decoding lysine, glutamine, and glutamic acid. Therefore, these enzymes are required for translation efficiency and/or fidelity of mRNAs containing 5′-NAA codons. We hypothesized that the impaired MBF activity that showed the mutants of this pathway could be due to problems in the translation machinery of some positive regulators of MBF, specially some regulator whose mRNA contains an elevated number of 5'-NAA codons. We decided to check the levels of Rep2 in absence of Trm112, since Rep2 is the only co-activator of MBF described so far. However, we did not detect any changes in amount of Rep2, although it is possible that reduced translational infidelity of Δ*trm112* mutant promotes protein errors due to decreased wobble base modifications. Future experiments could test the translation infidelity of Rep2, analysing the protein sequence by mass spectrometry. Also, it is possible that the translation of another, yet unknown regulator is controlled by this modification machinery. This mechanism could work as a checkpoint of protein quality control that verifies the functionality of the translational machinery allowing the cell cycle progression through the transition G1-S only when the translational processes are functional. To test this question, we could analyse the cell cycle of synchronous cultures of these mutants by FACS. If this hypothesis is correct, these mutants should have a delay in the cell cycle due to dysfunctional translational processes.

In previous works, it was described that defects in the introduction of inosine at U_{34} produced an arrest of the cell cycle during G1/S and G2/M transitions (Tsutsumi et al., 2007). Therefore, not only proteins enriched in Glu, Gln and Lys are important to maintain the homeostasis of the cell cycle. Moreover, Trm112 has also an active role in ribosome biogenesis (Sardana and Johnson 2012). These

results support the previous exposed idea about the existence of a protein quality control checkpoint. With all this data it would be possible to confirm that translational processes, and more specifically tRNA modifications, need to be fine-tuned in order to promote the MBF activity, and therefore the proper G1-S transition, probably ensuring the accurate translation of a yet undescribed positive regulator of the MBF complex.

COP9/Signalosome and Cul4-Ddb1Cdt2 regulate negatively MBF-dependent transcription

In the genetic screening we also discovered mutants with increased MBF activity. Apart of the co-repressors Yox1 and Nrm1, the deletion of two of the six components of COP9/signalosome complex (CNS), Csn1 and Csn2, and some components of the E3 ubiquitin ligase complex Cul4-Ddb1^{Cdt2} exhibited the highest levels of MBF activity. These complexes work together in the activation of the ribonucleotide reductase (RNR) at the beginning of S phase and during the DNA repair because they promote the degradation of the RNR inhibitor, Spd1. Outside of S phase, Spd1 anchors the small subunit of RNR, Suc22, in the nucleus, away from large subunit, Cdc22, in the cytoplasm. At the onset of S phase, or after DNA damage, Spd1 is ubiquitinated and degraded, and Suc22 is relocates to the nucleus to form an active complex with Cdc22, allowing the increase of dNTP levels (Liu et al., 2003). Therefore, the degradation of Spd1 is cell cycle regulated, and is mediated by components of CSN, the scaffold protein Cul4, the adaptor protein Ddb1, and the substrate recruiting factor Cdt2, which is a MBF regulated gene (Fig. 18) (Liu et al., 2003; Holmber et al., 2005; Liu et al., 2005; Moss et al., 2010; Salguero et al., 2012).

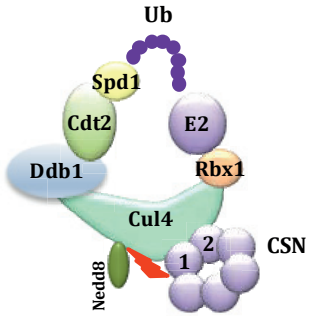

Figure 18. Cul4-Ddb1^{Cdt2} ubiquitin ligase system recognizes Spd1 and promotes its polyubiquitination and degradation. The cullin Cul4 serves as scaffold of the complex, Rbx1 is a RING finger protein that recruits the ubiquitin-conjugating E2 enzyme to ubiquitin the substrate Spd1, Ddb1 works an adaptor protein and Cdt2 is the substrate recognition factor that confers specify to the complex. The CSN regulates Cdt2 stability through Cul4 deneddylation (Nedd8).

The E3 Cul4-Ddb1^{Cdt2} complex has been widely related with the control of genome instability and DNA repair (Holmberg et al., 2005; Moss et al., 2010). To test if the activation of replicative checkpoint in these mutants was responsible for increasing the levels of MBF activity, we analysed the phosphorylation of Yox1. Yox1 is a co-repressor of MBF complex, which is phosphorylated by the effector kinase Cds1 when the replication checkpoint is triggered, leading to activation of MBF-dependent transcription (Ivanova et al., 2011). csn1 and csn2 mutants are unable to ubiquitinate and degrade the inhibitor Spd1, therefore the formation of RNR is prevented and the levels of dNTPs are downregulated. In consequence the DNA synthesis checkpoint is activated. The activation of the checkpoint could be reversed if we were able to restore the levels of dNTPs, eliminating the inhibitor Spd1. After eliminating the inhibitor Spd1 in these strains we could observe that the co-repressor Yox1 was not

phosphorylated, pointing that the depletion of dNTPs was the main cause that triggered the replicative checkpoint in mutants of Ddb1-Cul4Cdt2 ubiquitin complex, and therefore the MBF-dependent transcription was consequently up-regulated.

Surprisingly we noticed that although these mutants had the MBF-dependent transcription up-regulated and the replicative checkpoint activated, they were sensitive to replicative stress, and this sensitive was not recovered eliminating Spd1. This is consistent with previous studies that show UV sensitive and S-phase delay for csn1 and csn2 deletion mutants (Mundt et al., 2002; Bondar et al., 2003). Although Spd1 is a bona fide target of Ddb1-Cul4Cdt2, other targets have been described as well. The licensing protein Cdt1 and the heterochromatin regulator Epe1 have also been described as targets of this ubiquitin system (Ralph et al., 2006; Braun et al., 2011; Bayne et al., 2014), and they may be responsible of the sensitive to HU of these mutants.

Cdt1 is involved in the formation of the pre-replication complex (pre-RC), which has to be confined to G1 phase, ensuring that the origins are activated only once per cell cycle, avoiding re-replication processes. Cdt1 is degraded during S phase and also after DNA damage, to delay S-phase entry and presumably to prevent DNA replication until the DNA damage can be repaired (Higa et al., 2003; Ralph et al., 2006). In mutants of CSN-Ddb1-Cul4^{Cdt2}, Cdt1 levels remain high, and this fact may originate an efficient pre-RC formation leading to chromosome instability, or even to re-licensing events. In fact, Cdt1 is also a MBF-dependent gene, therefore in these mutants the levels of Cdt1 will be increased because the checkpoint is activated and the co-repressor Yox1 is phosphorylated. It is possible that an additive effect between the inhibition of degradation in these mutants and the activation of MBF-dependent transcription, may

produce unusual high levels of Cdt1. Previous studies have shown that overproduction of Cdt1 alone does not have a discernible effect on DNA synthesis, which reflects redundancy in mechanisms preventing re-replication (Gopalakrishnan et al., 2001; Ralph et al., 2006). The other replication origin-licensing factor that works with Cdt1, Cdc18, is also a MBF-dependent gene. Since in mutants of CSN-Ddb1-Cul4^{Cdt2} the MBF activity is up-regulated, it is possible that the increase of both licensing factors leads to inappropriate origin refiring and thereby cause genome instability. Previous studies have shown that hyperactivation of MBF induces elevated levels of Cdc18, leading to overreplication of DNA (Caetano et al., 2014). For future experiments, we could test this question analysing the DNA profile of these mutants to check if there is an increase in DNA content (>2C DNA content), which is an indicative of DNA overreplication.

The deletion of components of CSN-Ddb1-Cul4^{Cdt2} system also leads to increase the levels of Epe1. Epe1 is enriched at borders of heterochromatin, where catalyses the removal of H3K9 methylation counteracting the spreading of heterochromatin. CSN-Ddb1-Cul4^{Cdt2} is responsible to eliminate Epe1 from central heterochromatic domains, limiting its location to the boundaries. In absence of Ddb1-Cul4Cdt2, the chromosomal distribution of Epe1 is altered and the levels of H3K9me2 are reduced around heterochromatic loci (Braun et al., 2011). The redistribution of Epe1 may produce that the formation of heterochromatin is unpaired, and the DNA would be then more susceptible to DNA damage. This could be one possible explanation of the fact that double mutants Δ*spd1*Δ*ddb1* and Δ*spd1*Δ*cdt2* do not recover the phenotype in HU plates if the levels of dNTPs are restored. To confirm this question, we could check the phenotype in HU plates of a strain overexpressing Epe1, or a strain

that harbours H3K9R (unmethylated lysine 9) to check if mimics the effect of having high levels of Epe1. Also, we could check if the phenotypes are recovered in the following mutants ∆*epe1*∆*spd1*∆*ddb1* or ∆*epe1*∆*spd1*∆*cdt2*. It has been suggested that the degradation of Epe1 is cell cycle regulated, being limited to S phase to allow the assembly of the heterochromatin (Braun et al., 2011). Therefore, its degradation would coincide with the expression of Cdt2, supporting a role of Cdt2 in the formation of heterochromatin. Also it has been described that cells treated with HU show reduced levels of Epe1 (Braun et al., 2011), probably because the HU increases the MBF target gene Cdt2. It is possible that MBF controls the formation of heterochromatin through the expression of Cdt2 that is involved in the degradation of Epe1. In this sense, when MBF induces the transcription of Cdt2, the E3 ubiquitin system Ddb1-Cul4C^{dt2} removes Epe1 during S phase, allowing the assembly of heterochromatin.

INO80 complex promotes the transcription of MBF-regulates genes

Once we have described new proteins that indirectly regulate the transcription of MBF-dependent genes, we aimed to isolate new regulators that directly regulate the MBF-dependent transcription. With this aim, we decided to purify the MBF complex, tagging the core subunit Cdc10 with the epitope HA and we analysed the possible interactors using a proteomic approach which provides us a large-scale and high-throughput analyses for the detection and identification of new essential and non-essential MBF interactors. Among the peptides that we isolated, we found some members of the chromatin remodeler INO80 complex. In addition to the canonical four core histones (H2A, H2B, H3 and H4) that compose the

chromatin, there are histone variants with specific regulatory functions. H2A.Z (Pht1 in fission yeast) is a histone variant of H2A. The deposition of histone H2A.Z is under the control of SWR1 complex, and INO80 is in charge of its removal, redistributing the H2A.Z along the genome. Interestingly, H2A.Z is enriched at the -1 and +1 nucleosome of inducible genes, establishing the NDR between these both nucleosomes.

The co-IP experiments showed that Ies4 and Ino80, which are members of INO80 complex, interact directly with Cdc10, confirming the results obtained in the proteomic identification. We were able to detect both proteins located within MBF promoters, and its binding correlated with the expression of MBF-dependent genes. Cdc10, and therefore, MBF complex bind to target promoters constitutively along the cell cycle. The MBF promoters are characterized by having a NDR that is flanked by the -1 and +1 nucleosomes, and where MBF is located. Our ChIP results are consisting with previous works where INO80 recognizes and preferentially binds DNA that is depleted of nucleosomes, promoting the gene expression (Yen et al., 2013; Krietenstein et al., 2016), although its role in these regions is unknown.

We showed that different components of INO80 are important to maintain the MBF complex bound to their target promoters. In this sense, the deletion of the non-essential mutants of INO80 complex reduced the binding of Cdc10, and this fact is reflected in the reduction of the MBF-dependent transcripts. These results pointed that INO80 was important to maintain the structure of the chromatin around the NDR, since otherwise, the chromatin could be destabilized and probably the NDR would be altered, which ultimately will produce a decrease in MBF-dependent transcription. In fact, we could check that in strains that do not express H2A.Z, the

architecture of the NDR is altered, appearing a new nucleosome that may block or reduce the transcription. We also noticed that INO80 binds to MBF promoters 20 minutes before the onset of transcription, indicating that INO80 is required to prepare the chromatin to be transcribed.

Since acetylation of H2A.Z is associated to the induction of the transcription of the genes where it is deposited (Kim et al., 2009), we decided to analyse the acetylation of H2A.Z around MBF promoters in synchronous cultures. We showed that the acetylation of H2A.Z is cell cycle regulated in MBF-regulated genes, and it takes place around the onset of MBF-dependent transcription. We confirmed the importance of H2A.Zac with a mutated version which were unable to be acetylated (Pht1-KR): the unacetylable H2A.Z showed a strong decrease of MBF-dependent expression. This downregulation of the MBF activity was confirmed in synchronous cultures.

We have shown here that INO80 is important for the correct expression of MBF-dependent genes through mechanisms that are still not clear. But the data points that INO80 is important to maintain the NDR where the transcriptional machinery has to be coupled to initiate the transcriptional processes. The molecular mechanism by which H2A.Z contributes to regulation of transcription is still unknown. However, some studies point that the energy barrier of nucleosomes with histone H2A.Z is lower than nucleosomes with histone H2A, facilitating the progression of RNA pol II (Subramanian et al., 2015). Taking in consideration that H2A.Z is acetylated, and this acetylation can relax the structure of the chromatin, making it even accessible to the transcription machinery. This acetylation can also work as a recognition site for proteins with bromodomains. For example, it has been suggested that H2A.Zac may be recognized by the transcription factor Brd2 to mediate gene activation

(Subramanian et al., 2015). In the case of MBF, future experiments will be required to determine if H2A.Zac is only required to maintain the open chromatin or if it could act as signal for directing co-activators or general transcription factors to their appropriate positions.

Gcn5 is required for full activation of the G1-S transcriptional wave

Diverse molecular processes can be regulated through the different levels of DNA packaging. Among them, the transcription is one of the processes more tightly regulated by the state of the chromatin. The structure and composition of the chromatin can be regulated by chromatin remodelers or by histone modifiers. We have described above that the chromatin remodeler INO80 plays an active role in the activation of MBF-dependent transcription. We have also described that H2A.Zac is necessary to maintain a proper MBF activity. Histone acetylation is a modification typically related with the activation of gene expression, and it is under the control of HATs. As we continue with the search for activators that directly regulate the MBF-dependent transcription, we decided to perform a screening of the recruitment of HATs to MBF promoters. We decided to use HU to activate the MBF-dependent transcription, since HU activates the DNA replication checkpoint, releasing the co-repressor Yox1 from the chromatin, and promoting the MBF activity. We found that the HAT Gcn5 was actively recruited to MBF promoters when the transcription of MBF was activated. We also tested in synchronous cultures whether the binding of Gcn5 correlated with the activation of MBF complex. In this sense, Gcn5 was bound to MBF promoters during Mitosis, G1 phase and it was released at the end of S phase. Gcn5 is part of the transcriptional co-activator complex called SAGA.

Thus, we tested if Spt7, which is part of the core component of SAGA could bind to MBF promoters as well. Since MBF is located around promoters, we focused our interest in the study of Gcn5 and SAGA around MBF promoters, where we have also seen that is mostly located. SAGA complex has no known DNA-binding activity, and it has been suggested that its recruitment to UASs is a reflect of its recruitment by some DNA-binding proteins (Baptista et al., 2017). In this work, we showed by first time a specific role for co-activator Rep2, which physically interacts with Gcn5, and it is necessary to anchor SAGA complex to MBF promoters. Moreover, SAGA is recruited to some target genes through the recruitment module, Tra1 (Helmlinger et al., 2011). We also confirmed that Tra1 is important for the recruitment of Gcn5 to MBF target genes. However, in absence of Rep2 and Tra1, the recruitment of Gcn5 is the same that when any of two proteins is not present, indicating that both proteins work in the same pathway of recruitment. Gcn5 has a bromodomain to target acetylated proteins, and which helps to retention of SAGA on acetylated nucleosomes, and to promote nucleosome acetylation (Hassan et al., 2002; Li and Shogren-Knaak et al., 2009). Therefore, the remaining levels of Gcn5 that we detect in absence of Tra1 and Rep2 within MBF promoters could be due to its recruitment by the bromodomain. To discard this question, we should create in the future a truncated version of Gcn5 where the bromodomain will be eliminated; therefore, we could check in this situation if the binding of Gcn5 is decreased.

