Cytolocation of prosome antigens on intermediate filament subnetworks of cytokeratin, vimentin and desmin type

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Analysis by double-label indirect immunofluorescence of PtK1 and HeLa cells had previously demonstrated that prosome* antigens form networks that superimpose on those of the intermediate filaments of the cytokeratin type. We show here that in PtK1 cells various prosomal antigens also reside to a variable extent on intermediate filaments subnetworks of the vimentin type. In proliferating human fibroblasts the prosome and vimentin networks were found to coincide, while in proliferating myoblasts of the C2.7 mouse myogenic cell line the prosomal antigens seem to superimpose on the intermediate filaments of the desmin type. Thus, the prosomes, which are RNP particles of variable composition and subcomplexes of untranslated mRNP, and carry a multicatalytic proteinase activity, seem to co-localize with the specific kind of cytoplasmic intermediate filament in relation to the cell type. These results, which generalize the previous data, are discussed in view of possible role(s) for prosomes in mRNA metabolism and/or intermediate filaments remodelling.

Key words: prosome, multi-catalytic proteinase, MCP, proteasome, cytoskeleton, intermediate filament, cytokeratin, vimentin, desmin, RNP, mRNP, mRNA

INTRODUCTION

First observed as subcomplexes of untranslated messenger RNA in the cytoplasm of eukaryotic cells (Spohr et al., 1970; for review see Scherrer, 1990; Scherrer and Bey, 1994; Scherrer et al., 1988), prosomes are ubiquitous ribonucleoprotein particles (RNP) of about 720,000 relative molecular mass (Coux et al., 1992; Schmid et al., 1984) found in all eukaryotic cells, from yeast to human (Arrigo et al., 1987; Martins de Sa et al., 1986). These highly stable complexes are remarkably well-organized structures of uniform morphology (cylindrical particles of 12 nm diameter, about 17 nm long), composed of about 24-28 distinct proteins (Mr 20,000 to 36,000), some containing small RNA (70 to 120 nucleotides long) (Coux et al., 1992; Nothwang et al., 1992a). Interestingly, most of the prosomal proteins are highly conserved in evolution (Martins de Sa et al., 1986; Zwickl et al., 1992; Bey et al., 1993).

The precise physiological role(s) of prosomes is not clearly established, although several functions have been suggested (Goldberg and Rock, 1992; Scherrer, 1990). These particles were found to be components of repressed mRNP (Grainger and Winkler, 1987; Nothwang et al., 1992b; Schmid et al., 1984), which inhibit cell-free protein synthesis (Horsch et al., 1989; Kleinschmidt and Buhl, 1987) and are able to associate in vitro with viral mRNA (Horsch et al., 1990). The fact that disruption of some yeast prosomal protein genes is lethal strongly reinforces the notion that prosomes are involved in important cellular functions (Emori et al., 1991; Fujiwara et al., 1990; Heinemeyer et al., 1991).

To date, the best-characterized functional property of prosomes is their multicatalytic proteinase (MCP, ‘proteasomes’) activity (for review see Orlowski, 1990; Rivett, 1989) and their involvement in the ‘26 S proteasome’ responsible for the degradation of ubiquitinylated proteins (Eytan et al., 1989; Orlowski, 1990; Hersko and Ciechanover, 1992). Recent observations suggest, furthermore, the involvement of the prosome-MCP particle in the production of peptides presented as antigens within the major histocompatibility complex (MHC) at the cell surface to the T-lymphocytes (Brown et al., 1991; for review see Goldberg and Rock, 1992).

Biochemical and immunocytochemical studies on prosomes during embryogenesis and cell differentiation in various species (Martins de Sa et al., 1986; Ahn et al., 1991; Briane et al., 1992; Falkenburg and Kloetzel, 1989; Haass and Kloetzel, 1989; Pal et al., 1988; Yang et al., 1992) indicate that different

*We use here the term ‘prosome’ introduced by our laboratory (Schmid et al., 1984) for the then unknown particle and, when speaking of its protease activity, we use the term ‘multicatalytic proteinase or MCP’ according to the recommendation of the group of enzymologists concerned (Dahlmann et al., 1988; Orlowski and WiIk, 1988), in preference to the term ‘Proteasome’ suggested by Arrigo et al. (1988).
types of proosomes exist in multiple variants, on the basis of a variable subunit composition, and are probably implicated in different cellular processes. This hypothesis is strongly reinforced by the recent observation of a specific type of proosomes, the ‘LMP’ complex, which may be involved in antigen processing during the immune response (Brown et al., 1991; Yang et al., 1992).

