# IV./ Discussion

## IV.1. The discovery of UEV protein and its role in different cellular processes

In our studies of cell differentiation and cell cycle control, we have isolated a new gene that is downregulated upon cell differentiation. We have demonstrated that this gene, previously called CROC1 and considered a transcriptional activator of c-fos (Rothofsky and Lin, 1997), is highly conserved in phylogeny, and constitutes, by sequence relationship, a novel subfamily of the ubiquitin-conjugating, or E2, enzymes. We have demonstrated that these proteins are very conserved in all eukaryotic organisms and that they are very similar to the E2 enzymes in sequence and structure, but these proteins lack a conserved cysteine residue responsible for the catalytic activity of the E2 enzymes (Chen *et al*., 1993; Jentsch, 1992a). We have given these proteins the name UEV (ubiquitin-conjugating E2 enzyme variant). Work by other laboratories has shown that experimental mutagenesis of this cysteine in the catalytic center leads to the inactivation of the E2 enzyme activity, and the mutated protein can behave as a dominant negative variant (Banerjee *et al*., 1995; Sung *et al*., 1990; Madura *et al*., 1993). However, in our initial experiments with recombinant proteins, UEV did not behave as a negative regulator of ubiquitination (Sancho *et al*., 1998).

We have demonstrated also the existence of at least two different human UEV genes, one coding for UEV1/CROC-1, and the other coding for UEV2. The second protein has also been given different names by others, DDVit-1 (Fritsche *et al*., 1997).

The transcripts from the two human UEV genes differ in their 3'untranslated regions, and produce almost identical proteins. We focused our studies on the UEV1 gene, localized on chromosome 20q13.2. Our RT-PCR analysis showed that this gene is expressed as at least four isoforms by alternative splicing. By analyzing genomic sequence databases, we have detected the presence of pseudogenes or genes related to human UEV1 on chromosomes 1, 2, and 7.

In this study we show that the exogenous overexpression of UEV1 in HT-29M6 colon cancer cells leads to the accumulation of cells in $G_2$-M, to endoreduplication and to apoptotis in about 3% of the cells (Sancho *et al.*, 1998). Other studies have demonstrated the accumulation of cells in $G_2$-M (Russel *et al.*, 1995) when exposed to DNA-damaging agents such as UV irradiation or drugs in a process dependent on p53 (Waldman *et al.*, 1996; Chomczynski and Sacchi, 1987) and p21/WAF (Waldman *et al.*, 1996), and with the same damaging agents, the endoreplication in cells that lacks p53 or p21/WAF. The fact that HT-29 cells are mutant cells which lack functional p53 (Waldman *et al.*, 1996) indicate that the induced accumulation in $G_2$-M, endoreduplication and apoptosis by UEV1 are independent on p53.

Similar phenotypes have been seen in other experimental situations. For example, the deletion of cyclin B, the regulatory component of cdc2 leads to a round of replication in absence of mitosis (Hayles *et al.*, 1994). In mammalian cells the tyrosine kinase inhibitor K252a induces endodureplication (Usui *et al.*, 1991). The inhibition of the mitotic kinase cdk1 by the viral protein like human immunodeficiency Vpr (Bartz *et al.*, 1996; He *et al.*, 1995; Jowett *et al.*, 1995; Rogel *et al.*, 1995) or simian virus 40 large T (Scarano *et al.*, 1994), result in the arrest of cells in G2-M and DNA replication without mitosis (Bartz *et al.*, 1996). Thus it could be that some effects observed by overexpression of exogenous UEV1 were a consequence for the inhibition of the cdk1.

We have shown that this is indeed the case. In addition, we have reported that the expression of the endogenous UEV1A isoform is modulated during the cell cycle (Sancho *et al.*, 1998), reaching maximum levels in $G_1$-S and being undetectable in S and at the beginning of $G_2$-M. The cyclic expression of UEV1A supports a possible role in physiological regulation of cell cycle transitions. Our interpretation of these results is that, in untransfected cells, loss of expression of endogenous UEV1A at the end of the S phase would allow the appearance of active cdk1 and normal progression through $G_2$-M. However, continuous expression of the exogenous gene in transfected cells would block this transition by inhibiting cdk1 activity.

In the studies of postreplicative DNA repair, Saccharomyces cerevisiae mutants have been generated by deletion of the MMS2 (the homologue of hsUEV1 A in yeast). These mutant cells showed an increase in their sensitivity to DNA damaging agents as UV or MMS (methyl methanesulfonate). This sensitivity vas reduced when a copy of the MMS2 or HsUEV1A was introduced in mutant cells.

