Normal anterior pattern formation after barrier placement in the chick leg: further evidence on the action of polarizing zone

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SUMMARY

Impermeable barriers were inserted into the stage-20 to -21 leg bud to test whether or not such an interruption of diffusion of the proposed morphogen from the polarizing zone would result in failure of leg elements to develop anterior to the barrier. Tantalum foil was placed at somite levels 30/31 or mid-31 through the dorsoventral extent of the leg bud separating anterior from posterior mesoderm and ectodermal ridge. In the resulting legs, structures developed anterior to the level of the barrier. For example, legs with foil at the 30/-31-somite level developed digits 1 and 2. We conclude that either the barrier is not an effective block of diffusion of polarizing zone morphogen or that the influence of the polarizing zone is not required for determination of leg structures at these stages.

INTRODUCTION

Summerbell (1979) has shown that an impermeable barrier inserted to bisect the wing bud anteroposteriorly prevents outgrowth of structures which should form anterior to the position of the barrier. He proposed that the loss of wing structures resulted from blockage of a morphogen from the polarizing zone (zone of polarizing activity) in the mesoderm anterior to the barrier. We offered an alternative explanation, that the interruption of the apical ectodermal ridge by the barrier was responsible for deletions of wing structures anterior to the barrier. By removing posterior pieces of apical ectoderm, we demonstrated that in order for the apical ectodermal ridge anterior to the mid-19 somite level to function in allowing outgrowth of wing structures, it must be continuous with apical ridge posterior to that level (Rowe & Fallon, 1981). However, this was not the case for the leg bud. When posterior pieces of apical ridge were removed from the leg bud, structures expected to develop anterior to the level of the removal did so.

Posterior apical ridge removal in the wing resulted in the same deletions of anterior wing structures as produced by barrier insertion experiments. However, the ridge removal data did not demonstrate whether or not interruption

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Fig. 1. Diagram of the stage-19 leg bud with the maximum amount of ridge that could be responsible for outgrowth of each digit indicated: digit 1 – between somite levels mid-29 and 29/30, digit 2 – between somite levels 29/30 and 30/31, digit 3 – between somite levels 30/31 and mid-31, digit 4 – between somite levels mid-31 and 31/32. Derived from partial ridge removal data in Rowe & Fallon (1981).

of the ridge by the barrier was responsible for the anterior deletions seen in the barrier experiments. Because posterior ridge removal in the leg bud did not result in deletions of anterior leg structures, we considered it important to carry out the barrier experiment on the leg bud to examine the effect on structures which should develop anterior to the position of the barrier. When barriers were placed in the leg bud, the appropriate leg structures did develop anterior to the barrier.

MATERIALS AND METHODS

Fertilized White Leghorn eggs, 3½ to 4 days old, were candled and fenestrated. Embryos at Hamburger & Hamilton (1951) stages 20–21 were selected for experimentation. A fine glass needle was used to make a slit through the dorso-ventral extent of the right leg bud perpendicular to the anteroposterior axis at either the 30-/31- or mid-31-somite level. Into this slit a tantalum foil barrier was inserted, bisecting the entire leg bud. With the barrier at those levels at least digits 1 and 2 would be expected to develop from mesoderm anterior to the level of the barrier, as predicted from partial leg ridge removal data presented in a previous paper (Rowe & Fallon, 1981) and summarized in Fig. 1.

In one experimental group, the barrier was pinned in place as was done by Summerbell (1979). In the other group, the barrier was inserted, but not pinned. Control embryos either received no barrier after the slit was made or
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Fig. 2. (A) Photograph of the leg which most often results from insertion of a tantalum foil barrier (b) into the stage 20 leg bud at the 30-/31-somite level. Digits 1 and 2 can be seen, but digits 3 and 4 failed to develop. (B) Photograph of the leg after insertion of a tantalum foil barrier (b) into the stage-20 leg bud at the 30-/31-somite level. Digits 1, 2, and 4 can be seen, but digit 3 failed to develop.

the barrier was removed before stage 25, i.e., before the digits begin to be specified (Rowe & Fallon, 1982).

After the operation, eggs were sealed with cellophane tape and returned to the incubator. They were examined at 24 and 48 h, then re-incubated for 5 days, at which time the 11-day embryos were fixed, stained and cleared.