In previous investigations on the cytolocalization of prosomal antigens, three particularly interesting observations were made: (1) proosomes were not only found in the cytoplasm but also to a variable extent in the nucleus (Gautier et al., 1988; Grossi de Sa et al., 1988a; Pal et al., 1988); this finding was confirmed by others (Arrigo et al., 1988; Haass and Kloetzel, 1989; Kumatori et al., 1990; Tanaka et al., 1989). (2) Proosomes were found located on the nuclear matrix in the nucleus and, most interestingly, on the intermediate filaments (IF) of the cytokeratin type in the cytoplasm of epithelial cells (Grossi de Sa et al., 1988b; Arcangeletti et al., 1992; Olink-Coux et al., 1992). (3) Since Triton X-100 extracts a fraction of proosomes, it is evident that not all proosomes are bound to the cytoskeleton.

In order to clarify the functional relationship between proosomes and the cytoskeleton, it was important to know whether the observation of the interaction of proosomes with the cytokeratins could be generalized to other types of IF. Therefore, in non-epithelial cells we tested whether these particles were located on IF other than the cytokeratins. We show that in PtK1 cells, where superposition of the prosome and cytokeratin networks was first observed, these particles are, in addition, located to a certain extent on the vimentin filaments. Furthermore, in human fibroblasts, which have an IF network exclusively constituted of vimentin, the prosomal antigens probed were found to coincide fully with the vimentin fibers. Finally, in proliferating muscle cells of the C2.7 mouse myogenic cell line, where muscle-specific desmin is the predominant constituent of the IF, the prosomal antigens probed were found partially located on the desmin network.

MATERIALS AND METHODS

Cell culture
Monolayer cultures of rat kangaroo kidney epithelial cells (line 1, PtK1) were grown in DME (Gibco Laboratories, Grand Island, NY) containing 0.85 g/l of sodium bicarbonate supplemented with 10% fetal calf serum (FCS) (Bayer Diagnostic, Puteaux, France) and antibiotics (100 IU/ml penicillin, 50 µg/ml streptomycin). Monolayer cultures of C2.7 myoblasts (derived from the C2 mouse myogenic cell line of Jaffé and Saxel (1977), kindly given by C. Pinset and D. Montarras) proliferated in DME supplemented with 20% FCS and antibiotics. Monolayer cultures of 2002 Flow fibroblasts (human embryonic lung fibroblasts) were grown in Earle’s modified MEM (ICN Biomedical SpA, Via Lambro, Opera (MI), Italy) supplemented with 10% FCS (ICN Lab.), 1% L-glutamine (ICN Lab.), 1% non-essential amino acids (ICN Lab.), 1% vitamins (ICN Lab.) and antibiotics as in DME.

Prosome-specific monoclonal antibodies
Monoclonal antibodies directed against the prosomal proteins p25K (7A11), p27K (IBS), p29K (GD6), p31K (AA4) and p30-33K (62A32) were from mouse ascitic fluids (Organon Teknika, Turnhout, Belgium). Purified proosomes from duck erythroblasts or HeLa cells were used as antigens for mouse immunization, according to the procedure described previously (Grossi de Sa et al., 1988a).

Cell fractionation of PtK1 cells, 2002 Flow fibroblasts and C2.7 myoblasts
Exponential PtK1, 2002 Flow and C2.7 culture cells were trypsinized (trypsin/EDTA in PBS, Gibco Laboratories, Grand Island, NY), washed twice with isotonic buffer (140 mM NaCl, 10 mM Hepes, pH 7.4, 5 mM KCl, 1.5 mM MgCl2) and pelleted again by low-speed centrifugation (800 g) for 10 minutes. All the following steps were performed at 4°C. Cells were gently resuspended in 8 volumes of hypotonic buffer (10 mM triethanolamine, pH 7.4, 10 mM KCl, 1 mM MgCl2, 1 mM MnCl2, 5 mM 2-mercaptoethanol) and homogenized in a tight Dounce homogenizer. Lysis was monitored by phase-contrast microscopy and isotonicity was restored after 3-4 minutes by addition of sucrose 2 M (0.25 M final). Differential centrifugation was performed to obtain the fractions corresponding to nuclei, mitochondria, polyribosomes and free-mRNP as described by Grossi de Sa et al. (1988b) and Vincent et al. (1980). The nuclei were purified according to Reynaud et al. (1980). The mitochondria were washed twice in isotonic buffer (hypotonic buffer containing 0.25 M sucrose), centrifuged at 15,000 g for 20 minutes, resuspended in TEK buffer (10 mM triethanolamine, pH 7.4, 50 mM KCl, 5 mM 2-mercaptoethanol) and sonicated twice for 5 seconds on ice. All final pellets were resuspended in TEK buffer and equal amounts of each fraction were analyzed by SDS-PAGE (Laemmli, 1970) after protein quantification (Bradford, 1976).

