Regional differences in the morphology and motility of mesodermal cells from the early wing-bud of normal and talpid³ mutant chick embryos

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SUMMARY

A method of culturing has been employed to compare the properties of cells migrating from small mesodermal explants taken from different regions of normal and mutant limb-buds at different stages of development. An analysis by time-lapse cinematography of the morphology and mobility of cells migrating from explants defines a distal region within the limb-bud where these properties are distinct from those of cells from more proximal regions. In the normal wing-bud distal cells subjacent to the apical ectodermal ridge possess a characteristic multipolar morphology and translocate slowly in vitro. Cells from more proximal regions tend to be bipolar and translocate more rapidly. Distal and proximal cells also probably differ in their adhesive strengths. In the mutant, talpid³, distal and proximal cells do not differ in the above properties and cells from all regions of the limb-bud are multipolar, translocate slowly and are more adhesive than normal cells. A study of light micrographs and scanning electron micrographs suggests that these regional differences are found in the limb-bud in vivo and are not merely an effect produced by the in vitro culturing system.

INTRODUCTION

The development of the chick limb-bud has often been described (most recently by Saunders, 1977). It arises as an outgrowth of the somatic mesoderm at 3–3½ days – stage 17 of Hamburger & Hamilton (1951), with an ectodermal covering which becomes thickened and specialized distally slightly later (stage 20) to form an apical ectodermal ridge (AER), the presence of which is essential for the continued normal outgrowth and differentiation of the limb-bud. The mesodermal component has commonly been regarded as consisting, in the early stages, of an homogeneous mesenchyme within which the muscle and skeletal rudiments later become differentiated. Recent evidence now suggests that at least some of the musculature is derived from mesodermal cells which

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have migrated in from the somites (Christ, Jacob & Jacob, 1977; Chevallier, Kieny & Mauger, 1977). Some of the properties of these mesenchymal cells have been investigated and compared with those of the corresponding cells from the talpid3 mutant where the pattern of limb outgrowth and morphogenesis is highly abnormal (Ede & Agerbak, 1968; Ede & Flint, 1972, 1975a, b). Cells were obtained from pooled whole mesoderms taken from several wing-buds at stages 25–26 when skeletal and muscle cell differentiation has begun. Cell morphology, adhesivity and motility were observed in reaggregation and time-lapse cinemagraphy studies; the mutant cells were found to be less polarized than normal cells, more adhesive to each other and less motile. A model relating these cell properties to the abnormal morphogenesis of the talpid3 limb was proposed by Ede & Agerbak (1968) and developed in a computer simulation by Ede & Law (1969) and later by Wilby (1977).

Cairns (1977) has made comparable studies on wing-bud mesodermal cells obtained from normal embryos and from the mutant talpid3, whose general abnormalities are similar to those of talpid3 (but less extreme). Using small quantities of cells derived from particular regions of the bud he found that in the normal limb-bud, prior to any visible differentiation, cells taken from a distal strip extending about 100 μm in from the AER differed in their properties expressed in vitro from more proximal cells. Whereas the more proximal cells appeared as typical fibroblasts in culture, with distinct polarity, those from the distal strip were multipolar and less motile, and in fact generally resembled the properties found in talpid3 pooled mesenchyme cells much more closely than pooled normal cells. Kwasigroch & Kochar (1975) in similar but not so precisely localized studies on the mouse have shown that cells from the distal half of the mouse limb-bud are less motile than those from the proximal half, and it is possible that this difference is a significant and widespread feature of the developing limb in vertebrates.

The existence of these regional differences is clearly important for consideration of normal limb development, and it is of interest to see whether corresponding differences exist in the talpid3 mutant. In addition, in using the ‘micro’ method far less material is required and therefore younger stages may be used. The opportunity was therefore taken to study the morphology of the limb-buds at 3½ days (stages 19–21) as well as the properties of the cells of normal and mutant at these stages, well before any cellular differentiation has occurred.

