A Brief History of the *Premature Death* (*p*) Mutation

Ann C. Graveson and John B. Armstrong
Departments of Biology
Dalhousie University
Halifax, Nova Scotia B3H 4J1
and
University of Ottawa
Ottawa, Ontario K1N 6N5

The axolotl has long been a popular animal with developmental biologists. The size and quantity of their eggs, combined with the ease with which they can be obtained, maintained and manipulated, make them ideal for experimental studies. One potential advantage to working with the axolotl is the availability of a variety of developmental mutants, many of which have been but partially characterized (Armstrong, 1985).

One of these, the *premature death* (*p*) mutation, has slowly been revealing its potential as an invaluable tool in the study of several major developmental processes, including neural induction, and the segregation of the neural-crest-cell lineages.

The *premature death* mutation was first described by Tompkins, but his original stock was subsequently lost. A similar mutant phenotype was later found in spawnings of sibling animals donated to the University of Ottawa Axolotl Colony by R. R. Humphrey. The new mutant was designated because of its resemblance to Tompkins' description (Trottier and Armstrong, 1977).

Premature death is a recessive lethal mutation, which appears to affect only homozygotes. Mutant embryos are indistinguishable from wild-type embryos until after stage 37, when the gills consist of short, unbranched primary filaments, the heart has begun to beat, and the embryos respond to tactile stimulation. The embryos undergo developmental arrest at this stage, except for the continued differentiation of pigment cells. Their primary gill filaments acquire characteristic bulb-like structures distally, and epidermal blistering and disintegration begins within days of their identification. It should be noted that heartbeat and tactile reflexes continue in spite of extensive degeneration. A time of death is, therefore, extremely difficult to assign, but is usually deemed to occur approximately one week after identification. Although externally normal at stage 37, histological examination of mutant embryos has revealed a wide variety of internal abnormalities (Trottier and Armstrong, 1977). The major features include: a plug of undifferentiated tissue replacing the endocardial lining of the ventricle and conus arteriosus of the heart, abnormalities of the pharyngeal endoderm and branchial pouches, and underdeveloped liver and myotomes.

Despite the diversity of defects, all are dependent on endoderm for their development; the liver and pharyngeal pouches are derived from endoderm, while the heart, gills and myotomes require endoderm as an inductor. This common link led to the proposal that the mutation caused a general endodermal defect. This hypothesis was supported by the results of transplantation experiments of mutant tissues onto wild-type hosts (Mes-Hartree and Armstrong, 1980). Transplants of eyes, epidermis, and limbs, whose development is independent of endoderm, survived and developed, while gill primordia transplants did not. The survival of some mutant tissues also provided conclusive evidence that *p* was not an autonomous cell lethal mutation (its original classification, based on the results of parabiosis experiments).

As so many endodermal functions appeared to be affected in mutant embryos, it seemed reasonable to check whether the endoderm was also defective in another of its major roles—as the inducer of cartilage derived from cranial neural crest cells. This capacity could be tested only *in vitro*, as disintegration of mutant embryos occurs before chondrogenesis normally begins. Explant cultures of wild-type neural folds and pharyngeal endoderm almost always produce cartilage (Graveson and Armstrong, 1987), but, as expected, cartilage was never seen when mutant neural folds and endoderm were cultured together (Graveson and Armstrong, 1990). The cultures appeared healthy, and contained abundant melanocytes, which strengthened the argument that *p* does not directly affect all cells of the embryo.

Our view of the defects in *p* changed radically when mutant neural folds and mutant endoderm were individually tested in this *in vitro* system (Graveson and Armstrong, 1990). Mutant pharyngeal endoderm was able to elicit chondrogenesis from wild-type neural crest, but mutant neural crest was incapable

of responding to the inductive signals of wild-type endoderm. These unexpected results raised the possibility that other neural crest cell functions might also be affected by *p*. Unfortunately, *in vitro* testing is not feasible for most organ systems. Therefore, various *in vivo* neural crest manipulations were performed (Graveson and Armstrong, 1994). Unilateral homotopic transplantation of a branchial-level wild-type neural fold led to a partial rescue of the mutant embryo. Though survival of the embryo was not extended, the mutant characteristics, at this axial level, were dramatically improved. The primary gill filaments grew noticeably longer and did not develop the distal bulbs, secondary gill filament buds were often seen, and the morphology of the pharyngeal pouches appeared normal.

The extirpation of neural folds from wild-type embryos led to the production of *p* phenocopies, providing further evidence that neural crest is involved in the development of systems which are abnormal in the mutant. The gills of these embryos bore a striking resemblance to *p* gills, with short, unbranched primary filaments having distal bulbs, and no secondary filaments. The establishment of circulation was also affected, with the blood remaining in the blood islands, as is the case for *p* embryos. These *p* characteristics were not permanent, but we believe the gradual return to normalcy was the result of regulation, since the timing and extent appeared to be correlated with pigment cell regulation. Thus, it appears that most, if not all, of the abnormalities seen in the *p* mutant can be explained by defective neural crest, rather than by defective endoderm.

