The o mutant of the Mexican axolotl, an instructive lecture topic for a developmental biology course.

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I. Origin of the laboratory axolotl.

a. The name "axolotl" is specific to the Mexican species (*Ambystoma mexicanum*) (Smith, 1969).

1. Axolotls are neotenic, possibly because of a defect in TRF and the hypothalmus (Tassava, 1969).

b. Axolotls were passed on from Mexico to European dealers, to Dr. H. Gloor of Zurich, to Dr. H. Caspari of Cold Spring Harbor, to Dr. J. Holtfreter of University of Rochester to Dr. De Lanney of Wabash College, and finally to Dr. R. Humphrey of Indiana University. Selection pressure has resulted in survival of those larvae which adapted to glass bowls and laboratory conditions.

II. Discovery and characterization of the o mutant.

a. The o mutant was discovered by R. Humphrey (1966). His astute observations could have escaped a less critical eye.

b. A routine mating of two adult axolotls resulted in several hundred axolotl larvae which, when maintained together, often chew each other's legs off. Humphrey isolated these larvae and noted that 25% of the larvae did not regenerate the chewed legs.

c. When the 25% poor regenerators were grown to maturity and mated, it was observed that males were sterile and females, when mated to wild type males, produced defective oocytes.

d. These defective oocytes, when fertilized by wild type sperm

(i.e., carrying the + gene) arrested at gastrulation. Not a single embryo developed past gastrulation.

e. The original parents (IIb above) were then presumed to be heterozygous +/o and the 25% which regenerated poorly were o/o, homozygous recessive for the o gene (ova deficient) (Humphrey, 1966).

f. The fact that the mating: o/o female x +/+ male still resulted in 100% arrest, shows that the + gene does not correct at the time of fertilization. Thus the + gene must be a maternally active gene and the product of the + gene is present in oocytes of wild type females?

III. Cellular and biochemical studies of the o mutant.

a. Briggs and Cassens (1966) showed that the sap of the germinal vesicle (large oocyte nucleus) of +/+ females corrects the deficient oocytes of o/o females.

b. Briggs and Justus (1968) showed that the active substance of the GV sap has characteristics of a protein. This finding has been confirmed and the protein has been further purified by Malacinski and Brothers (1974).

c. Brothers (1976) showed that the + substance confirms a "stable change" on blastula nuclei.

IV. Some of the developmental concepts and ideas illustrated by the o mutant studies.

a. The + gene is differentially active. The + gene is expressed in oocytes during the lampbrush stage (Briggs, 1972). The + gene is expressed in oocytes of frogs as well (Briggs, 1972). The + gene is also expressed during spermatogenesis and possibly in cells of regenerating limbs.

IV. (cont.)

b. The mechanism of action of the + gene product may be to regulate gene activity during cleavage, i.e., to "program" blastula cells for gastrulation.

c. 25% of the progeny of a +/o x +/o mating, i.e., the o/o zygotes, are not totipotent but must be raised to adults to prove this point since the + gene is expressed only in gamete development.

d. The + gene product is an important component of the oocyte, but is only one of many components, i.e., germ plasm, grey crescent cytoplasm, masked mRNA (see Davidson, 1968).

V. Sample examination question to test a student's understanding of the o mutant. This question was actually used in an undergraduate course in animal development at The Ohio State University.

Question 1A. A female axolotl is mated with a male axolotl. (a) If the embryos all arrest at gastrulation, what are the possible genotypes of the female with regard to the o mutation?

_____, _____, _____: of the male?
_____, _____, _____.

[b] If all the embryos develop to adults, what are the possible genotypes of the female? _____, _____, _____.
of the male? _____, _____, _____.

B. In attempts to identify the corrective factor present in oocyte germinal vesicles of the "wild type" axolotls, Briggs and Justus carried out several biochemical tests and Malacinski has recently extended these tests by column chromatography. Briefly describe two of these tests, give the actual findings of the tests, the alternative findings, and the conclusions and alternative conclusions.

C. Having now identified the macromolecular identity of the + gene corrective factor, what are two additional questions which may now be answered with further work (assuming the "pure" substance can be obtained in reasonably large amounts?

1.

2.

Note: Questions concerning nuclear transplantations are an interesting challenge to students.

References

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POSITION AVAILABLE

A one or two year appointment for a research associate (Ph.D.) in biochemical embryology is available, beginning in the fall or winter, 1978, in G.M. Malacinski's laboratory. The appointee should have a knowledge of contemporary concepts of molecular biology, and skills in various biochemical fractionation and analytical techniques. Salary will be established at approximately the level of federal postdoctoral fellowships.

A general description of G.M.M.'s research program can be found in the following publications: Science 184:1142; J. Exp. Zool. 191:97; P.N.A.S. 72:1235; Eur. J. Biochem. 69:45; Dev. Biol. 56:24.

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