We noticed that the binding of Gcn5 along the cell cycle presented an inverse dynamic to the binding of co-repressors Nrm1 and Yox1. We confirmed that in absence of both co-repressors, Gcn5 was constitutively bound to MBF promoters, pointing that both co-repressors act as physical barrier for the recruitment of Gcn5. Since

Rep2 is constitutively bound to MBF promoters, we propose that the binding of the co-repressors Nrm1 and Yox1 mark the cell cycle-dependent regulation of Gcn5. However, we showed that in absence of both Rep2 and Nrm1 proteins, Gcn5 is unable to bind to MBF promoters, indicating that the component absolutely necessary for the binding of Gcn5 is the co-activator Rep2. Recently, we have described that Nrm1 is phosphorylated and degraded in Mitosis. And in the present work, we showed that Gcn5 binds to MBF promoters in mitosis. Therefore, all this data pointed that when Nrm1 is targeted to degradation in Anaphase, Gcn5 is able to bind to MBF promoters through its interaction with Rep2.

In previous works, it was shown that the main role of Gcn5 is to acetylate the histones H3K9, H3K14 and H3K18 (Nugent et al., 2010; Pai et al., 2014). In this work, we showed how Gcn5 is responsible for the acetylation of H3K9 and H3K18 within MBF promoters, and this acetylation is cell cycle regulated, peaking during the G1-S transcriptional wave. Although our experiments also showed a non-significant increase of acetylation of H3K14 when we treated cells with HU. The H3K14ac is under the control of both Gcn5 and Mst2 and it has been related with the DNA damage (Wang et al., 2012). This acetylation does not appear to have a relevant role in activation of MBF-dependent genes during unperturbed cell cycle.

Finally, we demonstrated that Gcn5 has an active role in the regulation of MBF-dependent transcription. This effect is specially highlighted in synchronous cultures, where we can observe that the peak of transcription of Δ*gcn5* does not reach wild type levels, and moreover, the peak has a delay between 10 and 20 min compare to the wild type strain. Early genes (*cdc18* and *cdt1*) present a longer delay than late genes (*cdt2* and *cdc22*). This effect seems to be in part by the acetylation of histones H3K9 and H3K18. We constructed

mutants where the lysine 9, lysine 18 or both lysines of histone H3 were mutated to arginine (K9R, K18R and K9RK18R), respectively. These mutations were introduced in a strain without two of the three copies of H3 *(Δh3.1 Δh3.3*). We observed that the mutant H3K9RK18R was as the same sensitive to HU as Δ*gcn5* cells, pointing that H3K9RK18R mimics the phenotype of the Δ*gcn5* strain. The quantitative analysis of mRNA of MBF-dependent genes showed that the double mutant H3K9RK18R presents the MBF-dependent transcription significantly downregulated. However, we do not see the same reduction for Δ*gcn5* mutant. A possible explanation for this effect is that in Δ*gcn5* mutant we still detect some acetylation of histone H3K9 and H3K18 as revealed the WB α-H3K9ac and α-H3K18ac, and therefore we cannot see the downregulation of transcription that we could detect that H3K9RK18R mutant, which is totally deacetylated. In synchronous cultures we could see like H3K9RK18R mutant presents a downregulation of MBF-dependent genes, confirming that the acetylation of both H3K9 and H3K18 is essential to maintain the levels of MBF transcripts. Further experiments to check the recruitment of chromatin modifiers in absence of Gcn5, will be required to determine if the acetylation performed by Gcn5 is essential to recruit other regulatory proteins.

We postulate that when the co-repressors Nrm1 and Yox1 are released from the chromatin in Anaphase, Gcn5 together with the SAGA complex bind to MBF promoters through the DNA-bound activator Rep2 and the Tra1 subunit. Gcn5 probably also interacts with acetylated histones H3 and H4, through its bromodomain, potentiating the cooperative nucleosome acetylation of H3 at lysine 9 and 18. Both acetylations can promote an open chromatin to recruit additional transcription factors and also the pre-initiation complex

(PIC). One of the modules of SAGA (composed by Spt3 and Spt8) is involved in the recruitment of the TATA-binding protein (TBP) to the promoters. Therefore, when Gcn5 acetylates neighbouring histones, opens the chromatin to promote the formation of PIC. Additionally, the acetylation serves as docking site for the recruitment of further chromatin remodelers that assist in the sliding or eviction of nucleosomes to allow the RNA pol II initiation of transcription. In fact, it has been described that after the initiation of transcription, SAGA colocalizes with RNA pol II in the body of genes, where probably assists the deubiquitination of H2B (Weake et al., 2011). Our ChIP experiments showed that Gcn5 was also recruited to coding region of MBF-dependent genes. In *S. cerevisiase*, SAGA is recruited to UASs of nearly all genes, similarly to TFIID, and both transcription factors are required for expression of all genes transcribed by RNA Pol II (Baptista et al., 2017; Warfield et al., 2017). In human and budding yeast, SAGA is involved in the acetylation of H3K9 around promoters and deubiqutination of H2Bub around gene bodies of all expressed genes (Bonnet et al., 2014). Traditionally, the studies about SAGA complex have related the expression of stress-regulated genes with the mediation of this complex (Basehoar et al., 2004)- However in these studies only the steady-state of mRNA is measured without considering that there are compensatory mechanisms, which results in mRNA level buffering. For example, the RNA pol II subunit Rpb4 deletion causes a great defect in mRNA synthesis that is compensated by down regulation of mRNA degradation (Schulz et al., 2014). Recent studies in budding yeast identified that the depletion of SAGA produced a compensatory increase of the half-life of the mRNAs that made very difficult to identify changes in steady-state mRNA levels in mutants of SAGA complex (Bonnet et al., 2014; Baptista et al.,

2017). To solve this question, the newly synthetized mRNA, (nascent mRNA), was measured, proving that the transcription of all RNA pol II-dependent genes was downregulated in Δ*gcn5* mutants. It is possible that the same buffering mechanism is acting in the MBF-dependent transcription when we analysed Δ*gcn5* mutants in asynchronous cultures, since we could not detect great changes. Or it could be merely an effect of having the 70% of the cells in G2, when the MBF-dependent transcription is downregulated. Future experiments, measuring the newly synthetized mRNA will be necessary to identify if SAGA is a general co-factor that regulate almost all genes in S. pombe as well. It is also important to mention that SAGA complex of S. cerevisiae y S. pombe are not exactly the same complex, since the Tra1 subunit is essential in *S. cerevisiae* but not in S. pombe. Another difference that we find in *S. pombe*, is that Gcn5 may also play a negative role in the transcription of meiotic and mating genes depending on the medium conditions (Helmlinger et al., 2008).

To summarize, we can integrate our results in the following model (Fig. 19): MBF remains always bound to MCB elements, which are UASs located within the NDR. H2A.Z containing nucleosomes flank the NDR on both sides, forming the -1 and +1 nucleosome. The activation of the complex depends on the release of co-repressors Yox1 and Nrm1. During G2 phase, the co-repressors Nrm1 and Yox1 remain bound to silent MBF promoters. However, during anaphase, Nrm1 is phosphorylated by CDK1, and both co-repressors are released from chromatin. At this moment (at the end of mitosis), INO80 is already bound to MBF promoters, probably to maintain the structure of NDR, or to assist in the organization of the promoter. The SAGA complex is recruited to MBF through the interaction of Rep2 with Gcn5 and with Tra1. Histone H3K9 and

H3K18 are acetylated at promoter-proximal regions, and these nucleosomes become much more mobiles. Bromodomain proteins, such as chromatin remodelers, recognize specific acetyl-lysine residues, and collaborates in the loss of +1 nucleosome. Therefore, the promoter core is exposed to the entire transcription machinery, including RNA pol II, which allows transcription of MBF-dependent genes during M, G1 and S phase. At the end of S phase, the co-repressors Nrm1 and Yox1 are re-loaded to MBF complex, blocking the binding of all histone modifiers and chromatin remodelers, and shutting down MBF-dependent transcription.

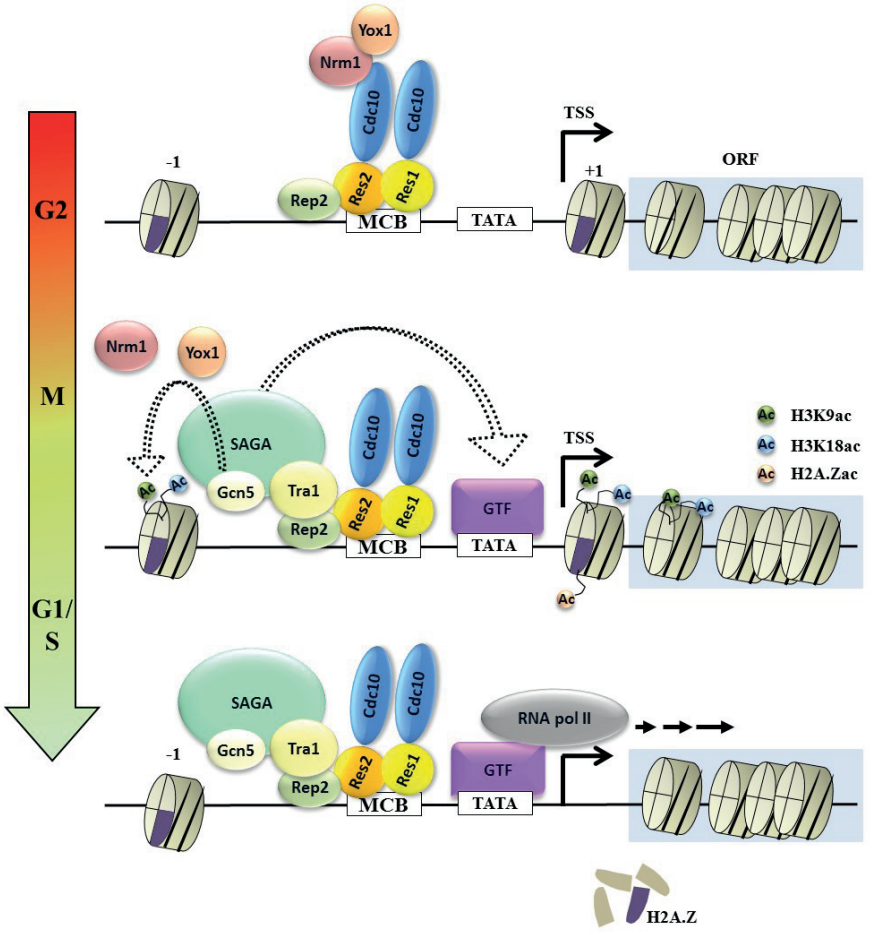

Figure 19. A schematic model illustrating the regulation of the MBF-dependent transcription along the cell cycle. MBF remains bound to MCB (MluI Cell Cycle Box) sequences along the cell cycle. The MCB motifs are located in a nucleosome depleted region just upstream of the TSS (transcription start site) that is flanked by the -1 and +1 nucleosomes. Both nucleosomes are enriched in histone variant H2A.Z (in purple). At the end of S phase, the co-repressors Yox1 and Nrm1 bind to MBF complex, inhibiting the MBF-dependent transcription. But during mitosis, the co-repressors are released from the chromatin, and the SAGA complex is brought to MBF promoters through direct interaction of the co-activator Rep2 with Gcn5 and Tra1. Gcn5 promotes the acetylation of H3K9 and H3K18 at promoter-proximal regions. The histone variant H2A.Z is also acetylated by Mst1. SAGA promotes the recruitment of GTF (general transcription factors) that bind around the TATA box. The GTF helps to recruit RNA pol II at the TSS, where the nucleosome +1 is evicted, promoting the transcription of MBF-dependent genes.

CONCLUSIONS

1. Cdc22-YFP can be used as a reporter system to monitor the variations of MBF activity.

2. tRNA methyltransferases and the Elongator complex promote MBF activity through an efficient and faithful translation.

3. CSN-Ddb1-Cul4^{Cdt2} regulate negatively the MBF-dependent transcription through the stabilization of the ribonucleotide reductase inhibitor Spd1.

4. INO80 complex physically interacts with MBF complex and is required to maintain MBF bound to their target promoters.

5. INO80 complex promotes the transcription of MBF-regulated genes.

6. The histone variant H2A.Z (Pht1) is important to maintain the architecture of NDR at MBF promoters.

7. The acetylation of H2A.Z is required for the proper expression of MBF-dependent genes.

8. Gcn5, which is part of SAGA complex, associates with MBF complex promoting the transcription of MBF-regulated genes.

9. The co-activator Rep2 is required for the association of Gcn5 with MBF promoters.

10. The co-repressors Nrm1 and Yox1 block the recruitment of Gcn5 to MBF promoters.

11. Gcn5 acetylates H3K9 and H3K18 of MBF promoters during G1-S transition.

REFERENCES

Abshiru, N., Rajan, R.E., Verreault, A., and Thibault, P. (2016). Unraveling Site-Specific and Combinatorial Histone Modifications Using High-Resolution Mass Spectrometry in Histone Deacetylase Mutants of Fission Yeast. J Proteome Res *15*, 2132-2142.

Agarwal, M., Papadopoulou, K., Mayeux, A., Vajrala, V., Quintana, D.M., Paoletti, A., and McInerny, C.J. (2010). Mid1p-dependent regulation of the M-G1 transcription wave in fission yeast. J Cell Sci *123*, 4366-4373.

Agris, P.F., Vendeix, F.A., and Graham, W.D. (2007). tRNA's wobble decoding of the genome: 40 years of modification. J Mol Biol *366*, 1-13.

Ahmad, K., and Henikoff, S. (2001). Centromeres are specialized replication domains in heterochromatin. J Cell Biol *153*, 101-110.

Ahn, S., Spatt, D., and Winston, F. (2012). The Schizosaccharomyces pombe inv1+ regulatory region is unusually large and contains redundant cis-acting elements that function in a SAGA- and Swi/Snf-dependent fashion. Eukaryot Cell *11*, 1067-1074.

Albert, I., Mavrich, T.N., Tomsho, L.P., Qi, J., Zanton, S.J., Schuster, S.C., and Pugh, B.F. (2007). Translational and rotational settings of H2A.Z nucleosomes across the Saccharomyces cerevisiae genome. Nature *446*, 572-576.

Aligianni, S., Lackner, D.H., Klier, S., Rustici, G., Wilhelm, B.T., Marguerat, S., Codlin, S., Brazma, A., de Bruin, R.A., and Bahler, J. (2009). The fission yeast homeodomain protein Yox1p binds to MBF and confines MBF-dependent cell-cycle transcription to G1-S via negative feedback. PLoS Genet *5*, e1000626.

Alonso-Nunez, M.L., An, H., Martin-Cuadrado, A.B., Mehta, S., Petit, C., Sipiczki, M., del Rey, F., Gould, K.L., and de Aldana, C.R. (2005). Ace2p controls the expression of genes required for cell separation in Schizosaccharomyces pombe. Mol Biol Cell *16*, 2003-2017.

Alper, B.J., Lowe, B.R., and Partridge, J.F. (2012). Centromeric heterochromatin assembly in fission yeast--balancing transcription, RNA interference and chromatin modification. Chromosome Res *20*, 521-534.

