Keratin and vimentin expression in early organogenesis of the rabbit embryo*

Christoph Viebahn¹, E. Birgitte Lane², and Frans C.S. Ramaekers³

¹ Institute of Anatomy, University of Bonn, Bonn, Federal Republic of Germany;
² Imperial Cancer Research Fund Clare Hall Laboratories, Potters Bar, Hertfordshire, United Kingdom;
³ Department of Pathology, University Hospital Nijmegen, The Netherlands

Summary. The expression of vimentin and keratins is analysed in the early postimplantation embryo of the rabbit at 11 days post conceptionem (d.p.c.) using a panel of monoclonal antibodies specific for single intermediate filament polypeptides (keratins 7, 8, 18, 19 and vimentin) and a "pan-epithelial" monoclonal keratin antibody. Electrophoretic separation of cytoskeletal preparations obtained from embryonic tissues, in combination with immunoblotting of the resulting polypeptide bands, demonstrates the presence of the rabbit equivalents of human keratins 8, 18, and vimentin in 11-day-old rabbit embryonic tissues. Immunohistochemical staining shows that several embryonic epithelia such as notochord, surface ectoderm, primitive intestinal tube, and mesonephric duct, express keratins, while others (neural tube, dermomyotome) express vimentin, and a third group (coelomic epithelia) can express both. Similarly, of the mesenchymal tissues sclerotomal mesenchyme expresses vimentin, while somatopleural mesenchyme (abdominal wall) expresses keratins, and splanchonpeleic mesenchyme (dorsal mesentery) expresses both keratins and vimentin. While these results are in accordance with most results of keratin and vimentin expression in embryos of other species, they stand against the common concept of keratin and vimentin specificity in adult vertebrate tissues. Furthermore, keratin and vimentin are not expressed in accordance with germ layer origin of tissues in the mammalian embryo; rather the expression of these proteins seems to be related to cellular function during embryonic development.

Key words: Vimentin – Keratin – Mesenchyme – Organogenesis – Embryo – Rabbit (Oryctolagus cuniculus)

In the light of their highly specific distribution in adult tissues, the expression of the 5 classes of intermediate filament proteins (keratins, vimentin, desmin, glial fibrillar acidic protein (GFAP), and neurofilament proteins) has been increasingly investigated in embryonic tissues. In the embryo, the expression of desmin, GFAP, and the neurofilament proteins has been demonstrated to be a sign of definite commitment of embryonic cells to become either muscle tissue, glial cells or neurons, respectively (Tapscott et al. 1981). In contrast, vimentin and keratin have been found in more primitive embryonic tissues and their specificity for either mesoderm (vimentin) or ectoderm and endoderm (keratins) in early embryonic tissues (Paulin et al. 1980; Jackson et al. 1981) opened up the possibility that during development the expression of vimentin could be an indication of mesodermal origin, and expression of keratin could be typical for endodermally or ectodermally derived tissues. Evidence in favour of such a hypothesis might be drawn from several investigations that describe intermediate filament protein patterns in developing organs of various species such as chick (Schmid et al. 1979), mouse (Kemler et al. 1981), rat (Lehtonen et al. 1985; Paranko et al. 1986; Paranko and Virtanen 1986), golden hamster (Viebahn et al. 1987), and human (Regauer et al. 1985). However, most of these reports are confined to the fetal period of organ development. Clearly, intermediate filament protein expression must be analysed during the embryonic period of organogenesis, i.e., from the early postimplantation period onwards up to the fetal stages of development, in order to demonstrate a relationship of vimentin expression with mesodermal origin, and of keratin expression with endodermal or ectodermal origin if tissues. Doubts about such a relationship have arisen recently from investigations on quail (Erickson et al. 1987) and chick (Page 1988) embryos in which an expression pattern for vimentin and keratin developing independently of the 3 germ layers was found. The present investigation, therefore, analyses keratin and vimentin expression at the beginning of organogenesis in the rabbit embryo in an attempt to fill the gap between investigation of early embryonic tissues and those of the fetal stages of organ development in mammals.

