Size matters

Workshop on Growth Control in Development and Disease

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Introduction

A recent workshop entitled Growth Control in Development and Disease brought together around 90 scientists in the beautiful village of Arolla in the Swiss Alps. During this meeting, growth control was discussed from the perspectives of evolution, metabolism, ageing and cancer biology. From formal presentations to poster sessions, and mountain hikes to late-night discussions, the participants indulged in multidisciplinary scientific exchanges that stimulated innovative insights and forged new collaborations. Here, I present a few highlights from the meeting.

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Systemic control of growth by hormones and growth factors

Whereas the growth of single-cell organisms, such as yeast, is mainly regulated by nutrient availability, the growth of higher eukaryotes is dependent on the integration of many cell-extrinsic cues, which include hormones, growth factors and nutrients (Kimball & Jefferson, 2000; Rohde *et al*, 2001). Indeed, the systemic control of metazoan growth by hormones and nutrients is a topic of intense investigation in the growth-control field.

F. Nijhout (Durham, NC, USA) discussed how the final body size of insects is determined by the size at which a larva stops feeding and switches on the pupal fate (Nijhout, 2003). This transition from larval to pupal development requires the decay of juvenile hormone (JH) and secretion of the moulting hormone ecdysone, the production of which, in turn, is regulated by the neurosecretory prothoracicotropic hormone (PTTH). Interestingly, different insects use diverse strategies to control the production of PTTH. In the tobacco hornworm *Manduca sexta*, the secretion of PTTH is induced by the cessation of JH, which, in turn, is tightly associated with the attainment of a well-defined 'critical weight'. In certain species of Hemiptera, such as the milkweed bug *Oncopeltus fasciatus*, PTTH secretion is controlled by abdominal stretch receptors that are activated when the animal reaches a particular size. By contrast, in the beetle *Onthophagus taurus*, PTTH secretion is triggered by the removal of its food supply, which in nature occurs when a larva exhausts the ball of food that is provided by its mother. Although a lot of research is still required to understand the molecular mechanisms that underpin these phenomena, it is reasonable to conclude that knowledge of both neural physiology and behaviour is necessary to understand size control at the organismal level.

Much progress has been made in recent years towards understanding the role of insulin-like molecules in promoting cell growth. Insulin-like molecules are present in worms, insects and mammals, and are part of an evolutionarily conserved signalling pathway that involves the insulin receptor (INR), insulin-receptor substrate (IRS), phosphatidylinositol-3-OH kinase (PI(3)K), phosphoinositide-dependent kinase 1 (PDK1) and the protein kinase AKT (Fig 1). Several presentations focused on the molecular dissection of this pathway. S. Leever (London, UK) discussed a genetic screen to identify new components of the insulin pathway in *Drosophila*. From this screen, she isolated a gene, *pixie*, which

Fig 1 | Cross talk between growth control and ageing pathways. Depicted are the insulin-signalling pathway, the nutrient-sensing TSC/TOR pathway and the caloric restriction-responsive sirtuins pathway. FOXO, forkhead transcription factor; INR, insulin receptor; IRS, insulin-receptor substrate; PDK1, phosphoinositide-dependent kinase 1; PI(3)K, phosphatidylinositol-3-OH kinase; PTEN, phosphatase and tensin homologue; RHEB, RAS homologue enriched in brain; TOR, target of rapamycin; TSC, tuberous-sclerosis complex.

