covered the carcass of an endangered white-tailed eagle, and they are trying to save another sick eagle. Prospects may be bleak for the area's 400 protected otters, says Szilagyi: "We don't see as many footprints and other signs that they are gathering at the usual places."

Downriver, in Szeged, residents are wondering if the annual appearance of "Tisza flowers"—colorful mayflies that briefly cover the river—will take place at all this spring. And in Szolnok, scientists are analyzing river samples to find out how much pollution remains and how many organisms have survived—or how few. "There isn't much life in the river right now," says microbiologist Eniko Szilagyi, who has examined poisoned plankton.

Damage control. In his office next to the palatial Hungarian Academy of Sciences in Budapest, Lang points to a map showing the tributaries of Hungary's rivers—with more than 90% of that water coming from Romania, Ukraine, Slovakia, and Austria. A thicket of mines, chemical plants, oil refineries, and other sources of pollutants line those tributaries. "The management of environmental security cannot be stopped at the borders," he says. The Hungarian government has filed lawsuits seeking monetary damages against the operators of the Baia Mare gold-extraction lagoon, and it has threatened to sue the Romanian government to help recover the cleanup costs. The accident has stoked bilateral tensions: Romanian officials accuse the Hungarian side of exaggerating the extent of the damage, while Hungarians assert that the Romanians are downplaying the spill.

The accident has also prompted soul-searching within Hungary. The government's top science official, physicist Jozsef Palinkas, told *Science* that he was unhappy with his country's procedures for dealing with such emergencies. He will try to convince the government to "develop an early-warning system for detecting and dealing with environmental disasters." But the World Wildlife Fund (WWF) and a dozen other groups that have banded together to form the "Tisza Platform" want a wider system and more enforceable international agreements on pollution. The WWF is also calling for emergency efforts to clean up the upper Tisza and Somes rivers and the surrounding watershed, it says, "because they will form the base from which the reintroduction of aquatic life in the contaminated waterways will start."

It may take months before Hungarian officials decide on a course of remediation, which could include seeding the river with pollutant-eating microbes. In the meantime, says hydrobiologist Oszkar Balazs, the algae and plankton that survived, as well as organisms that flow in from unaffected tributaries, will help breath new life into the Tisza. Tibor Müller, who heads the Hortobagy Fish Farm, thinks the river will heal within 5 years. Others expect a slower recovery. Biodiversity will suffer for decades, predicts the WWF's Gyorgy Gado. However, he says, "life will return to the Tisza, eventually." **–ROBERT KOENIG**

OBESITY RESEARCH

Tracing Leptin's Partners in Regulating Body Weight

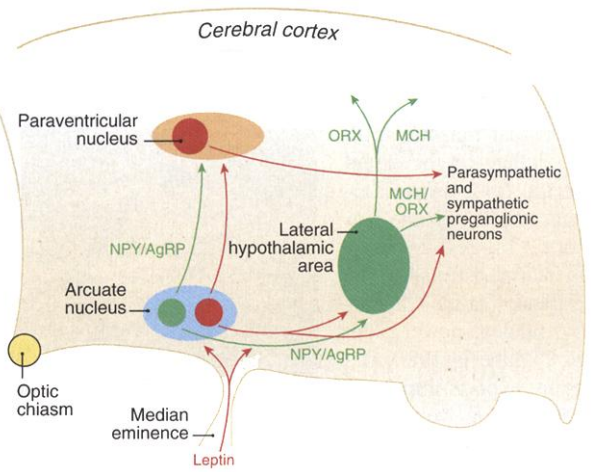
Although the hormone leptin hasn't turned out to be a "magic bullet" for obesity, it has partners in controlling weight—any of which may be antiobesity targets

When Jeffrey Friedman and his colleagues at The Rockefeller University in New York City discovered in 1994 that defects in a hormone called leptin make mice grossly fat, the news brought hope to millions of obese people. Could injections of a natural hormone do what the latest diet couldn't? The biotech firm Amgen Inc. of Thousand Oaks, California, was sufficiently impressed to plunk down $25 million for the rights to leptin, and the hormone seemed headed for the pharmaceutical big time. Since then, leptin's star has—perhaps predictably—fallen. The low point came last year when a clinical trial showed that high doses of leptin produced, at best, modest weight losses in a subset of obese patients. The hormone turned out to be anything but obesity's "magic bullet" (*Science*, 29 October 1999, p. 881).