Materials and methods

Embryos

Randomly bred female rabbits from crossings of various strains were artificially inseminated (Al-Hasani 1980) on day 0. At 11 days 0 hours post conceptionem females found pregnant at abdominal palpation were anaesthetized using
90 mg Nembutal i.v. followed by 100 mg Ketamine i.m. and embryos were collected by Caesarean section. 14 embryos out of 2 litters were included in the present study.

Antibodies

**Keratin antibodies.** The polypeptide-specific keratin monoclonal antibodies LE41, LE61, LP2K, RCK105, RCK106, CK18-2, RGE53, and the panepithelial antibody RCK102 have been described in detail before (Lane 1982; Ramaekers et al. 1987; Viebahn et al. 1987). The specificity for individual human keratins of the keratin antibodies used in this study, their original references, and the dilutions used for immunohistochemistry, are summarised in Table 1.

**Vimentin antibodies.** Monoclonal antibody RV202 was obtained after fusion of lymphocytes from a mouse immunised with bovine lens vimentin. The antibody recognises vimentin only (Ramaekers et al. 1987) and stains mesenchymal tissues. Monoclonal antibody V9, reacting with vimentin from human, rat and chick tissue (Osborn et al. 1984) was obtained from Boehringer, Mannheim (FRG).

**Immunocoujugated second antibodies.** For immunohistochemistry, FITC-labelled anti-mouse Ig antibody, produced in goat, was obtained from Nordic Immunologicals, Tilburg, Holland, and used at a dilution of 1:100. For the immunoblotting experiments peroxidase-conjugated goat anti-mouse Ig antibody, obtained as well from Nordic Immunocoujugated goat, was used at a dilution of 1:2000.

**Immunohistochemistry**

For immunohistochemical analysis, embryos were quickly dissected out of the uterus, freed from their membranes, and transferred into a drop of O.C.T. compound (Tissue Tek, Naperville, USA), which had been placed at the bottom of gelatine capsules (Balzers Union, Balzers, FL). Embryos were then covered with another drop of O.C.T. and rapid freezing was accomplished by immersion of the bottom of the gelatine capsule into liquid nitrogen. After the O.C.T. drop containing the embryo was completely frozen the whole capsule was gradually lowered into the liquid phase of nitrogen. This method of 2-phase freezing prevented splitting of the specimens (K. Fröjdman, personal communication). Immunohistochemical staining using the indirect immunofluorescence technique was carried out as described before (Viebahn et al. 1987). 4-6 μm thick frozen sections, cut in a cryostat (Cambridge Instruments, Heidelberg, FRG) were fixed in most cases by immersion in cold (−20°C) methanol for 10 min, immediately followed by 1 min in cold (−20°C) acetone. Unfixed sections had to be used only for stainings with monoclonal antibody LE41. After air drying, sections were incubated with the first antibody for 30 min at room temperature followed by three 5-min washes in phosphate-buffered saline (PBS). Incubations with secondary antibodies were 30 min at room temperature, again followed by three 5-min washes in PBS. 5 min in distilled water, 5 min 96% methanol and mounting in MOWIOL (Hoechst, Frankfurt, FRG) containing 2.5% (w/v) NaN₃ to retard fading of the fluorescence. For controls, the first antibody was omitted. Sections were examined with a Zeiss Universal microscope equipped with epi-illuminating optics.

**Table 1. Monoclonal antibodies applied in this study. Numbers of keratins refer to human keratins catalogued by Moll et al. (1982 a)**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IF-Protein</th>
<th>Dilution of culture supernatant</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>RCK105</td>
<td>Keratin 7</td>
<td>Undiluted</td>
<td>Ramaekers et al. (1987)</td>
</tr>
<tr>
<td>LE41</td>
<td>Keratin 8</td>
<td>Undiluted</td>
<td>Lane (1982)</td>
</tr>
<tr>
<td>RCK102</td>
<td>Keratins 5 and 8</td>
<td>Undiluted</td>
<td>Broers et al. (1986)</td>
</tr>
<tr>
<td>RCK106</td>
<td>Keratin 18</td>
<td>Undiluted</td>
<td>Ramaekers et al. (1987)</td>
</tr>
<tr>
<td>RGE53</td>
<td>Keratin 18</td>
<td>Undiluted</td>
<td>Ramaekers et al. (1983, 1987)</td>
</tr>
<tr>
<td>CK18-2</td>
<td>Keratin 18</td>
<td>Undiluted</td>
<td>Broers et al. (1986)</td>
</tr>
<tr>
<td>LE61</td>
<td>Keratin 18</td>
<td>Undiluted</td>
<td>Lane (1982)</td>
</tr>
<tr>
<td>LP2K</td>
<td>Keratin 19</td>
<td>Undiluted</td>
<td>Lane et al. (1985)</td>
</tr>
<tr>
<td>RV202</td>
<td>Vimentin</td>
<td>Undiluted</td>
<td>Ramaekers et al. (1987)</td>
</tr>
<tr>
<td>V9</td>
<td>Vimentin</td>
<td>1:10</td>
<td>Osborn et al. (1984)</td>
</tr>
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</table>

