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Previews

The Secret Life of Histones

Recent evidence reveals an unexpected role for the linker histone H1.2 in DNA damage-induced apoptosis. DNA double strand breaks induce translocation of nuclear H1.2 to the cytoplasm, where it promotes release of cytochrome c from mitochondria by activating the Bcl-2 family protein, Bak.

DNA damage presents a particularly acute threat to long-lived multi-cellular organisms because of the potentially calamitous consequences of genetic alterations such as cancer. One important line of defense lies in the multitude of repair mechanisms which correct damage and maintain genome stability. However, because complex organisms such as mice and men have the luxury of excess capacity in terms of individual cells, an alternative and more certain strategy is to eliminate all risk by extirpating the damaged cells through apoptosis. Dedicated specialists like p53 clearly play a vital role in this ruthless failsafe process (Oren, 2003). But more recent evidence suggests that some less glamorous components of the cell, such as histones and ribosomal proteins, also have the capacity—in addition to their better-known day jobs—to monitor damage and mediate apoptosis and stress responses.

In a recent development of this theme, Konishi and colleagues demonstrate a role for the linker histone H1.2 in triggering apoptosis in response to DNA damage (Konishi et al., 2003 [this issue of *Cell*]). The route to this surprising discovery was via biochemistry; cytosol from irradiated rat thymocytes was fractionated and assayed for induction of cytochrome c release from purified mitochondria in vitro. Remarkably, the most potent activity detected was due to H1.2, a conclusion confirmed using recombinant H1.2 protein. This activity of H1.2 was highly specific; other histone H1 isoforms were either inert or only very weakly active. Although such in vitro assays are obviously only surrogates for "real" cell death, the authors subsequently demonstrate a requirement for H1.2 for optimal apoptotic responses to DNA damage in vivo using both antisense RNA and gene disruption approaches to deplete H1.2 expression in living cells and mice. Both thymocytes and cells in the small intestine in H1.2-deficient mice show remarkable resistance to killing by X-rays. Thus, H1.2 emerges as something of a Jekyll and Hyde character—ordinarily an innocuous (and non-essential) component of chromatin, but capable of transforming into a killer.

So how does H1.2 trigger release of cytochrome c from mitochondria? A link with proapoptotic Bcl-2 family members and/or the outer mitochondrial membrane appears to hold the key. H1.2 induces conformational activation and oligomerization of Bak, and mitochondria from Bak-deficient mice were relatively resistant to the effects of H1.2, suggesting that Bak activation is required for cytochrome c release. Remarkably, H1.2 was as potent an activator of Bak as the BH3 domain-only protein Bid, while cytochrome c release by H1.2 was blocked by the anti-apoptotic Bcl-2 family protein Bcl-xL. Beyond this, the underlying mechanism remains obscure; unlike Bid, H1.2 lacks a BH3 domain and direct interactions with Bcl-2 family members were not detected. The authors speculate that H1.2 might activate Bak by disrupting the outer mitochondrial membrane directly; however, at present there is no evidence to support this mechanism. Perhaps all will become clear when the relevant domain(s) of H1.2 are mapped and compared with related but nonapoptotic histone H1 isoforms.

Since DNA damage does not lead to any change in the overall level of H1.2 expression, the next obvious question is; how is its apoptotic function activated? As one might predict for a nuclear protein which acts on mitochondria, the answer seems to lie in subcellular localization. While H1.2 is confined to the nucleus in undamaged cells (except perhaps during mitosis), irradiation induces a leptomycin B-sensitive accumulation of the protein in the cytoplasm. Exactly how this occurs is unclear. Numerous posttranslational modifications of histones—including phosphorylation, acetylation, methylation, and ubiquitination—have been shown to affect histone function (Spencer and Davie, 1999). However, H1.2 itself does not undergo any obvious posttranslational modification as a result of DNA damage (nor are such modifications required for mitochondrial cytochrome c release in vitro), and the nuclear and cytoplasmic forms are indistinguishable. Presumably DNA damage dislodges some H1.2 from chromatin, perhaps as a result of physical remodelling or damage-induced modification of other chromatin components. This dislodged population is then available for export from the nucleoplasm by an active process which may itself be stimulated by damage.

