Differential localization of $[^{35}\text{S}]$ sulfate within ectodermal basement membrane in relation to initiation of chick limb buds

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SUMMARY

This study examined the possible developmental relationship between differential amounts of glycosaminoglycans (GAG) within the ectodermal basement membrane and initiation of limb outgrowth. Chick embryos at stages 11–20 were labeled in ovo with $^{35}\text{SO}_4$, and processed for autoradiography. After exposure to sulfate for 15 min, the label was localized within the surface ectoderm whereas after 3 h the label was localized within the subjacent basement membrane. The label was sensitive to chondroitinase ABC. These experiments suggest that the labeled material was ectodermally derived chondroitin sulfate. At all stages examined, intense labeling of the basement membrane was associated with relatively undifferentiated, mitotically active tissue, e.g. limb-bud mesoderm. The labeling was less intense in regions with decreased mitotic activity, e.g. the flank. Thus, the labeling pattern of the basement membrane correlated with differential mitotic rates presumed to be associated with limb outgrowth. These observations support the hypothesis that communication between tissues of different origin resulting in altered mitotic behavior of limb and flank mesenchymal cells is facilitated by the ectodermal basement membrane.

INTRODUCTION

The well known epithelial–mesenchymal interactions of the limb and trunk appear to be mediated by the ectodermal basement membrane. Indirect evidence for this comes from the observation that mesenchymal cells do not directly contact cells of the ectoderm but only the intervening basement membrane (Ede, Bellairs & Bancroft, 1974; Kelly & Bluemink, 1974; Smith, Searls & Hilfer, 1975; Kaprio, 1977; Thesleff, Lehtonen & Saxen, 1978). Additional evidence is provided by the talpid$^3$ chick embryo in which the limb reduction anomaly is associated with fewer than normal contacts between mesenchymal cells and basement membrane (Ede et al. 1974). Since compositional changes of the basement membrane have been observed in developing tissues (Bernfield, Cohn & Banerjee, 1973; Thesleff, Stenman, Vaheri & Timpl, 1979), it may be that the basement membrane is more than passively involved in the signaling of...
new developmental events. Although our understanding of the synthetic function of the ectoderm and composition of the ectodermal basement membrane of early avian embryos has increased (Fisher & Solursh, 1977; Pintar, 1978; Sanders, 1979; Solursh, Fisher & Singley, 1979), the significance of the overall spatial and temporal alterations of the membrane has yet to be determined.

The present study makes use of the autoradiographic technique to describe the overall pattern of radiosulfate incorporation into the ectodermal basement membrane of chick embryos during stages 11–20. Specific interruptions of the general labeling pattern were observed which were temporally and spatially associated with the initiation of limb outgrowth.

**MATERIALS AND METHODS**

Fertile eggs of the Babcock strain, obtained locally, were incubated at 37 °C. Embryos, stages 11–20 inclusive (Hamburger & Hamilton, 1951), were labeled in ovo with 10 μCi Na₂³⁵SO₄ (New England Nuclear) for 3 h or with 100 μCi for 15 min. After labeling, the embryos were removed from the yolk, fixed in Carnoy’s solution containing 0.5% cetylpyridinium chloride, embedded in paraffin and serially sectioned at 7 μm in transverse and sagittal planes. Random sections were exposed for 1 h to chondroitinase ABC (Miles Laboratories) in enriched tris buffer according to Saito, Yamagata & Suzuki (1968), to buffer alone or left untreated. The slides were dipped in Kodak NTB-2 nuclear track emulsion, stored in the dark at 4 °C for 10 days, developed in Dektol and stained with nuclear fast red. Embryos thus processed were photographed and examined for differential labeling of the ectodermal basement membrane.

