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The Mexican Axolotl (*Ambystoma mexicanum*) as An Experimental Material for Studies in Embryology II. Developmental Genetics

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發生學研究用 實驗動物로서의 Mexican axolotl II. 發生遺傳學

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適 要

현재 약 40종의 유전인자가 Mexican axolotl에 알려져있다. 이 유전인자들은 모두 열성인자로서 발생에 미치는 영향과 시기에 따라 5군으로 분류되었다. 즉, 난자형성에 영향을 미치는 유전자군(maternal-effect gene), 인의 크기에 영향을 미치는군, 특수 조직이나 기관발생에 영향을 미치는군, 모든 세포와 조직에 치사 작용을하는 유전자군(autonomous lethals), 그리고 색소세포에 영향을 미치는 유전자군들이다. 본 논문은 이 유전자들의 표현형과 현재 추구되고 있는 실험방법들에 대하여 간단히 소개하였다.

INTRODUCTION

The Mexican axolotl, a neotonous salamander, provides experimental material for a variety of research projects in developmental biology. These include research on the pattern of early embryonic morphogensis, macromolecular synthesis in early development, and the formation of tissues and organs. The popularity of this organism is due in large part to the availability of mutant genes which permit the analysis of the developmental genetics of those research projects. At the present time, over 3 dozen mutant genes have been recognized in this amphibian species. The majority of these genes were identified by Dr. R.R. Humphrey, at Indiana University. They were detected, for the most part, by randomly mating animals with an animal of known genotype. The progeny from such a mating were then backcorssed with the parent which was originally being

tested, or alternatively, that progeny was used in random sib matings. Virtually all of these mutant genes are inherited as simple recessives, in a strict Mendelian fashion.

Several strains of the Mexican axolotl provided these mutant genes, including the so-called "Wistar", "English", "Dutch", "Holtfreter", or "Tompkins" lines. Those strains were obtained by Dr. R.R. Humphrey from various laboratories around the world, and are designated accordingly.

The various mutant genes have been categorized according to the developmental stage at which their phenotype is predominately expressed. Fig.1 contains a diagram of the life cycle of the axolotl, with a total of 5 groups of mutant genes illustrated. The purpose of this report is to review the phenotypes of those genes, and to briefly describe some of the current research applications of those genes. The first paper in this series (Chung and Malacinski, 1977) provided a general introduction to the biology of this organism. This paper will consider each of the 5 groups of mutant genes illustrated in Fig. 1 separately, and in the Discussion several comments will be made about the value of this experimental system.

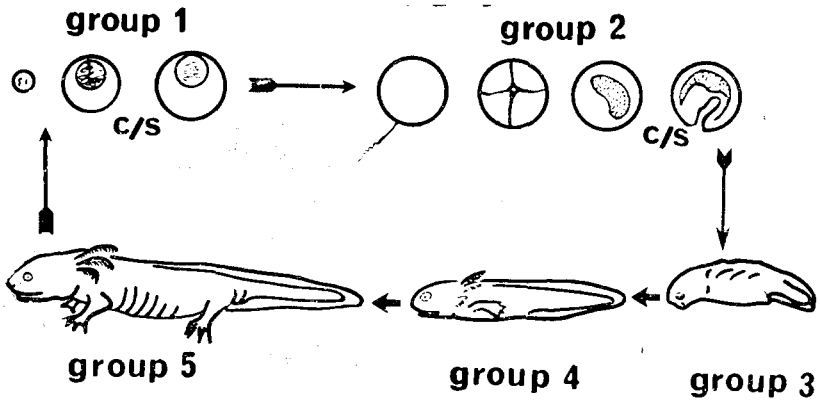

Fig. 1. The developmental life cycle of the Mexican axolotl, *Ambystoma mexicanum*. Mutant genes which affect the developmental life cycle are included in the group designations shown above. Group 1 : Oogenesis; Group 2 : early development; Group 3 : organogenesis; Group 4 : tissue and organ function; Group 5 : adult. (C/S=cross section).

Group I: Oogenesis

Evidence from experimental embryology indicates that information governing the pattern of early embryogenes is built into the egg during oogenesis. The exact biochemical nature of that information is, however, not known. In a few cases some of the macromolecules synthesized during oogenesis for deployment in early post-fertilization development have been identified. These include informational RNA (Davidson and Hough, 1971), ribosomal RNA (Brown and

Littna, 1964), and histones (Adamson and Woodland, 1974). In addition to those molecules, regulatory elements which directly control the gene expression of early development are probably also synthesized. Mutations in genes which are being expressed during oogenesis for the synthesis of components required for post-fertilization development should give rise to maternal effects. Several such maternal effect genes have been discovered in the axolotl. They are listed in Table 1, which also contains references to some of the publications which describe the mutant phenotypes in detail.