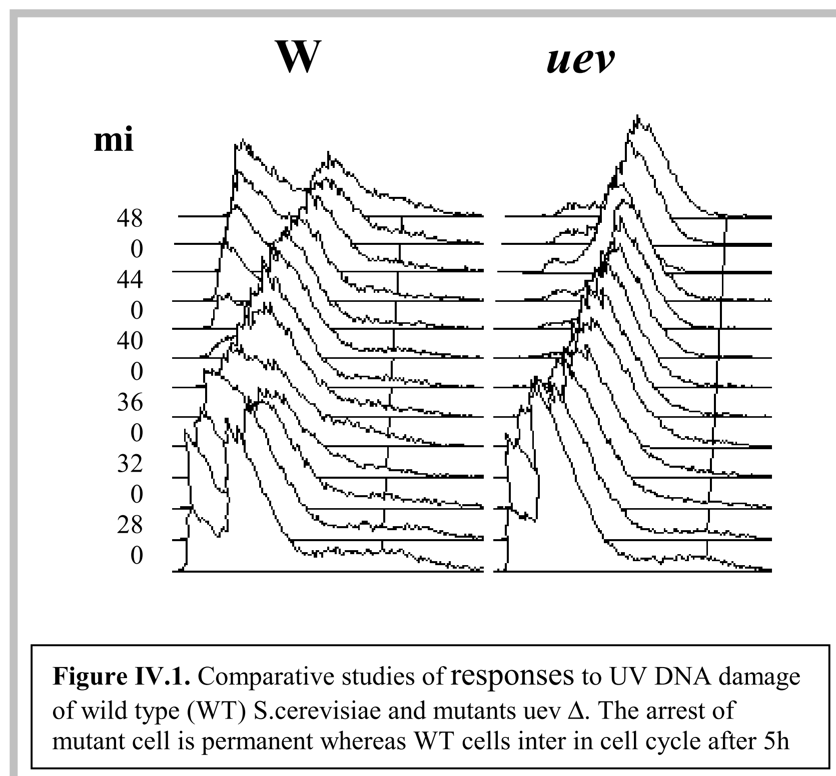


**Figure IV.1.** Comparative studies of responses to UV DNA damage of wild type (WT) S.cerevisiae and mutants uev Δ. The arrest of mutant cell is permanent whereas WT cells inter in cell cycle after 5h

In a separate work by our group (Thomson *et al*., 1998) and others (Bloomberg *et al*., 1998), the yeast orthologue of UEV, called also MMS2, was shown to function in a specific pathway for the repair of DNA damage (**Figure IV.1**). In epistatic analysis in S. cerevisiae, it was shown that UEV protein acts downstream in the RAD6-regulated pathways (Bloomberg *et al*., 1998). Posterior work has shown that UEV (or MMS2, which is the same as UEV2), forms a hetedimer with a enzymatically active E2, Ubc13 (Hoffman and Pickart, 1999).

It was also shown in a number of laboratories that a protein with a RING finger motif, Rad5, recuits the heterodimer Ubc13-Mms2 to a DNA repair complex formed by the proteins Rad6 and Rad18. Rad18 is also a RING-finger domain-containing protein. **Figure IV.2** is a good model to explain the involvement of UEV protein in the RAD6 postreplicative pathways (Ulrich and Jentsch, 2000). RAD6 is another E2 enzyme that is known to attach ubiquitin directly to substrate proteins, either with or without the help of a ubiquitin ligase (Jentsch *et al*., 1987; Dohmen *et al*., 1991; Sung *et al*., 1991). The heteromeric association of Rad18 with Rad5 could recruit the heteroduplex Ubc13-Mms2 for cooperation with Rad6 in error-free DNA repair. The resulting complex comprising the two distinct UBCs, Rad6 and Ubc13-Mms2, with the two chromatin-associated RING finger proteins could act in DNA repair mediating polyubiquitination variant at Lys63 (see below) of unknown substrates.

Finally, it has been shown that MMS2, like UBC13 and many other repair genes, is transcriptionally up regulated in response to DNA damage. This results support the idea that RAD5, UBC13 and MMS2 function as a unit of genetically and physically interacting repair factors within the RAD6 pathway which is coordinately affected by SRS2 (a suppressor of UV sensitivity) (Ulrich and Jentsch, 2001).

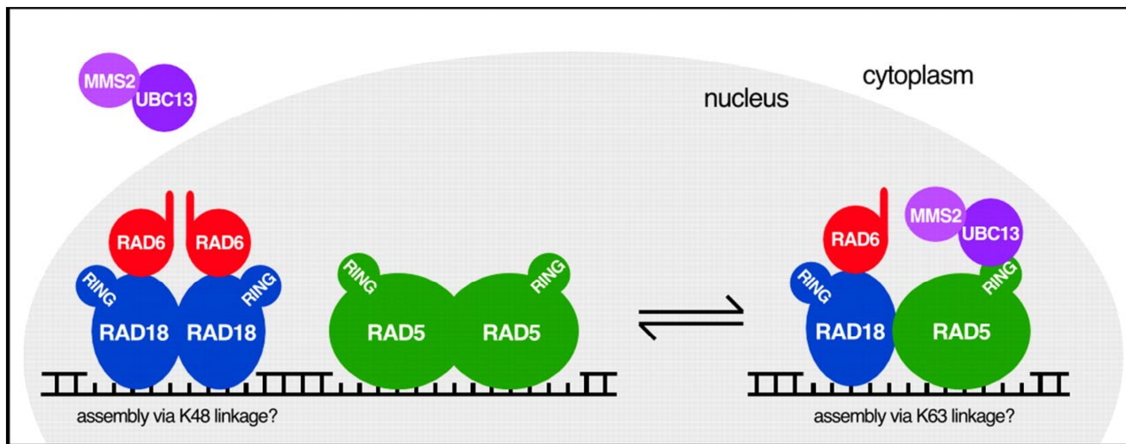


**Figure IV.2.** Model for alternative repair complexes within the RAD6 pathway of DNA repair. A schematic representation of the DNA repair proteins shows the distribution of RING finger–UBC complexes that would result from a competition between RAD18 and RAD5 homo- and heterodimerization. Homodimerization of RAD18 would lead to assemblies comprising RAD6 as the sole UBC, whereas heterodimerization with RAD5 would promote the recruitment of UBC13–MMS2 for the assembly of lysine (K) 63-linked multiubiquitin chains. (From Ulrich and Jentsch, 2001).

