Meetings

Mosaics in Venice: Report on a NATO Conference on the Use of Mosaic Systems in Developmental Biology

Genetic analysis, with its precision and clarity, has the potential for elucidating the mechanisms of development in higher organisms. A. H. Sturtevant and C. Stern recognized this almost half a century ago (*1*) and developed methods for producing individual fruit flies (*Drosophila*) composed of two genetically distinct cell lines. Such individuals are called mosaics, and they present a unique opportunity to study cell lineages and interactions. Considering the potential of such an approach, it is surprising that only during the last decade have developmental biologists begun to fully exploit it. At the moment, the field is enjoying rapid growth. Thus it was appropriate that a North Atlantic Treaty Organization (NATO) meeting (organized by S. Gartler, University of Washington, and E. Gandini, Università Degli Studi, Ferrara) convened in Venice from 25 September to 3 October 1972 to discuss the use of mosaic organisms for the study of ontogeny.

Drosophila, the original model system for mosaic analysis, remains of fundamental importance. Mosaics that are part male and part female (gynandromorphs) can be obtained by using a ring X chromosome that, for unknown reasons, is routinely lost in one of the two daughter nuclei following the first-cleavage division of the zygote. As a consequence, one daughter nucleus has two X chromosomes and gives rise to cells that autonomously express female characteristics in various parts of the body, while the other cell line has only a single X chromosome, which in *Drosophila* leads to maleness. This male cell line expresses X-linked recessive traits carried by the single X chromosome. These traits do not appear in the female cell line because of dominant (wild-type) genes carried on the ring X. This generates flies with female parts showing dominant genetic traits and male parts showing recessive genetic traits. P. Bryant (University of California, Irvine) described exploitation of this technique to determine the effect of recessive X chromosome lethals on developmental processes. In some cases, such lethals lead to missing structures, such as parts of the thorax, in the adult mosaic fly. In other instances, the presence of normal tissue in the organism leads to rescue of the otherwise doomed cell line. In both cases, the mosaic methodology offers the potential to pinpoint the mechanism of the many X-linked lethals collected and retained in various stocks for many years.

A. Garcia-Bellido (Instituto de Genética y Antropología, Madrid) outlined the technique that he and J. Merrian (University of California, Los Angeles) have developed for producing two-dimensional fate maps that relate adult structures to embryonic structures. The mapping, for which gynandromorphic flies are used, depends on three characteristics of *Drosophila* development:

1) The first-cleavage spindle is oriented randomly with respect to the axis of the future adult fly. This means that the male and female daughter cell nuclei formed in a mosaic can take up any position with respect to the axes of symmetry of the resultant adult fly.

2) The progeny of the original cell nuclei tend to adhere together rather than mix randomly in the egg. This produces large patches of male and female tissue in adult mosaics, corresponding to the clones derived from the original daughter nuclei.

3) All the nuclei eventually arrive at the outer border of the egg, where they are walled off into cells. In that position they are by some unknown process determined to form a specific part such as head, wing, or tail.

The mapping consists of obtaining the frequency with which the male-female cleavage line passes between two adult structures, such as left wing and left leg. From the three properties outlined above, it follows that this frequency is inversely related to how close together the two structural antecedents were in the embryo. The two-dimensional map represents the egg outer surface, and the map relationships obtained are internally consistent and additive over short distances, in analogy to one-dimensional chromosome maps. S. Benzer (California Institute of Technology) described the use of fate mapping to determine in what embryonic structure a particular behavioral trait originates. He and his colleagues have isolated various recessive X-linked traits that influence behavioral characteristics of the fly, such as leg shaking, wings up, and even a syndrome called drop dead. By correlating the behavioral trait with the mosaic composition of a variety of visible adult structures in many gynandromorphic flies, the trait can be located on the fate map. This localizes the origin of the behavior to a part of the embryo, and hence directs the search for biochemical and morphological correlates of the trait.