Previously, neural crest had not been known to be to be involved in several of the processes affected by the mutation, namely: pharyngeal pouch morphogenesis, primary and secondary gill filament morphogenesis, and the establishment of circulation. [Neural crest has recently been shown to invade the anterior regions of the heart in another amphibian (Sadaghiani and Thiebaud, 1987)]. Therefore, *p* mutant characteristics may serve as indicators of neural crest involvement.

Even more exciting is the potential for *p* to be used in the study of some of the early stages of embryogenesis. In *p* embryos, neural crest cells are produced in the proper location (the neural folds), and even the defective (chondrogenic) cells possess the ability to migrate along the pathways appropriate for their axial level (unpublished data). The differentiative capacity of some of the cells is normal (i.e., pigment cells and Rohon-Beard cells), while other functions are impaired (i.e., chondrogenic ability, and roles in gill and branchial pouch morphogenesis). Thus, *p* affects one subpopulation of neural crest cells. The segregation of affected and unaffected subpopulations must be an early event, completed by mid-neurula stages.

Although very little is known about the establishment and early (pre-migratory) development of the neural crest, only a few tissues can be involved in these processes. Neural induction, where involuting chordamesoderm induces overlying gastrula ectoderm to neuralize, appears to be a prerequisite for its formation. The ability of mutant chordamesoderm to induce neurectoderm formation, and the ability of mutant gastrula ectoderm to respond to this induction by producing chondrogenic neural crest were individually tested by implanting dorsal lip material into the blastocoeles of early gastrulae (stage 9), followed by explant culture of the resulting secondary neural folds to test for chondrogenic ability (Graveson and Armstrong, submitted). Mutant chordamesoderm was found to be able to initiate the processes leading to the production of chondrogenic neural crest from wild-type gastrula ectoderm. This would include neural induction itself, as well as any subsequent inductive signals from the mesoderm which might be required to establish the chondrogenic neural crest. However, mutant gastrula ectoderm was unable to respond properly to the normal signals provided by wild-type mesoderm; the secondary neural folds contained only non-chondrogenic cells.

The presumptive neural crest cells need not necessarily be the only target tissue of the *p* gene. Several of the proposed models for the establishment of the neural crest require interactions between neurectoderm and adjacent epidermal ectoderm, occurring after the neural induction signals (Graveson, 1993). Therefore, the mutation could also be affecting any combination of these tissues. Alternatively, the defective tissue could be an ectodermal inductor of neural crest, while the presumptive neural crest cells are affected only indirectly, through a lack of induction.

Although questions remain, the defect in *p* has now been localized to the ectoderm. The affected tissues include a major subpopulation of neural crest cells, and at least one derivative of the ectoderm adjacent to the neural folds, the placode-derived lateral line neuro-

masts (Smith et al., 1994). Further studies on the full extent of *p*'s effects on the ectoderm may help reveal the sequence of intra-ectodermal interactions involved in the formation of the affected cells.

References

Armstrong, J.B. 1985. The axolotl mutants. Dev. Gen. **6**:1-25.

Graveson, A.C. 1993. Neural crest: Contributions to the development of the vertebrate head. Amer. Zool. **33**:424-433.

Graveson, A.C. and Armstrong, J.B. 1987. Differentiation of cartilage from cranial neural crest in the axolotl (*Ambystoma mexicanum*). Differentiation **35**:16-20.

Graveson, A.C. and Armstrong, J.B. 1990. The *premature death* (*p*) mutation of *Ambystoma mexicanum* affects a subpopulation of neural crest cells. Differentiation **45**:71-75.

Graveson, A.C. and Armstrong, J.B. 1994. *In vivo* evidence that the *premature death* (*p*) mutation of *Ambystoma mexicanum* affects an early segregating subpopulation of neural crest cells. J. Exp. Zool. (in press).

Mes-Hartree, M. and Armstrong, J.B. 1980. Evidence that the *premature death* mutation (*p*) in the Mexican axolotl (*Ambystoma mexicanum*) is not an autonomous cell lethal. J. Emb. Exp. Morphol. **60**:295-302.

Sadaghiani, B. and Thiebaud, C.H. 1987. Neural crest development in the *Xenopus laevis* embryo, studied by interspecific transplantation and scanning electron microscopy. Dev. Biol. 124:91-110.

Smith, S.C., Graveson, A.C., and Hall, B.K. 1994. Evidence for a developmental and evolutionary link between placodal ectoderm and neural crest. J. Exp. Zool. (in press).

Trottier, T.M. and Armstrong, J.B. 1977. Experimental studies on a mutant gene (*p*) causing premature death of *Ambystoma mexicanum* embryos. J. Emb. Exp. Morphol. **39**:139-149.