Influence of chromatin remodeling in the processing of UVC induced lesions in CS cells

Wilner Martínez-López

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We have recently shown that transcription coupled repair (TCR) failure seems not to be responsible for the increased frequency of chromosomal aberrations observed in Cockayne's Syndrome (CS) simile cells exposed to UVC, since chromosome breakpoints were distributed more random in CS cells than in normal ones instead of being concentrated on the transcribed chromosome regions as expected (Martínez-López et al., 2010) (Paper is included in deliverables).

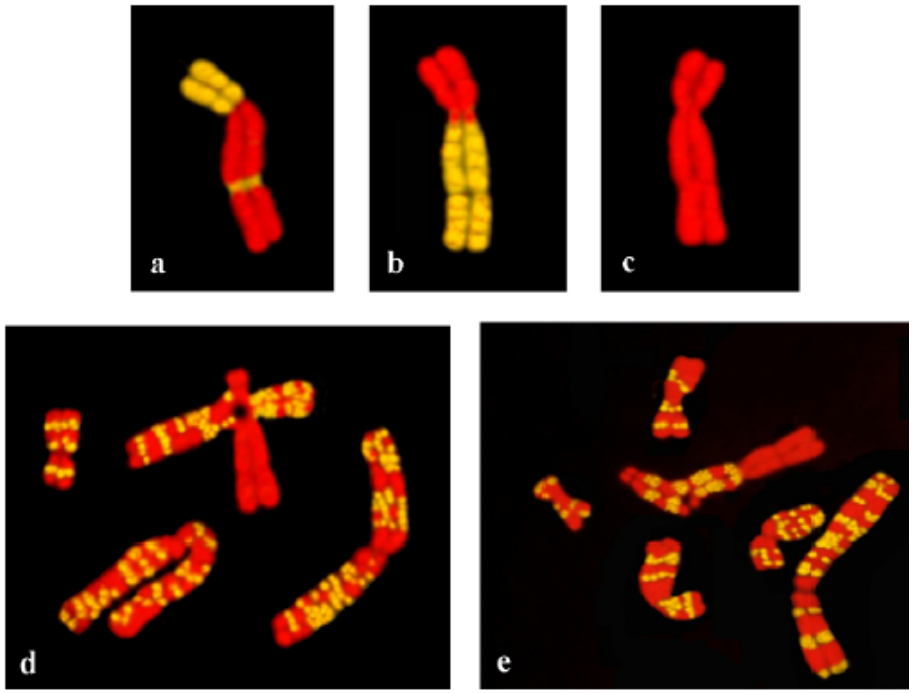

Table 3
Frequency of breakpoints found in the euchromatic (E) and heterochromatic (H) regions of the X chromosome from AA8 and UV61 cell lines exposed to 10 or 4 J/m², respectively, during early S-phase. Expected values and the statistical analysis (χ^2) of breakpoints distribution along the euchromatic and heterochromatic regions of the X chromosome from AA8 and UV61 cell lines are shown. The p-value obtained with the application of two-tailed two-proportion z-test is shown ($P_1 = 0.66$; $P_2 = 0.59$) (H0: $P_1 = P_2$; H1: $P_1 \neq P_2$).

		Breakpoints	Expected values	χ^2
AA8	E	66	45	9.80
AA8	H	34	55	8.02
Total		100	100	17.82*
UV61	E	59	45	4.36
UV61	H	41	55	3.56
Total		100	100	7.92**
Two-proportion z-test	p-Value	0.3066		

* $p < 0.001$.
** $p < 0.005$.

Fig. 1. BrdUrd immunolabelling (yellow) of the Chinese hamster X chromosome counterstained with propidium iodide (red). (a) ES: early S-phase; (b) LS: late S-phase; (c) UL: unlabeled. Chromosomal aberrations involving the X chromosome from ES metaphases: (d) quadrirradial and (e) trirradial involving the short arm of the X chromosome. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

In this respect, we have observed that changes in the histone acetylation level by treating normal cells with an histone deacetylase inhibitor like trichostatin A (TSA) produce a similar sensitivity to UVC than in CS cells, increasing chromosomal aberration frequency (Figure 1).

