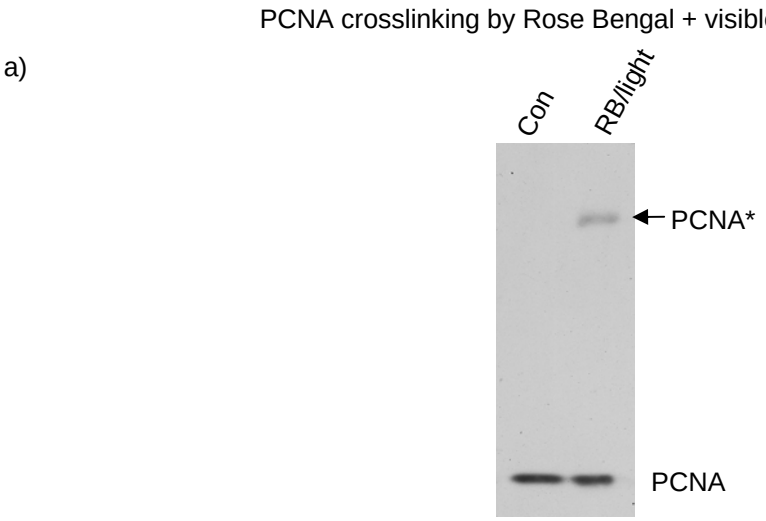
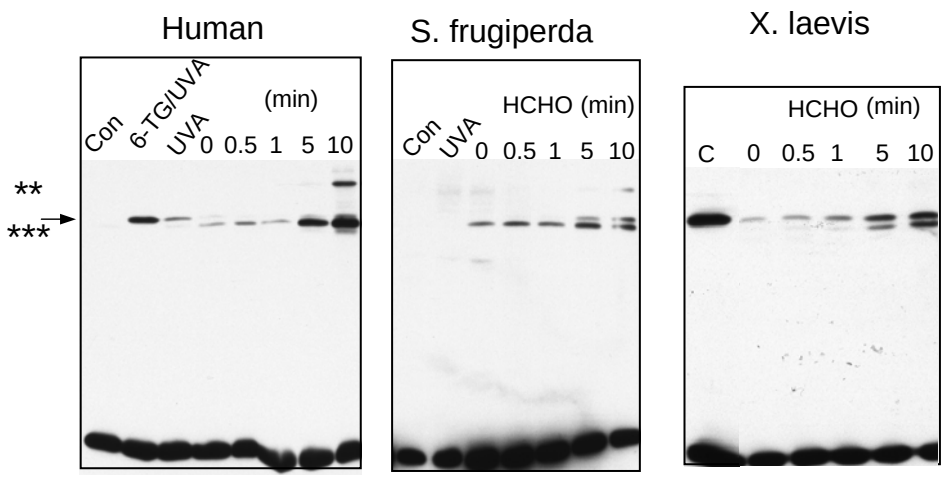
Supplementary Figure S2

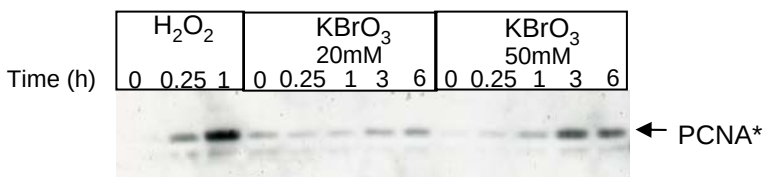
a)



b)



c)



Legends to supplementary figures

Figure S1 Formation of PCNA* by UVA and by 6-TG/UVA treatment in Raji, CCF-CEM, and A2780 cells.

a) Asynchronous cells were treated for 15min with the concentrations of 6-TG shown and UVA irradiated. Cell extracts were analysed by western blotting with the PC10 antibody.

Upper panel. Raji cells

Middle panel. CCF-CEM cells.

Lower panel. A2780 cells.

b) Raji cells pulse-labelled for 15min with 30μM 6-TG were returned to medium without 6-TG. Aliquots of cells were UVA irradiated (30kJ/m^2) at the indicated chase times after removal of 6-TG. Cell extracts were analysed by western blotting. [^{3}H]-TdR incorporation into TCA-insoluble material was also determined.

Figure S2 Chemical and photochemical PCNA crosslinking

a) Photochemical

Chromatin-associated proteins were extracted from HCT116 cells synchronized by growth in 1mM hydroxyurea and treated with rose bengal (0.12mM) and visible light (RB/light) or left untreated (Con). Illumination (10min) was delivered by a 60W domestic lightbulb at a distance of approximately 10cm from the protein solution which was maintained at ice temperature during the irradiation. PCNA was detected by western blotting with PC10.

b) Chemical

S. frugiperda (SF9) and human (HCT116) cells in PBS were treated with 1% formaldehyde at 20^o for the times indicated (min). Cells were harvested and extracts (harvesting and preparation time, approximately 5min) analysed by western blotting (6% SDS-PAGE) with PC10 antibody. Control cells were either untreated (Con) or irradiated with 300kJ/m^2 UVA (UVA). In an additional control (6-TG/UVA and C), HCT116 cells were grown for 24h in 1μM 6-TG and irradiated with 10kJ/m^2 UVA.

Photochemical and chemical treatments produce qualitatively somewhat different crosslinking patterns. In HCT116, 6-TG/UVA and UVA alone induces PCNA* the position of which is indicated by the arrow. The predominant initial product of formaldehyde treatment of human cells is a PCNA complex with slightly faster migration (***). A second more slowly migrating species (**) forms at later times. A third complex, which migrates at a similar position to PCNA* represents a minor reaction product. This pattern is also seen in HCHO treated SF9 cells and *X. laevis* extracts. Although the higher MW *X. laevis* form is not evident in this particular experiment, it can be seen in Figure 3c.

A high MW (200kDa) form of PCNA has been described previously and was ascribed to inter trimer crosslinking (Naryzhny et al, 2005). We also observe a complex with this mobility after high levels of photochemical crosslinking by 6-TG/UVA or UVA alone (see for example: A2780 30μM 6-TG + 30kJ/m^2 UVA (Supp. Fig S1a:): Raji 300kJ/m^2, S phase (Fig. 3a)).

UVA does not form PCNA complexes of SF9 or *Xenopus* PCNA. This is consistent with a requirement for His153 which is replaced by Gln in these species. Formation of the insect and amphibian PCNA forms (**) and (***) by formaldehyde is consistent with chemical crosslinking via the conserved aminoacids – including the primary amino groups of Lys110 and Arg146 (Naryzhny et al, 2005).

c) By oxidizing agents

Asynchronous CCF-CEM cells were treated with 0.03% H_2O_2 or $KBrO_3$ at the concentrations shown. Extracts were prepared at the indicated times and PCNA analysed by western blotting.