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Review

The developmental mutants of *Xenopus*

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Introduction

Among the developmental mutants found in Amphibians, those of the axolotl are the best known. They have been the subject of several reviews (Briggs, 1973; Malacinski and Brothers, 1974; Humphrey, 1975; Armstrong, 1985). In other Urodeles, a few mutants have also been isolated, especially in *Pleurodeles waltl* (Gallien and Collenot, 1964; Beetschen and Jaylet, 1965; Signoret *et al.*, 1966; Lacroix and Capuron, 1970; Jaylet *et al.*, 1980; Collenot and Collenot, 1985; Collenot *et al.*, 1989).

In Anura, in addition to the mutants found in *Rana* (reviews by Browder, 1975 and Dubois, 1979), an important number of developmental mutants have been identified in *Xenopus*, especially in our laboratory, the Geneva *Xenopus* Centre. They were described gradually as they were found; the first few have been listed previously (Fischberg *et al.*, 1964; Gurdon and Woodland, 1975). Recently, a list of *Xenopus* mutants, as complete as possible, has been published (Droin, 1991b), including those found by Krotoski *et al.* (1985). However, we thought it necessary to publish a more detailed review on these mutants, with the purpose of making them better known and utilized in more elaborate analyses as they constitute an important potential for research on developmental mechanisms.

This review begins with the historical background — i.e., the rationale that led to the discovery of these mutations — and their origin is discussed. A general characterization of the different mutant groups then precedes a brief individual description of the mutant phenotypes.

Historical background

In 1952, Briggs and King achieved the first successful nuclear transfer in *Rana pipiens*. They obtained normal feeding larvae from blastula nuclei transplanted into enucleated eggs. In 1958, Fischberg and his collaborators, working in Oxford, adapted this technique to *Xenopus* (Fischberg *et al.*, 1958, Elsdale *et al.*, 1960). The aim of these experiments was to test the ability of somatic nuclei to promote normal development; indeed it was important to know if the nucleus of a somatic cell could replace the zygote nucleus and hence, to determine whether somatic nuclei, in the course of differentiation, were submitted to irreversible changes in their genome or whether they remained totipotent. The first transfer experiments with blastula nuclei in *Xenopus* had shown that one could obtain adult and fertile frogs from such somatic nuclei. More transfers were then carried out in *Rana* and in *Xenopus*, by transplanting nuclei of more and more differentiated or specialized cells from larval stages up to adults, resulting in the development of adult frogs or larvae respectively, with a higher percentage of success in *Xenopus* than in *Rana* (see Tables 1 and 2, Gurdon, 1986). In addition, serial transfers were carried out which suggested an altered, but steady state of the nucleus since the whole range of abnormalities in a given experiment (stage attained, and types of malformation) tended to recur indefinitely in each serial experiment.

All these experiments demonstrated the totipotency of nuclei of embryonic and several types of differentiated cells, and the pluripotency of nuclei of larval and even adult cells, at least in a small proportion of transfers. Furthermore, it was concluded that abnormal development could be due to chromosomal aberrations resulting from an asynchrony between the cytogenetic events of the donor nucleus and the cytoplasm of the recipient egg (see reviews by Gurdon, 1986 and Di Berardino, 1987).

Genetic analysis

On the assumption that nuclei, when transplanted, might very often undergo irreversible alterations, we tried to determine whether phenotypically normal transplant frogs carried more recessive mutations in the heterozygous state than control frogs, and also to detect mutations carried by lethal transplant embryos. Fischberg (1961) proposed three methods of genetic analysis: 1) a classical analysis of untreated adult and phenotypically normal transplant frogs (F2 method); 2) an analysis of normal and abnormal transplant embryos by germ cell grafting prior to genetic analysis (F2 method); and 3) a shorter analysis of normal and abnormal transplant embryos (F1 method) consisting of serial transfers, germ cell grafting and crosses of clonal members (for detailed schemes and explanations, see Fischberg and Blackler, 1963).

A total of 21 normal *Xenopus* adults that had resulted from nuclear transplantation were genetically analyzed; 17 issued from endoderm nuclei of different developmental stages, from blastula to feeding stage, the remaining 4, from mesoderm of neurulae. Furthermore, 7 of these frogs belonged to 2 different clones resulting from serial transfers: one clone of 4 frogs issued from endoderm nuclei and one clone of 3 frogs issued from mesoderm nuclei. The analysis revealed that these frogs were indeed isogenic: the same mutation was carried by the 4 frogs of the endoderm clone and another 5 mutations were carried by each of the 3 males of the mesoderm clone (Fig. 1).

These frogs were analyzed by the first classical method consisting of producing an F_1 by crossing the transplant frog with a wild-type individual or, if possible, with one of the parents of the donor embryo used for the transfer of the nucleus. The F_1 individuals were then tested, either by backcross with the transplant frog or *inter se*. If the transplant frog is heterozygous for a recessive mutation, one in two backcrosses will produce 25% of homozygous embryos, or one in four in sibmatings. In addition, a testcross can be carried out between heterozygous F_1 individuals and the parents of the donor embryo (Fig. 4, Fischberg and Blackler, 1963).

