# Symposium Focuses on Genes in Development

*A recent symposium on the "Molecular and Genetic Basis of Growth and Development" surveyed progress in understanding the genetic control of development. Reflecting the sponsorship of the National Institute of Dental Research, the symposium, which was held at the Bethesda campus of the National Institutes of Health on 22 and 23 February, put special emphasis on the bones and connective tissue, but also featured more general aspects of development, including the role of growth factors and mechanisms of gene control.*

## Collagen Gene Mutations Cause Brittle Bones

Collagen, one of the most abundant proteins of the animal body, is the glue for holding the soft tissues together and the scaffolding for building bone. In fact, says Darwin Prockop of Jefferson Medical College in Philadelphia, "The shape of many complex organisms is defined not by their cell structure, but by the amount, size, and shape of their collagen fibrils." When the normal orderly structure of those fibrils is disrupted, the consequences can be serious, even fatal.

Osteogenesis imperfecta is a case in point. In this hereditary disease, the bones fail to develop normally and are brittle. In the worst cases they are so fragile that affected fetuses may be crushed to death in the womb. Individuals that do survive may have a lifelong susceptibility to fractures and, as result, deformed bodies.

About 9 years ago, Prockop decided to investigate whether osteogenesis imperfecta might be caused by gene mutations that alter the structure of type I collagen, the principal collagen of bone. He considered the quest to be something of a longshot, with perhaps a 10% chance of finding a positive result. Many genes, most of which are unknown, undoubtedly contribute to bone formation, Prockop notes. Moreover, two structural genes are needed for making type I collagen and both had to be cloned before the search for mutations could even begin.

Once this was done, however, the quest paid off. "Surprisingly," Prockop says, "80% or more of children with osteogenesis imperfecta clearly have mutations in the procollagen genes." Researchers in Prockop's laboratory and elsewhere have identified about 20 well-defined mutations in those genes in individuals who have the bone disease.

The locations of the mutations are related to the severity of the abnormalities they produce. During the assembly of a complete collagen molecule, two copies of the polypeptide chain encoded by one of the type I collagen genes and one copy of the chain encoded by the other, wind together, essentially forming a triple helix with loose, unwound ends. Both ends are then clipped from this procollagen, as it is called, to form the collagen molecules that eventually produce the fibrils.

The mutations with the worst consequences are usually those that alter the central, triple helical segments of the collagen polypeptides. These mutant chains can combine with normal collagen polypeptides to begin the assembly process, but the abnormal chains prevent the formation of the triple helix. Consequently, the aggregates are rapidly degraded, a result that Prockop calls "procollagen suicide." Such mutations, which greatly reduce the formation of collagen fibrils, are either lethal or produce severe osteogenesis imperfecta.

Mutations that block removal of the amino end of procollagen cause a less serious condition known as the Ehlers-Danlos—or "rubber man"—syndrome, which is characterized by dislocated joints and excessively elastic skin. "The abnormal collagens that result form very irregular fibrils that are adequate for some purposes," Prockop explains, "but not strong enough for large joint ligaments."

With the significance of collagen gene mutations now established for osteogenesis imperfecta, Prockop plans to look for mutations in other conditions that might be caused by abnormalities in collagen structure. These include osteoporosis, another brittle bone disease; osteoarthritis, in which the joint cartilage deteriorates; and aneurysms, in which blood vessel walls become distended and rupture. These conditions generally do not appear until fairly late in life, a circumstance that will make performing family studies much more difficult than those on the childhood disease osteogenesis imperfecta. But Prockop says, "We have the genes. We have the technology lined up. I think it's worth a try."

In a related development, Rudolph Jaenisch and his colleagues at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, have devised a method for reproducing osteogenesis imperfecta in mice. Jaenisch and his colleagues use a type I collagen gene with a mutation that prevents its protein product from forming the helical structure needed for the assembly of collagen fibrils.

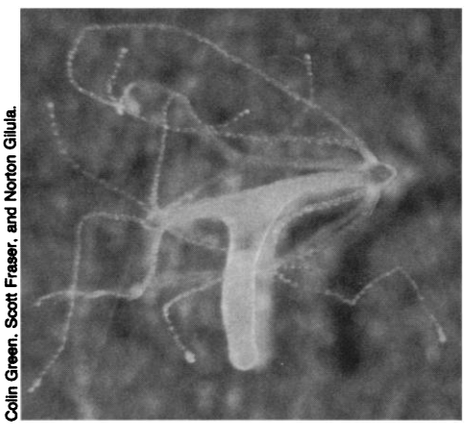
The Cambridge workers introduced the altered gene into otherwise normal mouse embryos with a viral vector. "The point mutation in this gene caused a dominant, lethal phenotype," Jaenisch told the meeting participants. This occurred even though the mice were still making normal procollagens. "As little as 10% of the mutant RNA is sufficient to reduce collagen production by 50% and produce the lethal effects," Jaenisch says. The situation is similar to the "procollagen suicide," described by Prockop.

Jaenisch suggests that the gene transfer method may be applicable to the study of other structural proteins and their functions. The various filaments and tubules that form the cell skeleton, for example, are also built up of orderly arrays of individual proteins. If the organization of these structures is disrupted by relatively small amounts of a mutant protein interloper, as collagen fibril assembly is, then researchers would effectively be able to produce mutations for studying the functions of the structural proteins.

## Gap Junctions Needed for Development

Without cell-to-cell communication normal embryonic development simply could not occur. The communication may sometimes be mediated by large molecules that are released from cells and act much as hormones do. In addition, small molecules that are transferred directly between adjacent cells by the intercellular channels known as gap junctions may also play a role. "Gap junction communication does have an influence on developmental processes, especially in the area of patterning," says Norton Gilula of the Research Institute of Scripps Clinic in La Jolla.

