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Review

The extracellular matrix and cell surface, mediators of cell interactions in chicken gastrulation

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The periplast ... which has hitherto passed under the names of cell wall, contents, and intercellular substance, is the subject of all the most important metamorphic processes, whether morphological or chemical, in the animal or the plant. By its differentiation every variety of tissue is produced.

T.H. Huxley (1853)
(Quoted by A.A. Moscona, 1974)

Introduction

Intercellular communication is a biological phenomenon of particular importance in understanding the developmental behavior of individual cells and tissue sheets brought into association by morphogenetic movements. It provides multicellular organisms with mechanisms that guarantee positional information to the cells and regulate intracellular activities, which ultimately lead to synchronized morphogenesis, growth and differentiation. The means by which cells and tissues communicate during embryonic development are of fundamental interest, and the old problem of "embryonic induction" stresses the importance that embryologists have attached to this field since the thirties (see Jacobson and Sater, 1988). Indeed, as soon as "Chemical Embryology" (Needham, 1931) was born, and as soon as the existence of an "inducing substance" (Holtfreter, 1934) or an "evocating substance" (Waddington *et al.*, 1936) was postulated, the question of the nature of this substance was raised. Nowadays, possibilities for re-investigating such problems related to "embryonic induction" have greatly increased due to the development of an ever-increasing number of analytical methods. Far from having identified *the* inducing substance (see Witkowski, 1985), our interest has shifted to concern with the subcellular events and molecular mechanisms underlying cell and tissue interactions, and to the identification of the multitude of factors mediating these interactions (for review: Gurdon, 1987). It now appears that some forms of communication require cell contact, whereas others involve transfer of information through the external milieu. The cell surface and the extracellular matrix are thus of major importance in regulating many developmental processes. Both are dynamic structures which, in response to genetic regulation and environmental stimuli, are able to undergo structural and functional changes that, in turn, may trigger intracellular events, including gene expression, in third cells (Bissell and Barcellos-Hoff, 1987).

Among the different vertebrate embryos that have been investigated in the past, the chicken embryo is probably one of the most widely used. Brachet (1974) very accurately noticed that "after man discovered artificial incubation, ...he must have had sufficient curiosity to break the shell immediately and look at the developing chick embryo". Advantages making the choice of gastrulating chicken blastoderms particularly attractive are the availability of eggs at any time of the year, the short duration of early development, the possibility of obtaining homogeneous populations of embryos of a required developmental stage by incubation of fertilized eggs for a given period, the possibility of delaying development by keeping the eggs cool, and the availability of a wide range of culture and microsurgery techniques that allow us to investigate developmental and cell biological processes (for review: Harrisson and Vakaet, 1989). Today the chicken embryo is a thoroughly described system as to structure and developmental fate, and topographical schemes and fate maps have been published (Vakaet, 1970, 1984b, 1985; Harrisson *et al.*, 1988a).

The mechanisms of intercellular communication are well-illustrated in chicken gastrulation (for review: Harrisson *et al.*, 1988a). Besides the several forms of junctional communication, the protrusive activity of the cell surface, and the physical fields measured in the vicinity of the germ layers, the chemical nature of the extracellular matrix and of the cell surface is central to understanding the molecular basis of cell and tissue interactions. Knowledge of the chemical nature of the factors mediating these interactions in gastrulation of the chicken embryo largely exceeds that of any other developmental system. It is, therefore, our aim to review thoroughly this information in the chicken blastoderm. However, for the sake of completeness or for the purpose of comparison, we will include information available from other gastrulating embryos as well as a brief discussion of factors that have not yet been investigated in the chicken embryo, but are presumably important in cell interactions. For convenience, this review of factors involved in cell interactions will successively deal with the extracellular matrix, the basement membrane and related structures, and the cell surface, but it is obvious that in practice it is often difficult to determine whether a given molecule should be regarded as an integral plasma-membrane molecule, as a molecule closely associated with the cell surface because linked to a mem-

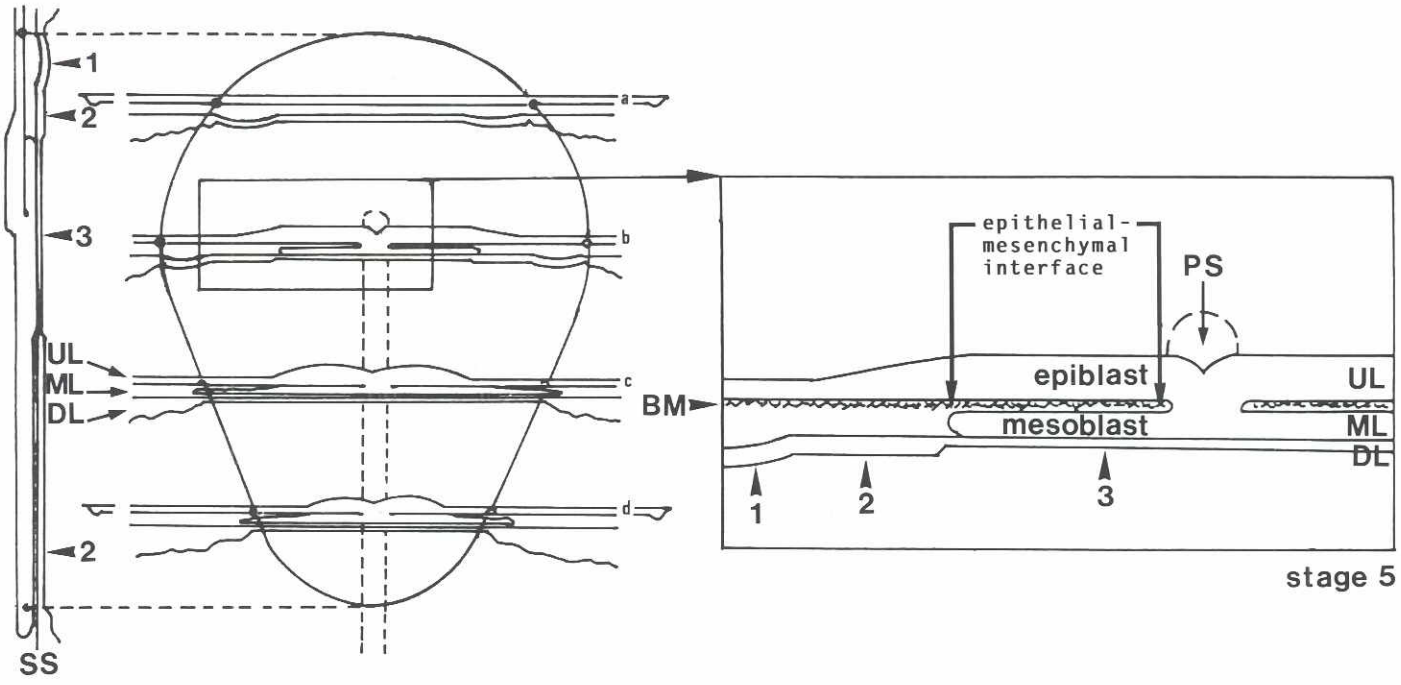


Fig. 1. Schematic representation of a stage-5 (Vakaet, 1970) chicken embryo, *to show the disposition of the different germ layers in a sagittal (SS) and in transverse sections (a-d). Part of transverse section b is depicted at a higher magnification, to represent the location of the basement membrane which, medially, forms an epithelial-mesenchymal interface. Abbreviations used in all figures: BM, basement membrane; DL, deep layer composed of endophyll (1), hypoblast (2), and definitive endoblast (3); ML, middle layer or mesoblast; PS, primitive streak; UL, upper layer or epiblast.*

brane receptor, as an integral component of the basal lamina, or simply as a matrix molecule of the intercellular milieu.

Note on terminology used

The terminology used to refer to the different germ layers and the series of stages used to identify a given developmental period of gastrulation are taken from Vakaet (1970; for review: Harrisson *et al.*, 1988a). Briefly, chicken gastrulation is divided into 10 stages, from 0 (the unincubated blastoderm) through 9, recognizable on the basis of changes in shape and morphology. During this period, the embryo progressively transforms from a two-layered germ to a three-layered germ. The germ layers are defined by the terms *upper layer* or epiblast, *middle layer* or mesoblast and *deep layer,* composed of endophyll, hypoblast and definitive endoblast. These terms have been introduced to refer to the relative position of the layers, without presuming their fate.

Finally, it should be mentioned that the terms *basement membrane* and *basal lamina* are equivalent in the chicken blastoderm, as the lamina fibroreticularis of typical adult basement membranes is missing during gastrulation. In this review, we use the term *basement membrane* to refer to the extracellular sheet, composed of a *lamina densa* and a *lamina lucida,* underlying the upper layer of the chicken blastoderm. In all other systems, a distinction between basement membrane and basal lamina (or its constituents) is made to distinguish results obtained by light microscopy from those obtained by electron microscopy.

A schematic representation of a primitive-streak stage-5 blastoderm is given in Fig. 1.

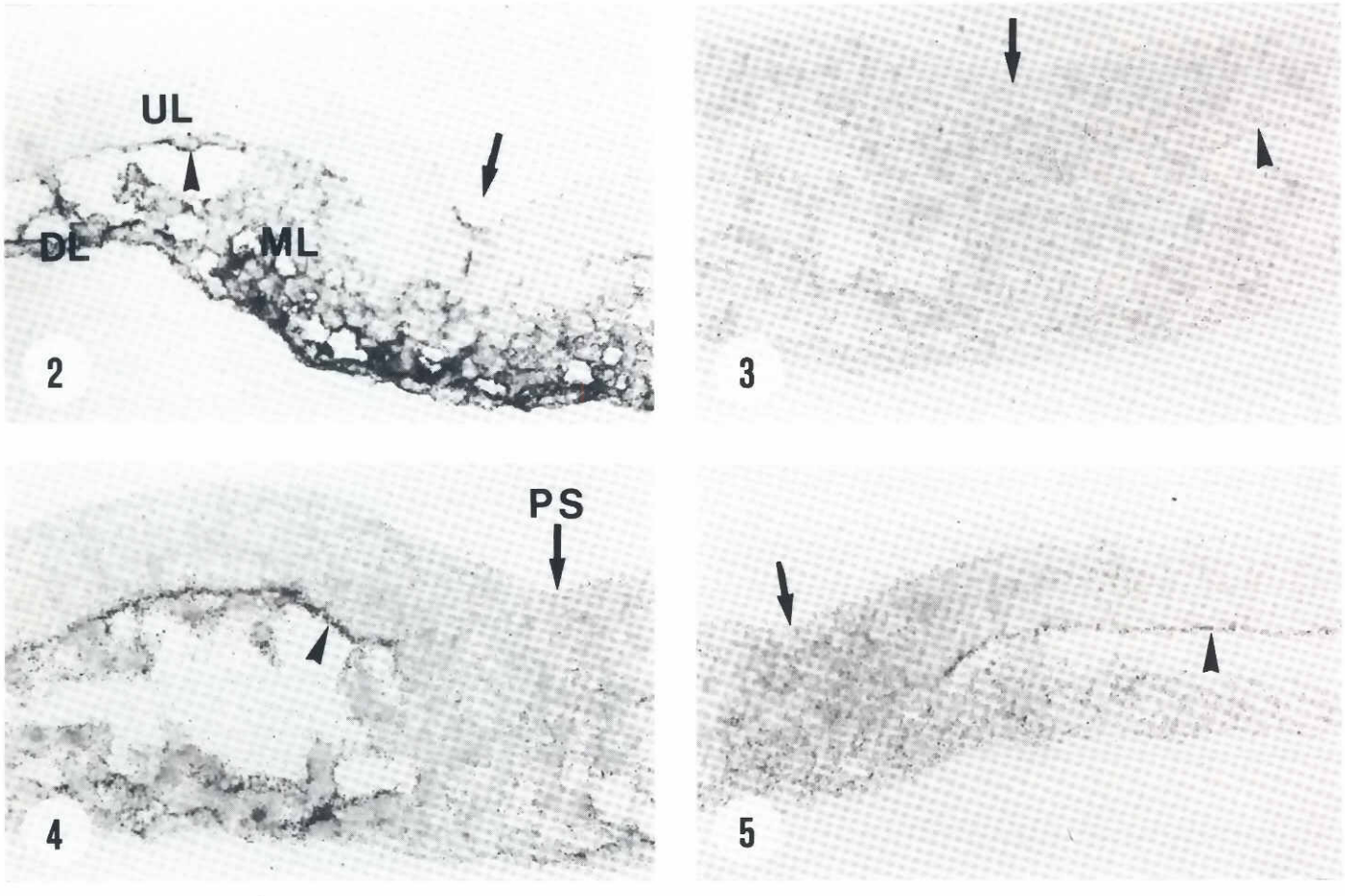
The extracellular matrix

The extracellular matrix of early embryos is composd of material that lies under epithelia, surrounds mesenchymal cells and comprises the substratum on which the cell or tissue may be attached *in vitro.* It may be divided into three main classes of macromolecules known as the glycosaminoglycans, the collagens and the non-collagenous glycoproteins. At the level of the cell surface, a structural and functional continuity probably exists between the cytoskeleton, the plasma membrane and the molecules of the matrix (for review: Burridge *et al.*, 1988), and it is therefore often difficult to locate a barrier between the plasma membrane proper and the extracellular matrix.