Prosome purification
Prosomes were purified from the free cytoplasmic mRNP complexes by sedimentation of the resuspended mRNP pellets in TEK buffer in the presence of 0.2% Sarkosyl. About 1 to 5 A260 units were loaded on a 5% to 21% (w/v) isotonic sucrose gradient containing 0.1% Sarkosyl and centrifuged at 150,000 g for 16 hours at 4°C (Beckman rotor SW41).

Western blotting
After electrophoresis, proteins were electrophoretically transferred onto nitrocellulose membrane (0.45 µm) according to the method of Towbin et al. (1979). The incubation of the nitrocellulose membranes with the antibodies was as described by Grossi de Sa et al. (1988a).

Indirect Immunofluorescence
PtK1 cells were grown on coverslips or on two-well glass culture chambers (ICN Biochemicals, Ltd., Bucks, England). They were seeded at low density and allowed to grow for 48 hours. C2.7 myoblasts were seeded at 2×104 cells/well and allowed to grow on coverslips or on two-well glass culture chambers in proliferating medium for 2-3 days. 2002 Flow fibroblasts were seeded at 8×104 cells/well in two-well glass chamber slides and allowed to grow for 48 hours.

Fixation protocol with methanol
C2.7 myoblasts were briefly rinsed with PBS (7 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.4, 137 mM NaCl, 2.7 mM KCl) at 37°C, immediately fixed with pure pre-cooled (~20°C) methanol for 10 minutes and air dried. The cells were then rehydrated with PBS for 5 minutes and processed for immunofluorescence as described below.

Triton X-100 extraction and fixation protocol
Exponential cultures of PtK1 cells and 2002 Flow fibroblasts were briefly rinsed with CSK buffer (10 mM Pipes, pH 6.9, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl2, 1 mM EGTA, 1.2 mM PMSF) at 37°C and extracted with CSK buffer containing either 0.3% Triton X-100 (Sigma Chemical Company, St Louis, MO) for 5 minutes at room temperature (Grossi de Sa et al., 1988b) or PtK1 cells, or 0.2% Triton X-100 for 2 minutes for 2002 Flow fibroblasts (37°C). Extracted cells were carefully washed with CSK buffer, fixed with 4% paraformaldehyde in CSK buffer for 20 minutes, washed three times for 10 minutes in PBS, and then processed for immunofluorescence as described below.
As 2002 Flow fibroblasts and C2.7 myoblasts are less adherent, an alternative extraction and fixation protocol was also used for these two cell lines. These cells were simultaneously Triton-extracted and fixed with paraformaldehyde for 20 minutes (0.5% Triton X-100 and 4% paraformaldehyde in CSK buffer, 37°C), then washed three times in PBS for 10 minutes, and finally processed for immunofluorescence as described below.

**Staining protocol**

The fixed cells were preincubated for 15 minutes with 1% BSA in PBS (to reduce background staining) and then incubated with the prosomal p-mAbs (anti-p25K, anti-p27K, anti-p29K, anti-p31K and anti-p30-33K diluted 1:20) in PBS containing 0.2% BSA (incubation buffer) for 1 hour at room temperature in a humid chamber. The cells were washed three times for 10 minutes with PBS and the fixed antibody was revealed by FITC- or (RITC)-conjugated anti-mouse IgG (ICN Biochemicals, Ltd, Bucks, England) diluted 1:40 in PBS/0.2% BSA. The cells were washed abundantly with PBS and mounted in Mowiol (Calbiochem AG, Lucerne, Switzerland) or in 50% glycerol in PBS, pH 8.5. Observations were made with a fluorescence microscope (Leitz Laborlux S, Wild-Leitz, Wetzlar-RFA) and photography was done on HP5 film (400 ASA; Ilford Ltd, Basilden, Essex, England). Negative controls were carried out by an identical procedure, except that the first antibody was replaced by the dilution buffer.

**RESULTS**

**Cytolocalization of prosomes in various subcellular fractions of PtK1 cells, human embryonic fibroblasts and C2.7 myoblasts**

The first series of results on cytolocalization of prosomal antigens in PtK1 cells have been reported (Grossi de Sa et al., 1988; Olink-Coux et al., 1992).

To substantiate and to extend these data to other cell systems, fractionation of human embryo lung fibroblasts (2002 Flow cells) and C2.7 myoblasts was carried out, and the prosome distribution of these two systems was compared with that in PtK1 cells. Furthermore, immunofluorescence studies were done on these three different cell types, paying attention, in particular, to the distribution of prosomal antigens on the...
intermediate filaments of the three different types (vimentin, cytokeratin, desmin) present in these cells. It was of interest to know if the notion of colocalization of prosomes and IF of the cytokeratin type could be extended to other types of IF and, possibly, generalized.

