emerge as the regulation of this cytoskeletal structure is unraveled.

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Selected Reading

RhoGAP: The Next Big Thing for Small Mice?

The size of an organism is determined by the number and size of its constituent cells. The insulin/IGF-1 signaling systems have been long recognized to play a critical role in the determination of body size. Now the generation of mice deficient for a RhoGAP suggests that this small G protein might also regulate the growth of animals.

In a time marked by impressive advances in the elucidation of both principles and details governing development, one of the most poorly understood processes is the determination of the mature size of organs and organisms. While we all accept the consistency by which human arms are bilaterally roughly equal in size and smaller than legs and that humans are invariably larger than mice, the molecular mechanisms that ensure the fidelity of these phenomena are only beginning to be understood. Thus, the report by Sordella et al. in this issue of Developmental Cell, pointing to a surprising pathway that regulates cell size, represents a potentially important contribution to the solution of this problem (Sordella et al., 2002).

Three fundamental processes principally regulate biological mass: cell proliferation, death, and growth (used here to indicate the determination of cell size irrespective of rates of division). Whereas the first two have received substantial attention in recent decades, only in the last several years have scientists begun to address the problem of regulation of cell growth. Perhaps the relatively constant size of most cells has caused many of us to forget that proliferation must be accompanied by a commensurate, perfectly matched increase in growth, or over time cell size will drift. The simplistic but rather tempting view that growth always follows passively as a direct result of proliferation has never fit the data particularly well, but only recently has it been refuted using modern cellular and genetic strategies (Day and Lawrence, 2000). Moreover, there are so many examples in which growth and proliferation are physiologically uncoupled that one is forced to at least consider the possibility that these two processes might generally be regulated independently. For example, reductive cleavages in most early embryos represent cell division without growth, whereas the opposite occurs in a hypertrophic muscle cell or a megakaryocyte, the giant bone marrow cell that sheds platelets. Interestingly, the latter cell represents an example of the rule that DNA content directly correlates with cell size, as it is the repeated rounds of DNA synthesis unaccompanied by cell division that accounts for the bulk of the megakaryocyte.

Another principle guiding the determination of organ size is that alterations in proliferation are compensated by inverse changes in cell size, such that the volume of the compartment remains unchanged. Thus, we are presented with an intriguing paradox: while the major factor responsible for differences in size among organisms is the number rather the size of the constituent cells, the primary determinant of organ size within an organism is the rate of growth and not proliferation.

Over the past several years, a number of lines of investigation have converged on the evolutionarily conserved insulin/IGF-1 signaling pathway as a critical regulator of cell growth (Day and Lawrence, 2000). Some of the most informative data derive from genetic experiments in the fruit fly, Drosophila melanogaster, though confirmatory information has begun to emerge in mammals. The critical signaling components include, in addition to the insulin receptor and its substrate chico (the fruit fly homolog of mammalian insulin receptor substrate 1), phosphoinositide 3'-kinase, phosphoinositide-dependent protein kinase 1, Akt/protein kinase B, and S6 protein kinase and PHAS1/4EBP1, though the latter two translational regulators might well be more responsive to nutritional cues than insulin. Suggesting a surprisingly linear cascade, overexpression of each member of this pathway yields a virtually equivalent phenotype, an increase in compartment size mediated by a disproportionate augmentation in cell growth. The lipid phosphatase PTEN functions as a negative regulator of the pathway.