Glycosaminoglycans (GAGs)

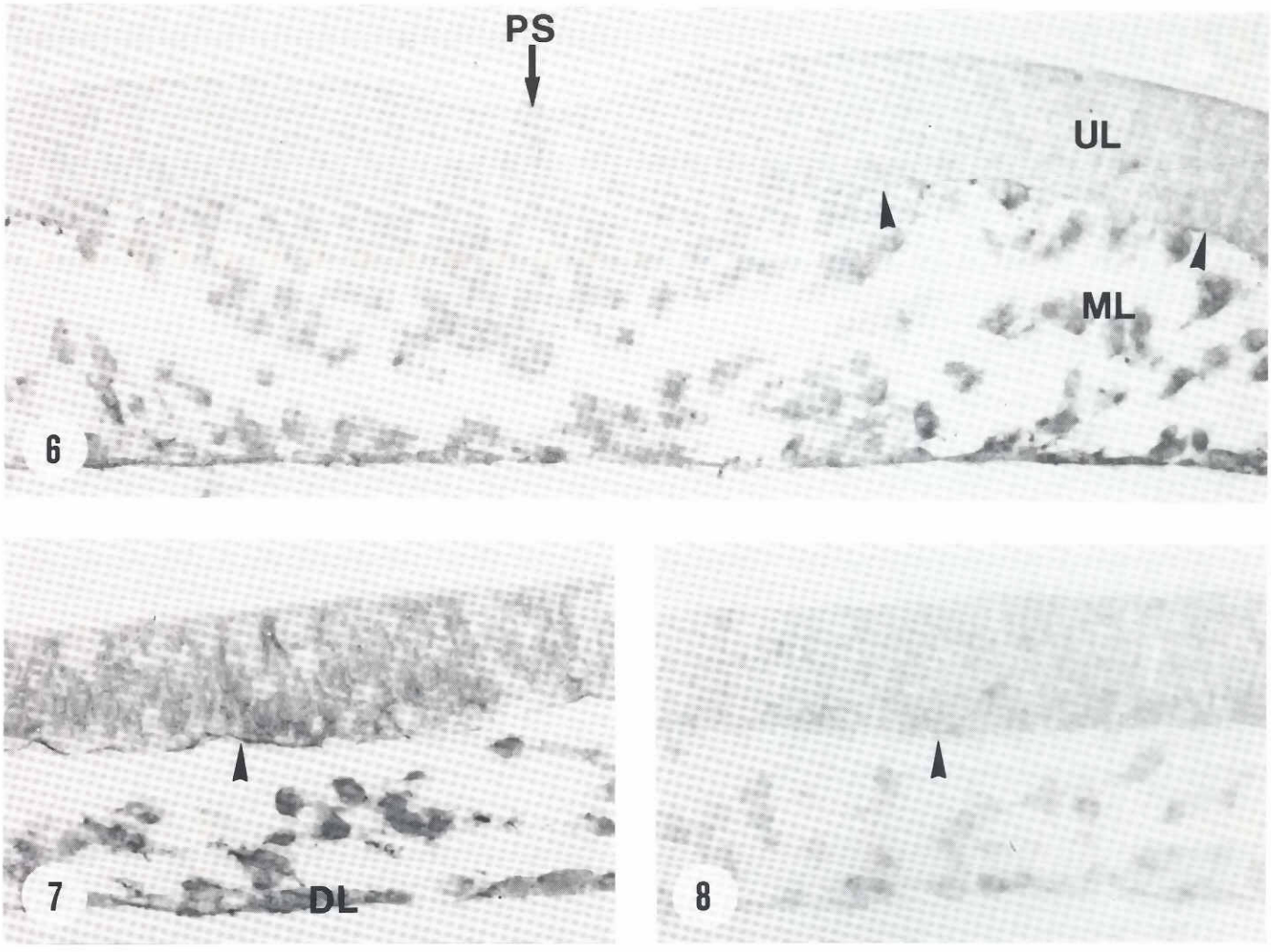
The GAGs are polyanionic carbohydrate chains composed of repeating disaccharide units. Hyaluronate differs from the other GAGs, such as chondroitin sulfates and heparan sulfate, in three respects: it is not sulfated, it is not linked to a protein and it has a much higher molecular weight than the proteoglycans (for recent reviews: Prehm, 1986; Ruoslahti, 1988b).

A large body of information is available on the expression, distribution, turnover and fate of GAGs in the blastoderm. Biochemically, GAGs labeled with ^{3}H-glucosamine or with ^{35}S-sulfate have been identified on the basis of their



Figs. 2-5. autoradiographic labeling of chicken blastoderm. (2) *Photomicrograph of a stage-6 chicken blastoderm after metabolic labeling with tritiated glucosamine for 30 min in culture. Autoradiographic labeling of glucosamine-containing macromolecules is present at the level of the primitive streak (PS, arrows), where upper-layer cells de-epithelialize, in migrating middle-layer cells (ML), in the deep layer (DL), as well as at the level of the basement membrane (arrowheads) of the upper layer (UL). Magnification: x420.* **(3)** *Shows that most of the autoradiographic labeling is sensitive to enzymatic digestion with testicular hyaluronidase, suggesting that the major incorporation of radiolabel occurs in GAGs. Magnification: x600.* **(4)** *Represents a transverse section at the level of the anterior half of the primitive streak (PS) after incorporation of tritiated glucosamine for 30 min and culture on a chase medium for 90 min. The absence of autoradiographic labeling at the level of the primitive streak reflects the rapid turnover of GAGs at that site. Magnification: x600.* **(5)** *Autoradiograph of a stage-4 blastoderm, showing a uniform incorporation of ^{35}S-sulfate in the three germ layers, and labeling of the basement membrane (arrowhead). From stage 8 on, labeling occurs mainly at the level of the developing notochord. Magnification: x420.*

electrophoretic mobility and of their sensitivity to GAG-degrading enzymes and, in the case of heparan sulfate, to nitrous acid. Hyaluronate appears to be the major GAG in gastrulation (Manasek, 1975; Solursh, 1976; Fisher and Solursh, 1977; Wakely and England, 1979; Vanroelen *et al.*, 1980a, b, c). Indeed, during stages 5-6 (stage 3+/4, Hamburger and Hamilton, 1951), hyaluronate represents the principal glucosamine-labeled electrophoretic peak, both in embryonic and extraembryonic tissues (Manasek, 1975), and this molecule represents 84% of the GAGs during gastrulation (Solursh, 1976). The synthesis of sulfated GAGs, which is not a unique characteristic of chondrogenic or other connective tissue cells, has been investigated after metabolic labeling with ^{35}S-sulfate. Early results of Johnston and Comar (1957), obtained by injection of the label in unincubated eggs and autoradiography after different incubation times, have shown that sulfated GAGs are present from the earliest stages and that regional differences in their distribution occur from late gastrulation on. In agreement with Manasek (1975), the major shortcoming of this method is, however, that each stage examined has been incubated for a different amount of time. Consequently, the label does not necessarily reflect synthesis, but rather final deposition of sulfated GAGs. A significant incorporation of sulfate into GAGs of the blastoderm has, however, been confirmed by Abrahamsohn *et al.* (1975), who reported that 41% of the label is incorporated in chondroitin 6-sulfate and 20% in chondroitin 4-sulfate, 38% being chondroitinase-resistant. Similar quantities have been measured by Solursh (1976), who found that, among the 10% of the GAGs that are sulfated, 55% are sensitive to testicular hyaluronidase (the chon-



Figs. 6-8. Photomicrographs of sections of stage-6 blastoderms stained with alcian blue at pH 2.2. *The results show that staining does not occur at the level of the primitive streak (PS, 6), whereas increasing amounts of GAGs are present in laterally migrating mesoblast cells and at the level of the basement membrane (arrowheads, 6-7). Pretreatment of serial sections of the same blastoderm with testicular hyaluronidase confirms that the alcian-blue staining reflects the presence of GAGs* **(8)**. *Magnification: x600.*

droitin sulfates) and 36%-39% are resistant to enzyme degradation, but sensitive to nitrous acid (heparan sulfate). No stage-related changes have been mentioned by Solursh (1976). Summarizing these results, it is thus reasonable to believe that about 94% of the GAGs that are synthesized during gastrulation represent hyaluronate (84%), chondroitin 6-sulfate (4%), chondroitin 4-sulfate (2%) and heparan sulfate (4%). The sulfated GAGs appear to be undersulfated (Manasek, 1975; Vanroelen *et al.*, 1980a), except in the area opaca where Manasek (1975) found fully sulfated chondroitin sulfates. Minute amounts of keratan sulfate and/or dermatan sulfate have also been detected in this area. Peculiarly, this author reported that chondroitin 4-sulfate is the only isomer whose synthesis was detected.

At the light-microscope level, information has been obtained by histochemical staining of GAGs with alcian blue and autoradiographic labeling with ^{3}H-glucosamine or with ^{35}S-sulfate, combined with enzymatic pretreatment of sections with several GAG-degrading enzymes. The autoradiographic approach has demonstrated a testicular hyaluronidase-sensitive fraction at the periphery of primitive streak cells, of middle-layer cells and of deep-layer cells, and a testicular hyaluronidase-resistant fraction at the ventral side of the epiblast, where a basement membrane is present (Figs. 2, 3) (Manasek, 1975; Vanroelen *et al.*, 1980a). These regional differences have been regarded as an early specialization of the extracellular compartment. Grain-density differences, obtained after various chase periods, have suggested the rapid renewal of the testicular hyaluronidase-sensitive fraction (Fig. 4) (Vanroelen *et al.*, 1980b). Using alcian-blue staining, accumulation of hyaluronate in extracellular spaces of deep-layer cells and of migrating middle-layer cells is evident

(Figs. 6-8), except at the level of the primitive streak (Fig. 6) (Fisher and Solursh, 1977; Vanroelen *et al.*, 1980c). Autoradiography of sulfate-labeled macromolecules has shown an equal grain density over all tissues during early gastrulation and labeling at the level of the basement membrane (Fig. 5) where, at least, chondroitin sulfates and N-sulfated heparan sulfate are present (Vanroelen and Vakaet, 1981). From stage 7 on, a remarkable labeling pattern is expressed in the head process (Johnston and Comar, 1957; Manasek, 1975; Vanroelen and Vakaet, 1981). Alcian-blue staining has confirmed the presence of sulfated and non-sulfated GAGs in the basement membrane (Fisher and Solursh, 1977; Vanroelen *et al.*, 1980c).

The relationship between the presence of GAGs and the migration of mesoblast cells has been investigated at the light and electron microscope levels after microinjection of GAG-degrading enzymes into the extracellular space of migrating mesoblast cells (Van Hoof *et al.*, 1984, 1986; for review: Harrisson *et al.*, 1988a). The removal of hyaluronate led to the loss of the mesenchymal aspect of the mesoblast, more particularly to compaction of mesoblast cells, retraction of cell processes and appearance of intercellular junctions. Consequently, the migrating mesoblast cells of experimentally manipulated embryos acquired the ultrastructural aspect of de-epithelializing cells at the level of the primitive streak of normal chicken blastoderms. We assumed that hyaluronate, which is a macromolecule that can be extensively hydrated, creates extracellular spaces, promotes detachment of ingressed cells and preserves the mesenchymal aspect of mesoblast cells during their lateral migration.

