Convergence of DNA repair and cell-cycle checkpoint control

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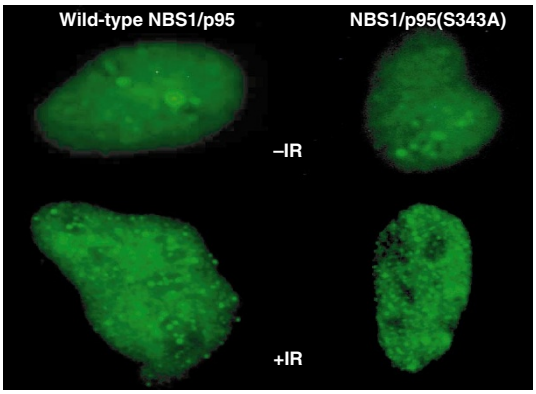
Eukaryotic cells have two pathways for DNA repair - the first, homologous recombination, depends upon the presence of homologous DNA, such as sister chromatids, whereas the second, non-homologous end joining, is capable of fusing any broken DNA ends. Deficiencies in a cell's ability to carry out DNA repair can cause susceptibility to cancer. Two human DNA-repair syndromes that fall under this umbrella are ataxia telangiectasia (AT) and Nijmegen breakage syndrome (NBS). In the mid-to-late 1990s, the genes responsible for these disorders were identified as *ATM* and *NBS1*, respectively. The ATM protein has been shown to be a serine/threonine kinase, but the function of NBS1/p95 is less clear. In a recent issue of *Nature* (**404,** 613–617; 2000), Lim and colleagues report the finding that the products of ATM and NBS1 are in fact sequential components of a pathway that repairs DNA damage caused by ionizing radiation.

A downstream target of the ATM pathway is the p53 tumour-suppressor protein. Lim and colleagues found that ionizing-radiation-dependent activation of p53 is unaffected in NBS cells, indicating that NBS1/p95 may not act upstream of ATM. Irradiation of normal cells resulted in phosphorylation of NBS1/p95; this was abrogated in AT cells, but not in cells lacking DNA-PKcs, a kinase involved in non-homologous DNA repair. The ability of ATM to directly phosphorylate NBS1/p95 was therefore examined. One site in the NBS1/p95 sequence, S343, was found to be phosphorylated by ATM *in vitro*, which is consistent with the finding that this site is phosphorylated *in vivo* in response to ionizing radiation.

The obvious next question is this – what effect does phosphorylation of NBS1/p95 have on its function? NBS1/p95 forms a complex with hMre11 and hRad50 that is thought to be involved in the identification and repair of DNA double-strand breaks. Formation of this complex can be followed by the appearance of foci after cells are irradiated. Rather disappointingly, in both AT cells and cells containing the NBS1/p95(S343A) phosphorylation mutant, focus formation was



not affected (see picture; IR, ionizing radiation).

One more property of NBS cells remained to be tested, however. In normal cells, exposure to ionizing radiation induces S-phase arrest, but in both AT and NBS cells the S-phase checkpoint is lost, resulting in 'radioresistant' DNA synthesis in the presence of DNA damage. Cells expressing the NBS1/p95(S343A) mutant also exhibited reduced S-phase arrest, indicating that phosphorylation of NBS1/p95 may be required for maintenance of the S-phase checkpoint.

These results contribute to the increasingly complex model of how ATM controls different checkpoints – its regulation of p53 phosphoryation influences G1 arrest; its phosphorylation of NBS1/p95 regulates S-phase arrest; and it may also inhibit the G2/M transition through interactions with hChk2 kinase. The findings of Lim and colleagues offer an intriguing glimpse of the function of the NBS1-hRad50-hMre11 complex in the control of cell-cycle checkpoints, although the details of its activity remain to be revealed.

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genesis of the upper longitudinal interface (modelled on that of α/β-tubulin) should disrupt nucleation if the template model is correct (but it may also affect interactions predicted by the protofilament model, as there is one important γ–α-tubulin longitudinal bond; Fig. 2). Mutagenesis of the 'left' lateral interface should disrupt nucleation only in the protofilament model.

Moritz *et al.*[7] have generated a tomographic reconstruction of the γTuRC, showing a dome-like cap. They have also generated tomographic reconstructions of microtubules nucleated by the γTuRC and showed that these have a dome or cap-like structure at one end, which they suggest is the γTuRC. From these results, they propose that the outer circular framework is defined by γ-tubulin subunits, whereas the dome is formed by associated proteins. Similar cap-like microtubule ends have been shown by Keating and Borisy and Wiese and Zheng. All three groups argue that the rounded end of the microtubule corresponds to the cap-like structure of the γTuRC. The original illustration of the protofilament model showed the helical γTuRC hanging off the minus end, attached by a short protofilament insertion into the microtubule. In this situation, the extending γTuRC could serve to attach the nucleated microtubule to the centrosome. However, these three electron-microscopic studies show no evidence of γTuRC spirals hanging off the end. A problem now for the protofilament model is to explain how γ-tubulin and its associated proteins wrap around the microtubule end to form the cap-like structure.

One clear distinction between the two models is in the orientation of the subunits in the γTuRC relative to their orientation in the microtubule (Fig. 1). In the template model, the outside of the γTuRC corresponds to the outside surface of the microtubule, whereas in the protofilament model, the opposite is the case; the surface corresponding to the outside of the microtubule faces the centre of the γTuRC. This is analogous to α/β-tubulin rings, which curl away from the microtubule, leaving the outside surface facing the centre of the ring[5].

Moritz and colleagues have obtained images of γTuRCs labelled with an antibody against the C-terminal peptide of γ-tubulin. They did not extend their interpretation to the orientation of the γ-tubulin subunits, but I believe that such an interpretation can be proposed. The last 19 amino acids of α-tubulin and β-tubulin are not visible in the atomic model[13], but the preceding amino acids are present at the 'front' (outside) surface of the microtubule. The C-terminal peptide may be disordered but is probably confined to this front surface, where it can serve as a binding site for microtubule-associated proteins (MAPs). Sequence similarity between β-tubulin and γ-tubulin extends into the beginning of the peptide that was used to raise the antibody, so it is likely that this antibody labels the outside surface of the microtubule. Moritz and colleagues found that the position of the antibody in the images was unequivocal, stating that "the outer diameter of labelled γTuRCs did not increase; instead, the lumen of the ring seemed to be partially filled with antibody". This labelling pattern matches exactly the prediction of the protofilament model and seems to be inconsistent with the template model.

The three articles in this issue proclaim the demise of the protofilament nucleation model, but in fact this model only requires

