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Cytokeratins in mesenchymal cells: impact on functional concepts of the diversity of intermediate filament proteins

JÜRGEN MARKL
Zoological Institute, University of Würzburg, Röntgenring 10, D-8700 Würzburg, Germany

Shape, motility, viscosity and organelle distribution of animal cells is regulated, or at least influenced, by fibrous proteins constituting a 'cytoskeleton' that involves the F-actin-based microfilaments (5-7 nm in diameter) and the microtubules (20-25 nm). In addition, most vertebrate cells contain a third type of highly organized protein bundle, the intermediate filaments (IF; 8-12 nm). Their specific role is less clear, but they certainly contribute to tensile strength of cells and, via their anchorages at desmosomes, to cell-cell interactions (for reviews, see Franke et al. 1982; Traub, 1985; Krohne and Benavente, 1986; Franke, 1987; Steinert and Roop, 1988; Nagle, 1988; Bershadsky and Vasiliev, 1988; Robson, 1989; Carmo-Fonseca and David-Ferreira, 1990). Ultrastructurally, the IFs of different cell types are very similar, but biochemical and immunological data have revealed in mammals at least seven distinct classes comprising, for example in humans, a total of approximately 40 different polypeptides: vimentin (predominantly, but not exclusively in mesenchymally derived cells), desmin (typical of most types of muscle cells), glial filament protein (GFP; predominantly in astrocytes), several different neurofilament proteins (in most neurons), peripherin (in certain neuronal cells), at least four different lamins (structural components of the nuclear lamina), and a whole panoply of cytokeratins (present in almost all epithelial cells). A given tissue is characterized by a specific pattern of IF polypeptides, which is extensively used for cell typing in tumor diagnosis (see Kartenbeck, 1989), and as a differentiation marker in embryogenesis (e.g. see LaFlamme and Dawid, 1990).

Also in invertebrates, in addition to the ubiquitous lamins, a widespread though not ubiquitous occurrence of cytoplasmic intermediate filaments has been reported, and at least two distinct cytoplasmic classes of invertebrate IF proteins have been identified to date (Bartnik and Weber, 1989). One is specific for neuronal cells and resembles vertebrate neurofilament protein, the other class is non-neuronal (Bartnik et al. 1987). Recently, two epithelial IF proteins from the gastropod Helix pomatia and two muscle IF proteins from the nematode Ascaris lumbricoides have been sequenced (Weber et al. 1988; Weber et al. 1989). It turned out that the four invertebrate IF proteins are more closely related to nuclear lamins than are vertebrate IF proteins, and neither of them could be specifically assigned to any of the mammalian IF protein classes. Thus, divergence of the different non-neuronal IF proteins as observed in mammals might be a specific feature of chordate or vertebrate tissues.

Orthologons to mammalian cytokeratin, vimentin, desmin and GFP also exist in birds, reptiles, amphibia and fish (e.g. see Fuchs and Marchuk, 1983; Quax et al. 1984; Balcarek and Cowan, 1985; Herrmann et al. 1989a, b; Frail et al. 1990). Recently, the primary structure of a cytokeratin from the goldfish Carassius auratus has been solved (Giordano et al. 1989). It is conclusive that this protein is structurally similar to human cytokeratin 8, demonstrating that at least one of the mammalian cytokeratin forms already existed, as a distinct protein, in the early period of vertebrate evolution. With a few reported exceptions, like, for example, the replacement of GFP by cytokeratins in optic nerve astrocytes of fish and amphibia (Giordano et al. 1989; Runnger-Brandle et al. 1989; Maggs and Scholes, 1990), the expression patterns of GFP and desmin seem to be comparable to those found in higher vertebrates (for references, see Markl and Franke, 1988). In contrast, expression patterns of cytokeratin and vimentin in fish differ fundamentally from those found in terrestrial vertebrates, as recently detected (Markl and Franke, 1988; Markl et al. 1989). The situation in amphibia may be somewhat intermediate (cf. Jahn et al. 1987; Runnger-Brandle et al. 1989). There is a conflict between such experimental observations and emerging theories on potential cause-and-effect roles of these patterns on cellular differentiation and/or function.

