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Cancer, type 2 diabetes, and ageing: news from flies and worms

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Summary

The tumour suppressor gene *PTEN* is, next to *p53*, the second most frequently mutated gene in human cancers. The genes *TSC1* and *TSC2* are mutated in the severe human syndrome called Tuberous Sclerosis. Patients with this disease have large benign tumours composed of large cells in the brain. The genetic dissection of pathways controlling the growth of cells, organs, and the entire organism in *Drosophila* has contributed to the understanding of the signalling pathways that are controlled by these two tumour suppressors. Together with studies on nutrient regulation of growth and ageing in the nematode *Caenorhabditis elegans*, evidence from these model organisms has moved the Insulin/IGF (IIS) and the Target Rapamycin (TOR) signalling pathway onto the centre stage of cellular growth control and made them attractive novel targets for cancer therapy. In this review, I will outline the contributions of model organism genetics to the understanding of these disease relevant pathways and highlight the evolutionary conservation of nutrient-dependent growth regulation.

Key words: Drosophila; insulin; TOR; development; body size

Cell growth and cell cycle control

Differences in size are often the most distinctive features between species, yet we still know little about the genetic basis of size regulation [1]. Over the past 20 years, developmental genetics in model organisms such as *Drosophila* and the nematode *C. elegans* has provided insight into the genetic networks that control the setting up of the main body axes, the patterning of limbs, and the guidance of axons. However, little is known about the mechanisms that underlie the control of cell, organ, or body size. How do the pairs of arms and legs end-up the same size? What controls that the arms in humans are always shorter than the legs? Organs must somehow be able to measure their final size, but how? These fascinating questions are still largely unresolved but developmental biologists have now turned to them and the first results begin to emerge.

Understanding cell growth is also important to understand the formation of tumours. Cell proliferation has often been equated with cell growth and thus large efforts in the past 20 years have gone into the elucidation of cell cycle progression. This research has lead to a detailed understanding of the regulatory networks associated with the progression through the cell cycle [2]. Obviously, for an organism to grow, cells do not only have to divide, they also have to grow: they have to engage in macromolecular synthesis. Macromolecular synthesis, which in most cells consists primarily of the synthesis of proteins, has to be coordinated with the cell cycle to ensure that the size of a particular cell type stays roughly constant. Almost 40 years ago Killander and Zetterberg observed that small cells took on average more time to undergo division than large cells. They suggested that cells pass an internal size check point before starting to divide [3]. Similar experiments in yeast also pointed to a strong influence of cell growth on cell proliferation [4, 5]. We were reminded of these results by experiments addressing the connection between cell growth and cell cycle progression in *Drosophila* imaginal discs [6, 7]. Neufeld and Edgar labelled individual cells in growing imaginal discs and determined, after a fixed time interval, the clonal growth of the progeny of the labelled cell by measuring the area occupied by the cell clone and by counting the number of cells in the clone. These results were compared with those obtained by inducing, at the time of marking the individual cell, expression of the cell cycle accelerator E2F or the cell cycle inhibitor Rb. The authors observed that the growth of the clones, measured by the clone area, stayed constant. E2F expressing clones possessed more, but smaller cells, whereas Rb expressing clones contained fewer, but larger cells. These

results suggested that, within the limits of this experiment, progression through the cell cycle does not induce growth but that growth is regulated independently. What are the regulatory networks that control cell growth during development? Systematic genetic screens and candidate gene approaches have led to the identification of the principle modulators of growth during *Drosophila* development.