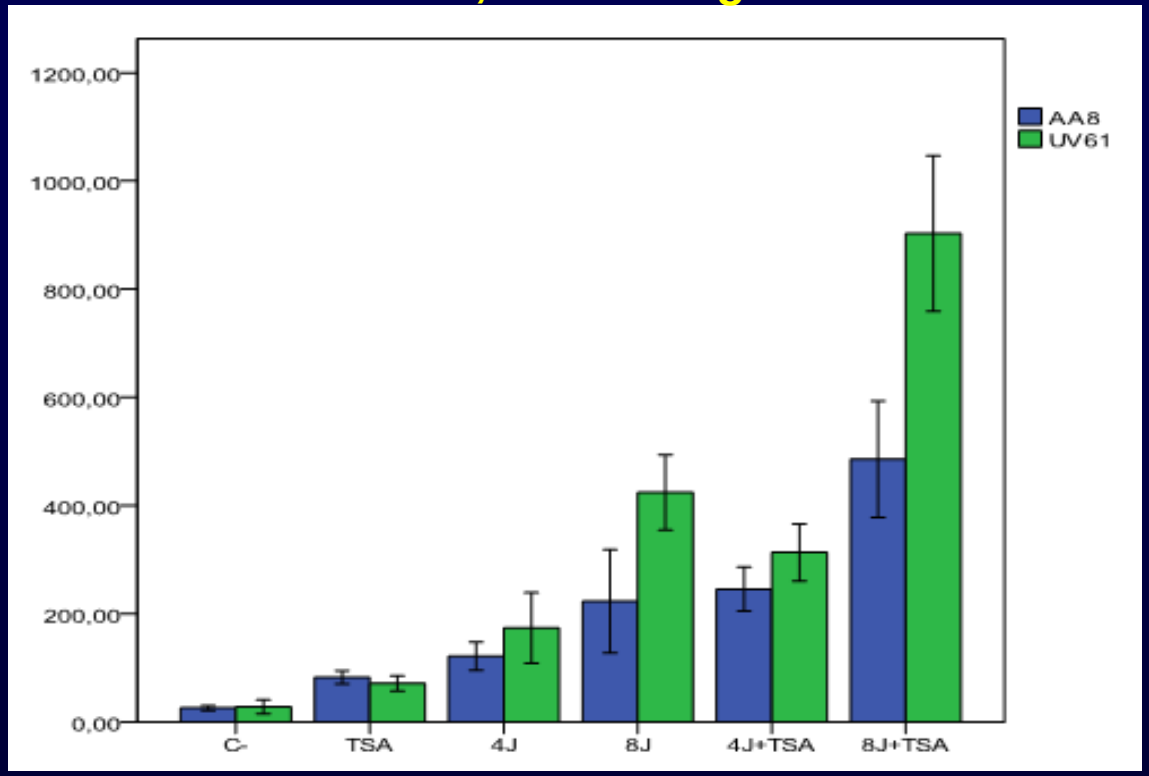

Figure 1.- A significant increase in the frequency of UVC-induced chromosomal aberrations was found in the presence of TSA (50 ng/ml) either in proficient or deficient CHO cells in TCR. C= Control; TSA= trichostatin A; 4J= 4 Joules; 8J= 8 Joules. Bars represent 2 x SE (standard error) from two independent experiments.

Besides, we have analyzed the kinetics of CPDs removal in normal and CS cells in the presence of TSA by using the comet assay plus the T4 endonuclease V which recognizes specifically CPDs introducing single strand breaks which increase DNA migration (Figures 2 and 3).....

