Insulin-like/mimetic signaling in lower eukaryotes.

Muller GA*

Helmholtz Diabetes Center (HDC) at the Helmholtz Center Munchen, Institute for Diabetes and Obesity, Oberschleissheim, Germany

Abstract

Functional responses of lower eukaryotes, to mammalian hormones and the operation of corresponding signal transduction cascades have been described for decades. Recent findings on the existence of components of insulin-like and mimetic signaling pathways, such as an insulin-binding protein, as well as their coupling to G-protein coupled receptors and metabolic pathways in lower eukaryotes, particular in *Neurospora crassa* and *Saccharomyces cerevisiae* will be presented. These data together with the knowledge about insulin-like/mimetic molecules in lower eukaryotes provide further evidence for the provocative view that the evolutionary roots of the vertebrate endocrine system may be far more ancient than is generally believed and that the expression of insulin-like/mimetic signalling pathways in lower eukaryotic model organisms can be useful for future drug discovery efforts.

Keywords: Glucagon-like peptides, G-protein-coupled receptors, insulin receptor, *Neurospora crassa*, *Saccharomyces cerevisiae*, type 2 diabetes.

Accepted on November 12, 2018

Introduction

Insulin effects on mammalian glucose homeostasis are well documented. Insulin may affect very primitive functions via ancient intracellular signaling systems. Literature citations spanning 15-20 years propose structural and functional homologs of vertebrate hormones in invertebrate and unicellular organisms. Indirect evidence includes metabolic responses, the existence of gene sequences for proteins and peptides, and their related controlling elements. The apparent demonstration of materials in unicellular eukaryotes which somehow resembles mammalian insulin according to structural criteria provokes the question as to whether these insulin-like molecules have the ability to induce some insulin-like effects on the cells of its origin or typical insulin target cells. In fact, the observations of insulin-like molecules and/or effects in unicellular eukaryotes prompted a few studies on the molecular mode of insulin-mimetic action in these organisms, which may potentially reveal interesting similarities between typical and non-typical insulin target cells. Detailed knowledge about the molecular basis of insulin signaling and action has been gathered during the last decades. Upon binding of insulin to the insulin receptor, which belongs to the IGF family of receptor tyrosine kinases, a cascade of tyrosine phosphorylation events at the so-called insulin receptor substrate (IRS) proteins, in particular at IRS-1, becomes initiated in the insulin target tissues, such as adipose, muscle and liver [1-3]. Phosphorylated IRS-1 then induces the upregulation of downstream signalling components, such as phosphatidylinositol 3-kinase (PI3K), protein kinase B (PKB/AKT), several (classical, atypical and novel) isoforms of protein kinase C (PKC) and mitogen-activated protein kinase (MAPK), which via engagement of additional adaptor proteins, enzymes, such as protein and lipid kinases and phosphatases, and second messengers, such as Ca^{2+} and cAMP, leads to the transmission of the insulin signal from the insulin receptor to the terminal insulin metabolic and mitogenic effector systems, such as the glucose transporter-4 (GLUT4) translocation machinery for stimulation of glucose uptake, the enzyme cascades for the conversion and storage of glucose into glycogen and triacylglycerol, the translation apparatus for elevated protein synthesis and specific gene expression elements for increased growth and mitogenesis [1,4].

Insulin-like signaling in C. elegans

An insulin receptor-like signaling pathway regulates the metabolism, development, and longevity of *C. elegans* [5,6]. This insulin receptor pathway is required for reproductive growth and normal metabolism. The predicted DAF-2 protein is 35% identical to the human insulin receptor and like the human insulin receptor DAF-2 contains several common structural domains. DAF-2 has a putative signal peptide, a cysteine-rich region in the ligand binding domain, a transmembrane domain and a tyrosine kinase domain showing 70% similarity to the human insulin receptor. In addition, the DAF-2 carboxy-terminal region may serve a similar function to that of the mammalian insulin receptor substrate, IRS. Consistent with a conservation of insulin-like signaling pathways, sequence analysis of another DAF gene revealed that DAF-23 encoded a protein that shared homology to the class 1A PI3K genes [7]. This is similar to the catalytic subunit of PI3K in mammalian cells. PI3Ks are one of the major downstream effectors of the insulin receptor suggesting that DAF-23 may regulate metabolism. On the basis of previous research on the interaction of mammalian IRS-1 with PI3K, YXXM motif at tyrosine1626 of DAF-2 is likely to mediate the interaction with DAF-23. Similar to the human insulin receptor, DAF-2 contains analogous phosphor-tyrosine binding sites for additional signaling proteins. It is possible that recruitment of DAF-23/PI3K to the DAF-2/insulin receptor-like peptide (IRL) activates its kinase

