

## Co-localisation of $\gamma$ -H2AX and 53BP1 to sites of DNA double strand breaks following low- and high-LET irradiation of mammalian cells

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When cells are exposed to ionising radiation lesions are introduced into the DNA including single strand breaks (SSBs), double strand breaks (DSBs) and base damage together with clustered DNA damage sites, a specific feature of radiation induced DNA damage. Complex DNA damage sites have indeed been hypothesised from modelling approaches to be critical determinants leading to loss of genome stability. Recent developments in immuno-histochemistry have allowed the detection, as foci, of repair/signalling proteins recruited to sites of DNA damage, even at doses <0.1 Gy or 1 or 2 particle tracks for high LET radiation. The aims of the project are to assess if the mechanisms of processing of certain types of radiation DNA damage, induced against a **high** background level of endogenous oxidative damage, are dependent on dose and thereby the extent of damage. Secondly we question if the mechanisms of rejoining of DSB induced by  $\gamma$ -radiation or  $\alpha$ -particles are different due to the damage complexity. Thirdly are the contributions of DSB rejoining by non-homologous end-joining (NHEJ) and homologous recombination (HR) modulated by dose and radiation quality?

An early step in the response of mammalian cells to radiation induced DSBs is the substantial phosphorylation of the histone H2AX ( $\gamma$ -H2AX) at sites of DNA DSBs. p53 Binding Protein 1 (53BP1), a protein proposed to be involved in the repair of DSB, has also been shown to localise to sites of radiation induced DNA DSBs. These foci co-localise with those of H2AX phosphorylation ( $\gamma$ -H2AX) which is required for the formation of 53BP1 foci at sites of DSB. The activation and foci formation of these proteins can be utilised to visualise the dose dependence for radiation induced DNA DSBs down to low doses using immunofluorescence.

Using immunofluorescence we have investigated the induction and repair of radiation induced DNA DSBs in exponentially growing, wild-type hamster cells (V79-4) and human fibroblast cells (HF19). We have assessed the effects of radiation quality on the induction and rejoining of radiation induced DNA DSBs with time, at a range of doses, using  $\gamma$ -H2AX and 53BP1 foci as early cellular markers of DSB formation.

A linear induction of  $\gamma$ -H2AX and 53BP1 foci in V79-4 cells was observed with doses in the range 20-2000mGy for  $\gamma$ -irradiation or 250-2000mGy for  $\alpha$ -irradiation. The loss of  $\gamma$ -radiation induced  $\gamma$ -H2AX foci follows the dynamics of rejoining of DSBs as demonstrated by PFGE; in contrast to different time course for loss of radiation-induced 53BP1 and  $\gamma$ -H2AX foci following a  $\gamma$ -radiation dose of 1 Gy where 50% of foci remain ~1 h and 3.5 h post irradiation for  $\gamma$ -H2AX and 53BP1 respectively. Radiation-induced 53BP1 foci reach maximal levels ~30-60 min post  $\alpha$ - or  $\gamma$ -irradiation and increased levels of 53BP1 foci were seen to persist at 24 h following  $\alpha$ -irradiation, possibly reflecting the increased complexity of DNA DSB damage. Following either  $\alpha$ - or  $\gamma$ -irradiation, 53BP1 and  $\gamma$ -H2AX foci co-localise, peaking at ~30 min post irradiation. Very few co-localised foci were detected in controls

indicating that co-localised foci are radiation-induced; consistent with the involvement of  $\gamma$ -H2AX in the recruitment of 53BP1 to sites of DNA DSB. The question arises as to whether the more complex DNA damage requires additional pathways to processes the damage prior to rejoining.

Non-homologous end-joining (NHEJ) and homologous recombination (HR) are the main pathways responsible for rejoining of the majority of DNA DSBs. NHEJ is active throughout the cell cycle and a key protein involved in this pathway is the protein kinase DNA-PKcs, which is phosphorylated when recruited to radiation-induced DSB. To date, DNA-PKcs foci have been detected using doses  $>2$ Gy however it is unknown if these foci can be visualised when lower doses of irradiation are used. We have investigated whether DNA-PKcs foci formation can be utilised to investigate NHEJ following low dose irradiation. We have preliminary indications of a dose-dependent increase in DNA-PKcs foci in exponentially growing HF19 human cells. These foci were detected following 100mGy of  $\gamma$ -irradiation, indicating that DNA-PKcs foci can be used to examine the role of NHEJ at low doses. Furthermore these foci co-localised with  $\gamma$ -H2AX so that DNA-PKcs can be used to follow NHEJ processing of DNA DSBs in contrast to RAD51 for HR.

We are presently developing approaches to be able to follow the rejoining of DSB at low doses and determine the phosphorylation levels of various proteins in different phases of the cell cycle.

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