Probing Gene Action During Development

For nearly 20 years, the Miami Winter Symposium has been bringing biologists to sunny Florida in the dead of winter to learn about the latest advances in some up-and-coming research area. This year's meeting, which was held on 9 to 13 February and attracted more than 800 participants, focused on "Advances in Gene Technology: The Molecular Biology of Development." The presentations emphasized the current surge of efforts to identify the genes that control the course of development in complex organisms. A selection of the results presented at the wide-ranging speaker and poster sessions are reported here.

The Good News—and the Bad—About Gene Therapy Prospects

A few years ago, researchers were very optimistic that newly developed viral vectors could soon be used to achieve the gene transfers needed for accomplishing the gene therapy of human genetic diseases. Those hopes have so far not been realized, however, because the genes transferred by the vectors into the cells of living organisms have usually been expressed poorly, if at all.

The good news as described by the participants at the Miami Winter Symposium is that the newer approaches to gene therapy that are now under development do work. The bad news is that the methods are still not efficient enough to try on human patients.

The most obvious targets for potential gene therapy are diseases that might be cured by the introduction of new genes into bone marrow cells, which can easily be removed from patients and then re-injected after the necessary gene transfers have been accomplished. Such diseases include a hereditary immune deficiency that is the condition most likely to be treated first. Researchers who attempted to test the efficacy of gene transfer into experimental animals soon learned, however, that the protein products of the newly introduced genes were often not made in the bone marrow cells even though the intact genes were present there.

One possible problem may have been the vector construction. In the vectors used for the early transfers, the genes were put under the control of viral regulatory elements present in the long terminal repeats (LTRs) at the end of the vectors. To ensure the continued presence of a transferred gene in the cells produced by the bone marrow, the genes must be introduced into the stem cells, which have the potential of giving rise to all types of bone marrow cells, including the red and white blood cells. Genes linked to the viral LTRs are apparently not expressed in the cells derived from stem cells that have been infected with a viral vector.

In the vectors used for the more recent work, the genes to be transferred either are left under the control of their own regulatory sequences or are combined with other cellular regulatory sequences of known activity. For example, Richard Mulligan of the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology described experiments in which Elaine Dzierzak of his group introduced a cloned human beta globin gene bearing its own regulatory sequences into the bone marrow of mice. "The gene was expressed to a reasonable degree," Mulligan says, "up to 5 to 10% of the expression of the endogenous beta globin gene."

Moreover, the expression was relatively tissue-specific, with the human beta globin being made primarily in the red blood cell lineages, where that gene ought to be expressed. "The results suggest that gene replacement therapy for beta thalassemia or sickle cell anemia may be more feasible than previously thought," Mulligan says. The two diseases in question are caused by defective beta globin genes. Mulligan notes, however, that expression of 20 to 50% of normal will probably be required to correct the beta globin deficiencies.

Beatrice Mintz, Robert Hawley, and Luis Covarrubias of the Fox Chase Cancer Center in Philadelphia have constructed vectors in which the viral LTRs are disabled and the two genes to be transferred (one the cellular version of the *myc* oncogene and the other a gene coding for antibiotic resistance) are connected with other active regulatory sequences. Both genes were expressed when they were introduced into cultured lymphoid cells.

In addition, the Fox Chase workers have used the vectors to transfer the genes into mice that have hereditary defects in bone marrow cell lineages. "Both of them worked quite well with regard to successful infection and propagation [of the bone marrow cells] for at least 8 months in the mice," Mintz says. "The evidence is quite convincing that we are repopulating stem cells." However, Mintz and her colleagues have not yet determined whether the foreign genes are expressed in the mice.

Theodore Friedman and his colleagues at the University of California at San Diego are not only tinkering with their viral vectors but are also beginning to develop a system for introducing new genes into liver cells, instead of bone marrow cells. Although adult liver cells are not susceptible to infection by the kinds of viruses commonly used as gene transfer vectors, the cells dedifferentiate and become immature for a few days when they are induced to grow in culture. They then differentiate again.

The UCSD workers have found that for a day or two during the period of dedifferentiation the cells can be infected with viral vectors and that the gene thus transferred is expressed, albeit at a low level in the case of vectors with disabled LTRs. Nevertheless, as Friedman told the symposium participants, "The result makes available in principle an attack on hepatic disease with retroviral vectors." Hereditary diseases that affect genes expressed in liver cells include those involving clotting factor deficiencies, such as hemophilia.