activity and results in the production of the lipidic second messenger PIP_3. Thus DAF-2, like mammalian insulin-mediated signaling is used to monitor and alter carbohydrate and lipid metabolism to face the surrounding environment. Mutations in the insulin/IGF-1 receptor homolog daf-2/IRL [5] or in the PI3K homolog age-1 [7] cause animals to arrest as dauers, shift metabolism to fat storage, and live longer [8-10]. This regulation of *C. elegans* metabolism is similar to the physiological role of mammalian insulin in metabolic regulation. Insulin controls glucose homeostasis by changing the subcellular localization and activity of key metabolic regulators, such as glucose transporters and metabolic enzymes [11]. Mutations in the Forkhead transcription factor DAF-16 completely suppress the dauer arrest, metabolic shift, and longevity phenotypes of daf-2 and age-1 mutants [12-14] indicating that DAF-16 is a negatively regulated downstream target of *C. elegans* insulin receptor-like signaling. Mammalian homologs of DAF-16 may similarly be regulated by insulin receptor signaling to mediate the transcriptional effects of insulin [14]. Molecules that couple the DAF-2 insulin receptor-like protein and the AGE-1 PI3K to the DAF-16 transcription factor have not been identified by previous extensive genetic screens Previously it was demonstrated that the *C. elegans* Akt/PKB homologs, akt-1 and akt-2, transduce arrest and that AKT-1 and AKT-2 signaling is indispensable for insulin receptor-like signaling in *C. elegans* [15]. Inactivation of Akt/PKB signaling causes a dauer constitutive phenotype. A loss-of-function mutation in the Fork head transcription factor DAF-16 relieves the requirement for Akt/PKB signaling, which indicates that AKT-1 and AKT-2 function primarily to antagonize DAF-16. An activating mutation in akt-1 revealed by a genetic screen as well as the increased dosage of wild-type akt-1 relieves the requirement for signaling from AGE-1 PI,3K, which acts downstream of the DAF-2 insulin/IGF-1 receptor homolog. Apparently, Akt/PKB signaling via its major downstream target DAF-16 is necessary for reproductive growth and metabolism [15]. A reversible arrest of C. elegans development at the metabolically less active dauer stage is triggered by a dauer-inducing pheromone [16]. This pheromone is detected by sensory neurons, which then signal by a complex pathway to target tissues, such as the germ line, pharynx, intestine, and ectoderm for remodeling and metabolic shifting. Genetic analysis of daf mutants, which arrest at the dauer stage or enter the reproductive life cycle independent of pheromone regulation, has revealed that parallel genetic pathways regulate distinct aspects of the dauer metamorphosis [17-19]. The pathway that included DAF-2 controls both reproductive development and normal senescence: DAF-2 mutant animals arrest development at the dauer larval stage and exhibit a marked increase in longevity [8,20]. Decreased DAF-2 signaling also causes an increase in life-span. Life-span regulation by insulin-like metabolic control is analogous to mammalian longevity enhancement induced by caloric restriction, suggesting a general link between metabolism, diapause, and longevity *C. elegans* harbors a homolog to the human tyrosine phosphatase PTEN, which is mutated in a wide variety of sporadic tumors and known as tumor suppressor gene. A strong loss-of-function allele of the PTEN homolog causes the deficient strain to fail in entering the dauer diapause [21]. An insulin-like PI3K signaling pathway regulates dauer-stage entry. Mutations in either the DAF-2/IRL gene or the age-1 encoded PI3K catalytic subunit homolog cause constitutive dauer formation and also affect the lifespan and metabolism of non-dauer animals. Strikingly, loss-of-function mutations in the age-1 PI3K and DAF-2 IRL genes are suppressed by loss-of-function mutations in the PTEN homolog, which is encoded by daf-18. This gene interacts genetically with the DAF-2/IRL AGE-1/PI3K signaling pathway and antagonizes PI3K function in vivo [21]. The *C. elegans* worm has a simple life cycle where under uncrowded conditions with plenty of food, the larvae develop rapidly through four larval stages (L1 to L4) to become adult worms with a lifespan of 2 to 3 weeks. By contrast, when the worms grow so numerous that they threaten their food supply, there is an increase in pheromone concentrations which triggers the individual worm's chemosensory alarms. This alerts the worm to the fact that there is a shortage of glucose available and this causes the worms to increase fat deposits around the intestine, and to thicken their cuticles. These signals push the *C. elegans* into an alternative third larval stage, called the dauer stage. Dauer larvae are developmentally arrested, non-feeding and resistant to desiccation and can endure harsh environmental conditions. However, when favorable conditions return, dauers recover and develop into adults to complete their normal lifespan, irrespective of how long they spent in the dauer stage. Many genes are involved in this switch between the two alternative developmental pathways, the so-called dauer formation (DAF) genes. Genetic screens have identified genes that are important in promoting longevity and/or affecting dauer larvae formation. DAF-2 and DAF-23/AGE-1 are two constitutive DAF genes that have been placed at the same level in the genetic pathway for dauer formation. DAF-2 and DAF-23/AGE-1 mutants do not become dauer but exhibit a marked increase in adult longevity [22]. During times of plenty, the worm may maintain a high level of an insulin-like hormone that binds to DAF-2. This, in turn, activates DAF-23 to propagate the downstream second messengers thus passing on the signal for metabolizing stored fuels since food is plentiful. More recently, the longevity phenotype of both genes was shown to be suppressed by mutations in the most downstream dauer gene in the pathway, DAF-16. DAF-16 was identified to encode for an HNF-3/fork head transcription factor family member [13]. This implies that mutations in DAF-2 and DAF-23/AGE-1 that promote longevity exert their effect not simply by changing the activities of pre-existing enzymes but in part by initiating a new genetic program. This new program is expected to include expression of particular genes that are specific for the dauer with the major consequence of longer life for these mutant worms [23-25]. In conclusion, there are several surprising parallels between signaling pathways in C. elegans and those mediating insulin action in mammals [26] and their mutual coupling to nutritional signaling and regulation of lifespan [25-27].