Table 1. Mutant genes which affect oogenesis

Gene	Phenotype	Reference
o	Embryos arrest at gastrulation due to cytoplasmic deficiency	Briggs and Cassens (1966)
cl	Embryos arrest during cleavage	Carroll and Van Deusen (1973)
f	Fluid accumulation in blastocoel	Humphrey (1960)
v	Arrest at blastula stage, variable	Justus and Humphrey (1964)
nc	Eggs do not cleave	Raff *et al* (1976)

These mutant phenotypes range from effects on cleavage (genes *cl*, *nc*) to gastrulation (gene *o*). Of particular interest is the gene *o*, since the mutant phenotype can be completely corrected by microinjection of normal egg cytoplasm into the eggs spawned by a female which is homozygous for the gene *o* (Briggs and Cassens, 1966). That observation provides dramatic proof that the gene *o* gives rise to a deficiency of the egg cytoplasm. A bio-assay based upon this correction has been developed and has been employed to demonstrate that the active component is a protein (Briggs and Justus, 1968). Substantial evidence exists that this protein has a regulatory function in early embryogenesis (Brothers, 1976). The other maternal effect mutations are not easily corrected. In a few cases, however, partial corrections have been reported. Cleavage can be promoted in gene *nc* eggs by microinjection of microtubule preparations (Raff *et. al.*, 1976). The maternal effect of the gene *v* is variable. The extent of development displayed by eggs shed by females homozygous for the *v* gene can be prolonged by low temperature beyond gastrulation, at which stage they would normally arrest, to larval stages (Justus and Humphrey, 1964).

Group II: Early Development

Genes included in this group affect the size of the nucleolus yielding, in each case, a nucleolus of reduced size. These mutant genes were discovered by cytological analysis of cells from animals of the appropriate genotype. In general,

it is not known what the molecular basis of the reduced nucleolar size is. In one case, however, the mutation apparently gives rise to a diminution in the number of copies of ribosomal DNA at the nucleolar organizer site. These genes, listed in Table 2, will be useful both as cytological markers, and for studies on the cell biology of the nucleolus.

Table 2. Mutant genes which display phenotypes during early development

Gene	Phenotype	Reference
n^1	Small nucleolus	Humphrey (1961)
n^2	Small nucleolus	Humphrey (1961)
n^3	Small nucleolus	Humphrey (1975)
n^4	Reduced amount of ribosomal DNA	Briggs (1973)

Group III: Organogenesis

Amphibia are favorable material for analysis of embryonic induction. Several organs and tissues are known to develop as a result of inductive interactions, including the eye, heart, pancreas, etc. Mutant genes which affect the development of specific tissues and organs have been identified in the axolotl. These genes are listed in Table 3. Some of these genes apparently lead to abnormal inductive interactions, so they should provide very important experimental material for embryological studies. The phenotypes of two of these, the genes *c* and *e*, will be briefly reviewed here. In embryos which are homozygous for the gene *c* heart development arrests. The heart never beats. In parabiosis with normal co-twins, these *c/c* embryos survive, indicating that when the mutant embryo is provided with a normal circulatory system (via parabiosis), its development is normal (Humphrey, 1972). A series of grafting experiments were carried out to localize the tissue which is responsible for the block to heart development. The results of reciprocal grafting experiments reveal that the mesodermal component of the presumptive heart is capable of responding to induction by normal endoderm. The inducing endoderm of mutant animals is, however, not capable of carrying out its function.

Embryos which are homozygous for the gene *e* display a complete absence of eyes. Histological analyses indicate that eye development is blocked very early. The results of a series of reciprocal grafting experiments demonstrate that the ectodermal tissue, which normally responds to induction by the chorda-mesoderm, is defective. It does not respond to normal inductive influences (Van Deusen, 1973). These two genes illustrate the tissue specificity of some of the effects of the mutant genes. The other genes listed in Table 3 appear to exert less specific

Table 3. Mutant genes which affect organogenesis

Gene	Phenotype	Reference
c	Heart fails to develop	Humphrey (1972)
e	Eyes do not develop	Van Deusen (1973)
an	Transitory anemia	Humphrey (1974)
micro	Microphthalmia (semilethal)	Signoret and Lefresne (1969)
s	Short toes, effects on renal system	Humphrey (1967b)
ph	Phocomelia, delay in formation of long bones	Humphrey (1975)
sp	Spastic swimming behavior of larvae	Ide and Tompkins (1975)
as	Larvae develop ascities	Humphrey (1975)

effects. These effects range from transitory anemia (gene *an*), to abnormal larval behavior patterns (gene *sp*).

