The proximodistal determination of skeletal parts in the developing chick leg

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SUMMARY

Currently the chick leg bud or its components are being used extensively to study questions in development. Although fate maps of the leg, similar to that developed by Saunders (1948) for the wing, have been available (Hampé, 1957a, 1959), no study of the proximodistal sequence of the specification of leg structures exists. Such a sequence developed for the wing by removal of the apical ectodermal ridge at successive stages (Saunders, 1948; Summerbell, 1974), has proven useful to the study of wing development. In this paper, we present a similar proximodistal sequence for the chick leg including the same developmental stage range as that of the wing sequence.

INTRODUCTION

The chick wing bud has been used extensively as a model for amniote limb morphogenesis (reviewed in Zwilling, 1961; Saunders, 1977). In addition, leg-bud components used in combination with those of the wing have been useful, for in the resulting composite limbs, leg and wing structures can be distinguished from one another (Kieny, 1964; Pautou & Kieny, 1973). Many similarities exist between the two limb buds. For example, the presence of a polarizing region along the posterior border (Saunders, 1972) and the requirement for a period of association with the somites for normal development (Kieny, 1969, 1970). Moreover, leg-bud ectoderm allows outgrowth of wing structures when combined with wing-bud mesoderm and vice versa (Zwilling, 1956, 1964; Saunders, Cairns & Gasseling, 1957). However, differences between leg and wing have been noted, including the timing of events leading to normal morphogenesis (Kieny, 1968, 1969, 1970) and the gross morphology of the leg and wing buds. This is particularly the case with the apical ectodermal ridge which, in the avian wing bud, is uniquely asymmetrical, thicker posteriorly than anteriorly (Zwilling, 1961). In contrast, the avian leg and the limb buds of other amniotes possess a ridge of symmetrical thickness anteriorly and

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posteriorly. Whether morphological differences reflect any mechanistic differences in the morphogenesis of the wing and leg is unknown.

Hampé (1956a, 1957a, 1959) has provided a map which shows the placement of the precursor cells of leg structures. This map, based upon experiments utilizing carbon markings and the development of isolated sections of the leg bud, allows localization in the bud of structures already determined, proximal to the proposed progress zone (Summerbell, Lewis & Wolpert, 1973), at the stages used for experimentation. However, the results of Hampé's experiments do not demonstrate the most distal structures determined at successive stages of development, as has been done in the wing by apical ridge removal (Saunders, 1948; Summerbell, 1974). Considering the increasing interest in the use of the leg as an experimental system, a sequence similar to that for the wing is necessary as a common reference. Therefore, in this paper, we present a proximo-distal sequence of determined elements for the leg at stages 17-29.

MATERIALS AND METHODS

Fertilized White Leghorn eggs were incubated at 38 °C for 2½–3 days. At that time they were candled and fenestrated according to the technique of Zwilling (1959). The stage of the embryo was determined by somite number at stage 17, by the ratio of the length to the width of the wing and leg buds for stages 18–21, and by other criteria as described by Hamburger & Hamilton (1951) for all stages. At each of the stages 17–29, the apical ectodermal ridge was removed from the right leg bud with a fine glass needle. The eggs of operated embryos were then sealed with Scotch tape and returned to the incubator. Embryos were checked at 24 h and those with incomplete ridge removals were discarded. A ridge may form after removal of the apical epithelium from stage-17 and -18 leg buds (Kieny, 1968; Fraser & Abbott, 1971) and such ridge formation could be distinguished from cases of incomplete ridge removal. Incomplete removal resulted in an irregularly shaped limb bud, while those legs with a complete ridge, which formed after healing of the epithelium, had a normal limb contour. The few embryos which exhibited normal ridges after stage-17 to -18 removal were allowed to develop with the embryos from which the ridge was completely removed. The embryos were allowed to develop until the 10–12 day of incubation. They were then fixed in 10 % formalin and stained with Victoria blue and cleared.

RESULTS

After the apical ectodermal ridge was removed from the leg bud, a truncated limb resulted. As the age of the embryo at the time of operation increased, the resulting legs became more complete distally. Typical examples of the legs which developed after apical ridge removal and a diagram summarizing the results appear in Figs. 1–11.
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Fig. 1. The leg elements resulting from removal of apical ridge at stage 18: the femur and head of the tibia/fibula.

Fig. 2. The leg elements resulting from removal of apical ridge at stage 19: the femur and about half of the tibia/fibula.

Fig. 3. The leg elements resulting from removal of apical ridge at stage 20: the femur and most of the tibia/fibula.

Fig. 4. The leg elements resulting from the removal of apical ridge at stages 21 and 22: the femur, tibia/fibula, and proximal tarsals fused to the tibia (arrow).

