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Commentary

Gatekeepers of organ growth

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As morphologically complex animals approach adulthood, how is it that each organ knows how to arrive at its appropriate size? This question has prompted relatively limited experimental inquiry during the molecular age of biological research. It did, however, spark considerable interest 30–40 years ago. Perhaps the most intriguing and controversial research on this topic came from scientists studying organ regeneration in rodents and putative substances claimed to be capable of selective control of organ growth.

When a significant part of the liver of a rat is removed, the remaining tissue is quickly triggered to regenerate via mitotic cell growth. The same process can happen with the kidney and certain other organs. Hepatectomized rats somehow know that their liver-to-body ratio is abnormally low and respond by the selective regeneration of just the right organ. Research carried out in the 1950s and 1960s, championed by W. S. Bullough (1, 2), sought to explore the phenomenon of balanced organ growth from an experimental perspective. Rat liver tissue was dissected, homogenized, and fractionated to separate soluble components from tissue debris. When injected into hepatectomized rats, this liver solute was observed to impede regeneration. Similar experiments carried out with kidney homogenates were reported to impede kidney regeneration. Remarkably, however, liver homogenates failed to block kidney regeneration and kidney homogenates could not block liver regeneration.

Based upon these findings Bullough hypothesized the existence of soluble factors whose job it was to control organ size. Such factors were dubbed "chalones" (Greek, to make slack), owing to the proposal by Shafer (3) that this term was preferable to "hormone" for the description of chemical messengers that function by a depressant mode of action. The idea was that a mature organ should produce, at its growth apex, a substance whose job it is to specifically limit further growth of its cognate tissue.

The assays required for biochemical purification of these hypothetical substances were obviously difficult. Research on chalones failed to progress, was maligned, and eventually forgotten by modern biologists. However, to the rescue, last spring tabloids hit the press describing "supermice," laboratory mice that display twice the normal amount of muscle mass. These animals were the outcome of studies by Alexandra McPherron and Se-Jin Lee of Johns Hopkins University Medical School, two of the last keepers of the chalone talisman.

Although all previous work on chalones may have been bogus, the concept provided McPherron and Lee with invaluable perspective. Using modern methods of gene cloning, Lee and colleagues identified a large family of genes encoding proteins designated growth and differentiation factors (GDFs). These proteins resemble transforming growth factor β (TGF-β) in primary amino acid sequence and encode secreted, disulfide-bonded polypeptides. Because TGF-β is known to display growth inhibitory properties (4), it was reasonable to hypothesize that the related GDFs might share this biological activity. Unlike TGF-β, however, certain of Lee's GDFs are expressed in a highly tissue-restricted manner. For example, the first to be studied by targeted gene disruption, GDF8, was known to be expressed selectively in skeletal muscle. Lo and behold, GDF8-deficient mice are endowed with musculature admirable even to Hollywood's most notable (5). The product of GDF8, now termed myostatin, can thereby be interpreted to function as a selectively governor of skeletal muscle mass in adult mice. In its absence, skeletal muscle mass expands far beyond its normal bounds.

Before returning to the chalone–myostatin connection, another flash of the Paparazzi cameras warrants summarization. Published in this issue of the *Proceedings*, McPherron and Lee (6) now recognize that cattle breeders beat reverse geneticists to the task of deriving myostatin-deficient animals. Two breeds of "double-muscle" cattle, Belgian Blue and Piedmontese, are herein shown to suffer inactivating mutations in the bovine gene encoding myostatin (6). Results similar to the findings of Lee were recently reported by Georges and colleagues (7), who pursued the double-muscle gene and phenotype via a forward genetic strategy. So widespread was the news of "supermice"—and so simple the concept—that a Montana rancher asked me in the spring of 1996 whether there might be a connection between supermice and beef production (tabloids do reach the grocery stores of even the most rural areas of America). Little did I know that skilled cattleman had observed the bovine phenotype and selected it for breeding decades ago, and that geneticists had been chasing down the relevant gene since the early 1980s!

How do GDFs work? On the more trivial, molecular side, we can safely assume that they will operate via cell surface receptors and trigger signaling cascades that ultimately control cell proliferation. Considering myostatin as an example, it can be predicted that the simplest regulatory loop will involve muscle-restricted expression of both myostatin (an observation that has already been well established) and its cognate receptor. Assuming that these predictions prove to be correct, a number of more perplexing questions arise. First, how is the system balanced? As an animal develops through embryogenesis and from adolescence toward adulthood, is there a slow and graded rise in the production of myostatin relative to body mass, such that peak levels and resulting homeostasis are reached only in the mature adult? Alternatively, saltatory production of myostatin, its receptor, or substances required for their function might account for the implementation of organ-specific growth regulation at the apex of development. These issues further pique the question of whether GDFs will operate systemically or locally? The early work on chalones might be interpreted to reflect systemic action. Indeed, one of the early chalone experiments purported to show that injection of plasma into an animal that had been hepatectomized could block the regenerative response, but that plasma taken from an animal that had itself been hepatectomized was not inhibitory. Taken at face value, these experiments suggest that the hepatic inhibitory factor of the rat circulates and that its levels drop after hepatectomy, perhaps due to the fact that its source had been resected. More recent and reputable work on organ transplants, reviewed by J. M. W. Slack in the context of Lee's

myostatin discovery (8), might also be interpreted to reflect the function of tissue-specific growth regulators that operate systemically. It should now be possible to address these issues by use of appropriate molecular reagents (antibodies, etc.) and experimental manipulations (transbiosis, organ transplants, etc.).

As final considerations, how widespread will the myostatin phenomenon be and what therapeutic implications might this work point toward? Regarding the first of these questions, Lee and colleagues (9–11) have discovered genes encoding numerous additional GDFs, many of which are expressed in a restricted distribution of adult mouse tissues. It is thus possible that the work on myostatin will prove to be a generally applicable paradigm describing how vertebrate organs know when they have reached their appropriate size. As to therapeutic potential, two ideas come to mind. If tumor cells derived from a specific tissue retained the receptor to the cognate GDF of the parent tissue, the relevant "statin" (GDF) might prove selectively useful in the containment of tumor growth. Second, antagonists of GDF action, such as antibodies or mutated GDF variants that could bind their cognate receptor without triggering biological response, might provide a means of facilitating organ growth (regeneration) in diseased patients suffering the wasting of specific organs. Might, for example, an antagonist of myostatin prove useful for the regeneration of muscle mass in patients suffering from muscular dystrophy?

Although chalones and the problem of tissue homeostasis skipped a scientific generation, it is safe to predict that this field will attract new-found attention in both academic and industrial research laboratories.

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