Benzer also reported on the analysis of a biological clock that determines activity patterns in adult flies. After mutants with altered clocks were isolated, gynandromorphs were constructed in which (by inference from external markers) one side of the brain had a fast clock genotype (19-hour cycle) and the other side had a slow clock genotype (22-hour cycle). These animals had rhythms that could be represented by the superimposition of the two activity cycles acting independently of each other. It is reasonable to conclude from these experiments that activity is neurologically dominant to nonactivity, and that each side of the brain has its own phenotypic clock. The search is under way for genetically determined visible cell markers that will allow direct correlation between behavioral traits and various brain structures. The consensus of the meeting was that this type of analysis has a promising future.

Mouse mosaics can be formed in a variety of ways. One approach, originally developed by A. K. Tarkowski and B. Mintz, is to place together two genetically distinct early embryos in vitro, and allow them to spontaneously aggregate. The mosaic reaches the blastocyst stage, and is then reimplanted into a foster mother and undergoes normal development. R. Gard-

ner and V. Papaioannou (Cambridge University) described a variant on this technique which can best be termed blastocyst engineering. By using micromanipulators, they are able to remove the inner cell mass (which is thought to form the embryo proper) from the outer layer of the blastocyst (the trophoblast). A new inner cell mass, bearing a different set of genetic markers, then can be reintroduced, and development is allowed to proceed. At various stages thereafter, the embryo and extraembryonic parts can be sampled and tested for phenotype. Although the experiments are still in progress, the conclusion seems to be that the inner cell mass, while necessary for extraembryonic parts to form, contributes very few, if any, cells to these structures. They are mainly derived from trophoblast cells.

Another variant on this technique, described by M. Lyon [Medical Research Council (MRC) Radiobiology Unit, Harwell, England] and R. Gardner, is to inject a single, genetically marked cell from a donor blastocyst into a blastocyst cavity and determine which tissues of the resultant mouse contain progeny of the injected cell. Roughly 60 percent of the injected embryos survive to adulthood, and about a quarter of these have evidence of donor cell contribution, usually in tissues derived from all three germ layers. In some cases, the donor cell type is the majority member of a given organ. These results indicate that a cell at this stage is not irreversibly determined (and may not be determined at all) to form a specialized cell type. They also point to a fundamental difference between fly and mouse mosaics. Early embryonic cells in the mouse mix much more randomly than they do in *Drosophila*, so that the mosaicism seen in the adult mouse is of a much finer grain.

Another way to obtain mouse mosaics is to take advantage of X chromosome inactivation. Female mice, and other female mammals as well, have two populations of cells, one in which the paternally derived X chromosome is genetically inactive and the other with an inactive maternally derived X. Only genes on the active X chromosome are expressed, leading to mosaicism in various tissues. Much discussion at the meeting was devoted to comparing the mosaicism seen in this situation with that in embryo aggregation mosaicism. B. Mintz (Institute for Cancer Research, Philadelphia) described her modified standard pattern of coat color stripes seen in the embryo aggregation mosaics. This consists of roughly 34 randomly distributed lateral slots where pigment cells can clone out during development. This type of pattern has now been seen by B. Cattanach (MRC Radiobiology Unit), using a type of X inactivation mosaic with coat color genes translocated to one of the X chromosomes. As judged by the lively discussion that ensued, the relation between this pattern and determinitive events in the embryos remains an open question. Both W. Whitten (Jackson Laboratory, Bar Harbor, Maine) and A. McClaren (University of Edinburgh) presented instances in which X inactivation mosaics had finer-grained mosaicism than did the aggregation mosaics. This was true for the pigmented tissues of the retina, inner ear, and tail. One possible explanation is that X chromosome inactivation takes place late in development relative to clone formation. Another explanation is that two cell lines with very different cell surface characteristics do not mix randomly, but instead tend to adhere to cells of their own type. Appropriate experiments should distinguish between these possibilities.

Since both types of mouse mosaics generally have two differential cell lines in all organs tested, it is tempting to build mathematical models based on the binomial and hypergeometric distributions that relate interorgan correlations of cell mix to the numbers of precursor cells forming these organs. M. Nesbitt (University of California, San Diego) presented such a model and used it to analyze data from X inactivation mosaics. She reached the tentative conclusion that the ectoderm and mesoderm have a common precursor pool size of about 20 cells. Both she and McClaren pointed out that further work is needed before the assumptions involved in making such models are proved correct. Nevertheless, the methods outlined promise much new information in the near future.