Collagens

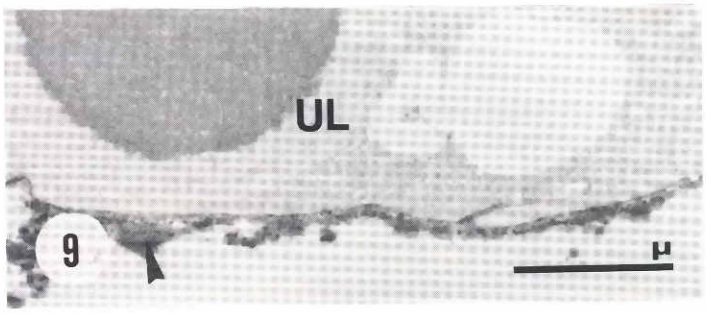
The collagens represent a family of glycoproteins that are found in all multicellular animals. The central feature of all collagen molecules is their triple-stranded helical structure composed of three polypeptide chains referred to as a-chains. So far, at least eleven distinct types of collagen have been defined (for review: Mayne and Burgeson, 1987). The major types are referred to as types I to V. After being secreted into the extracellular space, types I, II and III collagen molecules assemble into fibrillar polymers, which are cable-like structures clearly recognizable in electron micrographs. Types IV and V collagens do not polymerize into fibrils and have been localized to the basal lamina.

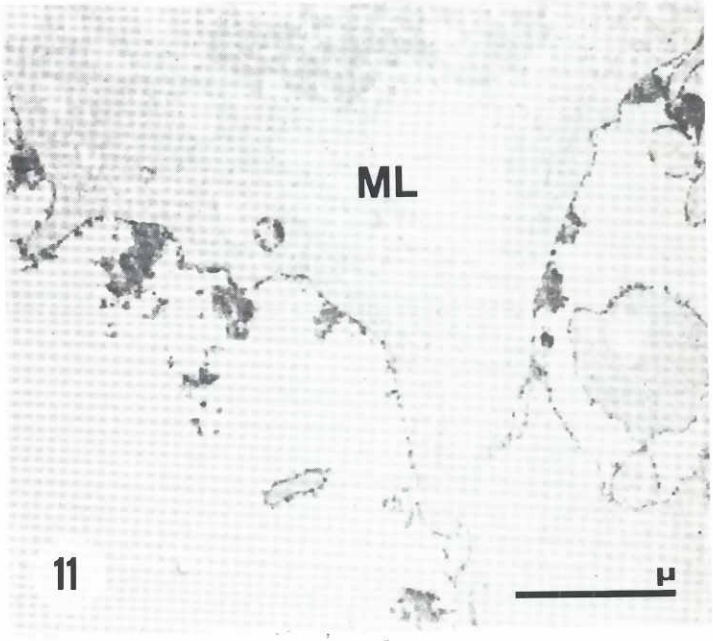
Little biochemical and immunocytochemical information is available during the period of gastrulation. Collagen synthesis has been examined by labeling primitive-streak chicken embryos with ^{3}H-proline, and subsequent analysis of the labeled proteins with cellulose chromatography (Manasek, 1975). The results of Manasek reveal that, at the time of formation of the primitive streak, both area opaca and area pellucida synthesize interstitial type I and type III collagens, and basal lamina type IV collagen, which represents approximately 5% of total collagen synthesis. These collagen types are widely distributed at the time of neural crest development (Duband and Thiery, 1987). Type II collagen is expressed somewhat later, at the time of formation of the notochord (Linsenmayer *et al.*, 1973). Indirect evidence for the presence of collagen in the basement membrane has been gained by inhibition of the extracellular deposition of procollagen by *cis*-hydroxyproline (Wakely and England, 1979). In embryos treated with this drug, no basement membrane was observed with SEM, and middle-layer cells did not spread. Type IV collagen has recently been localized to the entire basement membrane of gastrulating chicken embryos (unpublished data). Its distribution is correlated with the onset of formation of the basement membrane.

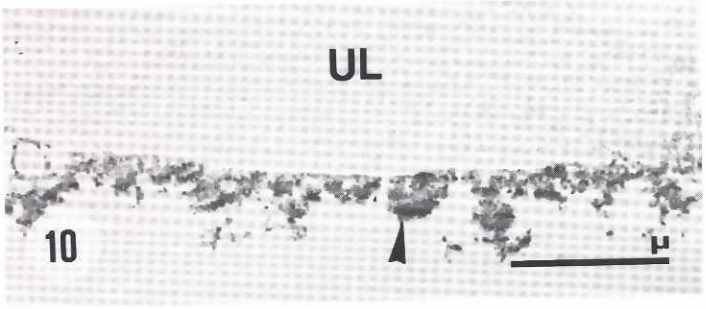
Non-collagenous glycoproteins

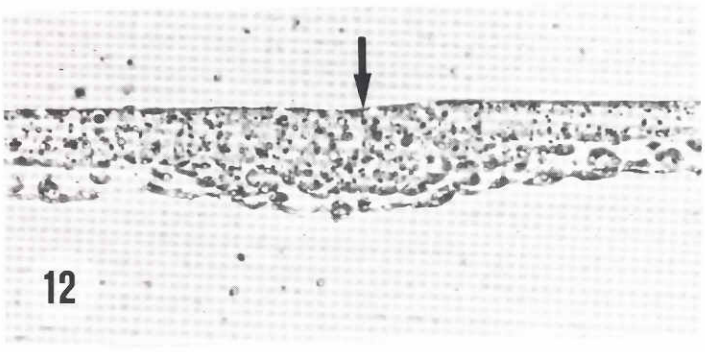
The non-collagenous glycoproteins represent a heterogeneous family of molecules that possess interesting binding interactions with other matrix constituents and with the cell surface. A number of reviews have evaluated in considerable detail the structure and function of fibronectin (for recent review: Mosher, 1989) and of laminin (for recent review: Campbell and Terranova, 1988), which are the best-characterized molecules. Fibronectin is a high-molecular-weight disulfide-bonded dimer (Mr=440-500kD), detected as immunologically cross-reactive forms in plasma and along cell surfaces. Although minor interspecific variations exist, all fibronectins are biochemically and immunologically related. Due to its functional domain structure, it interacts specifically with a number of biologically important extracellular molecules, hyaluronate includ-ed, and with the cell surface (Pierschbacher *et al.*, 1985). Laminin, first isolated from the basement membrane-secreting EHS sarcoma, is another high-molecular-weight glycoprotein (Mr=850-1000 kD) consisting of three disulfide bonded chains appearing most typically as a cross-shaped molecule on rotary shadowing images. Binding to type IV collagen, heparan sulfate proteoglycan, nidogen, as well as to a transmembrane 70-kD receptor, probably mediates the attachment of migrating individual cells and of epithelial cells to the basal lamina.

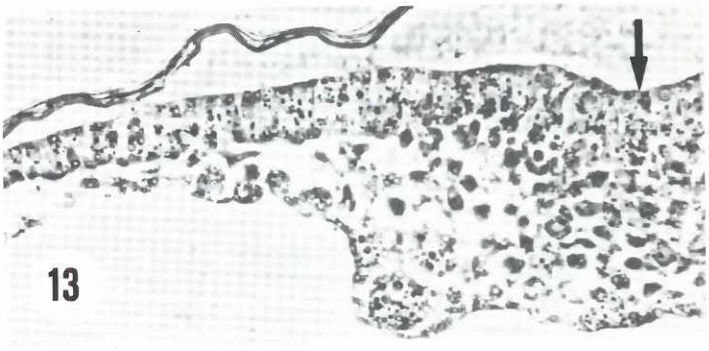
The role of several non-collagenous glycoproteins in cell adhesion and migration is nowadays largely recognized, and this prompted several authors to investigate their expression and distribution in early development. Although several glycoproteins have been described, only fibronectin and laminin have been the subject of investigations in the gastrulating chicken embryo (for review: Thiery, 1985; Thiery *et al.*, 1989). Using a whole-mount immunofluorescence technique, Critchley *et al.*, (1979) were first to demonstrate the presence of fibronectin along a band of parallel fibers that parallel the border of the area pellucida, at the level of the endophyllic crescent, along thin fibers running mainly at right angles to the embryonic axis, and in a meshwork situated at the outer blastoderm margin. On the basis of spatiotemporal correlations with morphogenetic events, these authors have implicated fibronectin, respectively, in a contact guidance system for the migration of primordial germ cells and mesoblast cells, and in the expansion of edge cells beneath the vitelline membrane (Critchley *et al.*, 1979; Wakely and England, 1979; England, 1981, 1982, 1983). From light-microscope studies, it has appeared that fibronectin is not present *in*

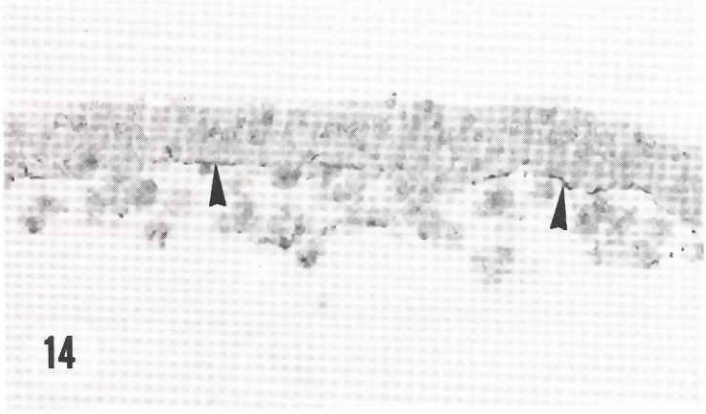


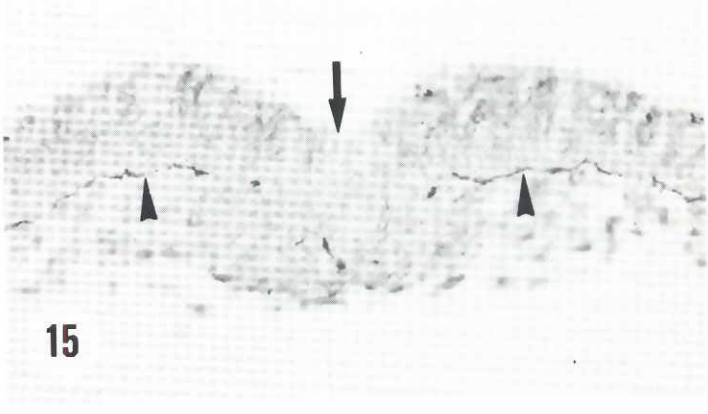












Figs. 9-11. *Ultrastructural localization of* **fibronectin** *(i) to the basal lamina and associated interstitial bodies (arrowheads), at the level of the endophyllic crescent* **(9)** *and of the primitive streak* **(10)**, *and (ii) to the cell surface and matrix of migratory mesoblast cells* **(11)** *of stage-8 blastoderms. Magnification: x20,000.*

Figs. 12-13. Semi-thin sections of epoxy-embedded stage-5 blastoderms *cultured for 5 h (control experiment:* **12***) or microinjected with anti-fibronectin antibodies and cultured for the same period* **(13)**. *In the latter case, note the enormous accumulation of de-epithelialized cells at the level of the anterior part of the primitive streak (arrow), and the absence of lateral migration, probably due to a lack of adhesion to similar cells and to the basement membrane. Magnification: x300.*

Figs. 14-15. *Immunocytochemical localizatin of* **laminin** *to the basement membrane of the upper layer. Note the presence of a discontinuous layer of laminin in stage-1 blastoderms* **(14)**, *and of a continuous sheet interrupted at the level of the primitive streak in stage-6 blastoderms* **(15)**. *Magnification: x600.*

utero, but is expressed soon after laying, from axis formation and early gastrulation on, in the whole basement membrane of the upper layer and sparsely in extracellular spaces of the deep layer (Duband and Thiery, 1982b; Mitrani, 1982; Mitrani and Farberov, 1982; Harrisson *et al.,* 1984b; Krotoski *et al.,* 1986).