Genetic approaches to identify genes involved in growth control

To identify genes involved in growth regulation in *Drosophila*, the simplest genetic approach is to search for mutations that alter the body size of flies. One such mutation is *chico*, meaning small boy in Spanish (figure 1). Flies homozygous for *chico* develop more slowly, are only half the size of wild-type flies, and females are sterile [8]. The size reduction is caused by a reduction in cell number and a reduction in the size of each individual cell. The *chico* gene is involved in the regulation of growth at the level of a single cell and at the level of the organism. This suggested that there is a class of genes in the genome that is dedicated to the regulation of cell growth independently of patterning and differentiation. The *chico* gene encodes the *Drosophila* homologue of the Insulin Receptor Substrate (IRS) proteins in vertebrates. Mammals contain four different IRS proteins (IRS1–4). As the name indicates, these proteins bind to the activated insulin and Insulin-like Growth Factor (IGF) receptors and become phosphorylated on tyrosine residues. As described below, IRS proteins are important adaptors that link these two receptors to downstream signalling pathways. Together with the previous observation that partial loss-of-function mutations in the *Drosophila* insulin receptor gene, *Inr*, also reduces body size, these results suggest that the insulin signalling pathway plays a critical role in growth regulation in *Drosophila* [9].

Figure 1

Mutations in the Drosophila gene *chico* encoding the homologue of IRS1–4 cause a proportional reduction in body size. The two flies on the left are homozygous for *chico*. Their body size is reduced by 50% compared with the two heterozygous flies on the right. The small size is caused by a reduction in the number and the size of the cells.

Figure 2

Mutations in genes involved in growth regulation are identified in the *pinhead/bighead* screen. The fly in the middle is wild-type and displays a normal head size. The fly on the left is a genetic mosaic. It is homozygous for a growth inhibiting mutation in the head and heterozygous for the same mutation in the rest of the body. As a consequence, the size of the head is reduced *(pinhead)*, yet bristles and eyes are differentiated normally. The head of the fly on the right is homozygous for a mutation in the tumour suppressor gene *PTEN*. Because of the lack of this growth inhibitor, head size is increased. Mutant flies with these phenotypes are selected, the mutated genes are mapped and molecularly characterised.

It is unlikely that the search for mutations reducing body size will identify all genes involved in cellular and organismal growth, since loss-of-function mutations in most of these genes are expected to be lethal. Screens devised to detect mutations affecting growth in clones of cells in which randomly induced mutations have been made homozygous by mitotic recombination, have helped to obtain a more complete picture of the genes involved in growth control. In one variant of these screens, genetically modified male flies are fed the mutagenic substance ethylmethanesulphonate (EMS) and mated to special strains of females. Owing to a tissue-specific recombination system that is active only in the eye and head progenitor cells, a portion (one chromosome arm) of the paternal genome carrying newly induced mutations is made homozygous [10]. In this way, mosaic flies are generated that are homozygous for newly induced mutations on one chromosome arm in the head but heterozygous for the same mutations in the rest of the body. Since each progeny fly is derived from a single sperm containing a unique set of mutations, the phenotype of these recessive mutations is displayed in individual F1 flies. Some mutations occur in genes whose function is essential for the growth or differentiation of these cells. Such mutations cause cell lethality and flies without a head die in the pupal case. Mutations in genes that either promote or inhibit growth, however, will result in flies whose heads are too small (mutation in a growth promoting gene) or too large (mutation in a growth inhibiting gene). Only flies with such phenotypes are backcrossed to the maternal strain and lines are established. We refer to this screen as the *pinhead/bighead* screen. An example of a pinhead and a bighead mutation is shown in figure 2. One advantage of this type of screen is the large number of flies that can be analyzed. Since EMS treatment generates at least one loss-of-function mutation per haploid genome, multiple coverage of the *Drosophila* genome containing approximately 15 000 genes is readily