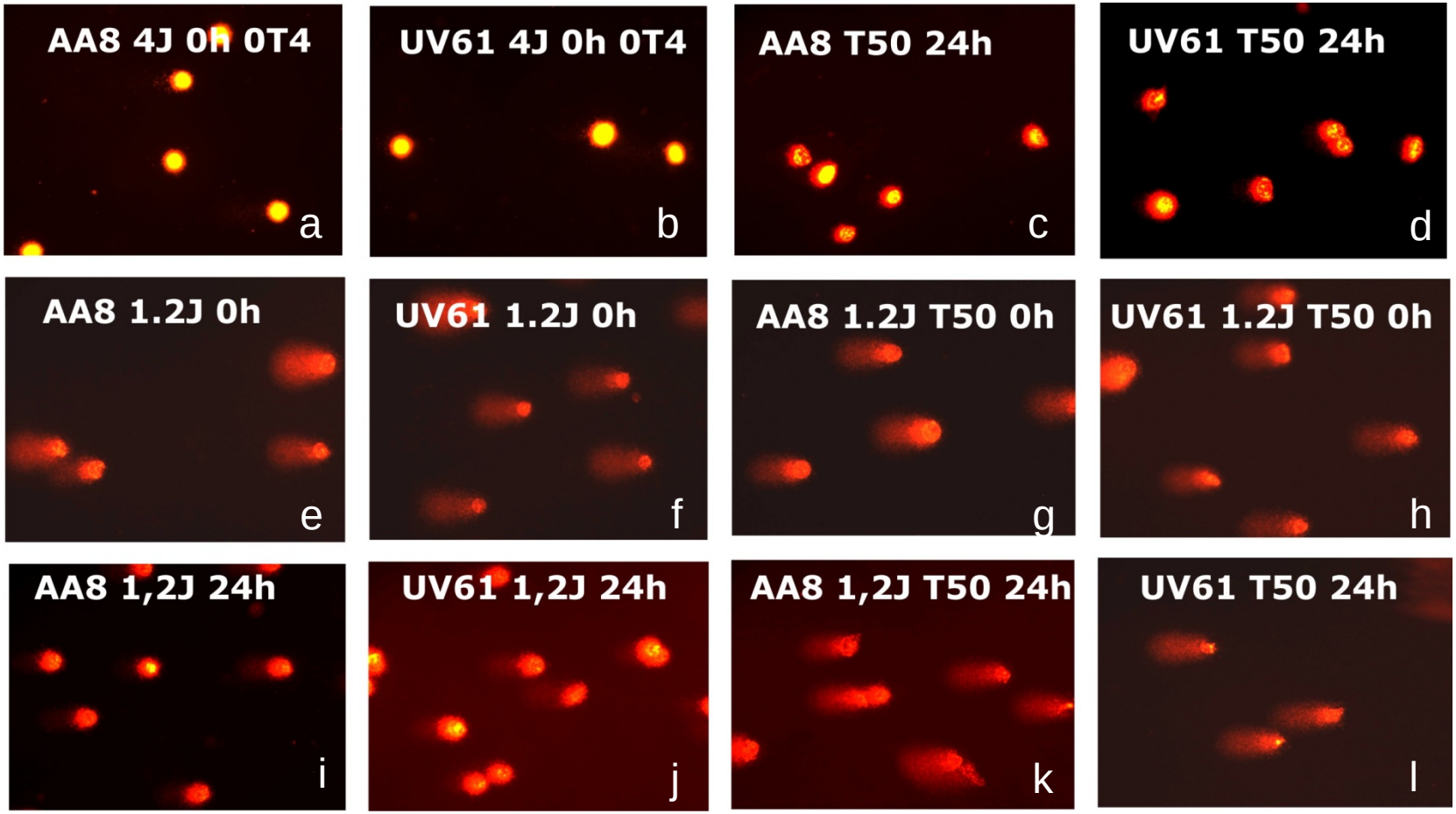

Figure 2.- Comet assay employing T4 endonuclease V in AA8 and UV61 cells exposed to 1,2 J/m2 of UVC in the presence or absence of TSA (50 ng/ml), and recovered at 0h and 24h after UVC-irradiation. (a-b) UVC-irradiated cells (4 J/m2) but not exposed to T4 endonuclease V. (c-l) All nucleoids were exposed to 1 μl of T4 endonuclease V diluted in 50 μl of NET buffer. Note that after 24 h recovery time after UVC-irradiation, primary lesions are still not removed in AA8 as well as in UV61 cells.