**Immunohistochemistry**

**Preparation of cytoskeletal material.** Immediately after opening of the uterus, embryos were transferred into cold (0-4°C) buffer A containing 5 mM EDTA, 0.4 mM phenylmethylsulfonyl fluoride (PMSF), and 10 mM Tris-HCl, pH 7.2. The primitive intestinal tube with its mesentery still attached was removed from 6 embryos and the resulting pieces of tissue were collectively lysed and extracted as described by Moll et al. (1982 b) in "high-salt" extraction buffer containing 1.5 M KCl, 0.5% Triton X-100 (TX-100, Sigma, Deisenhofen, FRG), 5 mM EDTA, 0.4 mM PMSF, and 10 mM Tris-HCl, pH 7.2. After 20 min of incubation at 4°C the preparation was centrifuged (8 min at 14500 g at 4°C), resuspended in buffer A, and centrifuged again. The pellet was then used for electrophoresis as described below.

**Gel electrophoresis and immunoblotting.** Sodium dodecyl sulfate (SDS)-containing polyacrylamide gel electrophoresis (PAGE) was performed according to Laemmli (1970) using 10% slab minigels (Matsudaïra and Burgess 1978): Cytoskeletal preparations were resuspended in 200 μl sample buffer (2% SDS, 0.1 M dithiothreitol, and 0.0625 Tris-HCl, pH 6.8) and solubilized by boiling for 2 min. Bovine serum albumin and rabbit muscle actin were used as molecular weight markers (67 kD and 42 kD, respectively). Gels were either silver stained (Heukeshoven and Dernick 1985) or used for immunoblotted. For immunoblotting electrophoretically separated polypeptides were transferred from gels to a nitrocellulose membrane, pore size 0.2 μm (No. BA85, Schleicher and Schüll, Dassel, FRG), between graphit plates, using a semi-dry electrobietter as described by Kyhse-Andersen (1984) with a constant current density of 0.8 mA/cm² for 90 min. The nitrocellulose sheets were stained with Ponceau (Serva, Heidelberg, FRG) and cut into vertical strips after the transferred protein bands had been marked. The proteins were detected by exposing them to the following incubation steps at room temperature under gentle shaking: 10 min PBS to wash out the Ponceau stain, 1 h blocking with PBS containing 3% bovine serum.
albumin (BSA) and 0.1% TX-100, 4 h with primary antibody as undiluted culture supernatant. Only monoclonal antibody V9 was diluted 1:100 in PBS containing 3% BSA and 0.1% TX-100. After 1 h washing in several changes of PBS containing 0.1% TX-100, the strips were exposed to a horseradish-peroxidase conjugated antibody diluted 1:2000 in PBS containing 0.1% TX-100 for 2 h and washed again for 1 h. Peroxidase activity was visualised with 3'-3'-diaminobenzidine-HCl (Sigma, Deisenhofen, FRG). For controls the first antibody was omitted.

Results

Immunochemistry

In the 11.0 d.p.c. rabbit embryo most of the organ anlagen have been laid down. The embryo has about 36 somites (Minot and Taylor 1905) of which the cranial ones down to the level of the hindlimb bud have each differentiated into dermatoome, myotome and sclerotome. The primitive intestinal tube and the abdominal wall are closed, except at the umbilical level. The genital ridges have formed and within them the mesonephric duct is clearly visible and mesonephric tubules are beginning to form. The schematic drawing in Fig. 1 illustrated where the organ anlagen are located in horizontal sections through the trunk region of an 11.0 d.p.c. rabbit embryo, such as shown in Figs. 2 and 3.