During the last 30 years, genetic analysis has continued, at first with the original transplant frogs, and later on with their offspring obtained by inbreeding, or outbreeding with wild-type animals. A total of 32 developmental mutations were found and are listed

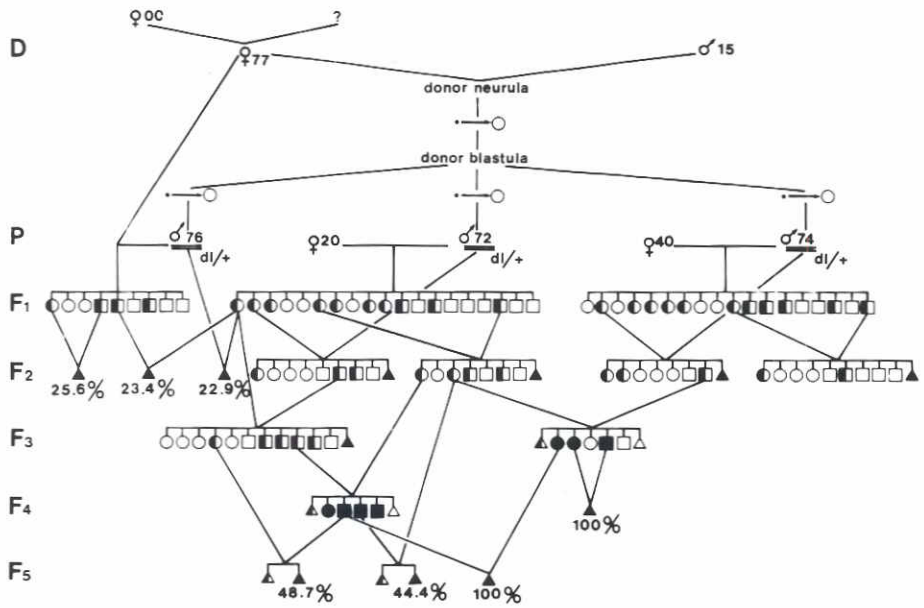

Fig. 1. Example of a genetic analysis by the first F_2 method. *The three transplant-males (♂76,♂72,♂74) form a clone resulting from a serial transfer experiment. The analysis has shown that the three males were heterozygous for the dl mutation (reproduced from Droin and Chavane, 1976, with permission of the editor).*

below (Table 1). In addition, as the mutation *anucleolate* (*0 nu*, see below) was used as a marker for the transplanted nucleus (Elsdale *et al.*, 1958), all the transplant frogs except 2 were heterozygous *(1nu)*, hence the homozygous *0 nu* mutants were present throughout all these analyses.

Origin of mutations

The genealogies of the families and the origin of the different mutations have been detailed in different publications. In summary, 15 of the 32 mutations were carried by transplant frogs; the origin of 8 of them could not be verified while, for the 7 others, back- or testcrosses have shown that they were carried either by the normal mate or by one of the parents of the donor embryo and could, therefore, be attributed to the wild-type stock.

Of the other 17 mutations, 8 originated in the stock and the remaining 9, although not tested, were probably also carried by wild-type frogs. These are the last-found mutations that occurred in subsequent generations of transplant frogs, most often obtained by crossing with wild-type individuals.

All of the mutations are recessive except the dominant *Screwy*; this is the only one that could have occurred during the transplantation process. It was expressed in the transplant male during its development, but it was not possible to determine whether it was due to a somatic mutation during the differentiation process of the donor embryo, or whether it was a consequence of the transplantation itself (Uehlinger, 1966).

It can be concluded from these analyses that the majority of the mutations were carried by wild-caught imported frogs or their laboratory-bred progeny, showing thus that somatic nuclei were not subjected to genetic changes during cell differentiation or transfers. It should be emphasized, however, that only adult transplant frogs were analyzed, which evidently represent a strong selection for total developmental capacity. In spite of the unknown origin of several mutations, one may infer that the 32 mutations found were carried by 20 different frogs, i.e., a percentage of 1.6 mutations per frog. These results are in accordance with those of Krotoski *et al.* (1985) who found 14 developmental mutations (one carried by 2 different frogs) by gynogenesis or inbreeding of 8 wild-type females, giving a frequency of 1.875 mutations per frog.

Mutant characteristics

The mutants listed in Table 1 are classified into 3 categories in analogy to those of the axolotl (Armstrong, 1985), but they will be described alphabetically in the last part of this review.

Maternal effect mutants (three mutants)

Maternal effect mutants are interesting and useful in providing information on the mechanisms of action of the genes which are active during oogenesis and which contribute to store metabolic, structural or regulatory products controlling early morphogenesis. Furthermore, rescue of the mutants can be used to attempt to identify the biochemical nature of the defect.

Two types of maternal effect mutants can be encountered, (a) the paternally rescuable ones in which the wild-type allele introduced by the male intervenes sufficiently early to allow normal embryogenesis and to correct an abnormal phenotype of the eggs laid by homozygous mutant mothers. Nine of the non-lethal types of homozygous mutant females have been tested but none of them presented any such maternal effect during early developmental stages.