**Materials and Methods**

Eggs were obtained by mating heterozygote birds carrying the talpid3 mutation (ta3) from a stock maintained at the West of Scotland College of Agriculture, Ayr. Normal (+/+ and +/ta3) and mutant (ta3/ta3) embryos at stages 19, 20 and 21 were selected after 3–4 days of incubation.

When used for preparing explants from the wing-buds the embryos were
Mesodermal cells from wing-bud of talpid³ chicks

Fig. 1. Diagrammatic representation of the chick limb-bud (stage 20) showing the regions from which explants were taken and cultured as referred to in the text. A–H, distal mesoderm; 2–5, proximal mesoderm; Do, dorsal proximal mesoderm; V, ventral proximal mesoderm; AER, apical ectodermal ridge.

Fig. 2. Diagram of the culturing system used for time-lapse cinéma­tography of explants. A, drop of culture medium containing explant; B, sterile glass coverslip supported on a plastic ring to provide an optically flat surface for filming; C, inverted phase contrast microscope; D, Falcon plastic Petri dish; E, ring of water; F, water soaked cotton wool to maintain humidity; G, culture chamber; H, inflow; I, outflow 5% CO₂/air mixture; J, light source.

placed in Tyrode solution (1910). The wing-buds were removed and placed into culture medium (Eagle Earle's Minimal Essential Medium supplemented with MEM amino acids and vitamins, 10% foetal calf serum, 100 units/ml penicillin and $1 \times 10^{-4} \text{g/ml streptomycin}$) in which all further manipulations were carried out. Fine tungsten needles were used to dissect small cubes of mesoderm (approximately $0.1 \times 0.1 \times 0.1 \text{mm}$) from the different regions of the wing-bud shown in Fig. 1. Each piece of mesoderm was transferred to a single drop of culture medium in a Falcon plastic Petri dish and cultured at $37 \, ^\circ \text{C}$
in a humidified 4% CO₂/air atmosphere. To provide an optically flat field for photographing and filming the set-up shown in Fig. 2 was used.

Cultures were filmed by attaching a Bolex H16 ciné camera to an inverted phase-contrast microscope. A single frame was taken every 15 sec and in the analysis of the film the position of the cell and its nucleus was recorded every fortieth frame, i.e. at 10 min intervals. The distance between the centre of the cell’s nucleus in each of these positions was measured and recorded as a single point on the histograms in Figs. 6 and 7. Only cells which had broken away from the explant were selected for measurement and those which made substantial or prolonged contact with other cells were not measured and neither were cells undergoing mitosis.

Six normal and six talpidae embryos at stage 21 were washed with Tyrode solution and then fixed with 2% glutaraldehyde in 0.05 M cacodylate buffer pH 7.2 at room temperature for 2 h. They were then washed with three changes of cold 0.05 M cacodylate buffer and post-fixed with 1% osmium tetroxide in 0.05 M cacodylate buffer at 4 °C for 1 h. The wing-buds were removed and dehydrated through an alcohol series (70%-90%-100%-100%-100%). Half of the wing-buds were then taken into amyl acetate through ethanol/amyl acetate mixtures (2/1-1/2 and finally three changes of amyl acetate). These limb-buds were dried in a critical point bomb by replacing the amyl acetate with liquid CO₂ and finally evaporating off the CO₂ at its critical temperature and pressure. The limb-buds were then carefully cut into anterior and posterior halves, vertically along the proximal-distal axis with a new double-edged razor blade as shown in Fig. 1. They were then coated with gold and viewed on a Philips scanning electron microscope.

The remaining limb-buds were embedded in TAAB embedding resin and 1 μm sections along the proximo-distal axis were cut with glass knives on a Reichart OMU3 ultratome and stained in 1% toluidine blue. Two different regions of the limb-bud (corresponding to D1 and 4, Fig. 1) were photographed with a ×100 oil immersion objective and prints made to give a total magnification of 2000×. These prints were used for a statistical analysis applying the sampling technique described by Bellairs (1959) for the analysis of electron micrographs, a modification of that described by Chalkley (1943). A transparent sheet of polythene divided by fine intersecting lines into 1 cm squares was placed over the print. The structure which lay at each intersection was noted and recorded as belonging to one of four different groups: (1) nucleus, (2) cytoplasmic process less than 0.5 μm diameter, (3) remaining cytoplasm, (4) intercellular space. Intersections which lay over ectodermal structures or blood vessels were not included in the analysis.
Fig. 3. Normal (A–C) and talpid³ (D–F) embryos at stage 19 (A, D), stage 20 (B, E) and stage 21 (C, F) of development. Note the eyes drawn towards the ventral mid-line, the elongated anterior-posterior axis of the wing-bud, the reverse cervical flexure and the small allantois of the mutant. wb, wing-bud; e, eye; al, allantois.
RESULTS