As mentioned above, the formation of the heterodimer UEV-UBC13 is necessary for conjugation of ubiquitin to the protein substrates by UBC13. However this heterodimer uses a new form of reaction between the glycine at the carboxy-terminal of the first ubiquitin and the lysine at position 63 (Lys63 or K63) of the second ubiquitin. This is a new form of ubiquitination does not involve the lysine, commonly used, at the position 48 (Lys48 or K48). This new modification is called "variant" polyubiquitination to distinguish it from the "canonical" polyubiquitination that uses the lysine at position 48 of the ubiquitin and that leads to the proteasome degradation. Contrary to the "canonical" polyubiquitin chains, the "variant" chains are not recognized by the proteasome and thus the modified proteins are not tagged for destruction (Deng *et al*., 2000; Finley, 2001). Rather, in the system in eukaryotic cells where this process was shown to be functionally relevant, signal transduction by TNFα and IL-1 receptors,

the UEV-Ubc13 dimer and variant polyubiquitination were shown to be essential for the activation of the pathway (Finley, 2001; Wang *et al*., 2001).

Several substrates for Lys63-linked polyubiquitin chains have been identified. The signal transducer TRAF6 regulates the activation of the NF-κB pathway in response to preinflammatory cytokines, and is the first known substrate for the heterodimer UEV-UBC13. The biochemical pathways that use the polyubiquitination variant (Lys63) mediated by this heterodimer have been well determined in the last two years (Deng *et al*., 2000; Wang *et al*., 2001; Finley, 2001). The heterodimer UEV-Ubc13 interact physically to the RING-finger domain of TRAF2 and TRAF6 and this interaction lead to the polyubiquitination by Lys63 of the substrates that activate the kinase IKKα and the phosphorylation of IκBα (Deng *et al*., 2000). The phosphorylation of IκBα, that forms a complex with NFκB-Rel (50-65), triggers its canonical polyubiquitination at Lys 48 and its degradation in the proteasome. The destruction of IκBα permits the free NFκB-Rel to enter to the nucleus and regulate gene transcription (Baeuerle and Baltimore, 1996) (**Figure IV.3**). This is a remarkable example of the involvement of the new form of polyubiquitination in pathways that do not result in protein degradation but rather in activation of proteins, in this case involved in the modulation of signal transduction (Finley, 2001). There are other potential substrates for variant polyubiquitination. The component of the large ribosomal subunit L28 is polyubiquitinated by a Lys63-linked chain in the intact ribosome. This postranslational modification allows a proper translation of new mRNAs. When this modification is absent, a hypersensitivity to translational inhibitors and polysome instability is observed (Spence *et al*., 2000). Also, the Lys63-polyubiquitination of the general amino acid permease (Gap1) (Springael *et al*., 1999), and the plasma membrane Fur4 (Galan*et al*., 1996) is necessary for its endocytosis followed by vacuolar degradation. So far, the

