Carcinogenesis vol.23 no.8 pp.1267–1271, 2002

The clastogenic response of the 1q12 heterochromatic region to DNA cross-linking agents is independent of the Fanconi anaemia pathway

E.Callén[1], M.J.Ramírez, A.Creus[1], R.Marcos[1], S.Frias[2], B.Molina[2], I.Badell[3], T.Olivé[4], J.J.Ortega[4] and J.Surrallés[1,5]

[1]Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Barcelona, Spain, [2]Cytogenetics Laboratory, Instituto Nacional de Pediatría, México D.F., Mexico, [3]Bone Marrow Transplantation Unit, Department of Paediatrics, Sant Pau Hospital, Barcelona, Spain and [4]Bone Marrow Transplantation Unit, Service of Paediatric Hematology-Oncology, Vall d'Hebron Hospital, Barcelona, Spain

[5]To whom correspondence should be addressed at: Group of Mutagenesis, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Cerdanyola del Vallès, Barcelona, Spain
Email: jordi.surralles@uab.es

Fanconi anaemia (FA) is a rare genetic syndrome of cancer susceptibility characterized by spontaneous and induced chromosome fragility, especially after treatment with cross-linking agents. Recent investigations showed interactions between FA proteins and chromatin remodelling factors. To investigate a potential uneven distribution of the FA pathway through the human genome depending on chromatin conformation, we have analysed chromosome breakage in the largest constitutively heterochromatic region in the human genome, the 1q12 band, in lymphocytes from FA patients, carriers and healthy controls after treatment with the cross-linking agents mitomycin-C (MMC) and diepoxybutane (DEB). As expected, a higher level of MMC-induced cytotoxicity and chromosome breakage was observed in cells from FA patients when compared with normal controls and carriers. However, the increase in 1q12 breakage after increasing concentrations of MMC was of a similar magnitude in FA patients, carriers and controls. Similarly, DEB induced a high level of overall genome chromosome fragility in cells from FA patients when compared with controls with no parallel increase in chromosome breaks specifically involving the heterochromatic band 1q12. We therefore conclude that, unlike the overall genome, the sensitivity of chromosome 1 constitutive heterochromatin to the chromosome breaking activity of cross-linking agents is independent of a functional FA pathway, indicating that the action of the FA pathway is unevenly distributed through the human genome.

Introduction

Fanconi anaemia (FA) is an autosomal recessive disease characterized by increased spontaneous and induced chromosome instability, a diverse assortment of congenital malformations, progressive pancytopenia and cancer susceptibility, especially acute myelogenous leukaemia but also solid tumours. The genetics of FA is highly heterogeneous with at least eight different genes involved (*FANCA*, *B*, *C*, *D1*, *D2*, *E*, *F* and *G*), all of them, but *FANCB* and *FANCD1*, cloned and characterized (reviewed ref. 1). Most of the FA proteins (A, C, E, F and G) assemble in a nuclear complex that is required for the activation, via monoubiquitination, of FANCD2. Activated FANCD2 interacts with the breast cancer susceptibility protein BRCA1 in mutagen-induced nuclear foci (2). Thus, all known FA proteins participate in the same cellular pathway, which is involved in the cellular response to DNA cross-linking agents such as mitomycin-C (MMC) or diepoxybutane (DEB). In addition, the FA pathway participates in a number of cytoplasmic and nuclear functions such as redox regulation, cell cycle, apoptosis and DNA repair (1–4). To this list, we have recently added a severe telomere dysfunction in FA, leading to short and hyperbreakable telomeres and chromosome end-fusions (5).

Chromatin structure is known to play a major role in the processing of DNA lesions by several DNA repair mechanisms. This is well documented for UV light and ionizing radiation but little is known about the role of chromatin conformation on the repair of DNA cross-links. The major pathway involved in the processing of DNA cross-links is the FA pathway, and recent experimental evidence has uncovered a molecular cross talk between FA proteins and chromatin remodelling factors including a subunit of the SWI/SNF complex (6), histone acetyltransferases (7) and FAZF (8). In addition, FA proteins are excluded from condensed mitotic chromosomes (9) and FANCD2 interacts with BRCA1 that in turn is involved in transcriptional control through modulation of chromatin structure (10).