Is leptin, then, the pharmaceutical equivalent of a fad diet—an overhyped solution to a serious personal and national problem? Not at all, say obesity researchers. Even as the clinical efforts were faltering, basic lab research—much of it stimulated by the discovery of leptin—has been pushing ahead, sketching out a much clearer picture of just how the body controls its weight. And this information is providing many potential new targets for antiobesity drugs. Researchers have identified a wealth of molecules involved in weight regulation, some of which cooperate with leptin to suppress appetite, while others blunt the hormone's fat-busting effects.

Neuroanatomists, meanwhile, are fast on the trail of how and where these molecules work. For the most part, the hot spot is the brain. But leptin also acts on muscle and fat tissue, and a cadre of endocrinologists is teasing out its effects there, too (see sidebar). "Compared to where we were even 3 to 4 years ago, we have an enormous base of new knowledge that we hadn't even a whiff of before," says endocrinologist Jeffrey Flier of Beth Israel Deaconess Medical Center and Harvard Medical School in Boston.

Researchers have been trying to solve the puzzle of what causes obesity for decades. Progress limped along, however, until Friedman's team set the field afire. They reported that mutations in the leptin gene cause hereditary obesity in a long-studied strain of mutant mice. Just 1 year later, Louis Tartaglia's team at Millennium Pharmaceuticals in Cambridge, Massachusetts, and their colleagues at Hoffmann–La Roche in Nutley, New Jersey, hunted down the receptor through which the hormone exerts its weight-suppressing effects. Those discoveries, says Tartaglia, "really provided some genetic entry points into these [obesity]



Wired. Once in the brain, leptin acts on the appetite-enhancing (green) and appetite-inhibiting (red) neurons of the arcuate nucleus. These in turn can influence other brain areas and also the sympathetic and parasympathetic neurons, which communicate with the body periphery.

SOURCE: *NEURON*, 22, 221 (1999)

Is Leptin a 'Thrifty' Hormone in Muscle and Fat?

Most Westerners find it much easier to put on pounds than to take them off—a problem that may have its roots in evolutionary history. While people in today's developed countries have a veritable glut of food, life for our hunter-gatherer ancestors was a constant struggle with famine. Thus, decades ago, researchers proposed a "thrifty genotype" hypothesis, which holds that early on, animals and people evolved mechanisms that allowed them to hoard calories—as fat—in times of abundance in preparation for famine later on. Recent work suggests that the hormone leptin, despite having won media fame for its role as a "fat buster" (see main text), may contribute to this metabolic thriftiness. "Leptin's role may really be as a regulator of the body's response to starvation," says endocrinologist Jeffrey Flier of Beth Israel Deaconess Medical Center and Harvard Medical School in Boston, whose group first proposed the idea back in 1996.

New evidence supporting that proposal comes from endocrinologist Luciano Rossetti and colleagues at the Albert Einstein College of Medicine in New York City. In August, the researchers reported that leptin injections given to rats that had fasted for 5 hours caused the animals to turn down the activity of the leptin gene in fat cells while turning it up from ground zero levels in muscle. The investigators determined this by measuring the cells' content of the messenger RNA (mRNA) that directs leptin synthesis.