Amon, A., Tyers, M., Futcher, B., and Nasmyth, K. (1993). Mechanisms that help the yeast cell cycle clock tick: G2 cyclins transcriptionally activate G2 cyclins and repress G1 cyclins. Cell *74*, 993-1007.

Anderson, M., Ng, S.S., Marchesi, V., MacIver, F.H., Stevens, F.E., Riddell, T., Glover, D.M., Hagan, I.M., and McInerny, C.J. (2002). Plo1(+) regulates gene transcription at the M-G(1) interval during the fission yeast mitotic cell cycle. EMBO J *21*, 5745-5755.

Aparicio, J.G., Viggiani, C.J., Gibson, D.G., and Aparicio, O.M. (2004). The Rpd3-Sin3 histone deacetylase regulates replication timing and enables intra-S origin control in Saccharomyces cerevisiae. Mol Cell Biol *24*, 4769-4780.

Armas-Pineda, C., Arenas-Huertero, F., Perezpenia-Diazconti, M., Chico-Ponce de Leon, F., Sosa-Sainz, G., Lezama, P., and Recillas-Targa, F. (2007). Expression of PCAF, p300 and Gcn5 and more highly acetylated histone H4 in pediatric tumors. J Exp Clin Cancer Res *26*, 269-276.

Arnold, A., and Papanikolaou, A. (2005). Cyclin D1 in breast cancer pathogenesis. J Clin Oncol *23*, 4215-4224.

Atanassov, B.S., Evrard, Y.A., Multani, A.S., Zhang, Z., Tora, L., Devys, D., Chang, S., and Dent, S.Y. (2009). Gcn5 and SAGA regulate shelterin protein turnover and telomere maintenance. Mol Cell *35*, 352-364.

Aves, S.J., Durkacz, B.W., Carr, A., and Nurse, P. (1985). Cloning, sequencing and transcriptional control of the Schizosaccharomyces pombe cdc10 'start' gene. EMBO J *4*, 457-463.

Avvakumov, N., and Cote, J. (2007). The MYST family of histone acetyltransferases and their intimate links to cancer. Oncogene *26*, 5395-5407.

Ayoub, N., Noma, K., Isaac, S., Kahan, T., Grewal, S.I., and Cohen, A. (2003). A novel jmjC domain protein modulates heterochromatization in fission yeast. Mol Cell Biol *23*, 4356-4370.

Ayte, J., Leis, J.F., and DeCaprio, J.A. (1997). The fission yeast protein p73res2 is an essential component of the mitotic MBF complex and a master regulator of meiosis. Mol Cell Biol *17*, 6246-6254.

Ayte, J., Leis, J.F., Herrera, A., Tang, E., Yang, H., and DeCaprio, J.A. (1995). The Schizosaccharomyces pombe MBF complex requires heterodimerization for entry into S phase. Mol Cell Biol *15*, 2589-2599.

Ayté, J., Schweitzer, C., Zarzov, P., Nurse, P., and DeCaprio, J.A. (2001). Feedback regulation of the MBF transcription factor by cyclin Cig2. Nat Cell Biol *3*, 1043-1050.

Badis, G., Chan, E.T., van Bakel, H., Pena-Castillo, L., Tillo, D., Tsui, K., Carlson, C.D., Gossett, A.J., Hasinoff, M.J., Warren, C.L., *et al.* (2008). A library of yeast transcription factor motifs reveals a widespread function for Rsc3 in targeting nucleosome exclusion at promoters. Mol Cell *32*, 878-887.

Balasubramanian, R., Pray-Grant, M.G., Selleck, W., Grant, P.A., and Tan, S. (2002). Role of the Ada2 and Ada3 transcriptional coactivators in histone acetylation. J Biol Chem *277*, 7989-7995.

Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C., and Kouzarides, T. (2001). Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature *410*, 120-124.

Baptista, T., Grunberg, S., Minoungou, N., Koster, M.J.E., Timmers, H.T.M., Hahn, S., Devys, D., and Tora, L. (2017). SAGA Is a General Cofactor for RNA Polymerase II Transcription. Mol Cell *68*, 130-143 e135.

Barbaric, S., Luckenbach, T., Schmid, A., Blaschke, D., Horz, W., and Korber, P. (2007). Redundancy of chromatin remodeling pathways for the induction of the yeast PHO5 promoter in vivo. J Biol Chem *282*, 27610-27621.

Bartek, J., Lukas, C., and Lukas, J. (2004). Checking on DNA damage in S phase. Nat Rev Mol Cell Biol *5*, 792-804.

Bartek, J., and Lukas, J. (2003). Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell *3*, 421-429.

Basehoar, A.D., Zanton, S.J., and Pugh, B.F. (2004). Identification and distinct regulation of yeast TATA box-containing genes. Cell *116*, 699-709.

Batsche, E., Yaniv, M., and Muchardt, C. (2006). The human SWI/SNF subunit Brm is a regulator of alternative splicing. Nat Struct Mol Biol *13*, 22-29.

Baum, B., Wuarin, J., and Nurse, P. (1997). Control of S-phase periodic transcription in the fission yeast mitotic cycle. EMBO J *16*, 4676-4688.

Bayne, E.H., Bijos, D.A., White, S.A., de Lima Alves, F., Rappsilber, J., and Allshire, R.C. (2014). A systematic genetic screen identifies new factors influencing centromeric heterochromatin integrity in fission yeast. Genome Biol *15*, 481.

Bean, J.M., Siggia, E.D., and Cross, F.R. (2005). High functional overlap between MluI cell-cycle box binding factor and Swi4/6 cell-cycle box binding factor in the G1/S transcriptional program in Saccharomyces cerevisiae. Genetics *171*, 49-61.

Bell, O., Tiwari, V.K., Thoma, N.H., and Schubeler, D. (2011). Determinants and dynamics of genome accessibility. Nat Rev Genet *12*, 554-564.

Bell, S.P., and Dutta, A. (2002). DNA replication in eukaryotic cells. Annu Rev Biochem *71*, 333-374.

Benito, J., Martin-Castellanos, C., and Moreno, S. (1998). Regulation of the G1 phase of the cell cycle by periodic stabilization and degradation of the p25rum1 CDK inhibitor. EMBO J *17*, 482-497.

Benson, L.J., Phillips, J.A., Gu, Y., Parthun, M.R., Hoffman, C.S., and Annunziato, A.T. (2007). Properties of the type B histone acetyltransferase Hat1: H4 tail interaction, site preference, and involvement in DNA repair. J Biol Chem *282*, 836-842.

Berger, I., Blanco, A.G., Boelens, R., Cavarelli, J., Coll, M., Folkers, G.E., Nie, Y., Pogenberg, V., Schultz, P., Wilmanns, M.*, et al.* (2011). Structural insights into transcription complexes. J Struct Biol *175*, 135-146.

Berndsen, C.E., Tsubota, T., Lindner, S.E., Lee, S., Holton, J.M., Kaufman, P.D., Keck, J.L., and Denu, J.M. (2008). Molecular functions of the histone acetyltransferase chaperone complex Rtt109-Vps75. Nat Struct Mol Biol *15*, 948-956.

Bertoli, C., Skotheim, J.M., and de Bruin, R.A. (2013). Control of cell cycle transcription during G1 and S phases. Nat Rev Mol Cell Biol *14*, 518-528.

Bhaumik, S.R., Smith, E., and Shilatifard, A. (2007). Covalent modifications of histones during development and disease pathogenesis. Nat Struct Mol Biol *14*, 1008-1016.

Bian, C., Xu, C., Ruan, J., Lee, K.K., Burke, T.L., Tempel, W., Barsyte, D., Li, J., Wu, M., Zhou, B.O.*, et al.* (2011). Sgf29 binds histone H3K4me2/3 and is required for SAGA complex recruitment and histone H3 acetylation. EMBO J *30*, 2829-2842.

Biddick, R., and Young, E.T. (2005). Yeast mediator and its role in transcriptional regulation. C R Biol *328*, 773-782.

Bjorklund, S., and Gustafsson, C.M. (2005). The yeast Mediator complex and its regulation. Trends Biochem Sci *30*, 240-244.

Blanco, M.A., Sanchez-Diaz, A., de Prada, J.M., and Moreno, S. (2000). APC(ste9/srw1) promotes degradation of mitotic cyclins in G(1) and is inhibited by cdc2 phosphorylation. EMBO J *19*, 3945-3955.

Bloom, J., and Cross, F.R. (2007). Multiple levels of cyclin specificity in cell-cycle control. Nat Rev Mol Cell Biol *8*, 149-160.

Blow, J.J., and Laskey, R.A. (1986). Initiation of DNA replication in nuclei and purified DNA by a cell-free extract of Xenopus eggs. Cell *47*, 577-587.

Bondar, T., Ponomarev, A., and Raychaudhuri, P. (2004). Ddb1 is required for the proteolysis of the Schizosaccharomyces pombe replication inhibitor Spd1 during S phase and after DNA damage. J Biol Chem *279*, 9937-9943.

Bonnet, J., Wang, C.Y., Baptista, T., Vincent, S.D., Hsiao, W.C., Stierle, M., Kao, C.F., Tora, L., and Devys, D. (2014). The SAGA coactivator complex acts on the whole transcribed genome and is required for RNA polymerase II transcription. Genes Dev *28*, 1999-2012.

Bottomley, M.J. (2004). Structures of protein domains that create or recognize histone modifications. EMBO Rep *5*, 464-469.

Brand, M., Moggs, J.G., Oulad-Abdelghani, M., Lejeune, F., Dilworth, F.J., Stevenin, J., Almouzni, G., and Tora, L. (2001). UV-damaged DNA-binding protein in the TFTC complex links DNA damage recognition to nucleosome acetylation. EMBO J *20*, 3187-3196.

Braun, S., Garcia, J.F., Rowley, M., Rougemaille, M., Shankar, S., and Madhani, H.D. (2011). The Cul4-Ddb1(Cdt)(2) ubiquitin ligase inhibits invasion of a boundary-associated antisilencing factor into heterochromatin. Cell *144*, 41-54.

Breeden, L. (1996). Start-specific transcription in yeast. Curr Top Microbiol Immunol *208*, 95-127.

Breeden, L., and Nasmyth, K. (1987). Similarity between cell-cycle genes of budding yeast and fission yeast and the Notch gene of Drosophila. Nature *329*, 651-654.

Brehm, A., Miska, E.A., McCance, D.J., Reid, J.L., Bannister, A.J., and Kouzarides, T. (1998). Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature *391*, 597-601.

Brown, C.E., Howe, L., Sousa, K., Alley, S.C., Carrozza, M.J., Tan, S., and Workman, J.L. (2001). Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. Science *292*, 2333-2337.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996). Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell *84*, 843-851.

Buck, V., Ng, S.S., Ruiz-Garcia, A.B., Papadopoulou, K., Bhatti, S., Samuel, J.M., Anderson, M., Millar, J.B., and McInerny, C.J. (2004). Fkh2p and Sep1p regulate mitotic gene transcription in fission yeast. J Cell Sci *117*, 5623-5632.

Bulmer, R., Pic-Taylor, A., Whitehall, S.K., Martin, K.A., Millar, J.B., Quinn, J., and Morgan, B.A. (2004). The forkhead transcription factor Fkh2 regulates the cell division cycle of Schizosaccharomyces pombe. Eukaryot Cell *3*, 944-954.

Burgess, R.J., Zhou, H., Han, J., and Zhang, Z. (2010). A role for Gcn5 in replication-coupled nucleosome assembly. Mol Cell *37*, 469-480.

Buscaino, A., Lejeune, E., Audergon, P., Hamilton, G., Pidoux, A., and Allshire, R.C. (2013). Distinct roles for Sir2 and RNAi in centromeric heterochromatin nucleation, spreading and maintenance. EMBO J *32*, 1250-1264.

Cadoret, J.C., Meisch, F., Hassan-Zadeh, V., Luyten, I., Guillet, C., Duret, L., Quesneville, H., and Prioleau, M.N. (2008). Genome-wide studies highlight indirect links between human replication origins and gene regulation. Proc Natl Acad Sci U S A *105*, 15837-15842.

Caetano, C., Klier, S., and de Bruin, R.A. (2011). Phosphorylation of the MBF repressor Yox1p by the DNA replication checkpoint keeps the G1/S cell-cycle transcriptional program active. PLoS One *6*, e17211.

Campsteijn, C., Wijnands-Collin, A.M., and Logie, C. (2007). Reverse genetic analysis of the yeast RSC chromatin remodeler reveals a role for RSC3 and SNF5 homolog 1 in ploidy maintenance. PLoS Genet *3*, e92.

Cao, Y., Cairns, B.R., Kornberg, R.D., and Laurent, B.C. (1997). Sfh1p, a component of a novel chromatin-remodeling complex, is required for cell cycle progression. Mol Cell Biol *17*, 3323-3334.

Cayrou, C., Coulombe, P., Vigneron, A., Stanojcic, S., Ganier, O., Peiffer, I., Rivals, E., Puy, A., Laurent-Chabalier, S., Desprat, R.*, et al.* (2011). Genome-scale analysis of metazoan replication origins reveals their organization in specific but flexible sites defined by conserved features. Genome Res *21*, 1438-1449.

Chai, B., Huang, J., Cairns, B.R., and Laurent, B.C. (2005a). Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. Genes Dev *19*, 1656-1661.

Chai, J., Charboneau, A.L., Betz, B.L., and Weissman, B.E. (2005b). Loss of the hSNF5 gene concomitantly inactivates p21CIP/WAF1 and p16INK4a activity associated with replicative senescence in A204 rhabdoid tumor cells. Cancer Res *65*, 10192-10198.

Chakrabarty, B., and Parekh, N. (2014). Identifying tandem Ankyrin repeats in protein structures. BMC Bioinformatics *15*, 6599.

Chakravarthy, S., Bao, Y., Roberts, V.A., Tremethick, D., and Luger, K. (2004). Structural characterization of histone H2A variants. Cold Spring Harb Symp Quant Biol *69*, 227-234.

Chang, D.Y., Shi, G., Durand-Dubief, M., Ekwall, K., and Lu, A.L. (2011). The role of MutY homolog (Myh1) in controlling the histone deacetylase Hst4 in the fission yeast Schizosaccharomyces pombe. J Mol Biol *405*, 653-665.

Chang, K.P., Wu, C.C., Chen, H.C., Chen, S.J., Peng, P.H., Tsang, N.M., Lee, L.Y., Liu, S.C., Liang, Y., Lee, Y.S.*, et al.* (2010). Identification of candidate nasopharyngeal carcinoma serum biomarkers by cancer cell secretome and tissue transcriptome analysis: potential usage of cystatin A for predicting nodal stage and poor prognosis. Proteomics *10*, 2644-2660.

Chen, C.C., Carson, J.J., Feser, J., Tamburini, B., Zabaronick, S., Linger, J., and Tyler, J.K. (2008a). Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. Cell *134*, 231-243.

Chen, E.S., Zhang, K., Nicolas, E., Cam, H.P., Zofall, M., and Grewal, S.I. (2008b). Cell cycle control of centromeric repeat transcription and heterochromatin assembly. Nature *451*, 734-737.

Chen, L., Wei, T., Si, X., Wang, Q., Li, Y., Leng, Y., Deng, A., Chen, J., Wang, G., Zhu, S.*, et al.* (2013). Lysine acetyltransferase GCN5 potentiates the growth of non-small cell lung cancer via promotion of E2F1, cyclin D1, and cyclin E1 expression. J Biol Chem *288*, 14510-14521.

Cheng, X., Collins, R.E., and Zhang, X. (2005). Structural and sequence motifs of protein (histone) methylation enzymes. Annu Rev Biophys Biomol Struct *34*, 267-294.