These findings would implicate that the action of the FA proteins is modulated by chromatin remodelling and transcriptional factors. Chromatin structure and transcriptional activity are known to be unevenly distributed between and within human chromosomes and therefore we have hypothesized that the action of the FA pathway could be non-homogeneously distributed through the human genome. In order to study the processivity of DNA lesions by the FA pathway in a heterochromatic region and to study a potential uneven chromosomal distribution of the FA pathway, we have analysed MMC- and DEB-induced breakage in the heterochromatic chromosome band 1q12 in comparison with the overall genome in lymphocytes from FA patients, carriers and controls. The 1q12 band is the largest constitutively heterochromatic region in the human genome, and is formed by hypermethylated DNA associated with underacetylated histones in a highly condensed chromatin conformation. 1q12 breakage was detected by multicolour tandem labelling fluorescence *in situ* hybridization (TL-FISH) (11,12).

Materials and methods

Individuals

Blood samples were obtained from seven FA patients, four FA carriers (parents of four of the FA patients) and seven healthy controls. Samples from the FA patients and their unaffected parents were obtained during routine clinic visits with informed consent. For technical reasons, the complementation group is

Abbreviations: Cyt-B, cytochalasin-B; DEB, diepoxybutane; FA, Fanconi anaemia; MMC, mitomycin-C; MN, micronuclei; TL-FISH, tandem labelling fluorescence *in situ* hybridization.

not known for all patients but at least the four FA patients tested for MMC response belong to the complementation group A (data not shown). Control blood samples were kindly provided by healthy volunteers. This study was approved by the University Ethics Committee on Human Research.

Cell culturing and treatments

Five millilitre cultures were set up following standard blood cell culturing procedures as we described previously (13). Twenty-four hours after the initiation of the cultures, lymphocyte samples from four FA patients, four carriers and three healthy controls were concurrently treated with increasing concentrations of MMC (0, 0.01, 0.05 and 0.1 μg/ml). Some of the cultures were subsequently incubated for 22 h, treated with colcemid for 2 h and fixed following standard cytogenetic methods (total culture time 48 h). Microscope slides were prepared and kept at –20°C until TL-FISH. Replicate cultures were incubated 48 h after MMC treatment (72 h in total) and received the cytokinesis blocking agent cytochalasin-B (Cyt-B) (6 μg/ml; Sigma, St Louis, MO) at 44 h after the initiation of the cultures (14,15). Cells were then harvested with no addition of colcemid to get cytokinesis-blocked binucleated interphase cells with well-preserved cytoplasm, as described previously (16). Three FA blood samples and four control samples received DEB (0 and 0.1 μg/ml; Sigma) at the beginning of the culture and were harvested at 72 h after 1 h of colchicine (Sigma) treatment. Metaphase spreads were prepared following standard cytogenetic procedures.

Cell-cycle kinetics

Cell-cycle delay after increasing concentrations of MMC was analysed in Cyt-B-exposed cultures by measuring the relative frequencies of undivided lymphocytes (mononucleated), lymphocytes divided once (binucleated) and lymphocytes divided more than once (multinucleated). A total number of 200 cells with well-preserved cytoplasm were counted per sample and classified according to the number of nuclei. The final value for each MMC concentration was expressed in a proliferation index as a percentage relative to the untreated culture.

Tandem labelling multicolour FISH

Chromosome breakage at the band 1q12 was analysed by tandem labelling multicolour FISH (11,12) as described in detail elsewhere (16,17), with the only difference that the chromosome 1 centromeric-specific α-satellite probe was generated and biotin-labelled by PCR (18). The 1q12 band is adjacent to the chromosome 1 centromeric DNA. Therefore, cells with a 1q12 signal non-adjacent to a chromosome 1 centromeric signal were interpreted as cells carrying a break in the 1q12 band (12). The suitability of this methodology to detect mutagen-induced 1q12 breakage has been proven elsewhere (11,12,16,19,20). Three MMC concentrations (0, 0.01 and 0.05 μg/ml) and two DEB concentrations (0 and 0.1 μg/ml) were evaluated for 1q12 fragility.