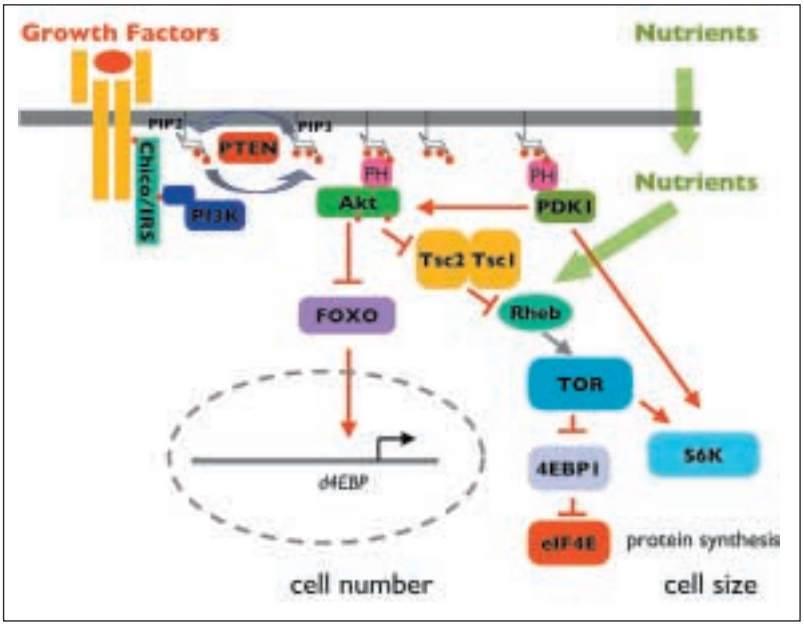

Figure 3

Diagram of the insulin/IGF signalling pathway. Indicated are the key elements of this pathway that have been identified and characterised genetically and biochemically. Connections indicated in red represent direct physical interactions. See text for detail.

obtained. This provides the unique opportunity to identify all genes in the genome that modulate growth and not patterning in a cell- or tissue-autonomous manner. Having screened approximately 300 000 flies for mutations on the four major arms of the autosomes we have reached a 20-fold coverage of the genome and identified approximately 50 genes whose products are involved in promoting or inhibiting cellular growth. The genes most frequently identified in this screen encode proteins that constitute the backbone of two linked signalling pathways, the insulin/IGF and the Target of Rapamycin (TOR) pathway. This indicates that these two pathways play a central role in the control of cell growth in *Drosophila* (figure 3)

Insulin/IGF signalling (IIS) in mammals

Because of its central role in glucose homeostasis, the signalling pathway controlled by the insulin receptor is probably one of the most intensely investigated cellular pathway. A large body of literature covers the role of various signalling components in development, physiology, and diseases in mammals. In the following section I will outline the salient features of the IIS pathway that have emerged from biochemical studies in tissue culture cells and knock-out studies in mice.

The main ligands that activate this signalling pathway are insulin produced by the pancreatic β-cells in the islets of Langerhans and the insulin-like growth factors 1 and 2 (IGF-1/2), mainly produced by the liver. The primary role of insulin is to maintain glucose homeostasis and thus it plays a central role in diabetes mellitus [11]. IGF-1/2 regulates organismal growth in response to growth hormone [12]. In addition, IGF-1 also exerts a neuroprotective function in the brain [13]. The mammalian insulin (IR) and IGF-1 receptors (IGFR) belong to the family of receptor tyrosine kinases and are activated by their corresponding peptide ligands, insulin and IGF-1/2, respectively. Ligand-binding induces dimerisation of the receptor and activates the cytoplasmic kinase domain resulting in autophosphorylation on tyrosine residues. Phosphotyrosine residues preceded by an arginine and a proline residue at position –3 and –2 (NPxY) in the juxtamembrane domain of the receptors are bound by the Phosphotyrosine Binding (PTB) domain of adaptor proteins of the Insulin Receptor Substrate (IRS) family. In addition to the PTB domain, these proteins contain an NH2-terminal PH (pleckstrin homology) domain and several phosphotyrosine motifs that serve as docking sites for SH2 (Src-Homology 2) domain containing proteins. SH2 containing proteins link the insulin/IGF signalling network to two major signalling pathways: The PI3K/Akt pathway via the SH2 containing regulatory subunit of PI3K, p85 (see below), and the Ras/MAPK pathway via the SH2 adaptors Grb2 and SHC [14].