The other type (b) consists of strict maternal effect mutants affecting the development of all the embryos of the homozygous mothers independently of the genotype of the father. Three such have been found, *pale eggs (pe)*, *partial cleavage* and *no cleavage*. These mutants (except *pe*) are difficult to maintain since homozygous males and females cannot be identified; one must resort to sibmatings to raise large numbers of tadpoles and frogs and wait until they reach sexual maturity in order to carry out test crosses.

Developmental lethals (nineteen mutants)

In the early reports concerning the mutants of axolotl (Humphrey, 1975; Malacinski, 1978), the term «autonomous lethals» or «cell lethals» was attributed to the mutants which could not survive,

TABLE 1

LIST OF MUTANTS[1]

Name of mutation	Symbol	Stage of expression[2]	Characteristic features
Maternal effect mutations			
no cleavage	*nc*	1	abnormal maturation abnormal fertilization absence of cleavage
pale eggs	*pe*	1-41	pale color of eggs, small amount of melanosomes
partial cleavage	*pc*	3-9	abnormal cleavage
Developmental lethals			
abnormal joints	*abj*	58	abnormal limb development
bent tail	*bt*	42-48	edemas, internal hemorrhages, tail bent up
bloated-3	*bl-3*	33-46	trunk edema, heart and gut abnormal
degenerative cement gland	*dcg*	30-45	cement gland, followed by generalized degeneration
delayed metamorphosis-2	*dm-2*	49-50	long delay of development until metamorphic climax
droopy tailtip	*dtp*	39-47	microcephaly, microphthalmy, edema, drooping of tail
dwarf-1	*dw-1*	48	arrest of development
dwarf-2	*dw-2*	47	arrest of development
dwarf-3	*dw-3*	47	arrest of development
dwarf-4	*dw-4*	46	arrest of development
flat head	*fh*	42-46	microphthalmy, head flattened
folded jaw	*fj*	42-48	deformation of lower jaw
muscle opacity (linked to *ry*)	*mo*	47-48	opacity of the ventral muscles of the head
edema	*oe*	52-postmeta-morphosis	edemas of lymph sacs
otolithless	*otl*	36-48	absence of otoliths, swollen ears
precocious edema	*p. oe*	39-47	generalized edema
Screwy	*S*	S/S	degeneration
		32-40	absence of cephalic mesenchyme
		S/+	subvital [3]
		52-66	spiral torsion of the tail
white lethal	*wl*	39-50	abnormal differentiation of chromatophores
yolky rectum	*yr*	31-42	microcephaly, microphthalmy, absence of endoderm differentiation
Developmental non-lethals			
abnormal limbs	*abl*	54-adult	semilethal; syndactyly polydactyly, brachydactyly
delayed metamorphosis	*dm*	49-58	subvital; delay of development until metamorphic climax
distended lungs	*dl*	49-66	semilethal; delay of development, distension of lungs
goître	*g*	meta-morphosis	semilethal?; hyperplastic goiter, delayed metamorphosis
immobile	*im*	23-41	lack of muscular contraction in early stages
kinky tailtip	*kt*	42-66	subvital; abdominal edema, kink of the tail
polydactyly	*pd*	54-adult	subvital; polydactyly, mainly of posterior limbs
rusty (linked to *mo*)	*ry*	43-49	viable; persistence of egg pigment
turner	*tr*	47-53	semilethal; abnormal morphology and position of otoliths
ventral edema	*v. oe*	42-66	semilethal; abdominal edema

[1]The majority of the mutants are still available. For further information, contact Dr. Ch. Thiébaud, Station de Zoologie expérimentale, route de Malagnou 154, 1224 Chêne-Bougeries, Geneva, Switzerland.

[2]Nieuwkoop and Faber, 1956

[3]subvital= 50-80% survival; semilethal= 2.5-50% survival (Hadorn 1955)

either fused in parabiosis with a wild-type embryo or after transplantation of tissue anlagen in a normal host. In 1980, Neff *et al.* developed a technique of explant culture of embryonic cells to test whether the cells of the lethal mutants exhibited the lethal traits and, if so, to analyze the cellular origin of these traits. On the basis of these three criteria, parabiosis, transplantation and explant cultures, Armstrong (1985) proposed three subclasses for these lethals — the rescuable cell lethals, the nonrescuable cell lethals, and the pseudo cell lethals to which «the tissue- and organ-specific» mutants are added (see Table 2, Armstrong, 1985).

The developmental lethals of *Xenopus* can be divided into two classes — the previously called «autonomous lethals» and the «tissue- and organ-specific» lethals. A few of them have been tested by parabiosis or by anlagen transplantation but none by explant cell cultures. It is therefore not possible to subdivide these lethals into the subclasses proposed for the axolotl.

In the «tissue- or organ-specific» category, death occurs as a direct consequence of the specific abnormalities — for example, abnormal swimming for *otl*, impediment of feeding for *fj* or destabilization of body equilibrium due to abnormal limbs for *abj*; in the majority of cases however, the causes of lethality are unknown.

