Developmental Control Gene Sequenced

The sevenless *gene, which controls the development of a nerve cell in the fruit fly eye, encodes a protein that resembles a growth factor receptor*

WITHIN the past few years researchers have begun to get a look at the genes governing the development of complex organisms. This week's *Science* features a recent case in point (p. 55). A research team led by Gerald Rubin of the University of California at Berkeley reports the cloning and sequencing of the *sevenless* (*sev*) gene, which is needed for the development of one of the cells of the fruit fly eye. The work indicates that the protein encoded by the gene serves as a receptor for receiving the incoming signal that determines the cell's developmental fate. "It is one of the first glimpses at the kinds of local cues that cells receive during development to specify the cells' fate," notes J. Michael Bishop of the University of California at San Francisco.

Moreover, the findings provide further support for the idea that developmental signals resemble growth factors and oncogenes, which can cause cells to become cancerous, in their mechanism of action. The *sev* protein has the characteristic features of a tyrosine kinase enzyme, similar to those of the *ros* and *src* oncogene proteins and of the well-studied receptor for epidermal growth factor (EGF).

The *sev* gene was originally identified on the basis of mutants discovered about 10 years ago in the laboratory of Seymour Benzer at the California Institute of Technology. The fruit fly eye has a very regular structure, consisting of a two-dimensional array of approximately 800 facets, called ommatidia. Each ommatidium in turn consists of 20 cells, including eight photoreceptor cells that convert light signals to nerve impulses.

In *sev* mutants, the photoreceptor cell designated R7 does not develop (thus the name *sevenless*). Instead the cell that ought to produce the photoreceptor cell develops into a cone cell, which produces the liquid-filled lumen underlying the lens. The *sev* mutations are therefore homeotic mutations, which are classically defined as mutations that cause cells to switch from one developmental fate to another.

Although immature cells can be genetically programmed to attain a particular developmental fate, this is apparently not the case for the R7 precursor cell. Evidence from several laboratories indicates that positional information, presumably obtained from neighboring cells in the developing ommatidia, serves as the signal that tells the R7 precursor to develop into that particular photoreceptor cell. "From that background we knew that the *sev* gene appeared to be involved in reading the positional information," says Rubin, who cloned and sequenced the gene with Berkeley colleagues Ernst Hafen and Konrad Basler, and Jan-

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Erik Edstroem of the European Molecular Biology Laboratory in Heidelberg, Germany. The *sev* gene has also been cloned by Benzer, Utpal Banerjee, and their Caltech colleagues, although these investigators have not yet determined the nucleotide sequence.*

Determination of the nucleotide sequence of the *sev* gene provides a good indication of how it might work in reading the positional signals. "We lucked out in that the gene sequence told us something," as Rubin puts it. The gene encodes a protein with a molecular weight of at least 220,000—and the characteristics expected for a membrane-spanning receptor.

Moreover, the amino sequence of the carboxyl end of the protein, which is likely to be inside the cell, turned out to resemble the tyrosine kinase segments of the EGF receptor and the protein products of the *ros* and *src* oncogenes. Tyrosine kinase enzymes add phosphates to proteins on residues of the amino acid tyrosine. Such phosphorylations are thought to be involved in transmission of the growth-stimulatory signals of the oncogene products and the activated EGF receptor to the cell interior, and the current work suggests that the developmental signal is transmitted in a similar fashion.

The *sev* gene therefore joins a handful of other developmental control genes thus far identified that encode products that apparently operate like growth factors at the cell membrane. These other genes include *lin-12*, a homeotic gene of the flatworm *Caenorhabditis elegans*, and *Notch*, a fruit fly gene that affects nerve tissue development. The proteins encoded by these two genes contain segments that strongly resemble EGF itself.

In addition, a protein encoded by the *decapentaplegic* gene complex of the fruit fly, which affects body pattern development in that organism, has a structural homology to transforming growth factor–β. These gene products may be sending developmental signals, however, whereas the *sev* protein appears to be the first example of a protein that receives local developmental cues.

The probable mode of action of the above-mentioned genes contrasts with that of still other developmental control genes that may operate directly at the gene control level. This latter group includes the homeo box–containing genes of the fruit fly, which are also necessary for the development of a normal, segmented body pattern in the insect. The homeo box is a conserved DNA sequence that may enable the products of the genes that contain it to bind to, and thereby regulate, the expression of other genes. Higher organisms, including humans and mice, also have homeo box–containing genes that may participate in developmental regulation.

A number of questions remain to be answered about how the *sev* protein works. For one, Rubin points out that a tyrosine kinase activity has been inferred for the protein from the sequence data, and has not been directly demonstrated. But he says, "It's as strong an argument as you can make from sequence homology. All the important amino acid residues are present."

How the *sev* gene itself is controlled is another unanswered question. Both the Benzer and Rubin groups have detected messenger RNA transcripts that indicate that the gene is active in the appropriate regions of the developing fruit fly eye at the appropriate time. Whether synthesis of the protein is limited to the R7 precursor cell is currently unclear, however. Benzer and his colleagues have produced an antibody that detects the *sev* protein and may help to resolve this issue.

