Cell Growth Control Comes Under Scrutiny

Some 800 researchers gathered in Keystone, Colorado, from 24 to 30 January for a pair of symposia devoted to the topic of cell growth control. One of them, entitled "Growth Factors and Their Receptors: Genetic Control and Rational Application," focused primarily on those genes and agents that stimulate cell division. The second symposium, on "Growth Inhibitory and Cytotoxic Polypeptides," looked at the inhibitory side of the growth control coin. But the ease and rapidity with which the attendees shuttled back and forth between the two meetings illustrated how entwined the two topics are.

Still Another Growth Factor Family

Sometimes growth factor families seem to proliferate as rapidly as cells do. A presentation at the growth factor symposium in which Rik Derynck of Genentech, Inc., in South San Francisco described the cloning of a gene for a protein called MGSA, for "melanoma growth stimulatory activity," provides a case in point. The work points to the identification of still another family of proteins that regulate cell division. As is the case with many other such regulatory proteins, the members of the family display a range of effects, including promotion of inflammatory responses and perhaps inhibiting as well as stimulating cell division.

Ann Richmond and her colleagues at Emory University and the Veterans Administration Medical Center in Atlanta had originally identified MGSA in 1981 in the fluid used to grow melanoma cells in culture. The cells secrete the protein, which then stimulates their growth. The protein is also made by several additional types of cells that proliferate abnormally, Richmond says, including cells of lung, kidney, prostate, and skin cancers.

After several tedious years of trying to isolate MGSA, the Emory group, in collaboration with Joachim Spiess at the Salk Institute in La Jolla, obtained enough of the pure material by last spring to determine a partial amino acid sequence. This sequence provided the information needed for cloning the gene, which Richmond did during the summer in collaboration with Derynck. When the researchers then determined the nucleotide sequence of the gene, they made an unexpected discovery. "The surprise," Derynck says, "was that the sequence is identical to another sequence that was published by Ruth Sager."

Sager and her colleagues at Harvard's Dana-Farber Cancer Institute identified the gene in question, which they call *gro* (for growth regulated), in a tumor-producing line of Chinese hamster cells. In these tumorigenic cells, large quantities of the *gro* protein are made all the time. Nontumorigenic cells of the same type, however, make lesser amounts of the *gro* product and then only when the cells are stimulated to divide.

In addition, the MGSA protein closely resembles the product of the KC gene, which was cloned and sequenced by Charles Stiles, also of the Dana-Farber Cancer Institute, and his colleagues. The KC gene is one of a group of "early response" genes that are turned on rapidly—within minutes—after cells are stimulated by growth factors. The products of the early response genes may bring about the changes that are necessary for cell division. The current results imply that the MGSA/*gro* protein may have a similar role, at least in some types of cells.

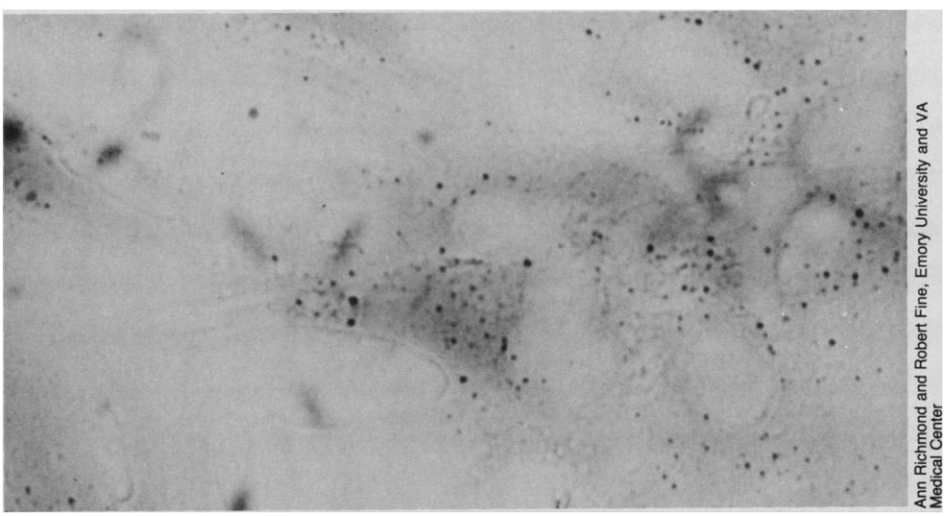
In others, according to Sager, the *gro* protein may inhibit growth. She and her colleagues find that expression of the *gro* gene is lower in some breast cancer cells than in normal breast epithelial cells. There are precedents for growth control proteins—transforming growth factor-β is one—that are stimulatory in some contexts, but inhibitory in others. "We have some players in the game that can yin today and yang tomorrow," as Sager puts it.