Developmental non-lethals (ten mutants)

These mutants suffer from specific abnormalities that do not impede their survival, although the number of survivors varies widely depending on the different mutations. These mutants are easy to maintain and to work with since one can get spawnings with 100% homozygous mutants. They offer the possibility of analyzing some restricted or precise mechanisms of development such as limb morphogenesis *(abl, pd)*, the importance of lungs during larval respiration *(dl)* or the comprehension of hormonal metabolism during premetamorphosis *(g, dm)*.

Other mutants

Nucleolar mutants

Since the occurrence of the first *anucleolate* mutation *(0 nu)* found by Elsdale *et al.* (1958), new cases of similar mutations have been described by Blackler (1968) and Krotoski *et al.* (1985). Miller and Gurdon (1970) have found other nucleolar mutants consisting of partial nucleoli *(p nu)* of various sizes. Nucleolar morphology, variability, rate of rRNA synthesis and number of rRNA genes have been summarized in Table 3, Gurdon and Woodland (1975). In addition, two *3-nucleolate* mutants have been found in *Xenopus borealis* (Jotterand and Fischberg, 1974) and one in *Xenopus fraseri* (Tymowska, 1977). In a comparative study of the karyotypes of various species of *Xenopus*, Tymowska (1991) considers that secondary constrictions associated with nucleolar organizer regions manifest a high degree of mutability.

Developmental mutants in other species

Several developmental mutants have also been found in three other species of *Xenopus*, all recessive and lethal and exhibiting mainly edematous phenotypes (Table 2): four in *Xenopus borealis* (Droin, 1978, 1985; Droin and Colombelli, 1982), one in *Xenopus muelleri* (Droin and Colombelli, 1982), analogous to a *X. borealis* mutant; and finally, one in *Xenopus tropicalis* (Droin and Colombelli, 1983). These are spontaneous mutations having occurred in the offspring of different populations of these species brought back from Africa by Fischberg and his collaborators.

Mutants found in other laboratories

In addition to the well-known *periodic albinism* (a^p) found in Moscow (Hoperskaya, 1975), Krotoski *et al.* (1985) have described 14 mutants obtained by gynogenesis or inbreeding. Several of them exhibit phenotypes similar to those of our mutants, especially *unresponsive*, *light lethal*, *balloon* and *arrest* which have some analogies with *immobile*, *white lethal*, *bent tail* or *ventral edema* and *dwarf* respectively. *Unresponsive* has been analyzed experimentally (Reinschmidt and Tompkins, 1984) and presents indeed a high degree of similitude with *immobile*.

Lately, two maternal effect mutants have been found in Japan, one leading to developmental arrest at gastrulation (Ikenishi and Tsuzaki, 1988; Shiokawa *et al.*, 1988), the other exhibiting abnormal cleavage furrows (Kubota *et al.*, 1991; Asada-Kubota and Kubota, 1991).

TABLE 2

MUTANTS IN OTHER *XENOPUS* SPECIES

Species	Name	Symbol	Stage of expression	Characteristic features
Xenopus borealis	*bloated-1*	*bl-1*	33-46	edema, heart and gut abnormal
	hooked tailtip	*htp*	36-47	edema, bending of the tail
	narrow head	*nh*	42-47	edema, lower jaw deformation
	wrinkled edema	*w.oe*	42-47	head edema, wrinkled tail fin
Xenopus muelleri	*bloated-2*	*bl-2*	33-46	edema, heart and gut abnormal
Xenopus tropicalis	*triple edema*	*tr.oe*	44-47	edema of coelomic cavity, head and anus

Conclusions

The analysis of these mutants has been conventional, descriptive, mainly morphological and histological; only a few of them were submitted to embryological, physiological or biochemical experiments. This has led to interesting conclusions but none have given any clues with regard to the site of gene action nor allowed a classification into housekeeping or regulatory genes. It is interesting to observe that the effects of many genes are exerted at major developmental stages during the life cycle: early morphogenesis, organogenesis, feeding stage, growth and metamorphosis. Further analysis could lead to a better understanding of these transitional events.

Xenopus developmental genetics may seem trivial in view of the results obtained in other organisms. The improvement in DNA technology, such as isolation of putative developmental genes, increased number of cloned genes, gene targeting, physical mapping, insertional mutagenesis, has led to spectacular advances in the understanding of the molecular mechanisms that regulate normal development, especially in *Drosophila* (Akam, 1987; Ingham, 1988) and in *Caenorhabditis elegans* (Coulson *et al.*, 1986, 1988). These techniques can now be applied to Vertebrates, the zebra fish and the mouse being the most appropriate for such studies (Kimmel, 1989; Rossant and Joyner, 1989; Rossant and Hopkins, 1992). Mutated genes have already been identified for several developmental loci in the mouse (Reith and Bernstein, 1991).

In *Xenopus*, valuable progress has been made in the genetic mapping, gene-centromere mapping as well as establishment of linkage groups (Du Pasquier and Kobel, 1979; Kobel and Du Pasquier, 1979; Kobel, 1981; Colombelli *et al.*, 1984; Thiébaud *et al.*, 1984; Reinschmidt *et al.*, 1985; Graf, 1989a,b; Graf and Kobel, 1991). Moreover, the genotype-to-phenotype approach of development has also been well-documented these last years.