Knockout experiments in mice helped to elucidate the role of the two receptors and the IRS proteins. Both IR and IGFR are required for embryonic and postnatal growth [15–17]. Whereas mice lacking IR function die shortly after birth due to severe defects in glucose homeostasis, mice lacking IGFR function are reduced in size and die at birth because of respiratory failure. Loss of both receptors increases the severity of the growth phenotype [18]. The functions of IRS-1 and IRS-2 appear to be largely complementary. Loss of *IRS-1* results in growth retardation and insulin resistance in peripheral tissues [19, 20]. *IRS-2* knock-out mice have only a small reduction in body weight but display insulin resistance in the periphery and a reduced β-cell mass [21, 22].

The main branch of signalling downstream of the IR/IGF-1R involves the lipid kinase phosphatidylinositol-3-OH-kinase (PI3K) [23]. Its regulatory subunit (p85) binds via its SH2 domain to phosphotyrosine motifs in IRS proteins, thereby recruiting the catalytic subunit (p110) to the plasma membrane. At the plasma membrane,

p110 phosphorylates its substrate, phosphatidylinositol-(4,5)-bisphosphate (PIP2), and thereby generates phosphatidylinositol-(3,4,5)-trisphosphate (PIP3). PIP3 acts as a second messenger in mediating the cellular responses to the activation of the IR and IGF-1R receptors. Its generation from PIP2 by PI3K is counteracted by the lipid phosphatase PTEN (phosphatase and tensin homologue on chromosome 10) that dephosphorylates the D3 position of the inositol ring [24]. The loss of PTEN in immortalised embryonic fibroblasts or embryonic stem cells results in a two to three-fold increase in PIP3 levels [25, 26]. Interestingly, PTEN is a tumour suppressor that is frequently mutated in a wide variety of human cancers [27].

Elevated levels of PIP3 recruit the PH-domain containing protein kinase Akt (also called PKB) to the plasma membrane and permit its further activation by the phosphoinositide-dependent kinase 1 (PDK1) [28]. Amongst the growing list of substrates phosphorylated by Akt are the metabolic enzymes GSK3 (glycogen synthase kinase 3) and 6-phosphofructo-2-kinase, proteins involved in cell survival such as BAD, transcription factors of the FOXO family FKHR, FKHRL1, and AFX, and the tumour suppressor protein Tuberous sclerosis 2 (TSC2) [29]. It has been very difficult to address the relative importance of these different substrates for the various *in vivo* functions of IIS. Mouse knock-out mutations in the genes coding for the three mammalian isoforms Akt1–3 show that *Akt1* mutant mice are reduced in size, *Akt2* mutants are normal in size but hyperglycaemic, and *Akt3* mutant are phenotypically normal [30]. Although compensatory upregulation of the remaining isoforms is observed there is a certain degree of functional specialisation in the three isoforms in a way similar to the IRS isoforms. IRS-1 is dedicated to growth, whereas IRS-2 is required for the regulation of glucose homeostasis [19, 21].

Akt kinases are not the only output of IIS. Insulin or IGF-1/2 stimulation also results in the activation of ribosomal protein S6 kinase (S6K) and the phosphorylation of the elongation factor 4E binding proteins (4EBP) [31]. Phosphorylation of the ribosomal protein S6 by S6K is thought to increase translation of mRNAs containing oligopyrimidine tracks in their 5' leaders [32]. These messages encode components of the translation machinery. Phosphorylation of 4EBP prevents its association with the translation initiation factor 4E thus freeing it to form translation initiation complexes. S6K activation and 4EBP phosphorylation in response to insulin involves the activity of the Target of Rapamycin kinase (TOR) since treatment with its inhibitor, Rapamycin, blocks these changes [31]. As in the case of its family member Akt, S6K also requires phosphorylation by PDK1 for its further TOR dependent activation. In summary, the cellular responses to insulin/IGF-1/2 signalling are highly complex. They include activation of the Ras and the PI3K signalling pathway, transcriptional regulation by phosphorylation of FOXO transcription factors, and modulation of translation via TOR.