(A) A comparison of normal and talpid^3 embryos at 3–4 days

The talpid^3 chick embryo has previously been described at stages later than 4½ days (stage 25) by Ede & Kelly (1964a, b). Three stages of talpid^3 embryos are shown in Fig. 3D–F, corresponding to stages 19, 20 and 21 of normal development (Fig. 3A–C). Both normal and talpid^3 embryos were staged according to the number of somites developed, being 39, 42 and 45 for the three different stages.
Mesodermal cells from wing-bud of talpid\textsuperscript{3} chicks

Several abnormalities that had previously been described by Ede & Kelly in later stages are also evident at these earlier stages. Thus the eyes are drawn together under the midline of the embryo. It is at stage 20 that the normal embryo turns onto its left side but this fails to occur in the mutant. The cervical flexure of the mutant is much reduced and is characteristically in the earlier stages in the opposite sense from normal. A median longitudinal groove is present along the dorsal ridge of the trunk of the mutant which appears very angular compared to the normal trunk. Deep pits separate the somites of the mutant. It may also be noted that the development of the allantois is delayed in the mutant – at stage 20 when the allantois is about the size of the hindbrain in the normal embryo it is barely evident in the mutant and at a stage later when it is being deformed around the snout of the normal embryo only a small structure is formed in the mutant.

During this period of development in talpid\textsuperscript{3} the outgrowth of the wing-bud is quite different from normal. The anterior-posterior length of the wing-bud is increased both in its actual length and in the number of somites over which it extends (Fig. 4). This broadening is maintained through all the early stages of limb development but the number of somites over which the limb extends is gradually reduced from seven to five somites in talpid\textsuperscript{3} and from five to four somites in normal embryos, over the three stages examined. Also the proximo-distal outgrowth of the limb for any particular stage is reduced in talpid\textsuperscript{3}. Differences in the hindlimb buds are less evident.

Finally the talpid\textsuperscript{3} embryo is most easily identified at the earliest stages of development studied by the presence of oedema in the peripheral circulation which is evident even when the embryo is in situ.

(B) Distal and proximal mesodermal explants

(1) Normal distal explants

Normally eight explants were taken from an anterior-posterior strip of mesoderm subjacent to the AER, from which the ectoderm had been removed, and cultured in a small drop of culture medium as described in Materials and Methods. A central distal explant (D1, Fig. 1) is shown in Fig. 5 and is typical of explants from this distal strip of mesoderm although there is some variation along the anterior-posterior border as described by Cairns (1975). No differences were observed between explants taken from embryos of different stages (19–21 and 25) and the properties described below were maintained in culture for several days.

The explants are initially irregularly shaped but round up soon after placement in culture and cells begin to migrate from the explant after about 6 h in culture. The cells form a loose but continuous sheet with the explant whose boundary becomes less well defined. Cells rarely break away from the explant and the motility of those that do is relatively low (Fig. 6A, B; Table 1). The
Fig. 5. Mesodermal explants taken from the wing-buds of stage-20 normal (A–E) and talpid³ (F–J) embryos. A and F, distal mesoderm (D1); B and G, proximal mesoderm (2); C and H, proximal mesoderm (3); D and I, dorsal proximal mesoderm (Do); E and J, ventral proximal mesoderm (V).
Fig. 6. Motility of cells in culture. Each measurement of motility is measured as the distance migrated by a single cell during a 10 min period. (A) Normal distal mesoderm with the AER removed, stage 20. (B) normal distal mesoderm with the AER present, stage 20. (C) normal proximal mesoderm, stage 19. (D) normal proximal mesoderm, stage 20.
Fig. 7. Motility of cells in culture as measured in Fig. 6. (A) *Talpid³* distal mesoderm with the AER removed, stage 19. (B) *Talpid³* proximal mesoderm, stage 19. (C) *Talpid³* proximal mesoderm, stage 20.

