An analysis of pigment cell development in the periodic albino mutant of *Xenopus*

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SUMMARY

The periodic albino mutant (avap) of *Xenopus* in which the development of melanophores is impaired, is further reported here to possess an aberrant pattern of iridophore differentiation. The development of mutant and wild-type neural crest explants isolated in vesicles derived from tissues from identical and different genotypes was examined to determine if the mutant effect resides in the pigment cells or is mediated by the environmental tissues. Mutant melanophores and iridophores cultured in either mutant or wild-type tissues exhibited mutant patterns of differentiation. Wild-type pigment cells cultured in both wild-type and mutant tissues exhibited wild-type patterns of differentiation. Hence the mutation affects the capacities of melanoblasts and iridoblasts to differentiate but not the ability of the environmental tissues to support pigment cell differentiation.

INTRODUCTION

Periodic albinism is a mutant (avap) of *Xenopus laevis* which affects melanin synthesis. Mutant oocytes lack melanosomes and premelanosomes (Bluemink & Hoperskaya, 1975) and melanoblast differentiation in mutant larvae is delayed until stage 43 (staging according to Nieuwkoop & Faber, 1967) when reduced numbers of faint, often punctate melanophores differentiate (Hoperskaya, 1975). In contrast, melanophores of wild-type larvae first appear at stage 33/34 in a dendritic form and rapidly become darkly pigmented.

Following a grafting analysis, Tompkins (1977) proposed that the mutant gene affects both the capacity of melanoblasts to differentiate and the ability of the environmental tissues to support melanoblast differentiation, a proposition based on his finding that orthotopic grafts of wild-type neural crest to mutant embryos gave rise to melanophores which at first appeared normal but by stage 45 had come to resemble the mutant melanophores of the host. However, on grafting mutant neural crest to wild-type embryos, Tompkins was unable to demonstrate any reciprocal enhancement of mutant melanoblast differentiation in wild-type tissues, as the tissues populated by donor melanoblasts became obscured by wild-type melanophores from the host neural crest. In view of this gap in

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evidence it seemed desirable to re-appraise the extent to which the mutant effect resides in pigment cells and the extent to which the effect is mediated by environmental tissues, by analysing experiments carried out under conditions designed to prevent host melanophores from obscuring the results.

In the course of this study it became apparent that the development of iridophores in mutant larvae was also abnormal. In wild-type larvae silver iridophores appear at stage 46–47 and within 24 h become golden and highly iridescent; in mutant larvae, however, silver iridophores appear at late stage 47 become only faintly golden and are much less iridescent than their wild-type counterparts. Hence the mutant effect on iridophores was also studied.

**MATERIALS AND METHODS**

Eggs of mutant \((a^p)\) and wild-type \((w^+)\) *Xenopus* were obtained by standard methods (New, 1966). Uniform portions of neural crest and underlying dorsal sector of neural tube were excised from the anterior trunks of stage-22 embryos. Such explants were cultured in vesicles derived from uniformly sized, square-shaped portions of lateral plate mesoderm and overlying epidermis. The techniques used in preparing and culturing vesicles have been described previously (MacMillan, 1976). Fourteen vesicles of wild-type crest in wild-type tissues, 15 vesicles of mutant crest in mutant tissues, 17 vesicles of wild-type crest in mutant tissues and 18 vesicles of mutant crest in wild-type tissues were cultured at 20 °C and examined at regular intervals for up to 3 weeks. The numbers and morphology of melanophores which subsequently differentiated in vesicles were recorded. To facilitate counting, melanophores were temporarily rendered punctate by placing vesicles in darkness for up to 1 h. It was not possible to count individual iridophores, as these cells tended to form networks or sheets; hence records of iridophore development were restricted to the time of appearance and morphology. Other stage-22 embryos (10 wild-type and 10 mutant) were allowed to develop for use as controls and to determine the developmental age of the vesicles.

**RESULTS**

The development of melanophores

(a) Culture of wild-type crest in (i) wild-type and (ii) mutant tissues

Dense populations of darkly pigmented, mainly dendritic melanophores (Fig. 2A) began to appear in all vesicles of both groups from stage 33/34. The morphology and intensity of pigmentation of melanophores in vesicles were similar to that of melanophores in wild-type control larvae (Fig. 1). These similarities persisted in both groups of vesicles throughout the period of examination. Deterioration of wild-type melanophores in mutant tissues did not occur.
Pigment cell development in Xenopus

Fig. 1. Wild-type and a" mutant control larvae. Late stage 47. Compare darkly pigmented, mainly dendritic melanophores of wild type (above) with faint, mainly punctuate melanophores of mutant (below). Although both larvae possessed eye and peritoneal iridophores, only the wild type exhibits areas of iridescence (arrows).

