Report on "Developmental Genetics of the Early Embryo" symposium.

A symposium on this subject was recently held on the campus of Indiana University in Bloomington on October 17-21, 1976. An international group of scientists participated in this conference and discussed various aspects of the molecular biology, cytology, and genetics of the development of the early embryo. A short summary of the meeting has been published in a recent issue of Bioscience* (April, 1977). What follows is a more detailed report of several of the topics discussed.

John B. Gurdon (M.R.C., Cambridge, England) reported on his attempts to observe the re-programming of gene activity in nuclei injected into amphibian oocytes. Oocytes were chosen as an experimental system, rather than fertilized eggs, so that gene expression could be more easily monitored in the absence of cell division. In one series of experiments Hela cell nuclei - up to 200 nuclei per oocyte - were injected. These nuclei were observed to swell, and the chromatin within them dispersed and their nucleoli enlarged. They were active in transcription for approximately 4 weeks. During this period protein synthesis was monitored and an apparent diminution in the synthesis of Hela cell-specific proteins was detected. A few dozen Hela marker proteins were characterized by two dimensional electrophoresis gels and fingerprint patterns, and the synthesis of all except approximately three proteins was apparently shut off in the oocyte.

Nuclei from cultured Xenopus kidney cells injected into Pleurodeles oocytes behaved similarly in that the synthesis of the half dozen marker proteins was either severely diminished or completely eliminated. The synthesis of 5 Xenopus oocyte specific marker proteins was, however, observed. This observation permitted the important conclusion that the Pleurodeles oocyte cytoplasm specified the expression of oocyte proteins encoded in the genome of the kidney cell line.

Employing an alternative experimental approach, Dr. Gurdon reported on the injection of various DNAs, including ribosomal DNA (28S,18S), 5S DNA, mouse satellite DNA, ϕ x 174 DNA, and histone DNA, into the oocyte cytoplasm. Those experiments were performed in collaboration with D.D. Brown. Using molecular hybridization (excess DNA on filters) as a probe, extensive transcription of several of the injected DNAs was observed. This experimental approach generated substantial discussion during the course of the conference, for it should provide a useful tool for analyzing transcription. In one experiment, for example, it was observed that supercoiled circles were better templates than linear DNA molecules.

Donald D. Brown (Carnegie Institution) elaborated on one goal of contemporary research in molecular genetics - the isolation of specific genes, for example the 5S RNA genes, and the analysis of their function under conditions which permit faithful in vitro transcription. Several features of the 5S gene system were reviewed. These genes actually represent a dual system. Somatic cells display a homogenous population of 5S RNA, whereas oocytes contain a heterogenous population including a major oocyte specific species, minor oocyte specific species, and a small amount of somatic type 5S RNA. To provide the large quantities of 5S RNA synthesized during oogenesis, a separate set of approximately 24,000 repeating copies of the 5S RNA genes are present in the genome. The primary transcript (120 nucleotides long) has been sequenced. Compared with the somatic type 5S RNA, the oocyte type has 6 nucleotide substitutions.

*Bioscience 27:279

Employing what Dr. Brown referred to aptly as "genetics by evolution" - that is, a comparison of the 5S genes from two closely related amphibians, Xenopus laevis and Xenopus borealis, direct comparison of two different oocyte 5S DNAs were carried out. Much of the work reported on was directed towards the spacer regions. Denaturation and restriction enzyme maps were compared, and the spacer sequences analyzed. Dr. Brown presented a very elegant gel electrophoresis procedure which permitted an almost direct reading of the base sequence of various regions of the gene. These studies demonstrated that the length heterogeneity of X. laevis 5S DNA is due to repeats in the AT rich portion of the spacer DNA. Repeat units were reported to differ from each other in the number of internal 15-mer repeats they contain. This observation was confirmed by attaching different restriction fragments to plasmids, cloning them in E. coli, and sizing them. With this plasmid technique it was also shown that all the 5S RNA genes are located on a single segment of DNA, rather than being dispersed.

The main objective of the 5S DNA work, discussed by Dr. Brown, is to attempt to achieve an understanding of the possible role the spacer region might play in the regulation of the transcription of 5S RNA.

The structure of rDNA from Drosophila was compared with the structure of Xenopus rDNA by Igor B. Dawid (Carnegie Institution). The rRNA genes are located on two different chromosomes in Drosophila (the X and Y chromosomes), as compared to Xenopus, in which the rRNA genes are all localized on one chromosome. Two basic types of repeating units were isolated from Drosophila rDNA. One type, with a length of approximately 11 kb and containing both a 38S rRNA precursor and a non-transcribed spacer, resembles the Xenopus rDNA unit. The second type contains an insertion within the 28S rRNA gene. It is not known whether this type of rRNA gene is actually transcribed in vivo.

Analysis of the restriction patterns of these rDNAs revealed that approximately one third of the X chromosomal nucleolar organizer DNA contains repeating units without insertions, while the remaining two thirds, have insertions. Ribosomal DNA from the Y chromosome, in contrast, does not contain any repeats with long insertions.