encodes a soluble ATP-binding cassette (ABC) domain protein. Data were presented that implicate Pixie in the general control of protein translation. E. Hafen (Zürich, Switzerland) discussed studies of the *Drosophila* homologue of the forkhead transcription factor FOXO, which in *Caenorhabditis elegans* has been shown to act downstream of AKT in the ageing pathway. FOXO seems to specifically mediate the reduction in cell numbers in insulin-signalling mutants through the transcriptional regulation of target genes, including the translation-initiation regulator 4E-BP (Jünger *et al*, 2003; Fig 1). B. Burgering (Utrecht, the Netherlands) and A. Brunet (Boston, MA, USA) discussed how the FOXO family function as central molecular sensors of the extracellular environment in mammalian cells (Burgering & Kops, 2002; Tran *et al*, 2003). These studies indicate that different patterns of phosphorylation and acetylation of FOXO proteins might specify various cellular responses that range from stress resistance to apoptosis. The co-regulation of FOXO proteins by AKT and protein deacetylases, such as sirtuins (see below), provides an important link between the two main longevity pathways of insulin signalling and caloric restriction (Fig 1). M. White (Boston, MA, USA) discussed the insights that have been obtained from studying mice in which *Irs2* is specifically disrupted in the pancreatic β -cells and the hypothalamus. This mouse model recapitulates many of the classic features of type 2 diabetes, including the development of diabetes and obesity. Restoring *Irs2* expression in the β -cells rescues the diabetic phenotype without reversing the obesity phenotype. Therefore, *Irs2* signalling in the hypothalamus seems to be important for the regulation of food intake.

Regulation of growth by nutrients

It has long been appreciated that nutrient availability is a crucial determinant of body size, but the molecular mechanisms that underlie this phenomenon are only just beginning to be elucidated. Several insulin-like peptides are expressed in *Drosophila* neurosecretory cells in the larval brain in a nutrient-dependent manner, which indicates that nutrients might promote growth through the regulation of insulin production (Ikeya *et al*, 2002). The fat body of *Drosophila*, which is an endocrine organ that is analogous to the mammalian liver, also produces a systemic signal that regulates larval growth in response to nutrients (Britton & Edgar, 1998). P. Leopold (Nice, France) presented molecular evidence for this fat-body-derived systemic signal (Colombani *et al*, 2003). The specific downregulation of amino-acid uptake in the fat body, through the inhibition of an amino-acid transporter called *slimfast*, causes a global growth defect similar to that seen in *Drosophila* that are raised under poor nutrient conditions. In the fat-body cells, *slimfast* regulates the production of a secreted cofactor of insulin-like molecules through a pathway that involves the tuberous-sclerosis complex tumour-suppressor proteins (Tsc1/Tsc2) and target of rapamycin (Tor) (for details of the general TSC/TOR pathway, see Fig 1). This cofactor, which is known as the acid-labile subunit (Als), then acts systemically to control the growth of peripheral tissues through the local regulation of insulin signalling. M. Galloni (Montpellier, France) presented evidence that the larval gut is also involved in the systemic control of growth in *Drosophila*. Mutations of *bonsai*, which is predominantly expressed in the gut, result in the retardation of systemic growth. Therefore, the ability to mediate the systemic control of organismal growth is probably not limited to the neurosecretory cells of the brain and the fat body.

Besides their systemic mode of action, nutrients also exert profound cell-autonomous effects on cell growth. The TOR-signalling pathway has emerged as a central pathway that couples nutrient availability to cellular growth. M. Hall (Basel, Switzerland) and D. Sabatini (Boston, MA, USA) discussed recent progress in the characterization of the TOR-signalling complex (TORC) in yeast (Loewith *et al*, 2002) and mammalian cells (Kim *et al*, 2002), respectively. TOR exists in two distinct functional complexes, with TORC1 mediating the rapamycin-sensitive pathway and TORC2 mediating the rapamycin-insensitive pathway. Hall also presented data indicating that TORC1 might control ribosome biogenesis through the RAS and cyclic AMP pathway in yeast. T. Radimerski (Basel, Switzerland) and D. Pan (Dallas, TX, USA) presented recent studies in *Drosophila* that have implicated Tsc1/Tsc2 and the small GTPase Ras homologue enriched in brain (Rheb) as upstream regulators of Tor (Garami *et al*, 2003; Saucedo *et al*, 2003; Stocker *et al*, 2003; Zhang *et al*, 2003). Collectively, these studies show that the TSC1/TSC2 protein complex functions as a GTPase activating protein (GAP) of RHEB, which, in turn, regulates TOR activity. Pan also presented evidence that during *Drosophila* development, the Tsc/Rheb pathway acts in parallel to, rather than downstream of, the insulin/PI(3)K/Akt pathway.