The MGSA/*gro* and KC genes are not the only members of the new gene family. Several proteins that participate in inflammatory responses, including platelet factor 4, connective tissue activating protein III, and γ-interferon inducible protein 10, are also structurally similar to the MGSA and KC proteins, although the resemblance is not as great as that between MGSA and KC themselves. Moreover, Uta Francke of Yale University School of Medicine has found that the MGSA gene maps to human chromosome 4, in the same region as the genes for some of the inflammatory peptides. This finding is consistent with the hypothesis that the various genes may have evolved by duplication of a common ancestral gene.

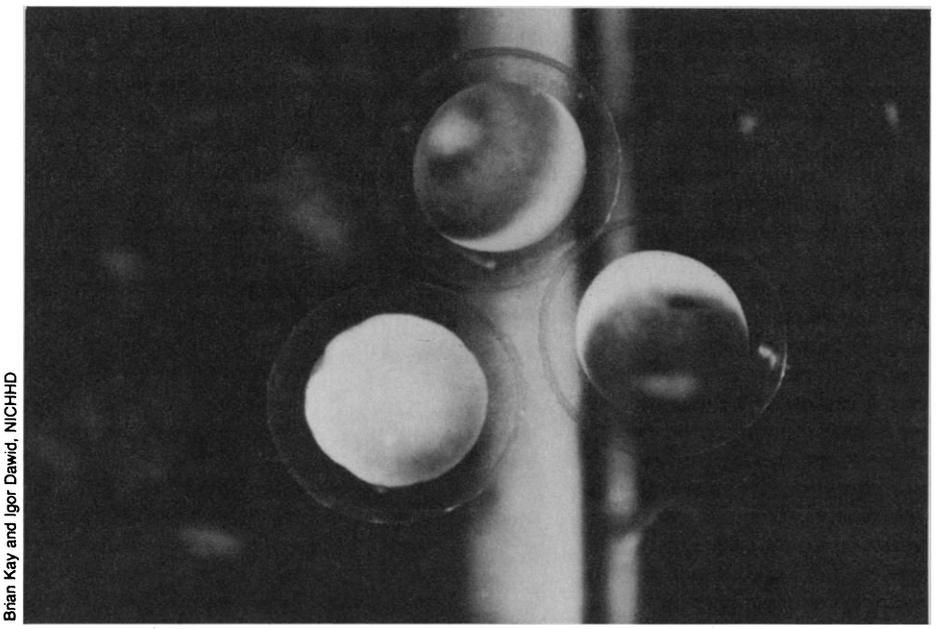
Researchers often find that growth-regulating activities go hand-in-hand with inflammatory properties. This is true, for example, for members of the transforming growth factor-β and fibroblast growth factor families, tumor necrosis factor, and certain interleukins. The family that includes the MGSA/*gro* and KC genes can now be added to this illustrious and probably still incomplete list.

TGF-β Family Linked to Development

The body makes very efficient use of its regulatory motifs. Researchers frequently find that the same agents that control cell growth in the mature organism are also active during embryonic development. For example, at the growth inhibition meeting



MGSA in a basal cell carcinoma. *In this view of cells from a basal cell carcinoma of the skin, the dark granules and dark staining in the cell cytoplasm indicate the presence of MGSA, which was detected with an antibody.*



Brian Kay and Igor Dawid, NICHHD

Xenopus eggs. *Nature has aided developmental researchers by giving a dark coloration to the animal pole of* Xenopus *eggs and a light coloration to the vegetal pole.*

David Tannahill of Harvard University described the identification by researchers in Douglas Melton's group of a possible "cytoplasmic determinant" in frog eggs. This molecule has turned out to be related to transforming growth factor-β (TGF-β), a protein that is already well known as a regulator of cell growth and inflammation.