The nontranscribed spacer at the end of each gene was found to be only somewhat variable in length. As well, the nontranscribed spacers appeared to be relatively homogenous. The 28S repeating units (with and without insertions) of the X chromosome nucleolar organizer were analyzed, and found to be interspersed in a random manner.

Those observations on length heterogeneity of rDNA repeats were compared for Xenopus and Drosophila, and various models for the evolution of randomly repeated genes were discussed.

Eric H. Davidson (Caltech) provided a detailed discussion of the heterogeneity of the structural gene sets which are active during sea urchin oogenesis and early embryogenesis. With regard to the "complex" class of mRNA, i.e., the class of RNA which comprises approximately 10% of the total mRNA of the embryo, and displays the highest degree of nucleotide sequence complexity, each cell of the 600 cell (blastula) embryo contains, on the average, one copy of each individual species of mRNA. The "prevalent" class of mRNA, on the other hand, was estimated to be present in an average of at least 50 copies per cell.

Could the complex class of mRNA, present at approximately one copy per cell, maintain steady-state levels of functional enzymes? A review of the literature values for mammalian liver, perhaps the best studied tissue in this regard provided the following insights: Of the 40 enzymes for which sufficient data exists, 8 could be maintained with such low levels of mRNA. The other would apparently require upwards of 100 copies per cell. The calculation fits well with the present knowledge of mRNA abundance classes of the sea urchin embryo.

Dr. Davidson presented data from his laboratory on the persistence of maternally inherited (oogenetic) mRNA sequences. The rate of disappearance of that RNA was followed from fertilization up through the pluteus stage. mRNA sequences found in the oocyte disappeared from total cytplasmic RNA at a slower rate than they did from polysomes. By the pluteus stage only a small percentage of RNA sequences present in the egg were still detectable by hybridization analyses.

An estimate was made of the minimum per gene transcription rate which would be required at the blastula/gastrula stage to maintain a steady state level of mRNA. That rate was estimated to be approximately 0.1-4.0 molecules/gene/hr. This rate was considered to be extremely slow, several orders of magnitude slower, in fact, than transcription rates calculated for globin or ovalbumin. The various active genes, including those represented in all classes of RNA, must still be transcribed only occasionally during the developmental stages of the sea urchin.

The synthesis during Xenopus oogenesis of both stable and unstable types of RNA was described by L. Dennis Smith (Purdue). Approximately 30% of the newly synthesized RNA of stage 6 oocytes is metabolically stable, and consists mainly of rRNA. Another 60% turns over relatively rapidly. The bulk of this RNA is "heterogenous" RNA, and can be divided into 3 kinetic classes. One class, which constitutes approximately 50% of the unstable RNA has a very short life of less than 30 min. A second class, which makes up another 45% of the unstable RNA, has a half life of more than 4 hours. And a third, minor component, is stable over the 24 hr course of his experiments. The shortest lived class appears, by virtue of its size, base composition, and nuclear location, to be similar to the hnRNA observed in somatic cells by many workers. The second class also appears to be localized in the nucleus. The minor component, which is relatively stable, appears to be localized in the cytoplasm. The bulk of the unstable RNA appears, therefore, to be turned over in the nucleus.

A comparison to stage 3 (lampbrush) oocytes was reported. The rate of hnRNA synthesis is approximately two-fold increased in stage 3 oocytes. The rate of synthesis of the stable component is increased, and the stable component which enters the cytoplasm represents approximately 9% of the total newly synthesized hnRNA.

With regard to the accumulation of maternal mRNA during oogenesis, the above data could be interpreted in several ways. Maternal RNA could accumulate in stage 3 oocytes, and hnRNA synthesized in subsequent stages could contribute a minor component to the mRNA population. Alternatively, the oocyte, at all stages, might be in a steady state during which virtually all species are turned over. From the observation and speculations an important point was made - hnRNA synthesis is occurring in virtually all stages of oogenesis, including the post-lampbrush stages.

The developmental significance of lampbrush chromosomes in amphibian oocytes was discussed by Joseph G. Gall (Yale). He pointed out that there are approximately 10,000-20,000 loops per haploid genome. If each loop were transcribing only a single species of mRNA, there should be at least 10,000 different mRNAs in the amphibian oocyte. The important question is "What exactly is being synthesized on a single loop?". Electron micrographs were shown which indicated that the loops contain ribonucleoprotein particles. As a model system, the 5S genes were chosen. A search for those genes was carried out. With in situ hybridization techniques it was found that 5S RNA hybridizes on the axes, rather than on the loops themselves. The sites of hybridization were close to the centromeres. Four of the centromeres of 11 chromosomes displayed grains with this method. Whether the loops are actually synthesizing 5S RNA was also examined by in situ hybridization. Iodinated 5S DNA was hybridized to nondenatured chromosomes. Hybridization was observed to loops which were located at or near the centromeres - those same four loci, in fact, which were previously labelled by hybridization. These loops were uniformly labelled. Dr. Gall pointed out the many gaps in our understanding of lampbrush loop function.