Table 1. Numbers (mean ± s.e.) of melanophores recorded in vesicles at various stages of development

<table>
<thead>
<tr>
<th>Stage of development of control larvae</th>
<th>Genotype of vesicle tissue (T) + neural crest implant (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>w^+T+w^+C</td>
</tr>
<tr>
<td>33/34</td>
<td>2.07±3.65</td>
</tr>
<tr>
<td>41</td>
<td>48.43±14.38</td>
</tr>
<tr>
<td>43</td>
<td>73.93±15.49</td>
</tr>
<tr>
<td>48</td>
<td>97.86±16.31</td>
</tr>
</tbody>
</table>

(b) Culture of mutant crest in (i) mutant and (ii) wild-type tissues

Sparse populations of faintly pigmented, mainly punctate melanophores (Fig. 2B) began to appear in most vesicles of both groups from stage 43; the remaining vesicles failed to exhibit melanophores. Throughout the period of examination, the incidence and intensity of pigmentation of melanophores in both groups of vesicles was in general less than that of melanophores in mutant larvae (Fig. 1).

The numbers of melanophores present in vesicles at various stages of development are recorded in Table 1.
The development of iridophores

(a) Culture of wild-type crest in (i) wild-type and (ii) mutant tissues

Silver iridophores began to differentiate in vesicles of both groups from stage 46–47 and within 24 h became golden and highly iridescent (Fig. 2C). The pattern of differentiation of iridophores in vesicles was similar to that of iridophores in wild-type larvae (Fig. 1).

(b) Culture of mutant crest in (i) mutant and (ii) wild-type tissues

Whitish-grey, non-iridescent ‘iridophores’ began to appear in both sets of vesicles from stage 47–48 (Fig. 2D) at which stage iridophores appeared in mutant larvae (Fig. 1). In a few vesicles of both groups some such cells became silvery and faintly iridescent; none became golden. Hence the differentiation
of iridophores in both groups of vesicles was somewhat less than that of irido-
phores in mutant larvae, which in general went on to become faintly golden.

DISCUSSION

The time at which melanophores and iridophores began to differentiate in
vesicles and the extent of their subsequent differentiation were found to depend
entirely on the genotype of the neural crest implant. Mutant pigment cells in
association with either mutant or wild-type tissues exhibited mutant patterns
of differentiation. Wild-type pigment cells in association with either wild-type
or mutant tissues exhibited wild-type patterns of differentiation. These results
indicate that the mutant gene is expressed within melanoblasts and iridoblasts,
reducing their inherent capacities for differentiation, and contradict Tompkin's
proposal that deficiencies in the immediate tissue environment contributes to
the faint punctate form of mutant melanophores. In the present study the
differentiation of mutant melanophores was not enhanced in wild-type tissues.
Furthermore, wild-type melanophores in mutant tissues maintained wild-type
characteristics throughout the period of observation. This result disagrees with
Tompkin's finding that wild-type melanophores in mutant larvae became
punctate at stage 45 and suggests that the change in morphology reported by
Tompkins arose in response to factors other than a genetically controlled
deficiency in the environmental tissues. In addition, the results indicate that the
mutant effect is unrelated to any hormonal control of pigment cell differentiation
as expression of the mutant genotype occurred in tissues isolated from larval
sources of hormones. This is in agreement with Tompkin's finding that para-
biosis and head transplants between mutant and wild-type embryos had no
effect on melanophore character.

The effect of the mutation on the development of iridophores is only apparent
on close examination of larvae and was not reported in the original description
of the mutant (Hoperskaya, 1975). Several mutations affecting more than one
type of pigment cell have been reported for Ambystoma mexicanum and Rana
pipiens. However, these mutations affect either melanophores and xanthophores
(Dumeril, 1870) or xanthophores and iridophores (Humphrey & Bagnara, 1967;
Richards, Tartof & Nace, 1969; Lyerla & Dalton, 1971). The \( \text{a}^\text{p} \) mutant of
Xenopus is the first mutation found to affect melanophores and iridophores and
may be a useful aid in investigating the cellular and biochemical relationships
of these pigment cells.

REFERENCES

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