Meeting report

DNA-damage signaling and apoptosis

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A report on the 674th meeting of the Biochemical Society, Dublin, Ireland, 11-12 July 2001.

In the host colloquium of this meeting, entitled 'DNA damage signaling and apoptosis', on which I will focus, speakers presented many approaches, ranging from yeast and mouse genetics, through biochemistry and genomics, to chemistry and structural biology, in a multidisciplinary attempt to enlighten this area of research. The multidisciplinary nature of this topic is not surprising when you consider that it is linked not only to DNA repair, cell-cycle control and growth arrest but also to transcription, telomere biology, immunology, development and even aging.

Steve Jackson (Wellcome/CRC Institute, Cambridge, UK) initiated proceedings by emphasizing the close relationship that is now becoming apparent between DNA-repair and DNA-damage signaling pathways, particularly in yeast. The tripartite yeast Mre11 complex, composed of Rad50 and Xrs2 in addition to Mre11, binds to double-stranded DNA breaks (DSBs) and is required for two DSB-repair pathways. It is therefore in an ideal position to function as a sensor of this particular DNA lesion. Consistent with this hypothesis, the normal cell-cycle delay (or checkpoint) that occurs in S phase when cells that are replicating DNA encounter a DSB is partially defective in *mre11* mutant cells. Developing this theme, I presented evidence for a general role for the Mre11 complex in all checkpoints, namely those in G1, S and G2/M phases of the cell cycle, after treatment of yeast cells with DSB-inducing agents. Both Jackson and I speculated that the Mre11 complex may function as a damage sensor that could amplify the checkpoint signal.

RAD9, the prototypical checkpoint gene believed to function in sensing DNA damage, also has roles in amplifying the checkpoint signal. I presented evidence that one form of the Rad9-containing protein complex functions like a solid-state catalyst: binding of inactive Rad53 molecules to phosphorylated residues on Rad9 facilitates their conversion to active forms of the Rad53 protein kinase. Yossi Shiloh (Tel Aviv University, Israel) referred to the critical role played by the Atm protein kinase in human cells in "sounding the DSB alarm". *ATM* is the gene mutated in the human DNA-repair-defective condition ataxia-telangiectasia. Indeed, as essentially all cellular responses to DSBs are defective in $ATM^{-/-}$ mutant cells, Atm must function upstream of all these responses; this conclusion has been reinforced by Shiloh's gene-profiling studies that demonstrated *ATM*-dependent regulation of genes involved in DNA repair and checkpoint regulation. These studies also emphasized a role for *ATM* in many other pathways. In fact, *ATM* appears to have multiple additional roles in cellular homeostasis, ranging from hormone and growth-factor regulation to membrane ruffling.

The principal downstream cellular consequence of DNA damage that the meeting focused on was apoptosis. Seamus Martin (Trinity College Dublin, Ireland) described the use of a Jurkat (T-cell) *in vitro* system to dissect the 'nuts and bolts' of caspase activation. Activation of these cysteine/aspartic acid-cleaving proteases (of which 14 mammalian family members are currently known) is triggered by many stimuli, including DNA damage; once caspase activation is triggered, the cell is irreversibly committed to cell death, whereas 'bystander' cells are spared. In this system, caspase 9 is the 'apical' caspase (the first in the cascade), being activated when cytochrome c is released from the mitochrondria. Cytochrome c binds the apoptosis-activating factor 1 (Apaf1) protein, leading to oligomerization of Apaf1 and caspase 9 into a large 'apoptasome', which then initiates a cascade of caspase activation. Although some non-caspase targets of caspase activation are known, the consequences of proteolysis of these targets are not well understood. Similarly, the events upstream of activation of the caspase cascade in response to DNA damage are not well known; in particular, it is not clear what regulates the decision to

undergo apoptosis or to arrest cell proliferation and repair the damage.

Jean Wang (University of California, San Diego, USA) addressed this question in the mouse and presented evidence of a role for the retinoblastoma (*RB*) tumor-suppressor gene and the c-*abl* proto-oncogene in this process. Rb protein is an inhibitor of c-Abl, which in turn is an activator of p73 (a homolog of the well-known tumor suppressor p53); p73 functions in parallel with p53 to contribute to apoptosis. Thus, to efficiently trigger apoptosis, Rb must be inactivated; this can be achieved by viral oncoproteins, by mutations in Rb, by phosphorylation of cell-cycle proteins (for example, once phosphorylated in S and G2 phase, c-Abl is released and can activate p73) or by proteolysis by caspases. These observations raise an interesting conundrum. Why should a proto-oncogene control apoptosis when apoptosis is an obvious tumor-suppression function? The answer is that, unlike c-Abl which continuously shuttles between the nucleus and the cytoplasm, oncogenic forms of Abl, such as Bcr-Abl, are exclusively cytoplasmic. If Bcr-Abl is trapped in the nucleus, it activates apoptosis. Essentially, an oncogene has thereby been converted into a tumor suppressor. Furthermore, this observation establishes the principle that the decision to undergo apoptosis can be regulated by the subcellular localization of c-Abl.

The cellular responses to DNA damage are clearly of great interest as possible targets for anti-cancer strategies. Both Marion Boland (University College Dublin, Ireland) and William Beck (University of Illinois at Chicago, USA) focused on topoisomerase inhibitors. Boland described how inhibitors such as mitoxantrone result in the activation of the transcription factor NFκB in a way that is dependent on topoisomerase and DNA damage. It is still not known, however, how components of the DNA-damage-response pathway interact with the NFκB pathway. The use of gene-array technology to identify differential gene regulation after treatment of cells with topoisomerase inhibitors was commented on by Beck: given the sheer number of genes that are either upregulated or downregulated, it is difficult to decide on the ones on which to focus. Beck also reported an enhanced interaction between topoisomerase IIα and Rb caused by some inhibitors, resulting in relocation of topoisomerase IIα to the nuclear periphery and activation of the c-Jun N-terminal kinase (JNK), followed by apoptosis.

Intriguingly, Doug Green (La Jolla Institute for Allergy and Immunology, San Diego, USA) reported on a role for p53 as an anti-rheumatoid-arthritis factor, which suggests that it might perform a similar role in other hyper-proliferative diseases. Rheumatoid arthritis occurs when hyperplastic tissue invades and destroys joints. Reactive oxygen species, released at joints as a result of inflammatory damage, should normally result in p53-dependent apoptosis of synoviocytes (joint cells). Cells from the joints of rheumatoid arthritis patients have mutations in p53, however, suggesting a requirement for p53 in the efficient removal of hyperplastic tissue. Crucially, in a mouse model of rheumatoid arthritis, absence of p53 results in worse arthritis.

Studies of DNA-damage signaling and apoptosis are clearly entering an exciting phase. Future work will no doubt result in more detailed mechanistic understanding of the pathways involved as well increased understanding of what determines the central decision of whether to die or not to die. This is clearly cell-type-specific, and most probably changes during development. Finally, improved therapeutic strategies will eventually result from our increased understanding of the DNA-damage response.