One assumption that cannot be made about embryo aggregation mosaics is that the two cell lines have an equal chance of contributing to a particular tissue in the adult animal. Mintz presented examples, from tissues as diverse as somite, blood, and gonad, that indicated that one parental cell line or the other may systematically predominate in one tissue, while the other cell line may predominate in another tissue. A particularly interesting case of cell selection involves germ cells in XX/XY mouse aggregation mosaics. Most of these individuals develop testicles, and breeding data indicates that only the XY cell line forms sperm when these mice become sexually mature. However, McClaren was able to show that in embryonic XX/XY mosaics, XX cells enter the embryonic gonad first, divide for a while, and then disappear. Apparently the XY cells then enter and completely fill the germ-line niche.

X inactivation mosaics in human females have been used to great advantage by P. Fialkow (University of Washington) and his colleagues in determining whether tumors arise from one or more than one cell. If the tumor arises only from one cell, it should express genes carried by only one of the two X chromosomes in the woman's genome. By this criterion, most tumors seem to have a single cell origin. An example is leiomyoma, a benign smooth muscle tumor of the uterus which frequently appears in multiple sites. Individual tumors express only one of the two X-linked alleles of glucose 6-phosphate dehydrogenase, although other leiomyomas in the same uterus can express the alternative allele. There are a few tumor types which by the same criterion have a multiple cell origin. One is the neurofibroma associated with von Recklinghausen's hereditary neurofibromatosis.

Mosaic mammals have long been used in the field of immunology as a model system for self-tolerance. By being exposed to foreign tissue early in development, the mature animal lacks a normal rejection response to that tissue. R. Ceppelini (Basel Institute for Immunology) described a human XX/XY hermaphroditic mosaic who has an interesting combination of abnormalities, including juvenile diabetes and dwarfism. He speculated that these abnormalities could be due to some anti-"self" immune reactivity. However, there was no evidence for autoimmune lymphocytes in the peripheral blood of this individual. T. Wegmann (Harvard University) discussed self-tolerance in adult mouse aggregation mosaics. In these animals, a population of cells in the spleen is capable of specifically suppressing autoimmune reactions, at least in vitro. This work, when coupled with previous experiments demonstrating autoimmune potential in these animals, suggests that self-tolerance may be a continuous, active process throughout the lifetime of the individual. R. Barnes (Clinical Research Center,

Harrow, England) has aggregated NZB embryos (which have spontaneous autoimmunity as adults) with normal embryos. Preliminary data seems to indicate that the appearance of autoimmunity is postponed. One of the four mice tested eventually lost one parental cell line and subsequently rejected a skin graft genetically identical to the lost cell line. From these experiments and others reported by K. Bechtol (Stanford University) on the analysis of immune response genes in aggregation mosaic mice, it is clear that mosaicism continues to be a useful tool for analysis of the immune system.

The meeting was generally useful because it brought together investigators who use similar methods to ask similar questions on quite different organisms. Until now their respective literatures have remained relatively nonoverlapping, and the meeting was a promising beginning in correcting that deficiency. The Venetian atmosphere itself made a considerable contribution to the meeting's success in unexpected ways. R. Steward (U.S. Department of Agriculture, Beltsville, Maryland), who reported on plant mosaics, found a number of them growing in Venice, as well as a mosaic ivy accurately depicted by Leonardo da Vinci in his painting of the Annunciation hanging in nearby Florence. S. Benzer and D. Benzer, acting on a suggestion by M. Delbrück, found that the pigeons who come to the Piazza San Marco to get fed at 9:00 a.m. each day tell time by an internal clock. This experiment was possible because Venice went off daylight saving time during the meeting. The following morning the pigeons arrived an hour early according to people time. But the very next day they had reset their internal clocks and arrived on time. The Benzers were last seen looking for a mosaic pigeon.