Insulin-like signaling in fungi

Human insulin has been found to exert effects on glucose metabolism in protozoa and microorganisms, among them fungi and *Tetrahymena pyriformis* [28-32]. However, so far the molecular mechanisms and pathways underlying these apparent insulin-like signaling activities in those lower eukaryotes have

been investigated in some detail only for *Tetrahymena pyriformis* [30] *Neurospora crassa* and *Saccharomyces cerevisiae*.

Neurospora crassa: Biological effects of mammalian insulins have been observed in a cell wall-less strain of *Neurospora crassa*. The addition of mammalian insulin to a nutritionally rich, chemically defined culture medium resulted in distinctively different morphology, enhanced growth, and extension of viability at the stationary phase in culture [33]. Bovine, porcine and recombinant human insulin had a similar influence on growth and morphology, while proinsulin, reduced insulin, and several other proteins were inactive. Insulin in the presence of excess anti-insulin antibody did not exert activity. These observations are consistent with the well-established role of insulin or the related IGF-1 and IGF-2 as a mitogen or differentiation-inducing factor in hormonally defined media for mammalian cells in culture. Growth promotion and prolonged viability imply that nutritional metabolism, especially carbohydrate utilization, may be altered by the hormone. Experiments with these cells revealed that treatment with bovine insulin produced several significant effects on glycogen metabolism [34-36]. For example, cells grown in the presence of 100 nm insulin possessed 40% more glycogen than did control cells. The incorporation of ^{14}C-labeled glucose into glycogen increased 41% ($p>0.01$) after a 30-min treatment with the same concentration of the hormone. Intracellular levels of the glycogen precursor, UDPG-glucose, determined by ^{31}P-nuclear magnetic resonance, were found to be increased in response to insulin. Furthermore, in intact *Neurospora crassa* cells, insulin-stimulated glucose and amino acid metabolism (glycolysis, glucose oxidation, glycogen synthesis [33] and alanine synthesis [36] and the growth rate. Flawia and Torres described the effects of bovine insulin on membrane-associated adenylate cyclase from *Neurospora crassa* [37-38]. Insulin at low doses (nm-range) was observed to significantly inhibit the specific activity of the fungal enzyme. Alternatively, glucagon, a known antagonist of insulin action under many physiological circumstances, stimulated adenylate cyclase activity [38].