Two especially interesting questions con-

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cern the identities of the agent recognized by the *sev* protein and of the cellular protein or proteins phosphorylated as a result of its tyrosine kinase activity. Growth and differentiation signals may be received at the cell membrane, but they almost certainly must be transmitted to the genes in the nucleus to produce their effects. The phosphorylation reaction presumably plays an integral role in that signal transfer.

Unfortunately, however, the researchers who have been studying the tyrosine kinases of the EGF receptor and oncogene proteins have been frustrated for years in their attempts to determine which of the cellular proteins phosphorylated by the kinases are critical to signal transfer to the nucleus.

Pinpointing the critical protein targets of the *sev* tyrosine kinase may be more feasible because the fruit fly is much more amenable to genetic analysis than the mammalian cells usually used for the growth factor and oncogene studies. It might be possible, for example, to identify mutants that do not produce the R7 cell because they lack the target proteins for the tyrosine kinase. However, Bishop, who has been studying the fruit fly equivalent of the *src* oncogene, cautions that such genetic studies will not necessarily be easy. The experience of the past few years has nonetheless shown that the riddles of development are beginning to yield. ■

JEAN L. MARX

A Free Electron Laser in the Visible

Researchers from Stanford and TRW use the high-quality electron beam from a superconducting linear accelerator and a novel energy-doubling scheme to make visible light

Most of the action in free electron laser research has come in the infrared and microwave regions of the electromagnetic spectrum. A joint effort by researchers from Stanford University and TRW to push into the visible paid off in February, when the investigators succeeded in generating green light with a wavelength of 5280 angstroms. The group, which includes H. Alan Schwettman and Todd Smith of Stanford and George Neil, John Edighoffer, and Steven Fornaca of TRW, is only the second to make a visible-wavelength free electron laser and the first to make one with an output power high enough for use in biomedical, materials, and other research.

In this day and age, there is nothing unique about a visible laser. Conventional instruments are commercially available that are tailored to have a specific combination of properties. They emit high-power radiation, are tunable over a range of wavelengths, generate ultrashort pulses of light, and so on. The advantage of the free electron laser is that it cuts a wide swath through the multidimensional "parameter" space whose coordinates are these properties, making it a highly flexible light source. In some cases, it may be unique. Visible wavelengths, for example, are a stepping-stone to the ultraviolet, where versatile commercial lasers are rare.

Two years ago, Congress directed the Strategic Defense Initiative Organization to initiate a program aimed both at developing free electron laser technology and at searching for applications in medical and materials research. The program is separate from the much larger free electron laser effort directed toward missile defense.

With a budget of $13.5 million this year, the program is managed by the Office of Naval Research and the Air Force Office of Scientific Research. In addition to the Stanford-TRW project, the program supports a far-infrared free electron laser facility at the University of California at Santa Barbara.

The short wavelength must be accompanied by an increase in electron beam brightness.

The program is also funding the construction of a second independent facility at Stanford, which now has a free electron laser operating in the near-infrared and later will have one in the visible and ultraviolet, and a machine at the National Bureau of Standards that will also cover the near-infrared, visible, and near-ultraviolet. Experimental halls adjacent to all four free electron lasers will give users from other institutions access to the light sources for their research.

Free electron lasers emit their light when a high-energy beam of electrons from an accelerator shoots through a special magnet known variously as an undulator or wiggler because it bends the straight electron beam into a sinusoidal trajectory. The wavelength of the light emitted by the undulating electrons is fixed by the electron beam energy, the magnetic field strength, and the period of the sinusoidal trajectory. In particular, it varies approximately as the inverse square of the beam energy. So, the first requirement for a visible free electron laser is an accelerator of sufficiently high energy.

With typical values of the undulator period and magnetic field, the required energy turns out to be something above 100 million electron volts (MeV). The venerable superconducting linear accelerator at Stanford can boost electrons to 60 MeV and has been used extensively to test free electron laser physics in the near-infrared at 1.5 micrometers. The Stanford-TRW group attacked the problem by developing a beam-recirculation system to nearly double the energy. As explained by Smith, a pulse of electrons enters the linear accelerator from an injector accelerator at an energy of 5 MeV. On emerging from the linac at 60 MeV, the pulse is guided by magnets through a beam pipe back to the front end of the linac. After the second pass, the pulse has an energy of 115 MeV, sufficient to generate green light in the undulator built at TRW.

For some applications, especially those requiring the generation of high-power laser light, the fraction of the electron beam energy converted to optical energy (efficiency) is a major factor in the economics of the free electron laser. Researchers at the Los Alamos National Laboratory demonstrated last year that it is possible to recover a large part of the energy from a pulse of electrons after it has passed through an undulator by decelerating the pulse. The recovered energy is then transferred through a waveguide to the radio-frequency cavities of the accelerator for use on a subsequent pulse. The free electron laser at Santa Barbara operates routinely in a similar mode with an electrostatic accelerator.

The Stanford-TRW group has used its beam-recirculating system to accomplish the same end by recovering the energy directly in the radio-frequency cavities. Whether a pulse is accelerated or decelerated in the linac depends on the timing (phase) of the pulse relative to the radio-frequency electric field in the cavities that drive the linac. By adjusting the pathlength of the recirculated