Group IV: Tissue and Organ Function

This group includes genes (Table 4) which exert lethal effects on all tissues or organs, in contrast with the genes included in group III. The onset of the effects of each of these genes varies from gene to gene. Usually, the first effects are observed between the prehatching to the larval stages. Each of the abnormalities is also unique. Identification of phenotypes belonging to this group depends primarily on parabiosis tests and tissue transplantation experiments. When tissues such as the limb or gill from larvae which are homozygous for a prospective cell lethal gene are grafted to a normal host larva, the results are predictable. If

Table 4. Mutant genes which affect cell and tissue function

Gene	Phenotype	Reference
p	Premature death	Humphrey (1975)
st	Stasis of blood circulation	Humphrey, (1975)
q	Quivering behavior pattern	Humphrey (1975)
t	Gills develop twisted morphology	Humphrey (1975)
ut	Slow growth and abnormal gills	Malacinski, *et al* (1977)
mi	Microphthalmic lethal	Humphrey (1975)
g	Irregular gills, plasma membranes abnormal	Tompkins (1970)
l	Small eyes	Chung and Briggs (1975)
r	Renal insufficiency	Humphrey (1964)
x	Fragile gills	Humphrey (1975)
h	Foot malformation, trunk curved	Humphrey (1975)
y	Limb development arrested	Humphrey (1975)
b	Slow development of forelimbs	Humphrey (1975)

the mutant gene is indeed a cell lethal the grafted tissues do not survive. As well, in parabiosis with normal embryos, mutant larvae do not survive. The mutant gene is, therefore, postulated to exert its effects on all cells in the organisms and is referred to as an "autonomous cell lethal".

Group V: Adult

Pigment mutants are included in this group (Table 5). Wild-type axolotls display three types of pigment cells: melanophores, xanthophores, and iridophores. These cells are derived from the neural crest and migrate during development to produce the characteristic pigmentation patterns of the axolotl. One of the most interesting genes is the gene *a* (albinism). This mutant gene was originally discovered in the tiger salamander and introduced into the axolotl by nuclear transplantation (Humphrey, 1967a). Other genes affect pigment synthesis (e.g. gene *ax*), or the number of pigmented cells (e.g. genes *d* and *m*).

Table 5. Mutant genes which affect pigmentation

Gene	Phenotype	Reference
d	White	Benjamin (1970)
a	Albino	Benjamin (1970)
m	Melanoid	Benjamin (1970)
ax	Axanthic	Lyerla and Dalton (1971)

In addition to the mutant genes listed in Tables 1-5, there exists a series of histocompatibility factors (DeLanney and Blackler, 1969). These factors control the acceptance or rejection of skin grafts. They are inherited as codominant alleles.

DISCUSSION

In no other vertebrate is such a large collection of mutant genes available. These genes are included in all phases of the developmental life cycle of the organism (Fig.1). The amphibian is a particularly useful organism for embryological analyses because the eggs and early embryos can tolerate a wide variety of manipulations. By combining the advantages of the amphibian embryo with the availability of such a large variety of mutant genes, novel approaches to historically important research problems should be possible. This has been appropriately documented in the case of the genes which affect the process of embryological induction (e.g. genes *e* and *c*) (Lemanski *et al.*, 1977). Biochemical analyses of the egg cytoplasm are possible with the maternal effect genes (e.g.

genes *o* and *nc*). As yet unknown experimental approaches should be possible with several of the other mutant genes.

SUMMARY

At present over 3 dozen mutant genes have been recognized in the Mexican axolotl. These genes, all recessives, are categorized in 5 groups according to the nature of their effects and the developmental stage at which their phenotype is predominately expressed. They are genes affecting the oogenesis (maternal-effect genes), genes affecting the size of the nucleolus, genes affecting the development of specific tissues and organs, genes exert lethal effects on all cells or tissues (autonomous lethals), and genes affecting pigment cells.

This report describes briefly the phenotypes and some of the current research applications of those genes.

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REFERENCES

Adamson, E.D. and H.R. Woodland, 1974. Histone synthesis in early development: histone and DNA synthesis are uncoordinated. *J. Mol. Biol.* **88** : 263—285.

Benjamin, C.P., 1970. The biochemical effects of the *d*, *m*, and *a* genes on pigment cell differentiation in the axolotl. *Devlop. Biol.* **23** : 62—85.

Briggs, R., and G. Cassens, 1966. Accumulation in the oocyte nucleus of a gene product essential for embryonic development beyond gastrulation. *Proc. Natl. Acad. Sci.* (U.S.) **55** : 1103.

Briggs, R. and J.T. Justus, 1968. Partial characterization of the component from normal eggs which corrects the maternal effect of a gene *o* in the Mexican axolotl (*Ambystoma mexicanum*). *J. Exptl. Zool.* **167** : 105.

Briggs, R., 1973. Developmental genetics of the axolotl. 31st Symp. Soc. Dev. Biol. pp.169—199. Academic Press, New York.