Prior to the immunofluorescence studies, the intracellular distribution of some prosomal antigens in the three cell lines was determined biochemically. Cell lysates were fractionated by differential centrifugation into nuclei, mitochondrial, polyribosomal and free mRNP fractions. The proteins in each of the subcellular fractions were analyzed by gel electrophoresis, Coomassie Blue staining and immunoblotting of PtK1 cells (Fig. 1A, A'), human embryo fibroblasts (Fig. 1B, B'), C2.7 myoblasts (Fig. 1C, C'). As shown in Fig. 1A', in PtK1 cells the 25K, 27K and 31K prosomal antigens are mainly present in the cytoplasm of PtK1 cells. Little if any reaction with the antibodies probed can be observed in the fractions corresponding to nuclei and mitochondria (Fig. 1A', lanes 2 and 4). Prosomes were predominantly detected in the untranslated mRNP fraction (Fig. 1A', lane 7). Contrary to previous results in HeLa cells (Grossi de Sa et al., 1988a), small amounts of the 25K and 27K prosomal antigens were also found in the polyribosomal fraction (Fig. 1A', lane 6); there was no significant amount in the cytosol fraction (Fig. 1A', lane 8). The intracellular distribution of the 25K and 27K prosomal antigens probed seemed to be identical, while a fainter reaction, which was limited to the untranslated mRNP fraction was detected for the 31K prosomal antigen.

Similar results were obtained using the same fractionation procedure in human embryo fibroblasts (Fig. 1B and B'), testing the 25K, the 27K and the 30-33K prosomal antigens. In this case also, prosomes were found predominantly in the untranslated mRNP fraction, although only a very faint reaction was detected for the p25K (Fig. 1B', lane 7). Little if any reaction can be observed in the fractions corresponding to the nuclei (Fig. 1B', lane 2), mitochondria (lane 4) and cytosol (lane 8). Small amounts of the p30-33K antigen and no significant amount of the p27K antigen were found in the polyribosomal fraction (Fig. 1B', lane 6).

But in this case, the cytoplasmic 27K prosomal antigen was not found exclusively in the untranslated mRNP fraction (lane 7). The 31K prosomal antigen was faintly detected only in the mRNP fraction (lane 6). The 31K prosomal antigen was faintly detected only in the mRNP fraction (lane 7), while no reaction was found in any fractions for the p25K antigen in these denaturating conditions. The apparent presence of prosomes in the polyribosomal fraction may reflect the abundance of mRNA of high Mr in muscle cells (myosin etc.), which, obviously, will co-migrate with ribosomes on a sucrose gradient; the genuine presence of prosomes at the level of polyribosomes can however not be excluded. Further work will have to be done to decide among these two interpretations.

In order to test whether the prosomal antigens observed in the cells are free proteins or part of prosome particles, the free mRNPs of human embryo fibroblasts were fractionated after dissociation on a sucrose gradient (Fig. 2A). Gel electrophoresis of proteins (Fig. 2B), and the corresponding immunoblot (Fig. 2C) of the gradient fractions revealed that prosomal proteins were absent at the level of the 4 S peak of the soluble proteins and found exclusively in the 19 S sedimentation zone of prosome particles. Similar results were obtained for PtK1 cells (Olink-Coux et al., 1992) and C2.7 cells.
(data not shown). This demonstrates that, in these cells, the immunofluorescence results shown below correspond to the distribution of the prosome particles and not of the free prosomal proteins.

**Distribution in between the cytokeratin and vimentin networks of prosome antigens in PtK₁ cells and human fibroblasts**

As pointed out in the Introduction, a major fraction of prosomes in the cytoplasm of HeLa cells (Grossi de Sa et al., 1988b) and PtK₁ cells (Olink-Coux et al., 1992) is insoluble to Triton X-100. Cells were systematically extracted by Triton X-100 prior to fixation, in order to extend the previous studies of the interactions between these Triton-resistant prosomes and the IF. It had also been shown that in PtK₁ cells prosomal antigens co-localize extensively with cytokeratin type IF, while their presence on the vimentin network seemed then to be insignificant (Grossi de Sa et al., 1988b). Since the vimentin

![Fig. 3](image)
filaments were found to participate in the distribution of prosomal antigens in other cell types (Arcangeletti et al., 1992), we reinvestigated, in PtK1 cells, the possibility of an interaction of prosumes with the vimentin network.

