HIGH-LIGHTS

APOPTOSIS

Wreck removal

Cells cope with DNA damage by repairing the damage, using sophisticated repair mechanisms, or by eliminating the damaged cell altogether. Several pro-apoptotic proteins of the Bcl2 family are known to function in DNA-damage-induced apoptosis. They transmit signals, by still-unknown mechanisms, from the nucleus to the mitochondria, causing the release of apoptogenic molecules, including cytochrome *c*. In *Cell*, Tsujimoto and colleagues now report the identification of an unexpected new player in DNA-damage-induced apoptosis – the linker histone H1.2.

Taking a biochemical approach, Tsujimoto and co-workers fractionated the cytosol of thymocytes from X-ray-irradiated rats and assayed for cytochrome *c* activity by incubating the cytosolic fractions with isolated mitochondria. They purified a 34-kDa protein, which was identified as rat H1.2, and confirmed that H1.2, but not any other H1 isoforms, has strong cytochrome-*c*-releasing activity.

To gain insight into the mechanism of H1.2-induced cytochrome *c* release, Tsujimoto and colleagues examined the role of Bak, a pro-apoptotic Bcl2 family member. Using an anti-Bak antibody specific for activated Bak, they showed that H1.2 caused the activation of Bak. And the mitochondria of Bak-deficient mice were relatively resistant to H1.2-induced cytochrome *c* release, which indicates that Bak activation is required for cytochrome *c* release.

H1.2 and other H1 isoforms are normally not detected in the cytosol but, as the authors show, accumulate in the cytosol after X-ray irradiation. Given the essential role of p53 in DNA-damage-induced apoptosis in many cell types, Tsujimoto and co-workers tested whether the cytoplasmic increase of H1.2 is p53 dependent. It is indeed, as no increase in H1.2 was seen in the cytosol of thymocytes from irradiated $p53^{-/-}$ mice. The irradiation-induced increase in cytosolic H1.2 levels was inhibited in cells treated with leptomycin B – an inhibitor of nuclear export. So, the cytoplasmic accumulation of H1.2 seems to be due to increased nuclear release, rather than new synthesis in the cytoplasm.

Using an antisense approach to reduce H1.2 expression, Tsujimoto and colleagues found that, after X-ray irradiation, cytochrome *c* release was prevented and apoptosis inhibited. In addition, they showed that reduced H1.2 expression had no effect on UV-induced cytochrome *c* release and apoptosis, or on other forms of apoptosis, including p53-induced apoptosis. There was one exception though – *H1.2*-antisense-DNA-transfected cells showed increased resistance to treatment with etoposide (an inhibitor of topoisomerase II) that also causes double-stranded DNA breaks. These findings were confirmed using H1.2-deficient mice, which were resistant to X-ray-induced and etoposide-induced apoptosis. So, the authors concluded that H1.2 is specifically involved in apoptosis induced by DNA double-stranded breaks.

But how does H1.2 monitor DNA damage and signal apoptosis? Although it's too early to answer this question, the subcellular localization of H1.2 and a link with the Bcl2-family protein Bak might provide two important clues to the underlying mechanism. One priority in future studies will be to identify the domain of H1.2 that mediates its signalling function.

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H1.2
http://ca.expasy.org/cgi-bin/niceprot.pl?P15865

Bak
http://ca.expasy.org/cgi-bin/niceprot.pl?O08734

p53
http://ca.expasy.org/cgi-bin/niceprot.pl?P02340

References and links

ORIGINAL RESEARCH PAPER Konishi, A. *et al.* Involvement of histone H1.2 in apoptosis induced by DNA double-strand breaks. *Cell* **114**, 673–688 (2003)