**RESULTS**

After labeling for 15 min with ³⁵SO₄⁻, the isotope was localized within the ectoderm but not the basement membrane of stage-11 to stage-20 chick embryos. The intensity of the label for different regions of the embryo depended on the stage examined. The general labeling pattern was similar in chick embryos exposed to the isotope for 3 h except that the label was localized predominantly within the basement membrane (Figs. 1–4). This suggests that basement membrane labeled materials originated from the ectoderm (Solursh et al. 1979).

Labeled materials were sensitive to chondroitinase ABC treatment. During stages 11–13, prior to limb initiation, the ectodermal basement membrane overlying axial and lateral plate mesoderm labeled differentially with ³⁵SO₄⁻. Relative to anterior regions (Fig. 1a), labeling of the basement membrane in posterior regions (e.g. segmental plate and most posterior somites, Fig. 1b) was more intense. The anteroposterior gradient of increasing labeling intensity was also observed in parasagittal sections (Fig. 1c). This general
Fig. 1. Stage 11. The ectodermal basement membrane (EBM) overlying the fifth pair of somites (a) did not label intensely relative to the more posterior EBM overlying the segmental plate (b). In sagittal section (c) an anteroposterior gradient of increased labeling intensity is shown. ×132.
Fig. 2. Stage 14. The axial EBM overlying the tenth pair of somites (a) did not label intensely relative to the more posterior EBM overlying the sixteenth pair of somites, or presumptive wing region (b). Relative to the lateral plate EBM in anterior regions of the embryo (a) the lateral plate EBM of the wing (b) was intensely labeled. Labeling was intense within both the lateral plate and axial EBM at the segmental plate, or flank region (c). Note the cell-free space (arrow) in the flank (c) but not in the wing (b) region. ×132.
Fig. 3. Stage 16. Labeling of the lateral plate EBM anterior to the wing bud (a) was light, whereas labeling of the wing EBM (b) was intense. Labeling of the axial EBM was light in these two regions. Relative to that of the flank (c) labeling of axial and lateral plate EBM at the leg level was intense (d). ×132.
labeling pattern was similar throughout stages 11–13, i.e. decreased labeling intensity of the ectodermal basement membrane was observed in more mature, anterior regions.

At stage 14, during the initial outgrowth of the wing bud (Searls & Janners, 1971), the labeling intensity of the basement membrane overlying axial mesoderm was light anterior to the presumptive wing region (somites 1–14, Fig. 2a), moderate at the presumptive wing region (somites 15–20, Fig. 2b) and intense at the presumptive flank and leg regions (somites 21–22 and segmental plate, Fig. 2c). Labeling of lateral plate basement membrane in the posterior regions of the embryo (wing and flank, Fig. 2b, c) was intense relative to more anterior regions (Fig. 2a).

Outgrowth of the wing bud continued and initial outgrowth of the leg bud occurred during stages 15–16. The segmental plate had given rise to somites in the flank and anterior part of the leg regions. The labeling pattern of the basement membrane overlying the embryonic axis was similar to that observed in younger embryos, i.e. light anterior to the wing region (Fig. 3a), moderate within the wing and flank region (Fig. 3b, c), and intense within the leg region.
Stage: 11 14 16 18
Somites: 13 22 28 34

Fig. 5. The changing $^{35}$SO$_4^{--}$ labeling pattern of the ectodermal basement membrane (EBM) overlying axial and lateral plate mesoderm as the lateral plate differentiates into wing (W), flank (F), and leg (L) tissues. Intense labeling is indicated by a solid line; less intense labeling by a broken line.

(Fig. 3d). The basement membrane overlying lateral plate mesoderm was similarly labeled except that the labeling intensity of the wing basement membrane was not decreased from that of stage 14 (Fig. 3b).

During stages 17–20, outgrowth of the wing and leg buds continued and the apical ectodermal ridge (AER) developed. The labeling intensity of the axial basement membrane overlying the somites of the wing, flank and leg region was light (Fig. 4a–c). The basement membranes of the wing and leg, formerly lateral plate, were intensely labeled, particularly in the vicinity of the AER (Fig. 4a, c). In the flank region (Fig. 4b), the basement membrane overlying lateral plate mesoderm was lightly labeled.

