Minireview

Is the 65 kDa protein a direct signal for the nuclease release from nuclear matrix, starting the apoptotic cascade ?

Jan Szopa and Rajmund Adamiec

Institute of Biochemistry, University of Wrocław, S. Przybyszewskiego 63, 50–148 Wrocław, Poland

The accumulated data [1] clearly suggest that apoptosis plays a crucial role in physiological and pathological processes as diverse as embryogenesis, haemopoiesis and neoplasia. A wide variety of physical and chemical stimuli induce apoptosis. Though the molecular mechanism of selection of the biochemical pathway of cell death is not fully understood it is becoming clear that the death programme could be switched on by an independent initiator and mediated by a wide variety of messengers and second messengers.

The first irreversible and rate limiting step shared by all cells undergoing apoptosis is stimulation of nuclease activity leading to internucleosomal DNA degradation. It is not known whether this is due to the expression of the nuclease gene or to the activation of the constitutively expressed nuclease gene product. It is suggested that nuclease activation results from the release of the enzyme from nuclear matrix.

In a great variety of cell systems, the primary proliferating and differentiating cells die after a limited number of cell divisions depending upon the cell kind and its age. The exception are the transformed cells, practically immortal, unable, however, to undergo differentiation. Like other major processes in cell biology, cell death is initiated by perception of defined stimuli which leads to a sequence of intracellular changes and culminates in final events. Re-cent studies lead to the conclusion that two major processes in cell death can be discerned. The first, called necrosis, involves unrepaired damage to the cell membrane. In the second, referred to as apoptosis, prominent nuclear changes occur. Both these types of death were initially distinguished on morphological grounds, but recently the intracellular molecular background has been evidenced.

The data accumulated strongly suggest that apoptosis is under intracellular control, thus providing a good system for studying molecular events of the death process [1, 2].

The characteristic features of apoptotic cells

Compared to necrotic death apoptosis, recently defined as a gene-directed or programmed cell death, is a relatively slow process and involves a cascade of regulated events. It appears to proceed by a complex, multistep pathway. The cells undergoing apoptosis within tissues lose contact with their neighbours. Apoptosis of lymphocytes is characterized by nuclear condensation, DNA fragmentation and formation of the chromatin-containing membranous vesicles called apoptotic bodies [3, 4]. These bodies are usually the targets of phagocytosis by the neighbouring cells. Alternatively, apoptotic bodies may be liberated into the lumen of epithelium-lined cavities. In the cytoplasm, organelles become aggregated, but initially do not lose their structural integrity.

However, it is characteristic that the synthesis of mitochondrial ATP is decreased.

It should be pointed out that apoptosis depends upon RNA and protein synthesis [3, 5] in lymphocytes treated with glucocorticoids, and in thymus when cell death was induced by perturbation of the T-cell receptor complex or by deprivation of interleukin-3.

The signals initiating apoptosis

The programmed cell death occurs in a number of physiological circumstances including embryonic development, clonal selection, in response to hormonal stimuli and in the process of T cell killing. The well-defined zones of apoptosis which appear at predictable periods of the development of bird wings can serve as an examples, since these zones are responsible for deletion of interdigital webs. Certain avian mutants have genetic lesions associated with abnormally either low or high levels of apoptosis during development of the wing. This results respectively, in clumsy paddle-shaped wing or development of only vestigial wing tissue (for review see [6]).

The developing nervous system provides another example of the significant role of apoptosis (for review see [7]). Motor neurons are initially generated in excess in the spinal cord and their number falls sharply during the few days when neuromuscular connections are established. It appears that all the motor neurons are doomed to die at this time, unless they receive a signal from the associated muscle.

One of the most important signal comes from glycoproteins which activate self-destructive pathway of programmed cell death manifested in lymphoma cell lines and thymocytes in the afore mentioned changes (for review see [8]).

Activation of nucleases seems to be the first common step in the apoptosis cascade induced by various agents

It is clear that cells select their own apoptotic pathway which depends both, on the cell type and the agent inducing the programmed cell death. Thus, it was observed that apoptosis induced by glucocorticoids involving the glucocorticoid receptor [9, 10] does not share the cAMP-induced cell death which appeared to be initiated by activation of the cAMP-dependent protein kinase A and is independent of the calcium-binding protein gene expression [11 -

14]. However, in most cases the onset of apoptosis is manifested by an early induction of the calcium-binding protein synthesis.

There is, however, an event shared by different cell death pathways and this is a nuclease induction, resulting in chromatin cleavage at internucleosomal sites. The activation of DNA cleavage precedes cell death. Nuclease induction results in generation of a series of DNA fragments appearing in 180 - 200 base pairs increments. In case of glucocorticoid treated cells, this internucleosomal DNA degradation can be blocked by the inhibitors of transcription and translation [15]; hence, apoptotic cells appear to be responsible for their own death.