Chica, N., Rozalen, A.E., Perez-Hidalgo, L., Rubio, A., Novak, B., and Moreno, S. (2016). Nutritional Control of Cell Size by the Greatwall-Endosulfine-PP2A.B55 Pathway. Curr Biol *26*, 319-330.

Choi, E.S., Cheon, Y., Kang, K., and Lee, D. (2017). The Ino80 complex mediates epigenetic centromere propagation via active removal of histone H3. Nat Commun *8*, 529.

Chow, K.N., and Dean, D.C. (1996). Domains A and B in the Rb pocket interact to form a transcriptional repressor motif. Mol Cell Biol *16*, 4862-4868.

Chu, Z., Eshaghi, M., Poon, S.Y., and Liu, J. (2009). A Cds1-mediated checkpoint protects the MBF activator Rep2 from ubiquitination by anaphase-promoting complex/cyclosome-Ste9 at S-phase arrest in fission yeast. Mol Cell Biol *29*, 4959-4970.

Chuang, R.Y., and Kelly, T.J. (1999). The fission yeast homologue of Orc4p binds to replication origin DNA via multiple AT-hooks. Proc Natl Acad Sci U S A *96*, 2656-2661.

Ciccia, A., and Elledge, S.J. (2010). The DNA damage response: making it safe to play with knives. Mol Cell *40*, 179-204.

Collado, M., Blasco, M.A., and Serrano, M. (2007). Cellular senescence in cancer and aging. Cell *130*, 223-233.

Connolly, T., and Beach, D. (1994). Interaction between the Cig1 and Cig2 B-type cyclins in the fission yeast cell cycle. Mol Cell Biol *14*, 768-776.

Costanzo, M., Nishikawa, J.L., Tang, X., Millman, J.S., Schub, O., Breitkreuz, K., Dewar, D., Rupes, I., Andrews, B., and Tyers, M. (2004). CDK activity antagonizes Whi5, an inhibitor of G1/S transcription in yeast. Cell *117*, 899-913.

Coudreuse, D., and Nurse, P. (2010). Driving the cell cycle with a minimal CDK control network. Nature *468*, 1074-1079.

Creamer, K.M., Job, G., Shanker, S., Neale, G.A., Lin, Y.C., Bartholomew, B., and Partridge, J.F. (2014). The Mi-2 homolog Mit1 actively positions nucleosomes within heterochromatin to suppress transcription. Mol Cell Biol *34*, 2046-2061.

Creanor, J., and Mitchison, J.M. (1996). The kinetics of the B cyclin p56cdc13 and the phosphatase p80cdc25 during the cell cycle of the fission yeast Schizosaccharomyces pombe. J Cell Sci *109 (Pt 6)*, 1647-1653.

Dai, J., Chuang, R.Y., and Kelly, T.J. (2005). DNA replication origins in the Schizosaccharomyces pombe genome. Proc Natl Acad Sci U S A *102*, 337-342.

de Bruin, R.A., Kalashnikova, T.I., Chahwan, C., McDonald, W.H., Wohlschlegel, J., Yates, J., 3rd, Russell, P., and Wittenberg, C. (2006). Constraining G1-specific transcription to late G1 phase: the MBF-associated corepressor Nrm1 acts via negative feedback. Mol Cell *23*, 483-496.

de Bruin, R.A., Kalashnikova, T.I., and Wittenberg, C. (2008). Stb1 collaborates with other regulators to modulate the G1-specific transcriptional circuit. Mol Cell Biol *28*, 6919-6928.

de Bruin, R.A., McDonald, W.H., Kalashnikova, T.I., Yates, J., 3rd, and Wittenberg, C. (2004). Cln3 activates G1-specific transcription via phosphorylation of the SBF bound repressor Whi5. Cell *117*, 887-898.

Deckert, J., and Struhl, K. (2002). Targeted recruitment of Rpd3 histone deacetylase represses transcription by inhibiting recruitment of Swi/Snf, SAGA, and TATA binding protein. Mol Cell Biol *22*, 6458-6470.

DeGregori, J., and Johnson, D.G. (2006). Distinct and Overlapping Roles for E2F Family Members in Transcription, Proliferation and Apoptosis. Curr Mol Med *6*, 739-748.

Dick, F.A., and Rubin, S.M. (2013). Molecular mechanisms underlying RB protein function. Nat Rev Mol Cell Biol *14*, 297-306.

Diehl, J.A. (2002). Cycling to cancer with cyclin D1. Cancer Biol Ther *1*, 226-231.

Dischinger, S., Krapp, A., Xie, L., Paulson, J.R., and Simanis, V. (2008). Chemical genetic analysis of the regulatory role of Cdc2p in the S. pombe septation initiation network. J Cell Sci *121*, 843-853.

Djupedal, I., and Ekwall, K. (2009). Epigenetics: heterochromatin meets RNAi. Cell Res *19*, 282-295.

Dorn, E.S., and Cook, J.G. (2011). Nucleosomes in the neighborhood: new roles for chromatin modifications in replication origin control. Epigenetics *6*, 552-559.

Dror, V., and Winston, F. (2004). The Swi/Snf chromatin remodeling complex is required for ribosomal DNA and telomeric silencing in Saccharomyces cerevisiae. Mol Cell Biol *24*, 8227-8235.

Durand-Dubief, M., Sinha, I., Fagerstrom-Billai, F., Bonilla, C., Wright, A., Grunstein, M., and Ekwall, K. (2007). Specific functions for the fission yeast Sirtuins Hst2 and Hst4 in gene regulation and retrotransposon silencing. EMBO J *26*, 2477-2488.

Dutta, C., Patel, P.K., Rosebrock, A., Oliva, A., Leatherwood, J., and Rhind, N. (2008). The DNA replication checkpoint directly regulates MBF-dependent G1/S transcription. Mol Cell Biol *28*, 5977-5985.

Dutta, C., and Rhind, N. (2009). The role of specific checkpoint-induced S-phase transcripts in resistance to replicative stress. PLoS One *4*, e6944.

Eaton, M.L., Prinz, J.A., MacAlpine, H.K., Tretyakov, G., Kharchenko, P.V., and MacAlpine, D.M. (2011). Chromatin signatures of the Drosophila replication program. Genome Res *21*, 164-174.

Ebbert, R., Birkmann, A., and Schuller, H.J. (1999). The product of the SNF2/SWI2 paralogue INO80 of Saccharomyces cerevisiae required for efficient expression of various yeast structural genes is part of a high-molecular-weight protein complex. Mol Microbiol *32*, 741-751.

Egger, G., Liang, G., Aparicio, A., and Jones, P.A. (2004). Epigenetics in human disease and prospects for epigenetic therapy. Nature *429*, 457-463.

Eguchi, T., Takaki, T., Itadani, H., and Kotani, H. (2007). RB silencing compromises the DNA damage-induced G2/M checkpoint and causes deregulated expression of the ECT2 oncogene. Oncogene *26*, 509-520.

Ekwall, K. (2005). Genome-wide analysis of HDAC function. Trends Genet *21*, 608-615.

Enserink, J.M., and Kolodner, R.D. (2010). An overview of Cdk1-controlled targets and processes. Cell Div *5*, 11.

Epstein, C.B., and Cross, F.R. (1992). CLB5: a novel B cyclin from budding yeast with a role in S phase. Genes Dev *6*, 1695-1706.

Eser, U., Falleur-Fettig, M., Johnson, A., and Skotheim, J.M. (2011). Commitment to a cellular transition precedes genome-wide transcriptional change. Mol Cell *43*, 515-527.

Espinosa, M.C., Rehman, M.A., Chisamore-Robert, P., Jeffery, D., and Yankulov, K. (2010). GCN5 is a positive regulator of origins of DNA replication in Saccharomyces cerevisiae. PLoS One *5*, e8964.

Fernandez Sarabia, M.J., McInerny, C., Harris, P., Gordon, C., and Fantes, P. (1993). The cell cycle genes cdc22+ and suc22+ of the fission yeast Schizosaccharomyces pombe encode the large and small subunits of ribonucleotide reductase. Mol Gen Genet *238*, 241-251.

Ferrezuelo, F., Colomina, N., Futcher, B., and Aldea, M. (2010). The transcriptional network activated by Cln3 cyclin at the G1-to-S transition of the yeast cell cycle. Genome Biol *11*, R67.

Fillingham, J., Recht, J., Silva, A.C., Suter, B., Emili, A., Stagljar, I., Krogan, N.J., Allis, C.D., Keogh, M.C., and Greenblatt, J.F. (2008). Chaperone control of the activity and specificity of the histone H3 acetyltransferase Rtt109. Mol Cell Biol *28*, 4342-4353.

Fischer, M., and Muller, G.A. (2017). Cell cycle transcription control: DREAM/MuvB and RB-E2F complexes. Crit Rev Biochem Mol Biol *52*, 638-662.

Fishburn, J., Mohibullah, N., and Hahn, S. (2005). Function of a eukaryotic transcription activator during the transcription cycle. Mol Cell *18*, 369-378.

Fisher, D.L., and Nurse, P. (1996). A single fission yeast mitotic cyclin B p34cdc2 kinase promotes both S-phase and mitosis in the absence of G1 cyclins. EMBO J *15*, 850-860.

Flury, V., Georgescu, P.R., Iesmantavicius, V., Shimada, Y., Kuzdere, T., Braun, S., and Buhler, M. (2017). The Histone Acetyltransferase Mst2 Protects Active Chromatin from Epigenetic Silencing by Acetylating the Ubiquitin Ligase Brl1. Mol Cell *67*, 294-307 e299.

Ford, J., Odeyale, O., and Shen, C.H. (2008). Activator-dependent recruitment of SWI/SNF and INO80 during INO1 activation. Biochem Biophys Res Commun *373*, 602-606.

Freeman-Cook, L.L., Gomez, E.B., Spedale, E.J., Marlett, J., Forsburg, S.L., Pillus, L., and Laurenson, P. (2005). Conserved locus-specific silencing functions of Schizosaccharomyces pombe sir2+. Genetics *169*, 1243-1260.

Furnari, B., Rhind, N., and Russell, P. (1997). Cdc25 mitotic inducer targeted by chk1 DNA damage checkpoint kinase. Science *277*, 1495-1497.

Gabellini, C., Del Bufalo, D., and Zupi, G. (2006). Involvement of RB gene family in tumor angiogenesis. Oncogene *25*, 5326-5332.

Gambus, A., Jones, R.C., Sanchez-Diaz, A., Kanemaki, M., van Deursen, F., Edmondson, R.D., and Labib, K. (2006). GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. Nat Cell Biol *8*, 358-366.

Garg, A., Futcher, B., and Leatherwood, J. (2015). A new transcription factor for mitosis: in Schizosaccharomyces pombe, the RFX transcription factor Sak1 works with forkhead factors to regulate mitotic expression. Nucleic Acids Res *43*, 6874-6888.

Georgakopoulos, T., and Thireos, G. (1992). Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. EMBO J *11*, 4145-4152.

Giangrande, P.H., Zhu, W., Rempel, R.E., Laakso, N., and Nevins, J.R. (2004). Combinatorial gene control involving E2F and E Box family members. EMBO J *23*, 1336-1347.

Godfrey, M., Touati, S.A., Kataria, M., Jones, A., Snijders, A.P., and Uhlmann, F. (2017). PP2A(Cdc55) Phosphatase Imposes Ordered Cell-Cycle Phosphorylation by Opposing Threonine Phosphorylation. Mol Cell *65*, 393-402 e393.

Gomar-Alba, M., Mendez, E., Quilis, I., Bano, M.C., and Igual, J.C. (2017). Whi7 is an unstable cell-cycle repressor of the Start transcriptional program. Nat Commun *8*, 329.

Gómez-Escoda, B., Ivanova, T., Calvo, I.A., Alves-Rodrigues, I., Hidalgo, E., and Ayté, J. (2011). Yox1 links MBF-dependent transcription to completion of DNA synthesis. EMBO Rep *12*, 84-89.

Gomez, E.B., Espinosa, J.M., and Forsburg, S.L. (2005). Schizosaccharomyces pombe mst2+ encodes a MYST family histone acetyltransferase that negatively regulates telomere silencing. Mol Cell Biol *25*, 8887-8903.

Gopalakrishnan, V., Simancek, P., Houchens, C., Snaith, H.A., Frattini, M.G., Sazer, S., and Kelly, T.J. (2001). Redundant control of rereplication in fission yeast. Proc Natl Acad Sci U S A *98*, 13114-13119.

Govind, C.K., Yoon, S., Qiu, H., Govind, S., and Hinnebusch, A.G. (2005). Simultaneous recruitment of coactivators by Gcn4p stimulates multiple steps of transcription in vivo. Mol Cell Biol *25*, 5626-5638.

Govind, C.K., Zhang, F., Qiu, H., Hofmeyer, K., and Hinnebusch, A.G. (2007). Gcn5 promotes acetylation, eviction, and methylation of nucleosomes in transcribed coding regions. Mol Cell *25*, 31-42.

Grallert, A., Boke, E., Hagting, A., Hodgson, B., Connolly, Y., Griffiths, J.R., Smith, D.L., Pines, J., and Hagan, I.M. (2015). A PP1-PP2A phosphatase relay controls mitotic progression. Nature *517*, 94-98.

Grant, P.A. (2001). A tale of histone modifications. Genome Biol *2*, REVIEWS0003.

Grant, P.A., Eberharter, A., John, S., Cook, R.G., Turner, B.M., and Workman, J.L. (1999). Expanded lysine acetylation specificity of Gcn5 in native complexes. J Biol Chem *274*, 5895-5900.

Grewal, C., Hickmott, J., Rentas, S., and Karagiannis, J. (2012). A conserved histone deacetylase with a role in the regulation of cytokinesis in Schizosaccharomyces pombe. Cell Div *7*, 13.

Grewal, S.I., and Jia, S. (2007). Heterochromatin revisited. Nat Rev Genet *8*, 35-46.

Groth, A., Corpet, A., Cook, A.J., Roche, D., Bartek, J., Lukas, J., and Almouzni, G. (2007). Regulation of replication fork progression through histone supply and demand. Science *318*, 1928-1931.

Grunberg, S., Henikoff, S., Hahn, S., and Zentner, G.E. (2016). Mediator binding to UASs is broadly uncoupled from transcription and cooperative with TFIID recruitment to promoters. EMBO J *35*, 2435-2446.

Guarente, L. (1988). UASs and enhancers: common mechanism of transcriptional activation in yeast and mammals. Cell *52*, 303-305.

Hahn, S., and Young, E.T. (2011). Transcriptional regulation in Saccharomyces cerevisiae: transcription factor regulation and function, mechanisms of initiation, and roles of activators and coactivators. Genetics *189*, 705-736.

Haldar, D., and Kamakaka, R.T. (2008). Schizosaccharomyces pombe Hst4 functions in DNA damage response by regulating histone H3 K56 acetylation. Eukaryot Cell *7*, 800-813.

Haldar, S., Saini, A., Nanda, J.S., Saini, S., and Singh, J. (2011). Role of Swi6/HP1 self-association-mediated recruitment of Clr4/Suv39 in establishment and maintenance of heterochromatin in fission yeast. J Biol Chem *286*, 9308-9320.

Hampsey, M., and Reinberg, D. (2003). Tails of intrigue: phosphorylation of RNA polymerase II mediates histone methylation. Cell *113*, 429-432.

Hanigan, C.L., Van Engeland, M., De Bruine, A.P., Wouters, K.A., Weijenberg, M.P., Eshleman, J.R., and Herman, J.G. (2008). An inactivating mutation in HDAC2 leads to dysregulation of apoptosis mediated by APAF1. Gastroenterology *135*, 1654-1664 e1652.