Overall genome chromosome fragility

The frequency of micronuclei (MN) was measured as marker of overall genome chromosome breaking activity of MMC, as the number of metaphases were very low in FA samples after MMC treatment. In most cases, a total of 1000 binucleated cells in Cyt-B-exposed cultures were analysed, per treatment point and donor, for the presence of MN in Giemsa-stained slides. Replicated slides from the cultures treated with 0 and 0.1 μg/ml DEB were analysed for chromosome aberrations in Wrigth/Giemsa-stained slides. The average number of breaks per cell was obtained by analysing the presence of structural chromosomal aberrations, including chromosome and chromatid breaks, fragments, dicentrics, rings and radials, in 25 metaphases per concentration and sample.

Statistics

The effect of MMC and DEB on the different end-points was measured by the unpaired Student's *t*-test for independent variables.

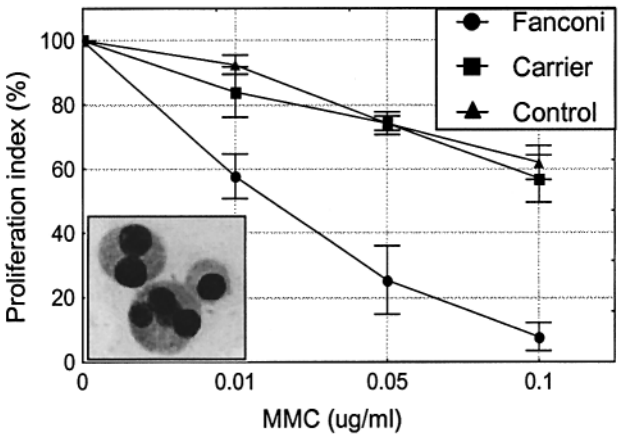

Fig. 1. MMC-induced cell-cycle delay in peripheral blood cells from FA patients, carriers and controls as measured by the cytokinesis-blocked proliferation assay. Mean and standard deviation (bars) are indicated. The panel inside the graph illustrates cells with different number of nuclei after cytokinesis blockage: an undivided mononucleated cell, a binucleated cell divided once and a tetranucleated cell divided twice.

Results

Table I and Figure 1 show the results on MMC-induced cell-cycle delay in four FA patients, four carriers and three normal controls. In this study we have used a novel approach to measure MMC-induced cell-cycle delay in FA lymphocyte cultures. It is based on the analysis of the average number of cell divisions undergone under increasing concentrations of MMC. This was achieved by blocking cytokinesis with Cyt-B so that those cells divided once are easily recognized by their binucleated appearance after cytokinesis blockage, whereas undivided cells and cells divided twice appear mononucleated and multinucleated, respectively. As expected, no differences in sensitivity to MMC were observed between controls and carriers, whereas a much higher sensitivity to MMC was obtained in cells from FA patients. The MMC concentration required to reduce by 50% the cell proliferation in controls and carriers was ~6-fold higher than in FA patients.

The same slides were used to measure MMC-induced overall genome chromosome breakage by using the MN assay. The data are summarized in Table I and graphically represented in Figure 2a. As expected, MMC induced a much higher frequency of MN in FA samples when compared with controls and carriers, highlighting the well-known chromosome hypersensitivity to MMC in FA cells. Concurrently grown cultures from the same individuals were tested for 1q12 chromosome fragility by TL-FISH. TL-FISH gives a nuclear pattern consisting of two adjacent red (1q12) and green (1cen) signals (two double signals). A break in 1q12 results in three red 1q12 signals, one of them non-adjacent to the centromeric 1cen signals. Results on 1q12 fragility are detailed in Table I and summarized in Figure 2b. A statistically significant increase ($P < 0.001$) in the frequency of 1q12 breaks by increasing concentrations of MMC was observed in FA patients, carriers and controls. At both MMC concentrations tested (0.01 and 0.05 μg/ml), we observed a similar increase in the frequency of 1q12 breaks in FA patients, carriers and normal controls, when compared with untreated cultures. It is therefore concluded that the sensitivity of the heterochromatic band 1q12 to the chromosome breaking activity of MMC is independent of the FA genotype.