Fig. 3 reports double-label immunofluorescence studies carried out with the anti-p27K prosomal monoclonal antibody (p-mAb), and with polyclonal antibodies directed against cytokeratin or vimentin. In A, A’ and C, C’ the network stained by the anti-p27K p-mAb superimposes largely on the cytokeratin fibers. Furthermore, as can be seen at low magnification in B and B’, the p27K prosomal antigen seems to co-localize with the vimentin network also. However, the correspondence is incomplete; it is restricted to the filamentous pattern outlined by arrows in the higher magnification of Fig. 3D. Furthermore, the observed co-localization with vimentin is apparently limited to some areas of the cell.

That the vimentin network participates in prosome antigen distribution was confirmed in human fibroblasts. Proliferating fibroblasts are known to contain exclusively vimentin-type IF (Traub, 1985b). Therefore, double-label indirect immunofluorescence was carried out on human embryo lung fibroblasts (Flow 2002 cells). The results indicate that in these cells, at low and high magnification, prosomes including the p27K protein co-localize extensively with the vimentin network (Fig. 4). B and B’ of Fig. 4 show the correspondence of prosome and vimentin networks at high magnification, and also provide an important internal control. Indeed, as demonstrated previously on PtK1 cells (Grossi de Sa et al., 1988b), the p-mAbs stain the filaments in a more granular pattern than does the polyclonal antibody specific for vimentin. This difference in the staining of prosomes and IF by specific antibodies argues against a mere cross-reaction of the p-mAbs with vimentin.

As fibroblasts easily detach from their substratum upon detergent treatment, the permeabilization-fixation procedure was modified for processing cells prior to immunofluorescence staining. As described in Materials and Methods, the significant difference was that cells were permeabilized and fixed simultaneously before staining.

Panels C, C’ and D, D’ of Fig. 4 show double-label staining of the vimentin network (C, D) and the 27K prosome antigens (C’, D’) using this procedure. The cytoskeleton seems to be better preserved using the new technique and, furthermore, no significant difference in the results was observed when this procedure was adopted for human fibroblasts, and also for the myoblasts (see below), where the same problem arose.

All these data (Fig. 4) confirm the extensive co-localization of the 27K prosomal antigen with the vimentin network. Similar results were obtained when another p-mAb, the anti-p25K mAb, was probed (Fig. 5A’, B’, C’) in double-staining immunofluorescence experiments together with the antivimentin polyclonal antibody (Fig. 5A, B, C). Again, the standard permeabilization fixation procedure (A, A’, B, B’) and the new one (C, C’) gave comparable results. We conclude, therefore, that there is an extensive presence of prosomes on the vimentin network, which is possibly involved in their cytodistribution.

The presence of prosomal antigens on desmin fibers in murine myoblasts

As the third system, the desmin-type of IF, the C 2.7 mouse myogenic cell line, originally established by Yaffé and Saxel (1977), was chosen as the biological model. In a medium inducing differentiation these myoblasts are able to form myotubes; this model thus allows the study of prosome-IF association during the reorganization of the cytoskeleton leading to myotube formation. The data reported here deal only with proliferating myoblasts, i.e. cells in myogenic stages of differentiation that are sufficiently advanced to contain a well-developed desmin network but not yet forming myotubes.

As can be seen in Fig. 6, in methanol-fixed myoblasts stained directly with p-mAbs without prior Triton X-100 extraction, the prosomal networks probed with anti-p25K, anti-p29K and anti-p31K p-mAbs superimpose on the desmin fibers. This indicates that this type of IF also participates in the cytoplasmic distribution of prosomes. Furthermore, the existence of prosomal networks, stained with different p-mAbs, even in cells not previously extracted by Triton X-100, strongly reinforces the notion that the observation of correspondence between prosomes and the IF is genuine, and not due to redistribution of prosomal antigens as a result of Triton X-100 extraction of the cells, or to the fixation procedure used.

In order to compare the data on C 2.7 myoblasts with those obtained with PtK1 cells and human embryo fibroblasts, C 2.7 cells were also permeabilized with Triton X-100 and fixed simultaneously with paraformaldehyde, according to the technique developed with human fibroblasts. The results shown in Fig. 7 indicate that prosomes, including the p25K (D’), the p27K (C’), and the p31K antigens (A’ and B’), co-localize largely with the desmin network (A, B, C, D).

Interestingly, the degree of co-localization, in relation to the prosomal antigen probed seems to be variable. In particular, only some prosomes superimpose on desmin fibers when p25K (Fig. 7D, D’) and p27K (Fig. 7C, C’) are used, while a more extensive co-localization is detected when the p31K antigen is visualized (Fig. 7A, A’, B, B’). In this case an important additional observation was made: although the correspondence between the p31K-including prosomes and desmin fibers is very extensive, an additional peripheral network is stained with the anti-p31K mAb (Fig. 7B, B’, arrows), in a cell area where apparently few desmin fibers are present. Preliminary observations indicate that, in such areas, prosomes might colocalize with actin filaments, but their presence also on very faint vimentin fibers cannot be excluded at present (work in progress).