Hartwell, L.H. (1978). Cell division from a genetic perspective. J Cell Biol *77*, 627-637.

Hartwell, L.H., and Weinert, T.A. (1989). Checkpoints: controls that ensure the order of cell cycle events. Science *246*, 629-634.

Hassan, A.H., Prochasson, P., Neely, K.E., Galasinski, S.C., Chandy, M., Carrozza, M.J., and Workman, J.L. (2002). Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. Cell *111*, 369-379.

Hayles, J., Fisher, D., Woollard, A., and Nurse, P. (1994). Temporal order of S phase and mitosis in fission yeast is determined by the state of the p34cdc2-mitotic B cyclin complex. Cell *78*, 813-822.

Hegde, M.L., Hazra, T.K., and Mitra, S. (2008). Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res *18*, 27-47.

Helmlinger, D., Marguerat, S., Villen, J., Gygi, S.P., Bahler, J., and Winston, F. (2008). The S. pombe SAGA complex controls the switch from proliferation to sexual differentiation through the opposing roles of its subunits Gcn5 and Spt8. Genes Dev *22*, 3184-3195.

Helmlinger, D., Marguerat, S., Villen, J., Swaney, D.L., Gygi, S.P., Bahler, J., and Winston, F. (2011). Tra1 has specific regulatory roles, rather than global functions, within the SAGA co-activator complex. EMBO J *30*, 2843-2852.

Hennig, B.P., Bendrin, K., Zhou, Y., and Fischer, T. (2012). Chd1 chromatin remodelers maintain nucleosome organization and repress cryptic transcription. EMBO Rep *13*, 997-1003.

Henry, K.W., Wyce, A., Lo, W.S., Duggan, L.J., Emre, N.C., Kao, C.F., Pillus, L., Shilatifard, A., Osley, M.A., and Berger, S.L. (2003). Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. Genes Dev *17*, 2648-2663.

Higa, L.A., Mihaylov, I.S., Banks, D.P., Zheng, J., and Zhang, H. (2003). Radiation-mediated proteolysis of CDT1 by CUL4-ROC1 and CSN complexes constitutes a new checkpoint. Nat Cell Biol *5*, 1008-1015.

Hoeijmakers, J.H. (2009). DNA damage, aging, and cancer. N Engl J Med *361*, 1475-1485.

Hofmann, J.F., and Beach, D. (1994). cdt1 is an essential target of the Cdc10/Sct1 transcription factor: requirement for DNA replication and inhibition of mitosis. EMBO J *13*, 425-434.

Hogan, C.J., Aligianni, S., Durand-Dubief, M., Persson, J., Will, W.R., Webster, J., Wheeler, L., Mathews, C.K., Elderkin, S., Oxley, D.*, et al.* (2010). Fission yeast Iec1-ino80-mediated nucleosome eviction regulates nucleotide and phosphate metabolism. Mol Cell Biol *30*, 657-674.

Holmberg, C., Fleck, O., Hansen, H.A., Liu, C., Slaaby, R., Carr, A.M., and Nielsen, O. (2005). Ddb1 controls genome stability and meiosis in fission yeast. Genes Dev *19*, 853-862.

Holt, L.J., Tuch, B.B., Villen, J., Johnson, A.D., Gygi, S.P., and Morgan, D.O. (2009). Global analysis of Cdk1 substrate phosphorylation sites provides insights into evolution. Science *325*, 1682-1686.

Horita, D.A., Ivanova, A.V., Altieri, A.S., Klar, A.J., and Byrd, R.A. (2001). Solution structure, domain features, and structural implications of mutants of the chromo domain from the fission yeast histone methyltransferase Clr4. J Mol Biol *307*, 861-870.

Hsu, J.M., Huang, J., Meluh, P.B., and Laurent, B.C. (2003). The yeast RSC chromatin-remodeling complex is required for kinetochore function in chromosome segregation. Mol Cell Biol *23*, 3202-3215.

Hu, F., and Aparicio, O.M. (2005). Swe1 regulation and transcriptional control restrict the activity of mitotic cyclins toward replication proteins in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A *102*, 8910-8915.

Huang, J., Hsu, J.M., and Laurent, B.C. (2004). The RSC nucleosome-remodeling complex is required for Cohesin's association with chromosome arms. Mol Cell *13*, 739-750.

Huarte, M., Lan, F., Kim, T., Vaughn, M.W., Zaratiegui, M., Martienssen, R.A., Buratowski, S., and Shi, Y. (2007). The fission yeast Jmj2 reverses histone H3 Lysine 4 trimethylation. J Biol Chem *282*, 21662-21670.

Hui, A.M., Li, X., Makuuchi, M., Takayama, T., and Kubota, K. (1999). Over-expression and lack of retinoblastoma protein are associated with tumor progression and metastasis in hepatocellular carcinoma. Int J Cancer *84*, 604-608.

Huisinga, K.L., and Pugh, B.F. (2004). A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in Saccharomyces cerevisiae. Mol Cell *13*, 573-585.

Hutchison, C.J., Cox, R., Drepaul, R.S., Gomperts, M., and Ford, C.C. (1987). Periodic DNA synthesis in cell-free extracts of Xenopus eggs. EMBO J *6*, 2003-2010.

Ivanova, T., Alves-Rodrigues, I., Gomez-Escoda, B., Dutta, C., DeCaprio, J.A., Rhind, N., Hidalgo, E., and Ayte, J. (2013). The DNA damage and the DNA replication checkpoints converge at the MBF transcription factor. Mol Biol Cell *24*, 3350-3357.

Iyer, V.R. (2012). Nucleosome positioning: bringing order to the eukaryotic genome. Trends Cell Biol *22*, 250-256.

Iyer, V.R., Horak, C.E., Scafe, C.S., Botstein, D., Snyder, M., and Brown, P.O. (2001). Genomic binding sites of the yeast cell-cycle transcription factors SBF and MBF. Nature *409*, 533-538.

Jiang, C., and Pugh, B.F. (2009). Nucleosome positioning and gene regulation: advances through genomics. Nat Rev Genet *10*, 161-172.

Johnsson, A., Xue-Franzen, Y., Lundin, M., and Wright, A.P. (2006). Stress-specific role of fission yeast Gcn5 histone acetyltransferase in programming a subset of stress response genes. Eukaryot Cell *5*, 1337-1346.

Jones, P.A., and Baylin, S.B. (2002). The fundamental role of epigenetic events in cancer. Nat Rev Genet *3*, 415-428.

Kalocsay, M., Hiller, N.J., and Jentsch, S. (2009). Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. Mol Cell *33*, 335-343.

Kanu, N., Gronroos, E., Martinez, P., Burrell, R.A., Yi Goh, X., Bartkova, J., Maya-Mendoza, A., Mistrik, M., Rowan, A.J., Patel, H.*, et al.* (2015). SETD2 loss-of-function promotes renal cancer branched evolution through replication stress and impaired DNA repair. Oncogene *34*, 5699-5708.

Kaykov, A., and Nurse, P. (2015). The spatial and temporal organization of origin firing during the S-phase of fission yeast. Genome Res *25*, 391-401.

Kelly, T.J., and Brown, G.W. (2000). Regulation of chromosome replication. Annu Rev Biochem *69*, 829-880.

Kelly, T.J., Martin, G.S., Forsburg, S.L., Stephen, R.J., Russo, A., and Nurse, P. (1993). The fission yeast cdc18+ gene product couples S phase to START and mitosis. Cell *74*, 371-382.

Kennedy, B.K., Barbie, D.A., Classon, M., Dyson, N., and Harlow, E. (2000). Nuclear organization of DNA replication in primary mammalian cells. Genes Dev *14*, 2855-2868.

Kim, H.S., Vanoosthuyse, V., Fillingham, J., Roguev, A., Watt, S., Kislinger, T., Treyer, A., Carpenter, L.R., Bennett, C.S., Emili, A.*, et al.* (2009). An acetylated form of histone H2A.Z regulates chromosome architecture in Schizosaccharomyces pombe. Nat Struct Mol Biol *16*, 1286-1293.

Kitajima, S., and Takahashi, C. (2017). Intersection of retinoblastoma tumor suppressor function, stem cells, metabolism, and inflammation. Cancer Sci *108*, 1726-1731.

Kizer, K.O., Phatnani, H.P., Shibata, Y., Hall, H., Greenleaf, A.L., and Strahl, B.D. (2005). A novel domain in Set2 mediates RNA polymerase

II interaction and couples histone H3 K36 methylation with transcript elongation. Mol Cell Biol *25*, 3305-3316.

Klopf, E., Schmidt, H.A., Clauder-Munster, S., Steinmetz, L.M., and Schuller, C. (2017). INO80 represses osmostress induced gene expression by resetting promoter proximal nucleosomes. Nucleic Acids Res *45*, 3752-3766.

Knudson, A.G., Jr. (1971). Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A *68*, 820-823.

Knutsen, J.H., Rein, I.D., Rothe, C., Stokke, T., Grallert, B., and Boye, E. (2011). Cell-cycle analysis of fission yeast cells by flow cytometry. PLoS One *6*, e17175.

Kobor, M.S., Venkatasubrahmanyam, S., Meneghini, M.D., Gin, J.W., Jennings, J.L., Link, A.J., Madhani, H.D., and Rine, J. (2004). A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. PLoS Biol *2*, E131.

Koch, C., Moll, T., Neuberg, M., Ahorn, H., and Nasmyth, K. (1993). A role for the transcription factors Mbp1 and Swi4 in progression from G1 to S phase. Science *261*, 1551-1557.

Kolupaeva, V., and Janssens, V. (2013). PP1 and PP2A phosphatases--cooperating partners in modulating retinoblastoma protein activation. FEBS J *280*, 627-643.

Kominami, K., and Toda, T. (1997). Fission yeast WD-repeat protein pop1 regulates genome ploidy through ubiquitin-proteasome-mediated degradation of the CDK inhibitor Rum1 and the S-phase initiator Cdc18. Genes Dev *11*, 1548-1560.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell *128*, 693-705.

Krietenstein, N., Wal, M., Watanabe, S., Park, B., Peterson, C.L., Pugh, B.F., and Korber, P. (2016). Genomic Nucleosome Organization Reconstituted with Pure Proteins. Cell *167*, 709-721 e712.

Krogan, N.J., Keogh, M.C., Datta, N., Sawa, C., Ryan, O.W., Ding, H., Haw, R.A., Pootoolal, J., Tong, A., Canadien, V.*, et al.* (2003). A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol Cell *12*, 1565-1576.

Kruger, M.K., Pedersen, S., Hagervall, T.G., and Sorensen, M.A. (1998). The modification of the wobble base of tRNAGlu modulates the translation rate of glutamic acid codons in vivo. J Mol Biol *284*, 621-631.

Kuo, M.H., Brownell, J.E., Sobel, R.E., Ranalli, T.A., Cook, R.G., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996). Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. Nature *383*, 269-272.

Kurdistani, S.K., and Grunstein, M. (2003). Histone acetylation and deacetylation in yeast. Nat Rev Mol Cell Biol *4*, 276-284.

Lafon, A., Taranum, S., Pietrocola, F., Dingli, F., Loew, D., Brahma, S., Bartholomew, B., and Papamichos-Chronakis, M. (2015). INO80 Chromatin Remodeler Facilitates Release of RNA Polymerase II from Chromatin for Ubiquitin-Mediated Proteasomal Degradation. Mol Cell *60*, 784-796.

Lammens, T., Li, J., Leone, G., and De Veylder, L. (2009). Atypical E2Fs: new players in the E2F transcription factor family. Trends Cell Biol *19*, 111-118.

Lang, S.E., McMahon, S.B., Cole, M.D., and Hearing, P. (2001). E2F transcriptional activation requires TRRAP and GCN5 cofactors. J Biol Chem *276*, 32627-32634.

Lantermann, A.B., Straub, T., Stralfors, A., Yuan, G.C., Ekwall, K., and Korber, P. (2010). Schizosaccharomyces pombe genome-wide nucleosome mapping reveals positioning mechanisms distinct from those of Saccharomyces cerevisiae. Nat Struct Mol Biol *17*, 251-257.

Lee, J., Choi, E.S., Seo, H.D., Kang, K., Gilmore, J.M., Florens, L., Washburn, M.P., Choe, J., Workman, J.L., and Lee, D. (2017). Chromatin remodeller Fun30(Fft3) induces nucleosome disassembly to facilitate RNA polymerase II elongation. Nat Commun *8*, 14527.

Lee, K.K., Sardiu, M.E., Swanson, S.K., Gilmore, J.M., Torok, M., Grant, P.A., Florens, L., Workman, J.L., and Washburn, M.P. (2011). Combinatorial depletion analysis to assemble the network architecture of the SAGA and ADA chromatin remodeling complexes. Mol Syst Biol *7*, 503.

Lengronne, A., and Schwob, E. (2002). The yeast CDK inhibitor Sic1 prevents genomic instability by promoting replication origin licensing in late G(1). Mol Cell *9*, 1067-1078.

Li, Q., Zhou, H., Wurtele, H., Davies, B., Horazdovsky, B., Verreault, A., and Zhang, Z. (2008). Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. Cell *134*, 244-255.

Li, S., and Shogren-Knaak, M.A. (2009). The Gcn5 bromodomain of the SAGA complex facilitates cooperative and cross-tail acetylation of nucleosomes. J Biol Chem *284*, 9411-9417.

Lim, K.K., Nguyen, T.T.T., Li, A.Y., Yeo, Y.P., and Chen, E.S. (2018). Histone H3 lysine 36 methyltransferase mobilizes NER factors to regulate tolerance against alkylation damage in fission yeast. Nucleic Acids Res *46*, 5061-5074.

Lindahl, T., and Barnes, D.E. (2000). Repair of endogenous DNA damage. Cold Spring Harb Symp Quant Biol *65*, 127-133.

Liu, C., Poitelea, M., Watson, A., Yoshida, S.H., Shimoda, C., Holmberg, C., Nielsen, O., and Carr, A.M. (2005). Transactivation of Schizosaccharomyces pombe cdt2+ stimulates a Pcu4-Ddb1-CSN ubiquitin ligase. EMBO J *24*, 3940-3951.

Liu, C., Powell, K.A., Mundt, K., Wu, L., Carr, A.M., and Caspari, T. (2003a). Cop9/signalosome subunits and Pcu4 regulate ribonucleotide reductase by both checkpoint-dependent and -independent mechanisms. Genes Dev *17*, 1130-1140.

Liu, X., Tesfai, J., Evrard, Y.A., Dent, S.Y., and Martinez, E. (2003b). c-Myc transformation domain recruits the human STAGA complex and requires TRRAP and GCN5 acetylase activity for transcription activation. J Biol Chem *278*, 20405-20412.

Lorenz, D.R., Meyer, L.F., Grady, P.J., Meyer, M.M., and Cam, H.P. (2014). Heterochromatin assembly and transcriptome repression by Set1 in coordination with a class II histone deacetylase. Elife *3*, e04506.

Lorenz, D.R., Mikheyeva, I.V., Johansen, P., Meyer, L., Berg, A., Grewal, S.I., and Cam, H.P. (2012). CENP-B cooperates with Set1 in bidirectional transcriptional silencing and genome organization of retrotransposons. Mol Cell Biol *32*, 4215-4225.

Lowndes, N.F., McInerny, C.J., Johnson, A.L., Fantes, P.A., and Johnston, L.H. (1992). Control of DNA synthesis genes in fission yeast by the cell-cycle gene cdc10+. Nature *355*, 449-453.