Microinjections of various molecules (DNA constructs, antisense RNA, homeobox mRNA genes, antibodies or other markers) have greatly improved the understanding of the action of gene products during early development (De Robertis *et al.*, 1990, 1991; Vize *et al.*, 1991; Slack and Tannahill, 1992). The existence of developmental mutants in *Xenopus*, though limited in number, might incite developmental geneticists to approach the phenotype-to-genotype strategy. Among them, a choice must be made in order to pick out the most suitable ones for a more complete analysis at the cellular, biochemical and molecular level — particularly the morphogenetic maternal mutants, those appearing in the early developmental stages or those affecting specific organs, in particular limb development.

Krotoski *et al.* (1985) have shown that the screening of wild-caught females by gynogenesis allows one to recover recessive mutants at diploid loci in the first generation. Inbreeding is however necessary for the discovering of maternal effects and for the establishment of the inheritance pattern of the mutants. Their results are in agreement with our data and demonstrate that wild-type populations of *Xenopus laevis* constitute an important source of mutations affecting development. In view of the difficulty of applying molecular techniques to promote mutagenesis in *Xenopus*, gynogenesis is indeed a relatively easy manipulation that tends to result in the finding of new interesting mutants.

Alphabetical list of mutants

A description of the mutants found by Krotoski *et al.* has been published in *J. Exp. Zool. 233:* 443-449, 1985.

Abnormal joints (abj). The homozygous mutants' limbs are normally developed but abnormally positioned. Instead of being stretched away from the body as in wild-type tadpoles, both the posterior and anterior *abj* limbs are nearly parallel to the body due to the abnormal articulation between girdles and limbs and between the different elements of the limbs. These mutants represent a typical case in which lethality is probably a direct consequence of the anomaly. Death occurs during metamorphosis, due to a lack of equilibrium between a changing body and a regressing tail, which, due to the abnormal position of *abj* limbs, cannot be compensated as it is by the limbs of normal metamorphosing tadpoles (Droin, 1988a)

Abnormal limbs (abl). In these mutants, the forelimbs are more affected than the hindlimbs. Various kinds of abnormalities are encountered but mainly brachymely, syndactyly, polydactyly and brachydactyly. During metamorphosis, the mutant animals develop edemas of the lymph sacs and most of them die, a minority becoming adults. This mutation is not allelic to *polydactyly* (Droin and Fischberg, 1980).

Bent tail (bt). This is one among several non-allelic mutants with more or less similar phenotypes consisting in edemas of various body regions appearing around st. 40 to 42 and followed by general degeneration and death around st. 46 to 48. Each one of these mutants however presents certain characteristics facilitating their distinction. The *bt* homozygotes are identified by a very pronounced bending up of the tail (Droin *et al,* 1970).

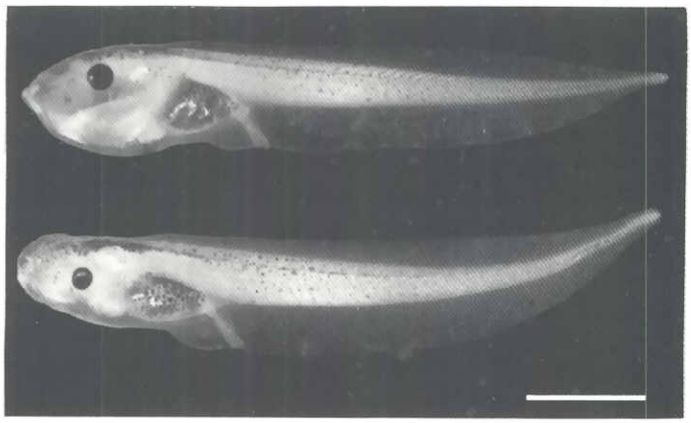

Fig. 2. Above: *Normal tadpole aged 8 days (st. 46).* **Below**: *fh mutant of the same age (st. 45/46) exhibiting microphthalmy and flattening of the head. Scale bar= 2 mm.*

Bloated-3 (bl-3). This is one of the three analogous mutations found in different species of *Xenopus: bloated-1* in *X. borealis*, *bloated-2* in *X. muelleri* and *bloated-3* in *X. laevis*. The mutations are not allelic but the phenotypes are very similar. They are characterized by a large edema of the body cavity and abnormal heart and gut morphology. When fused in parabiosis with normal embryos, the anomalies of *bl-3* individuals are corrected, which indicates that the gene product acts at a physiological, i.e., supracellular, level (Droin, 1988b).

Degenerative cement gland (dcg). The homozygous mutants are easily identified at st. 29/30 by a lack of elongation and stickiness of the cement gland. In subsequent stages the gland degenerates as well as probably all other tissues, and death occurs at st. 45. Reciprocal mutant/normal cement gland grafts and parabiosis experiments have shown that this degeneration is an intrinsic property of the mutant cells (Droin, 1981). As the cement gland is a simple organ, very accessible to work with, it could be a useful tool for analyzing the mechanism of action of this mutant gene.