Another interesting characteristic of the C 2.7 myoblasts is that, in these cells, in the growth conditions applied, a well-developed desmin network coexists with a very limited vimentin system. In order to confirm this peculiarity of C 2.7 cells, double-label immunofluorescence experiments were done using combinations of polyclonal and monoclonal antibodies specific for vimentin and desmin (Fig. 8A, A’). As can be seen, no clear-cut vimentin network can be observed (A’, showing only a diffuse pattern) while a quite distinct desmin network is present (A). This experiment apparently provides a negative answer to the question of whether, in the C 2.7 myoblasts, vimentin also participates to a major extent in the cytodistribution of prosomes.

Altogether, the results obtained with the three cell lines show that prosomes co-localize with all types of IF tested, i.e. the cytokeratin, vimentin and desmin networks. This may thus allow us to extend the previous observations of the selective...
interaction between prosomes and the cytokeratins to all types of IF.

**DISCUSSION**

The aim of this work was to determine whether IF other than cytokeratins could carry prosomal antigens in non-epithelial cells. Therefore, in addition to the already explored cytokeratin-type IF (Olink-Coux et al., 1992; Arcangeletti et al., 1992), we analyzed two further cytoskeletal systems: the vimentin network in PtK1 and LLC-MK2 cells, which co-exists with the cytokeratin-type IF, the exclusively vimentin-type IF in human lung fibroblasts and the desmin-type IF in C2.7
myoblasts. The latter are cells in a myogenic stage of differentiation that is sufficiently advanced to already contain a well-developed desmin network prior to myotube formation.

Most importantly, it was established here that in all the cell systems tested all types of prosomal antigens, as part of the prosome particles, co-localize with the respective IF networks. Immunofluorescence on the three IF systems studied showed that for all antigens probed (p25K, p27K, p29K and p31K) a cytoplasmic fraction of prosomes was always found on Triton-resistant IF networks. Superimposition of prosomes was observed with vimentin-type IF in PtK1 cells and human fibroblasts, and with desmin-type IF in muscle cells. In the

Fig. 5. Cytolocalization of the p25K antigen on the intermediate filaments of vimentin type in human fibroblasts. The cells were first Triton-extracted, then fixed with paraformaldehyde (A,A',B,B'), or simultaneously fixed and permeabilized (C,C'). Finally they were double-labeled with the anti-p25Kp mAb (A',B',C') and a polyclonal anti-vimentin antibody (A,B,C), added simultaneously. (D,D') Negative controls, using the first procedure except that the first antibody was replaced by 0.2% BSA in PBS. Immunoreactivity was revealed as described for Fig. 4. Bars: 25 μm (A,A',C,C'); 10 μm (B,B').
latter cell system, the fact that such correspondence was even found in cells directly stained after fixation with methanol indicates that the interaction of prosomes with the IF is not the result of a redistribution of these particles following the Triton extraction procedure. These data suggest that prosomes interact with IF of any kind. Furthermore, the superposition seems to be IF specific, i.e. little correspondence of prosomes with the other filament systems of the cytoskeleton, such as actin or tubulin, was observed in Triton-extracted cells (Grossi de Sa et al., 1988b; Olink-Coux et al., unpublished observations).

Another interesting deduction arose from the studies on transformed cells in culture containing more than one system of IF. In cells in which vimentin is co-expressed with the tissue-specific component of IF, prosomes seem to be associated with both IF networks. However, these particles apparently bind to the tissue-specific IF with a higher frequency.

Fig. 6. Co-localization in C 2.7 myoblasts of the p25K, p29K and p31K prosomal and IF antigens of desmin type by double-label immunofluorescence. The cells were fixed with methanol and double-labeled with anti-desmin (A,B,C,D) and anti-p25K (A′,B′), anti-p31K (C′) or anti-p29K (D′) primary antibodies, added simultaneously. The fixed prosomal antibodies were secondarily detected with a FITC-conjugated sheep antibody (anti-mouse IgG). The anti-desmin antibody was revealed with a RITC-labeled sheep antibody (anti-rabbit IgG). Bars, 20 μm.
than to the co-expressed vimentin. For instance, our data for PtK₁ cells showed that the p27K antigen co-localizes much more extensively with the cytokeratin network than with the vimentin one. This only partial superposition with vimentin led, in a previous study, to the conclusion that the interaction with prosomes was limited to the cytokeratin that is specific to these cells (Grossi de Sa et al., 1988b). However, several new data indicate that this partial co-localization of prosomes with vimentin is not artefactual. (i) Even at high magnification, a perfect superposition of some prosomal antigens with the vimentin network was found in some sections of the cytoplasm (this paper; and Olink-Coux et al., 1992). (ii) Similar results showing localization of prosomes on vimentin-type IF in addition to cytokeratin-type IF were obtained in