Luo, J., Su, F., Chen, D., Shiloh, A., and Gu, W. (2000). Deacetylation of p53 modulates its effect on cell growth and apoptosis. Nature *408*, 377-381.

Macdonald, J.I., and Dick, F.A. (2012). Posttranslational modifications of the retinoblastoma tumor suppressor protein as determinants of function. Genes Cancer *3*, 619-633.

Maes, T., Mascaro, C., Tirapu, I., Estiarte, A., Ciceri, F., Lunardi, S., Guibourt, N., Perdones, A., Lufino, M.M.P., Somervaille, T.C.P.*, et al.* (2018). ORY-1001, a Potent and Selective Covalent KDM1A Inhibitor, for the Treatment of Acute Leukemia. Cancer Cell *33*, 495-511 e412.

Malumbres, M., and Barbacid, M. (2001). To cycle or not to cycle: a critical decision in cancer. Nat Rev Cancer *1*, 222-231.

Maqbool, Z., Kersey, P.J., Fantes, P.A., and McInerny, C.J. (2003). MCB-mediated regulation of cell cycle-specific cdc22+ transcription in fission yeast. Mol Genet Genomics *269*, 765-775.

Marguerat, S., Jensen, T.S., de Lichtenberg, U., Wilhelm, B.T., Jensen, L.J., and Bahler, J. (2006). The more the merrier: comparative analysis of microarray studies on cell cycle-regulated genes in fission yeast. Yeast *23*, 261-277.

Marquardt, S., Escalante-Chong, R., Pho, N., Wang, J., Churchman, L.S., Springer, M., and Buratowski, S. (2014). A Chromatin-Based Mechanism for Limiting Divergent Noncoding Transcription. Cell *158*, 462.

Marquez-Vilendrer, S.B., Rai, S.K., Gramling, S.J., Lu, L., and Reisman, D.N. (2016). Loss of the SWI/SNF ATPase subunits BRM and BRG1 drives lung cancer development. Oncoscience *3*, 322-336.

Martin-Castellanos, C., Blanco, M.A., de Prada, J.M., and Moreno, S. (2000). The puc1 cyclin regulates the G1 phase of the fission yeast cell cycle in response to cell size. Mol Biol Cell *11*, 543-554.

Martin, S.G., and Berthelot-Grosjean, M. (2009). Polar gradients of the DYRK-family kinase Pom1 couple cell length with the cell cycle. Nature *459*, 852-856.

Massague, J. (2004). G1 cell-cycle control and cancer. Nature *432*, 298-306.

Masumoto, H., Hawke, D., Kobayashi, R., and Verreault, A. (2005). A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. Nature *436*, 294-298.

Matzuk, M.M., McKeown, M.R., Filippakopoulos, P., Li, Q., Ma, L., Agno, J.E., Lemieux, M.E., Picaud, S., Yu, R.N., Qi, J.*, et al.* (2012). Small-molecule inhibition of BRDT for male contraception. Cell *150*, 673-684.

McInerny, C.J. (2011). Cell cycle regulated gene expression in yeasts. Adv Genet *73*, 51-85.

McInerny, C.J., Kersey, P.J., Creanor, J., and Fantes, P.A. (1995). Positive and negative roles for cdc10 in cell cycle gene expression. Nucleic Acids Res *23*, 4761-4768.

Mechali, M., Yoshida, K., Coulombe, P., and Pasero, P. (2013). Genetic and epigenetic determinants of DNA replication origins, position and activation. Curr Opin Genet Dev *23*, 124-131.

Mikheyeva, I.V., Grady, P.J., Tamburini, F.B., Lorenz, D.R., and Cam, H.P. (2014). Multifaceted genome control by Set1 Dependent and Independent of H3K4 methylation and the Set1C/COMPASS complex. PLoS Genet *10*, e1004740.

Mitra, D., Parnell, E.J., Landon, J.W., Yu, Y., and Stillman, D.J. (2006). SWI/SNF binding to the HO promoter requires histone acetylation and stimulates TATA-binding protein recruitment. Mol Cell Biol *26*, 4095-4110.

Mitsuzawa, H., and Ishihama, A. (2002). Identification of histone H4-like TAF in Schizosaccharomyces pombe as a protein that interacts with WD repeat-containing TAF. Nucleic Acids Res *30*, 1952-1958.

Miyamoto, M., Tanaka, K., and Okayama, H. (1994). res2+, a new member of the cdc10+/SWI4 family, controls the 'start' of mitotic and meiotic cycles in fission yeast. EMBO J *13*, 1873-1880.

Mohibullah, N., and Hahn, S. (2008). Site-specific cross-linking of TBP in vivo and in vitro reveals a direct functional interaction with the SAGA subunit Spt3. Genes Dev *22*, 2994-3006.

Mohrmann, L., and Verrijzer, C.P. (2005). Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. Biochim Biophys Acta *1681*, 59-73.

Monahan, B.J., Villen, J., Marguerat, S., Bahler, J., Gygi, S.P., and Winston, F. (2008). Fission yeast SWI/SNF and RSC complexes show compositional and functional differences from budding yeast. Nat Struct Mol Biol *15*, 873-880.

Mondesert, O., McGowan, C.H., and Russell, P. (1996). Cig2, a B-type cyclin, promotes the onset of S in Schizosaccharomyces pombe. Mol Cell Biol *16*, 1527-1533.

Moore, J.D., Kirk, J.A., and Hunt, T. (2003). Unmasking the S-phase-promoting potential of cyclin B1. Science *300*, 987-990.

Moreno, S., Hayles, J., and Nurse, P. (1989). Regulation of p34cdc2 protein kinase during mitosis. Cell *58*, 361-372.

Morgan, D.O. (1997). Cyclin-dependent kinases: engines, clocks, and microprocessors. Annu Rev Cell Dev Biol *13*, 261-291.

Morris, S.A., Shibata, Y., Noma, K., Tsukamoto, Y., Warren, E., Temple, B., Grewal, S.I., and Strahl, B.D. (2005). Histone H3 K36 methylation is associated with transcription elongation in Schizosaccharomyces pombe. Eukaryot Cell *4*, 1446-1454.

Moseley, J.B., Mayeux, A., Paoletti, A., and Nurse, P. (2009). A spatial gradient coordinates cell size and mitotic entry in fission yeast. Nature *459*, 857-860.

Moss, J., Tinline-Purvis, H., Walker, C.A., Folkes, L.K., Stratford, M.R., Hayles, J., Hoe, K.L., Kim, D.U., Park, H.O., Kearsey, S.E.*, et al.* (2010). Break-induced ATR and Ddb1-Cul4(Cdt)(2) ubiquitin ligase-dependent nucleotide synthesis promotes homologous recombination repair in fission yeast. Genes Dev *24*, 2705-2716.

Motamedi, M.R., Verdel, A., Colmenares, S.U., Gerber, S.A., Gygi, S.P., and Moazed, D. (2004). Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. Cell *119*, 789-802.

Muhar, M., Ebert, A., Neumann, T., Umkehrer, C., Jude, J., Wieshofer, C., Rescheneder, P., Lipp, J.J., Herzog, V.A., Reichholf, B.*, et al.* (2018). SLAM-seq defines direct gene-regulatory functions of the BRD4-MYC axis. Science *360*, 800-805.

Mundt, K.E., Liu, C., and Carr, A.M. (2002). Deletion mutants in COP9/signalosome subunits in fission yeast Schizosaccharomyces pombe display distinct phenotypes. Mol Biol Cell *13*, 493-502.

Nagy, Z., and Tora, L. (2007). Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. Oncogene *26*, 5341-5357.

Nakashima, N., Tanaka, K., Sturm, S., and Okayama, H. (1995). Fission yeast Rep2 is a putative transcriptional activator subunit for the cell cycle 'start' function of Res2-Cdc10. EMBO J *14*, 4794-4802.

Nakayama, J., Rice, J.C., Strahl, B.D., Allis, C.D., and Grewal, S.I. (2001). Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science *292*, 110-113.

Narasimha, A.M., Kaulich, M., Shapiro, G.S., Choi, Y.J., Sicinski, P., and Dowdy, S.F. (2014). Cyclin D activates the Rb tumor suppressor by mono-phosphorylation. Elife *3*.

Navarro, F.J., and Nurse, P. (2012). A systematic screen reveals new elements acting at the G2/M cell cycle control. Genome Biol *13*, R36.

Neumann, F.R., Dion, V., Gehlen, L.R., Tsai-Pflugfelder, M., Schmid, R., Taddei, A., and Gasser, S.M. (2012). Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination. Genes Dev *26*, 369-383.

Neuwald, A.F., and Landsman, D. (1997). GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. Trends Biochem Sci *22*, 154-155.

Newlon, C.S., and Theis, J.F. (1993). The structure and function of yeast ARS elements. Curr Opin Genet Dev *3*, 752-758.

Ng, H.H., Robert, F., Young, R.A., and Struhl, K. (2003). Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol Cell *11*, 709-719.

Nicolas, E., Yamada, T., Cam, H.P., Fitzgerald, P.C., Kobayashi, R., and Grewal, S.I. (2007). Distinct roles of HDAC complexes in promoter silencing, antisense suppression and DNA damage protection. Nat Struct Mol Biol *14*, 372-380.

Noma, K., Allis, C.D., and Grewal, S.I. (2001). Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. Science *293*, 1150-1155.

Noma, K., and Grewal, S.I. (2002). Histone H3 lysine 4 methylation is mediated by Set1 and promotes maintenance of active chromatin states in fission yeast. Proc Natl Acad Sci U S A *99 Suppl 4*, 16438-16445.

Nugent, R.L., Johnsson, A., Fleharty, B., Gogol, M., Xue-Franzen, Y., Seidel, C., Wright, A.P., and Forsburg, S.L. (2010). Expression profiling of S. pombe acetyltransferase mutants identifies redundant pathways of gene regulation. BMC Genomics *11*, 59.

Nurse, P., and Bissett, Y. (1981). Gene required in G1 for commitment to cell cycle and in G2 for control of mitosis in fission yeast. Nature *292*, 558-560.

Nurse, P., and Thuriaux, P. (1977). Controls over the timing of DNA replication during the cell cycle of fission yeast. Exp Cell Res *107*, 365-375.

Nurse, P., Thuriaux, P., and Nasmyth, K. (1976). Genetic control of the cell division cycle in the fission yeast Schizosaccharomyces pombe. Mol Gen Genet *146*, 167-178.

Oliva, A., Rosebrock, A., Ferrezuelo, F., Pyne, S., Chen, H., Skiena, S., Futcher, B., and Leatherwood, J. (2005). The cell cycle-regulated genes of Schizosaccharomyces pombe. PLoS Biol *3*, e225.

Orpinell, M., Fournier, M., Riss, A., Nagy, Z., Krebs, A.R., Frontini, M., and Tora, L. (2010). The ATAC acetyl transferase complex controls mitotic progression by targeting non-histone substrates. EMBO J *29*, 2381-2394.

Owen, D.J., Ornaghi, P., Yang, J.C., Lowe, N., Evans, P.R., Ballario, P., Neuhaus, D., Filetici, P., and Travers, A.A. (2000). The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. EMBO J *19*, 6141-6149.

Pai, C.C., Deegan, R.S., Subramanian, L., Gal, C., Sarkar, S., Blaikley, E.J., Walker, C., Hulme, L., Bernhard, E., Codlin, S.*, et al.* (2014). A histone H3K36 chromatin switch coordinates DNA double-strand break repair pathway choice. Nat Commun *5*, 4091.

Pai, C.C., Kishkevich, A., Deegan, R.S., Keszthelyi, A., Folkes, L., Kearsey, S.E., De Leon, N., Soriano, I., de Bruin, R.A.M., Carr, A.M.*, et al.* (2017). Set2 Methyltransferase Facilitates DNA Replication and Promotes Genotoxic Stress Responses through MBF-Dependent Transcription. Cell Rep *20*, 2693-2705.

Paolinelli, R., Mendoza-Maldonado, R., Cereseto, A., and Giacca, M. (2009). Acetylation by GCN5 regulates CDC6 phosphorylation in the S phase of the cell cycle. Nat Struct Mol Biol *16*, 412-420.

Papadopoulou, K., Chen, J.S., Mead, E., Feoktistova, A., Petit, C., Agarwal, M., Jamal, M., Malik, A., Spanos, A., Sedgwick, S.G.*, et al.* (2010). Regulation of cell cycle-specific gene expression in fission yeast by the Cdc14p-like phosphatase Clp1p. J Cell Sci *123*, 4374-4381.

Papadopoulou, K., Ng, S.S., Ohkura, H., Geymonat, M., Sedgwick, S.G., and McInerny, C.J. (2008). Regulation of gene expression during M-G1-phase in fission yeast through Plo1p and forkhead transcription factors. J Cell Sci *121*, 38-47.

Papamichos-Chronakis, M., Watanabe, S., Rando, O.J., and Peterson, C.L. (2011). Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity. Cell *144*, 200-213.

Pardee, A.B. (1989). G1 events and regulation of cell proliferation. Science *246*, 603-608.

Parker, L.L., Atherton-Fessler, S., and Piwnica-Worms, H. (1992). p107wee1 is a dual-specificity kinase that phosphorylates p34cdc2 on tyrosine 15. Proc Natl Acad Sci U S A *89*, 2917-2921.

Partridge, J.F., Mikesell, G.E., and Breeden, L.L. (1997). Cell cycle-dependent transcription of CLN1 involves swi4 binding to MCB-like elements. J Biol Chem *272*, 9071-9077.

Patil, A., Chan, C.T., Dyavaiah, M., Rooney, J.P., Dedon, P.C., and Begley, T.J. (2012). Translational infidelity-induced protein stress results from a deficiency in Trm9-catalyzed tRNA modifications. RNA Biol *9*, 990-1001.

Patrick, K.L., Ryan, C.J., Xu, J., Lipp, J.J., Nissen, K.E., Roguev, A., Shales, M., Krogan, N.J., and Guthrie, C. (2015). Genetic interaction mapping reveals a role for the SWI/SNF nucleosome remodeler in spliceosome activation in fission yeast. PLoS Genet *11*, e1005074.

Peng, X., Karuturi, R.K., Miller, L.D., Lin, K., Jia, Y., Kondu, P., Wang, L., Wong, L.S., Liu, E.T., Balasubramanian, M.K.*, et al.* (2005). Identification of cell cycle-regulated genes in fission yeast. Mol Biol Cell *16*, 1026-1042.

Penn, M.D., Galgoci, B., and Greer, H. (1983). Identification of AAS genes and their regulatory role in general control of amino acid biosynthesis in yeast. Proc Natl Acad Sci U S A *80*, 2704-2708.

Petersen, J., and Hagan, I.M. (2005). Polo kinase links the stress pathway to cell cycle control and tip growth in fission yeast. Nature *435*, 507-512.

Petersen, J., and Nurse, P. (2007). TOR signalling regulates mitotic commitment through the stress MAP kinase pathway and the Polo and Cdc2 kinases. Nat Cell Biol *9*, 1263-1272.

Petty, E., and Pillus, L. (2013). Balancing chromatin remodeling and histone modifications in transcription. Trends Genet *29*, 621-629.

Piggott, J.R., Rai, R., and Carter, B.L. (1982). A bifunctional gene product involved in two phases of the yeast cell cycle. Nature *298*, 391-393.

Pokholok, D.K., Harbison, C.T., Levine, S., Cole, M., Hannett, N.M., Lee, T.I., Bell, G.W., Walker, K., Rolfe, P.A., Herbolsheimer, E.*, et al.* (2005). Genome-wide map of nucleosome acetylation and methylation in yeast. Cell *122*, 517-527.