The architecture of the insulin signalling pathway in *Drosophila* and *C. elegans*

In spite of the large body of literature on the responses of mammalian cells to insulin and IGF-1/2 stimulation, genetic dissection of this signalling pathway in model organisms has contributed in at least three different ways to our understanding of IIS. First, genetic screens have identified novel essential components in the pathway. Second, the analysis of the function of the various signalling branches under physiological condition has provided insight into their relative contribution to cell growth and cell physiology. Third, studying IIS in model organisms has pointed to the evolutionary ancient role of this pathway in nutrient sensing and the regulation of lifespan. I will first outline the salient features of the IIS in *C. elegans* and in *Drosophila* and then address the different physiological functions of this pathway.

Although the overall architecture of the insulin signalling pathway in *Drosophila* and in *C. elegans* is simpler than in vertebrates, these organisms contain a large number of genes encoding insulin-like peptides. The *C. elegans* genome harbors more than 30 distantly related insulin-like genes. Whether all of these encode ligands for the single insulin receptor is however unclear [33]. In *Drosophila*, there are seven insulin-like peptide (dilp) genes that are expressed in a highly dynamic pattern [34]. Three of the seven genes are expressed in two paired clusters of 7 neurosecretory cells in the brain [35]. These cells project axon terminals to the corpora cardiaca, a component of the major endocrine gland, and to the aorta, from where Dilps are released in to the insect blood, the haemolymph. Ablation of these cells results in a strong reduction in body size. Dilp expression and release from these cells is regulated by nutrients and haemolymph glucose levels suggesting that these cells are functionally equivalent to the β-cells of the mammalian pancreas [36]. Moreover, a hormone related to glucagon called adipokinetic hormone is produced by cells in the corpora cardiaca and its release is controlled by an ATP-dependent potassium channel and a sulphonylurea receptor that are homologous to those controlling the release of glucagon in α cells in the pancreas [37, 38]. This suggests that the endocrine

regulation of glucose homeostasis in vertebrates and in invertebrates relies on the same pair of hormones and that this insect model may be used to study some aspects of diabetes.

The single insulin-like receptors in *Drosophila* and *C. elegans* connect to a signalling cascade that, with the exception of two Akt homologues in *C. elegans*, consists of single orthologues of the mammalian signalling components. This makes the genetic dissection of this pathway in these simple organisms readily accessible. In *C. elegans*, insulin signalling mediates the starvation-induced developmental arrest at the stage of a long-lived dauer larva. Loss-of-function mutations in the genes encoding the insulin receptor or PI3K cause a constitutive dauer phenotype. This phenotype is completely suppressed by mutations in the *daf-16* gene. *Daf-16* encodes the *C. elegans* FOXO homologue. This was the first evidence that FOXO transcription factors are essential downstream targets of the insulin signalling pathway [39]. Akt, activated by high insulin levels, phosphorylates FOXO transcription factors and promotes their binding to 14–3–3 proteins, thereby anchoring them in the cytoplasm and rendering them inactive [40, 41]. In the absence of Akt activity, FOXO is dephosphorylated, enters the nucleus, and activates target genes many of which are involved in the protection against cellular stress. In *C. elegans*, flies, and mammals, FOXO transcription factors are sensors for various cellular stress conditions [42, 43]. Intriguingly, cellular growth phenotypes associated with insulin pathway mutants in *Drosophila* are conspicuously absent in *C. elegans*. Although *C. elegans* TOR mutants have been identified they show no obvious connection to dauer formation or insulin signalling, although it has been reported that TOR also affects longevity in nematodes [44].