Insulin signalling, caloric restriction and ageing

The insulin-signalling pathway not only controls the growth of an organism, but also exerts a profound influence on metabolism, reproduction and longevity. Several talks focused on the molecular and physiological aspects of ageing. In *C. elegans*, a decrease in signalling through the insulin receptor DAF-2 results in a DAF-16 (a FOXO orthologue)-dependent increase in longevity. C. Kenyon (San

Francisco, CA, USA) and G. Ruvkun (Boston, MA, USA) discussed recent efforts to identify the transcriptional targets of DAF-16 in *C. elegans* (Lee *et al.*, 2003; Murphy *et al.*, 2003). Collectively, these studies show that DAF-16 functions both as a transcriptional activator and a transcriptional repressor. Downstream targets of DAF-16 include genes that are implicated in diverse cellular processes, such as metabolism, stress response and antimicrobial defence. It seems that individual targets have a relatively minor effect on lifespan and that all DAF-16 targets act additively to extend longevity.

Caloric restriction slows the pace of ageing and increases the maximum lifespan of species as diverse as yeast, worm, flies and mammals. Two presentations at the meeting provided exciting insights into this phenomenon. L. Partridge (London, UK) reported that in *Drosophila*, dietary restriction (DR) prolongs lifespan by reducing the short-term risk of death (Mair *et al.*, 2003). She noted that DR in adult flies of any age generates equal protection from mortality, irrespective of previous dietary history. These results indicate that caloric restriction does not increase longevity by simply reducing the accumulation of cellular damage. However, although it could be inferred from these studies that there is no urgent need to start good eating habits early in life, Partridge cautioned that comparable experiments have yet to be performed in mammals. D. Sinclair (Boston, MA, USA) discussed the discovery of small molecules that mimic the effects of caloric restriction (Howitz *et al.*, 2003). These molecules, including resveratrol—which is a plant polyphenol that is commonly found in red wine—stimulate the enzymatic activity of a family of NAD⁺-dependent protein deacetylases called sirtuins, which, in turn, extend the lifespan of yeast and mammalian cells. Therefore, sirtuin activators might be exploited for the therapeutic intervention of ageing-related human diseases.

Regulation of growth at the tissue level

Studies of the insulin and the TSC/TOR pathways indicate that environmental cues, such as nutrients, are important in determining the final size of an organ. However, there must be intrinsic counting mechanisms that stop growth when an organ reaches its final size. This phenomenon was the subject of many intense discussions at the meeting and how an organ (or organism) stops growing remains an open question.

D. Ingber (Boston, MA, USA) discussed how mechanical forces might be transduced into alterations in cellular biochemistry. He noted that extracellular matrix (ECM)-dependent changes in cell shape affect the decision of a cell to proliferate, differentiate, die or migrate (Ingber, 2002). His presentation reinforced the importance of considering size control at the tissue level and reminded us of the important contribution of the ECM, which is arguably a little-studied aspect in the growth-control field.

During the growth of *Drosophila* imaginal discs, cells compete for limited survival factors. As a consequence, when slow-growing cells are juxtaposed with those that are fast growing, the slow-growing cells are actively eliminated by cell death—a phenomenon that is known as cell competition. L. Johnston (New York, NY, USA) and K. Basler (Zürich, Switzerland) offered molecular insights into this process. They reported that the *myc* oncogene has a key role in cell competition. Elevated Myc levels are sufficient to transform cells into 'super competitors'. Conversely, the manipulation of several other growth pathways, such as insulin/PI(3)K and cyclin D, does not result in altered cell competition. This differential effect is probably owing to the fact that the downstream targets that are activated by Myc are

different to those that are activated by insulin/PI(3)K. B. Edgar (Seattle, WA, USA) has investigated these downstream targets and his studies show that Myc preferentially stimulates ribosome biogenesis.