cells have a stellate morphology and are well flattened and from time-lapse films can be seen to be multipolar with ruffled membranes along more than one edge. Time-lapse films also show that these cells rarely change from this characteristic shape unless by rounding up to undergo mitosis.
Table 1. The mobility of cells migrating from explants taken from different regions of the normal and talpid3 wing-bud

The mobility is the mean displacement (µm) of a cell's nucleus during a 10 min interval averaged for the number of such measurements made. Only cells that had broken away from the explant and made no substantial contacts with other cells were measured.

<table>
<thead>
<tr>
<th>Type of explant</th>
<th>Number of measurements</th>
<th>Mobility microns/10 min</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal distal - AER</td>
<td>129</td>
<td>0.38</td>
<td>±0.28</td>
</tr>
<tr>
<td>Stage 20</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Normal distal + AER</td>
<td>74</td>
<td>0.61</td>
<td>±0.45</td>
</tr>
<tr>
<td>Stage 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal proximal</td>
<td>101</td>
<td>1.65</td>
<td>±1.13</td>
</tr>
<tr>
<td>Stage 19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal proximal</td>
<td>196</td>
<td>1.51</td>
<td>±1.15</td>
</tr>
<tr>
<td>Stage 20</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normal proximal</td>
<td>149</td>
<td>1.18</td>
<td>±0.79</td>
</tr>
<tr>
<td>Stage 21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talpid3 distal - AER</td>
<td>183</td>
<td>0.94</td>
<td>±0.76</td>
</tr>
<tr>
<td>Stage 19</td>
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<tr>
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<td>1.24</td>
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<tr>
<td>Stage 19</td>
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<td></td>
<td></td>
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<tr>
<td>Talpid3 proximal</td>
<td>187</td>
<td>0.59</td>
<td>±0.48</td>
</tr>
<tr>
<td>Stage 20</td>
<td></td>
<td></td>
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<tr>
<td>Talpid3 proximal</td>
<td>139</td>
<td>0.58</td>
<td>±0.43</td>
</tr>
<tr>
<td>Stage 21</td>
<td></td>
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</tbody>
</table>

(2) Normal proximal explants

Explants taken from the central proximal strip of mesoderm of the wing-bud (Fig. 1: 2, 3, Do and V) and cultured for 11 h are shown in Fig. 5B–E. In contrast to distal explants proximal explants maintain their irregular shape in culture for much longer. The explants have well-defined boundaries and individual cells break away after only 3–4 h and migrate away from the explant rapidly making little or no contact with other cells. These cells also had a very high motility compared to any of the other cells studied (Fig. 6C, D and Table 1). The cells were polarized with ruffled membranes only at the ends of their elongated bodies and appeared to make contact only at these points as suggested by their mode of locomotion. A cell would detach at one end and the main cell body would spring back to the one remaining point of contact and the cell would then extend a filopodium in a new direction. Any cell might frequently change its shape becoming elongated or round and perhaps even multipolar. These characteristics would be maintained in culture for 24 h by which time occasional myoblast-like cells were observed.
Fig. 8. Examples of photomicrographs from which the grid analysis was made. (A) Normal distal mesoderm. (B) Normal proximal mesoderm. (C) Talpid\textsuperscript{3} distal mesoderm. (D) Talpid\textsuperscript{3} proximal mesoderm, all from stage-21 embryos.