Internucleosomally cleaved DNA is detectable in thymus shortly after glucocorticoid administration, prior to alterations in cell viability [3, 16]. It was shown that aurintricarboxylic acid, a well known nuclease inhibitor, blocked internucleosomal DNA cleavage and apoptosis induced by different agents. Based on the prevalent observations that internucleosomal degradation of DNA occurs in all the reported instances of apoptosis, it could be hypothesized that DNA degradation is the first irreversible process in programmed cell death.

Is the 32 kDa endonuclease involved in apoptotic cell death?

It was suggested that at least two nucleases with molecular mass of 18 kDa and 30 kDa were involved in apoptosis. The reported correlation between the occurrence of 18 kDa nuclease and DNA degradation made this protein a candidate for the apoptotic nuclease [16, 17].

The most intriguing question concerns the mechanisms by which apoptotic agents could activate internucleosomal DNA cleavage in intact cells. Two possibilities may be considered. First, induction of nuclease gene; and second, activation of the constitutive endogenous endonuclease. The increase in nuclease gene expression is rather unlikely since it was shown that, apparently, protein synthesis is required only in the case of the glucocorticoid-induced DNA cleavage. Moreover, it has been shown that the inhibitors of protein synthesis have no effect on activation of DNA cleavage in target cells by cytotoxic T-lymphocytes [17, 18]. It was also recently reported that cycloheximide, an

inhibitor of protein synthesis had no effect on the novobiocin-induced DNA cleavage in apoptotic lymphocytes [17]. These data suggest that activation of DNA cleavage is not mediated by transcriptionally induced endonucleases.

In this context we have proposed that activation of a constitutive endogenous endonuclease is a key event in the apoptotic cascade [8]. Our suggestion is consistent with the recent hypothesis of Alnemri & Litwack [17], who suggest the presence of inactive nuclease in intact cells in which chromatin is in compact form. They have also postulated that this endonuclease is able to cleave only that DNA region which becomes accessible.

We believe that the 32 kDa endonuclease purified in our laboratory is the best candidate for a significant role in the apoptotic cascade [19, 20]. The enzyme is mainly localized in nuclear matrix in proliferating or growth stimulated cells in which it is involved in DNA synthesis since IgG anti-nuclease inhibited incorporation of deoxyribonucleotides into trichloroacetic acid precipitable material [21]. The enzyme, when bound to nuclear matrix shows specificity towards the DNA structure. It introduces a single nick and only supercoiled DNA is a substrate for the enzyme [22]. On the contrary, the free enzyme cleaved DNA non-specifically [20]. We have proposed earlier [8] that the release of nuclease from nuclear matrix is a critical step in the apoptotic cascade. We do not yet know the mechanism by which the enzyme is released, we do know, however, that the 32 kDa enzyme is bound to nuclear matrix through a 65 kDa protein and that this polypeptide modulates the nuclease function. Thus, we hypothesize that apoptosis starts in nuclear matrix and the mutation in 65 kDa protein gene in its regulatory or open reading frame sequence could lead to its incorrect expression, nuclease release and initiation of irreversible chromatin cleavage.

It could be also suggested that mutation in the 65 kDa protein gene occurs in a tissue specific manner.

Our hypothesis is strongly supported by the results of the last findings that Bcl-2 protein, which protects various cells from apoptosis, is associated with nuclear envelope [23], and that in addition to Bcl-2, other proteins are involved in controlling cell death [24, 25].