A final fascinating, if perplexing, snippet is that relocalization of H1.2 from the nucleus to the cytoplasm is p53 dependent. Why this should be is unclear. Perhaps p53-mediated DNA repair is involved in displacing H1.2 from chromatin. Alternatively, given that p53 itself undergoes active nucleo-cytoplasmic shuttling (Liang and Clarke, 2001), p53 could conceivably play a more direct role in regulating H1.2 translocation after damage. So does this relationship extend to a functional collaboration? The answer to this is—perhaps. In overexpression experiments, p53 showed no obvious dependence on H1.2. Furthermore, activation of the apoptotic target genes Bax, Perp, and Puma by endogenous p53 occurs normally in H1.2-depleted cells after irradiation. However, cell death is attenuated in such cells, suggesting that p53 and H1.2 may cooperate under certain circumstances. Clearly further research is needed to unravel the details.

Regardless of the precise mechanism, the clear implication is that H1.2 is inherently proapoptotic if it reaches the cytoplasm, but that this is normally prevented by sequestration of the protein in the nucleus through incorporation into chromatin. This may explain why H1.2

only contributes to apoptosis in response to certain damaging agents, such as X-rays and etoposide, which induce mainly DNA double-strand breaks and thus extensive chromatin remodeling, but has little or no effect on cell death induced by UV (which results mainly in base modification) or non-genotoxic agents such as tumor necrosis factor and staurosporine. In essence the authors are suggesting that the level of cytoplasmic H1.2 could provide the cell with a means of measuring the scale of certain forms of DNA damage.

Viewed from this perspective, H1.2 may be acting in a manner analogous to that recently suggested for the ribosomal protein L11, which has the potential to activate p53 in response to ribosomal stress (Lohrum et al., 2003; Zhang et al., 2003). It has been proposed that perturbations of normal ribosome assembly lead to the release of free L11 which then binds MDM2 and inhibits p53 degradation. Although the circumstances under which this response is triggered are unclear, it appears that specific functional or structural components of cellular organelles are also sometimes capable of acting as effectors of apoptosis or stress signaling. Perhaps this strategy enables cells to mount appropriate biological responses to the widest range of adverse conditions. Interestingly, recent studies provide evidence that histones can also be important for the faithful repair of damaged chromosomes. Mice deficient for H2AX exhibit defects in the efficiency and/or fidelity of DNA double strand break repair which, in the absence of p53 function, leads to massive genetic instability and a dramatically increased incidence of cancer (Bassing et al., 2003; Celeste et al., 2003). The two responses to DNA damage—repair or death—seem almost diametrically opposed, but both are likely to contribute to the inhibition of tumor development. H2AX has already been shown to exhibit the properties of a tumor suppressor and, although H1.2-deficient mice are developmentally normal (Fan et al., 2001), it will be interesting to determine whether these mice show any similar predisposition to cancer.

An emerging theme therefore is that at least some supposedly workaday components of the cell actually play a vital role in monitoring and signaling various forms of damage and abnormalities in partnership with professionals like p53. The evolution of this strategy certainly makes sense—who better to detect a crack in the mine than the workers at the pit face? Like Superman, maybe many unassuming housekeeping proteins lead secret lives as guardians of cellular life and death.

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Amino Acids and the Humoral Regulation of Growth: Fat Bodies Use Slimfast

The mechanisms by which animals coordinate the growth of different tissues in response to nutrient levels is poorly understood. In this issue of *Cell*, Columbani et al. demonstrate that amino acid-responsive TOR signaling in the *Drosophila* fat body modulates insulin signaling and growth in peripheral tissues.

The ability to sense and respond to nutrients is vital during development. Yeast cells monitor the levels of amino acids and other nutrients via the protein kinase TOR and alter their growth accordingly. This cell-based nutrient-sensing mechanism has been retained through evolution. For example, if cultured mammalian cells are transferred to amino acid-free medium, they immediately suppress TOR signaling and protein synthesis. However, multicellular organisms also need to organize their growth globally via humoral mechanisms so that their component tissues can grow to the required overall size and shape. In an article published in this issue of *Cell,* Pierre Léopold and colleagues report that in addition to sensing nutrients and controlling growth in individual cells, *Drosophila* TOR (dTOR) can influence peripheral tissue growth via a humoral mechanism (Columbani et al., 2003).

The larvae of the fruit fly *Drosophila* have been used extensively to study growth regulation. The bulk of the *Drosophila* larva is made up of endoreplicating tissues (ERTs) that are histolyzed during the pupal phase to support metamorphosis. During metamorphosis, mitotic tissues known as imaginal discs are reorganized into the adult fly. The *Drosophila* insulin signaling pathway has been shown to promote growth in both these tissue types.

One ERT that is particularly sensitive to nutrients is the fat body. This tissue alters its morphology dramatically in response to amino acid starvation (Britton and Edgar, 1998). Several earlier observations have suggested that the fat body can modulate the growth of