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References

1. C. Stern, *Genetic Mosaics and Other Essays* (Harvard Univ. Press, Cambridge, Mass., 1968).

Amyotrophic Lateral Sclerosis: Summary of a Conference

Amyotrophic lateral sclerosis (motor neuron disease) is a rapidly progressive neurological disease. The disease accounts for 1/1000 of the adult deaths around the world, the same frequency as the more familiar neurological disease, multiple sclerosis. The disease appears between ages 50 and 60 years, with a duration of 3 to 5 years. The majority of the cases are sporadic. However, there are rare families in which a similar disease is inherited as a dominant condition. In addition, there are two geographic foci, Guam and the Kii Peninsula in Japan, where the incidence is many times the expected rate. A group of investigators representing the fields of epidemiology, neurovirology, neurochemistry, neuropathology, immunology, and clinical neurology met at Johns Hopkins University School of Medicine on 27 December 1972 to review the current state of research relative to this disease.

The conference started with a discussion of the clinical criteria for the diagnosis of amyotrophic lateral sclerosis. It became apparent that in a majority of the patients the disease fits the classical forms, such as progressive bulbar palsy, progressive muscular atrophy, and the combined form resulting in involvement of both upper and lower motor neuronal pathways. However, in a significant number of patients, perhaps as high as 20 to 25 percent, the illness does not fit the usual pattern and is aberrant either in manner of clinical presentation or in duration.

Jacob Brody (National Institute of Neurological Diseases and Stroke) reviewed the experience on Guam, where the incidence among the Chamorros is approximately 100 times the expected rate. Ten years of study of the Guamanian population have indicated that the disease process is not inherited in any known genetic pattern. In addition, attempts at linking the disease with any particular toxin, environmental factor, or infectious process have not been successful. In particular, the *Cycas circinalis* (cycad) nut is probably not the factor, because the incidence of the disease has not decreased even though the consumption of this nut as a staple in the diet has virtually disappeared. The high incidence of the disease on Guam is continuing despite changes in environment, standards of living, and diet. It is Brody's feeling that the disease on Guam is typical of amyotrophic lateral sclerosis as it is seen elsewhere, and that the Guamanian population may have genetic susceptibility to an exogenous factor such as an infectious agent. This population is a valuable one for study, particularly in terms of rapid evaluation of any therapeutic measures.

The high incidence of the disease on the Kii Peninsula in Japan was also briefly discussed. Studies of this population have not been as complete as those of the Guamanian population. It was pointed out that these two foci of amyotrophic lateral sclerosis are among the few foci of chronic neurological disease for which the environmental or etiological factors have not been demonstrated.

Richard Johnson (Johns Hopkins) and Clarence J. Gibbs, Jr. (National Institute of Neurological Diseases and Stroke) reviewed the current status of the neurovirological approaches to amyotrophic lateral sclerosis. In particular, the data obtained by Soviet scientists in the early 1960's and their recent follow-up studies were presented by Gibbs, as well as his unsuccessful attempts to confirm the Russian studies. He also reviewed the attempts to isolate an agent by means other than transmission to a primate. These newer methods include explants of human tissue obtained by biopsy or from recent autopsy, inoculation of involved brain into cultures of human fetal brain, and techniques for rescue of latent virus. It was the feeling of Gibbs and others that these techniques should be exploited fully in an attempt to further elucidate the possible infectious etiology of this disease.

Murray Gardner and Earle Officer (University of Southern California) presented a murine model for chronic motor system disease associated with C-type particle viruses. This disease occurs spontaneously in wild colonies of mice caught around farms outside the Los Angeles area. These mice develop lymphoma and also a peculiar form of paralysis, the incidence of both conditions increasing with age. A marked accumulation of viral particles is seen in the anterior horn cells of the animals with paralysis. This is not an exact model of amyotrophic lateral sclerosis, because the human disease has a specific age peak and viral particles are not found in the anterior horn cells of patients. Nevertheless, the murine disease is a slow virus infection that appears to be selectively affecting anterior horn cells, involving the lower part of the cord first.