Cytoplasmic determinants are egg molecules that, after an egg is fertilized and begins to divide, influence the developmental fates of the embryonic cells. A critical, early stage in development is formation of the three germ layers, known as the endoderm, the ectoderm, and the mesoderm, from which the specific tissues of the organism later differentiate. The TGF-β relative identified by Melton, Daniel Weeks, and their colleagues at Harvard may be a signal for inducing mesoderm formation in embryos of the frog *Xenopus laevis*.

In *Xenopus* eggs, the ectodermal cells form at the dark-colored "animal pole" and the endodermal cells form at the light-colored vegetal pole. The mesoderm then develops between them, apparently because the endodermal cells produce a signal that induces ectodermal cells to differentiate into mesodermal cells. Although the Harvard workers have not definitively proved that the TGF-β relative, which they call Vg1 (for vegetal pole 1), is a mesoderm-inducing factor, it has characteristics consistent with that role.

The results of the Melton group indicate that the TGF-β relative is made at the appropriate site, the cells of the vegetal pole of the egg, and at the right time in development for mesoderm induction. Moreover, the similarity to TGF-β is an encouraging finding as other investigators have implicated TGF-β activity in that event.

David Kimelman and Marc Kirschner of the University of California, San Francisco, have found that mammalian TGF-β itself potentiates the effects of another growth factor, fibroblast growth factor, in inducing mesoderm formation in *Xenopus* embryos. Anita Roberts and Michael Sporn of the National Cancer Institute (NCI) in collaboration with Igor Dawid of the National Institute of Child Health and Human Development also have recent evidence indicating that a TGF-β–like molecule participates in mesoderm induction in *Xenopus*.

Not only may members of the TGF-β family be involved in early development, but there is also evidence that they contribute in the later stages. For example, Richard Cate and his colleagues at Biogen Research Corporation in Cambridge, Massachusetts have found that Müllerian inhibitory substance, which causes the regression of female reproductive structures in genetic males, is structurally related to TGF-β.

Lastly, Roberts, Sporn, and their coworkers have detected high levels of expression of the TGF-β gene in mouse embryos, principally in tissues of mesodermal origin, including cartilage, bone, and connective tissue. The activity of the gene is greatest when those tissues are undergoing remodeling, as during limb bud and heart valve formation.

TGF-β has a number of specific biochemical actions that foster the formation of collagen and connective tissue during inflammation and wound-healing. Possibly those same actions are deployed during developmental remodeling.

Two Receptors Identified for PDGF

Evidence presented at the growth factor symposium has complicated life for researchers who are studying the action of platelet-derived growth factor (PDGF). Charles Hart of ZymoGenetics, Inc., in Seattle, and, independently, Carl-Henrik Heldin of the Ludwig Institute for Cancer Research in Uppsala, Sweden, have now found that there are two varieties of the cell surface receptor to which the growth factor must bind to produce its effects.

The discovery that there are two receptors for the growth factor helps to explain why previous examinations of PDGF's effects often produced results that were hard to reproduce. In fact, Hart began a systematic survey of PDGF receptor properties because of his own difficulties in obtaining consistent results when studying the activities of the growth factor.

"In the past, people have thought that there was only a single PDGF receptor," Hart says. "This was sort of naïve because there are three forms of PDGF." Two genes encode PDGF proteins, which are designated "A" and "B" and have different, although related, structures. A given molecule of the growth factor may contain either two A proteins, two B proteins, or an A plus a B. Different cells make the three PDGFs in different proportions. The AA form may predominate in some, for example, or the BB form in others.

By using pure preparations of either the AA, BB, or AB types of PDGF, Hart and his colleagues showed that there is one receptor that can recognize all three PDGF varieties and a second that binds only the BB variety. Heldin's findings are similar.

Although cells generally can make both versions of PDGF receptor, the ratios of the two may vary widely from one cell type to another, Hart says. Usually the BB receptor predominates, however. Considering that both the composition of PDGF preparations and the receptor content of the cells used for assaying the growth factor's activities may vary, it is no wonder that researchers have on occasion experienced difficulty in reproducing results.

A great deal still remains to be sorted out concerning the PDGF receptors. Although Hart expects that the existence of two receptor forms is biologically important and that they have different functions, this has not been demonstrated. Also unknown is whether the two forms of PDGF receptor are encoded by the same gene or different genes. The tools are now available for answering these questions, however. ■

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