RESULTS

In all 15 control embryos the right leg developed normally. At 24 h the two experimental groups, pinned and unpinned, were very similar and the leg
mesoderm was growing out anterior to the barrier. By 48 h, the pinned barriers usually were located more proximally in the developing leg, while the unpinned barriers were located between the forming digital cartilage condensations. Leg structures could be seen developing anterior to the barrier.

Eleven-day embryos exhibited similar right legs whether or not the barrier was pinned. Legs with a barrier inserted at the 30-/31-somite level usually (11 of 15 cases) developed digits 1 and 2, but not 3 and 4 (Fig. 2A). Three of 15 legs were missing only digit 3 (Fig. 2B). In one case, digit 2 was deleted and the barrier was located at the level of that digit. However, digit 1 did develop anterior to the barrier. Those legs with a barrier inserted at the mid-31-somite level developed digits 1, 2, and 3 in all 12 cases, but all or part of digit 4 was missing.

DISCUSSION

Tantalum foil barriers inserted into the stage-20 to -21 chick leg did not cause deletions of structures developing anterior to the barriers. However, in most cases they did cause deletion of structures which should develop at or posterior to the level of the insertion. Although it was common for structures posterior to the barrier to fail to develop, it was possible to obtain deletions only at the level of the barrier. Thus the failure of a digit to develop may have been the result of interruption of a level specific functioning of the ridge. However, a local effect of the barrier on the mesoderm as a cause of the deletions cannot be ruled out. The failure to cause deletion of anterior leg structures occurs in contrast to the results of barrier insertion in the wing (Summerbell, 1979), but is consistent with the results of removal of posterior pieces of apical ridge in the wing and leg (Rowe & Fallon, 1981). Removal of posterior apical ridge or barrier insertion into the wing bud resulted in deletion of wing structures anterior to the level of the removal or insertion. However, removal of posterior pieces of apical ridge from the leg bud did not result in comparable anterior deletions of leg elements.

Apical ridges of stage 20–21 chick wing and leg buds do not exhibit the same gross morphology. The apical ridge of the wing bud is asymmetrical, higher posteriorly than anteriorly, while that of the leg is symmetrical anteroposteriorly. Whether or not the morphological difference is related to a functional difference and, thus, to the difference seen in barrier and partial apical ectoderm removal experiments, we have not determined.

At stages 20–21, the femur, fibula, and much of the tibia have been determined (Rowe & Fallon, 1982). According to the morphogen theory for polarizing zone (Summerbell, 1979), the barrier should block diffusion between anterior and posterior leg mesoderm, resulting in failure of the specification of parts anterior to the barrier. At these stages those parts would include anterior metatarsals and digits. Since such deletions do not occur, it must be concluded that either barriers are not an effective block to the influence of polarizing zone
on the anterior leg bud or that polarizing zone is not required for normal de-
velopment at the time that the distal leg elements are being specified.

The latter conclusion may be the case despite a large body of data that demon-
strate that polarizing zone is competent at limb bud stages to influence anterior
limb bud mesoderm in the formation of a polarized outgrowth (Saunders &
Gasseling, 1959, 1968; Tickle, Summerbell & Wolpert, 1975; Summerbell, 1981;
Fallon & Crosby, 1975a, 1977). In these experiments, a graft of polarizing zone
tissue to an anterior limb bud site or a 180° rotation of the limb bud results in
formation of a polarized supernumerary limb. The conclusion that polarizing
zone is not required at limb bud stages for normal limb development is sup-
ported by the experiments of A. B. MacCabe, Gasseling & Saunders (1973),
where polarizing zone was removed from stage 15–24 chick wing buds and
normal wings developed. Further, Fallon & Crosby (1975b) demonstrated that
polarizing zone did not regenerate after removal, and this was recently con-
firmed by MacCabe, Knouse & Richardson, (1981). Considering these data,
Fallon & Crosby (1975b, 1977) suggested that polarizing zone acts at early
pre-limb bud stages and is residual at later stages. This is still a reasonable
possibility. As yet, there are no data that unequivocally demonstrate that
polarizing zone is required at limb bud stages during normal development.

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