Using different fixation methods, it has been shown that aqueous fixatives allow the demonstration of fibronectin in the whole basement membrane, but that fixation in ethanol or in aqueous solutions containing cetylpyridinium chloride, which preserves GAGs, causes masking of fibronectin immunoreactivity at the epithelial-mesenchymal interface (Harrisson *et al.,* 1984b, 1985a). This masking is reversible, since pretreatment of the sections with testicular hyaluronidase reveals fibronectin at that site. The masking thus appears to be the consequence of an interaction of GAGs and fibronectin in that particular area of the basement membrane that is underlaid by middle-layer cells. After microinjection of GAG-degrading enzymes in the living blastoderm, it has become clear that this particular site contains fibronectin that is masked *in vivo* by, at least, hyaluronate (Harrisson *et al.,* 1984a). Since this masking is correlated with mesoblast migration at all stages of gastrulation (Harrisson *et al.,* 1984b), it is suggested that remodeling of the interaction between GAGs and fibronectin in the basement membrane of the epiblast occurs as mesoblast cells migrate over it.

At the electron-microscope level, Sanders (1982) and Harrisson *et al.* (1985c) have demonstrated that the fibronectin content of the basal lamina increases as development proceeds, and that binding sites of anti-fibronectin occur in associated structures, such as interstitial bodies and fibrils (Figs. 9-10). Cell surfaces of mesoblast cells (Fig. 11) and of deep-layer cells are, in different degrees, binding sites of anti-fibronectin antibodies. Cell-surface labeling has been observed, most peculiarly, in coated pits, a specialized domain of the plasma membrane that has been implicated in the uptake of yolk or yolk protein (MacLean and Sanders, 1983). Shedding of fibronectin-coated, plasma membrane-limited vesicles with a diameter of 30-35 nm has been visualized in the area pellucida of gastrulating chicken embryos after fixation in a solution containing tannic acid, a mordant that enhances the subsequent staining of the unit membrane by heavy metals (Harrisson *et al.,* 1988b). The results suggested a cellular origin of the vesicles, and their presumptive recycling, since they were found in the vicinity of coated vesicles. We postulated that extracellular materials of the cell surface are packed in plasma membrane-coated vesicles and shed transiently in the environment where they may function in cell-cell and/or cell-matrix interactions. At the level of the area opaca, Monnet-Tschudi *et al.* (1985) and Kucera and Monnet-Tschudi (1987) observed fibronectin along radially oriented fibrils, and they found differences in the distribution of fibronectin that is associated with non-moving and moving cells. In the former case, the epithelial cells presented fibronectin staining exclusively at the level of their basement membrane, whereas in the latter case, edge cells of the margin of overgrowth and mesenchymal cells of the area vasculosa showed immunoreactive material around their entire surface and within the cytoplasm. Soon after gastrulation, fibronectin has been localized along pathways of migration of neural crest cells (Duband and Thiery, 1982a; Thiery *et al.,* 1982a) and of expansion of the vascular mesenchyme (Mayer *et al.,* 1981). Labeling of all basement membranes is a common feature in older chicken embryos (Linder *et al.,* 1975).

The assumption that fibronectin mediates migration of mesoblast cells during gastrulation has been based on spatial and temporal correlations between distribution of the molecule and migration, but not on experimental evidence. This prompted us to test the ability of anti-fibronectin antibodies to inhibit mesoblast migration in cultured blastoderms. Microinjection of affinity-purified anti-plasma fibronectin antibodies (generous gift of Prof. J.M. Foidart, Liège, Belgium) between upper layer and deep layer showed that binding of antibodies to the cell surface of mesoblast cells and to the basement membrane inhibits the lateral migration of these cells, but not the de-epithelialization of cells at the level of the primitive streak. Consequently, blastoderms microinjected with antibodies and cultured for some hours, presented very thick primitive streaks, especially at the level of Hensen's node where de-epithelialization is most active (Figs. 12-13). Ultrastructural analysis of microinjected embryos suggested that the de-epithelialized cells failed to emigrate from the primitive streak as a consequence of a lack of adhesion of cells to each other and to the basement membrane.

Laminin, another non-collagenous glycoprotein, has been localized by immunofluorescence from early gastrulation on, and seems to be restricted to the basement membrane (Mitrani and Farberov, 1982; Krotoski *et al.,* 1986; Duband and Thiery, 1987; Monnet-Tschudi and Kucera, 1988). Immunoperoxidase labeling after pepsin pretreatment of the sections demonstrates that laminin is, in fact, expressed shortly before laying, and is a marker of basement-membrane development (Figs. 14-15; Bortier *et al.,* 1989).

Finally, it is worthwhile to note that a 140-kD cell-surface receptor complex involved in adhesion to fibronectin and laminin has been observed outlining the circumference of most cells of the blastoderm, and along the basal surface of the epiblast, where a basement membrane is present (Duband *et al.,* 1986; Krotoski *et al.,* 1986).

The basement membrane and related structures

In addition to serving as a matrix around the germ layers, the extracellular macromolecules may also be assembled into specialized structures known as basement membranes (for recent review: Inoue and Leblond, 1988). In adult tissues, these extracellular sheets underlie epithelial cell layers, and surround individual muscle cells, adipocytes and Schwann cells (Vracko, 1978). As a consequence of the presence of heparan sulfate proteoglycan, of type IV and type V collagens, and of several non-collagenous matrix components (for recent review: Timpl, 1989) that are constantly present in evolution (Fessler *et al.,*

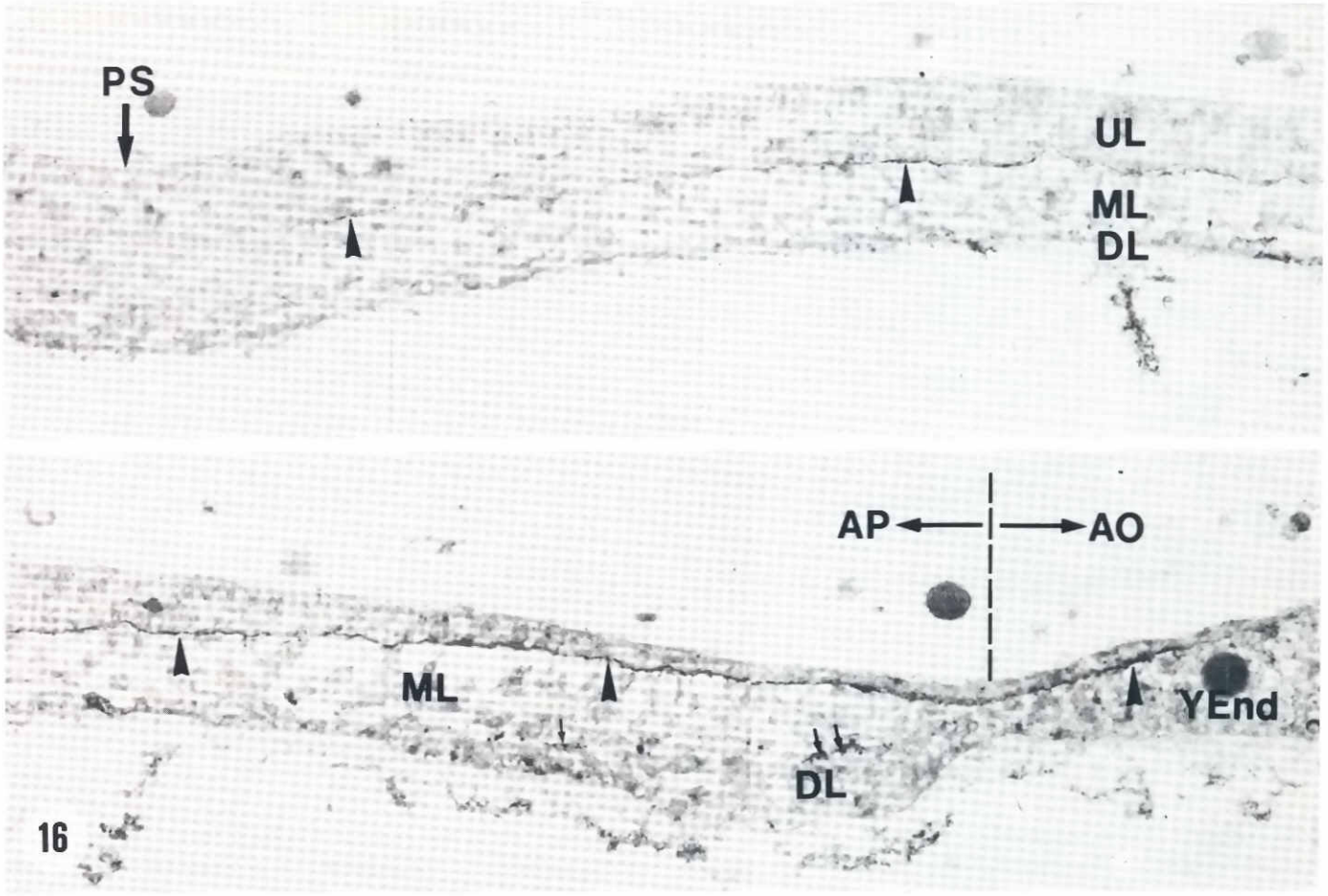


Fig. 16. Gradient of fibronectin immunoreactivity. *Transverse section at the level of the primitive streak of a stage-7 blastoderm fixed in aldehyde solution and immunostained for fibronectin. Note the gradient of fibronectin immunoreactivity in the basement membrane (arrowheads), increasing from the primitive streak (PS) to the edge of the area pellucida (AP). During late gastrulation, fibronectin is also expressed along the dorsal surface of the hypoblast (small arrows). Magnification: x600.*

1984), basement membranes have been implicated in migration and positioning of embryonic cells and cell layers (for review: Sanders, 1983).

Structure and chemical composition

In the chicken blastoderm, the basement membrane is composed of a lamina densa that is anchored to the basal surface of the epiblast by short strands running through a lamina lucida of about 60 nm thickness (for review: Sanders, 1983). Both layers taken together have a thickness ranging from 80 to 100 nm, depending on the preparative technique that has been used. As in the glomerular basement membrane, a pars fibroreticularis, composed of collagen fibrils and associated material, is not present at the time of gastrulation. Although non-striated fibrils have been reported at primitive-streak stages (Low, 1968; Sanders, 1979), it has not been demonstrated that interstitial types I, II, or III collagens are associated with these fibrils. Striated fibrils occur first at the six-somite stage, during heart development (Johnson *et al.*, 1974), but well-developed, mature connective tissue fibrils do not occur until the tenth day of incubation (O'Connell and Low, 1970).

The development of the basement membrane and associated structures has been investigated by Low (1967) and later on by Sanders (1979), who added tannic acid to the fixative. A primordium, consisting of a discontinuous basement membrane, is already present in the freshly-laid egg (Low, 1967). Little hyaluronidase-sensitive material (Sanders, 1979), little fibronectin (Duband and Thiery, 1982b; Harrisson *et al.*, 1984b) and little laminin (Mitrani and Farberov, 1982; Bortier *et al.*, 1989) are associated with the basement membrane of unincubated blastoderms and during the first hours of incubation. The amount of these materials progressively increases as gastrulation proceeds, to form a continuous sheet underlying the epiblast, except where polyingression of upper-layer cells occurs and, at the level of the primitive streak, where cells de-epithelialize. From stage 4 on, a gradient of fibronectin immunoreactivity, increasing from medial to lateral, is