(3) Talpid\textsuperscript{3} distal explants

Small explants taken from the distal strip of mesoderm of the mutant wing-bud were cultured in the same way as normal explants and a representative example taken from the centre of this distal strip is shown in Fig. 5F. These explants resemble those of normal distal mesoderm in many ways and round up soon after being placed in culture medium with the first cells emerging from the edge of the explant after about 6 h. The cells appear stellate and well flattened with ruffled membranes around much of their periphery but retain loose contact with cells around them to a greater extent than is observed in normal cultures. The motility of the cells is relatively low (Fig. 7A, Table 1) and the spread of motilities is much the same as for normal distal cells. It was also observed that there was not such great variation along the anterior-posterior border as was found for the normal limb-bud.
Fig. 9. Scanning electron micrographs of limb mesoderm from stage-20 embryos. (A) normal distal mesoderm – note the smooth edges of the cells and the long extracellular processes (P). (B) normal proximal mesoderm – note the presence of a fuzzy extracellular material on the surface of the cells (ecm) and long extracellular processes. (C) Talpid3 distal mesoderm. (D) Talpid3 proximal mesoderm. Note the smooth surfaces and many fine microvilli (mv) of talpid3 cells.
Table 2. Chi-squared tests on the grid sampling data from photomicrographs taken of 1 μm sections of mesoderm from different regions of the normal and talpid3 wing-buds of stage-21 embryos

<table>
<thead>
<tr>
<th>Type of cytoplasm</th>
<th>Nuclei</th>
<th>Cytoplasmic processes ≤ 0.5 μm</th>
<th>Remaining cytoplasm</th>
<th>Intercellular space</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal distal</td>
<td>20.4</td>
<td>7.9</td>
<td>45.7</td>
<td>25.8</td>
</tr>
<tr>
<td>Normal proximal</td>
<td>27.0</td>
<td>3.2</td>
<td>57.4</td>
<td>12.3</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.010</td>
<td>P &lt; 0.005</td>
<td>0.010</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.005</td>
<td>P &gt; 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Talpid</em>3 distal</td>
<td>26.1</td>
<td>4.7</td>
<td>52.7</td>
<td>16.6</td>
</tr>
<tr>
<td><em>Talpid</em>3 proximal</td>
<td>28.4</td>
<td>4.1</td>
<td>52.5</td>
<td>15.1</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.025</td>
<td>P &gt; 0.750</td>
<td>0.100</td>
<td></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.010</td>
<td>P &gt; 0.050</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P* is the probability of chance value that the two populations compared by the χ² test are similar.

(4) *Talpid*3 proximal explants

Blocks of mesoderm were taken from the same proximal positions as for the normal wing-bud but in contrast to normal proximal explants closely resembled normal and *talpid*³ distal explants (Fig. 5G–J). Also unlike normal proximal explants there was very little variation along the proximo-distal axis. Explants from the very earliest stages studied (stage 19) however did not show quite the same morphological characteristics as those from later stages. Cells from later stages were stellate, well flattened and began to migrate from the explant after about 6 h forming a continuous sheet of cells with many close contacts. The cells had motilities much the same as those of distal explants (Fig. 7B, C and Table 1) with a narrow distribution possessing a slight tail at the higher values, apparently a characteristic of all *talpid*³ cell populations. The motility of *talpid*³ proximal cells was significantly different from that of normal proximal cells at all the stages studied; however, the slightly higher mobility of the cells of the earliest stage studied was also reflected in their different morphology.

(C) Histological analysis and scanning electron microscopy of limbs

Normal and *talpid*³ limb-buds from stage-21 embryos were prepared for scanning electron microscopy and thin sectioning as described in Materials and Methods. Scanning electron micrographs are shown for the proximal and distal regions of the limb in Fig. 9. The results from the grid sampling analysis performed on photomicrographs (Fig. 8) as described in Materials and Methods
Mesodermal cells from wing-bud of talpid\textsuperscript{3} chicks

are presented in Table 2 and were for similar regions as those from which the scanning micrographs were taken.