The molecular link between insulin and TOR signalling has been pioneered in *Drosophila*. First, TOR mutants have been identified in the screen for growth mutations described above [45, 46]. Second, the *Drosophila* homologues of the tumour suppressor proteins TSC1 and TSC2 were shown to couple insulin signalling to the TOR pathway [47]. Tuberous Sclerosis is a hereditary syndrome in humans associated with benign tumours that contain large cells in the brain. It is associated with mutations in two genes, *TSC1* and *TSC2*, whose products form a complex [48]. Mutations in the *Drosophila* homologues where found in screens for mutations that cause a cell overgrowth phenotype [49–52]. Genetic interaction studies indicated a tight but complex connection to insulin signalling. The lethality associated with loss of insulin receptor function in *Drosophila* is rescued dominantly by mutations in *TSC2/1* suggesting that the TSC complex is an essential downstream component of insulin signalling. On the other hand, cells doubly mutant for *TSC* and *PTEN* are almost twice the size of cells having lost either TSC or PTEN function. This suggests an additive function of these two tumour suppressors [49]. In addition to the genetic evidence for a placement of TSC1/2 in the insulin signalling pathway, biochemical studies in vertebrates and in *Drosophila* showed that TSC2 is phosphorylated by Akt and that this phosphorylation decreases the stability of TSC2 or its complex formation [53–55]. Furthermore, loss of TSC robustly activates S6K suggesting that the TSC complex controls the TOR/SK6 branch of the pathway. How TSC function is repressed by the upstream insulin pathway is still controversial. Although direct phosphorylation and destabilisation of TSC by Akt has been demonstrated in mammals and in *Drosophila*, rescue experiments in *Drosophila* show that a mutant form of TSC2 lacking all consensus Akt phosphorylation sites is able to rescue the null mutant phenotype [56]. Thus, TSC inactivation by Akt may be particularly relevant in the context of unnaturally high Akt activity levels caused by the loss of PTEN or the oncogenic activation of Akt and may thus contribute to the oncogenic potential of Akt.

Although the upstream regulation of TSC is still not entirely clear, the small GTP-binding protein Rheb (Ras-homologue expressed in brain) has been shown to be the direct downstream target of the GAP (GTPase-activating-Protein) domain of TSC2. Loss of Rheb function in *Drosophila* reduces cellular growth in a way reminiscent of mutations in *chico* and other components in the insulin pathway [57–60]. Furthermore, the lethality associated with loss of TSC1 function is rescued by reducing Rheb function. Rheb function is required for TOR and S6K activation [59]. Although Rheb was first identified in vertebrates as a small G-protein closely related to Ras, its function remained elusive until it was placed in the TSC/TOR pathway by genetic means in *Drosophila*. This triggered a flurry of studies in mammalian cells corroborating the findings from *Drosophila* that Rheb is indeed a critical upstream activator of TOR in all mammalian cells [61–64]. The question of how Rheb regulates TOR has not yet been resolved. Although phosphorylation of 4EBP and activation of S6K are the best-known outputs of TOR, it is likely that other effectors also exist. Indeed, it has recently been demonstrated that, like in yeast, TOR exists in two separate protein complexes, only one of which is sensitive to Rapamycin [65–67]. The Rapamycin insensitive complex in yeast regulates the actin cytoskeleton and is not involved in growth. It is interesting to note that *Rheb* mutant cell clones surrounded by wild-type cells possess an abnormally elongated shape raising the interesting possibility that Rheb may also be involved in the regulation of the cytoarchitecture of the cell [68].

In yeast, nutrient deprivation sets in motion a complex genetic programme involving the altered expression of amino acid transporters and the regeneration of biosynthetic building blocks by degrading macromolecular components by a process called autophagy [69]. All these programs

are dependent on TOR function. A genetic link between TOR and autophagy has also been established in *Drosophila* [70, 71]. This process is independent of S6K activation and therefore opens a third branch point downstream of TOR.

Role of the IIS network in the control of cell size and cell number

The size of an organ and an organism depends on the number of cells and on the size of each individual cell. Mutations in the insulin pathway in *Drosophila* alter both cell size and cell number. Flies homozygous for *chico* are smaller because they possess 30% fewer cells and because the size of each individual cell is reduced by approximately 10% in the case of epithelial cells in the wing or up to 50% in the case of the highly specialized photoreceptor cells in the eye [8]. Conversely, cell number and cell size is increased by elevated insulin signalling or by the loss of the negative regulators PTEN or TSC1/2. Changes in cell size and cell number have also been observed in response to altered activity levels of the IIS pathway in mammals. In particular, loss of PTEN function in mice results in increased cell proliferation and cell size in neural stem cell populations. In postmitotic differentiated neuronal cells, PTEN loss causes an autonomous increase in cell size [72]. This increase in cell size is dependent on TOR function since it is suppressed by Rapamycin treatment.