**Fig. 7.** Co-localization in C 2.7 myoblasts of the p31K, p27K and p25K prosomal antigens and IF of desmin type by double-label immunofluorescence. The cells were simultaneously Triton-extracted and fixed with paraformaldehyde. Then the cells were double-labeled with: (A, B) the anti-p31K; (C) the anti-p27K; (D) the anti-p25K p-mAbs; and (A, B, C, D) a polyclonal anti-desmin antibody, added simultaneously. The anti-prosomal antibodies were secondarily detected with RITC-labeled goat anti-mouse IgG; the anti-desmin antibody was detected with FITC-labeled goat anti-rabbit IgG. Bars, 20 μm.
monkey kidney epithelial cells (Arcangeletti et al., 1992). (iii) In human fibroblasts, all prosomal antigens were found on vimentin-type IF. The degree of co-localization of prosomes with vimentin filaments seems, therefore, to vary according to the cell type and the p-mAbs used. The variation of the prosome-vimentin interaction observed in human fibroblasts and PtK₁ cells might result from a variable protein composition of the prosomes, or from possible differences in the vimentin IF structure and organization in epithelial and fibroblast cell lines. In permanently growing cells like PtK₁ cells, vimentin is co-expressed with cytokeratin-type tissue-specific IF, while it is normally synthesized in fibroblasts as an exclusive IF protein component (Traub, 1985a). One may thus suppose that the regulation of vimentin expression and organization differs for cells of mesenchymal origin compared with those that are transformed and/or in culture conditions (Isaacs et al., 1989).

When combined, these results demonstrate that prosomes are, to a very high degree, preferentially co-localized with the tissue-specific IF of a given type of cell.

In addition, in cells containing more than one type of IF, the extent of co-localization of prosomes with IF depended on the prosomal antigens tested. The pattern of prosome distribution on the cytokeratin networks in PtK₁ cells seems not to be the same when different types of p-mAbs are used (Olink-Coux et al., 1992); similar observations were made on the C 2.7 myoblasts, where some prosome filaments extend beyond the desmin network. Since the different cytokeratins present in PtK₁ cells may form subnetworks of IF, specific types of prosomes may, in these cells, recognize and interact with them differentially.

Moreover, this variable staining suggests the existence of different kinds of prosomes, each having a specific protein composition and distribution relative to the IF subnetworks. This notion of different types of prosomes is in agreement with previous immunocytochemical and biochemical data showing variable localizations of specific prosomal antigens in several tissues during embryonic development (Pal et al., 1988) and cell maturation (Grossi de Sa et al., 1988a), and prosomal protein pattern changes during cell differentiation (Ahn et al., 1991; Haass and Kloezel, 1989). Furthermore, a specific type of prosome, including the p31K antigen, was found localized selectively close to the bile canaliculi in the adult rat liver (Briane et al., 1992), whilst prosomes detected by a polyclonal antibody were found distributed throughout the cytoplasm of differentiated rat hepatocytes (Fourier et al., unpublished observation). Recent biochemical data (Yang et al., 1992) from HeLa cells demonstrate a difference in the protein pattern of the prosomes extracted from the microsomal fraction and those from the cytoplasmic supernatant, and changes in these patterns upon interferon γ induction.

**Possible implications for the selective interaction of prosomes with intermediate filaments**

The observation of cytoskeletal prosomes selectively on the IF raises several questions about their role(s) in different proposed

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**Fig. 8.** Cytolocalization in C 2.7 myoblasts of the IF antigens of vimentin and desmin type by double-label immunofluorescence. The cells were simultaneously permeabilized and fixed as described for Fig. 7 and then double-labeled with: (A) a monoclonal anti-desmin antibody; and (A') a polyclonal anti-vimentin antibody. The anti-desmin antibody was secondarily detected with FITC-labeled goat anti-mouse IgG; the anti-vimentin antibody was revealed with RITC-labeled goat anti-rabbit IgG. (B,B') Negative controls. Bars, 20 μm.
functions for prosomes in the cell. Since prosomes are described as subcomplexes of untranslated mRNPs, one may suggest the possible involvement of IF in the selective transport and positioning of these particles and, therefore, of untranslated mRNAs in the cytoplasm (Scherrer and Bey, 1994; Scherrer et al., 1988).