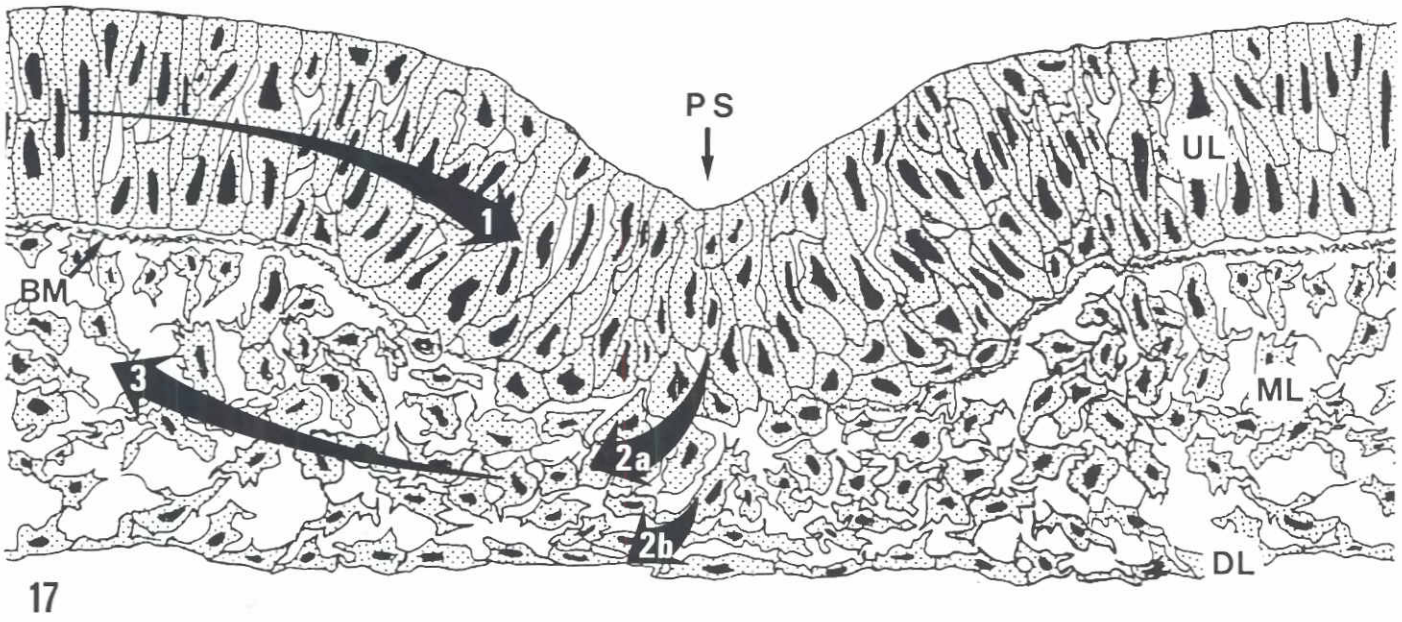


Fig. 17. Schematic representation of the morphogenetic movements leading to the formation of mesoblast. *The arrows indicate (1) a phase of movement of polarized upper-layer cells towards the primitive streak (PS), (2) a phase of de-epithelialization, which gives rise to middle-layer (ML, 2a) cells and to definitive endoblast in the deep layer (DL, 2b), and (3) a phase of lateral migration of individual mesoblast cells.*

clearly visible in the basement membrane (Fig. 16). This observation favors the idea that the basement membrane is used as a substrate for haptotactic migration of mesoblast cells. This hypothesis is supported by the fact that fibronectin possesses a chemotactic domain (Albini *et al.*, 1983) and chemotactic and haptotactic activities (Watanabe *et al.*, 1988). Similar observations pointing to the involvement of haptotactic mechanisms in the directional migration of individual cells have also been made in other systems (McCarthy *et al.*, 1983; McCarthy and Furcht, 1984; Zackson and Steinberg, 1987).

Two types of basement membrane specializations occur in the early chicken embryo. These are:

(1) the interstitial bodies, which appear as masses of extracellular materials closely related to the basement membrane (Low, 1970). They are especially numerous soon after gastrulation, where the neural tube separates from the upper layer (Low, 1970), and in the area vasculosa, where they are suspected to represent an adequate substrate for migration of the vascular mesenchyme (Mayer *et al.*, 1981). Fibronectin is, indeed, a constituent of these structures (Mayer *et al.*, 1981; Sanders, 1982; Harrisson *et al.*, 1985c). The possibility that interstitial bodies represent turnover products of the basement membrane to which they are attached, has been suggested by Sanders (1984).

(2) Fibronectin-rich fibrils (Low, 1968; Wakely and England, 1979; Andries *et al.*, 1985), which may guide directional migration of single primordial germ cells and of mesoblast cells (for review: England, 1981, 1982), as well as neural crest and sclerotome cells (Ebendal, 1977). Based on enzyme-digestion experiments, Frederickson and Low (1971) supported the hypothesis that these microfibrils, at least those associated with the notochordal basement membrane, are glycosamino-glycan-protein precursors of larger collagen-rich fibrils.

Role in mesoblast migration

The basement membrane may be regarded as an interface between the upper layer and the underlying tissue. In this respect, it has a double function. First, it serves as a scaffold underlying the upper layer and it contributes to the determination of the apicobasal polarity of upper-layer cells during their movement toward the primitive streak, where the basement membrane is interrupted to allow de-epithelialization of bottle-shaped cells. Second, due to its chemical composition, it serves as a natural substrate for adhesion and lateral migration of individual mesoblast cells (Jacob *et al.*, 1974; Revel, 1974; Ebendal, 1976; England, 1981; for review: Sanders, 1983). Consequently, three successive phases can be distinguished in the morphogenetic movements leading to the formation of mesoblast (Fig. 17). These are: (1) a phase of movement of polarized epithelial cells gliding as a tissue sheet towards the primitive streak (Vakaet, 1962); (2) a phase of de-epithelialization of upper-layer cells at the level of the primitive streak (Vakaet, 1984a); and (3) a phase of lateral migration of individual mesenchymal cells (Sanders,

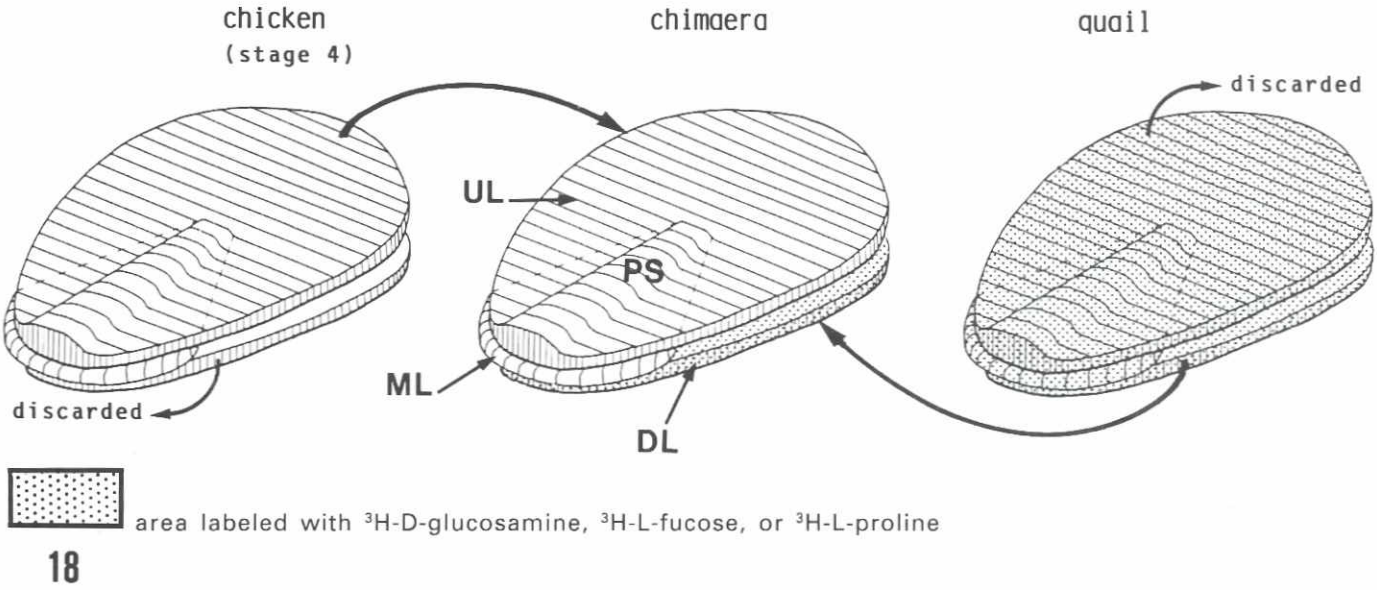


Fig. 18. Schematic representation of the experimental procedure *used for the preparation of radiolabeled stage-4 chimerae.*

1986). Especially the second and the third phases largely depend on cell-cell and cell-matrix interactions, and it has become increasingly evident that at least hyaluronate and fibronectin mediate in several ways the shape, ultrastructure and behavior of motile cells. Indeed, the phase of de-epithelialization occurs at a site where synthesis of hyaluronate is high (Fig. 2; Vanroelen *et al.*, 1980b), but accumulation is low (Fig. 6; Vanroelen *et al.*, 1980c). The cells are densely packed and linked together by intercellular junctions. The protrusive activity is low (Van Hoof *et al.*, 1986). This phase is independent of the presence of fibronectin, as shown after microinjection of anti-fibronectin antibodies (Fig. 13). During the final phase of lateral migration, middle-layer cells possess very few intercellular junctions, and they extend filopodia towards neighboring cells and toward the basement membrane. Increasing amounts of hyaluronate are present (Fig. 7) and it is believed that this GAG, by its physicochemical properties, plays an essential role in creating spaces (Toole, 1982). In the chicken blastoderm, this has been confirmed experimentally by Fisher and Solursh (1977) and by Van Hoof *et al.*, 1984, 1986), who observed compaction of the middle layer, appearance of intercellular junctions and retraction of cell protrusions after microinjection of hyaluronidases, and by Sanders and Chokka (1987), after treatment of the blastoderm with monensin. Fibronectin plays a major role in adhesion of middle-layer cells to each other and to the basement membrane, as evidenced by microinjection of anti-fibronectin antibodies. It is present along the cell surface of migrating cells, and it is a structural component of the whole basement membrane, which serves as a substratum for mesoblast migration and positioning. It is regarded as a permissive molecule (see Saxén, 1977) which may have the capability of influencing particular cell migratory events through (1) an interaction with other basement membrane components (Harrisson *et al.*, 1984a, 1985a) and/or (2) a haptotactic activity or adhesion gradient (Fig. 16). Finally, it should be indicated that little is known about the factors that govern the arrest of migration of mesoblast cells at their final location.

Cellular origin of components

In spite of the large variability in the chemical composition and function of basement membranes, it is generally believed that the cells which rest upon a basement membrane are responsible for the biosynthesis of the basal-lamina components, whereas the pars fibroreticularis originates from the connective tissue. In recent years, however, evidence for a contribution of underlying or adjacent tissue to the biosynthesis of the basal-lamina components has been provided (Brownell *et al.*, 1981; Kühl *et al.*, 1984; Sariola *et al.*, 1984a, b; Warburton *et al.*, 1984; Kimata *et al.*, 1985; Simon-Assmann *et al.*, 1988; Rescan *et al.*, 1989). Such evidence pointing to a dual cellular origin of basal-lamina components has partly been gained from the study of the chicken blastoderm (Harrisson *et al.*, 1985b; Harrisson 1986). This system has, indeed, the advantage of possessing a basement membrane composed of a lamina densa and a lamina lucida only, without pars fibroreticularis (Sanders, 1982; Harrisson *et al.*, 1985c). The transplantation experiments that have led to this conclusion are described here in some detail and, for the sake of completeness, some unpublished data have been added.

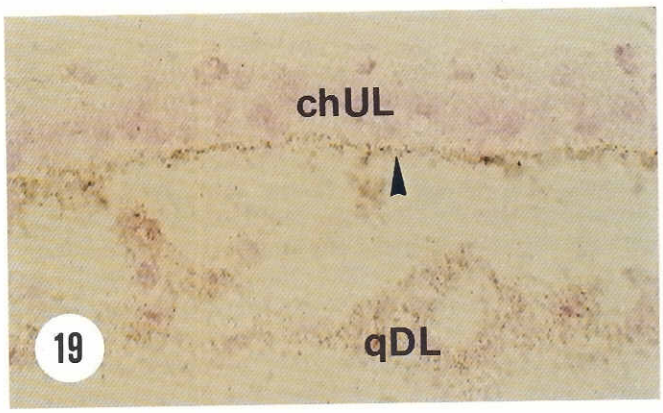
To test the hypothesis that the chicken basement membrane could be of dual cellular origin, we used chicken-quail chimeras (Fig. 18). The transfer of macromolecules from the deep layer (or from the middle layer) to the basement membrane of the upper layer was investigated

after transplantation of quail deep layers (with or without middle-layer cells), previously metabolically labeled for 2 h, into chicken blastoderms deprived of their own deep layer. The chimeras were allowed to grow for 5 h in culture before fixation and processing for autoradiography. The radiochemicals used for metabolic labaeling were D-glucosamine, which incorporates in GAGs and glycoproteins; L-fucose, which is specific for glycoproteins; and L-proline, which is predominantly present in collagens. The ability to distinguish chicken from quail cells on the basis of their different nuclear distribution of heterochromatin after Feulgen staining made it possible to determine the origin of the cells in the chimeras.