Delayed metamorphosis (dm). The *dm/dm* tadpoles are smaller than wild-type ones, develop more slowly and take, on the average, six times longer to reach metamorphic climax. About 80% of them become fertile adults. Their iodine metabolism was shown to be attenuated and it was suggested that the *dm* gene interferes with the hypophysial activation of the thyrotropic, gonadotropic, melanotropic and growth functions (Droin and Buscaglia, 1978).

Delayed metamorphosis-2 (dm-2). Recently, an analogous mutation was found — not allelic with *dm* — exhibiting a more accentuated expression with an even longer delay before reaching metamorphosis, and with a higher mortality.

Distended lungs (dl). The phenotype of the *dl/dl* tadpoles is characterized from st. 49/50 onwards by the dumpy appearance of the body, distension of the lungs, abnormally red blood vessels and heart, wavy tail and slackening of growth. The lungs are histologically abnormal; erythrocyte numbers and hematocrit values abnormally increased, with a peak at st. 52. A small percentage of mutant individuals reaches the adult stage and reprodution. Analysis of this mutant has shown the importance of the lungs in larval respiration (Droin and Chavane, 1976).

Droopy tailtip (dtp). The phenotype of *dtp* is very similar to that of *bent tail*, with edemas, microcephaly and microphthalmy but, in this case, the tail droops down (Droin, 1976).

Dwarf-1, dwarf-2, dwarf-3, dwarf-4, (dw-1, dw-2, dw-3, dw-4). These four mutations are analogous but not allelic. The mutant syndrome consists of a slackening followed by an arrest of growth varying from st. 46 to 48, according to the mutants. They remain small, do not feed well and die usually at the age of 3 to 4 weeks (Droin, 1974;1988b).

Flat head (fh). The phenotype of this recently found mutant is characterized by the appearance, at st. 43/44, of a lateral edema, an abnormal insertion of the dorsal fin and a flattening of the head. Histological analysis has revealed areas of cell degeneration in the anterior brain and mainly in its dorsal part which could explain the head deformation. In the following stages, other tissues degenerate too; the tadpoles cannot feed, and die between the 10th and the 15th day (Fig. 2).

Folded jaw (fj). The phenotype is characterized by a deformation of the cartilages and muscles of the lower jaw appearing at st. 42 and rendering the head narrow and the cement gland folded. The mutants cannot feed and die at st. 47 (Droin *et al.*, 1968).

Goiter (g). The genetics of this mutation was difficult to ascertain, its expression much depending on environmental conditions. Affected tadpoles present 4 characteristics: a variable delay in metamorphosis, greater size than normal tadpoles, proportional to the lag of metamorphosis, a high mortality rate and the formation of a hyperplastic goiter resulting from functional hypothyroidism (Uehlinger, 1965; Buscaglia, 1968, 1972).

Immobile (im). The *im/im* embryos do not present any muscular contractions from the neurula stage onwards except heart beat. In later stages, deformation of jaw and tail are observed. In the first matings carried out, the mutants could not move at all, could not feed and died at st. 47. In more recent matings, however, several mutant tadpoles recovered, exhibiting firstly tail tremor and then more or less normal swimming; a very few of them became fertile adults. This phenotype seems to be very similar to the *unresponsive* phenotype described by Reinschmidt and Tompkins (1984). Muscular tissue is histologically normal down to its banding pattern; cholinesterase and ATPase are present and glycerol-extracted myoblasts contract after addition of ATP (Droin and Beauchemin, 1975). An electrophysiological analysis of these noncontracting muscles has shown that they display the normal range of electrical properties, these results suggesting that the lesion lies at the level of the excitation-contraction coupling (Warner, 1981). Moreover, Armstrong *et al.* (1983) have used this mutant to demonstrate that the cholinergic stimulation-induced loss of gap junctions during development does not depend on muscular contraction.

Kinky tailtip (kt). These mutants are identified around st. 37 by a slight bending of the tail extremity which in later stages is transformed into a kink of the tailtip. In addition, in prefeeding stages, fluid accumulation takes place in the coelom but usually regresses before metamorphosis. The expression of the phenotype is variable; the most affected tadpoles die but the majority become adult fertile frogs (Uehlinger and Reynaud, 1965).

Muscle opacity (mo). The main abnormality of the *mo/mo* mutants appears at st. 47. An opacity of the ventral muscles of the head is due to degeneration of the muscular bundles. Arrest of growth, general degeneration and death ensue. Genetically, this mutant is interesting because of its linkage to the *rusty* locus with a map distance of 6.1% (Droin, 1991a).

No cleavage (nc). Maternal effect mutant. In the eggs laid by *nc/nc* females, the processes of maturation and activation are abnormal. Germinal vesicle breakdown does occur but no meiotic spindle is formed, only one or several cytoplasmic asters containing chromosomes, or spindle-like cytoplasmic structures. Furthermore, cortical granule exocytosis does not take place when these eggs are fertilized, which leads to polyspermy. Finally, although the nuclear mitotic cycle seems to proceed more or less normally, cytokinesis is never observed. Partial correction of the anomaly can be obtained with transfers of cytoplasm from wild-type matured eggs to premature abnormal oocytes. These experiments favor indeed, in the abnormal eggs, the formation of spindles instead of asters, but mainly cytoplasmic and not cortical ones (manuscript in preparation).