To check whether this effect extends to other cross-linking agents, we next analysed three FA patients and four controls for overall genome and 1q12 hypersensitivity to DEB. Results are shown in Table II. As expected, both spontaneous and DEB-induced overall genome chromosome fragility were statistically higher in FA patients when compared with controls ($P < 0.001$). The spontaneous level of breaks per cell was 0.31 ± 0.23 in FA and 0.01 ± 0.02 in controls. DEB (0.1 μg/ml) induced no chromosome breaks in control cells whereas $>6.61 \pm 3.74$ breaks per cell were obtained in FA at the same DEB concentration. Thus, DEB induced a 21-fold increase in overall chromosome breakage in FA but no increase in control samples. Thus, and as expected, the overall genome

in FA cells is much more sensitive than control cells to the chromosome breaking activity of DEB. Replicated slides from the same cultures were tested for spontaneous and DEB induced breaks specifically involving the heterochromatic region 1q12 by TL-FISH. Results are shown in Table II. In spite of the 21-fold increase in overall chromosome fragility observed in FA cells after DEB treatment, no parallel increase in 1q12 breakage was detected in the same patients in the presence of DEB ($P > 0.05$). In conclusion, and resembling the MMC data, while the overall genome in FA cells is hypersensitive to DEB, the 1q12 heterochromatic band is not.

Discussion

The 1q12 heterochromatic band resulted to be equally sensitive to the chromosome breaking activity of cross-linking agents in lymphocytes from FA patients, carriers and controls, in spite of the obvious overall cellular and chromosomal hypersensitivity of FA cells to MMC and DEB observed in replicated slides from the same blood samples. This result indicates that the sensitivity of this highly condensed chromosomal domain to cross-linking agents is independent of a functional FA pathway and therefore that the action of the FA proteins is unevenly distributed through the human genome.

There are increasingly evident connections between the FA pathway and chromatin remodelling and transcriptional activity: (i) the key player in the FA pathway, FANCD2, interacts with BRCA1 (2), a protein involved in DSB repair by homologous recombination (the DSB repair pathway has been recently reviewed in ref. 21). BRCA1 is also involved in transcriptional control through modulation of chromatin structure (10) and is essential for transcription–coupled repair of oxidative damage (22) such as 8-oxoguanine (23). Interestingly, the response to oxidative stress is highly impaired in FA resulting in a high level of 8-oxoguanine (24). (ii) FA

Table I. MMC-induced 1q12 fragility and cell-cycle delay in peripheral blood cells from FA patients (–/–), carriers (+/–) and controls (+/+)

Individual code	Disease status	MMC (μg/ml)	1q12 breakage		Overall genome breakage		
			Cells scored	1q12 breaks	Cells scored	MN/1000	Proliferation index (%)
1	FA	0	1000	9	792	15	100
		0.01	1000	19	612	37	35
		0.05	1000	25	296	108	3
		0.1	NS	–	NS	–	2
2	FA	0	1000	10	1000	18	100
		0.01	1000	6	1000	53	65
		0.05	1000	16	849	193	14
		0.1	NS	–	NS	–	2
3	FA	0	1000	11	1000	23	100
		0.01	1000	16	1000	47	61
		0.05	1000	25	675	87	50
		0.1	NS	–	NS	–	20
4	FA	0	1000	3	1000	16	100
		0.01	1000	6	1000	33	57
		0.05	1000	10	541	170	25
		0.1	NS	–	NS	–	7
5	Carrier	0	1000	3	1000	8	100
		0.01	1000	7	1000	12	75
		0.05	1000	14	1000	51	64
		0.1	NS	–	NS	–	53
6	Carrier	0	1000	8	1000	20	100
		0.01	1000	10	1000	18	69
		0.05	1000	8	1000	69	76
		0.1	NS	–	NS	–	38
7	Carrier	0	1000	11	1000	5	100
		0.01	1000	10	1000	11	110
		0.05	1000	31	1000	22	80
		0.1	NS	–	NS	–	67
8	Carrier	0	1000	8	1000	11	100
		0.01	1000	9	1000	28	77
		0.05	1000	17	1000	38	74
		0.1	NS	–	NS	–	57
9	Control	0	1000	2	1000	13	100
		0.01	1000	4	1000	15	93
		0.05	1000	5	1000	58	68
		0.1	NS	–	NS	–	56
10	Control	0	1000	10	1000	7	100
		0.01	1000	12	492	12	100
		0.05	1000	18	787	21	76
		0.1	NS	–	NS	–	78
11	Control	0	1000	3	1000	4	100
		0.01	1000	14	1000	10	90
		0.05	1000	20	1000	26	73
		0.1	NS	–	NS	–	64

Individuals 5–8 are the parents of the patients 1–4, respectively. NS: not scored.

