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The distribution of the polarizing zone (ZPA) in the legbud of the chick embryo.

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SUMMARY

The stage-21 to 22 legbud polarizing zone (ZPA) was mapped by transplanting small blocks of posterior marginal mesenchyme preaxially into stage-20 to -22 chick wing buds and assessing the degree of duplication of the wing digital skeleton produced in the host. Blocks taken from the posterior flank, from the angle between posterior flank and the proximal base of the limb bud, and from the most anterior distal position chosen (under the AER), all had very low activity. Blocks taken from the posterior margin of the legbud, plus the next distal block under the posterior part of the AER, all had high activity. We consider that barrier and amputation results on wing and legbud, when interpreted in the light of maps of the ZPA in both limb buds, are consistent with the hypothesis that both leg and wing have their growth and anteroposterior axis of pattern formation controlled by the ZPA.

INTRODUCTION

There is now substantial evidence that the zone of polarizing activity (ZPA) plays a role in the control of both outgrowth of the chick wingbud and its anteroposterior axis of differentiation (Wolpert 1981, Summerbell 1979, reviews Hinchliffe & Johnson 1980, Hinchliffe & Gumpel-Pinot 1983). The classic ZPA experiment is to graft it preaxially to a second wingbud which is provoked into limb duplication. In the wingbud, the ZPA has been mapped by MacCabe, Gasseling & Saunders (1973) and more recently by Summerbell & Honig (1982) and Honig & Summerbell (in press). These maps have proved most useful to those performing grafting experiments on the wingbud.

The more inaccessible chick legbud has been the subject of less experimentation. However, it too has been shown to possess a ZPA, which will provoke duplication of either wing or legbud when transplanted preaxially (Saunders & Gasseling 1968; Summerbell & Tickle 1977). This leg ZPA grafting has been carried out without the benefit of a map, and the work described here represents the first attempt to map quantitatively the intensity of ZPA duplication properties along the posterior border of the chick legbud. Small pieces of legbud mesoderm (with ectodermal

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covering) have been transplanted preaxially to the chick wingbud, and the degree of duplication produced has been assessed.

A map of the legbud ZPA is important in relation to one further issue. Fallon (Rowe & Fallon 1981, Fallon, Rowe, Frederick & Simandl 1982) has recently argued on the basis of barrier and apical ridge removal experiments that the legbud develops in a fundamentally different way from the wingbud. He considers the legbud may not require the presence of the ZPA and that the apical ectodermal ridge (AER) in the legbud has quite different properties from AER in the wingbud. In our view, the ZPA distribution which our studies reveal in the legbud (and in particular the marked anterior extensions of the legbud ZPA under the apical ectodermal ridge) explain the barrier experiments on the legbud and suggest that in the legbud, as in the wingbud, development is under the control of the ZPA.

MATERIALS AND METHODS

Stage-20–22 (Hamburger & Hamilton 1951) wingbuds were used as hosts, stage-21–22 legbuds as donors. Six regions along the posterior border of the legbuds were selected for assay (Fig. 1). Small blocks of test tissue (mesoderm plus ectoderm) from these areas were excised using fine tungsten needles in Biggers medium, and these were then pinned using small platinum pins into a correspondingly sized hole cut preaxially (in contact with the anterior AER) in the wingbud (Summerbell 1974). Blocks from 5 and 6 are both covered apically by thick AER, while the distal part of block 4 has at its apex attenuated posterior end of the AER. After grafting the host embryos were resealed and incubated: they were examined at 24 h after operation (at this stage the degree of preaxial AER thickening and mesenchymal excess is a good guide to the eventual degree of duplication) and the wings fixed at about 6 days after operation. The wing skeletons were fixed and stained with methylene blue by the Van Wijhe (Hamburger, 1960) method.

118 operations were performed, and 72 were analysed as to degree of duplication (Table 1). The remainder died before such analysis was possible. In the duplicated skeleton, digits were identified on morphological grounds as digit **2**, **3** or **4**. In some cases, anterior to the most anterior wing digit a toe, usually thin, was found (Fig. 2B), as in the results of Summerbell & Tickle (1977). Toes were recognized by their having more phalangeal elements than fingers, and by the absence of feather follicles. Sometimes a recognizable wing metacarpal element was capped by typical toe phalanges (Fig. 2B) (i.e. metacarpal 4 might have two phalanges instead of its usual one). Such elements were scored as wing digits. Scoring of the degree of duplication was by the Honig, Smith, Hornbruch & Wolpert system (1981) in which a duplicated digit **2** scores 1, digit **3** scores 2, digit **4** scores 3, but only the highest scoring digit nearest the graft is scored so that the maximum score for a duplication is 3.

When it had been discovered that area 5 under the AER, provoked good duplication, this region was further tested by preaxial grafts of blocks whose AER had been stripped off.