That was the first inkling that leptin might play a role in muscle. A clue to what it does there came in a second set of experiments. When Rossetti's group injected leptin into rats on a low-calorie diet, the hormone, surprisingly, had little or no effect on leptin mRNA production by fat cells, but sharply increased its synthesis by muscle cells. Apparently, Rossetti speculates, muscle cells amplify leptin production in times of food deprivation to guide that tissue toward burning fat instead of depleting its protein and carbohydrate stores. Work by Roger Unger's group at the University of Texas Southwestern Medical Center in Dallas and others indicates that Rossetti is on the right track. The Southwestern team found that leptin boosts the activity of nearly all the genes involved in lipid oxidation. "You need to protect the protein and glycogen that composes the muscle fibers so you would be able to undergo fight or flight," Rossetti says.

But when the investigators injected leptin into rats given a high-fat, high-calorie diet, they saw a different picture. Leptin mRNA levels plummeted in fat cells but did not change dramatically in muscle cells. This suggests, Rossetti says, that the leptin produced after a hearty meal curbs its own production in fat cells, apparently so that the animals will eventually eat again and increase their fat stores. At the same time, muscle will likely burn less fat than in the animals on the low-calorie diet with their higher leptin production. "This gives us a hint that constant overfeeding limits the effective leptin response," Rossetti says. He suggests that this tendency to brake leptin's action may sow the seeds of leptin resistance in overweight people.

Whether that's so remains to be seen. "Right now, nobody knows whether this is a trivial biological phenomenon or an important action of leptin," Flier says. The answers will come, he says, from experiments in which rodents are genetically engineered so that their leptin or leptin-receptor genes are knocked out only in specific tissues. That way, the hormone's effects in muscle and fat can be separated from those in brain. **–T.G.**

pathways that biochemical strategies failed to identify."

As other investigators threw their data into the pool, leptin's role in obesity evolved into that of a lipostat: Fat stores rise, and so do levels of leptin, which is manufactured mainly by fat cells. The hormone then signals the brain to eat less and the body to do more. But clinicians soon learned that whereas defects in either leptin or its receptor cause obesity in certain mutant strains of mice and rats, defects in those genes very rarely cause obesity in humans. So far, geneticists have fingered only two individuals with defects in their leptin genes, and none with mutations in the genes encoding the leptin receptor. Indeed, because leptin is churned out in proportion to the size of the fat deposits, many obese people have high levels of the hormone in their bloodstreams. But for some perplexing reason, they fail to respond to it.

Both puzzled and intrigued, researchers figured that they might get at leptin resistance by tracing out the components of the brain pathways through which the hormone exerts its effects. They knew the brain was a likely site of its action because earlier studies in which researchers had destroyed various brain regions of animals had identified at least four areas—mainly in the hypothalamus—involved in appetite control.

Leptin's partners

The first brain molecule found to interact with leptin was neuropeptide Y (NPY), a small protein that had long been known to boost appetite when injected into animals. Studies that involved crossing leptin-deficient mice with mice whose NPY gene had been knocked out showed that some—but not all—of leptin's appetite-dampening effects are due to its inhibition of NPY activity. Since then, researchers have unearthed at least a dozen more molecules that interact with leptin in the brain to control appetite. Perhaps the best studied is a member of the melanocortin family of proteins called α-melanocyte-stimulating hormone (α-MSH).

Although α-MSH is best known for orchestrating the production of brown pigment by skin cells, researchers found that the neuropeptide functions differently in the brain: It blunts appetite. The clue that tipped them off to this new role came from studies of a mutant mouse strain, called *agouti*, that has a striking gold-colored coat and is grossly obese. These mice continuously crank out copious amounts of a protein, also called agouti. The protein, researchers learned, blocks α-MSH's action on both skin cells and in the brain, thus accounting for the animals' obesity as well as their lack of dark pigmentation (*Science*, 7 February 1997, p. 751). The link between α-MSH and leptin came with the finding that leptin-deficient mutant mice make very little α-MSH. The discovery suggests that leptin stimulates α-MSH production, which then turns down appetite.

