Evidence that the premature death mutation (p) in the Mexican axolotl (Ambystoma mexicanum) is not an autonomous cell lethal

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SUMMARY

Cell-lethal developmental mutations, which are presumed to affect the viability of all cells in a mutant embryo, have been distinguished from other developmental lethals on the basis of the results of parabiosis and transplant experiments. Premature death (p), previously classified as a cell lethal, does not survive parabiosis. However, transplants involving mutant eye, flank epidermis and primordial limb tissue all survived on a normal recipient. The mutant, therefore, cannot be considered a true cell lethal, though it suffers from serious and widespread abnormalities that cannot be corrected by parabiosis. In addition, transplants of mutant branchial mound tissue did not develop into normal gills on a normal recipient. These transplants were the only ones involving mutant endoderm, and their failure supports our hypothesis that the mutation leads to a specific endoderm defect.

INTRODUCTION

More than 30 mutant genes affecting development of the Mexican axolotl, Ambystoma mexicanum, have been described by the late R. R. Humphrey and his colleagues (Briggs, 1973; Malacinski & Brothers, 1974; Humphrey, 1975). These genes have been divided into five groups. One group includes genes, such as the 'cardiac lethal' (c), which seem to affect specific organs or tissues. The heart of a cardiac mutant embryo fails to begin beating at the usual time (Humphrey, 1972), and appears to be abnormal in its ultrastructure (Lemanski, 1976). However, other organs or parts appear normal and survive as transplants, and mutants survive when fused with a normal embryo, though they depend on the normal embryo for circulation (Humphrey, 1972).

A second group are the so-called cell or autonomous lethals, which are believed to carry metabolic defects affecting all cells of the embryo. Mutant embryos united in parabiosis with normal fail to survive, and their death may result in the death of the normal co-twin. Transplants of gill or limb primordia to a normal host also fail to survive. Though a large number of mutants have been assigned to this group (Humphrey, 1975), many have not been studied in...
sufficient detail to establish that they are true ‘cell lethals’, with metabolic or other defects affecting the survival of all cells of the embryo. For example, the mutant gene \( st \) (stasis) was originally classified as an autonomous lethal (Malacinski & Brothers, 1974; Humphrey, 1975) and does not survive parabiosis. However, gill and limb transplants do survive on normal recipients (Humphrey & Chung, 1977).

The premature death mutation (gene \( p \)) leads to a complex of abnormalities first evident at about stage 37 of prehatching development. These include abnormalities of heart, liver, gill and muscle development (Trottier & Armstrong, 1977). Though \( p \) was originally classified as an autonomous lethal (Briggs, 1973; Malacinski & Brothers, 1974; Humphrey, 1975), histological examination of mutant embryos led us to propose that the defect might only involve the endoderm. The discovery that transplants of certain donor tissues survive on normal recipients, reported in this paper, indicates that the mutant is not a cell lethal, and further strengthens our hypothesis of an endoderm defect.

**METHODS**

The animals carrying the \( p \) mutation were either 3719-2 and 3719-5, originally from R. R. Humphrey (Trottier & Armstrong, 1977), or their progeny, bred in our lab. Spawnings were induced by injecting mature females intramuscularly with 4 mg follicle-stimulating hormone, and intraperitoneally with 50 µg luteinizing hormone, 12 h prior to mating. Embryos were raised at either 10° or 18 °C in 25 % Holtfreter's saline.

Approximately 200 embryos from several spawnings were divided approximately equally between parabiosis (or telobiosis) and transplant experiments. Parabiosis and telobiosis were performed, by the method of Rugh (1962), on embryos at stages 23–24 of the Schreckenberg & Jacobson (1975) series. Both heterotopic and reciprocal gill transplants, and eye transplants, were done by the method of Rugh (1962) on stage 30–31 embryos. Heterotopic limb transplants were performed at stages 33–34 as described by Slack (1977). Flank ectoderm transplants were also performed at this stage.

Operations were all carried out in sterile plastic Petri plates lined with 2 % agar. The operations were performed in sterile calcium-free 100 % Holtfreter's saline. After sufficient time had been allowed for healing, the calcium-free solution was gradually replaced with regular Holtfreter's containing 100 mg/l each of penicillin and streptomycin.