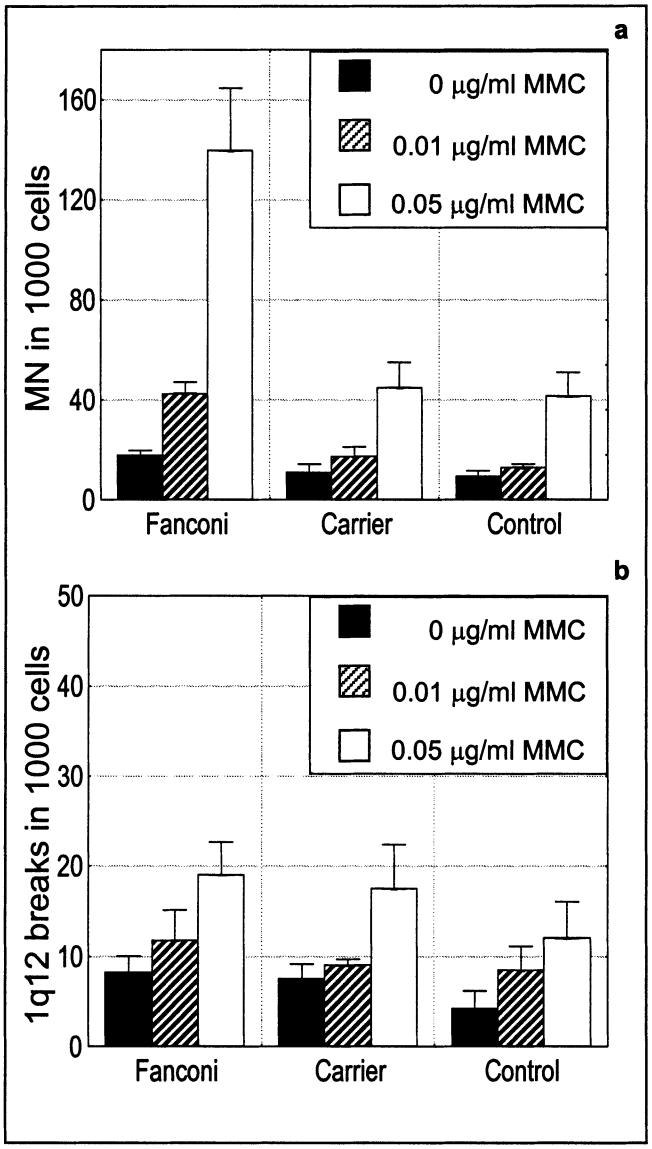

Fig. 2. MMC-induced overall genome (**a**) and 1q12 breakage (**b**) in peripheral blood cells from FA patients, carriers and controls as measured by the micronucleus assay and tandem labelling multicolour FISH, respectively. Mean (histograms) and standard deviation (bars) are indicated. MMC concentrations used were 0 (black), 0.01 (striped) and 0.05 (white) μg/ml.

proteins interact with a number of chromatin modifier factors such as BRG1, as a subunit of the SWI/SNF complex (6) and histone acetyltransferases, as evidenced in the yeast two-hybrid system (7); (iii) FANCC may be linked to a transcriptional repression pathway involved in chromatin remodelling through interaction with FAZF (8); and (iv) the FA proteins bind to chromatin and nuclear matrix but are excluded from condensed mitotic chromosomes (9). Thus, an interesting cross talk between the FA gene products, DSB repair proteins and transcription and chromatin modifying factors is coming into the light. Although our results cannot be directly extrapolated to all heterochromatic regions, this molecular cross-talk probably explains the results obtained in the present investigation as 1q12 is the largest heterochromatic and transcriptionally silent domain in the human genome. In a previous study we reported a low level of DNA excision repair in the same heterochromatic band (16) that was well explained by the transcription-coupled DNA nucleotide excision repair. Other DNA repair mechanisms

Table II. DEB-induced 1q12 and overall genome chromosome fragility in peripheral blood cells from FA patients, carriers and controls

Individual code	Disease status	DEB (μg/ml)	1q12 fragility		Overall fragility (breaks/cell)
			Cells scored	1q12 breaks	
12	FA	0	507	10	0.24
		0.1	709	11	4.40
13	FA	0	1000	14	0.56
		0.1	1000	15	4.50
14	FA	0	1000	13	0.12
		0.1	1000	16	10.92
15	Control	0	1000	10	0.04
		0.1	1000	14	0.00
16	Control	0	1000	3	0.00
		0.1	1000	9	0.00
17	Control	0	1000	6	0.00
		0.1	1000	10	0.00
18	Control	0	1000	4	0.00
		0.1	1000	13	0.00

are also coupled to transcription and are modulated by chromatin remodelling factors (25,26). Transcription takes place in the same substrate as repair, replication and recombination and it is therefore not surprising that these processes are physically and functionally connected (27). Further experiments will probably unravel whether this is also the case in the FA pathway.