The results indicated that after transplantation of a D-glucosamine-labeled quail deep layer into an unlabeled chicken blastoderm, autoradiographic silver grains were present not only over the quail tissue as expected, but also at the ventral side of the chicken epiblast where a basement membrane is present (Fig. 19). Chase experiments excluded the possibility that unprocessed, tritiated D-glucosamine was transferred to the basement membrane, suggesting a participation of underlying tissue to the assembly of the basement membrane Enzymatic digestion of the tissue sections of the chimeras using chondroitinase ABC, *Streptomyces* hyaluronidase, or testicular hyaluronidase (Fig. 20) before Feulgen staining and dipping of the sections in nuclear emulsion suggested that at least one family of glycoproteins of the basement membrane originates from the deep layer, since GAGs had been removed enzymatically (Harrisson *et al.*, 1985c). This assumption was confirmed by the transfer of L-fucose-containing macromolecules from the quail deep layer to the chicken basement membrane (Fig. 21; Harrisson 1986). The transplantation of L-proline-labeled deep layers did not allow the visualization of any autoradiographic labeling at the level of the basement membrane This is in contradiction with the results of Nayyar *et al.* (1980) who used tritiated L-proline to show that epithelial as well as endothelial cells play a role in the formation of the glomerular basement membrane. We also observed that (1) the presence of labeled quail middle-layer cells was not necessary to obtain transfer of labeled macromolecules to the upper layer (Fig. 22), (2) the presence of unlabeled chicken middle-layer cells (Fig. 23) or of labeled quail middle-layer cells (Fig. 24) between the basement membrane and the labeled quail deep layer did not interfere with the final result, i.e. the labeling of the basement membrane after culture of the chimeras, and (3) a gradient of autoradiographic labeling, increasing from medial to lateral, was clearly observed using D-glucosamine or L-fucose as a radiolabel (unpublished data). This gradient correlates with the presence of fibronectin in the chicken blastoderm, as observed after immunolabeling of this glycoprotein (Fig. 16).

We concluded that at least one class of glycoproteins is synthesized by the deep layer and deposited in the basement membrane of the upper layer. Although the nature of these glycoproteins has to be investigated further, it is worth noting that carbohydrate analyses of the collagen component of several basement membranes did not reveal the presence of L-fucose (Kefalides *et al.*, 1979), and that, in our experiments, the use of L-proline as a precursor did not allow the visualization of a transfer of macromolecules from the deep layer or from the middle layer to the basement membrane of the upper layer. This would imply that a class of non-collagenous glycoproteins originates from one epithelium and is deposited in another. In this respect, it should be noted that chicken deep layer and upper layer but not middle layer in culture have the ability to synthesize at least fibronectin and laminin (Sanders, 1980; Mitrani and Eyal-Giladi, 1982), and that both non-collagenous glycoproteins are present in the basement membrane. We postulate that the deep layer is jointly responsible for the production of fibronectin and its deposition in the basement membrane of the epiblast, since the mesenchyme is not able to produce this molecule in culture (Sanders, 1980). On the basis of the results obtained after enzymatic degradation of GAGs, we believe that these glycoconjugates are produced by the upper layer itself, as in the enamel organ, where the GAGs are also of epithelial origin (Frank *et al.*, 1979). A participation of underlying tissue into the assembly of a basement membrane has been shown by several authors (Kühl *et al.*, 1984; Sariola *et al.*, 1984a, b; Warburton *et al.*, 1984; Kimata *et al.*, 1985; Simon-Assmann *et al.*, 1988; Rescan *et al.*, 1989). In particular, Brownell *et al.* (1981), Osman and Ruch (1981) and Hurmerinta *et al.* (1986) noted that fibronectin, which is produced by dental papilla mesenchyme, can be isolated from the basement membrane of enamel organ epithelial explants which do not make fibronectin themselves. Fibronectin in the base-

Figs. 19-26. Xenograft experiments. (19-24) *Photomicrographs of Feulgen-stained sections of xenografts in which the quail (q) deep layer (DL) has been metabolically labeled previous to transplantation in a chicken (ch) blastoderm, as represented in Fig. 18. After transplantation of a glucosamine-labeled quail deep layer, the autoradiographs demonstrate silver grains at the level of the basement membrane (arrowheads) of the chicken upper layer (UL)* **(19)**. *This radiolabeling is not sensitive to treatment with testicular hyaluronidase, suggesting the incorporation of tritiated glucosamine in glycoproteins, and not (or very few) in GAGs* **(20)**. *Similar results, namely the transfer of labeled macromolecules from the quail deep layer to the chicken upper layer, are obtained by the use of tritiated fucose as a radiolabel. This is demonstrated in* **(21)**, *a photomicrograph taken at the level of the primitive streak (arrow).* ***(22)*** *through* ***(24)*** *demonstrate that the absence of middle layer (ML) at the level of the endophyllic crescent* **(22)**, *the presence of unlabeled chicken middle layer* ***(23)***, *or the presence of labeled quail middle layer* **(24)** *do not influence the final result, i.e. the labeling and transfer of fucose-containing macromolecules to the basement membrane (arrowheads) of the chicken upper layer. Magnification: x600.* **(25-26)** *Photomicrographs of Feulgen-stained sections of chimeric blastoderms obtained by orthotopic transplantation of a piece of glucosamine-labeled quail (q) upper layer (UL) into the upper layer of an unlabeled stage-4 chicken (ch) blastoderm, and cultured for 6 h* **(25)**, *which represents the lateral edge of the graft of quail origin, shows autoradiographic labeling of the grafted tissue and along the ventral side of the chicken and quail upper layers, where a basement membrane is present. The extracellular space of the chicken middle layer (ML) is also labeled.* **(26)**, *which represents the medial edge of the graft, shows no labeling at the chicken epithelial-mesenchymal interface. Magnification: x600.*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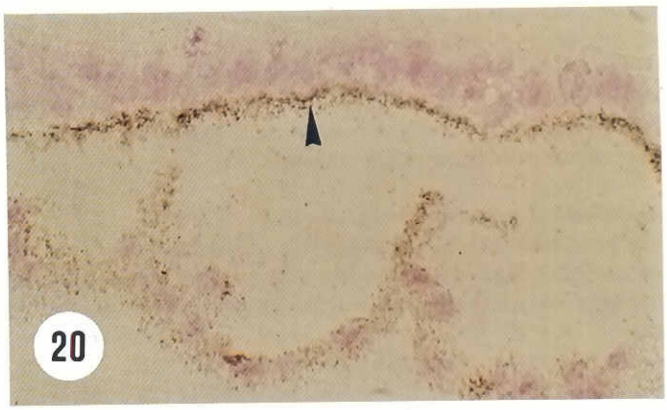
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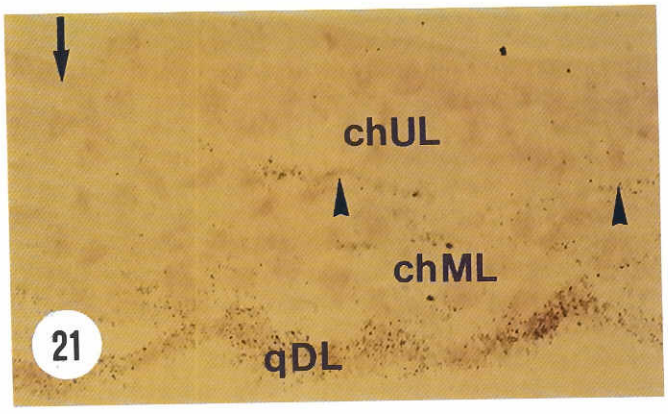
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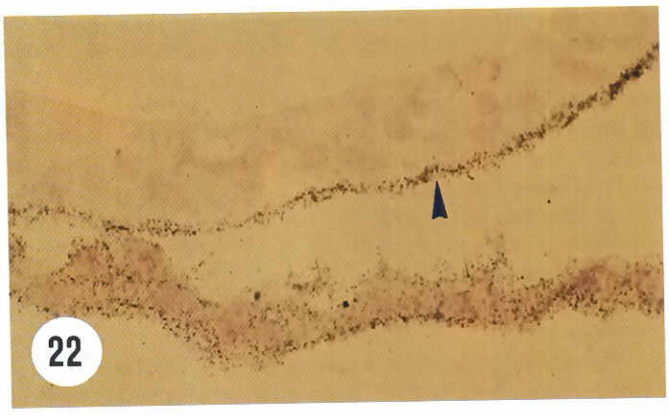
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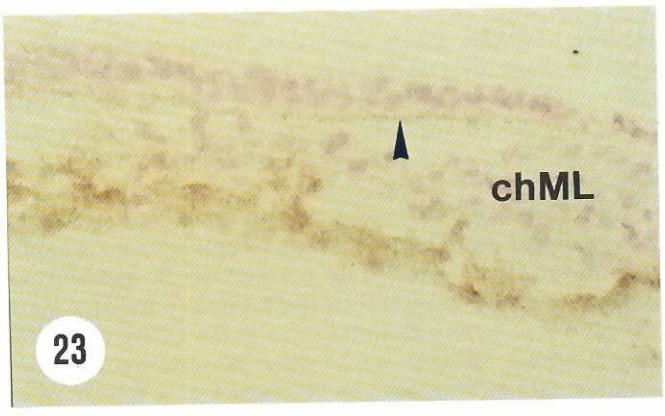
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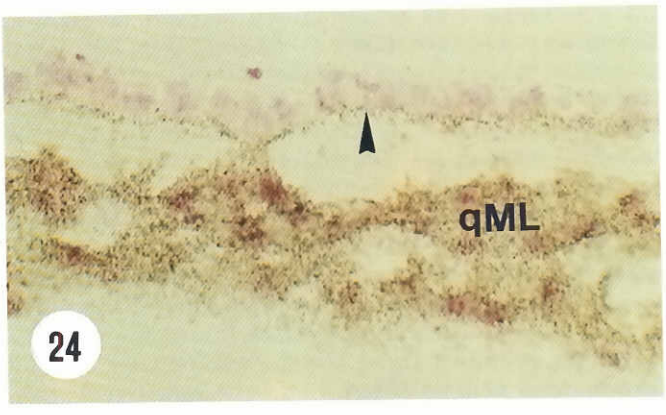
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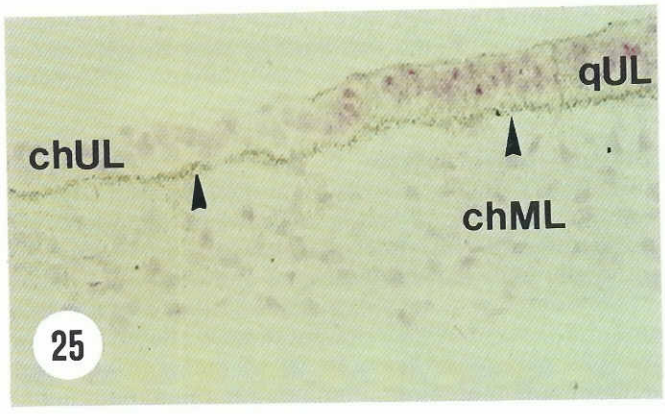
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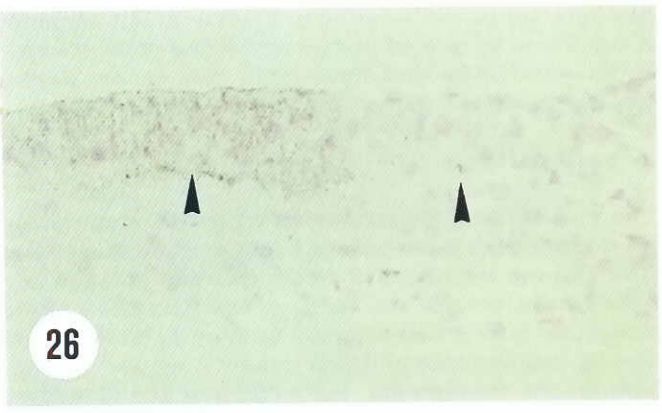
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ment membrane of the developing tooth is produced exclusively by the differentiating mesenchymal cells.