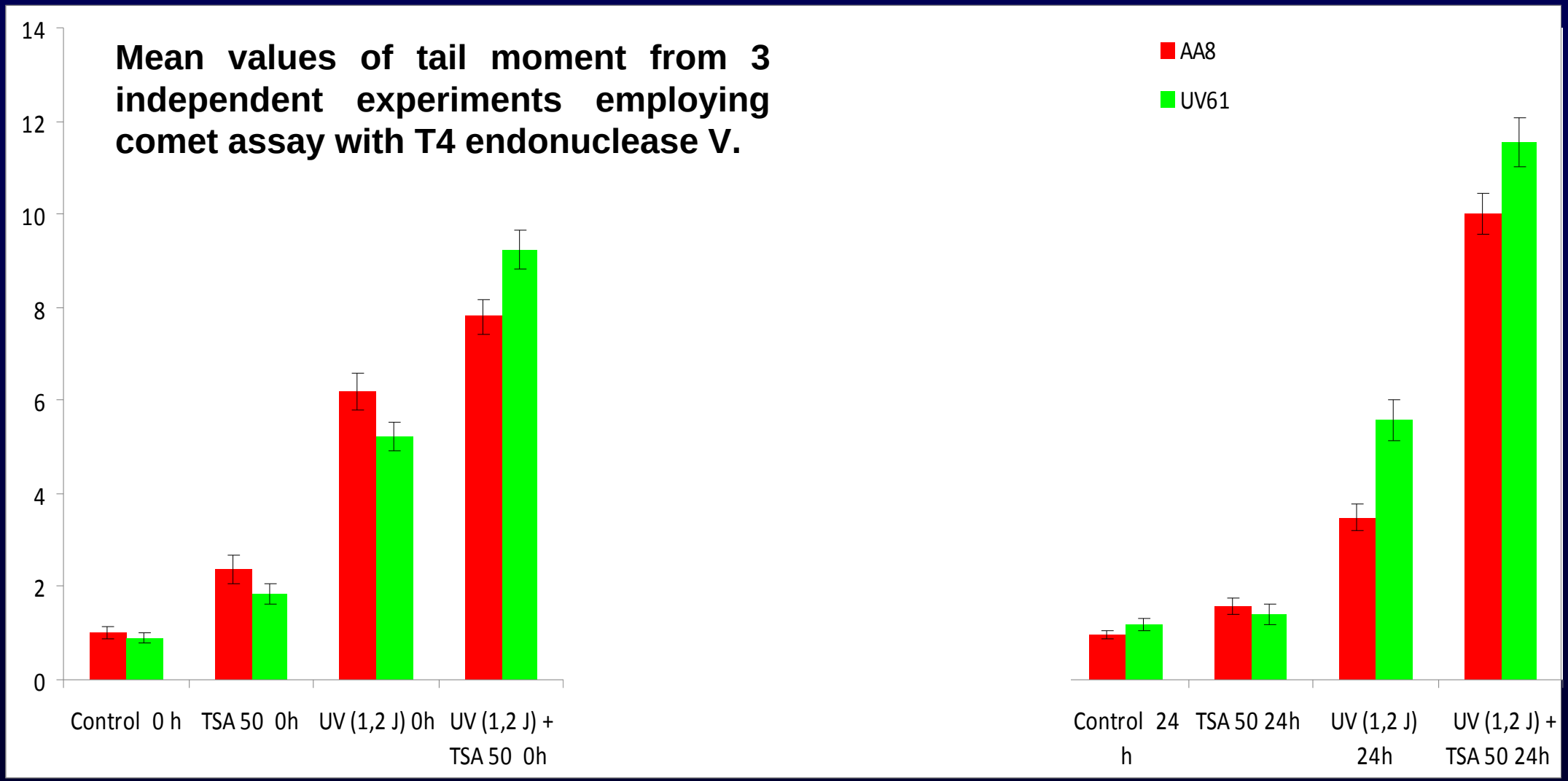

Figure 3.- Graphic representation of the effect of TSA in combination with UVC related to an impair produced by the TSA on the removal of UVC-induced primary lesions. Bars represent the standard deviation.

.....as well as immunolabeling techniques employing antibodies against CPDs and revealed with a secondary antibody conjugated with FITC (Figure 4). An impair of CPDs removal in the presence of TSA was observed with both techniques.