The figures in Table 2 for normal distal and proximal mesoderm differ quite considerably from each other, the most striking differences being observed in the number of fine cytoplasmic processes and the amount of intercellular space, both of which are more than doubled for distal mesoderm compared to proximal mesoderm. A comparison of the same regions of the talpid\textsuperscript{3} limb only reveals small and insignificant differences. In addition the figures for talpid\textsuperscript{3} do not resemble those for either normal distal or proximal mesoderm but are of intermediate value. A study of the scanning electron micrographs confirms these observations, talpid\textsuperscript{3} cells from any region resemble each other and are characterized by short spikey microvilli all around their edges (Fig. 9C, D) as previously described by Ede, Bellairs & Bancroft (1974). Normal distal cells however are quite different from normal proximal cells and appear flattened with a smooth surface and long cytoplasmic processes making contact with other cells at a considerable distance. Proximal cells are much more rounded and have a slightly fuzzy appearance at their cell surface but do also possess long cytoplasmic processes interconnecting with other cells although the statistical analysis suggests that their frequency is much reduced. These long cytoplasmic processes are rarely seen in talpid\textsuperscript{3} cells.

DISCUSSION

Previously studies by Ede and others (Ede & Kelly, 1964\textit{a, b}; Ede & Agerbak, 1968; Ede & Flint, 1972, 1975\textit{a, b}; Ede \textit{et al.} 1974; Ede, Flint, Wilby & Colquhoun, 1977) of the talpid\textsuperscript{3} mutation have been made at moderately late stages of limb development (stage 25). The present work shows that the mutation affects limb development from its initial stages and possibly even affects the field defining the region of outgrowth in the somatopleural mesoderm of the body wall as this region appears to be lengthened in the talpid\textsuperscript{3} mutant.

The analyses by Ede \textit{et al.} (1968, 1971, 1974, 1975\textit{a, b}) of the cellular properties affected by the talpid\textsuperscript{3} mutation of cell morphology, cell adhesion and cell motility, which probably reflect changes at the cell surface, were also carried out at these later stages of limb development when differentiation of the two major tissue types of the limb, cartilage and muscle, is occurring. Thus it is not clear whether these altered properties are a result of the abnormal differentiation of the mutant or whether they are its cause as Ede suggests in his model of limb development (Ede, 1971; Ede & Flint, 1975\textit{b}). The present study shows that the cell properties analysed by Ede \textit{et al.} are affected at even the earliest stages of limb development, well before any overt signs of differentiation, and therefore support their model.

By using small explants it has been possible to make a more detailed analysis of the above \textit{in vitro} cellular properties, and analyse their variation in different
regions of the limb. Cairns (1975, 1977) first used this method to demonstrate that the cells from a distal strip of mesoderm subjacent to the AER are characterized by a stellate morphology while those from proximal regions are more polarized. Kwasigroch & Kochar (1975) have observed a similar situation in the developing mouse limb. Cells taken from the distal half of the limb have a stellate morphology while those from the proximal half are more polarized and also are more motile. We have confirmed Cairns' observations but have also shown that the proximally derived cells are more motile than those derived from the distal mesoderm. Differences in the proximal and distal mesoderm of the limb of the normal chick embryo are also apparent in vivo as the histological and scanning electron microscope studies show.

In the talpid3 limb however there do not appear to be any differences between cells of proximal and distal mesoderm either in their morphology and motility in culture or by the analyses of histological and scanning electron microscope preparations. In culture all talpid3 cells resemble normal distal cells more closely than normal proximal cells having a stellate morphology and low motility. They do not however appear identical to normal distal cells since in cultured explants the cells appear to retain closer contacts, as also they appear to do in histological sections, a situation reminiscent of Ede & Flint's observation (1975a) that talpid3 cells tend to wrap around each other when forming aggregates. The histological analysis also shows that normal distal mesoderm possesses a great deal of extracellular space compared to talpid3 and also that talpid3 cells are characterized by many short spikey microvilli. Thus the talpid3 mutation is not acting by merely increasing the proportion of distal-type cells within the limb.

From where are the two cell populations derived within the normal limb and how are the two populations modified in the talpid3 limb? It is possible that the proximal cells are precursors to later differentiated cell types and that their different characteristics even at the earliest stages of limb development reflect their later fate. In this respect it is of interest that despite the homogeneous appearance of the early limb-bud it has been suggested that the musculature is derived from somitic mesoderm whilst the tendons and connective tissue are of somatopleural origin (Christ, et al. 1977; Chevallier et al. 1977). It is possible that the cells of somitic origin may correspond to the distinctive polarized cell type of the normal proximal mesoderm and that perhaps the invasion of the limb by this cell type is blocked by the talpid3 mutation.