This review has shown that, in addition to a large variability in chemical composition and function of basement membranes, a diversity in cellular origin and mode of assembly of components is evident.

Remodeling of macromolecules

Osman and Ruch (1981) were probably the first to demonstrate that the mesenchyme not only controls the synthesis of epithelially-derived D-glucosamine-labeled material (the GAGs, see Frank *et al.*, 1979) in the dental basement membrane, but also its degradation. This has been confirmed by Smith and Bernfield (1982), who demonstrated that the mesenchyme of developing salivary glands degrades basal lamina GAGs and provokes a rearrangement of components (for review: Bernfield *et al.*, 1984). In an attempt to visualize the remodeling of basement membrane components during gastrulation, Van Hoof and Harrisson (1986) used chimeric embryos resulting from the orthotopic transplantation of pieces of D-glucosamine-labeled quail epiblast into unlabeled chicken blastoderms. After a 5-7 h period of culture, they observed the presence of silver gains not only in the grafted quail tissue and at the level of its basement membrane, but also lateral to the graft, at the level of the chicken basement membrane (Fig. 25). This labeling extended as far as the edge of the area pellucida, i.e. in a region of chicken tissue situated more laterally than the initial position of the graft, and it was present only in areas where the chicken basement membrane was underlaid by chicken mesoblast. Labeling was absent medially to the graft (Fig. 26). Silver grains were noted in the extracellular matrix of migrating chicken mesoblast cells as well. Taking into account the movement of epiblast cells towards the primitive streak where they ingress, and the counter movement of mesoblast cells along the basement membrane of the epiblast, which is used as a substrate, Van Hoof and Harrisson (1986) hypothesized that chicken mesoblast cells picked up D-glucosamine-labeled materials in the basement membrane of the quail graft and left them behind during their lateral migration. These results, supported by the observation that the primitive streak and the middle layer present hyaluronidase activity (Stern, 1984), suggest that mesenchymal cells mediate the redistribution of basement membrane components during gastrulation. This observation could also be related to the remodeling of the interaction between GAGs and fibronectin that occurs during mesoblast positioning (Harrisson *et al.*, 1984b, 1985a).

The cell surface

The cell surface appears to be of prime importance, since it represents the interface between the extracellular matrix and the interior of the cell. Its importance is evidenced by the fact that the ability of cells to adhere to the extracellular matrix is shared in a number of phenomena, including maintenance of tissue integrity, wound healing, morphogentic movements, cell migration and spreading and metastasis. Several factoras at the cell surface may govern such phenomena or, at least influence cell behavior. In particular, the alignment of extracellular fibrils containing fibronectin or basement membrane heparan sulfate proteoglycan with focal contacts and intracellular actin (Heggeness *et al.*, 1978; Hynes and Destree, 1978; Singer, 1979; Hynes, 1981; Singer *et al.*, 1984, 1987b) and vinculin (Singer and Paradiso, 1981; Singer, 1982a), along with the organizing effect of exogenous fibronectin on the cytoskeleton (Singer, 1982b; Singer *et al.*, 1987a), has led to the hypothesis that transmembrane receptors exist that link the cytoskeleton to the extracellular matrix (Tamkun *et al.*, 1986; Lacy and Underhill, 1987; in development: Hay, 1982, 1983, 1984). It is not our aim to overview in this paragraph the extensive literature on this matter, but we feel that an introduction to those aspects that are of general relevance to intercellular communication, and of particular relevance to the study of the extracellular matrix in the chicken embryo, is necessary.

Cell-surface receptors

In recent years, a series of cell-surface receptors for extracellular matrix molecules have been identified (von der Mark *et al.*, 1984; von der Mark and Kühl, 1985; Hynes, 1987; Smalheiser and Schwartz, 1987; Underhill *et al.*, 1987; De Strooper *et al.*, 1988; for reviews: Buck and Horwitz, 1987a; Juliano, 1987; Ruoslahti, 1988a). Among these, one of the first molecules to be identified and characterized extensively as putative receptor for extracellular matrix molecules has been the 140-kD avian integrin, also known as CSAT antigen (Horwitz *et al.*, 1985; Buck and Horwitz, 1987b), which possesses binding properties for both fibronectin and laminin. This multifunctional extracellular matrix receptor has now been localized on several cell types, and it appears to be present in the gastrulating chicken embryo (Duband *et al.*, 1986; Krotoski *et al.*, 1986) where it codistributes with its ligands. According to Hynes (1987), and based on structural and functional similarities, this receptor belongs to a family of cell-surface receptors, the integrins, composed of a common ß -subunit, with which the CSAT monoclonal antibodies react, and a set of variable a-subunits (2 a-chains in avian CSAT antigen). In recent years, several surprising developments have emerged which suggest the existence of a family of cell-surface receptors for adhesion proteins, including fibronectin and vitronectin, all of which recognize the Arg-Gly-Asp sequence of the cell-binding domain (for reviews: Ruoslahti and Pierschbacher, 1986; Juliano, 1987; Yamada and Kennedy, 1987; Ruoslahti 1988a).

Glycosyltransferases

Besides the existence of cell-surface receptors for extracellular materials, which is central to understanding the link between extracellular and intracellular molecules, it is generally believed that adhesive and migratory behavior of embryonic cells also depends on other surface properties. The nature of the oligosaccharide chains exposed at the cell surface appears to be of major importance to several cell biological phenomena; the function of the

polysaccharide antennae exposed at the cell surface could be to sense chemical changes in the pericellular environment via the interlocking of complementary molecules which could interact in a lock-and-key fashion. Cell-cell and cell-substrate interactions could take placae between a protein and the carbohydrate portion of a cell-surface molecule. These may involve the glycosyltransferase enzymes. Although the existence of these enzymes at the cell surface has sometimes been a matter of debate (Keenan and Morré, 1975; Deppert and Walter, 1978), a cell-surface adhesion/recognition system has been postulated (Roseman, 1970; Roth, 1973; Shur and Roth, 1975). Recognition and communication between cells or between cells and substrates result from interactions between cell-surface glycosyltransferases and their glycosyl acceptors on apposing cell surfaces or on migration substrates. In this model, the cell-surface enzyme catalyzes the transfer of a monosaccharide from its uridine diphosphate donor to the terminus of the acceptor saccharide chain in three steps. First, a surface glycosyltransferase on cell A binds to its acceptor molecule on cell B (or on a migration substrate), leading to adhesion of the cells to each other (or to the substrate). Second, the enzyme on cell A glycosylates the cell surface of cell B (or the substrate) when it is provided with the appropriate sugar-nucleotide synthesized in the cytoplasm of cell A or B, leading to an intercellular modification of the antennae. And third, cells A and B (or cell A and its substrate) dissociate, the surface properties of cell B (or of the substrate) being changed after glycosylation. The binding of a new saccharide is postulated to result in modulation of cell adhesive characteristics. The gastrulating chicken embryo has been the subject of detailed examination for seven classes of endogenous cell-surface glycosyltransferase activities (Shur, 1977a, b), which have been named according to the monosaccharide that is transferred from the donor uridine diphosphate to the acceptor sugar. It appears that the autoradiographic patterns of galactosyl-, N-acetylglucosaminyl-, fucosyl- and sialyltransferases are most intense on ingressing primitive-streak cells, primordial germ cells and cranial neural crest cells. On the contrary, glucosyl-, N-acetylgalactosaminyl-, and glucuronyltransferases are relatively inactive under the assay conditions. Based on the adhesion/recognition model of Roseman (1970), Shur suggested that embryonic cells migrate over carbohydrate substrates via surface transferase binding to exposed oligosaccharide chains. This is supported by observations on mesenchyme migration-defective mouse T/T mutants, which possess galactosyltransferase only (Shur, 1982), and on the coincidence between the appearance of galactosyltransferase immunoreactivity and the onset of cell migration (Sato *et al.*, 1984).

Endogenous lectins

Alternatively to glycosyltransferase, endogenous lectins, which may bind to the carbohydrate portion of glycoproteins, proteoglycans and glycolipids of apposing cell surfaces, represent another class of carbohydrate-binding molecules acting as candidates for intercellular adhesion between cells (for reviews: Rutherford and Cook, 1984; Zalik and Milos, 1986). Lectins, by definition, are carbohydrate-binding proteins or glycoproteins of non-immune origin that generally have the property of agglutinating cells by reaction with cell-surface carbohydrate determinants (Nicolson, 1974). Besides the fact that exogenous lectins have become usual probes for mapping the distribution of surface saccharides in the chicken blastoderm (Zalik and Cook, 1976; Höök and Sanders, 1977; Sanders and Anderson, 1979) and for studying their role in cell migration (Lee *et al.*, 1976, 1978), we must be aware of the fact that a number of endogenous lectins have been found associated with embryonic cell surfaces. In the chicken, for example, a ß-D-galactose-binding lectin is already present in extracts of preincubated blastoderms (Cook *et al.*, 1979). This lectin has been isolated from primitive-streak embryos (Zalik *et al.*, 1983), and it has been involved in Ca^{++}-independent adhesion of extraembryonic endoderm cells (Milos and Zalik, 1982, 1983). *In vitro* studies with lectins have shown their ability to influence cell attachment and spreading, suggesting such a role *in vivo* (Milos and Zalik, 1981, 1986), and dissociated cells have galactose-bearing receptors at their surfaces, as shown by the agglutinability of these cells by plant lectins (Phillips and Zalik, 1982). Moreover, immunocytochemical localization of this lectin in cultured cells has demonstrated its presence intracellularly and in extracellular material deposited by moving cells (Zalik *et al.*, 1982). Recently, Zalik *et al.* (1987) have demonstrated the expression of lectin activity specific for ß-D-galactoside groups, not only by cells of the extraembryonic deep layer, but also at the lowest portions of the primitive streak, where cells emigrate laterally to form the definitive endoblast, and by primordial germ cells of the proximal area opaca. Lectin activity increases significantly in older embryos, during the spreading of the yolk sac, in particular in the area vasculosa (Mbamalu and Zalik, 1987). The results of Zalik and co-workers thus indicate that this lectin is expressed predominantly in areas that are undergoing cell spreading.

Besides this ß-D-galactose-binding lectin, a mannan/fucan-binding protein has been localized not only in plasma membranes, but also in the vitelline membrane where it could be involved in the attachment of cells to the vitelline membrane (Rutherford and Cook, 1981). This putative function does not hold for a lectin with an affinity for certain sulfated polysaccharides, which has been localized by Cook *et al.* (1985) in the outer layer of the vitelline membrane. According to these authors, this lectin is not involved in adhesion of the blastoderm to the vitelline membrane, nor is it involved in the expansion of the blastoderm nor in maintaining the strength of the membrane. With the exception of this last lectin, endogenous lectins from the surface of embryonic cells have generally been considered to function in cell adhesion and migration.