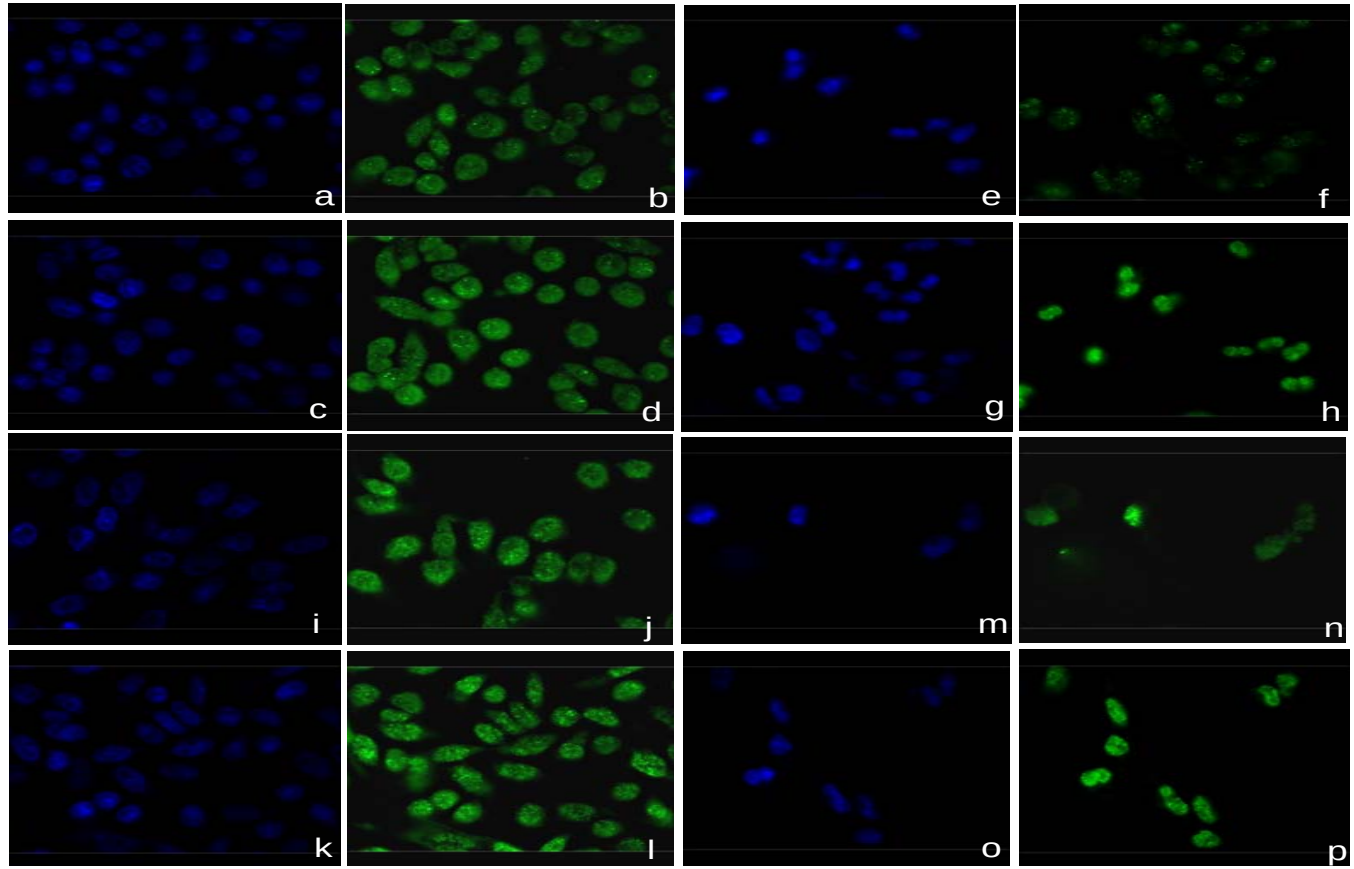

Figure 4.- Immunolabeling of cyclobutane pyrimidine dimmers (CPDs) employing anti-CPDs mouse antibodies revealed with an anti-mouse-FITC (green) and counterstained with DAPI (blue) on AA8 (a-h) or UV61 (i-p) nucleous. (a-b, i-j) Nucleous fixed with paraformaldehyde at 0 h after UVC irradiation (4 J/m2), (c-d, k-l) Nucleous fixed with paraformaldehyde at 0 h after UVC irradiation (4 J/m2) from cells pre-treated (4 h) with TSA, (e-f, m-n) Nucleous fixed with paraformaldehyde at 20 h after UVC irradiation (4 J/m2), (g-h, o-p) Nucleous fixed with paraformaldehyde at 20 h after UVC irradiation (4 J/m2) from cells pre-treated (4 h) with TSA, which produce an impair on CPDs removal by nucleotide excision repair

Although we have shown that TSA (an histone deacetylase inhibitor) is able to produce an impair in the removal of UVC-induced lesions in normal cells as it happens in CS cells, we could not find an increase in histone acetylation pattern in CS cells. On the contrary, we have observed in CS simile cells (UV61, the Chinese hamster homologue of the Cockayne's Syndrome B or CSB) a less decondensed chromatin triggered by UVC irradiation evidenced by *in situ* nick translation technique (Figures 5 and 6).