**RESULTS**

The diagnostic scheme for characterizing cell lethals (Malacinski, 1978) has generally involved reciprocal limb and gill transplants, as well as parabiosis. The results of such transplants, and others, detailed below, are summarized in Table 1.
Table 1. Summary of transplant experiments

<table>
<thead>
<tr>
<th>Type of transplant</th>
<th>Number performed</th>
<th>Number developing</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill: Reciprocal, mutant to wild type (Fig. 1)</td>
<td>4</td>
<td>0</td>
<td>Transplants failed to develop past stage 37</td>
</tr>
<tr>
<td>Gill: Reciprocal, wild type to mutant</td>
<td>4</td>
<td>4</td>
<td>Transplants developed to stage 40 while recipients remained at an apparent stage 37</td>
</tr>
<tr>
<td>Gill: Heterotopic, wild type to mutant (Fig. 3)</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Limb: Heterotopic, mutant to wild type (Fig. 4)</td>
<td>2</td>
<td>2</td>
<td>One was reduplicated, the other normal</td>
</tr>
<tr>
<td>Eye: Heterotopic, mutant to wild type</td>
<td>1</td>
<td>1</td>
<td>Development appeared normal</td>
</tr>
<tr>
<td>Flank epidermis: Mutant to wild type (Fig. 6)</td>
<td>6</td>
<td>6</td>
<td>Transplanted area retained donor pigmentation</td>
</tr>
</tbody>
</table>

In all experiments, control transplants (wild type to wild type) developed normally.

Gill transplants involved removal of complete branchial mound from the donor, including the endoderm as well as the overlying ectoderm and mesoderm. In reciprocal transplants, the mutant gill developed on a normal recipient only as far as an apparent stage 37 and retained the characteristic bulb at the distal end (Fig. 1). The mutant gill was resorbed with time, leaving the recipient with only one gill (Fig. 2). Such animals showed compensatory growth of the remaining gill.

In contrast, a normal gill on a mutant recipient developed to a more advanced stage than the mutant’s remaining gill. Though no circulation was established, the transplant reached a degree of differentiation which was slightly less than that in stage-40 controls, while the mutant embryo did not develop past stage 37. In heterotrophic transplants of a normal branchial complex to a mutant recipient, the normal gill again developed to a more advanced stage (Fig. 3).

Since homozygous \( p \) mutants die before limbs develop, reciprocal transplants were not possible. Instead, the presumptive limb area, consisting of ectoderm and underlying mesoderm, was transplanted from embryos of a spawning expected to segregate \( p/p \) mutants to the flank of embryos from a wild-type spawning. The wound, resulting from the removal of tissue, healed before stage 37, when donors were classified as \( p/p \) or wild type. Though only two transplants of the desired combination were obtained, the grafted limb primordia developed into limbs in both cases. One was single and one reduplicated (Fig. 4), as observed by Slack (1977) and ourselves for transplants involving wild-type donors. No signs of degeneration were seen as the recipients grew older (Fig. 5).

Eye transplants were also performed, and involved the entire optic vesicle and overlying ectoderm, but no endoderm. The eye region was transplanted to a region near the spine, just behind the branchial mound. Though only one
Fig. 1. Ventral view of a wild-type embryo, at about stage 39, which had its left branchial mound removed at stage 31 and replaced by that from a mutant embryo. × 13.

Fig. 2. Dorsal view of the animal shown in Fig. 2, about 3 months of age. The left gill has been totally resorbed. × 2·5.

Fig. 3. Heterotopic gill transplant of normal gill tissue to the belly region of a p/p mutant. The photograph was taken 4 days after the mutant had reached an apparent stage 37. × 20.
Mutant gene (p) in Ambystoma embryos

Fig. 4. Ventral view of a wild-type axolotl, about 5 weeks old, which has an extra limb on its left side resulting from a heterotopic transplant of the limb bud from a p/p animal. ×6.

Fig. 5. Same animal as in Fig. 4, at an age of about 1½ years. ×0·55.