RESULTS

The degree of duplication provoked by the different areas of legbud assayed is given in Table 1 and Fig. 1. Typical results are illustrated including full wing digit duplication (Fig. 2A) and duplication in which chimaeric wing/leg digits are formed (Fig. 2B). The intensity of duplicative activity in the six regions assayed is given (Table 1, Fig. 1), expressed as a percentage. This represents the sum of the individual scores (maximum for one individual graft is 3) as a percentage of the total possible score for each selected area.

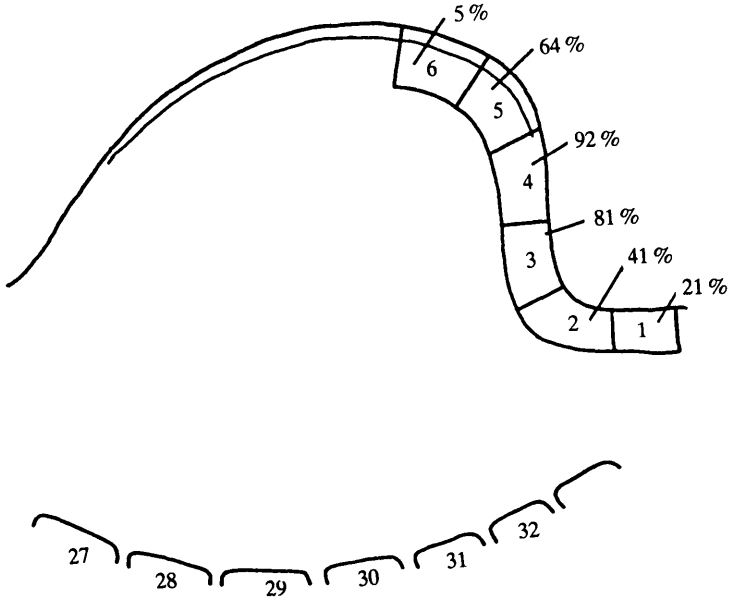

Fig. 1. Stage-22 legbud, drawn (from tracings of photographs) in relation to the numbered somites, showing relative activity of six areas along the posterior margin. (For method of assay see text.)

Table 1. *Response of host wing buds to grafts of test areas from the legbud.*

Digits in host wing	Area of legbud grafted						
	1	2	3	4	5	5 (–AER)	6
2,3,4 (normal)	5	2	1	1	3	5	10
2,2,3,4	2	5	1		(3)*	3	2
4,3,3,4			2	2		1	
4,3,2,3,4		1	2	7	1		
4,3,2,2,3,4	1	1	3	3	5		
Totals of grafts	8	9	9	13	12	9	12
Index of duplication	21%	41%	81%	92%	64%	22%	5%

The position of the areas is shown in Fig. 1. Duplicated digits are underlined. The numbers represent the grafts giving the particular digital pattern. (*One of these had two additional digits **2**, one on either side of the graft: scored as 1. The other two were **3 2 2 3 4**: scored as 2.) The scoring system on which the index of duplication is calculated is described in the text.

The results show a gradient of activity along the posterior border of the legbud. The posterior flank is almost negative, the angle between legbud and flank has low digit - **2** - inducing activity, while the two posterior border areas each have high activity, with most grafts provoking a high grade duplication (digits **4** and **3**). Rather surprisingly, the posterior mesoderm under the AER usually also produces

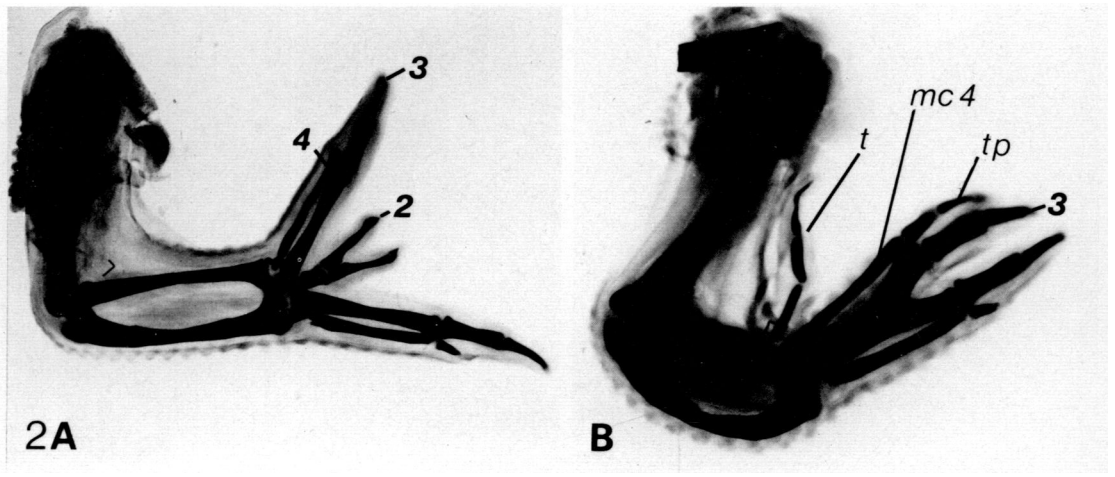

Fig. 2. (A), Full duplication obtained in response to a preaxial graft of legbud ZPA from region 4. (B), Duplication (scored as 3) obtained in response to a preaxial graft of region 4 legbud ZPA. A thin leg digit has developed (*t*), while metacarpal 4 (*mc 4*) is capped by toe phalanges (*tp*) (**2, 3, 4** duplicated wing digits).

high-grade duplication, but is much less effective in this, if its AER has been removed. Anterior to this region, area 6, with its AER, is almost completely negative.