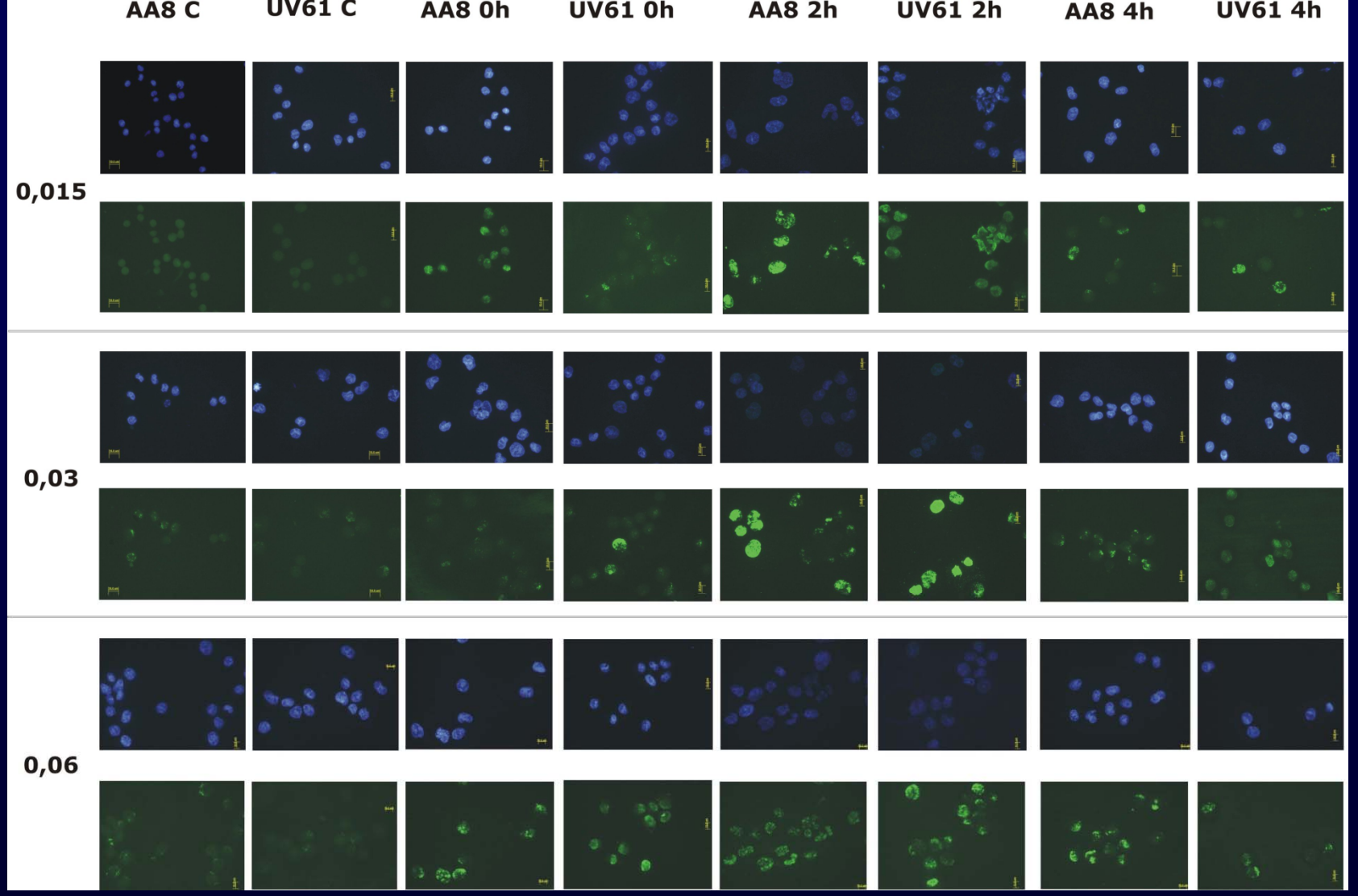

Figure 5.- *In situ* nick translation panel of fixed G1 cells from AA8 and UV61 irradiated with 4 J/m2 of UV-C and recovered at 0h, 2h and 4h post-irradiation, exposed to mixtures containing different doses of DNase I (0.015, 0.03 and 0.06 units/ml). Green fluorescence correspond to the incorporation of labeled nucleotides by polymerase I revealed with an antibody coupled with FITC. Blue fluorescence represent DAPI counterstaining.

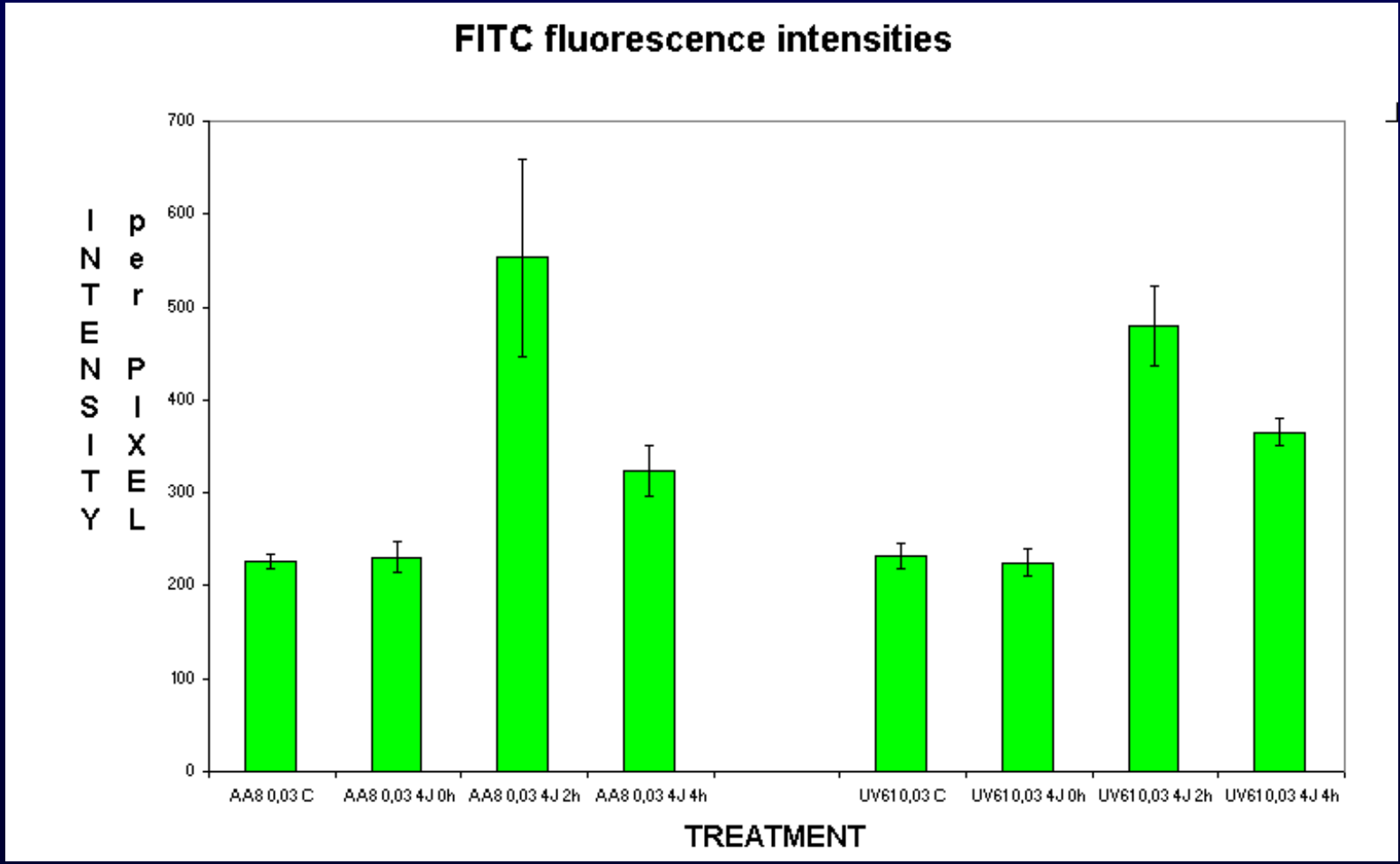

Figure 6.- Graphic representation of FITC fluorescence intensity per pixel from 50 fixed nuclei exposed to 0.03 units/ml of DNase I. Note that at 2 h after UVC-irradiation either AA8 or UV61 cells are decondensed being in AA8 more intense.