When using the viral vectors, researchers have no control over the final location of a transferred gene in the recipient cell genome. Mario Capecchi and his colleagues at the University of Utah in Salt Lake City have set themselves the difficult task of targeting genes to specific sites in the mammalian genome. If a gene could be targeted to its normal genomic site it might work better, especially with regard to control, than if it integrated just anywhere in the genome.

Capecchi and his colleagues have not yet achieved this kind of specific gene replacement. "The distance between our experiments and human somatic gene therapy is enormous," he points out. However, the Utah workers have shown that they can correct a defective gene that they had previously inserted in the genome of cultured cells by injecting the cells with another copy of the gene.

Despite the encouraging gene transfer results reported at the symposium, one finding has raised a strong cautionary note, especially with regard to expectations about the ultimate success of gene therapy involving the introduction of genes into bone marrow cells. Work from Mintz and Mulligan, among others, suggests that blood cell formation may result from the sequential use of small numbers of different stem cell clones, rather than from a static contribution of the entire stem cell pool.

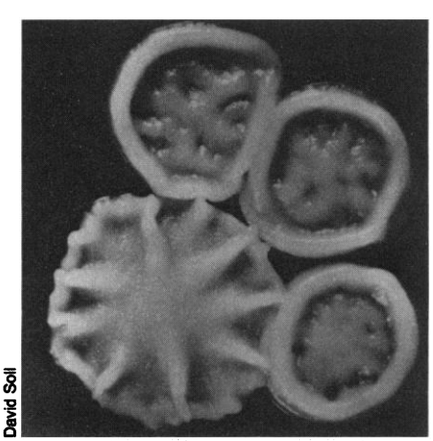
This would put a high premium on infecting all or nearly all of the stem cells in a bone marrow sample when it is exposed to a gene transfer vector. Otherwise the progeny of a stem cell that had been successfully infected could well be supplanted by the progeny of a stem cell that had not been.

Moreover, even if several stem cells contain the transferred gene, the integration sites are likely to be different, in which case the expression levels might vary in the stem cell progeny. The road to successful gene therapy in human patients may well be a long one.

"Switching" in Yeast and Slime Molds

Yeast infections are becoming increasingly common. In otherwise healthy people, they are usually annoying, but localized, infections of the mouth or vagina. However, in individuals with defective immune systems, including AIDS, cancer, and organ transplant patients, pathogenic yeast strains can invade any body tissue to produce life-threatening infections. Both the localized and disseminated infections often resist therapy. Recent work from David Soll's laboratory at the University of Iowa in Iowa City may help to explain what makes yeast such a versatile and hard-to-treat pathogen.

The Iowa workers found that cells of the common pathogenic yeast *Candida albicans*, when grown under certain laboratory conditions, can change their characteristics to produce any of seven readily distinguishable colony types. This "switching" of colony type naturally occurs in about one cell in every 10,000, but treatment with sublethal doses of ultraviolet radiation increases the frequency to about one cell in 100. Once a cell has switched, the frequency of change remains high in its progeny.



David Soll

Switching in yeast: *Three of the yeast colonies are of the "ring" type. The fourth, which arose as a result of switching induced by ultraviolet radiation, is of the star type.*

Some switches dramatically alter the yeast cells. The changes may include variations in antigenic composition and responsiveness to therapeutic drugs and in the ability to make hyphae, the growing filaments that allow yeast cells to invade tissues. All of these changes could influence the pathogenicity of yeast. "It's hard not to believe that switching has something to do with pathogenesis," Soll maintains. "Anything that gives a pathogen that kind of variability is going to be selected for."

Although a link between switching and pathogenicity has not yet been directly demonstrated, Soll, James Hicks of the Research Institute of Scripps Clinic in La Jolla, and their colleagues have shown that switching is not just a laboratory artifact. It occurs at high frequency in *C. albicans* and also in *C. tropicalis* isolated from the recipient of a bone marrow transplant. Moreover, the *C. albicans* strains isolated from 9 of 11 women with vaginal yeast infections also display switching, which apparently occurs at the infection site.