In the present report, Sordella et al. have generated...
mice deficient for one of the two known 190 kDa Rho GTPase-activating proteins, p190-B RhoGAP (Sordella et al., 2002). Rho is itself a small GTPase capable of influencing a number of important biological processes, most notably organization of the actin cytoskeleton but also vesicular trafficking and transformation. Like all G proteins, Rho is active when associated with GTP, but constantly recycles between this state and the inactive, GDP-bound form. Thus, since p190-B RhoGAP catalyzes the conversion of Rho-GTP to Rho-GDP, it is not unanticipated that cells derived from the null animals demonstrate constitutive activation of Rho. Much more surprising, however, are two conspicuous aspects of the phenotype of the p190-B RhoGAP mice. First, these animals bear a striking resemblance to mice deficient in the transcription factor CREB, which binds to the canonical cyclic AMP response element (Rudolph et al., 1998). More direct evidence of the link between the two knockout models is provided by the decreased phosphorylation at a crucial regulatory serine residue in CREB evident in most tissues from the p190-B RhoGAP null mice. Second, the latter mice are about 30% smaller than their wild-type littermates at birth and die soon thereafter. These data immediately suggest a model in which an important determinant of organismal size is CREB phosphorylation, and that Rho regulates this process. Even more unexpected is the mechanism by which body size is reduced; in virtually all organs examined, there was a decrease in cell size, as demonstrated most convincingly by a reduction in the ratio of protein to DNA (Sordella et al., 2002). One oddity of the phenotype is that, with the exception of the thymus, most organs are proportionally reduced in size, even though a number of tissues extracts do not reveal reduction in phospho-CREB immunoreactivity. These data might be hinting at a presently obscure noncell-autonomous component to regulation of cell growth by p190-B RhoGAP.

What then is the mechanism by which activation of Rho secondary to a loss of p190-B RhoGAP leads to phosphorylation of CREB? Here again, Sordella et al. provide a plausible and tantalizing model. Unlike most protein tyrosine kinase receptors, insulin and IGF-1 do not assemble signaling complexes on the receptor itself, but rely on a family of scaffolding proteins, the insulin receptor substrates (IRS1–4). The hormone-bound receptor phosphorylates IRS on a number of tyrosine residues, which serve to dock key signaling molecules via src homology 2 (SH2) domains present in the latter. Interestingly, phosphorylation of IRS on serine and threonine residues tends to inhibit subsequent tyrosine phosphorylation, and thus downregulate the system. The recent indication that Rho kinase (ROK) can function as an IRS serine/threonine suggests a mechanism linking hyperactivity of Rho to decreased insulin/IGF-1 signaling (Farah et al., 1998) (see Figure). Using fibroblasts derived from p190-B RhoGAP null mice, Sordella et al. show this to be the case, and, moreover, demonstrate that insulin signaling can be rescued by a ROK inhibitor (see Figure). Most of the interest in inhibitory IRS serine/threonine phosphorylation has been driven by the idea that this process is an etiological factor in the insulin resistance of type 2 diabetes mellitus (Birnbaum, 2001); thus, though the p190-B RhoGAP knockout die before they have the opportunity to develop diabetes, it will be interesting to see whether increased Rho activity is associated with insulin resistance in other mouse models or human diabetes.

The data of Sordella et al. present some intriguing questions that should be answerable in the near future. First, the connection between early insulin signaling and CREB phosphorylation remains unclear. Though Sordella et al. show the phosphorylation of several insulin-dependent kinases to be reduced in tissues from the p190-B RhoGAP null mice, Akt/PKB must be regarded as the prime suspect, given its well-established role in the regulation of cell growth in both Drosophila and mammals (Tuttle et al., 2001; Verdu et al., 1999). In fact, mice deficient in Akt1/PKBα are small at birth and remain so throughout life (Cho et al., 2001). Moreover, Akt/PKB has been shown to phosphorylate CREB directly (Du and Montminy, 1998). Much more perplexing is how CREB is affecting cell growth. For example, in the mammalian liver, insulin and cyclic AMP are antagonistic in the regulation of glucose production, so it is difficult to imagine how the same signaling paths could cooperate for cell growth. Last, much more information is needed as to whether reduction in insulin/IGF-1 signaling alone is sufficient to disproportionately reduce cell growth in mammals. Though IGF-1 receptor knockout mouse are small at birth and remain so throughout life (Liu et al., 1993). Whether alternative mechanisms compensate for loss of IGF-1 signaling in this
model, or Rho acts on targets in addition to IRS to suppress growth, the discovery of a role for p190-B RhoGAP in cell size regulation is certain to provoke a wealth of new and informative experimentation.

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