Summing up, we provide molecular cytogenetic evidence that the sensitivity of human chromosome heterochromatic band 1q12 to cross-linking agents is independent of a functional FA pathway suggesting that the action of the FA proteins is unevenly distributed through the human genome. Further studies are needed to uncover a modulating role of chromatin conformation and transcriptional activity in the FA pathway as a potential explanation to our results.

Acknowledgements

We would like to thank all FA families and volunteers that have participated in this study. This work was partially funded by the Fanconi Anemia Research Fund (USA), the Spanish Ministry of Health and Consumption (FIS, 99/1214) and the Commission of the European Union (Euratom Programme, F1S5-1999-00071). E.C. was supported during this work by a FPI fellowship awarded by the Universitat Autònoma de Barcelona (UAB). J.S. is supported by a 'Ramón y Cajal' project on Genome Stability and DNA Repair (Spanish Ministry of Science and Technology-UAB).

References

1. Joenje,H. and Patel,K.J. (2001) The emerging genetic and molecular basis of Fanconi anaemia. *Nature Rev. Genet.*, **2**, 446–457.
2. Garcia-Higuera,I., Taniguchi,T., Ganesan,S., Meyn,M.S., Timmers,C., Hejna,J., Grompe,M. and D'Andrea,A.D. (2001) Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol. Cell*, **7**, 249–262.
3. Cumming,R.C., Lighfoot,J., Beard,K., Youssofian,H., O'Brien,P.L. and Buchwald,M. (2001) Fanconi anemia group C protein prevents apoptosis in hematopoietic cells through redox regulation of GSTP1. *Nature Med.*, **7**, 814–820.
4. Grompe,M. and D'Andrea,A. (2001) Fanconi anemia and DNA repair. *Hum. Mol. Genet.*, **10**, 2253–2259.
5. Callén,E., Samper,E., Ramírez,M.J., Creus,A., Marcos,R., Ortega,J.J., Olivé,T., Badell, I., Blasco,M.A. and Surrallés,J. (2002) Breaks at telomeres and TRF2-independent end-fusions in Fanconi anemia. *Hum. Mol. Genet.*, **11**, 439–444.
6. Otsuki,T., Furukawa,Y., Ikeda,K., Endo,H., Yamashita,T., Shinohara,A., Iwamatsu,A., Ozawa,K. and Liu,J.M. (2001) Fanconi anemia protein, FANCA, associates with BRG1, a component of the human SWI/SNF complex. *Hum. Mol. Genet.*, **10**, 2651–2660.