Edema (oe). Two types of syndrome can affect the mutant tadpoles — a premetamorphic one which appears around st. 53 and is characterized by the successive formation of edemas in the heart, the limb and the eye regions. In the late or postmetamorphic syndrome, edemas are formed in all the subcutaneous lymph sacs. The mutants usually die a few weeks after metamorphosis. This mutation is recessive but is not transmitted as a simple Mendelian factor (Uehlinger and Beauchemin, 1968).

Otolithless (otl). The homozygous mutants can be identified at st. 35 by the absence of otolith formation and by a turgescence of the inner ear. Morphogenesis and general structure of the ear is normal none the less, as is general development. The tadpoles behave abnormally, rotating in circles with quick movements. They can feed but eventually die around st. 50. It is not known why calcium deposits do not take place in the early stages of ear formation while they do occur normally in the endolymphatic sacs at more advanced stages (Droin, 1967).

Pale eggs (pe). Maternal effect mutant. The eggs laid by the homozygous mothers (*pe/pe*) exhibit a yellow-to-beige color of the animal hemisphere instead of the dark brown tinge of the wild-type eggs. Sections have shown that such eggs contain fewer melanosomes than normal ones. Development of these eggs is completely normal, as is the second larval phase of melanization which occurs from st. 33 onwards; one can thus infer that oocyte melanization occurring during oogenesis, and larval melanization, are regulated by different genes. These eggs can be useful as markers in experimental procedures (Droin and Fischberg, 1984).

Partial cleavage (pc). Maternal effect mutant. In eggs laid by *pc/pc* mothers, the first two cleavages proceed normally. An anomaly becomes evident during the third cleavage in which one or two blastomeres show incomplete furrows or do not cleave at all. This abnormal process goes on until blastula stage and results in a very irregular cleavage pattern, varying greatly from one egg to another. The blastopore never forms. When sectioned, the eggs exhibit a cleaved animal area, a syncytial equatorial zone with multiple nuclei in an undivided cytoplasmic mass, and a vegetal part mainly undivided. In addition, one observes abnormal cytological features and an abnormal distribution of yolk platelets. This latter feature may possibly play a major role in the formation of this abnormal cleavage pattern (Droin and Fischberg, 1984). It has not been possible to carry out rescue experiments with these eggs.

Polydactyly (pd). The limb malformation can be recognized at st. 53 by an asymmetry and abnormal orientation of the limb buds. The posterior limbs are more affected than the anterior ones. There is great variability in the expression of the mutation, hindlimbs ranging from one supernumerary digit to 10 or 12 digits. Severely affected tadpoles cannot survive, but the majority of those with a weak expression become fertile adult individuals (Uehlinger, 1969). Graf has established a linkage map of the *Xenopus laevis* genome using isozyme markers and restriction fragment length polymorphism. He has shown that the *pd* locus was segregating with linkage group III (Graf, 1989a; 1989b).

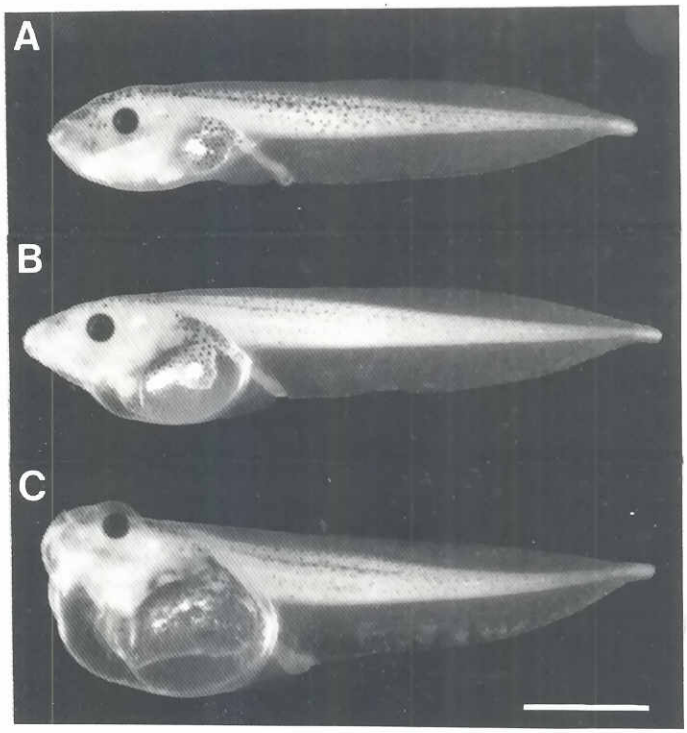

Fig. 3. Three tadpoles aged 6 days at st. 45/46; (A) *wild-type tadpole;* **(B)** *v.oe mutant exhibiting a small edema of body cavity;* **(C)** *v.oe mutant with voluminous edema representing the strong expression of the syndrome. Scale bar= 2 mm.*

Precocious edema (p. oe). This is the third mutation expressing a multiple edematous syndrome (similar to *bt* and *dtp).* The mutants exhibit not only external edemas but also internal ones, especially in the brain and the pronephros. This phenotype is not corrected by parabiosis (Droin, 1974).