Although cell competition represents an important mechanism for regulating local cell–cell interactions, this does not seem to account for the 'mass checkpoint' that stops growth when an organ reaches its final size. *Drosophila* genetics might provide a powerful tool to probe the molecular mechanism of this elusive checkpoint. G. Halder (Houston, TX, USA), I. Hariharan (Boston, MA, USA) and Pan reported recent findings that implicate the tumour-suppressor genes *hippo*, *salvador* and *warts* in this process (Harvey *et al.*, 2003; Udan *et al.*, 2003; Wu *et al.*, 2003). The inactivation of any of these genes results in the increased transcription of the cell-cycle regulator *cyclin E* and the cell-death inhibitor *diap1*, which leads to increased proliferation and reduced apoptosis. These tumour suppressors define a new kinase cascade in which Hippo phosphorylates Salvador and Warts, and Salvador potentiates the kinase activity of Hippo towards Warts. By analogy to other kinase cascades, it is not unreasonable to speculate that this pathway might be triggered by an unknown ligand. It can be envisioned that this ligand might be present at low concentrations during the exponential growth of imaginal discs and that its concentration increases as the disc approaches its final size. When it reaches this point, the Hippo pathway is fully activated, and growth is arrested by the triggering of cell death and the inhibition of cell proliferation. Another example of the coordination of cell proliferation and death was provided by N. Baker (New York, NY, USA), who showed that EGF-receptor signalling mediates both processes in the *Drosophila* eye (Baker & Yu, 2001).

Growth and cancer

Cancer occurs when growth control goes awry. J. Lees (Boston, MA, USA) described a genetic screen for tumour-suppressor genes in zebrafish that is being conducted in collaboration with N. Hopkins (Boston, MA, USA). A surprising finding from the screen is that heterozygous mutations for many ribosomal proteins predispose animals to tumour formation. The underlying mechanisms for the involvement of ribosomal proteins in tumorigenesis are unknown. T. Tlsty (San Francisco, CA, USA) discussed a cancer-progression model that uses human mammary epithelial cells (HMECs) (Romanov *et al.*, 2001). She described the stereotypical molecular changes in this cell-culture model, including the inactivation of p16 and the failure to maintain genomic instability. She also discussed how such changes mimic many of those seen in premalignant lesions in breast cancer, and how observations of this model could identify potential targets for prevention and therapy.

A growing trend in cancer therapy is to develop drugs that specifically target the oncogenic or tumour-suppressor pathway that is dysregulated in a particular tumour. This approach is based on the concept that tumours often become 'addicted' to certain dysregulated signalling pathways and hence become hypersensitive (compared with normal cells) to their downregulation (Weinstein, 2002). Therefore, anticancer drugs that target such dysregulated pathways present the best opportunity to achieve high efficacy at low toxicity. A. Matter (Basel, Switzerland) spoke about the history of the development of Gleevec, which is a small-molecule inhibitor of the oncogenic tyrosine kinase BCR-ABL for the treatment of chronic myelogenous leukaemia (CML). The potency of Gleevec against other tyrosine kinases, including c-KIT and the platelet-derived growth factor (PDGF) receptor, is also exploited for the treatment of several other diseases.

Concluding remarks

Impressive progress has been made in recent years in elucidating certain molecular aspects of growth control in metazoan development. However, our knowledge remains rudimentary. At the cellular level, components of many growth-regulating pathways have yet to be identified. At the tissue level, the mass checkpoint that stops growth when an organ reaches its final size remains elusive. At the organismal level, we are far from understanding the regulatory interplay among the tissues that modulate growth in intact animals. The multidisciplinary approach of this meeting certainly reminded the participants to think about the 'bigger picture' of growth control from a range of perspectives, including evolution, ecology, physiology, genetics and cell biology. With the integration of several approaches, the field of growth control is destined to progress rapidly in the coming years. Hopefully, a future reunion in the scenic Arolla village will show how much the field has accomplished.

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Duoqia Pan