Other recent work suggests that defects involving α-MSH can lead to obesity in humans. Greg Barsh, a geneticist at Stanford University, estimates that mutations in the gene encoding the brain receptor for α-MSH, a protein called MCR-4, account for 2% to 3% of severe obesity cases, presumably because they prevent the peptide from exerting its appetite-suppressing effects.

In addition, α-MSH is synthesized as part of a precursor protein called POMC (for pro-opiomelanocortin), which is chopped into fragments by a cellular enzyme to produce α-MSH plus several other peptide hormones. Two years ago, a group at Humboldt University in Berlin, Germany, identified mutations in the POMC gene as the cause of a rare human hereditary syndrome featuring severe obesity, red hair, and adrenal insufficiency. "The melanocortins must be exceedingly important, because upsetting any part of that system gives you obesity," says obesity researcher Joel Elmquist of Beth Israel Deaconess Medical Center and Harvard Medical School.

Still, mutations affecting leptin and α-MSH account for only a few percent of all

cases of human obesity, and so researchers are looking at other factors that might be involved. One of them is a molecule linked to obesity by endocrinologist Terry Maratos-Flier's group at the Joslin Diabetes Center and Harvard Medical School.

About the time leptin was discovered, Maratos-Flier was looking for molecules that might contribute to weight control. Using a technique called differential display, she homed in on a neuropeptide called melanin-concentrating hormone (MCH), whose gene is two to three times more active in the brains of *ob/ob* mice—one of the obese leptin-deficient strains—than in normal mice. In another variation on the obesity-pigmentation theme, MCH had originally been discovered as a hormone that lightens the color of fish scales. But Maratos-Flier's finding suggested that increased expression of the MCH gene—possibly in response to the *ob/ob* mouse's leptin deficiency—might also contribute to the animal's obesity.

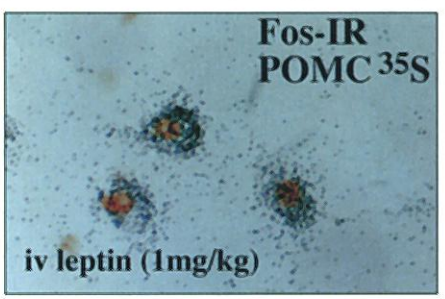
Indeed, when Maratos-Flier and her colleagues injected MCH into the brains of rats, the animals' food consumption shot up as the dose escalated. Conversely, when her group in collaboration with that of her husband, Beth Israel's Jeffrey Flier, knocked out the MCH gene in mice, the resulting animals ate less during the night—normal dinnertime for mice—and ended up 15% to 25% skinnier than their normal counterparts. What's more, the group found that the neurons making the peptide, which are located in the lateral hypothalamus, project to neurons in the cortex region of the brain, including those that orchestrate smell.

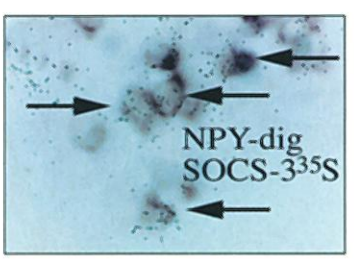
Based on these results, Maratos-Flier proposes that MCH causes what she calls "the pizza effect." When a person is satiated, neuropeptides like MCH are at their lowest levels, she says. But the smell of something tasty might trigger their release. "Even though you are not hungry and you don't need the calories, you still eat the pizza because you know it will taste good," Maratos-Flier explains.

So far, however, researchers have identified no MCH defects that might explain leptin resistance in obese people. But they are exploring other possibilities. One is that leptin may somehow be blocked from entering the brain in obese people. To see if that might be the case, Flier's group at Beth Israel is trying to track down the elusive molecular ferry that ships leptin across the blood-brain barrier. The investigators have found an attractive candidate: a shortened form of the leptin receptor that is presumably not tethered to the cell membrane, because it lacks the peptide segment that would normally hold it in place there.