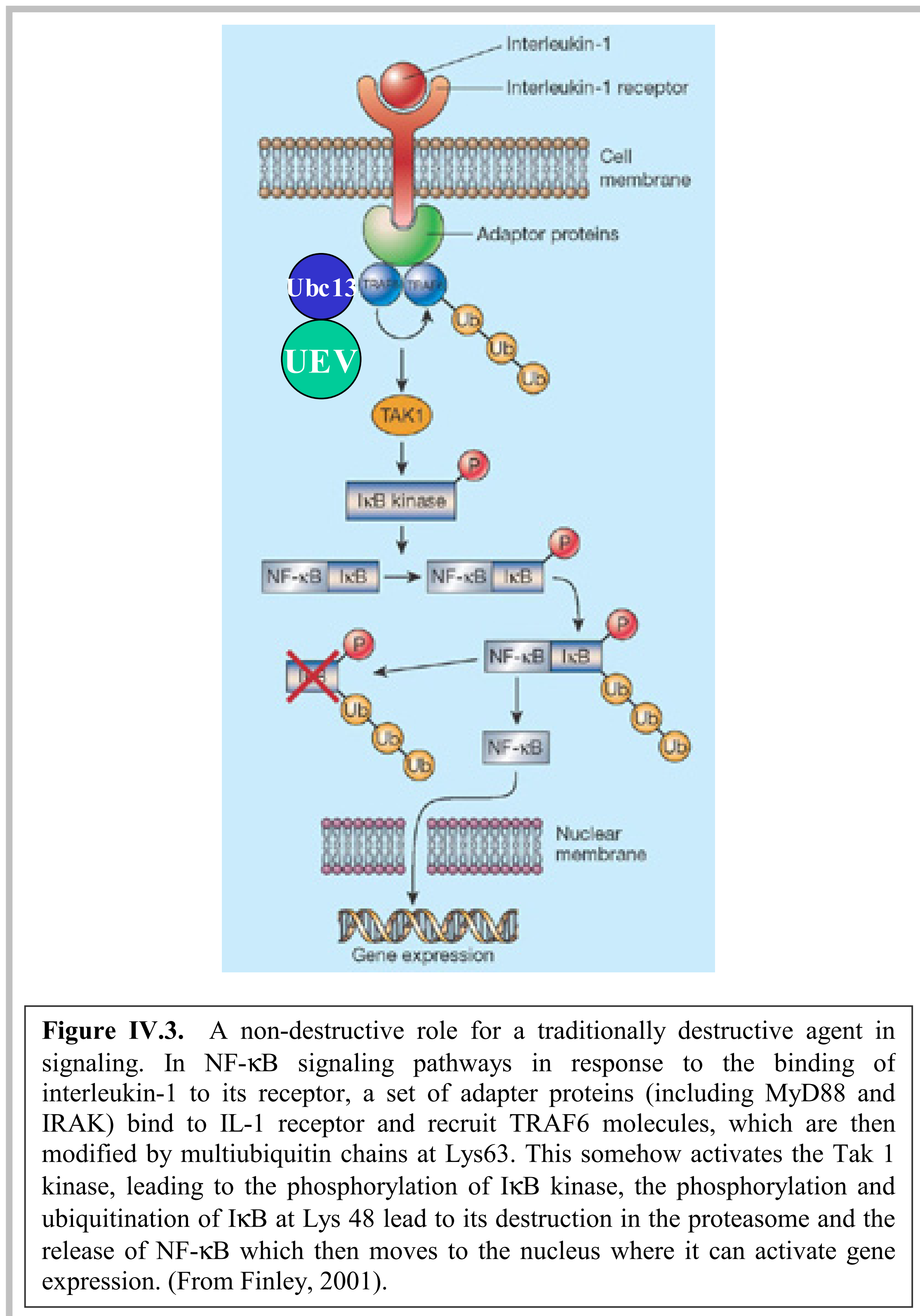


**Figure IV.3.** A non-destructive role for a traditionally destructive agent in signaling. In NF-κB signaling pathways in response to the binding of interleukin-1 to its receptor, a set of adapter proteins (including MyD88 and IRAK) bind to IL-1 receptor and recruit TRAF6 molecules, which are then modified by multiubiquitin chains at Lys63. This somehow activates the Tak 1 kinase, leading to the phosphorylation of IκB kinase, the phosphorylation and ubiquitination of IκB at Lys 48 lead to its destruction in the proteasome and the release of NF-κB which then moves to the nucleus where it can activate gene expression. (From Finley, 2001).

heteroduplex UBC13-UEV has not been shown to be involved in the polyubiquitination at Lys63 of L28, Gap1 or Fur4. Since UEV and Ubc13 are ubiquitously expressed, and several RING finger domain proteins interact with this heterodimer (Plans *et al*., personal communication), we predict that this new form of postranslational modification of proteins will be an important form of regulation of many relevant biochemical and cellular processes.

## IV.2. Evolution of UEV gene introns and the discovery of the Kua gene

We have analyzed the genomic organization of the UEV gene in all the organisms for which sequences are available in public databases.

The exons for the UEV gene were predicted by applying gene prediction algorithms (Guigó *et al*., 1992; Solovyev *et al*., 1994; Burge and Karlin, 1997) to DNA sequences from genomic cosmid clones, and validated by stringent alignment with expressed sequences tags (ETS). We have compared the sequences and the arrangement exon-intron of the UEV gene in *Saccharomyces serevisiae*, *Schizosaccharomyces pombe*, *Plasmodium falciparum*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Homo sapiens*. The results indicate an extraordinary conservation in the exon-intron stucture, between UEV genes in all these organisms. In particular, when introns are present, their position within the corresponding UEV genes is identical, regardless of the organism. The exon arrangements confirms the structure for the human UVE1 gene that was determined experimentally (Sancho *et al*., 1998), with three exons coding for the common domain of the protein (C domain), one for the

domain specific of isoform UEV1 A (A domain), and two for the domain specific for UEV 1 B domain (B domain).

In our previous work we had identified two major isoforms for UEV1, that we called UEV1A and UEV1B, and we had proposed that they were generated by alternative splicing and by the use of two independent promoters (Sancho *et al*., 1998). In our comparative analysis of UEV genes, we did not find any sequences equivalent to those coding for the human “B domain” in any other organism. Since the sequence and the structure of the UEV genes are so well conserved throughout phylogeny, we reasoned that the presence of an additional domain in the human protein and the corresponding exons reflected the *de novo* acquisition of these sequences in the human UEV1 gene. Therefore, we began a search for “B domain”-like sequences in genomic DNA databases in order to identify the origin of this domain.

We found two small fragments of significant similarities to the human “B domain” within *Caenorhabditis elegans* clone Y53C10 (86 Kb), localized in chromosome 1, and *Drosophila melanogaster* clone DS00863 (78 Kb), in chromosome 2L. These genomic clones do not contain any UEV-like sequences. In fact, in the *C. elegans* genome, the gene for UEV is in clone F39B2 also in chromosome 1, but approximately 2.5 Mb away from the "B domain"-like sequence in the Y53C10 clone (http://www.sanger.ac.uk), whereas in *D.melanogaster* UEV gene and the "B domain"-like sequence are localaized in different chromosomes, 3L and 2L, respectively. Therefore, in contrast to the "B domain" sequences *in H. sapiens* where they are part of the UEV gene and are expressed as alternative UEV transcripts, the "B domain"-like sequences in *C. elegans* and *D. melanogaster* do not appear to be part of the corresponding UEV genes.

The highly significant similarities observed between the "B domain"-like sequences of *C. elegans, D. melanogaster* to the sequences in *H. sapiens* led us to hypothesize that, in these organisms, these sequences belonged to a second gene, unrelated to UEV gene.

Mapping of known cDNAs and ESTs onto the genomic sequences indicated the presence of known genes upstream and downstream from the 1B like sequence in Y53C10 and DS00863. TBLASTX searches (Altschul *et al*., 1990) and dot-plot analyses between the segments in Y53C10 and DS00863 that lie within these positions (denoted Y53C10-B and DS00863-B) indicated that they shared other conserved segments in the vicinity of the conserved "B domain"-like sequence.