In humans as well as in the teleost fish, the rainbow trout Salmo gairdneri, only a single vimentin polypeptide has been detected (Fig. 1). In contrast, cytokeratins in both species represent a large multigene family comprising, in humans, a catalog of 20 different polypeptides (Fig. 1; Moll et al. 1982, 1990). A similar complexity of at least 15 distinct cytokeratins that cross-react immunologically with mammalian cytokeratins has recently been reported for the trout (Fig. 1; Markl et al. 1988). Ten additional human cytokeratins that are restricted to epidermal appendages such as hair- and nail-forming cells and certain tongue papillae (Fig. 1; Heid et al. 1988), appear to have no equivalent in fish, as deduced from immunological data (Markl and Franke, 1988). Human

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simple epithelia express cytokeratins 7, 8, 18 and 19, whereas 15 other cytokeratins are typical and exclusive to human stratified epithelia. Correspondingly, some internal epithelial cells and tissues in the trout express a specific set of S (simple epithelial) cytokeratins, whereas trout stratified epithelia contain completely different, i.e. E (epidermal) cytokeratins (Fig. 1). An interesting exception is the simple digestive tract mucosa, which in trout expresses a mosaic of S and E cytokeratins that more or less excludes S1, S4 and S9, the immunological correlates of mammalian cytokeratins 8 and 18 (Markl and Franke, 1988; Markl et al. 1989).

Cytokeratin polypeptides fold into three different domains, which are the common structural motif of all IF proteins: a highly conserved α-helical rod domain (interrupted by two non-helical spacers) is flanked by hypervariable, non-helical head and tail domains. The central rod domain is composed of a seven-residue (heptad) quasi repeat, which forms a coiled-coil structure, the basic core of the IF (for a scheme, see e.g. Bershadsky and Vasiliev, 1988; Nagle, 1988; Kartenbeck, 1989). This conserved backbone is surrounded by protruding head and tail domains. The size of cytokeratins is effectively determined by the size of the hypervariable subdomains of their heads and tails, which in humans increase as a function of the complexity of the epithelium in which they are expressed: cytokeratins typical of simple epithelia and early embryonic cells (cytokeratins 8 and 18 and, more restricted, 7 and 19) are among the smallest of the type II and type I polypeptide subsets, respectively (Fig. 1). It was therefore speculated that, in response to the need for IFs to perform more specialized functions, additional residues have been progressively introduced (Steinert et al. 1985). Moreover, the 'more-specialized' or 'late' cytokeratins of human stratified squamous epithelia and terminally differentiated epidermal cells are enriched, in their end domains, in glycine and serine residues that frequently occur as tandem repeats. In contrast, the 'less-specialized' or 'early' cytokeratins of human simple epithelia and embryonic cells possess differing repeats rich in serines and threonines (see Steinert and Roop, 1988). The intermediate isoelectric point of early human cytokeratins, compared to the more acidic and basic isoelectric points, respectively, of late human cytokeratins, might be a consequence of such differences in primary structure (Fig. 1). Trout cytokeratins have not yet been sequenced, but their narrow isoelectric pH and molecular weight ranges imply a 'limited' complement of less-diversified cytokeratins as compared to a more specialized complement of diverse cytokeratins in human. Indeed, as discussed below, trout cytokeratins (notably the S cytokeratins: Fig. 1) are expressed and presumably can function in a broader range of tissue types than their human counterparts.

It is widely accepted that cytokeratins are usually restricted to epithelium-derived tissues, whereas vimentin is typically found in cells of mesenchymal origin. This has been elaborated in several mammals, notably man, cow, rat and mouse, and in birds. Recently, however, it has been shown that in adult amphibia, e.g. the African clawed toad Xenopus laevis, some abundant non-epithelial tissues such as vascular endothelia and certain smooth muscle cells express cytokeratins in addition to vimentin (Godsave et al. 1988; Jahn et al. 1987; Achtstätter et al. 1989; Rungger-Brändle et al. 1989). In mesenchymal cells of the regenerating newt limb also, cytokeratins are transiently expressed (Ferretti et al. 1988). This pattern in which cytokeratins 8 and 18 are co-expressed with other IFs resembles that prevailing in early amphibian embryo genesis (Fouquet et al. 1988). In the adult trout, a really dramatic extension of cytokeratin expression in cells and tissues of mesenchymal origin has been described. Moreover, most of these tissues do not even seem to co-express vimentin. Such cells include the endothelium, scale-associated dermal cells, pillar cells of gills, certain vascular smooth muscle cells, and many interstitial cells, notably fibroblasts (Markl and Franke, 1988; Markl et al. 1989). Also the ocular lens epithelium and optic nerve astrocytes, which in mature mammals express vimentin, in the trout synthesize cytokeratins. The latter has also been found in the goldfish optic nerve, and in amphibia as already discussed. The cytokeratin complement present in such nonepithelial cells of the trout is surprisingly complex, as it not only comprises the putative equivalents of human cytokeratins 8 and 18, but up to a total of eight distinct cytokeratins (the S cytokeratins: Fig. 1). In some trout cells, e.g. the fibroblastoïd cultured cell line RTG-2, the whole panel is expressed (Markl et al. 1989). Expression of vimentin in this fish has been noted in ocular lens tissue, white blood cells and egg cells that all seem to lack cytokeratin (Markl, Fouquet and Franke, to be published). In trout, vimentin is co-expressed with cytokeratin, for example, in trout chondrocytes and chromatophores and, interestingly, with neurofilament protein in trout retinal photoreceptor cells. Red blood cells that are a rich vimentin source in higher vertebrates, including amphibia, do not seem to contain vimentin or any other IF protein in the trout; the same appears to be true for trout spermatozoa and spermatids (Markl, Fouquet and Franke, to be published). From the present results one could speculate that there exists a cytokeratin/vimentin shift of expression patterns in vertebrate evolution, as well as a shift towards higher and lower isoelectric points, respectively (Fig. 1). However, the data are still too limited to be