REFERENCES

1. Wyllie, A.H. (1987) Cell death. *Int. Rev. Cytol. Suppl.* **17**, 755 - 785.
2. Wyllie, A.H., Kerr, J.F.R. & Currie, A.R. (1980) Cell death: The significance of apoptosis. *Int. Rev. Cytol.* **68**, 251 - 306.
3. Cohen, J.J. & Duke, R.C. (1984) Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. *J. Immunol.* **132**, 38 - 42.
4. McConkey, D.J., Nicotera, P., Hartzell, P., Bellomo, G., Wyllie, A.H. & Orrenius, S. (1989) Glucocorticoids activate a suicide process in thymocytes through an elevation of cytosolic Ca^{2+} concentration. *Arch. Biochem. Biophys.* **269**, 365 - 370.
5. Ishida, Y., Agata, Y., Shibahara, K., Honjo, T. (1992) Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J.* **11**, 3887 - 3895.
6. Hinchliffe, J.R., Griffiths, P.J. (1984) in *Cell ageing and cell death* (Davies, I. & Sigee, D.C., eds.) pp. 223 - 242, Cambridge University Press, Cambridge.
7. Oppenheim, R.W. (1984) Naturally occurring and induced neuronal death in the chick embryo *in vivo* requires protein and RNA synthesis: Evidence for the role of cell death genes. *Dev. Biol.* **101**, 35 - 39.
8. Rzepecki, R., Szmidziński, R. & Szopa, J. (1991) The role of endogenous endonucleases in programmed cell death. *Post. Biochem.* **37**, 18 - 23 (in Polish).
9. Huet-Minkowski, M., Gasson, J.C. & Bourgeois S. (1982) Glucocorticoid resistance in lymphoid cell lines; in *Drug and hormone resistance in neoplasia* (Bruchowsky, N. & Goldie, J.H., eds.) vol. 1, pp. 80 - 94, CRC Press, Boca Raton, Fla.
10. Bourgeois, S. & Newby, R.F. (1977) Diploid and haploid states of the glucocorticoid receptor gene of mouse lymphoid cell lines. *Cell*, **11**, 423 - 430.
11. Insel, P.A., Bourne, H.R., Coffino, P. & Tomkins, G.M. (1975) Cyclic AMP-dependent protein kinase: pivotal role in regulation of enzyme induction and growth. *Science*, **190**, 896 - 898.
12. Friedrich, U. & Coffino, P. (1977) Mutagenesis in S49 mouse lymphoma cells: induction of

resistance to onabain, 6-thioguanine and dibutyryl cyclic AMP. *Proc. Natl. Acad. Sci. U.S.A.* **74**, 679 - 683.

13. Dowd, D.R. & Miesfeld, R.L. (1992) Evidence that glucocorticoid and cyclic AMP-induced apoptotic pathways in lymphocytes share distal events. *Mol. Cell. Biol.* **12**, 3600 - 3608.

14. Dowd, D.R., McDonald, P.N., Komm, B.S., Haussler, M.R. & Miesfeld, R. (1991) Evidence for early induction of calmodulin gene expression in lymphocytes undergoing glucocorticoid-mediated apoptosis. *J. Biol. Chem.* **266**, 18423 - 18426.

15. Giannakis, C., Forbes, I.J. & Zalewski, P.D. (1991) Ca^{2+}/Mg^{2+}-dependent nuclease: tissue distribution relationship to inter-nucleosomal DNA fragmentation and inhibition by Zu^{2+}. *Biochem. Biophys. Res. Commun.* **181**, 915 - 920.

16. Gaido, M.L. & Cidlowski, J.A. (1991) Identification, purification and characterization of a calcium-dependent endonuclease (NUC18) from apoptotic rat thymocytes. *J. Biol. Chem.* **266**, 18580 - 18585.

17. Alnemri, E.S. & Litwack, G. (1990) Activation of internucleosomal DNA cleavage in human CEM lymphocytes by glucocorticoid and novobiocin. *J. Biol. Chem.* **265**, 17323 - 17333.

18. Sen, S. & D'Incalci, M. (1992) Apoptosis. Biochemical events and relevance to cancer chemotherapy. *FEBS Lett.* **307**, 122 - 127.

19. Rudnicki, R., Rzepecki, R. & Szopa, J. (1988) Rearrangement of the nuclease-inhibitor complex components within cells of White bush treated with phytohormone. *J. Plant Physiol.* **132**, 658 - 663.

20. Rzepecki, R., Szmidziński, R., Bode, J. & Szopa, J. (1992) The 65 kDa protein affected endonuclease tightly associated with plant nuclear matrix. *J. Plant Physiol.* **139**, 284 - 288.

21. Rzepecki, R., Bułaj, G. & Szopa, J. (1989) Endonuclease tightly associated with plant nuclear matrix affected DNA synthesis *in vitro*. *J. Plant Physiol.* **134**, 364 - 369.

22. Szopa, J., Backer, A.J., Sikorski, A.F. & Kozubek, A. (1993) Does the nuclear matrix endonuclease show specificity towards DNA topoisomers? *J. Plant Physiol.* **141**, 172 - 175.

23. Jacobson, M.D., Burne, J.F., King, M.P., Miyashita, T., Reed, J.C. & Raff, M.C. (1993) Bcl-2 blocks apoptosis in cells lacking mitochondrial DNA. *Nature (London)* **361**, 365 - 369.

24. Cuende, E., Ales-Martinez, J.E., Ding, L., Gonzalez-Garcia, M., Martinez, A.C. & Nunez, G. (1993) Programmed cell death by bcl-2-dependent and independent mechanisms in B lymphoma cells. *EMBO J.* **12**, 1555 - 1560.

25. Piacentini, M., Fesus, L. & Melino, G. (1993) Multiple cell cycle access to the apoptotic death programme in human neuroblastoma cells. *FEBS Lett.* **320**, 150 - 154.