The frozen section shown in Fig. 2 is stained with monoclonal antibody RV202, specific for vimentin. Dorsally, the strongly RV202-positive neural tube is easily recognised, and lateral to it equally positive neural crest cells are seen on their migratory path within the sclerotomal mesenchyme. Of the differentiating tissues of the somite, only the dermatoome is strongly positive throughout. In the myotome the RV202-signal appears weak in most cells (see also Fig. 4a). However, there are a few cells in the dorsal and ventral extremities of the myotome which show a strong RV202-signal with a plaque-like appearance (right hand side in Fig. 2). Sclerotomal cells appear only weakly RV202-positive. Within the abdominal wall, RV202-positive cells are confined to blood vessel walls and further examples of such RV202-positive blood vessel walls in Fig. 2 are the umbilical veins, the aorta and the posterior cardinal veins in the genital ridge (see als Fig. 4a). The mesenchyme within the abdominal wall (Fig. 2) and within the limb buds (data not shown) is, in contrast to these blood vessels, virtually RV202-negative. This staining pattern produces a clear separation of the RV202-positive dermatoome and myotome. The coelomic epithelium appears RV202-negative covering the inner aspect of the abdominal wall and most of the lateral aspect of the genital ridge (Figs. 2, 5a). However, the coelomic epithelium covering the medial aspect of the genital ridge and the mesentery contains a few RV202-positive strands (Figs. 5a, 6a). Within the genital ridge the blastema of the mesonephros is RV202-positive whereas the mesonephric duct is negative (Fig. 5a). Other epithelial structures such as the surface endoderm, the remnants of the notochord, and the gut epithelium are distinctly RV202-negative. With respect to intracellular distribution, the vimentin staining varies between tissues: in epithelial structures such as the neural tube and the dermatoome, a fibrillar staining covering most of the cytoplasm predominates (Figs. 2, 4a) whereas in the mesenchyme and the coelomic epithelium, plaque-like and cable-like structures are outlined by the vimentin signal (Fig. 6a). Monoclonal antibody V9, the other vimentin antibody tested in this study, produces staining results identical to those obtained with monoclonal antibody RV202 (not shown).

Using keratin antibodies an almost complementary picture as compared to that seen with vimentin antibodies emerges (Fig. 3). The surface ectoderm, the remnants of the notochord, the epithelium of the primitive intestinal tube, and the mesonephric duct are all positive for antibody RCK106, which is specific for human keratin 18. A few RCK106-positive strands are seen in the ventral aspect of the neural tube but the remainder of the neural tube is negative. In the ventral extremity of the dermatoome there are a few RCK106-positive spots, apparently situated in the apices of dermatoome cells (Fig. 4b). Apart from this, there are no reactions with antibody RCK106 in the dermatoome or the myotome. Sclerotomal mesenchyme, too, is RCK106-negative. However, the mesenchyme within the abdominal wall and the dorsal mesentery, and the coelomic epithelium covering them, show strongly positive reactions with monoclonal antibody RCK106. Reminiscent of the staining picture obtained with vimentin antibodies there is a clear separation between the lateral border of the RCK106-negative dermatoome and myotome and the RCK106-positive mesenchyme of the abdominal wall (cf. Fig. 4a, b). A similarly clear separation is generated between RCK106-positive mesenchyme of the dorsal mesen-
Fig. 2. Horizontal frozen section through an 11 d.p.c. rabbit embryo at a level just below the forelimb buds. Indirect immunofluorescent staining with monoclonal antibody RV202, specific for vimentin. Positions of organ anlagen are illustrated in Fig. 1. × 170

tery and the RCK106-negative sclerotomal mesenchyme near the root of the dorsal mesentery. The mesenchyme within the limb buds, too, is strongly RCK106-positive (data not shown). Mesonephric blastema is RCK106-negative except for some positive cable-like structures which align to form a connection between the mesonephric blastema and the coelomic epithelium ventrally (Fig. 5b). The intracellular distribution of the keratin staining, too, varies between tissues. Mesenchyme and coelomic epithelium show single keratin-positive cable-like structures, which are connected to a strongly positive semi-circle near the nucleus, while in the epithelium of the primitive intestinal tube a network of fine fibrils covering most of the cytoplasm is outlined by the keratin antibody staining (Fig. 6b).