Rusty (ry). The egg pigment, due to melanosomes, is formed in the oocytes during oogenesis. In wild-type embryos and tadpoles, these intracellular melanosomes go through progressive stages of migration and concentration at the apical end of the cells. They are eventually eliminated by expulsion at various stages depending on the tissues concerned. In the *rusty* mutant individuals migration and expulsion do not take place; the non-elimination of the egg pigment from the skin accounts for the characteristic rusty color of the mutant, which persists until st. 48. In later stages, the melanosomes are diluted by the division of the cells in the growing tissues so that the mutants cannot be identified any more. During larval stages, the three types of chromatophores differentiate as in wild-type tadpoles and the mutants develop normally (Uehlinger and Droin, 1969; Uehlinger *et al.*, 1971). Homozygous mutants can be used as markers in embryological procedures. Recently, the *rusty* locus was shown to be linked to the *mo* mutant gene (Droin, 1991b).

Screwy (S). As it is a dominant mutation, two phenotypes are observed — a heterozygous and a homozygous one. The heterozygous phenotype can be identified from st. 52 onwards by an abnormal, twirling type swimming of the tadpoles due to the torsion of the caudal fins around the tail axis, like a screw. This abnormal behavior disappears during metamorphosis and the majority of the mutants reach the adult stage and become fertile.

The homozygous syndrome appears very early during development (st. 29/30) and is characterized by an asymmetric differentiation of the tailbud so that the embryos assume a «comma» shape. This is followed by the formation of wrinkles of head ectoderm and finally by a complete disintegration at st. 40. Absence of loose cephalic mesenchyme, abnormal differentiation of neural and endodermal tissues, and notochord hypertrophy are the main histological features observed (Uehlinger, 1966).

Turner (tr). This is the second mutation affecting the ears. Contrary to *otl*, the otoliths are present in the homozygotes but their shape and localization are abnormal, especially of the saccular otoliths, the utricular ones being more or less normally positioned. From st. 48 onwards, the otoliths become round and situated at the bottom of the sacculus instead of being elongated against the internal saccular wall, as in wild-type tadpoles. This anomaly leads to abnormal behavior, with the tadpoles turning around and around. This effect becomes attenuated in advanced stages with the growing of the otoliths. The majority of the mutants die before metamorphosis but the surviving ones become fertile adults (Droin, 1971).

Ventral edema (v.oe). The phenotype of this recently found mutant is similar to the *kt* one, except that there is no kink of the tail in *v.oe*. The two genes are not allelic. In *v.oe*, a ventral edema also appears in the prefeeding stage but the expression of the syndrome is quite variable: the edemas either become very voluminous and lead to the death of the mutants or, in the minority of cases, they regress and the mutant individuals grow normally until sexual maturity, and reproduce (Fig. 3).

White lethal (wl). Whereas *pe* and *ry* were concerned with scarcity or elimination of egg pigment, *wl* is a mutation interfering with larval pigmentation. The differentiation of the three types of chromatophores is affected. Melanophores appear at. st. 33 but stay pale grey and do not proliferate after st. 41; the rare xanthophores present contain only a few pterinosomes, and the iridophores consist of non-iridescent white dots. Furthermore, after st. 48, the mutants take on an abnormal shape, do not feed well and eventually die around st. 50. Reciprocal grafts have shown that the defect is intrinsic to the neural crest cells (Droin, 1992).

Yolky rectum (yr). This mutant is characterized by the early appearance of the defects (st. 31/32), microcephaly, microphthalmy and curvature of the axial organs. This is followed by the arrest of tissue differentiation (head, eyes, pronephros and gut) leading to death in a few days (Reynaud and Uehlinger, 1965).

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Summary

The 32 developmental mutants found in our laboratory have appeared in the course of the genetic analysis of adult *Xenopus laevis* issued from nuclear transfers, and of their progeny. These experiments originally were devised more than thirty years ago to test whether somatic nuclei had undergone irreversible changes during differentiation or whether they had remained totipotent.

In the majority of cases, the mutations were carried by wild-caught imported frogs or their laboratory-bred progeny; however, the precise origin of several mutations has not been determined. The mutants have been subdivided into three classes:

Maternal effect mutants

Three such mutants have been found; they affect the development of all the embryos from homozygous mothers independently of the genotype of the father.

Developmental lethals

This class comprises 19 mutants, among which are included the previously called «autonomous lethals» caused by general metabolic defects that affect all parts of the embryos as well as the tissue-and organ-specific lethals, in several of which death occurs as a direct consequence of the anomalies.

Developmental non-lethals

These are 10 mutants, suffering from specific defects not essential for survival.

In addition, nucleolar mutants as well as mutants found in different species of *Xenopus* and mutants of *Xenopus laevis* found in other laboratories are also mentioned. The last part consists of an alphabetical description of the mutant phenotypes including more detailed analyses which have been carried out on several of them.

KEY WORDS: *developmental genetics, Xenopus*

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