The stringent alignment with ESTs from non-redundant databases, and with the aid of computational gene identification programs, allowed us to predict the exonic structure for a gene in Y53C10-B and DS00863-B. The prediction in *C.elegans* gives a new gene with seven exons with a potential to encode a 319 amino acid protein (Thomson *et al*., 2000). The same procedure in *D.melanogaster* predicted a new gene in DS00863-B with five exons and with a potential to encode a 326 amino acid protein (Thomson *et al*., 2000).

The existence of ESTs corresponding to the newly predicted *C. elegans* gene confirmed that the prediction was correct. However, we did not find any expressed sequences in the databases for the equivalent *D. melanogaster* gene that supported our prediction. We resolved this question by RT-PCR experiments with RNAs from adult and larval *D. melanogaster*, and sequencing. In this way, we proved that the newly predicted gene in D. melanogaster was expressed, and thus our predictions for the new gene with the B domain-like sequences were confirmed.

Comparing the new gene from *C. elegans* and *D. melanogaster* with a sequence in PAC clone dJ1185N5 from chromosome 20 that contains the UEV gene, we found within this clone a conserved sequence with a high similarity to the new predicted gene. Human and mouse ESTs matched strongly with this region. These analyses, together with the application of gene prediction programs, allowed us to predict the presence of a gene in humans in this region, equivalent to the new genes predicted in *C. elegans* and *D. melanogaster*. This gene was predicted to have six exons and a potential to code for a protein with 270 amino acids (Thomson *et al*., 2000). All these predictions were confirmed experimentally by RT-PCR in several cell lines, which gave full-length transcripts of the size expected (964 nucleotides) for this new gene. We gave this new gene the name Kua.

The sequence of the RT-PCR products confirmed that Kua contained the B domain that was identified as part of the isoform UEV1B, and that these sequences lie 5' to UEV1. These sequences correspond to exons 4 and 5 of Kua. Since we had shown that the same sequences were also part of the UEV gene (Sancho *et al*., 1998; Rothofsky and Lin, 1997), we hypothesized that that the B domain-like sequences could be part of more than one gene and more than one type of transcript. We confirmed this hypothesis by RT-PCR and sequencing experiments performed with forward primers from the predicted Kua and reverse primers from UEV1 in different cell lines.

These experiments showed that the Kua-UEV locus can generate three classes of transcripts: a hybrid Kua-UEV transcript, a "Kua-only" transcript, and "UEV-only" transcripts. The latter contain at their 5' end sequences of the first exon of UEV1, that we had called the "A domain" (Sancho *et al*., 1998) and therefore correspond to the isoform UEV1A (Sancho *et al*., 1998). We therefore interpret the generation of these three classes of transcripts as an alternative splicing with the use of two different

promoters. We have isolated and sequence the mouse UEV1 locus, and have shown that, like in the human locus, it also contains the genes for Kua and UEV1 as closely linked genes, and have shown that also in this mammal there are hybrid Kua-UEV transcripts, in addition to Kua-only or UEV1-only transcripts (B. Cormand, V. Plans, M. de los Ríos and T. Thomson, unpublished observations). In other organisms, such as *Arabidopsis thaliana*, *Trypanosoma cruzi*, *Caenorhabditis elegans* and *Drosophila melanogaster*, Kua and UEV are unlinked genes and we have found no evidence for the existence of Kua-UEV hybrid transcripts.

As a result of our analysis, we predict that the locus for H.sapiens UEV1 on chromosome 20q13.2 has two contiguous genes, Kua and UEV1, each with its own promoter. The two genes can be expressed as two independent transcripts and proteins, Kua and UEV1A, or as a hybrid transcript and protein Kua-UEV. For the hybrid transcript to be functional the last exson of the Kua gene (exon 6), containing a 3' UTR, and the first exon of the UEV1 gene (exon A), containing a 5' UTR, are removed by splicing. The generation of three different classes of transcripts from the Kua-UEV locus represents a unique strategy aimed at the modular expression of two genes, coding either for two separate polypeptides, or as a combination of both to yield a single two-domain polypeptide.

Many other genes have been found to be fused during the evolution, and this fusion often indicates a functional interaction between the protein product of the fused genes. There must be selective pressure for certain genes to be fused over the course of evolution (Enright *et al*., 1999; Marcotte *et al*., 1999) because often the transcript fusion between two adjacent genes, although not infrequent, are observed only in a subset of closely associated gene pairs. We performed yeast two-hybrid experiments with Kua and UEV proteins from different organisms, which did not give any evidence

for direct interaction between UEV protein and the carboxyl or the amino terminal domain of Kua. However it remains possible that these two proteins perform functions in the same biochemical pathway, such that a requirement for functional interaction would provide a driving force for their evolutionary fusion.

By analyzing the sequence of the Kua proteins, we predict that they contain two two-pass transmembrane domains. When we expressed either GFP fusion proteins or epitope-tagged in Cos-7 or Rat-1 cells, we observed that UEV1A localizes mainly in the nucleus, whereas Kua and the hybrid Kua-UEV proteins are perinuclear, localized in cytoplasmic structure, probably in the endoplasmic reticulum. Therefore, one of the functional consequences of the fusion of *Kua* with *UEV* in humans is that the hybrid protein is redirected for localization to cytoplasmic structures, rather than the nucleus. As a consequence of their different subcellular localization, one possibility is that nuclear (UEV1A) and cytoplasmic (Kua-UEV) forms of UEV1 could target different substrates for variant (Lys63) polyubiquitination. Thus, an endomembrane-associated form of UEV1 could preferentially direct the variant polyubiquitination of substrates closely associated with the cytoplasmic face of the ER **(Figure.IV.4)**, possibly, although not necessarily, in conjunction with membrane-bound ubiquitin-conjugating enzymes (Sommer and Jentsch, 1993).