Fig. 6. A white axolotl, about 5 weeks old, with a dark patch of skin behind the left forelimb. The patch of skin originated from the flank of a dark p/p embryo. ×6.
Table 2. Parabiosis and telobiosis experiments

<table>
<thead>
<tr>
<th></th>
<th>Normal-normal</th>
<th>Normal-mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parabiosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibs*</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Non-sibs</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>Telobiosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibs</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Non-sibs</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

* Sibs indicates that both embryos were from a mating of +/p animals, where 1/4 of the progeny are expected to be p/p. Non-sibs means that one of the pair was from a mating of two heterozygotes, while the other was from a wild-type mating.

such transplant involved a mutant donor, an eye developed. Externally the eye appeared normal; internal morphology and possible function were not examined.

Transplants of ectodermal flank tissue from several mutants were also successful. The recipients in these experiments were normal white embryos and the dark transplanted patch of skin could easily be seen (Fig. 6).

Because, in our previous study (Trottier & Armstrong, 1977), only four parabiotic twins of the desired combination were obtained, and in view of the success of some of the transplants, we felt that additional parabiosis experiments were necessary. Since a mutant can only be recognized in a parabiosis experiment if correction does not occur, the only way we can demonstrate that correction never occurs is to show that the normal-mutant pairs occur in the correct Mendelian ratio. This time, six normal-mutant pairs were obtained; all showed the characteristics previously described. Though the normal embryo initially continued to develop, its red blood cells appeared to become trapped in the mutant's blood islands, which remained distinct. R. R. Humphrey (personal communication) suggested that the proximity of the mutant blood islands to the site of joining might cause the red blood cells of the normal twin to be trapped. Therefore, additional pairs were joined head to tail (telobiosis). However, all of 17 normal to mutant combinations exhibited the same characteristics as the parabiotic twins, including the trapping of the red blood cells.

Table 2 shows the total number of operations performed and the number of normal and mutant combinations. In these experiments, 112 embryos were from matings of two +/p animals, and 23 (21%) were p/p. The difference from the expected 1/4 is not significant ($P = 0.25$; $\chi^2$ test) and we feel this rules out the possibility that the mutant syndrome had been corrected in some cases by fusion with a wild-type embryo.


DISCUSSION

The criteria for deciding that a mutant is an autonomous or cell lethal have been that parabiosis fails to rescue the mutant and that transplants of mutant tissue fail to survive on a normal recipient (Briggs, 1973; Malacinski & Brothers, 1974; Humphrey, 1975; Malacinski, 1978). If only the failure of parabiosis and gill transplants were considered, premature death would have to be classified as a cell lethal. However, the success of transplants involving the eye, flank epidermis and primordial limb tissue indicates that not all mutant cells or tissues are defective. Though the number of successful transplants was small, we feel the results are unequivocal. The transplant experiments are not destructive to the donor, and we can easily recognize that the donor is a mutant at stage 37. Positive results, therefore, cannot arise from the transplant having been taken from a wild type instead of a mutant donor. The eye and limb transplants were heterotopic, so there should be no argument that we have failed to remove sufficient recipient tissue, as there might be in an orthotopic transplant, while the skin transplants relied on a difference in pigmentation.

If premature death is not a cell lethal, why, then, does parabiosis fail? Presumably because the mutant has sufficiently serious defects that the mere exchange of nutrients is not sufficient to rescue it. The failure might also be related to the trapping of the blood from both embryos in the mutant, though in spite of this, the normal twin continues to develop for several days. Our efforts to prevent the trapping of the blood failed. Nevertheless, we believe that the total absence of development of parabiosed mutant animals indicates that this trapping is not the sole reason parabiosis fails to rescue the mutant. The possibility that some mutants were corrected, but not others, seems to be ruled out by our observation that mutant embryos were found in the parabiosis experiments in a frequency not significantly lower than the expected 1/4.

The fact that the gill transplants were the only ones that did not succeed, and were the only transplants that included donor endoderm, further supports our proposal that the mutant has a specific endoderm defect (Trottier & Armstrong, 1977). Further study of the mutation may lead to important new insights into inductive interactions involving the endoderm.

This work, and the axolotl colony at the University of Ottawa, were supported by grants from the Natural Sciences and Engineering Research Council, Canada.

REFERENCES


(Received 7 February 1980, revised 29 April 1980)