An alternative explanation which also accounts for the apparent constancy in the depth of the distal strip of mesoderm of approximately 100 microns, despite the continued mitosis as the limb develops, is that there is a continual conversion of distal type cells to proximal type. Wolpert has suggested that cells in the developing limb receive a positional value according to the number of mitoses undergone within a hypothetical 'progress zone' at the distal tip.
Mesodermal cells from wing-bud of talpid³ chicks

(Summerbell, Lewis & Wolpert, 1973). Possibly the distal-proximal conversion corresponds to the transition of distal cells from this 'progress zone' and that this transition is disturbed in the talpid³ limb. Interestingly, a similar conversion of cell types occurs within the developing somitic mesoderm. Cells from unsegmented somitic mesoderm closely resemble those of normal distal mesoderm, having low motility and a stellate morphology, while cells from segmented mesoderm appear much more polarized (Bellairs & Portch, 1977).

The observation of only a single cell type within the talpid³ limb may be because either the superimposed effects of the mutation obscure the normal distal-proximal characteristics, or there is a genuine shift in the proportion of the two cell types. Interestingly, Cairns (1977) reported that the talpid² mutant does not show the two different cell types; however, this mutation is less severe in its effects upon the embryo than is the talpid³ mutation and therefore its effect upon the cells may be less easy to detect. The effect upon the cells may also be different, as Niederman & Armstrong (1972) were unable to detect any differences in the adhesive strength of talpid² cells by the indirect assay of cell sorting in mixed aggregates; however, their measurements may reflect a different cell property from those of Ede who measured the adhesive strength using a couette viscometer (Ede & Flint, 1975b). Thus it appears possible that the severity of the mutation affects the proportions of the two cell types, possibly by altering their sensitivity to some factor.

The AER seems a likely source for such a factor. It is known to affect the viability of cells underlying it since its removal from the normal limb in vivo leads to a wave of necrosis in this region (Saunders, 1948; Barasa, 1960; Janners & Searls, 1971; Summerbell, 1974). Also distal explants with the AER removed and proximal explants as well as cell aggregates undergo necrosis in culture (Cairns, 1975; Ede & Flint, 1972). A gradient of ridge factor along the distal-proximal axis and the sensitivity of the cells to this factor may define a threshold value at which the transition of distal to proximal cell types occurs and similarly may also define the 'progress zone'. Below this threshold value the concentration of factor is normally sufficient to maintain cell viability except in certain regions of the limb where cell death naturally occurs, such as the anterior and posterior necrotic zones. Removal of the source of factor, the AER, also leads to a drop in its concentration below this critical value with the resulting wave of necrosis.

The talpid³ mutation by acting at the cell surface, as suggested by their increased adhesiveness (Ede & Flint, 1975b) and altered cell surface charge (unpublished), not only affects the properties of cell morphology and motility but may also increase their sensitivity to the ridge factor. In this way their threshold value will occur at a lower concentration and therefore a greater proportion of the limb will consist of distal type cells. It also means that the concentration is less likely to drop below the critical value required for cell viability and indeed necrosis is reduced in talpid³ explants (unpublished) and
aggregates (Ede & Flint, 1972). Also the usual necrotic zones are absent from the \textit{talpid}\textsuperscript{3} limb (Hinchliffe & Ede, 1967).

Thus a hypothesis is suggested to relate the \textit{talpid}\textsuperscript{3} mutation to a defect at the cell surface the consequences of which lead to the altered properties of \textit{talpid}\textsuperscript{3} cells both \textit{in vivo} and \textit{in vitro}, to the loss of a cell type programmed in normal limb development and to the abnormal differentiation and patterning of the \textit{talpid}\textsuperscript{3} limb.

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REFERENCES


Mesodermal cells from wing-bud of talpid³ chicks


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