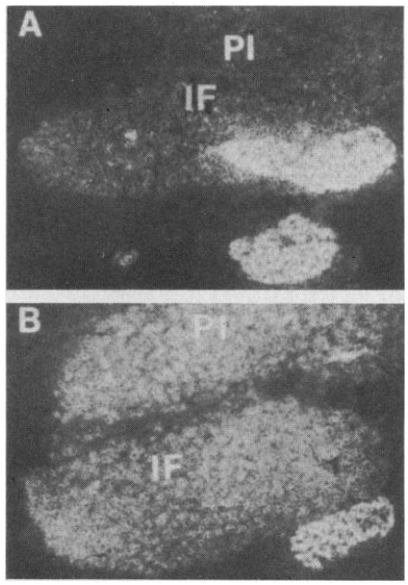
The Iowa workers have not yet pinpointed the cause of switching in yeast. Its characteristics indicate that it may be caused by transposable elements, segments of DNA that can move about in the genome and thereby alter gene expression.

Yeast is not the only organism that displays switching. It also occurs in the cellular slime mold *Dictyostelium discoideum*. Under normal conditions, slime molds are independently living, amoeba-like cells. But when the cells are starved, they form an aggregate, which then goes through a series of developmental changes culminating in production of a fruiting body that produces spores. According to Soll, switching alters the timing of these developmental events. Other than that, switching in the slime mold resemble those in yeast.

Making Contacts in the Developing Embryo

During the development of complex organisms, embryonic cells often have to break contacts with their old cellular partners and associate with new ones. A few years ago, researchers learned that cells are able to recognize the correct partners at the various stages of development by means of recognition molecules carried on the outer cellular surfaces. Synthesis of these molecules appears to be very closely regulated during development.

Masatoshi Takeichi of Kyoto University in Japan described his group's findings regarding the molecules known as "cadherins"



NCAM turn-on in embryonic muscle. *NCAM expression in embryonic muscle coincides with the branching of axons from the main nerve trunks to form connections with muscle fibers. In (A), before the branching, the developing chick thigh muscles (PI and IF) contain little NCAM. The bright patches are the two trunks of the sciatic nerve, which do contain it. In (B), when extensive axonal branching is occurring, NACM expression in the muscles is high.* [*Courtesy of* Developmental Biology, **114**, *437 (1986), © Academic Press*]

(for calcium-dependent cell-cell adhesion molecules). The three cadherins identified so far, which are designated "E" for epithelial, "N" for neural, and "P" for placental, are cell surface proteins with molecular weights ranging from 118,000 to 127,000. According to Takeichi, the cadherins play a crucial role both in maintaining associations between cells of the same type and in fostering the separation of different cell types during tissue and organ formation.

The Kyoto workers have found that, in the test tube, cells that bear E-cadherin preferentially associate with one another but do not adhere to cells carrying N-cadherin. The converse is also true. "This shows that the cadherins are very important for selective cell adhesion," Takeichi says.

In developing mouse or chick embryos, E-cadherin is the first to appear on cells. Although it is found on all the cells of early, preimplantation embryos, this situation changes as development progresses. According to Takeichi, the E- and N-cadherins have mutually exclusive distribution patterns in older embryos, with E-cadherin occurring on epithelial cells and N-cadherin on nerve and muscle cells. This situation reflects changes in expression of the cadherins that occur when those tissues are being formed.

For example, when the single cell layer of which the preimplantation embyro is composed separates into the two cell layers known as the ectoderm and mesoderm, the cells that form the mesoderm stop producing E-cadherin and make the neuronal type instead. The formation of nervous tissue from the ectoderm is also marked by a switch from E-cadherin to N-cadherin production. Takeichi concludes that the switch in cadherin expression may be essential if old cellular connections are to be broken and new ones made during organ formation in the embryo. P-cadherin is needed for making the embryonic and uterine cell connections of the placenta.

Takeichi and his colleagues have compared some of the structural characteristics, including partial amino acid sequences, of E-cadherin from mouse liver and of N-cadherin from chicken brain. The results indicate that the molecules are sufficiently related to belong to a "cadherin family." The number of family members is still unknown.

The molecule called NCAM (for neural cell adhesion molecule) is another protein that aids the formation of cellular associations, principally those involving nerve cells, during development. NCAM's cellular distribution is similar to that of N-cadherin, and NCAM-mediated interactions, like those of N-cadherin, require the molecule's presence on both partners. The two adhesion proteins are different, however.

Whereas the cadherins appear to act as classic cell-sorting molecules in the early embryo, NCAM has a more regulatory role in intercellular interactions, according to Urs Rutishauser of Case Western Reserve University School of Medicine in Cleveland. Variations in expression of the molecule help to create adhesive preferences between cells during a diverse set of developmental events, especially those involving nerve cells.