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Fig. 1. Schematic catalog of type II (open symbols) and type I (filled symbols) cytokeratin polypeptides of man (□, ●) and the rainbow trout (○, ●) as separated on two-dimensional polycrylamide gels. Cytokeratin filaments are heteropolymers comprising type I and type II polypeptides in equal proportions. (□, ●) Additional set of human trichocytic cytokeratins (Hs, acidic; Hb, basic). Vh, human vimentin; Vt, trout vimentin. According to Möll et al. (1982, 1986), Heid et al. (1988) and Markl et al. (1989). Note the comparatively narrow isoelectric pH and molecular weight range of trout cytokeratins (broken rectangle).
synthesized coherently into such a hypothesis. This awaits investigation of more species and of other chordate classes.

Interestingly, recent results from several species have demonstrated that in mammals also restriction of cytokeratins to epithelia is not without exceptions. Specifically, cytokeratins 8 and 18, occasionally accompanied by 19, can be seen, often transiently, in certain non-epithelial tissues, especially embryonal ones, certain non-epithelium-derived tumors, and in a rare subtype of human endothelial cells (for references, see Jahn et al. 1987; Franke et al. 1989). The appearance of cytokeratins 8 and 18 has also been noted in several lines of transformed fibroblasts of human and rodent origin as a rare, spontaneous event (Knapp and Franke, 1989; Knapp et al. 1989; see further references therein). After treatment of the cultured cells with 5-azacytidine, the frequency of such cells being positive for cytokeratins was often greatly increased. This shows that the synthesis of cytokeratins 8 and/or 18 and the formation of cytokeratin IFs can be controlled at different levels of a regulatory cascade, including DNA methylation (Semat et al. 1986; Knapp and Franke, 1989). Seen in this context, the observations made in the trout might suggest that the normal long-term inactivation of cytokeratin genes in fibroblasts of higher vertebrates does not occur in this, nor probably in other, teleost fish. This provides valuable information on an open field, the evolution of the regulation of cell type specificity of gene expression.

The cytoplasmic network formed by IFs extends from the nuclear periphery to the plasma membrane and structurally interacts with most cell organelles, other components of the cytoskeleton, ribonucleoproteins and nucleic acids. It has been speculated that besides having a mere structural role like 'mechanical integrators of cellular space', IFs could be involved in information transfer or cytoskeleton-dependent control of gene expression (see Traub, 1985; Carmo-Fonseca and David-Ferreira, 1990). In this context it is a crucial, and currently widely debated, question whether functional differences can result from different types of IF proteins, or whether cell function is independent of the specific complement of IF proteins (nuclear lamins are excluded: they provide the cell with a putative karyoskeleton for chromatin attachment). The 'unusual' expression of cytokeratins (instead of vimentin) in fish optic nerve astrocytes, for example, might correlate with the unique 'epithelial' as well as 'embryonic' character of this nerve, which is ribbon-shaped and capable of regenerating (e.g. see Giordano et al. 1989; Maggs and Scholes, 1990). It has been proposed that the head and tail domains, which protrude from the rod domain scaffolding and are hypervariable in both size and sequence, specify the function of the IF in which they occur (e.g. see Steinert et al. 1985). However, the recently detected widespread occurrence of cytokeratins in mesenchymal cells provides a caveat for such indications of functional differences between cytokeratin and vimentin or, more generally, between different cytoplasmic IF protein subsets. For example, trout fibroblasts contain a complex network of up to seven distinct cytokeratins, while in mammalian fibroblasts a 'simple' vimentin lacework fulfills comparable needs. Even comparison of different mammals (and one should bear in mind that only man, cow, rat and mouse have been carefully investigated to date) yielded fundamental interspecies differences in expression patterns of a given cell type (Owariie et al. 1988). It will be very difficult to provide explanations and theories that account for this in functional terms, and to date a comprehensive functional concept of the impressive diversity of IF proteins is not in sight.

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