Amongst the other keratin antibodies tested in this study, all antibodies specific for human keratin 18 (mono-
clonal antibodies CK18-2, RGE53 and LE61), and the antibody specific for human keratin 8 (monoclonal antibody LE41) as well as the pan-epithelial antibody RCK102 (specific for human keratin 5 and 8) give immunohistochemical staining patterns identical to the ones obtained with monoclonal antibody RCK106 as presented above. Monoclonal antibodies RCK105 (specific for human keratin 7) and LP2K (specific for human keratin 19), however, do not react with fixed or unfixed 11 d.p.c. embryonic rabbit tissues.

**Immunohistochemistry**

Electrophoretic separation of the cytoskeletal preparation obtained from mesentery and gut tissues of 11 d.p.c. rabbit
embryos produces 4 distinct polypeptide bands of approximate molecular weights of 40 kD, 45 kD, 54 kD, and 57 kD, respectively (Fig. 7, lane 1). Following electrotransfer to nitrocellulose paper, antibody RCK102 (specific for human keratins 5 and 8) recognises the polypeptide of 54 kD and shows a weak reaction with the polypeptide of 57 kD (lane 2), antibody RCK106 (specific for human keratin 18) recognises the polypeptide of 45 kD (lane 3), and vimentin antibody RV202 recognises the polypeptide of 57 kD (lane 4). The control incubation, with second antibody only, is negative (lane 5). While antibody V9 (specific for vimentin of several species) also reacts with the band at 57 kD, all the other keratin antibodies tested in this study do not react in our immunoblotting experiments of embryonic rabbit tissues.

Discussion

Most of the monoclonal intermediate filament antibodies used in this study were originally characterised on human material. In our immunochemical experiments on SDS-denatured cytoskeletal material from the rabbit embryo, however, four of these antibodies specifically react with single rabbit proteins of molecular weights corresponding to that of the appropriate human intermediate filament protein. This is in agreement with the findings of Schermer et al. (1986) who show that the keratins of rabbit can be considered to be homologous to human keratins. Only the band recognised by the monoclonal antibody RCK102, which is specific for human keratins 5 and 8, has a slightly higher molecular weight (54 kD) in rabbit embryonic tissues than its human equivalent, keratin 8, which has a molecular weight of 52.5 kD (Moll et al. 1982a). However, the paired expression of the two rabbit keratins within the same tissues, as detected in our immunoblotting study, is another similarity with the human keratins 8 and 18, which are typical partners in forming pairs in human keratin filaments (Quinlan et al. 1983). The weak reaction of the monoclonal antibody RCK102 with the band of 57 kD molecular weight could represent either cross-reactivity with vimentin (although such cross-reactivity is not seen in tissue sections, e.g. in the neural tube) or an immunoreaction with a rabbit keratin comigrating with rabbit vimentin. Immunoblotting experiments using 2-dimensional gel electrophoresis of rabbit keratins would solve this question.

Vimentin, desmin and glial fibrillary acidic protein (GFAP) show a remarkably high degree of amino acid sequence homology (reviewed in Steinert et al. 1985). One might speculate that antibodies against these intermediate filament subunits show increased cross-reactivity. However, in the present study it seems unlikely that either monoclonal antibody RV202 or monoclonal antibody V9 react with GFAP or desmin, as GFAP is commonly expressed later during embryonic development and is confined to glial cells (Tapscott et al. 1981; Levitt et al. 1983). Desmin, on the other hand, is confined to the heart and the myotome, in the 11-day-old rabbit embryo, and as immunohistochemically determined using monoclonal antibodies, seems to show a different distribution pattern in comparison to vimentin within these tissues (Viebahn, in preparation).
assume, therefore, that the rabbit equivalents of human keratin 8, keratin 18, and vimentin, respectively, are specifically recognised in the rabbit embryo by the antibodies used in this study.