Rutishauser and his colleagues have shown, for example, that the propensity of NCAM-positive cells to adhere to one another helps guide the optic nerve from the eye to its final destination in the brain. The nerve cells, which make NCAM, grow along the outer surface of the brain, following a trail of glial cell projections known as "end feet," which also make the molecule.

In addition, NCAM participates in the formation of the synaptic connections between nerve and muscle cells and possibly in the establishment of gap junctions, which are direct molecular links between cells. "NCAM is not a part of these specialized connections," Rutishauser says, "but it helps to bring cells together so that they can form." The importance of NCAM in mediating these cellular interactions is indicated by studies showing that antibodies to the molecule alter the route taken by the optic nerve and disrupt synapse and gap junction formation.

The synthesis of NCAM, like that of the cadherins, is carefully regulated during these developmental events. The glial end feet make NCAM just as the optic nerve axons pass by. Formation of nerve-muscle synapses and of gap junctions also correlates with NCAM expression at the appropriate sites. Finding out what is regulating the expression of the genes for NCAM and the cadherins will be a major goal for the future. ■

JEAN L. MARX

Japanese Super-Sequencer Poised to Roll

"In the 21st century, we foresee that DNA-sequencing supercenters will be set up in several countries," says Akiyoshi Wada of the University of Tokyo. Such centers would be symbols of human intellectual endeavor, he adds, being biology's equivalent of "large particle accelerators, and far-reaching programs of space research."

The stepping stone to this vision of the future is Japan's current effort to establish in the very near future a "factory" that will be able to run through a million bases a day. The project was conceived 5 years ago when Japan's Science and Technology Council formed a committee specifically to study the issue of automation of DNA sequencing. Wada heads that committee.

In the 26 February issue of *Nature*, Wada outlined his country's progress on its venture, including a projected cost of between 10 and 17 cents per base sequenced. This figure would represent close to an order of magnitude improvement over current costs.

The emphasis of the Japanese approach is to automate well-established techniques rather than develop new ones. This contrasts to some extent with a lot of the research endeavor in the United States where several research groups are developing substantially new approaches to sequencing.

For instance, the sequencing in the Japanese super-sequencer will be done, for the most part at least, by using the Sanger technique. And in spite of the recent development of fluorescent labeling and laser detection techniques for identifying the bases as they are separated by gel electrophoresis, Wada says that initially the super-sequencer will rely on the established method of radioactive labeling of bases and autoradiographic identification. The reason, he says is that the old approach is cheaper. "Specific labeling of the four nucleotide bases with characteristic fluorescent dyes may be advantageous in an automated system," he acknowledges, but only "if their cost-performance becomes attractive."

The prime goal of the project is to be able to sequence a million bases a day, not to produce a fully automated sequencing system, comments Wada. To this end, the sequencing task is broken up into discrete units, each of which is being automated by one or more prominent Japanese companies. For instance, Seiko is modifying its existing sequencing machine, which is based on the Maxam-Gilbert technique and charts up one base every 14.4 seconds, to produce a machine based on the Sanger method. A prototype machine, which was developed in collaboration with scientists at the Institute of Physical and Chemical Research at Wako, Saitama, now appears capable of an output of close to 300,000 bases a day, or one base every 0.28 second. With continuous operation and with "a relatively modest improvement," this machine would readily achieve the goal of 1 million bases a day.

The radiographic detection system is being handled by Fuji Photo Film Company, which has developed an acetate-covered gel film that can be exposed and developed much more rapidly than is typically done in most molecular biology laboratories. Fuji is already capable of producing sufficient quantities of this special film to cope with a 1-million-base-a-day throughput.

The autoradiographic pattern on the films will be read by one of the automatic scanners that have already been developed independently by Hitachi Software and by Seiko. So far these scanners can read at a rate of 1.4 bases a second, which is not especially fast compared with a skilled human practitioner. However, the machine can run 24 hours without interruption, giving a total of 60,000 bases a day. An order of magnitude improvement in reading rate through better optical processing and higher grade computing is easily attainable. The current 1% error rate can be virtually eliminated by reading two identical or complementary strands.

These, then, are the various components of the system, which will be linked together, says Wada, by "skilled human operators [who] would play a crucial role in monitoring the accuracy of the results." The project is poised to move into action, he adds. "We have now reached the point in the planning at which all the elements of a mass production line exist, together with the interfaces between them." The super-sequencer is therefore expected to be up and running in about a year. ■ ROGER LEWIN