To summarize these observations, at all stages examined the ectodermal basement membrane overlying the posterior, less differentiated axial regions of the embryo, e.g. segmental plate and newly formed somites, labeled more intensely with $^{35}$SO$_4^{--}$ than the more mature anterior regions (Fig. 5). The labeling pattern of the lateral plate basement membrane was similar to that of the embryonic axis except that within fore and hind limb regions the basement membrane continued to label intensely as the embryo progressed from stage 11–20.

DISCUSSION

The observations presented in this paper suggest that mitotically active, relatively undifferentiated mesoderm of chick embryos is associated with
Basement membrane GAG and limb outgrowth

Accumulation of sulfate-containing materials, presumably chondroitin sulfate (Cohn, Banerjee & Bernfield, 1977; Fisher & Solursh, 1977), into the ectodermally derived, expanding basement membrane. As each embryonic region matures in an anteroposterior direction, the accumulation of labeled products within the overlying basement membrane appears to decrease. A similar observation was made in younger embryos where the ectoderm at the level of the heart labeled more intensely with radiosulfate at stage 8 than at stage 12 (Manasek, 1970). Whether the distribution of isotope observed in the present study can be attributed to differences in rates of synthesis, pool sizes, or rates of degradation is not clear.

At stage 14 the axial basement membrane overlying somites 15–20 (level of the wing, Figs. 2b, 5) did not label as intensely with sulfate as it did earlier (Fig. 1b, 5). The time of onset of decreased labeling coincides with increased production of matrix hyaluronate and initiation of neural-crest-cell migration at the wing level (Pintar, 1978). Also at the wing level, cells of somite origin begin migrating at this stage (Chevallier, 1978; Jacob, Christ & Jacob, 1978; Chevallier, 1979) to give rise to the myogenic cells of the limb and flank (Chevallier, Kieny & Mauger, 1977). To what extent the observed compositional changes of the ectodermal basement membrane of the embryonic axis are associated with the initiation of such cellular behavior is not known.

A pattern of labeling (anterior-posterior) similar to that observed within the basement membrane overlying axial mesoderm was also observed within the basement membrane overlying the lateral plate mesoderm. An important difference, however, was that labeling of wing and leg basement membranes did not decrease as the wave of decreasing activity proceeded in a posterior direction. Rather, as mesodermal cells accumulated and became intimately associated with the ectoderm of the presumptive wing (Fig. 2b), the ectodermal basement membrane of this region continued to label intensely as in younger embryos. The difference between limb and flank regions in this regard confirms observations by Smith et al. (1975) that the composition of the subectodermal matrix in the limb differs from that of the flank. Furthermore, intense labeling of the limb basement membrane corresponds with the continued high mitotic activity of the wing mesoderm (Searls & Janners, 1971) associated with early limb outgrowth. Both mitotic activity and basement membrane labeling of the wing (Searls, 1965) decrease after stage 22. The decreased labeling of the flank basement membrane at stages 15–16 corresponds with decreasing mitotic activity of flank mesoderm (Searls & Janners, 1971).

In conclusion, our observations suggest the active participation of the basement membrane in limb and trunk development, that compositional alterations of the membrane and subjacent matrix may signal differing mesodermal activities such as mitosis or migration. It is conceivable that signals originating at the basement membrane–mesoderm interface may spread throughout the mesoderm by way of junctional complexes which are present in the mesoderm.
(Kelley & Fallon, 1978). On the other hand, our observations may merely reflect the necessary remodeling of the basement membrane during expansion of the ectoderm in mitotically active areas. It has been suggested that the expanding ectoderm of stage 23–25 limbs may promote the high mitotic activity within the distal mesoderm by providing space into which cells proliferate (Summerbell & Wolpert, 1972). Further work is needed to more clearly elucidate the functional role of the basement membrane in limb development.

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REFERENCES


Basement membrane GAG and limb outgrowth


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