Poli, J., Gerhold, C.B., Tosi, A., Hustedt, N., Seeber, A., Sack, R., Herzog, F., Pasero, P., Shimada, K., Hopfner, K.P.*, et al.* (2016). Mec1, INO80, and the PAF1 complex cooperate to limit transcription replication conflicts through RNAPII removal during replication stress. Genes Dev *30*, 337-354.

Qin, S., and Parthun, M.R. (2006). Recruitment of the type B histone acetyltransferase Hat1p to chromatin is linked to DNA double-strand breaks. Mol Cell Biol *26*, 3649-3658.

Racine, A., Page, V., Nagy, S., Grabowski, D., and Tanny, J.C. (2012). Histone H2B ubiquitylation promotes activity of the intact Set1 histone methyltransferase complex in fission yeast. J Biol Chem *287*, 19040-19047.

Raisner, R.M., Hartley, P.D., Meneghini, M.D., Bao, M.Z., Liu, C.L., Schreiber, S.L., Rando, O.J., and Madhani, H.D. (2005). Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. Cell *123*, 233-248.

Ralph, E., Boye, E., and Kearsey, S.E. (2006). DNA damage induces Cdt1 proteolysis in fission yeast through a pathway dependent on Cdt2 and Ddb1. EMBO Rep *7*, 1134-1139.

Rando, O.J., and Winston, F. (2012). Chromatin and transcription in yeast. Genetics *190*, 351-387.

Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z.W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C.P., Allis, C.D., *et al.* (2000). Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature *406*, 593-599.

Reddy, B.D., Wang, Y., Niu, L., Higuchi, E.C., Marguerat, S.B., Bahler, J., Smith, G.R., and Jia, S. (2011). Elimination of a specific histone H3K14 acetyltransferase complex bypasses the RNAi pathway to regulate pericentric heterochromatin functions. Genes Dev *25*, 214-219.

Reeves, W.M., and Hahn, S. (2005). Targets of the Gal4 transcription activator in functional transcription complexes. Mol Cell Biol *25*, 9092-9102.

Reisman, D.N., Sciarrotta, J., Wang, W., Funkhouser, W.K., and Weissman, B.E. (2003). Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis. Cancer Res *63*, 560-566.

Reymond, A., Marks, J., and Simanis, V. (1993). The activity of S.pombe DSC-1-like factor is cell cycle regulated and dependent on the activity of p34cdc2. EMBO J *12*, 4325-4334.

Rhee, H.S., and Pugh, B.F. (2011). Comprehensive genome-wide protein-DNA interactions detected at single-nucleotide resolution. Cell *147*, 1408-1419.

Richmond, T.J., and Davey, C.A. (2003). The structure of DNA in the nucleosome core. Nature *423*, 145-150.

Ringel, A.E., Cieniewicz, A.M., Taverna, S.D., and Wolberger, C. (2015). Nucleosome competition reveals processive acetylation by the SAGA HAT module. Proc Natl Acad Sci U S A *112*, E5461-5470.

Rizzardi, L.F., Dorn, E.S., Strahl, B.D., and Cook, J.G. (2012). DNA replication origin function is promoted by H3K4 di-methylation in Saccharomyces cerevisiae. Genetics *192*, 371-384.

Roberts, C.W., and Orkin, S.H. (2004). The SWI/SNF complex--chromatin and cancer. Nat Rev Cancer *4*, 133-142.

Roguev, A., Schaft, D., Shevchenko, A., Aasland, R., Shevchenko, A., and Stewart, A.F. (2003). High conservation of the Set1/Rad6 axis of histone 3 lysine 4 methylation in budding and fission yeasts. J Biol Chem *278*, 8487-8493.

Roguev, A., Shevchenko, A., Schaft, D., Thomas, H., Stewart, A.F., and Shevchenko, A. (2004). A comparative analysis of an orthologous proteomic environment in the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe. Mol Cell Proteomics *3*, 125-132.

Ropero, S., Fraga, M.F., Ballestar, E., Hamelin, R., Yamamoto, H., Boix-Chornet, M., Caballero, R., Alaminos, M., Setien, F., Paz, M.F*., et al.* (2006). A truncating mutation of HDAC2 in human cancers confers resistance to histone deacetylase inhibition. Nat Genet *38*, 566-569.

Rowland, B.D., and Bernards, R. (2006). Re-evaluating cell-cycle regulation by E2Fs. Cell *127*, 871-874.

Ruan, K., Yamamoto, T.G., Asakawa, H., Chikashige, Y., Kimura, H., Masukata, H., Haraguchi, T., and Hiraoka, Y. (2015). Histone H4 acetylation required for chromatin decompaction during DNA replication. Sci Rep *5*, 12720.

Russell, P., and Nurse, P. (1987). Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog. Cell *49*, 559-567.

Rustici, G., Mata, J., Kivinen, K., Lio, P., Penkett, C.J., Burns, G., Hayles, J., Brazma, A., Nurse, P., and Bahler, J. (2004). Periodic gene expression program of the fission yeast cell cycle. Nat Genet *36*, 809-817.

Salguero, I., Guarino, E., Shepherd, M.E., Deegan, T.D., Havens, C.G., MacNeill, S.A., Walter, J.C., and Kearsey, S.E. (2012). Ribonucleotide reductase activity is coupled to DNA synthesis via proliferating cell nuclear antigen. Curr Biol *22*, 720-726.

Sanso, M., Lee, K.M., Viladevall, L., Jacques, P.E., Page, V., Nagy, S., Racine, A., St Amour, C.V., Zhang, C., Shokat, K.M*., et al.* (2012). A positive feedback loop links opposing functions of P-TEFb/Cdk9 and histone H2B ubiquitylation to regulate transcript elongation in fission yeast. PLoS Genet *8*, e1002822.

Sanso, M., Vargas-Perez, I., Quintales, L., Antequera, F., Ayte, J., and Hidalgo, E. (2011). Gcn5 facilitates Pol II progression, rather than recruitment to nucleosome-depleted stress promoters, in Schizosaccharomyces pombe. Nucleic Acids Res *39*, 6369-6379.

Santamaria, D., Barriere, C., Cerqueira, A., Hunt, S., Tardy, C., Newton, K., Caceres, J.F., Dubus, P., Malumbres, M., and Barbacid, M. (2007). Cdk1 is sufficient to drive the mammalian cell cycle. Nature *448*, 811-815.

Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J., and Kouzarides, T. (2002). Active genes are tri-methylated at K4 of histone H3. Nature *419*, 407-411.

Sardana, R., and Johnson, A.W. (2012). The methyltransferase adaptor protein Trm112 is involved in biogenesis of both ribosomal subunits. Mol Biol Cell *23*, 4313-4322.

Schneider, A., Chatterjee, S., Bousiges, O., Selvi, B.R., Swaminathan, A., Cassel, R., Blanc, F., Kundu, T.K., and Boutillier, A.L. (2013). Acetyltransferases (HATs) as targets for neurological therapeutics. Neurotherapeutics *10*, 568-588.

Schulz, D., Pirkl, N., Lehmann, E., and Cramer, P. (2014). Rpb4 subunit functions mainly in mRNA synthesis by RNA polymerase II. J Biol Chem *289*, 17446-17452.

Seeber, A., Dion, V., and Gasser, S.M. (2013). Checkpoint kinases and the INO80 nucleosome remodeling complex enhance global chromatin mobility in response to DNA damage. Genes Dev *27*, 1999-2008.

Segurado, M., de Luis, A., and Antequera, F. (2003). Genome-wide distribution of DNA replication origins at A+T-rich islands in Schizosaccharomyces pombe. EMBO Rep *4*, 1048-1053.

Sermwittayawong, D., and Tan, S. (2006). SAGA binds TBP via its Spt8 subunit in competition with DNA: implications for TBP recruitment. EMBO J *25*, 3791-3800.

Shandilya, J., and Roberts, S.G. (2012). The transcription cycle in eukaryotes: from productive initiation to RNA polymerase II recycling. Biochim Biophys Acta *1819*, 391-400.

Shankaranarayana, G.D., Motamedi, M.R., Moazed, D., and Grewal, S.I. (2003). Sir2 regulates histone H3 lysine 9 methylation and heterochromatin assembly in fission yeast. Curr Biol *13*, 1240-1246.

Sharma, S., Kelly, T.K., and Jones, P.A. (2010). Epigenetics in cancer. Carcinogenesis *31*, 27-36.

Shelby, R.D., Monier, K., and Sullivan, K.F. (2000). Chromatin assembly at kinetochores is uncoupled from DNA replication. J Cell Biol *151*, 1113-1118.

Shen, X., Mizuguchi, G., Hamiche, A., and Wu, C. (2000). A chromatin remodelling complex involved in transcription and DNA processing. Nature *406*, 541-544.

Shevchenko, A., Roguev, A., Schaft, D., Buchanan, L., Habermann, B., Sakalar, C., Thomas, H., Krogan, N.J., Shevchenko, A., and Stewart, A.F. (2008). Chromatin Central: towards the comparative proteome by accurate mapping of the yeast proteomic environment. Genome Biol *9*, R167.

Shim, E.Y., Hong, S.J., Oum, J.H., Yanez, Y., Zhang, Y., and Lee, S.E. (2007). RSC mobilizes nucleosomes to improve accessibility of repair machinery to the damaged chromatin. Mol Cell Biol *27*, 1602-1613.

Shim, Y.S., Choi, Y., Kang, K., Cho, K., Oh, S., Lee, J., Grewal, S.I., and Lee, D. (2012). Hrp3 controls nucleosome positioning to suppress non-coding transcription in eu- and heterochromatin. EMBO J *31*, 4375-4387.

Shimada, K., Oma, Y., Schleker, T., Kugou, K., Ohta, K., Harata, M., and Gasser, S.M. (2008). Ino80 chromatin remodeling complex promotes recovery of stalled replication forks. Curr Biol *18*, 566-575.

Shivaswamy, S., and Iyer, V.R. (2008). Stress-dependent dynamics of global chromatin remodeling in yeast: dual role for SWI/SNF in the heat shock stress response. Mol Cell Biol *28*, 2221-2234.

Shukla, A., Bajwa, P., and Bhaumik, S.R. (2006). SAGA-associated Sgf73p facilitates formation of the preinitiation complex assembly at the promoters either in a HAT-dependent or independent manner in vivo. Nucleic Acids Res *34*, 6225-6232.

Siddiqui, K., On, K.F., and Diffley, J.F. (2013). Regulating DNA replication in eukarya. Cold Spring Harb Perspect Biol *5*.

Simanis, V., Hayles, J., and Nurse, P. (1987). Control over the onset of DNA synthesis in fission yeast. Philos Trans R Soc Lond B Biol Sci *317*, 507-516.

Simanis, V., and Nurse, P. (1989). Characterization of the fission yeast cdc10+ protein that is required for commitment to the cell cycle. J Cell Sci *92 (Pt 1)*, 51-56.

Sinha, I., Wiren, M., and Ekwall, K. (2006). Genome-wide patterns of histone modifications in fission yeast. Chromosome Res *14*, 95-105.

Sjoblom, T., Jones, S., Wood, L.D., Parsons, D.W., Lin, J., Barber, T.D., Mandelker, D., Leary, R.J., Ptak, J., Silliman, N.*, et al.* (2006). The consensus coding sequences of human breast and colorectal cancers. Science *314*, 268-274.

Skotheim, J.M., Di Talia, S., Siggia, E.D., and Cross, F.R. (2008). Positive feedback of G1 cyclins ensures coherent cell cycle entry. Nature *454*, 291-296.

Stern, B., and Nurse, P. (1996). A quantitative model for the cdc2 control of S phase and mitosis in fission yeast. Trends Genet *12*, 345-350.

Sterner, D.E., and Berger, S.L. (2000). Acetylation of histones and transcription-related factors. Microbiol Mol Biol Rev *64*, 435-459.

Sturm, S., and Okayama, H. (1996). Domains determining the functional distinction of the fission yeast cell cycle "start" molecules Res1 and Res2. Mol Biol Cell *7*, 1967-1976.

Su, D., Hu, Q., Li, Q., Thompson, J.R., Cui, G., Fazly, A., Davies, B.A., Botuyan, M.V., Zhang, Z., and Mer, G. (2012). Structural basis for recognition of H3K56-acetylated histone H3-H4 by the chaperone Rtt106. Nature *483*, 104-107.

Suarez, M.B., Alonso-Nunez, M.L., del Rey, F., McInerny, C.J., and Vazquez de Aldana, C.R. (2015). Regulation of Ace2-dependent genes requires components of the PBF complex in Schizosaccharomyces pombe. Cell Cycle *14*, 3124-3137.

Subramanian, V., Fields, P.A., and Boyer, L.A. (2015). H2A.Z: a molecular rheostat for transcriptional control. F1000Prime Rep *7*, 01.

Sudarsanam, P., Cao, Y., Wu, L., Laurent, B.C., and Winston, F. (1999). The nucleosome remodeling complex, Snf/Swi, is required for the maintenance of transcription in vivo and is partially redundant with the histone acetyltransferase, Gcn5. EMBO J *18*, 3101-3106.

Sugiyama, A., Tanaka, K., Okazaki, K., Nojima, H., and Okayama, H. (1994). A zinc finger protein controls the onset of premeiotic DNA synthesis of fission yeast in a Mei2-independent cascade. EMBO J *13*, 1881-1887.

Sugiyama, T., Cam, H.P., Sugiyama, R., Noma, K., Zofall, M., Kobayashi, R., and Grewal, S.I. (2007). SHREC, an effector complex for heterochromatic transcriptional silencing. Cell *128*, 491-504.

Surana, U., Robitsch, H., Price, C., Schuster, T., Fitch, I., Futcher, A.B., and Nasmyth, K. (1991). The role of CDC28 and cyclins during mitosis in the budding yeast S. cerevisiae. Cell *65*, 145-161.

Suzuki, S., Kato, H., Suzuki, Y., Chikashige, Y., Hiraoka, Y., Kimura, H., Nagao, K., Obuse, C., Takahata, S., and Murakami, Y. (2016). Histone H3K36 trimethylation is essential for multiple silencing mechanisms in fission yeast. Nucleic Acids Res *44*, 4147-4162.

Svotelis, A., Gevry, N., and Gaudreau, L. (2009). Regulation of gene expression and cellular proliferation by histone H2A.Z. Biochem Cell Biol *87*, 179-188.

Swaffer, M.P., Jones, A.W., Flynn, H.R., Snijders, A.P., and Nurse, P. (2016). CDK Substrate Phosphorylation and Ordering the Cell Cycle. Cell *167*, 1750-1761 e1716.

Taatjes, D.J. (2017). The Continuing SAGA of TFIID and RNA Polymerase II Transcription. Mol Cell *68*, 1-2.

Tahara, S., Tanaka, K., Yuasa, Y., and Okayama, H. (1998). Functional domains of rep2, a transcriptional activator subunit for Res2-Cdc10, controlling the cell cycle "start". Mol Biol Cell *9*, 1577-1588.

Takahashi, Y., Rayman, J.B., and Dynlacht, B.D. (2000). Analysis of promoter binding by the E2F and pRB families in vivo: distinct E2F proteins mediate activation and repression. Genes Dev *14*, 804-816.

Takayama, Y., and Takahashi, K. (2007). Differential regulation of repeated histone genes during the fission yeast cell cycle. Nucleic Acids Res *35*, 3223-3237.

Takeda, D.Y., and Dutta, A. (2005). DNA replication and progression through S phase. Oncogene *24*, 2827-2843.

Talbert, P.B., Ahmad, K., Almouzni, G., Ausio, J., Berger, F., Bhalla, P.L., Bonner, W.M., Cande, W.Z., Chadwick, B.P., Chan, S.W.*, et al.* (2012). A unified phylogeny-based nomenclature for histone variants. Epigenetics Chromatin *5*, 7.