As it has been demonstrated that UVC irradiation triggers a genome-wide histone hyperacetylation at both histones H3 and H4 (Yu et al., 2005), we have investigated the histone H4 acetylation level after UVC exposure of AA8 and UV61 cells by means of western blot using antibodies against specific histone H4 acetylation sites. Since di, tri and tetra-acetylation on lysines 12, 8 and 5, respectively, from the amino terminal tail of histone H4 are considered as hyperacetylation, we have analyzed the di, tri and tetra-acetylations patterns of AA8 and UV61 after UVC irradiation.

Mono-acetylation is unchanged after UVC-irradiation either in AA8 or UV61 and it is shown as loading control (Figure 7). The di-acetylation increase after 2 and 4 h after UVC irradiation in AA8 but only at 4 h in UV61 (Figure 8). However, tri-acetylation and tetra-acetylation increase also at 2 and 4 h after UVC irradiation in AA8 but almost no increase is observed in UV61 (Figures 9 and 10), indicating that CS cells could not sufficiently increase the histone acetylation level triggered by UVC irradiation as it occur in normal cells

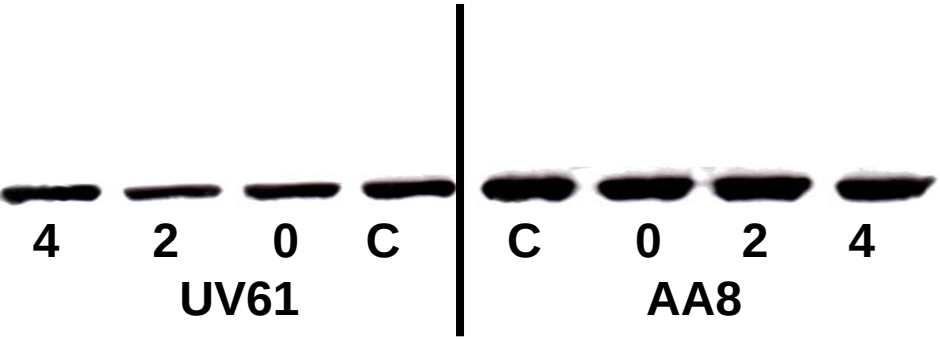

Figure 7.- Western blot showing the histone H4 acetylation pattern of H4K16 (mono-acetylated histone) after 0, 2 or 4 h post-UVC irradiation. Note that there is no differences in the mono-acetylation pattern among AA8 or UV61 samples.

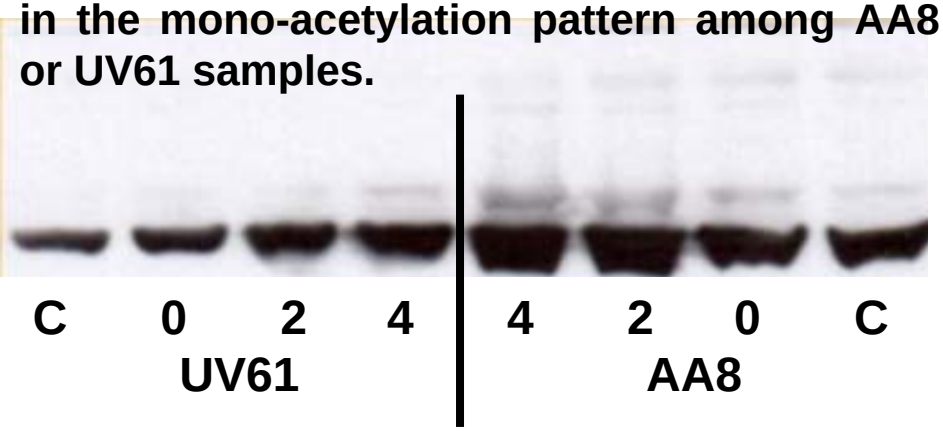

Figure 9.- Western blot of histone H4 acetylation pattern of H4K8 (tri-acetylated histone) after 0, 2 or 4 h post-UVC irradiation. Note that in AA8 cells there is an increase in tri-acetylation at 2 and 4 h after UVC while there is almost no tri-acetylation increase in UV61 cells.