In *Drosophila*, cell size and cell number appear to be controlled partly by two separate branches of the insulin signalling network. Cell size but not cell number is reduced in mutants lacking S6K [73]. Conversely, the reduction in cell number observed in *chico* mutants is dependent on the *Drosophila* FOXO transcription factor, dFOXO [74]. Intriguingly, although there is only a single *dFOXO* gene in *Drosophila*, loss-of-function mutations in this gene are viable under standard culture conditions [74, 75]. Thus the dFOXO branch of the pathway does not contribute to the regulation of final organ or body size under culture condition that permit normal levels of insulin signalling. Under these conditions, dFOXO appears to be fully phosphorylated by Akt and is retained in the cytoplasm [76].

The universal role of IIS in controlling cell size and cell number raises the question whether the IIS is indeed a central element in determining species specific cell, organ, and body size or whether it is merely a universal modulator of this genetic growth programme. The fact that normal body size is attained in flies lacking dFOXO, one of the central regulators in IIS, does in fact suggest that IIS plays a regulatory role. As we will see, insulin signalling plays a central role in adjusting growth under limited nutrient conditions.

Insulin signalling – a conserved mechanism to adjust growth rates and lifespan to environmental conditions

The ability to survive periods of low nutrients is a central function that was selected for in single cell organisms and in metazoa. Therefore signalling pathways that relay nutrient information to cellular metabolism must have evolved early and are highly conserved. Several lines of evidence suggest that the IIS and TOR signalling network primarily serves this function. In yeast, plants, and in multicellular organisms TOR activity regulates cellular metabolism in response to nutrients [77]. As outlined above, TOR regulates the major cellular anabolic and catabolic pathways including translation and autophagy. In contrast to higher organisms, yeast cells lack clear homologues of the IIS pathway such as PI3K, PTEN, and Akt. This pathway appears to have evolved with the need to communicate and coordinate growth in response to the nutrient status in multicellular organisms. IIS signalling is tightly linked to nutrients in vertebrates and invertebrates. In *Drosophila*, starvation produces small flies with fewer and smaller cells and causes sterility in females. These starvation-induced phenotypes are very similar to the phenotype observed by reducing insulin signalling. Indeed, starvation reduces the expression of two of the three insulin-like peptides in the main neurosecretory cells thereby establishing a direct link between nutrient availability and insulin signalling in *Drosophila* [35].

In *C. elegans*, insulin signalling mediates starvation-induced dauer formation and longevity thus providing us with another important and universal connection: the link between nutrient availability and lifespan. In all organisms studied thus far, from yeast to primates, a reduction in the amount of calorie intake by approximately 50% significantly extends lifespan (30% in mammals) [78]. In *C. elegans*, partial loss-of-function mutations in genes encoding insulin signalling components do not enter the dauer stage but develop to adulthood. The lifespan of these adults is up to three-fold higher than in the wild type [39]. Like in the case of the dauer formation, extended lifespan is entirely dependent on the Daf-16 FOXO

transcription factor. Given the universality of the effect of caloric restriction on lifespan it was not surprising that the connection between reduced IIS in *C. elegans* and lifespan extension also holds for other organisms. In *Drosophila*, *chico* and *Inr* mutant flies live longer [79, 80]. Furthermore, lifespan is extended in mice heterozygous for IGF-1R or in mice lacking IGF-R1 function in adipose tissues [81, 82]. Reduced insulin signalling does not only reduce growth but also activates cellular stress protective programmes that contribute to the lifespan extension. Studies in *Drosophila* have shown that caloric restriction extends lifespan not by slowing down the ageing process but by markedly delaying the onset of the ageing process associated with an increased mortality rate [83]. There is obviously a great excitement in the prospect of identifying amongst FOXO target genes the downstream effectors that contribute to the extension of lifespan.