Talluri, S., and Dick, F.A. (2012). Regulation of transcription and chromatin structure by pRB: here, there and everywhere. Cell Cycle *11*, 3189-3198.

Tamburini, B.A., and Tyler, J.K. (2005). Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand DNA repair. Mol Cell Biol *25*, 4903-4913.

Tanaka, A., Tanizawa, H., Sriswasdi, S., Iwasaki, O., Chatterjee, A.G., Speicher, D.W., Levin, H.L., Noguchi, E., and Noma, K. (2012). Epigenetic regulation of condensin-mediated genome organization during the cell cycle and upon DNA damage through histone H3 lysine 56 acetylation. Mol Cell *48*, 532-546.

Tanaka, K., Okazaki, K., Okazaki, N., Ueda, T., Sugiyama, A., Nojima, H., and Okayama, H. (1992). A new cdc gene required for S phase entry of Schizosaccharomyces pombe encodes a protein similar to the cdc 10+ and SWI4 gene products. EMBO J *11*, 4923-4932.

Tanny, J.C., Erdjument-Bromage, H., Tempst, P., and Allis, C.D. (2007). Ubiquitylation of histone H2B controls RNA polymerase II transcription elongation independently of histone H3 methylation. Genes Dev *21*, 835-847.

Taubert, S., Gorrini, C., Frank, S.R., Parisi, T., Fuchs, M., Chan, H.M., Livingston, D.M., and Amati, B. (2004). E2F-dependent histone acetylation and recruitment of the Tip60 acetyltransferase complex to chromatin in late G1. Mol Cell Biol *24*, 4546-4556.

Thaminy, S., Newcomb, B., Kim, J., Gatbonton, T., Foss, E., Simon, J., and Bedalov, A. (2007). Hst3 is regulated by Mec1-dependent proteolysis and controls the S phase checkpoint and sister chromatid cohesion by deacetylating histone H3 at lysine 56. J Biol Chem *282*, 37805-37814.

Thomas, M.C., and Chiang, C.M. (2006). The general transcription machinery and general cofactors. Crit Rev Biochem Mol Biol *41*, 105-178.

Tjeertes, J.V., Miller, K.M., and Jackson, S.P. (2009). Screen for DNA-damage-responsive histone modifications identifies H3K9Ac and H3K56Ac in human cells. EMBO J *28*, 1878-1889.

Tong, K., Keller, T., Hoffman, C.S., and Annunziato, A.T. (2012). Schizosaccharomyces pombe Hat1 (Kat1) is associated with Mis16 and is required for telomeric silencing. Eukaryot Cell *11*, 1095-1103.

Toselli-Mollereau, E., Robellet, X., Fauque, L., Lemaire, S., Schiklenk, C., Klein, C., Hocquet, C., Legros, P., N'Guyen, L., Mouillard, L.*, et al.* (2016). Nucleosome eviction in mitosis assists condensin loading and chromosome condensation. EMBO J *35*, 1565-1581.

Trewick, S.C., Minc, E., Antonelli, R., Urano, T., and Allshire, R.C. (2007). The JmjC domain protein Epe1 prevents unregulated assembly and disassembly of heterochromatin. EMBO J 26, 4670-4682.

Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M.E., Borchers, C.H., Tempst, P., and Zhang, Y. (2006). Histone demethylation by a family of JmjC domain-containing proteins. Nature *439*, 811-816.

Tsutsumi, S., Sugiura, R., Ma, Y., Tokuoka, H., Ohta, K., Ohte, R., Noma, A., Suzuki, T., and Kuno, T. (2007). Wobble inosine tRNA modification is essential to cell cycle progression in G(1)/S and G(2)/M transitions in fission yeast. J Biol Chem *282*, 33459-33465.

Uchida, C. (2016). Roles of pRB in the Regulation of Nucleosome and Chromatin Structures. Biomed Res Int *2016*, 5959721.

Udugama, M., Sabri, A., and Bartholomew, B. (2011). The INO80 ATP-dependent chromatin remodeling complex is a nucleosome spacing factor. Mol Cell Biol *31*, 662-673.

van Attikum, H., Fritsch, O., and Gasser, S.M. (2007). Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. EMBO J *26*, 4113-4125.

Verges, E., Colomina, N., Gari, E., Gallego, C., and Aldea, M. (2007). Cyclin Cln3 is retained at the ER and released by the J chaperone Ydj1 in late G1 to trigger cell cycle entry. Mol Cell *26*, 649-662.

Verreault, A., Kaufman, P.D., Kobayashi, R., and Stillman, B. (1996). Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. Cell *87*, 95-104.

Versteege, I., Sevenet, N., Lange, J., Rousseau-Merck, M.F., Ambros, P., Handgretinger, R., Aurias, A., and Delattre, O. (1998). Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. Nature *394*, 203-206.

Vogelauer, M., Rubbi, L., Lucas, I., Brewer, B.J., and Grunstein, M. (2002). Histone acetylation regulates the time of replication origin firing. Mol Cell *10*, 1223-1233.

Voichek, Y., Bar-Ziv, R., and Barkai, N. (2016). Expression homeostasis during DNA replication. Science *351*, 1087-1090.

Vorobyeva, N.E., Mazina, M.U., Golovnin, A.K., Kopytova, D.V., Gurskiy, D.Y., Nabirochkina, E.N., Georgieva, S.G., Georgiev, P.G., and

Krasnov, A.N. (2013). Insulator protein Su(Hw) recruits SAGA and Brahma complexes and constitutes part of Origin Recognition Complex-binding sites in the Drosophila genome. Nucleic Acids Res *41*, 5717-5730.

Waldholm, J., Wang, Z., Brodin, D., Tyagi, A., Yu, S., Theopold, U., Farrants, A.K., and Visa, N. (2011). SWI/SNF regulates the alternative processing of a specific subset of pre-mRNAs in Drosophila melanogaster. BMC Mol Biol *12*, 46.

Walfridsson, J., Bjerling, P., Thalen, M., Yoo, E.J., Park, S.D., and Ekwall, K. (2005). The CHD remodeling factor Hrp1 stimulates CENP-A loading to centromeres. Nucleic Acids Res *33*, 2868-2879.

Walfridsson, J., Khorosjutina, O., Matikainen, P., Gustafsson, C.M., and Ekwall, K. (2007). A genome-wide role for CHD remodelling factors and Nap1 in nucleosome disassembly. EMBO J *26*, 2868-2879.

Walworth, N., Davey, S., and Beach, D. (1993). Fission yeast chk1 protein kinase links the rad checkpoint pathway to cdc2. Nature *363*, 368-371.

Wang, B., Liu, K., Lin, F.T., and Lin, W.C. (2004a). A role for 14-3-3 tau in E2F1 stabilization and DNA damage-induced apoptosis. J Biol Chem *279*, 54140-54152.

Wang, H., Gari, E., Verges, E., Gallego, C., and Aldea, M. (2004b). Recruitment of Cdc28 by Whi3 restricts nuclear accumulation of the G1 cyclin-Cdk complex to late G1. EMBO J *23*, 180-190.

Wang, J., Reddy, B.D., and Jia, S. (2015). Rapid epigenetic adaptation to uncontrolled heterochromatin spreading. Elife *4*.

Wang, J., Tadeo, X., Hou, H., Tu, P.G., Thompson, J., Yates, J.R., 3rd, and Jia, S. (2013). Epe1 recruits BET family bromodomain protein Bdf2 to establish heterochromatin boundaries. Genes Dev *27*, 1886-1902.

Wang, L., and Dent, S.Y. (2014). Functions of SAGA in development and disease. Epigenomics *6*, 329-339.

Wang, Y., Kallgren, S.P., Reddy, B.D., Kuntz, K., Lopez-Maury, L., Thompson, J., Watt, S., Ma, C., Hou, H., Shi, Y.*, et al.* (2012). Histone H3 lysine 14 acetylation is required for activation of a DNA damage checkpoint in fission yeast. J Biol Chem *287*, 4386-4393.

Wang, Z., Weaver, M., and Magnuson, N.S. (2005). Cryptic promoter activity in the DNA sequence corresponding to the pim-1 5'-UTR. Nucleic Acids Res *33*, 2248-2258.

Warfield, L., Ramachandran, S., Baptista, T., Devys, D., Tora, L., and Hahn, S. (2017). Transcription of Nearly All Yeast RNA Polymerase II-Transcribed Genes Is Dependent on Transcription Factor TFIID. Mol Cell *68*, 118-129 e115.

White, S., Khaliq, F., Sotiriou, S., and McInerny, C.J. (2001). The role of DSC1 components cdc10+, rep1+ and rep2+ in MCB gene transcription at the mitotic G1-S boundary in fission yeast. Curr Genet *40*, 251-259.

Williams, S.K., Truong, D., and Tyler, J.K. (2008). Acetylation in the globular core of histone H3 on lysine-56 promotes chromatin disassembly during transcriptional activation. Proc Natl Acad Sci U S A *105*, 9000-9005.

Wiren, M., Silverstein, R.A., Sinha, I., Walfridsson, J., Lee, H.M., Laurenson, P., Pillus, L., Robyr, D., Grunstein, M., and Ekwall, K. (2005). Genomewide analysis of nucleosome density histone acetylation and HDAC function in fission yeast. EMBO J *24*, 2906-2918.

Wittenberg, C., and Reed, S.I. (2005). Cell cycle-dependent transcription in yeast: promoters, transcription factors, and transcriptomes. Oncogene *24*, 2746-2755.

Wolfel, T., Hauer, M., Schneider, J., Serrano, M., Wolfel, C., Klehmann-Hieb, E., De Plaen, E., Hankeln, T., Meyer zum Buschenfelde, K.H., and Beach, D. (1995). A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science *269*, 1281-1284.

Wu, P.Y., and Nurse, P. (2009). Establishing the program of origin firing during S phase in fission Yeast. Cell *136*, 852-864.

Wuarin, J., Buck, V., Nurse, P., and Millar, J.B. (2002). Stable association of mitotic cyclin B/Cdc2 to replication origins prevents endoreduplication. Cell *111*, 419-431.

Wurtele, H., Kaiser, G.S., Bacal, J., St-Hilaire, E., Lee, E.H., Tsao, S., Dorn, J., Maddox, P., Lisby, M., Pasero, P.*, et al.* (2012). Histone H3 lysine 56 acetylation and the response to DNA replication fork damage. Mol Cell Biol *32*, 154-172.

Xhemalce, B., and Kouzarides, T. (2010). A chromodomain switch mediated by histone H3 Lys 4 acetylation regulates heterochromatin assembly. Genes Dev *24*, 647-652.

Xhemalce, B., Miller, K.M., Driscoll, R., Masumoto, H., Jackson, S.P., Kouzarides, T., Verreault, A., and Arcangioli, B. (2007). Regulation of histone H3 lysine 56 acetylation in Schizosaccharomyces pombe. J Biol Chem *282*, 15040-15047.

Xu, D., Bai, J., Duan, Q., Costa, M., and Dai, W. (2009). Covalent modifications of histones during mitosis and meiosis. Cell Cycle *8*, 3688-3694.

Xu, F., Zhang, K., and Grunstein, M. (2005). Acetylation in histone H3 globular domain regulates gene expression in yeast. Cell *121*, 375-385.

Yamada, T., Fischle, W., Sugiyama, T., Allis, C.D., and Grewal, S.I. (2005). The nucleation and maintenance of heterochromatin by a histone deacetylase in fission yeast. Mol Cell *20*, 173-185.

Yamamoto, T., Poon, D., Weil, P.A., and Horikoshi, M. (1997). Molecular genetic elucidation of the tripartite structure of the Schizosaccharomyces pombe 72 kDa TFIID subunit which contains a WD40 structural motif. Genes Cells *2*, 245-254.

Yamano, H., Kitamura, K., Kominami, K., Lehmann, A., Katayama, S., Hunt, T., and Toda, T. (2000). The spike of S phase cyclin Cig2 expression at the G1-S border in fission yeast requires both APC and SCF ubiquitin ligases. Mol Cell *6*, 1377-1387.

Yao, G., Lee, T.J., Mori, S., Nevins, J.R., and You, L. (2008). A bistable Rb-E2F switch underlies the restriction point. Nat Cell Biol *10*, 476-482.

Yen, K., Vinayachandran, V., and Pugh, B.F. (2013). SWR-C and INO80 chromatin remodelers recognize nucleosome-free regions near +1 nucleosomes. Cell *154*, 1246-1256.

Yin, Y.W., Jin, H.J., Zhao, W., Gao, B., Fang, J., Wei, J., Zhang, D.D., Zhang, J., and Fang, D. (2015). The Histone Acetyltransferase GCN5 Expression Is Elevated and Regulated by c-Myc and E2F1 Transcription Factors in Human Colon Cancer. Gene Expr *16*, 187-196.

Yoo, E.J., Jin, Y.H., Jang, Y.K., Bjerling, P., Tabish, M., Hong, S.H., Ekwall, K., and Park, S.D. (2000). Fission yeast hrp1, a chromodomain ATPase, is required for proper chromosome segregation and its overexpression

interferes with chromatin condensation. Nucleic Acids Res *28*, 2004-2011.

Yu, R., Wang, X., and Moazed, D. (2018). Epigenetic inheritance mediated by coupling of RNAi and histone H3K9 methylation. Nature 558, 615-619.

Zannini, L., Delia, D., and Buscemi, G. (2014). CHK2 kinase in the DNA damage response and beyond. J Mol Cell Biol *6*, 442-457.

Zhang, H., Roberts, D.N., and Cairns, B.R. (2005). Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell *123*, 219-231.

Zhang, K., Fischer, T., Porter, R.L., Dhakshnamoorthy, J., Zofall, M., Zhou, M., Veenstra, T., and Grewal, S.I. (2011). Clr4/Suv39 and RNA quality control factors cooperate to trigger RNAi and suppress antisense RNA. Science *331*, 1624-1627.

Zhou, Z.X., Zhang, M.J., Peng, X., Takayama, Y., Xu, X.Y., Huang, L.Z., and Du, L.L. (2013). Mapping genomic hotspots of DNA damage by a single-strand-DNA-compatible and strand-specific ChIP-seq method. Genome Res *23*, 705-715.

Zhu, Y., Takeda, T., Whitehall, S., Peat, N., and Jones, N. (1997). Functional characterization of the fission yeast Start-specific transcription factor Res2. EMBO J *16*, 1023-1034.

Zilio, N., Codlin, S., Vashisht, A.A., Bitton, D.A., Head, S.R., Wohlschlegel, J.A., Bahler, J., and Boddy, M.N. (2014). A novel histone deacetylase complex in the control of transcription and genome stability. Mol Cell Biol *34*, 3500-3514.

Zofall, M., Yamanaka, S., Reyes-Turcu, F.E., Zhang, K., Rubin, C., and Grewal, S.I. (2012). RNA elimination machinery targeting meiotic mRNAs promotes facultative heterochromatin formation. Science *335*, 96-100.

LIST OF ABBREVIATIONS

CDK: Cyclin-dependent kinase

RNA pol II: RNA polymerase II

TSS: Transcription start site

TBP: TATA-binding protein

PIC: Preinitiation complex

GTF: General transcription factors

UAS: Upstream activation sequence

URS: Upstream repressing sequence

TAF: TATA-binding protein associated factor

SSB: Single-strand break

DSB: Double-strand break

HU: Hydroxyurea

MMS: Methyl methanesulfonate

NDR: Nucleosome depleted region

RNR: Ribonucleotide reductase

HAT: Histone acetyltransferase

HDAC: Histone deacetylase

CSN: COP9/Signalosome