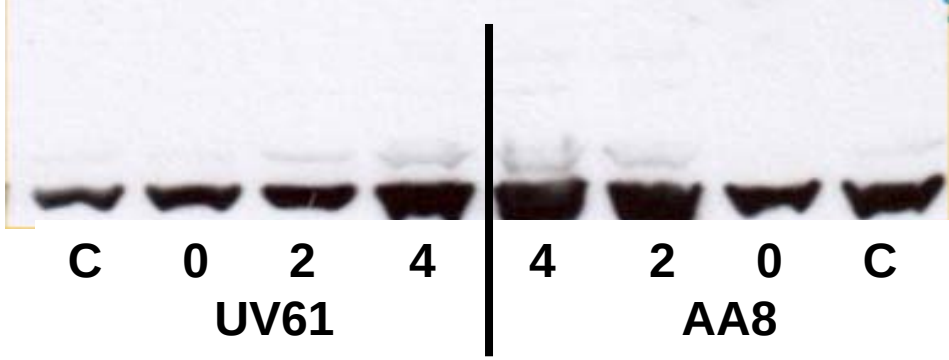

Figure 8.- Western blot showing the histone H4 acetylation pattern of H4K12 (di-acetylated histone) after 0, 2 or 4 h post-UVC irradiation. Note that in AA8 cells there is an increase in di-acetylation at 2 and 4 h after UVC while just at 4 h in UV61 cells.

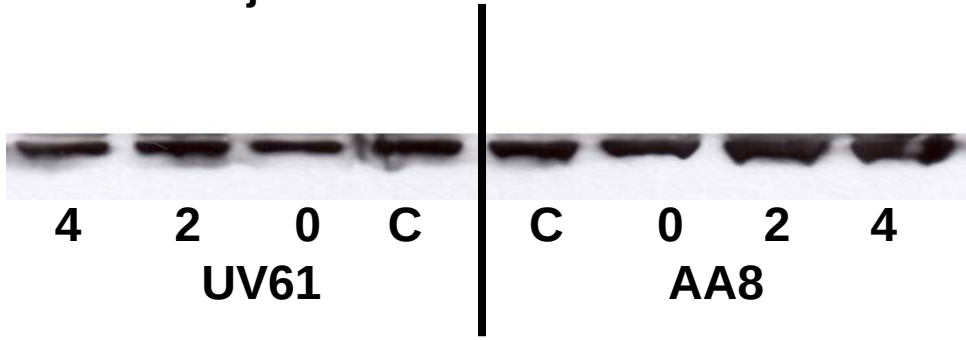

Figure 10.- Western blot of histone H4 acetylation pattern of H4K5 (tetra-acetylated histone) after 0, 2 or 4 h post-UVC irradiation. Note that in AA8 cells there is an increase in tetra-acetylation at 2 and 4 h after UVC while there is almost no tetra-acetylation increase in UV61 cells.

Summary of results obtained during last year of the MC project (Returning Phase)

We have confirmed that TSA impair NER activities for CPDs removal in normal cells after 24 h of UVC irradiation by using antibodies anti-CPDs either in nucleous or chromosomes from AA8 cells, obtaining a direct evidence of the lack of UVC-induced lesions removal.

Western blot analysis of the four sequentially acetylated sites at the N-terminal tail of the histone H4 in normal and CS simile cells showed that CS cells carry a defect in the global histone acetylation level triggered by UVC-irradiation.

At present, we are confirming our data obtained in hamster cell lines on human derived CS and XP cells in order to confirm our assumption that a differential nucleosome arrangement occur in CS cells after UVC irradiation that could explain the impair in the NER activity.

Summary of results obtained during the whole MC project

Survival assays showed higher sensitivity to UVC or TSA in UV61 cells but combined treatments of UVC in the presence of TSA affected much more AA8 cells than UV61.

TSA alone is able to induce apoptosis in both cell lines which can be increased after UVC irradiation, specially in UV61. Interestingly, the highest TSA concentration employed in our work affect much more AA8 than UV61 for inducing apoptosis.

TSA impair NER activities for CPDs removal in AA8 cells after 24 h of UVC irradiation evidenced either by the comet assay plus T4 endonuclease V or employing a specific antibody against CPDs.

Western blot analysis of the four sequentially acetylated sites at the N-terminal tail of the histone H4 in normal and CS simile cells showed that CS cells carry a defect in global histone acetylation triggered by UVC-irradiation.

Discussion

The CSB protein (Cockayne's Syndrome B protein) has a key role as coupling factor to attract the histone acetyltransferase p300 and nucleotide excision repair proteins (Citterio et al., 2000). Therefore, the 5 fold increase in chromosomal aberrations after UVC exposure (Proietti De Santis et al., 2001) could be caused by an acetylation imbalance since nucleotide excision repair proteins could not properly interact with DNA lesions in a less decondensed chromatin environment.

FINAL REMARK

As a conclusion, it seems that an adecquate nucleosome remodeling process has an important role in DNA repair, because its failure could strongly hamper the correct DNA damage accessibility or interaction by DNA repair proteins.

However, further experimental evidence is necessary to confirm the hypothesis of differential chromatin folding in Cockayne syndrome cells as a possible factor contributing to a higher sensitivity to UVC irradiation.