Last year, Flier's team showed that the cells that form the blood-brain barrier produce higher levels of the mRNA that encodes the shortened form of the receptor, called OBR-A, than any other cells in the body. The researchers have since shown that the protein is necessary to transport leptin across membranes formed by the cells in culture dishes. Whether transport problems spur leptin resistance in people, however, remains to be tested.

Leptin resistance might also stem from molecules that interfere with signaling by the leptin receptor, such as one identified last year by Flier's group, in collaboration with Elmquist's. The researchers found that when they injected leptin into normal animals, the hormone rapidly juiced up production of a protein called SOCS3 (for suppressor of cytokine signaling–3) in cells in the hypothalamus that bear leptin receptors. SOCS3, in turn, bit the hand that fed it by halting the leptin receptor from further signaling. This is presumably part of the normal mechanism for halting leptin signaling when the hormone has done its job. But in addition, Flier says, the finding "raises the possibility that this inhibitor might be mediating the resistance to leptin in obese people." He and his colleagues plan to look for defects in OBR-A and SOCS3 function in obese patients.





Shade of difference. As indicated by the brown staining for the Fos protein, which comes on in active brain neurons, leptin induces activity in neurons that make POMC (blue grains), source of the appetite inhibitor α-MSH. But neurons that make the appetite stimulator NPY (dark stain) respond to the hormone by making SOCS3 (dark grains), an inhibitor of the leptin receptor.

CREDIT: JOEL K. ELMQUIST

Making the connections

With all these new actors coming onto the stage, the most daunting task is to cast them together in one leptin-conducted performance. Among those tackling the problem are Elmquist and his colleagues. They've identified two sets of neurons in one of the brain's feeding hot spots, the arcuate nucleus of the hypothalamus, that respond in opposite ways to leptin stimulation. One population produces appetite-inhibiting peptides such as α-MSH, and this neuron group responds to leptin in the expected fashion—by expressing genes that signal activation. In contrast, the other population makes two appetite-boosting proteins, NPY and agouti-related protein (AgRP), the human equivalent of the mouse agouti protein, and these appear to shut off in response to leptin.

Elmquist attributes the difference to the fact that the neurons that make the appetite-stimulating peptides, but not the others, respond to leptin by producing, among other molecules, the leptin-receptor inhibitor SOCS3. But however it happens, the net result is a profound suppression of appetite. "It's intriguing that the same leptin receptor in the same nucleus could promote two distinct physiologic responses," Elmquist says.

And the neural circuitry reaches even farther. Studies by the Beth Israel team show that both sets of neurons also send projections to the neurons in the lateral hypothalamus that produce the appetite stimulator MCH. The MCH neurons, while probably inhibited by those producing α-MSH and perhaps stimulated by the NPY/AgRP neurons, also project into smell centers in the cerebral cortex and other nervous system regions that are responsible for complex behavior including feeding.

In addition, the arcuate neurons are wired to another population of neurons in the lateral hypothalamus that spew out potent peptides that are called orexins, because they stimulate appetite. The orexins have been linked to arousal in mice, dogs, and people, and defects in such peptides cause a narcolepsy-like state. "These [MCH and orexin] neurons are really in a powerful position to regulate broad areas of the central nervous system," Elmquist says. And at the center of it all sits leptin, regulating both appetite and feeding in the brain and possibly activity and calorie burning in the body.

Obesity researchers admit that they still have a lot of work to do before they trace out all the molecules involved in weight control. But they already have a number of promising targets for antiobesity drugs. For example, if SOCS3 does down-regulate leptin-receptor activity, SOCS3 inhibitors might heighten leptin signaling and help curb appetite. And MCH has already caught the eyes of several pharmaceutical companies, which are scrambling to find small molecules that block its action.

Indeed, obesity researchers can expect a glut of research leads to feast on. "There is so much data, it is turning out to be a remarkable story," Elmquist says. **–TRISHA GURA**

Trisha Gura is a writer in Cleveland, Ohio.