IF are dynamic structures distributed throughout the cytoplasm from the nucleus towards the plasma membrane. Although the function(s) of the IF has still not been elucidated, IF are believed to represent a tracking system for targeting specific molecular information, like specific mRNAs, to defined intracellular compartments (for review see Scherrer, 1990; Skalli and Goldman, 1991). Indeed, the concept that specific mRNAs are not randomly distributed in the cytoplasm is to date largely accepted (Bruckenstein et al., 1990; Melton, 1987; Singer et al., 1989). Although some data indicate that the actin-containing microfilaments carry the ribosomes and the mRNAs that are actively translated in polyribosomes (Hesketh and Pryme, 1991; Ramaekers et al., 1983), specific mRNAs seem also to have the capacity to bind to the IF (Murti and Goorha, 1989).

On the basis of data presented here and elsewhere, prosomes may thus have a role in the specific transport of mRNA on the IF. (i) Prosomes migrate from the cytoplasm to the nucleus and vice versa, as shown in studies during late oogenesis, embryogenesis and development (Gautier et al., 1988; Pal et al., 1988), and moreover in pathological cells (Kumatori et al., 1990). Although a possible model for the nuclear translocation of the prosomal proteins has been proposed (Tanaka et al., 1990), the mechanism of prosome migration between the two cellular compartments is still unknown. Nevertheless, the dynamic aspects of the IF extending from the nucleus towards the plasma membrane (Albers and Fuchs, 1989; Eriksson et al., 1992; Monteiro and Cleveland, 1989; Vikstrom et al., 1989), together with the presence of IF, as well as microfilaments, close to the nuclear pore complexes (Agutter, 1988; Carmo-Fonseca et al., 1987), make the IF a likely candidate for the intracellular transport and translocation of prosomes in both directions. (ii) Prosomes are distributed to very specific areas of the cell, as illustrated by the specific localization of prosomes including the p31K antigen in rat liver hepatocytes; a specific kind of prosome was found selectively close to the plasma membrane de-limiting the bile canaliculi of fetal and adult rat liver hepatocytes (Briane et al., 1992). (iii) Finally, the variable but also specific intracellular distribution of prosomes supports the notion that prosome particles, although having uniform morphology, are probably not identical in their composition (Ahn et al., 1991; Briane et al., 1992; Brown et al., 1991; Falkenberg and Kloetzl, 1989; Pal et al., 1988). The results of Brown et al. (1991) on the MHC-linked 'LMP' complex, which presents high immunological and structural similarities with the prosome particles, reinforce these findings. Indeed, their results indicate that prosomes, involved in antigen processing during the immune response, represent only a fraction of the particles present in the same cell. Thus, prosomes may constitute a population of particles that vary in composition, intracellular localization and, possibly, function.

In parallel to the possible role of prosomes as a transporter complex for mRNA distribution, the interaction of prosomes with the IF may also be related to the proteolytic activities of these particles. Since prosomes are found in the nucleus and cytoplasm, and at the plasma membrane, and since IF constitute a network connecting the nucleus to cell surface, these filaments may represent a tracking system for the prosome-MCP particles. One may suppose that, in general, enzymatic complexes are transported and concentrated within specific cell compartments. For instance, the prosome-related LMP particles, involved in antigen processing, are likely to be positioned close to the endoplasmic reticulum. In the latter compartment, the peptides, suggested to be cleaved by the prosome-MCP particles, associate with the MHC I complex (DeMars and Spies, 1992).

Another aspect of prosome-IF interaction might implicate MCP activities in IF remodelling. The IF organization is known to be completely modified during the cell cycle (Chou et al., 1990; Rosevear et al., 1990). Interestingly, Ohta et al. (1988) reported the presence of ubiquitin on cytokeratin IF. Since prosomes are able to associate with other structures to form the '26 S proteasome', involved in the selective processing of ubiquitylated proteins (Eyton et al., 1989), it is therefore possible that prosomes, via their multicatalytic protease activity, are involved in IF remodelling or turnover of IF components. Similarly, Nelson and Traub (1982) have previously characterized a specific Ca2+-activated protease that selectively modifies the IF components. Assays to determine the capacity of prosomes to degrade the IF proteins selectively are actually in progress.

In conclusion, this new perspective of selective interactions of prosomes with subnetworks of tissue-specific IF offers several non-exclusive possibilities for studying their role(s) in cell function. Correspondence between prosomes and IF observed in the different cellular systems tested concerned only a fraction of these particles in the cell, suggesting that the ability of prosomes to interact with IF is highly regulated. Since IF are precisely the elements of the cytoskeleton with which prosomes interact specifically, such interactions, related to the different functions proposed for prosomes, probably have important implications for cell behaviour.

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