Glycosphingolipids

What is the nature of the saccharide-bearing molecules of the cell surface, and what is their relation to the extracel-

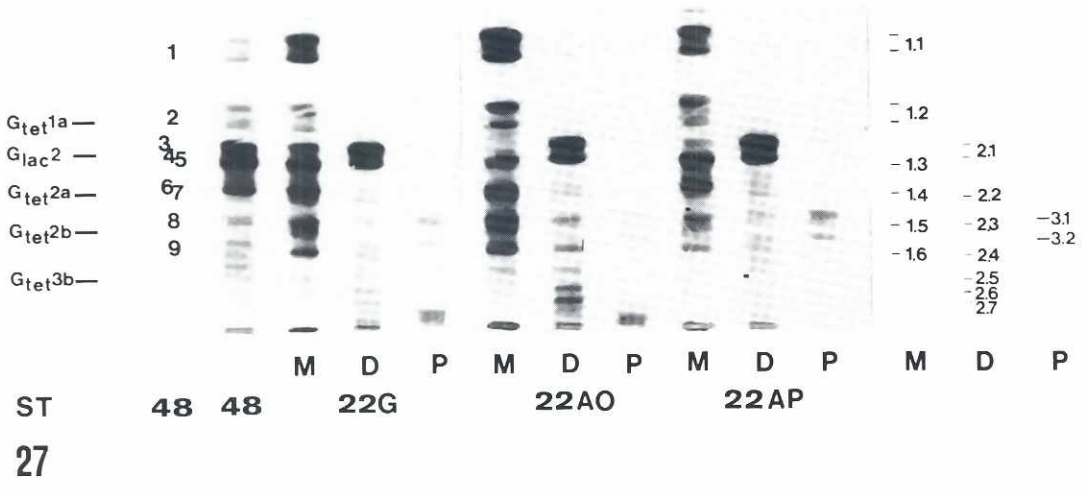

Fig. 27. High-performance thin-layer chromatography analysis of metabolically ^{3}H-labeled acidic fraction glycolipids *in early chicken embryos. The embryonic tritiated glycolipids were detected by autoradiofluorography.* Lane ST, *running properties of acidic standard glycolipids from human brain.* Lane 48, *tritiated acidic standard glycolipids of the area pellucida of 48-h embryos, with the following components: 1, $G_{gal}1$, $G_{lac}1$; 2, $G_{tet}1a$, nLc_4-derivatives; 3-5, $G_{lac}2$; 6-7, $G_{tet}1a$-derivative; G_{lac}-derivative, nLc_4-derivative; 8, nLc_4-derivative; 9, G_{lac}-derivatives (see Felding-Habermann* et al., *1986).* Lane 22G, *tritiated acidic glycolipids of 22-h embryos, area opaca and area pellucida together, without vitelline membrane.* Lane 22AO, *tritiated acidic glycolipids of 22-h embryos, area opaca.* Lane 22AP, *tritiated acidic glycolipids of 22-h embryos, area pellucida. M = monosialofraction; D = disialofraction; P = polysialofraction. Separation into mono-, di- and polysialogangliosides according to Chou* et al. *(1982). Abbreviations used: $G_{gal}1$, NeuAc-GalCer; $G_{lac}1$, II^3NeuAc-LacCer; $G_{lac}2$, $II^3(NeuAc)_2$-LacCer; $G_{tet}1a$, II^3NeuAc-Gg_4Cer; $G_{tet}2a$, IV^3NeuAc-, II^3NeuAc-Gg_4Cer; $G_{tet}2b$, $II^3(NeuAc)_2$-Gg_4Cer; $G_{tet}3b$, IV^3NeuAc-, $II^3(NeuAc)_2$-Gg_4Cer (according to Wiegandt, 1985).*

lular matrix? By virtue of their distribution in the plasma membrane of the cell, glycosphingolipids have been studied thoroughly. They appear to act in many ways to regulate the interactions of the cell with its environment, and to mediate cell-to-cell recognition and communication. An analysis of the function of glycolipids is beyond the scope of this overview (for review: Curatolo, 1987), but it is worth noting that the expression and composition of glycosphingolipids on the cell surface change with cell differentiation (Yates, 1986; Felding-Habermann and Wiegandt, 1987; Rösner and Rahmann, 1987).

Binding to extracellular matrix proteins has been reported several times. On the one hand, laminin has been shown to bind with high affinity to erythrocyte sulfatide (Roberts *et al.*, 1985) and to brain gangliosides (Laitinen *et al.*, 1987), a binding that is inhibited by gangliosides (Cheresh *et al.*, 1986; Roberts *et al.*, 1986; Litinen *et al.*, 1987). On the other hand, fibronectin has been shown to bind to gangliosides on the surface of cultured cells, resulting in inhibition of various fibronectin-mediated cell-adhesion processes *in vitro* (Kleinman *et al.*, 1979; Yamada, 1981, 1983; Morely *et al.*, 1987). Moreover, fluorescent gangliosides incorporate into cultured fibroblasts and organize into a network that codistributes with extracellular fibrillar fibronectin (Spiegel *et al.*, 1984, 1985, 1986). According to Griffiths *et al.* (1986), gangliosides are not essential for retention of a fibronectin matrix or for spreading of cultured cells on fibronectin-coated substrates. Thus, sulfatide and gangliosides may play a role in the interaction of cells with the extracellular matrix, although caution in the interpretation of results should be exerted.

Extensive biochemical analysis of the acidic (gangliosides and sulfatide) and neutral glycosphingolipids of unincubated, 22-h and 48-h chicken embryos has been performed by Felding-Habermann *et al.* (1986) and, more recently, by Pini, Harrisson and Wiegandt (Figs. 27-28, to be published). Thin-layer chromatography of chicken embryos metabolically labeled with D-glucosamine and D-galactose for 2 h in culture demonstrated the presence of a panoply of molecules belonging to the globo series (globoside, Forssman glycolipid), the lacto series (lactoneotetraosylceramide, nLc_4Cer, and two nLc_4Cer-based gangliosides, a monosialo and a disialo species), and the ganglio series (ganglioside $G_{tet}1a$ and higher sialated derivatives). These compounds are already present in both the area pellucida and the area opaca of unincubated blastoderms. A restriction on the expression of glycosphingolipids seems to occur as differentiation proceeds, leaving present only some characteristic molecules in adult tissues. Although the presence of particular glycosphingolipids in adult tissues is frequently correlated with their origin from the mesoderm (globo series), endoderm (lacto series), and ectoderm (ganglio series), they may not strictly be regarded as lineage-related markers (for review: Felding-Habermann and Wiegandt, 1987). Forssman antigen, for example, occurs in the inner cell mass of the mouse blastocyst and in its derivative tissues, the epiblast and the primary embryonic endoderm, before restriction to the endoderm cells (Stinnakre *et al.*, 1981). Based on their putative role in cell recognition and in cell-to-cell interactions, the working hypothesis is that glycosphingolipids may play a role in cell commitment and positioning of germ layers before the restriction occurs.

Heparan sulfate proteoglycans

Another category of saccharide-bearing molecules of the cell surface that have been implicated in cell-cell and cell-matrix interactions is composed of heparan sulfate proteoglycans and heparan sulfate-chondroitin sulfate hybrids (David and Van den Berghe, 1985; Rapraeger *et al.*, 1985; for review: Fransson, 1987). Indeed, the membrane ectodomains of several members of this category of mole-

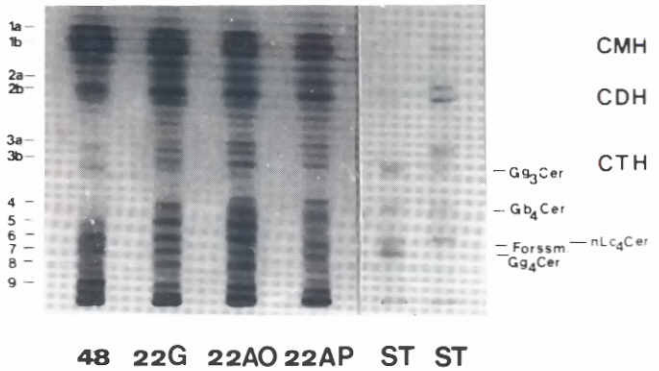

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Fig. 28. High-performance thin-layer chromatography analysis of metabolically ^3H-labeled neutral fraction glycolipids of early chicken embryos. *The embryonic tritiated glycolipids were detected by autoradiography. Standard glycolipds (ST) were visualized by orcinol spray.* Lane 48, *tritiated neutral standard glycolipids of the area pellucida of 48-h embryos, with the following components: 1a and 1b, Gal-Cer and Glc-Cer; 2a, Lac-Cer; 2b, Lac-Cer and Gal_2-Cer; 3a, fraction with a terminal ß-galactoside, probably a member of the muco-series glycolipid family; 3b, Lac_3-Cer or Gg_3-Cer; 4, Gb_4-Cer; 5, nLc_4-Cer; 6-7, Forssman glycolipid (see Felding-Habermann et al., 1986).* Lane 22G, *tritiated neutral glycolipids of 22-h embryos, area opaca and area pellucida together.* Lane 22AO, *tritiated neutral glycolipids of 22-h embryos, area opaca.* Lane 22AP, *tritiated neutral glycolipids of 22-h embryos, area pellucida.* Lane ST, *neutral standard glycolipids. At the right: CMH, CDH, CTH, nLc_4-Cer from human spleen. At the left: Gg_3-Cer from Guinea pig erythrocytes, Gb_4Cer from human erythrocytes, Forssman glycolipid from sheep erythrocytes, and Gg_4-Cer. Abbreviations used: CMH, ceramide monohexoside; CDH, ceramide dihexoside; CTH, ceramide trihexoside; nLc_4-Cer, lactoneotetraosyl-ceramide; Gg_4-Cer, gangliotetraosyl-ceramide*

cules serve as multivalent matrix receptors (Höök *et al.*, 1984; Rapraeger *et al.*, 1985) and link the extracellular matrix to the cytoskeleton (Rapraeger and Bernfield, 1982). So far, these presumptive receptors for matrix molecules have not been localized in early development.

Cell adhesion molecules

Finally, an impressive number of (glyco)proteins mediating cell-cell adhesion, collectively named cell adhesion molecules, has been reported in the literature (for reviews: Öbrink, 1986a, b). These compounds, regarded as integral membrane molecules or molecules "closely associated" with the cell surface, have been implicated in morphogenesis, as their distribution changes with development (for reviews: Edelman, 1984, 1985, 1986). Three cell adhesion molecules, isolated from liver (L-CAM) and neural tissue (Neural -CAM and Neuron-glia-CAM) have been extensively mapped in the chicken embryo, on the assumption that they are implicated in cell and tissue reorganizations during gastrulation and neurulation (Edelman *et al.*, 1983; Thiery *et al.*, 1982b, 1984, 1985). Briefly, L-CAM has already been found in pregastrulation embryos and, in the adult, it is found in epithelia derived from the three germ layers. Moreover, during gastrulation, the molecule disappears transiently from the mesoblast and the definitive endoblast, and remains detectable in the epiblast only. During neurulation, L-CAM also disappears from the neural ectoblast, in which immunostaining for N-CAM increases rapidly. N-CAM appears early in neural crest cells, but disappears during their migration on fibronectin. The distribution of L-CAM is similar to that of N-cadherin, a cell adhesion molecule localized in chicken embryos by Hatta and Takeichi (1986) and Hatta *et al.* (1987). Ng-CAM is expressed in the nervous system of 3-day-old chicken embryos. The functional characteristics of these adhesion molecules are, however, rather unclear and their implication in the regulation of developmental processes is so far based only upon spatial and temporal correlations between distribution and morphogenetic events. Experimental studies should be initiated to clarify their role in early embryogenesis and organogenesis.

Concluding remarks

Cells within an embryonic organism require extrinsic messages in order to express their developmental capacities. At the organismal and organ levels, positional information is required (for review: Wolpert, 1977). At the tissue and cell levels, each cell must exchange information with both like and unlike cells, directly from cell to cell, or indirectly, via the extracellular matrix. A large number of studies have documented the importance of such cell-cell and cell-matrix interactions in morphogenesis of several systems, the chicken embryo included. None of the systems that have been investigated so far has been fully understood, and it has become clear that we should not search for unifying, simplified concepts nor for common, general mechanisms underlying morphogenetic events in different systems, but rather analyze each system separately and thoroughly until basic cell biological mechanisms are highlighted. For example, the analysis of extracellular matrices, basement membranes and cell surfaces has demonstrated their implication in a variety of cell processes including cell-to-cell and cell-to-substrate adhesion, specific binding to biological macromolecules and to receptors of the cell surface, promotion of cell migration and positioning, transmembrane triggering of intracellular events, and modulation of cell shape. This analysis of extrinsic factors has without doubt contributed to our understanding of the mechanisms underlying morphogenetic movements in the chicken blastoderm. How cells express their developmental capacities at the right time and at the right place remains, however, a mystery.

Summary

This article reviews the factors that are involved in cell-cell and cell-matrix interactions during chicken gastrula-

tion. The chemical nature of the extracellular matrix, the structure, composition, cellular origin and remodeling of the basement membrane, and the nature of the cell surfaces are successively analyzed in relation to a variety of cell biological processes, such as cell-to-cell and cell-to-substrate adhesion, specific binding to biological macromolecules and to receptors of the cell surface, promotion of cell migration and positioning, transmembrane triggering of intracellular events, and modulation of cell shape. These processes are the cellular basis of morphogenesis in the chicken blastoderm.

KEY WORDS: *extracellular matrix, cell surface, basement membrane, cell interactions, gastrulation, chicken embryo*

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