IIS and cancer

The central role of IIS and TOR as modulators of cellular growth and the fact that the two tumour suppressors PTEN and TSC1/2 are essential to control this pathway, highlights the importance of components in this pathway as potential therapeutic targets in cancer therapy. The fact that growth of cells is impaired in the same way and to a similar extent by mutation in the genes coding for the insulin receptor, PI3K, Akt, Rheb and TOR suggests that each of these proteins is a key regulator of cellular growth and thus a potential target for therapeutic intervention. Apart from ongoing programmes in various pharmaceutical and biotech companies to develop inhibitors that block the catalytic activity of PI3K or Akt, genetic experiments in *Drosophila* suggest that targeting the interaction between the PH domain of Akt and PIP3 at the membrane may be a sensible alternative. In *Drosophila*, loss of PTEN function, a condition found in many tumours, causes lethality presumably because of increased levels of PIP3. This *PTEN* associated lethality is rescued by a specific mutation in *dAkt* that causes an amino acid substitution in the PH domain and reduces the affinity for PIP3 [84]. The most advanced strategy in targeting the IIS for cancer therapy is the inhibition of TOR activity by Rapamycin. This natural antibiotic has been in clinical use as an immunosuppressant after organ transplantation without the knowledge of its molecular target. Genetic experiments in yeast showed that Rapamycin inhibits TOR [85, 86]. Because of the genetic and biochemical evidence that TOR is a central component of the IIS pathway and a key regulator of cellular growth, Rapamycin has been tested for its effectiveness as an anti-cancer agent. In animal models it has proven effective particularly in PTEN deficient tumours. Clinical trials for the use of Rapamycin in tumour therapy and in restenosis in heart valves to prevent proliferation of endothelial cells are well underway [87, 88].

Outlook

Recent progress in understanding the function and regulation of IIS has come from surprisingly separate fields and biological questions. The different research programmes range from the characterisation of IIS as a clinically relevant pathway in diabetes and growth disorders, to studying the control of cellular growth in yeast and organ size in *Drosophila* and studying ageing in *C. elegans*. Results from each of these programmes have contributed to our present understanding of IIS in a unique way. It is expected that future progress in the understanding of this disease relevant pathway will continue to come from an integrated approach involving model system genetics and vertebrate models. It is likely that in the not too distant future this knowledge will provide new therapies not only for cancer, but also for type 2 diabetes. Furthermore, it may also provide insight into how lifespan can be extended. Not so much to reach older age, but to postpone the onset of age-related diseases such as cancer and neurodegenerative disorder.

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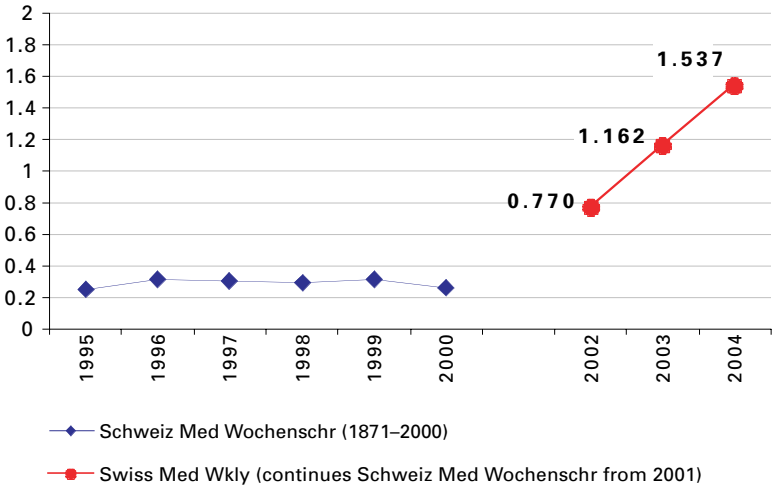

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