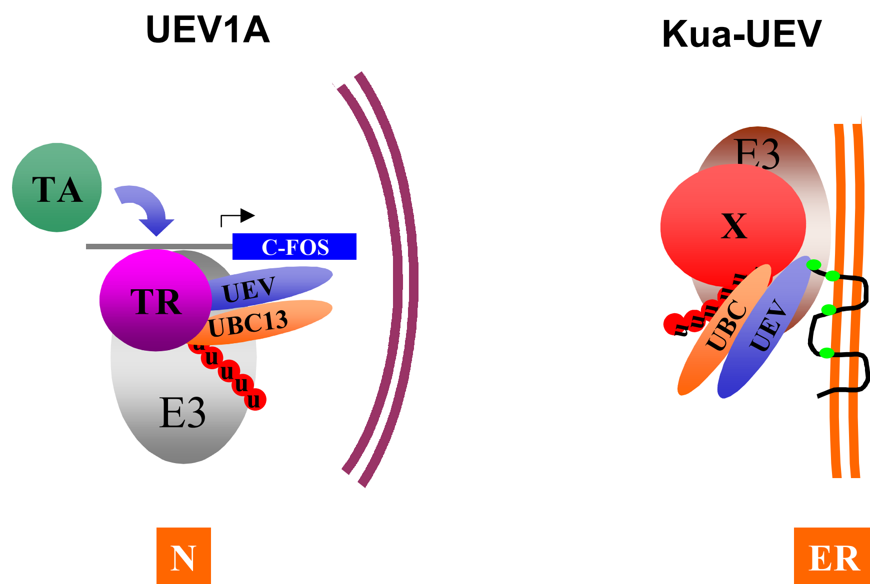


**Figure IV.·4** Hypothetical action of the hybrid protein Kua-UEV. The trabsmembrane localization of Kua protein localizes Kua-UEV in the cytoplasm far from nuclear substrates and close to cytoplasmic targets for UEV protein. Different subcellular localization for UEV and Kua-UEV lead to different targets.

## IV.3. Possible horizontal gene transfer of Kua between eukaryotes and prokaryotes

Search for genes homologues to Kua in all organisms for which sequences were available in the databases initially indicated the presence of this gene in most eukaryotic organisms (except yeasts and fungi) but not in prokaryotes. Very recently, however, a single prokaryote was found with a gene coding for a protein with sequence showing a very strong similarity to animal Kua proteins. This organism is the δ-proteobacterium *Myxococcus xanthus*. The gene and protein from *M. xanthus* called CarF is highly homologous to all animal and protozoan Kua proteins, including human, fly, worm, *Trypanosoma* and *Leishmania*, and is more distantly related to Kua proteins from plants.

Our analysis strongly suggests the occurrence of horizontal gene transfer (HGT) from eukaryotes to prokaryotes, as opposed to evolution through common ancestry, or "vertical" evolution. This conclusion is based primarily on the facts that (1) *M. xanthus* CarF is very similar to, and clearly clusters with, animal and protist Kua proteins, and (2) so far no other prokaryote investigated contains sequences even remotely similar to Kua/CarF. If the gene had originated at the root between eukaryotes and prokaryotes, subsequently following a "vertical" mode of evolution, multiple losses in the latter would need to be invoqued in order to explain the absence of the gene in all prokaryotes except Myxobacteria.

Several theories support the hypothesis that probably all the organic beings which have ever lived on this earth have descended from one primordial form, into which life was first breathed. In recent years, many authors (Ouzounis and Kyrpides, 1996; Aravind *et al*., 1999; Lazcano and Forterre, 1999; Diruggiero *et al*., 1999) have

used comparisons of the gene contents of modern genomes in attempts to reconstruct the proteome of the universal ancestor, which some call “the last universal common ancestor” or “LUCA”, and some call “the cenancestor”. Other researchers, however, have cast the rooting of the universal tree on which such analyses ultimately depend into doubt (Philippe and Forterre, 1999; Brinkmann and Philippe, 1999; Forterre and Philippe, 1999) or have claimed that lateral transfer of genes between species, phyla or domains is so frequent that all reconstruction attempts are doomed to failure (Doolittle, 1999; Martin, 1999).

The widely endorsed universal tree of life recognizes three primary domains (bacteria, archaea and eukaria), first defined by sequences of SSU (small subunit) rRNAs. The most often accepted rooting of this tree, based on paralogous protein-coding gene families, has its deepest division (earliest branching) separating bacteria, from a lineage that later diverged into archaea and eukaryotes (**Figure IV.5**). The parsimony reasoning on this tree tells us that - barring lateral gene transfer (LGT) between species - any gene present in organisms on both sides of the deepest branching was probably present in the universal ancestor (Harvey and Pagel, 1991). Genes present only on one side, that is only in bacteria or only in archaea plus eukarya, could be either recent (invented on that side) or ancestral (lost on the other). Genes restricted to a group of related organisms within a domain are most likely to be recently invented.

