Innovations

Unforeseen developments

Ontogeny, Inc.

For pharmaceutical companies the word 'development' is no longer just a suffix to the words 'research and'. A small but vociferous group of companies, including Ontogeny, Inc. of Cambridge, Massachusetts, has been promoting the joys of fruit flies, worms and mice, and the proteins that drive their development.

"The focus [for Ontogeny] is to identify the key molecules that are involved in building up the body, and to use them to reactivate the same processes in the adult," says Doros Platika, President and CEO of Ontogeny. Platika is convinced that many remodeling processes — which could potentially cure degenerative diseases — are merely dormant, waiting for the correct inducing signal. One example he gives is angiogenesis: when a clot blocks one of the heart's blood vessels, the body cannot reconstruct a new vessel, and yet a tumor producing the right factors can coax blood vessels to grow in its direction.

Hedgehogs as pharmaceuticals

The key proteins for Ontogeny are the three hedgehogs: Sonic (Shh), Indian (Ihh), and Desert (Dhh). Hedgehog (Hh) was first discovered in the fruit fly, as a molecule that defines the front and back of body segments. The discovery of the three mammalian genes was announced in 1993 by Philip Ingham, then at the Imperial Cancer Research Fund in Oxford, and Andrew McMahon of Harvard University. (Both scientists are now members of Ontogeny's scientific advisory board.)

If expressed appropriately the three proteins can substitute for each other, but, in wild-type animals, their distinct distributions result in unique activities. Shh controls the polarity of limb growth, directs the development of neurons in the ventral neural tube, and patterns the somites. Ihh controls one type of bone development, and Dhh is necessary for spermiogenesis.

The sound of nerve regrowth

With the help of Biogen, Inc. (Cambridge, MA), Ontogeny hopes to use Shh as a treatment for various degenerative disorders of the nervous system. The aim is to restore the lost neurons, whether it be basal forebrain neurons (Alzheimer's disease), motor neurons (amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease), or dopaminergic neurons of the substantia nigra (Parkinson's disease).

"[Shh] is important enough that it justifies going for it in a major way," says Ali Hemmati-Brivanlou of Rockefeller University. He believes, however, that timing may be critical. Tissues that respond to molecules like Shh in the embryo will not always respond in the adult.

Ontogeny is off to a promising start with Parkinson's disease. They recently reported that Shh promotes the survival of cultured dopaminergic neurons and neurons that produce the inhibitory neurotransmitter GABA. The latter neurons could keep the substantia nigra neurologically quiet while it is rebuilding itself, further protecting the critical dopaminergic neurons.

At least in a culture dish, Shh prevents the death of dopaminergic neurons after application of MPP^+, a highly specific neurotoxin. The designer drug MPP^+ — a distant relative of speed — causes a temporary high by killing dopaminergic neurons and so releasing the neurotransmitter dopamine. But as soon as a day later, severe Parkinson's disease sets in.

Moving from the laboratory to the clinic will not be easy. "Any drug that addresses the brain has a distribution problem right off the bat," admits Platika, and for Shh the only solution at present is holes in the skull. Ontogeny is proceeding with Shh animal trials, using direct injection into the brain, and a decision on human trials will be made in the first half of 1998.

Amgen: the company to emulate

Amgen, Inc. (Thousand Oaks, CA) is already involved in clinical trials of glial cell line-derived neurotrophic factor (GDNF) for Parkinson's. GDNF restores motor performance in animal models of the disease.

The economic leader in developmental inducing molecules, Amgen owns two molecules that between them generate ~$2 billion in revenue per year. Epogen (erythropoietin) generates red blood cells in the face of renal failure, and Neupogen (granulocyte-colony stimulating factor) stimulates the production of blood-clotting neutrophils after chemotherapy.

Regeneron Pharmaceuticals (Tarrytown, NY) is working with Amgen to test neurotrophin-3 for diabetic neuropathy, and Genentech (South San Francisco, CA) is trying out nerve growth factor for the same application. Regeneron has stopped trials of ciliary neurotrophic factor (CNTF) and subcutaneous brain-derived neurotrophic factor (BDNF), although trials continue for the injection of BDNF into the central nervous system of ALS patients.

Boning up on fracture repair

Lots of people break bones, and lots of them do it in such spectacular ways that more than a plaster cast is needed. Ihh is a molecule that could give regrowing bone a helping hand.

Bones grow by one of two mechanisms. Membranous bones such as the skull and clavicle form when fibroblasts develop directly into osteoblasts. In endochondral ossification, however, the cells that are the precursors of long bones first develop into cartilage.

Innovations is a monthly column that surveys the science done at biotechnology companies.