DISCUSSION

These results show a clear gradient of duplicating capacity in the chick legbud, with maximum intensity possessed by the posterior border of the legbud. The distal, progress zone mesoderm under the most posterior part of the AER is also positive, but this activity rapidly falls away anteriorly. The map of activity which emerges has similarities to that by Summerbell & Honig (1982) of the stage-21 chick wingbud, since both wing and leg have high activity along the posterior border and in addition both buds have low-grade duplicating capacity from the angle of the limb into the posterior flank.

One interesting difference is that distal legbud mesoderm under the posterior part of the AER (i.e. block 5) has good duplicating ability. The previous analysis of ZPA distribution in the wingbud by MacCabe *et al* (1973), though not representing the AER in the drawings, indicates at stages 20–22 that activity does not extend under the posterior AER, and in fact suggests a sharp cut-off point for activity in passing from posterior border to AER. Honig & Summerbell (in preparation) show that in the wingbud the posterior border block immediately proximal to the end of the AER (i.e. corresponding to legbud block 4) has only 50 % activity. The much greater distal extension of ZPA activity in the legbud is the most important difference from its distribution in the wingbud.

In block 5 in the legbud activity declined sharply if the AER is removed. Usually the limb ZPA has the same duplicating activity following preaxial grafting whether or not it has been deprived of its ectoderm, as demonstrated by Honig & Hornbruch (1982) and by Hornbruch (personal communication), but in this case a reason for the loss of activity may be suggested. This area, deprived of its AER, may not survive well when grafted, as a number of studies show that AER removal from distal wingbud mesoderm is followed by substantial mesenchymal cell death (Kaprio 1979, Rowe, Cairns & Fallon 1982, Saunders 1977).

One of the critical questions in recent analysis of the control of pattern formation has been the role in limb development of the ZPA in the control of growth and the anteroposterior axis of differentiation (Wolpert 1981, Tickle 1980, Summerbell & Honig 1982, Hinchliffe & Gumpel-Pinot 1983). The similarities in ZPA distribution in wing and legbud suggest that both these may possess similar developmental mechanisms. However, according to some workers the ZPA role in *normal* wing development has not been established (Saunders 1977, Saunders & Gasseling 1983, Fallon & Crosby 1975, Iten 1982). Furthermore, in a number of papers Fallon has argued that leg development is different from the wing, on the basis of experiments showing i) that wing and leg AERs behave differently when transected and ii) that barriers inserted in the legbud permit normal development on either side (Rowe & Fallon 1982). Fallon contrasts this result with two sets of experiments on the wingbud, which provide evidence that in this case the ZPA is essential for normal development. In one series of experiments, Summerbell (1979) inserted impermeable barriers in the wingbud and found that the anterior part, isolated from its ZPA, developed poorly. In another series, Hinchliffe & Gumpel-Pinot (1981) carried out controlled ZPA amputations on the wingbud, and found that in the absence of the ZPA the remaining anterior distal mesoderm immediately regressed and failed to form the skeletal parts appropriate to its normal fate, while if a portion of the ZPA was left, it developed normally (Hinchliffe & Griffiths 1984).

Barrier insertions made in the legbud in posterior positions, at the level of intersomite 30/1 or mid 31 (fig.1, Rowe & Fallon 1982) permit mosaic development of the legbud parts on both sides of the barrier. Rowe & Fallon conclude that either the barriers are not effective blocks, or 'the polarizing zone is not required for normal development at the time that the distal leg elements are being specified'. Based on our ZPA maps, our explanation of these mosaic results is that barrier insertion at the level of somite 31 may well include ZPA activity *anterior* to the barrier, and that at the level of 30/1 ZPA activity anterior to the barrier is a possibility. Our own experimental barrier insertions in the legbud in more anterior positions at intersomite 29/30 (or amputation of the posterior half) result in regression of the anterior half of the legbud which then makes little skeletal contribution (Hinchliffe & Griffiths 1984, Griffiths & Hinchliffe, in preparation). These results are very similar to those obtained by barrier insertion or posterior half amputation in the wing (Summerbell 1979, Hinchliffe & Gumpel-Pinot 1981, Hinchliffe & Griffiths 1984). Barrier and amputation experiments on wing and

legbud, when interpreted in the light of the respective maps of ZPA, are consistent with the hypothesis that both these limb buds have their growth and anteroposterior axis of pattern formation controlled by the ZPA.

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