Brothers, A.J., 1976. Stable nuclear activation dependent on a protein synthesized during oogenesis. *Nature* **260** : 112—115.

Brown, D.D. and E. Littna, 1964. RNA synthesis during the development of *Xenopus laevis*, the South African clawed toad. *J. Mol. Biol.* **8** : 669—687.

Carroll, C.R. and E.B. Van Deusen, 1973. Experimental studies on a mutant gene (*cl*) in the Mexican axolotl which affects cell membrane formation in embryos from *cl*/*cl* females. *Develop. Biol.* **32** : 155—166.

Chung, H-M. and R. Briggs, 1975. Experimental studies on a lethal gene (*l*) in the Mexican axolotl, *Ambystoma mexicanum*. *J. Exptl. Zool.* **191** : 33—48.

Chung, H-M. and G.M. Malacinski, 1977. The Mexican axolotl (*Ambystoma mexicanum*) as experimental material for studies in embryology I. General introduction. *Korean J. Zool.* **20** : 49—56.

Davidson, E.H. and B.R. Hough, 1971. Genetic information in oocyte RNA. *J. Mol. Biol.* **56** : 491—506.

DeLanny, L. E. and M. K. Blacker, 1969. Acceptance and regression of a strain-specific lymphosarcoma in Mexican axolotls. *In* "Biology of Amphiban Tumors", Special supplement, Recent Results in Cancer Research, Springer-verlag, Berlin and New York.

Humphrey, R. R., 1960. A maternal effect of a gene (*f*) for a fluid imbalance in the Mexican axolotl. *Develop.* Biol. **2** : 105—128.

Humphry, R.R., 1961. A chrosomal deletion in the Mexican axolotl (*Siredon mexicanum*) involving the nucleolar organizer and the gene for dark color. *Amer. Zool.* **1** : 361.

Humphrey, R R., 1964. Genetic and experimental studies on a lethal factor (*r*) in the axolotl which induces abnormalities in the renal system and other organs. *J. Exptl. Zool.* **155** : 139—150.

Humphrey, R. R., 1967a. Albino axolotls from an albino tiger salamander through hybridization. *J. Hered.* **58** : 95—101.

Humphrey, R. R., 1967b. Genetic and experimental studies on a lethal trail ("short toes") in the Mexican axolotl (*Ambystoma mexicanum*). *J. Exptl. Zool.* **164** : 281—296.

Humphrey, R. R., 1972. Genetic and experimental studies on a mutant gene (*c*) determining absence of heart action in embryos of the Mexican axolotl (*Ambystoma mexicanum*). *Develop. Biol.* **27** : 365—375.

Humphrey, R. R., 1975. The axolotl, *Ambystoma mexicanum, In:* Handbook of Genetics, (R.C. King, editor) Plenum Press, New York.

Ide, C.F. and R. Tompkins, 1975. Development of locomotor behavior in wild type and spastic (*sp/sp*) axolotls, Ambystoma mexicanum. *J. Exptl. Zool.* **194** : 467—478.

Justus, J. T. and R. R. Humphrey, 1964. The effect of sodium, potassium, and calcium ions on certain expressions of the semilethal gene *v* in the Mexican axolotl, *Ambystoma* (*Siredon*) *mexicanum. Develop. Biol.* **9** : 255—268.

Lemanski, L. F., B. S. Mark and C.S. Hill, 1977. Evidence for abnormal heart induction in cardiac-mutant salamanders (*Ambystoma mexicanum*). *Science.* **196** : 894—896.

Lyerla, T. A. and H.C. Dalton, 1971. Genetic and developmental characteristics of a new color variant, axanthic, in the Mexican axolotl, *Ambystoma mexicanum* Shaw. *Develop. Biol.* **24** : 1—18.

Malacinski, G. M., Humphrey, R. R. and H-M. Chung, 1977. Developmental studies of a mutant gene (*ut*) in the Mexican axolotl (*Ambystoma mexicanum*). Manuscript in preparation.

Raff, E. C., A. J. Brothers and R. A. Raff, 1976. Microtubule assembly mutant. *Nature* **260** : 615—617.

Signoret, J. and J. Lefresne, 1969. Description et interpretations nouvelle mutation "microphtalmie aleatoise" chez l'axolotl (*Ambystoma mexicanum Shaw*). *C. R. Acad. Sci.* **262** : 699.

Tompkins, R., 1970. Biochemical effects of the gene *g* on the development of the Maxican axolotl (*Ambystoma mexicanum*). *Develop. Biol.* **22** : 59—83.

Van Deusen, E.B., 1973. Experimental studies on a mutant gene (*e*) preventing the differentiation of eye and normal hypothalamus primordia in the axolotl. *Develop. Biol.* **34** : 135—158.