Restructuring and Extrusion of Nuclear Ribonucleoproteins (RNPs) During Apoptosis

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Abstract. To investigate the fate of nuclear ribonucleoprotein (RNP) during apoptosis, we performed a cytochemical and immunocytochemical study of apoptotic mammalian cells by fluorescence and electron microscopy using specific antibodies which recognize different RNP-associated proteins. Light and electron microscopy showed that during apoptosis, nuclear RNPs are rearranged, with the formation of fibrogranular heterogeneous clusters which are extruded from the nucleus into the cytoplasm, and finally released at the cell surface, as apoptotic blebs. Restructuring and extrusion of nuclear RNPs apparently determine the arrest of RNP maturation, thus effectively blocking protein synthesis in apoptotic cells.

Key words: Apoptosis — Cytometry — Electron microscopy — Immunocytochemistry — Ribonucleoproteins (RNPs)

Apoptosis has been described years ago as a particular form of physiological cell death occurring via a stereotypic sequence of cytoplasmic and nuclear events (Wyllie et al. 1980, Kerr et al. 1994). Cytoplasm condensation and blebbing, and chromatin condensation into discrete granular masses, with the final formation of membrane-bounded apoptotic bodies, are the main morphological signs of apoptosis.

The nuclear events of this process have been widely investigated, with special reference to the (possibly) causal relationship between caspase-mediated proteolytic-endonucleolytic processes and chromatin condensation-fragmentation (Earnshaw 1995).

In contrary, the fate of nuclear RNP-containing structures during apoptosis has received much less attention until very recent years, although the amount of cellular RNA decreases in apoptotic cells, while RNA synthesis is often necessary for apoptosis to take place (Kroemer et al. 1995).

To investigate in detail changes in the composition and distribution of nuclear RNPs during apoptosis, we performed a cytochemical and immunocytochemical study by fluorescence and electron microscopy. The occurrence of apoptosis was monitored by morphological (Kerr et al. 1994) and cytometric criteria (Pellicciari et al. 1993). Specific monoclonal antibodies (MoAb) were used, which recognize different proteins associated to either the...
Figure 1. Immunodetection of hnRNPs in human EUE cells after serum deprivation. In non-apoptotic cells, FITC immunopositivity (a) was observed only in the nucleus, whereas in apoptotic cells (large arrow), hnRNPs were detected in the cytoplasm, sometimes in the form of discrete spots (small arrows). At electron microscopy (b), these cytoplasmic dots correspond to heterogeneous clusters of fibrogranular material where hnRNPs (gold grains) are associated to morphologically recognizable interchromatin granules (N = nucleus). c) Dual parameter flow scattergrams of DNA contents (after propidium iodide staining, in abscissa) versus FITC-immunopositivity for hnRNP proteins (ordinate) in sub-G1 apoptotic cells (arrow), the immunofluorescence values are lower than in non-apoptotic cells.

In fluorescence microscopometry, the immunolabeling for RNPs was lower in apoptotic than in non-apoptotic cells, with residual positivity persisting in nuclear areas of non-condensed chromatin, in apoptotic cells only, the immunolabeling was also observed in
the cytoplasm and in apoptotic blebs (Fig 1a). At electron microscopy, RNP containing structures (i.e., perichromatin granules, perichromatin fibers, interchromatin granules, and nucleolar components) were observed to segregate during apoptosis, in the interchromatin space and to cluster into heterogeneous aggregates of granules which we called HERDS (for Heterogeneous Ectopic RNP-Derived Structures Biggiogera et al. 1998). HERDS are extruded from the nucleus and finally released at the cell surface, as membrane-bounded fragments. RNP complexes within HERDS are always recognized by specific antibodies (Fig 1b), which demonstrates that their proteolytic degradation (if any) might be only partial. Flow cytomteric measurements of DNA content versus immunopositivity (Fig 1c) showed a decrease in RNP amount in apoptotic cells, likely due to the release of RNPs by cytoplasm bleeding.

This RNP restructuring (Pellicciari et al. 1996, Biggiogera et al. 1997a,b, 1998) should likely induce a severe impairment in protein synthesis during apoptosis.

Similar phenomena were observed to occur spontaneously in mouse spermiogenesis (Courtens and Loir 1981), and during erythrocyte maturation in the bone marrow (Biggiogera et al., in preparation), when a blockade of RNA synthesis takes place.

We hypothesize that segregation of RNPs into heterogeneous granule clusters could be a common feature of cells undergoing transcriptional arrest, either physiologically or as a consequence of exogenous stimulation.

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References:

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