Restructuring and Extrusion of Nuclear Ribonucleoproteins (RNPs) During Apoptosis

C Pellicciari, M G Bottone and M Biggiogera

Dipartimento di Biologia Animale and Centro di Studio per l'Istochimica del CNR, Piazza Botta 10, I-27100 Pavia, Italia

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

Correspondence address C Pellicciari, Dipartimento di Biologia Animale and Centro di Studio per l'Istochimica del CNR, Piazza Botta 10, I-27100 Pavia, Italia
E-mail pelli@ipv36.unipv.it

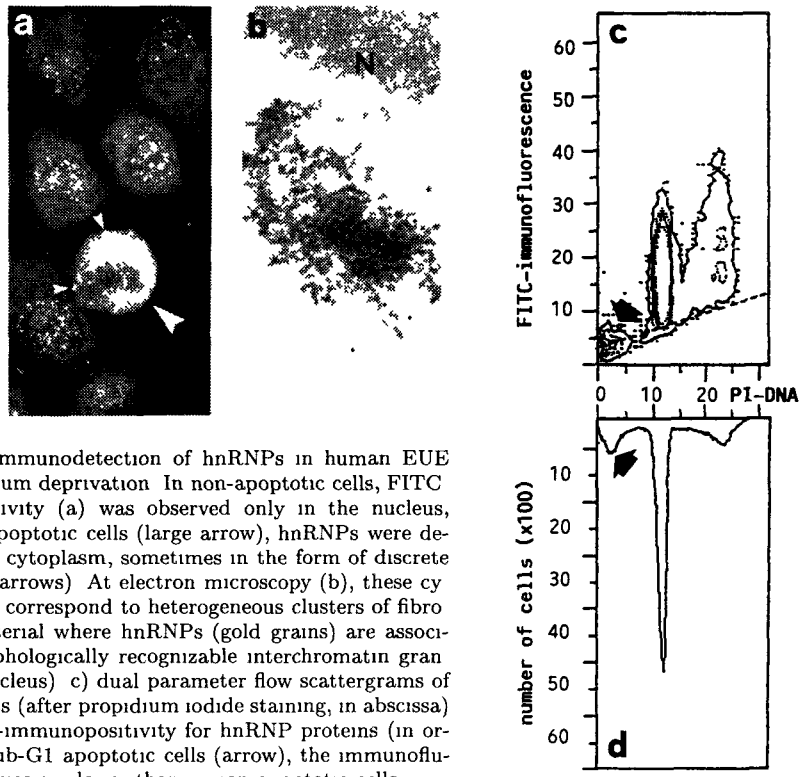

Figure 1. Immunodetection of hnRNPs in human EUE cells after serum deprivation In non-apoptotic cells, FITC immunopositivity (a) was observed only in the nucleus, whereas in apoptotic cells (large arrow), hnRNPs were detected in the cytoplasm, sometimes in the form of discrete spots (small arrows) At electron microscopy (b), these cytoplasm 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 (in ordinate) in sub-G1 apoptotic cells (arrow), the immunofluorescence values are lower than in non apoptotic cells

snRNP complexes, or the hnRNP core group polypeptides, or the nucleolus In particular we used a MoAb recognising the Sm antigen associated with U1, U2, U5 and U4/U6 snRNP complexes and a MoAb against hnRNP core group polypeptides (gift of Dr Terence Martin University of Chicago, USA), and a MoAb against the P0P1P2 proteins of the major ribosomal subunit (gift of Dr Toshio Uchiumi, Shinshu University, Japan) Dual-parameter flow cytometric measurements were performed of the cellular DNA content (after propidium iodide staining) versus the immunopositivity (using FITC-labelled secondary antibodies)

Different cell types were used as model systems (normal rat thymocytes and several mammalian cell lines EUE, human embryonic epithelium, HEP2, from a human larinx carcinoma, C6, from a rat glioma) In the case of thymocytes, apoptosis occurs spontaneously during the selection of the T cell repertoire, in addition, apoptosis was induced in vitro by serum deprivation, hypertonicity, or some antitumour drugs

For electron microscopy, a Zeiss EM900 was used operating at 80 kV and equipped with a 30 æm objective aperture Cytometric measurements were taken with a FACStar (Becton Dickinson) For fluorescence microphotography, a BX50 Olympus microscope was used

In fluorescence microscoscopy, 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 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 cytometric 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 blebbing

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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