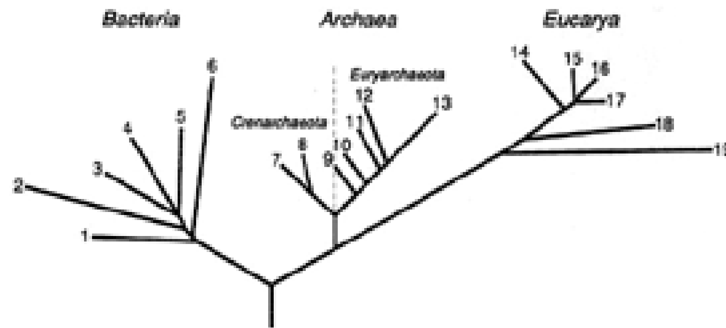


**Figure IV. 5.** Universal phylogenetic tree in rooted form, showing the three domains. Branching order and branch lengths are based upon rRNA sequence comparisons. The position of the root was determined by comparing sequences of pairs paralogous genes that diverged from each other before the three primary lineages emerged from their common ancestral condition. The numbers of on the branch tips correspond to the following groups of organisms. **Bacteria**: 1, Thermotogales; 2, flavobacteria and relatives; 3, cyanobacteria; 4, purple bacteria; 5, Gram-positive bacteria; and 6, the green nonsulfur bacteria. **Archae*: the kingdom Crenarchaeota***: 7, genus Pyrodictium; and 8, genus Thermoproteus; and kingdom Euryarchaeota: 9, Thermococcales; 10 Methanococcales; 11, Methanobacteriales; 12, Methanomicrobiales; and 13, extreme halophiles. **Eucarya**: 14, animals; 15, ciliates; 16, green plants; 17 fungi; 18, flagellates; and 19, microsporidia. (From Woese *et al*., 1990)

The SSU rRNA gives a big help to the construction the current universal tree. This molecule is superior to cytochromes or ferredoxins as a ”molecular chronometer “ for many reasons. It is abundant, it is coded by organellar as well as nuclear and prokaryotic genomes, it has slow and fast-evolving portions, and it has universally conserved structure (Woese, 1987). Two other factors that contribute to the confident use of SSU rRNA are its obviously ancient and essential fundamental function in the cellular economy and its interaction with many (well over 100) other evolved cellular RNAs and proteins (Green and Noller, 1997). These last features would seem to make rRNA genes the least liable of all genes to experience interspecific lateral gene transfer (LGT). However, although accepting the usefulness of SSU rRNA in the distinction between the three kingdoms of life, many have casted doubts on its capacity to distinguish important branching events within each kingdom, or to identify horizontal

transfers between kingdom or between organisms within the same kingdom (for example, Philippe and Forterre, 1999). One of the proposals for more reliable organismal trees is to build them upon all possible orthologous proteins, rather than a tree constructed on the basis of a single gene. Such trees maintain the basic three-kingdom scheme built on SSU rRNA, but provide new insights into the relationships between phyla, families and species (Woese et al., 1990; Doolittle, 1999).

With independence of the type of phylogenetic tree being contemplated, it is difficult to reconcile the topology of the tree for Kua/CarF with evolution of this gene through common ancestry. As discussed, a horizontal mode of evolution is a more parsimonious scenario. Horizontal gene transfer appears to be very common between prokaryotes, and it constitutes an important factor in the evolution of these organisms (Phelippe et al., 2000). But this mode of gene evolution does not seem to be so prevalent in eukaryotes. In fact, very few cases of proposed horizontal gene transfer that involved eukaryotes have stood the test of subsequent sequence and phylogenetic analyses (Salzberg et al., 2001). Only very recently a strong case has been made of evolution through horizontal transfer between eukaryotes and prokaryotes (Salzberg et al., 2001). The case of Kua/CarF, presented by us, is the first in which functional studies and inferences of the involved proteins are presented together with a simple sequence and phylogenetic analysis, with all the data supporting the conclusion of a conserved function of a gene transferred from an eukaryote to a prokaryote.

Our previous study had shown that Kua is predicted to contain two two-pass transmembrane domains, and that this conferred this protein the signals for its localization in endomembranes (Thomson *et al*., 2000). Here we show that *M. xanthus* CarF can be expressed efficiently in mammalian cells, and that it co-localizes with human Kua in cytoplasmic structures in most transfected cells. Although the results are

not provided in the accompanying manuscript, we have also found that another *M. xanthus* protein, CarR, is also expressed in Cos-7 cells, and co-localizes both with human Kua and *M. xanthus* CarF. *M. xanthus* CarR is a membrane-spanning protein, that acts as an anti-σ factor which in the dark sequesters the protein CarQ to the membrane (Gorham *et al*., 1996). CarQ is a regulator necessary for the transcription of the CarQRS operon (Nicolas *et al*., 1994). Illumination of the cells results in loss of CarR, so that Car Q is now free to activate CarQRS and crtI promoters (Gorham *et al*., 1996). In its absence, the gene crtI and the carQRS complex are expressed at high levels both in the dark and in the light. Our finding that CarR co-localizes with CarF and also with Kua allows us to propose the scheme presented in **Figure IV.6**. In *M. xanthus*, Kua/CarF would be a sensor for blue light located at the membrane of the bacterium. Upon exposure to light, CarF would interact with CarR, and as a consequence the latter would be inactivated or destroyed through unknown mechanisms (Gorham *et al*., 1996). The loss of CarR activity now frees CarQ, which can now activate transcription of the CarQRS operon. Evidences for most aspects of this sequence of events have been provided by different laboratories (Fontes *et al*., 1993; Nicolas *et al*., 1994; Gorham *et al*., 1996).

At this time we do not know the function of Kua. However, the finding of an ortholog in a bacterium should be very helpful for assigning biochemical functions to this protein. One possibility is that Kua functions in eukaryotes in a way that is similar to CarF in *M. xanthus*, that is, it could be a sensor of changes in membranes or their immediate vicinity induced by stimuli such as light. One of the changes induced by light is the variation of the redox status of the cellular media. This could influence the electronic state of metal atoms that presumably coordinate His and other residues on Kua/CarF, thus influencing the activity and/or interactions with other proteins. In this

sense, we speculate that Kua could be a general redox sensor. The consequences of this potential function on the activities of the chimeric Kua-UEV proteins could be the subject of yet further speculation.