Last year, Clifford Tabin (of Harvard Medical School and the Ontogeny scientific advisory board) reported that Ihh keeps endochondral bone precursors in a proliferating, undifferentiated state. When differentiation occurs too early there is a loss of bone mass. "When you knock out *Ihh*," says Platika, "you get a pretty nicely formed skeleton but in miniature. It's the bonsai of skeletons."

After a fracture, the healing of membranous bones and endochondral bones is identical. But broken bones that are immobilized and closely apposed heal by the membranous route, whereas mobile or separated bone fragments rejoin by endochondral growth. Tabin believes that Ihh could aid both forms of repair. Ihh turns on the production of several of the bone morphogenetic proteins (BMPs), which can directly create ectopic bone. And Ihh could be used to delay the premature differentiation that often occurs in fractures that cannot be completely set, thus encouraging full closure of the gap between bones.

Beware of false bone healers

The BMPs are not only the mediators of the Ihh signal, but also competitors for the same pharmaceutical niche. Genetics Institute (Cambridge, MA) and Creative Biomolecules (Hopkinton, MA) have begun clinical trials with BMP-2 and osteogenic protein-1 (OP-1 or BMP-7), respectively. Starting from behind, Ihh will need to show a clear clinical advantage over the BMPs to warrant further development.

"The burden of proof is still on us," admits Platika. But he hopes that Ihh, as master controller, will prove superior. "By stimulating at the top of the cascade — by pushing down the first domino — everything happens at the appropriate time in the appropriate way," he says. Rik Derynck, a growth-factor researcher at the University of California, San Francisco, says that the pathway's complexity may make such considerations irrelevant. "It's a little bit like a Swiss watch," he says. "If you turn one thing everything will turn."

The complexity of bone formation makes the success of Ihh hard to predict. Monetary support is, however, now in place following a deal with Boehringer Mannheim.

A reproductive desert

Harvard's McMahon reported in *Current Biology* last year that Dhh is produced by the Sertoli cells in the testes and is required for the maturation of sperm. This has sparked Ontogeny's interest, and Platika predicts that both Dhh (as a fertility drug) and an inhibitor of Dhh (as a contraceptive) could be useful pharmaceuticals. This project is at an early stage: Platika does not know, for example, if inhibition of Dhh starting in the adult would block sperm production.

Other early-stage projects include the transplantation of pancreatic precursor cells to treat diabetes, and alteration of elements downstream of Hh to target basal cell carcinoma (BCC). A non-metastatic but potentially disfiguring skin cancer, BCC is the most common cancer known, and its incidence is rising from increased sun exposure. Current treatment involves surgery. Ontogeny has the rights to treatments that boost the activity of Patched, the Hh receptor defective in BCC, but a more logical treatment may be inhibitors of Smoothened, the protein downstream of Patched that keeps the Hh pathway turned off. Rights to the latter treatments have not been resolved.

Ontogeny is seeking to decrease its reliance on the hedgehog proteins with OntoScreen, a series of assays that determine the level, timing and location of the expression of genes. Genes that show very specific expression patterns are investigated for possible roles in development.

An excellent evolutionary approach

One company that is young and yet not wedded to any one set of protein targets is Exelixis, Inc., which has recently moved to South San Francisco, CA. "I think Exelixis is going to be one of the best biotech companies," says Hemmati-Brivanlou. "For the first time a company has adopted an academic approach and is making use of evolution."

Exelixis uses developmental model organisms — fruit flies, worms, mice and frogs — to flesh out important cellular pathways. For example, a transgenic fly expressing the human oncogene *ras* in the eye has deformed eyes. Fly mutations that increase or decrease this effect can be easily identified and are often in genes that are part of the *ras* pathway. The aim is to identify proteins, such as enzymes and G-protein-coupled receptors, that are better targets for chemical inhibitors.

Exelixis will pursue multiple targets simultaneously, developing these projects to the point that animal and clinical trials can begin before selling the project to a pharmaceutical company. The starting points for mutation screens include the presenilins (Alzheimer's), glutamate receptors (excitatory neurotoxicity), the cell cycle targets p21, E2F and APC (cancer), the insulin and leptin receptors (diabetes and obesity), Notch (stem cell development for transplants), and netrins (nerve cell regeneration). With the speed of working with model organisms, says Geoffrey Duyk, Chief Scientific Officer, "you can get a lot of things started with relatively few people."

Distortions in a fly eye are a giant leap from earlier work on inducing molecules. Studying the experimentally accessible and clinically relevant blood system was an obvious start, but, with these new companies on the warpath, any interesting protein is fair game.

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