Fig. 5. The leg elements resulting from removal of apical ridge at stage 23: the femur, tibia/fibula, proximal (arrow) and distal tarsals (arrow head).

Fig. 6. The leg elements resulting from removal of apical ridge at stage 24: the femur, tibia/fibula, proximal tarsals fused to the tibia (arrow) and distal tarsal fused to the metatarsals (arrow head), and the proximal ends of the three leg metatarsals.
At stages 17–19, a morphologically identifiable apical ridge had not yet formed in the leg. When the apical epithelium was removed from stage-17 and -18 leg buds, normal limbs resulted in a small number of cases, three of ten at stage 17, and three of twelve at stage 18. In the remainder of cases, embryos from which the apical epithelium was removed at stage 17 developed legs with pelvic girdles and incomplete femurs. In the majority of cases, a femur approximately 90% of the length of the embryos left femur was the most distal element formed. Nine of twelve operations performed on stage-18 embryos yielded legs which were truncated at a level just distal to the condylar end of the tibia and head of the fibula (Fig. 1). When the apical epithelium had been removed
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Fig. 11. A drawing of the chick leg. The lines intersect the leg at the approximate level at which the leg is truncated after removal of apical ridge at the stages indicated. On the left is the pelvic girdle to which is attached the femur. Articulating with the femur are the tibia and fibula, with the smaller fibula lateral to the tibia. Fused to the distal end of the tibia are the proximal tarsals; these fused structures comprise the tibiotarsus. Distal to the joint is the distal tarsal which is fused with the metatarsals 2, 3, and 4, comprising the tarsometatarsals. The first digit originates from the plantar surface articulating with the very small first metatarsal. Digit 2 articulates with metatarsal 2 and has three phalangeal elements, digit 3 articulates with metatarsal 3 and has 4 phalangeal elements and digit 4 articulates with metatarsal 4 and has 5 phalangeal elements.

At stage 19 (Fig. 2), all embryos developed legs with the distal-most element consisting of about half of the tibia/fibula. In some of these cases, the fibula failed to develop.

By stage 20, removal of the ridge (ten cases) resulted in a range of outgrowth of the tibia from a level just slightly distal to half of its normal length to a level about three-quarters of its normal length (Fig. 3). Legs resulting from ridge removals at stages 21 (twelve cases) and 22 (ten cases) (Fig. 4) were very similar and extended distally to a level just distal to the proximal tarsals (which are fused with the tibia to make up the tibiotarsus), while those from removals at stage 23, 15 cases (Fig. 5) included the distal tarsal (which, in the normal leg, is fused with the metatarsals to make up the tarsometatarsals). Embryos from which ridge was removed at stages 24 (14 cases) and 25 (10 cases) developed increasing lengths of the three major metatarsals of the leg (Figs. 6, 7).

Between stages 26 and 29 (30 cases), removal of ridge resulted in elimination of decreasing numbers of phalangeal elements (Figs. 8–11) until at stage 29, removal affected only the distal-most phalanges.

DISCUSSION

In this paper, we present a proximodistal sequence of the determination of skeletal elements for the chick leg during stages 17–29. Legs resulting from stage-17 and -18 apical epithelium removal were normal in a low percentage
of cases, comparable to results shown by other investigators (Kieny, 1968; Fraser & Abbott, 1971). It is possible that the conditions for induction of a functional ridge in the healed epithelium remain in the mesoderm of a very few stage-17 and -18 leg buds (Kieny, 1968). The remainder of removals from stage 17 and 18 and removals at all other stages demonstrated the proximodistal sequence of the determination of leg structures at stages 17–29.

In an extensive series of experiments, Hampé (1956a–c, 1957a, b, 1958, 1959, 1960) developed fate maps for the structures in the mesoderm of the leg through stage 23. Hampé’s fate maps (1957a, 1959) do allow prediction of which structures have been determined proximally and where their precursors exist in the mesoderm. In contrast, the representation of the proximodistal sequence of development presented here shows the most distal structures laid down during leg bud stages 17–29. Comparable representations which have been developed for the wing (Saunders, 1948; Summerbell, 1974) have proven useful to investigators using the wing as an experimental model. Summerbell (1974) has shown that in the wing the specification of carpal elements spans four stages. It is interesting to note that this time requirement is similar for the tarsals and proximal metatarsals of the leg which are specified during stages 21–24. Further experiments are required to explain why this area takes longer to be specified than the rudiment of a long bone.

With the increasing use of the leg for experimentation, a proximodistal sequence of the leg will be required for analysis of results and can be used as that of the wing has been. The combination of Hampé’s fate maps and the proximodistal sequence of determination presented here will be a useful tool for analysis of future experiments on the leg.

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REFERENCES

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