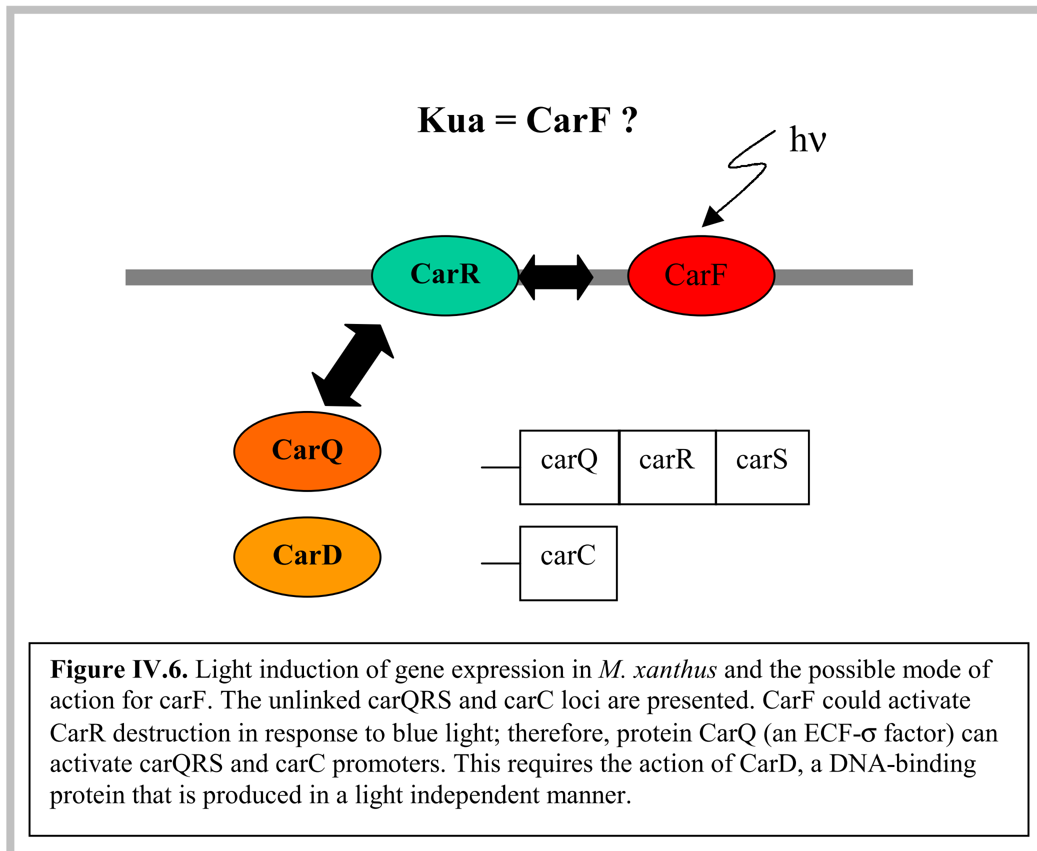


**Figure IV.6.** Light induction of gene expression in *M. xanthus* and the possible mode of action for carF. The unlinked carQRS and carC loci are presented. CarF could activate CarR destruction in response to blue light; therefore, protein CarQ (an ECF-σ factor) can activate carQRS and carC promoters. This requires the action of CarD, a DNA-binding protein that is produced in a light independent manner.

# V./ Conclusions

**1.** We have identified a new family of proteins that are variants of the ubiquitin conjugating enzymes, devoid of an intrinsic catalytic center. We have given these proteins the name UEV. We have shown that these proteins are highly conserved in all eukaryotes. We have also shown that UEV participates in a diverse array of cellular processes, including cell cycle control and differentiation. We have assigned the gene for UEV1 to chromosome 20q13.2, determined its intron-exon structure, and shown the expression of several forms of alternatively spliced transcripts.

**2.** We have shown that the architecture of the *UEV* genes, and in particular the position of the introns, when present, is extremely well conserved in all organisms. We have demonstrated that, in humans, the gene for the UEV1 protein is fused with a new second gene, that we have called *Kua*. In other organisms, such as *Drosophila melanogaster* and *Caenorhabditis elegans*, the genes *Kua* and *UEV* are in separated loci. In the human composite *Kua-UEV1* locus, three major classes of transcripts are produced, as a consequence of the use of two independent promoters and of alternative splicing. These transcripts are translated into three major classes of proteins, UEV1A, Kua and the hybrid Kua-UEV. The protein UEV1A localizes mostly inside the cell nucleus, whereas Kua is a predicted transmebrane protein that localizes in cytoplasmic structures. The localization of the hybrid Kua-UEV protein follows the subcellular localization of the Kua protein, and thus the addition of the Kua domain confers a new function to the UEV1 protein.

**3.** Our phylogenetic analysis of the *Kua* gene and predicted protein strongly suggests that this gene was subject of an event of horizontal transfer between eukaryotes and the bacterium *Myxococcus xanthus* or its ancestors. In this organism, the ortholog of Kua is the CarF protein, a sensor for blue light regulating the expression of enzymes of carotenoid biosynthetic pathways. Bacterial CarF and human Kua co-localize when expressed in mammalian cells. In early embryonic development in the mouse, Kua is expressed initially mainly in the central nervous system, with a pecular pattern in structures such as the developing eye. In latter stages, Kua is expressed in a more wide range of tissues. By analogy with CarF, we speculate that Kua could be a sensor for intracellular redox changes.

# VI./ References

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