Several parameters which affect laboratory viability and the usefulness of Xenopus gametes were introduced by Antonie W. Blackler (Cornell). Techniques for the storage of gametes were presented. These methods should be useful for preparing eggs for nuclear transplantation. A method (heat shock) for the induction of triploidy was also discussed. The proportion of a population of heat shocked eggs which develop as haploids, triploids, and tetraploids was reviewed.

George M. Malacinski (Indiana) described a series of experiments in which ultraviolet irradiation (U.V.) was used as a biophysical probe for the components of the newly fertilized amphibian egg which are required for subsequent neural induction. Mapping experiments have demonstrated that the future dorsal side of the egg is most sensitive to irradiation. Although U.V. can affect several morphogenetic processes of early development, a decrease in the capacity for invagination during gastrulation and diminution in the inducing capacity of the primary embryonic organizer were found to account for defective neurulation in irradiated embryos. Those findings were discussed in terms of reducing the problem of induction of the primary embryonic axis to several discrete steps which can be individually examined.

Various manipulations which bring about a correction of the defects caused by U.V. were reviewed. The most effective method described is a series of embryological grafting techniques. Replacement of either the dorsal lip or the primary organizer of an irradiated embryo with the analogous part from a non-irradiated embryo provided a complete correction.

Preliminary results of irradiation experiments in which U.V. of various wavelengths was employed to determine the action spectrum of the target were presented. Both dose effect curves and U.V. penetration data are now available. It should be possible, in the near future, to determine whether the target has the characteristics of more of a protein or of a nucleic acid.

The coding of nerve cell connections were analyzed, in the amphibian embryo, by R. Kevin Hunt (Johns Hopkins University). Current notions of how ganglion

cells of the retina send their axons into the brain to form synaptic connections in the midbrain optic tectum were reviewed. Ganglion cells apparently acquire, during differentiation, the ability to discriminate among several possible connections to reach the currect map locus. Microsurgical procedures which disrupt the development of the proper signals were outlined. Various perturbations gave rise to different types of scrambled retinotectal maps. An analysis of the results indicated that a combination of several elements specify the correct locus for each individual ganglion cell. These elements include rostrocaudal, mediolateral, and depth components. This information was related to general models in which the nervous system, not unlike other embryonic systems, develops as the result of a series of progressive determinations.

Karl Illmensee (Institute for Cancer Research) reviewed current experiments on early embryonic dtermination in Drosophila. He discussed three approaches to this research area. First, the results of nuclear transplantation experiments on cleavage, blastula, and gastrula stage nuclei indicate that these nuclei are not restricted in their developmental potential. This was proven by obtaining functional germ cells from these nuclei. When nuclei from tissue culture lines were transplanted into early embryos, no germ line cells were, however, obtained so their totipotency remains unproven. In a second set of experiments, cytoplasmic localizations for primordial germ cells were demonstrated to exist at the posterior tip of the egg. Cytoplasmic transfers of this region to other parts of the embryo were made, and the induction of pole cells was observed. Thus, the cellular determination of germ cells can be attributed to a specific region of the egg cytoplasm.

Finally, Dr. Illmensee described a third set of experiments in which he transplanted blastoderm cells in reciprocal combinations from the anterior, mid-ventral, and posterior regions in order to investigate whether those cells were able to integrate with their new surrounding cells to form viable tissue masses. Both anterior and posterior cells were not able to integrate at opposite ends, but ventral cells apparently could form some structures at either tip. Dr. Illmensee speculated that these results could be very important, for they could be interpreted to mean that anterior and posterior cells are determined in their fate when they first form. This interpretation might suggest that unique types of cytoplasmic factors might be responsible for those very early determinations.

Anthony Mahowald (Indiana) reported on the current status of experiments designed to clarify the nature of the germ cell determinants of the Drosophila egg. In collaboration with Dr. Illmensee he discovered that the germ plasm is functionally complete prior to the completion of oogenesis. This observation clearly indicates that the germ cell determinants are produced during oogenesis. They also showed that in nuclear-cytoplasmic hybrids the nuclear body, a structure which is unique to the pole cells or primordial germ cells, is characteristic of the cytoplasmic type. The type of polar granule formed is, however, dependent upon the type of nuclear genome. Electron microscopic analyses of these mutations affecting pole cell formation were also presented. No common pattern of defect could be found.

Progress on the isolation of polar granules was detailed. The clusters of ribosomes which are seen on polar granules in situ are also found on granules which have been partially purified. Using pole cells as starting material, relatively clean polar granules have been isolated. Preliminary data indicate that most of the granule is composed of a single protein of approximately 100,000 MW.

In addition to these major reports, several workshops on various aspects of nuclear/cytoplasmic interactions, and the developmental genetics of amphibia, especially the Mexican axolotl, were held. A substantial proprotion of the 200 registrants participated in either the main talks or in these workshops. From the workshops came an understanding of how various mutant genes in the axolotl are being employed by various laboratories. As well, updates were given on the progress being made by several groups on the characterization of cytoplasmic components, and the analyses of nuclear function in early pre-gastrula development.

George M. Malacinski
Ann Janice Brothers
Department of Zoology
Indiana University
Bloomington, Indiana 47401