Supplementary Data

Detection of DNA-protein crosslinks (DPCs) by novel direct fluorescence labeling methods: distinct stabilities of aldehyde and radiation-induced DPCs

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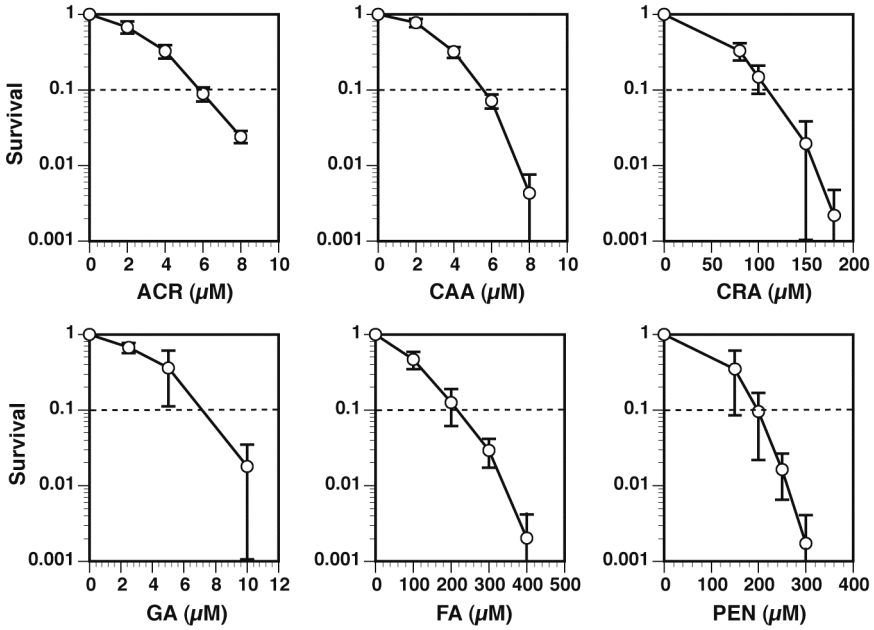
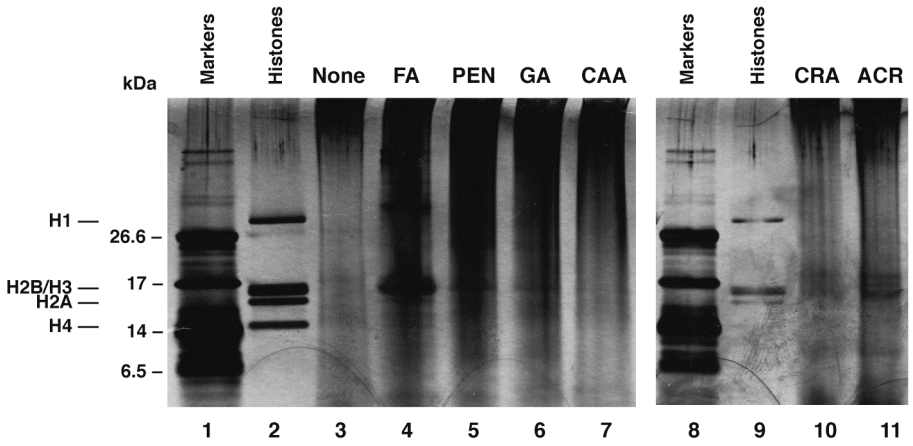


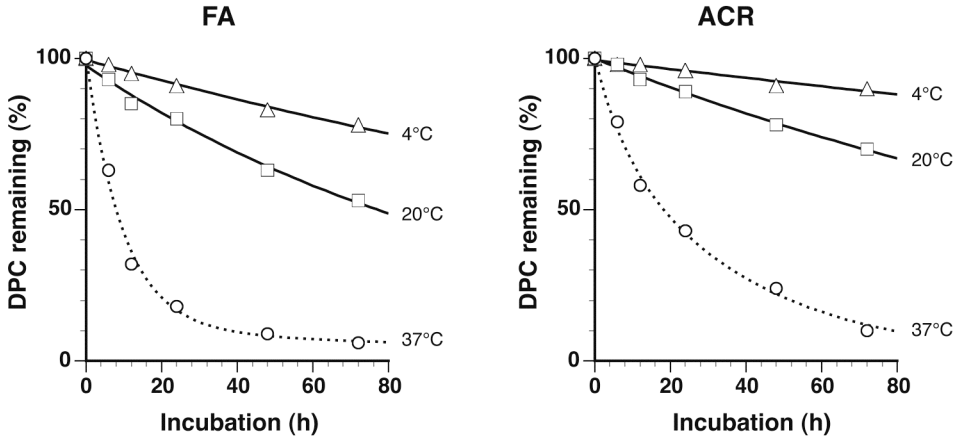
Supplementary Figure S1.

Survival curves of MRC5-SV cells following treatment with aldehydes. Cells were treated with the aldehydes at the indicated concentrations for 3 h, and washed twice with fresh medium and allowed to form colonies for about 7 days. The dashed line indicates 10% survival.



Supplementary Figure S2.

SDS-PAGE analysis of proteins in aldehyde-induced DPCs. MRC5-SV cells were treated with aldehydes at LD_{10} (Table 1), and DNA was isolated from the cells immediately after treatment. DNA (ca. 40 μg) was heated at 70°C for 6 h to liberate CLPs. After heating, samples were separated by 16.5% SDS-PAGE. Protein size markers (lanes 1 and 8) and histones (H1, H2A, H2B, H3, and H4; lanes 2 and 9) were run alongside. The aldehydes used for DPC induction are indicated at the top of the gels. DNA from untreated control cells is shown in lane 3. DPCs induced by FA and ACR (lanes 4 and 11) contained the core histones H2B and/or H3, which were not separated under the present conditions. DPCs induced by FA may also have contained modified histone H1. The heat liberation of CLPs varies with the aldehydes used and so it is possible that not all CLPs appeared in the gels.



Supplementary Figure S3.

In vitro kinetics of the elimination of FA- and ACR-induced DPCs at different temperatures. The remaining DPCs were quantified as described in the legend to Figure 4. The data at 37°C were taken from Figure 4.

Supplementary Table S1. Estimated loss of DPCs during DNA purification[a]

DNA purification	Loss of FA-DPCs (%)			Loss of ACR-DPCs (%)		
	20°C	4°C	total	20°C	4°C	total
one centrifugation[b]	7	8	15	4	4	8
two centrifugations[c]	14	14	28	7	8	15

[a] The percentages of the loss of DPCs at 20°C (ultracentrifugation step) and 4°C (dialysis and sample handling steps) were estimated from the corresponding decay curves in Supplementary Figure S3.
[b] 7 h at 20°C and 24 h at 4°C
[c] 14 h at 20°C and 43 h at 4°C