Immunohistochemically, some epithelia of the rabbit embryo such as surface ectoderm, endoderm, and the differentiating mesonephric ducts of the rabbit embryo seem to express keratins but not vimentin. Other epithelia, such as the neural tube and the myotome, express vimentin but not keratins, while a third type of epithelia, such as the coelomic epithelium and the dermatome shows coexpression of keratins and vimentin in some parts. Mesenchymal tissues express either keratin or vimentin, except for the mesenchyme of the mesentery, which expresses both. Most epithelial cells show a fibrillar intracellular distribution of the keratin or vimentin signal, while in many mesenchymal cells single cable-like structures and a perinuclear cap are outlined by the intermediate filament staining. Similar cap-like structures have been described before in vimentin stainings of the neural tube of chick embryos (Tapscott et al. 1981). These results make the rabbit embryo the first mammal in which intermediate filament expression can be demonstrated in intraembryonal organs other than the intestinal tube and the notochord at such an early developmental stage, and they pose the question whether the paucity of the immunohistochemically determined intermediate filament expression in comparable developmental stages of the mouse (Kemler et al. 1981) is due to lack of expression of the proteins or rather to reduced affinity of the antibodies used.

The unexpected expression of keratins in mesenchymal tissues coincides with the pattern of intermediate filament expression in quail (Erickson et al. 1987) and chick (Page 1988) embryos of similar developmental age. In these species, too, keratins are expressed in undifferentiated mesenchyme of the lateral plate mesoderm, which is the precursor of the mesenchyme in the dorsal mesentery and in the abdominal wall, during embryonic development. Equally surprising is the virtual absence of vimentin expression in mesenchymal tissues of the rabbit embryo such as in the mesenchyme of the abdominal wall. Vimentin expression in epithelia of vertebrate embryos, however, has to be regarded as a common phenomenon during development. Expression of only vimentin has, to date, been described in the neural tube of the chick (Tapscott et al. 1981), quail (Erickson et al. 1987) and rabbit (this paper), in the dermo-

Fig. 5a, b. Genital ridge (comprising mesonephric blastema and mesonephric duct) shown in Figs. 2 and 3 at higher magnification. Indirect immunofluorescent staining with monoclonal antibody RV202, specific for vimentin (a), and with monoclonal antibody RCK106, specific for keratin 18 (b). Dashed lines indicate surface of the coelomic epithelium. AW abdominal wall; CE coelomic epithelium; MB mesonephric blastema; MD mesonephric duct. × 450
myotome of the quail (Erickson et al. 1987) and rabbit (this paper), in the mesonephric duct of the golden hamster (Viebahn et al. 1987), and in embryonic human kidney (Holthöfer et al. 1984). Even more examples exist of coexpression of vimentin and keratins in embryonic epithelia (reviewed in Viebahn et al. 1987 and Van Muijen et al. 1987) to which the recent finding of a coexpression of vimentin and keratin 8 in the primitive intestinal tube of the 11.5 d.p.c. rat embryo (Paranko, personal communication) has to be added. All of these findings show that the characteristic specificity of keratins for epithelial and vimentin for non-epithelial tissues, which is normally found in the adult vertebrate body, is not as rigidly enforced in embryonic tissues. Moreover, taking into account the derivation of the embryonic tissues mentioned above from one of the 3 germ layers, these results also speak against a specificity of keratins for only organs of ectodermal or endodermal origin, or of vimentin for only mesodermally derived organs in the mammalian embryo. For the avian embryo, Erickson et al. (1987) and Page (1988) have already demonstrated such germ layer-independent expression of keratins and vimentin. However, earlier stages of embryonic development in the rabbit will have to be investigated in order to show intermediate filament expression in the developing germ layers themselves. Such investigations, in particular studies on the histogenesis of the somite (see below), might settle the question as to whether or not a relationship exists between intermediate filament expression and cell lineage.