7. Reuter,T., Herterich,H., Hoehn,H. and Gross,H.J. (2000) On our way to the function of the FA-proteins: yeast two hybrid FA-interactors are involved in chromatin modification. 12th Annual International Fanconi Anemia Symposium, Abstracts book, p. 27.
8. Hoatlin,M.E., Zhi,Y., Ball,H., Silvey,K., Melnick,A., Stone,S., Arai,S., Hawe,N., Owen,G., Zelent,A. and Licht,J.D. (1999) A novel BTB/POZ transcriptional repressor protein interacts with the Fanconi anemia group C protein and PLZF. *Blood*, **94**, 3737–3747.
9. Qiao,F., Moss,A. and Kupfer,G.M. (2001) Fanconi anemia proteins localize to chromatin and the nuclear matrix in a DNA damage and cell cycle-regulated manner. *J. Biol. Chem.*, **276**, 23391–23396.
10. Bochar,D.A., Wang,L., Beniya,H., Kinev,A., Xue,Y., Lane,W.S., Wang,W., Kashanchi,F. and Shiekhattar,R. (2000) BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell*, **102**, 257–265.
11. Eastmond,D.A., Rupa,D.S. and Hasegawa,L.S. (1994) Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence *in situ* hybridization with DNA probes. *Mutat. Res.*, **322**, 9–20.
12. Rupa,D.S., Hasegawa,L. and Eastmond,D.A. (1995) Detection of chromosomal breakage in the 1cen-1q12 region of interphase human lymphocytes using multicolor fluorescence *in situ* hybridization with tandem DNA probes. *Cancer Res.*, **55**, 640–645.
13. Surrallés,J., Hande,M.P., Marcos,R. and Lansdorp,P.M. (1999) Accelerated telomere shortening in the human inactive X chromosome. *Am. J. Hum. Genet.*, **65**, 1616–1622.
14. Fenech,M. and Morley,A.A. (1985) Measurement of micronuclei in lymphocytes. *Mutat. Res.*, **147**, 29–36.
15. Surrallés,J., Jeppesen,P., Morrison,H. and Natarajan,A.T. (1996) Analysis of loss of inactive×chromosome in interphase cells. *Am. J. Hum. Genet.*, **59**, 1091–1096.
16. Surrallés,J., Darroudi,F. and Natarajan,A.T. (1997) Evidence for low level of DNA repair in human chromosome 1 heterochromatin. *Genes Chromosomes Cancer*, **20**, 173–184.
17. Ramírez,M.J., Surrallés,J., Galofré,P., Creus,A. and Marcos,R. (1999) FISH analysis of 1cen-1q12 breakage, chromosome 1 numerical abnormalities and centromeric content of micronuclei in buccal cells from thyroid cancer and hyperthyroidism patients treated with radioactive iodine. *Mutagenesis*, **14**, 121–127.
18. Lengauer,C., Dunham,I., Featherstone,T. and Cremer,T. (1994) Generation of alphoid DNA probes for fluorescence *in situ* hybridization (FISH) using polymerase chain reaction. In Choo,K.H.A. (ed.) *Methods in Molecular Biology*, Vol.22: *in situ Hybridization Protocols.* Humana Press Inc., Totowa, NJ.
19. Murg,M.N., Schuler,M. and Eastmond,D.A. (1999) Persistence of chromosomal alterations affecting the 1cen-q12 region in a human lymphoblastoid cell line exposed to diepoxybutane and mitomycin C. *Mutat Res.*, **446**, 193–203.
20. Puerto,S., Ramírez,M.J., Marcos,R., Creus,A. and Surrallés,J. (2001) Radiation-induced chromosome aberrations in human euchromatic (17cen-p53) and heterochromatic (1cen-1q12) regions. *Mutagenesis*, **16**, 291–296.
21. Khanna,K.K. and Jackson,S.P. (2001) DNA double-strand breaks: signalling, repair and the cancer connection. *Nature Genet.*, **27**, 247–254.
22. Gowen,L.C., Avrutskaya,A.V., Latour,A.M., Koller,B.H. and Leadon,S.A. (1998) BRCA1 required for transcription–coupled repair of oxidative DNA damage. *Science*, **281**, 1009–1012.
23. Le Page,F., Randrianarison,V., Marot,D., Cabannes,J., Perricaudet,M., Feunteun,J. and Sarasin,A. (2000) BRCA1 and BRCA2 are necessary for the transcription–coupled repair of the oxidative 8-oxoguanine lesion in human cells. *Cancer Res.*, **60**, 5548–5552.
24. Degan,P., Bonassi,S., De Caterina,M., Korkina,L.G., Pinto,L., Scopacasa,F., Zatterale,A., Calzone,R. and Pagano,G. (1995) *In vivo* accumulation of 8-hydroxy-2′-deoxyguanosine in DNA correlates with release of reactive oxygen species in Fanconi's anaemia families. *Carcinogenesis*, **16**, 735–741.
25. Surralles,J., Puerto,S., Ramirez,M.J., Creus,A., Marcos,R., Mullenders,L.H.F. and Natarajan,A.T. (1998) Links between chromatin structure, DNA repair and chromosome fragility. *Mutat. Res.*, **404**, 39–44.
26. Meijer,M. and Smerdon,M.J. (1999) Accessing DNA damage in chromatin: insights from transcription. *Bioessays*, **21**, 596–603.
27. Aguilera,A. (2001) The connection between transcription and genomic instability. *EMBO J.*, **21**, 195–201.

Received February 26, 2002; revised and accepted April 9, 2002