Gilula and his colleagues have found that blocking gap junction communication produces developmental abnormalities in species as diverse as the hydra, the frog, and the mouse. The simple hydra, for example, is essentially a tube with a tentacled head and a foot with which it attaches itself to a surface. Normally, a second head will not develop



Collin Green, Scott Fraser, and Norton Gilula.

**A two-headed hydra** *may be obtained if gap junction communication is blocked.*

from a graft of head tissue to the hydra body, presumably because the original head produces an inhibitory molecule that prevents another one from forming.

However, Gilula and his colleagues have found that a second head will develop if the grafted animals are treated with antibodies that recognize and bind to the protein that forms gap junctions. This treatment apparently prevents the passage of the inhibitory molecule from cell to cell through gap junctions.

The antibodies also interfere with frog and mouse embryogenesis. When they are injected into a particular cell of the eight-cell frog embryo, the tadpoles that result are often asymmetrical. The brain and eye on the right side either fail to develop at all or are irregular in shape or position. Injecting early mouse embryo cells has a different result, the separation of the injected cells from the other embryonic cells. "The embryos seem to eliminate cells that cannot communicate," Gilula says.

Gilula and his colleagues are also getting a look at gap junction structure. They have cloned the genes for the gap junction proteins of human liver and of frog embryos. Determination of the nucleotide sequences of the cloned genes indicates that the proteins they encode are likely to contain four alpha helices each. These helices, which have similar amino acid sequences in the two proteins, are probably embedded in the cell membrane. The remaining segments of the proteins, which do not resemble one another, project to the cell interior and may be involved in regulating gap junction activities, Gilula says.

According to the model for gap junction structure that Gilula described at the development meeting, six copies of the protein assemble in a ring to form a channel. One of the four helices of each protein lines the channel, with the other three acting to stabilize the protein's position in the membrane. This assemblage then lines up with a similar arrangement in the membrane of an adjoining cell to form a complete gap junction that allows small molecules to pass between cells. The next step is to identify the ones that influence development.

## Dissecting Receptor Structures

The cloning of the genes for growth factor and hormone receptors is enabling researchers to obtain a better understanding of how the receptors work in transmitting the hormonal signals to the cell interior. Specific alterations can be introduced into the cloned genes, and the activities of the mutant receptors can then be compared with those of the normal receptors. This provides a means of determining which parts of the molecule are performing what functions. The development meeting provided examples of two, very different receptors for which this is now being done.

Keith Yamamoto of the University of California, San Francisco, described his group's results with the receptor for the steroid hormones known as the glucocorticoids. In contrast to the situation with most other receptors, the pathway by which the receptors for the steroid hormones transmit signals to the cell nucleus is well understood. The hormones diffuse through the cell membrane, bind to receptor molecules in the cytoplasm, and then the complex moves into the nucleus where it alters gene expression.

"We wanted to understand what it is that the hormone does to make the receptor functional," Yamamoto says. The San Francisco group found that the receptor has two specific regions that are needed for entering the nucleus. This movement can occur, however, only after the hormone binds, presumably changing the receptor shape and exposing the nuclear localization sites.

Once inside the nucleus, the receptor complex turns some genes on and turns others off. Genes that are activated have a common regulatory region to which the complex binds. Yamamoto and his colleagues have identified the segment of the receptor that binds to this positive regulatory sequence and find that it is distinct from the nuclear localization sites. Studies of mutants indicate that the DNA-binding segment contains separate sites for gene activation and suppression, although additional portions of the receptor also appear to contribute to its gene regulatory effects.

The sites to which the receptor binds on the genes that it turns off have not yet been characterized in detail. Nevertheless, Yamamoto speculates that the negative regulatory sites induce a change in the three-dimensional structure of the receptor complex that may account for the different outcome of the binding. "A DNA sequence may be an allosteric effector of the structure of the receptor," Yamamoto explains. "As a result, it may no longer act as a positive regulator, but may act as a repressor instead."

If the ways in which the glucocorticoid receptor regulates gene expression are now being worked out, the manner in which signals are transmitted to the cell nucleus from the membrane receptor for epidermal growth factor (EGF) remains unknown. Dissection of the activities of the receptor itself is well under way, however, as reported by Joseph Schlessinger of the Weizmann Institute in Rehovot, Israel, and Rorer Biotechnology, Inc., in Philadelphia.

The EGF receptor can be divided into three main parts: one projects into the cell cytoplasm and has tyrosine kinase activity, that is, it acts as an enzyme that transfers phosphate groups from adenosine triphosphate to the amino acid tyrosine in acceptor proteins; the second spans the membrane; and the third extends outside the cell and binds the growth factor. Schlessinger and his colleagues have introduced mutations into each of these regions to see how this affects receptor function.

The tyrosine kinase activity, for example, is thought to be instrumental in transmitting EGF's growth-stimulatory signal to the cell nucleus. The Schlessinger group's results bear out that hypothesis. They find that mutations that prevent the EGF receptor from catalyzing the phosphate transfer also block the enhancement of cell division in response to the growth factor.

These mutations have another consequence. Those receptors that have bound EGF are normally taken into cells where both the receptor and the EGF are degraded. If the receptors lack tyrosine kinase activity, however, they are not broken down but are recycled to the cell surface, although the EGF is still degraded. "The kinase plays a role in receptor trafficking, but how we don't know." Schlessinger says. The finding raises the possibility that the cellular location of the receptor kinase may influence its ability to stimulate cell growth, although that remains to be demonstrated.

The mutations introduced into the transmembrane region of the receptor had little effect on the receptor functions. Finally, the Schlessinger group's results point to a portion of the outer part of the EGF receptor that is bounded by two regions with a high content of the amino acid cysteine as important for binding the growth factor. ■

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