Meeting Report – Toronto, 1977

Developmental Genetics of the Mexican Axolotl

The symposium was convened by George Malacinski with some general remarks and continued with a paper by R. R. Humphrey. Humphrey had demonstrated that in many dihybrid crosses of recessive lethal genes in the axolotl, the double homozygous recessive embryos died earlier than either of the single homozygous recessive embryos. These epistatic effects suggested that these lethals have deleterious effects upon the embryo earlier than can be detected morphologically in single homozygous recessive embryos. This supports George Malacinski's idea that analysis of the biochemical effects of lethal genes at the time of the appearance of the mutant syndrome of abnormalities may in fact be a study of the physiology of death. The important lesions have set in motion a complex pleiotrophy at a much earlier stage in development.

Janice Brothers reviewed the work on the o gene maternal effect. Of particular interest was her recent demonstration of the mitotic hereditability of nuclear changes caused by the o corrective substance. By nuclear transplantation from normal embryos to eggs of o/o females, she demonstrated that the o corrective substance effects a change in the nuclei at mid-blastula stages; transplants from earlier embryos develop the maternal effect, transplants from mid-blastulae develop normally in the absence of further exposure to the corrective substance. Brothers also reported correction by treatment in vitro of nuclei from the offspring of o/o females, which then promoted normal development in the eggs of oo females.

Larry Etkin reviewed his work on maternal effects on gene regulation. Ambystoma texanum liver nuclei injected into A. mexicanum oocytes synthesized RNA. A. texanum lactate dehydrogenase but not alcohol dehydrogenase was synthesized. Thus, a gene which normally functions in oocytes, LDH, is expressed whereas one which is not normally expressed in oocytes, ADH, is not. Since the donor cells were synthesizing ADH in the liver, the oocyte cytoplasm was effecting a specific control over this gene. Etkin reviewed his work on A. texanum x mexicanum hybrids wherein he has shown that the paternal genomes ADH are expressed at a later time than the maternal ones. This suggests that abnormal interactions between egg regulatory factors and the foreign genome retard the expression of this differentiated cell function. Finally Etkin discussed his current efforts to study the regulation of plasmid-borne sea urchin histone genes in Xenopus oocytes.

George Malacinski discussed his and John Sinclairs' work on the small nucleolar mutants of the axolotl. One of these has been shown to be the result of a reduction in rRNA cistrons. The identification of cells containing these markers is somewhat difficult, but they may become useful as tissue markers in chimerae.

Raff & Raff reviewed their work on axolotl tubulin and microtubules. They noted heterogeneity in tubulins from different strains and between different stages. Of particular interest they reported that nc oocytes contain a normal tubulin pool and demonstrated that the lesion in the maternal effect can be partially corrected with heterologous microtubule fragments.

Leonard Epp discussed the eyeless mutation. By grafting analysis he showed that the prospective eye and hypothalamic ectoderm of e/e embryos is unable to respond to normal merodermal signals. He also demonstrated an epistatic effect of r/r on the e locus. Ee/rr animals have abnormal eyes.

R. Brun also reported on the eyeless mutation. He demonstrated that normal ectoderm placed over the prospective eye cup region led to the development of eyes in the e/e embryos. This suggests that the prospective eye ectoderm is not deficient and that the prospective epidermis of e/e embryos is affected. In normal embryos and in the above chimerae, head mesenchyme is cleared from the region between the prospective eye cup and the epidermis and the two ectodermal tissues become closely associated. This does not occur in the e/e embryos and this failure may be the basis of the eyeless condition.

R. K. Hunt reviewed his work on axis determination in the eye and reinnervation of the tectum by the retinal fibers. Based on this he predicted the types of mutants that might be found in amphibians.

C. Ide reviewed his work on the spastic mutation. Neurophysiological, anatomical and behavioral evidence support the conclusion that the cerebellum is affected by this mutant. Probably abnormal cell migration patterns during cerebellur morphogenesis leads to an abnormally constructed organ incapable of normal function.

P. Model reported on her studies on the axolotl Mauthner's cells. These cells are determined in the gastrula stage but their axes are not determined until later, as shown by rotation grafts. Extra otic vesicles synapse on the normal Mauthner's cells and do not evoke new

ones. This scribe was reminded of Fankhauser's unpublished work on these cells. Although increasing ploidy decreases the number of cells in the nervous system all polyploid embryos have the normal two Mauthner's cells. However, haploidy results in approximately twice the number of cells, each one half normal size. In haploids there are four Mauthner's cells.

Lou Delanney reviewed his work on the axolotl immune system. Although the axolotl has well developed thymi, the B-cell system and its characteristics relative to the T-cells are not well defined. Of particular interest was his finding that lymphocytes may acquire specificity within an allograft without going back to lymph nodes or other lymphopoietic tissues.

Joe Bagnara presented an overview of amphibian pigmentation. The biochemical bases of pigment lesion in the axolotl color mutant were discussed.

Jerry Justus presented the history of the cardiac mutant and related his findings to those of Humphrey. Lemanski presented his biochemical analysis of the cardiac. He showed that cardiac hearts have actin and myosin but virtually no tropomyosin. Few of these muscle elements present are organized in a normal fashion. Further, he showed that the c/c anterior endoderm, which Humphrey demonstrated to be incapable of normally inducing heart muscle, differs from normal. It appears to be more than normally differentiated at the time of heart induction and thus the cardiac effect may be a result of a timing lesion. It is worth noting that most of the molecules characteristic

of heart muscle appear in c/c embryo's hearts. This suggests that heart induction is a complex of interactions and that the mutant c affects only some of them.

Kulikowski and Manasek showed a very interesting time lapse film of cardiac lethal hearts which clearly demonstrated that these hearts do beat in vitro, but not in vivo. These results support the suggestion of Justus that the cardiac lethal results from an ion imbalance in the heart region.

John Armstrong presented his methods for mutagenesis in the axolotl. Immersion of males in solutions of EMS gave positive results by the dominant lethal test. However, this test may not truly reflect mutation rates and the results of inbreeding of offspring to recover recessives must be awaited. He suggested that parthenogenesis may aid in rapidly identifying recessive mutants.

Tompkins reviewed the cryptic metamorphosis of the axolotl and its relation to induced external metamorphosis. The gene an affects the larval-to-adult hemoglobin transition in the axolotl; an/an animals become anemic at the time their normal sibs are developing adult hemoglobin. Since thyroxine suppresses the anemic effect, this mutant may affect the sensitivity of genes controlling this cryptic metamorphic event.

The meeting was summarized by G. Malacinski and he discussed the analyses of cell-lethal genes. He stressed the importance of bringing new methods, such as cell culture, into these analyses.

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