In their study of intermediate filament expression in the parietal endoderm of the early mouse embryo, Lane et al. 1983 suggest that vimentin expression is a sign of motile cells. This view was supported recently by Erickson et al. (1987) and Page (1988) in their studies of early avian embryos. Our results are in accordance with this, since in the rabbit embryo, too, many motile cells such as in the neural tube, the neural crest, the dermatome, and the myotome show expression of vimentin. In particular, the virtual absence of vimentin expression in the mesenchyme of the abdominal wall, next to the vimentin-containing dermatome and myotome, illustrates how the different expression of vimentin reflects different migratory properties of the cells involved. Transplantation experiments using the quail-chick marker system have shown that dermomyotome cells have extensive migratory abilities, while somatopleuric cells retain their position, following orthotopic transplantation (Wachtler et al. 1982).

The expression of keratin in the mesenchyme of the abdominal wall, and of the dorsal mesentery of the rabbit embryo, however, suggests that keratin expression is a feature of undifferentiated mesenchymal cells, which provide support for motile components. The mesenchyme of the abdominal wall forms the pathway for axons, neural crest cells, and cells from the dermatome and myotome, and the mesenchyme of the dorsal mesentery is traversed by axons, neural crest cells, and primordial germ cells, for example. Keratin expression could, therefore, have a stabilizing and
mesonephric peritoneal funnel (Torrey et al. 1954), or whether it marks a cellular contribution of the mesonephros to the gonad (reviewed by Byskov 1986) which, at later stages, will develop in the angle between mesonephros and dorsal mesentery.

The intracellular appearance of the staining signals obtained in various tissues of the rabbit embryo in the present study raises two questions: Firstly, could the similar intracellular appearance of keratin and vimentin expression in some parts of the coelomic epithelium and in the underlying mesenchyme be a sign common to all cells which comprise the developmental "unit" formed by the mesenchyme and coelomic epithelium (Gruenwald 1942)? Secondly, do the fibrillar appearances of the intermediate filament staining, at one end of the scale, and the coarser, cable-like or cup-like staining structures, at the other extreme, reflect the degree up to which the intermediate filament cytoskeleton is connected to the network of intercellular contacts? The weakly keratin-positive spots in the apical parts of the cells in the ventral extent of the dermatome are indications of such a relation to apical cell contacts, since Mestres and Hinrichsen (1976) describe numerous cell contacts in chick dermomyotomes of comparable developmental stages. Further investigations are needed in order to clarify why the intracellular distribution of the staining signal of either vimentin or keratin antibodies in epithelia such as the neural tube, the dermatome, or the intestinal tube is different from the staining pattern observed in mesenchymal tissues.

The differential expression of vimentin in the dermatome on the one hand and in the myotome on the other, and within the myotome itself, seems to reflect the differentiation of myogenic cells. However, other specific signs of early myogenesis in vivo include myosin expression (Holtzer et al. 1957), acetylcholinesterase activity (Tennyson 1971; Miki and Mizuno 1982; Edde and El-Gadi 1986), and the expression of desmin (Tapscott et al. 1981: Danto and Fishman 1984; Solursh and Meier 1986), the muscle-specific intermediate filament protein. Only simultaneous analysis of the exact sequence of the expression of all these muscle-specific molecules during myogenesis in vivo will clarify how changes in intermediate filament protein expression are related to myotome differentiation. Furthermore, the origin of myotome cells, which still arouses controversy (reviewed in Edde and El-Gadi 1986), could be monitored with antibodies against these molecules.

In summary, our results show differential expression of the intermediate filament proteins vimentin and keratin in embryonic tissue to be more closely correlated with varying cell functions than with epithelio-mesenchymal organisation or germ layer-origin of tissues. This makes them seem to be more similar to the other groups of intermediate filament proteins (desmin, GFAP, and neurofilaments), which are expressed upon functional differentiation of cells during development.

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References


Minot CS, Taylor E (1905) Normal plates of the development of the rabbit (Lepus cuniculus L.). In: Keibel P (ed) Normentafeln zur Entwicklungsgeschichte der Wirbeltiere. Fischer, Jena, Fünfötel Heft


Wiebahn C, Lane EB, Ramaekers FCS (1987) The mesonephric (Wolfian) and paramesonephric (Mullerian) ducts of golden hamsters express different intermediate-filament proteins during development. Differentiation 34:175–188


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