Yeast: Some time ago, the surprising finding was reported that human insulin exerts marked and specific effects on oxidative and non-oxidative glucose metabolism in *Saccharomyces cerevisiae* [39]. For instance, glycogen storage under conditions of growth limitation in glucose medium (i.e. transition of intact cells from late logarithmic to stationary phase or incubation of spheroplasts in synthetic glucose medium) was found to be markedly elevated in the presence of human insulin. Thus insulin seems to increase the sensitivity of glycogen synthase (GS) for activation by glucose as well as of glycogen phosphorylase (GP) for inactivation by glucose. The activity of both enzymes is regulated by phosphorylation/dephosphorylation. It is assumed that their phosphorylation state is controlled by a complex interplay between serine/threonine-specific phosphatases, like protein phosphatase 2A (PP2A) [40] which are activated directly or indirectly by the SNF1 kinase and cAMP-dependent protein kinase A (PKA) [41] SNF1 kinase was demonstrated previously to be induced under conditions of glycogen accumulation in yeast [42] which subsequently was reported to be further enhanced in the presence of insulin [44-46]. In agreement with the proposed linkage between glycogen storage and the activities of SNF1, PP2A and PKA, subsequent studies revealed (i) increased PP2A activity in both glucose-induced spheroplasts and glucose-exhausted intact cells (ii) slightly but significantly reduced PKA activity in glucose-exhausted intact cells and (iii) considerable elevated cAMP-specific phosphodiesterase (cAMP-PDE) activity in glucose-exhausted intact cells in response to insulin. In addition to degradation of cytosolic cAMP by cAMP-PDE, a direct inhibition of PKA by an insulin-dependent but cAMP-independent covalent and/or allosteric mechanism is likely to operate based on the kinetic differences in inhibition of PKA and activation of cAMP-PDE by human insulin The existence of allosteric effectors of protein phosphatases in mammalian cells has been postulated albeit there is indirect experimental evidence available so far, only. The molecular basis for the rather provocative finding that human insulin stimulates glycogen synthesis in *Saccharomyces cerevisiae* was further characterized. Apparently, it is based on both activation of GS and inhibition of GP [46]. For this, the effect of human insulin on the activities of PP2A, PKA and cAMP-PDE was studied under two different conditions of growth limitation which support glycogen storage in yeast. The data obtained suggest that these enzymes may function as key regulators in mediating the insulin-like effects on glycogen metabolism in yeast [45]. The molecular mechanisms by which nutrients control yeast physiology, in general, and glycogen metabolism, in particular, are far from clear. Genetic and biochemical evidence suggests that in yeast as invertebrates, GP and GS activities can be modulated at the transcriptional level [47-49] and in addition, through both allosteric and phosphorylation/de-phosphorylation-dependent mechanisms [50-52]. Phosphorylation causes inactivation of GS and activation of GP, whereas de-phosphorylation exerts the opposite effects. The activity of GSY-2, the rate-limiting enzyme of the two isoforms of GS increases during the growth cycle in batch culture on glucose medium in parallel with the accumulation of glycogen [53]. GSY-2 is thought to be regulated post-translationally by phosphorylation at three sites close to the carboxy-terminus of the protein [54]. Mutation of these serine residues to alanine or truncation of the protein to remove all three sites leads to a constitutively hyperactive GSY-2 protein that will result in overaccumulation of glycogen in a wild-type background by bypassing the phosphorylation controls. These findings indicate the importance of phosphorylation/de-phosphorylation in the regulation of GSY-2. In addition to protein phosphatase type 1 (PP1), PP2A has been implicated in the regulation of GSY-2. In addition to PP1, PP2A has been implicated in the regulation of glycogen metabolism in higher eukaryotic cells [55] and in fact, a type 2A activity was recognized in vitro as a GS phosphatase in yeast [56]. In *Saccharomyces cerevisiae,* two genes (PPH21 and PPH22) encode closely related proteins that are highly similar to the catalytic subunits of mammalian PP2A [57,58] and account for most of the PP2A activity in yeast cells [57]. As in mammals, the yeast catalytic subunits probably interact with at least two regulatory subunits, encoded by the genes TPD3 (similar to the mammalian A subunit) and CDC55 (similar to the mammalian B subunit) [58-60] The decrease in total PP2A activity by progressive and regulated depletion of the PPH22 gene product in a mutant yeast strain, in which the PPH21 gene was deleted, correlated with reduced accumulation of glycogen and a more pronounced inactivation of GS. On the other hand,

GP became more resistant to inactivation [40]. In addition, deletion of the SIT4 gene, which codes for a protein distantly related to the catalytic subunit of mammalian PP2A, resulted in activation of GP and inactivation of GS [61]. These observations suggest a role of PP2A in controlling the activation states of both enzymes in yeast The cAMP pathway also has been implicated in coupling glycogen storage to the supply of nutrients in yeast. It is assumed that it does so by controlling the phosphorylation states of GSY-2 and GP and, thus, by counteracting PP2A action. For instance, defects in the BCY1 gene, which encodes the regulatory subunit of the PKA, lead to a constitutively active kinase and result in hypersensitivity to starvation and inability to sporulate. In contrast, mutants with an attenuated cAMP pathway (e.g. in a ras2 genetic background) sporulate even in rich media [62]. These strains also display aberrant glycogen accumulation, with ras2 mutants showing hyperaccumulation and bcy1 mutants being unable to synthesize glycogen. Consequently, it has been suggested that the glycogen accumulation phenotypes of cAMP pathway mutants reflect posttranslational controls of yeast GS by PKA. A 53-kDa plasma membrane protein has been identified previously in *Saccharomyces cerevisiae* which specifically binds human insulin with Kd-values at 0.3 to 0.7 µM. Surprisingly, in response to insulin, this insulin-binding protein undergoes phosphorylation on serine residues, exclusively [44]. This modification may be due to auto-phosphorylation or, alternatively, to a distinct insulin-responsive serine-specific kinase activity contained in the binding protein preparation. These properties clearly differ from those of the mammalian insulin receptor [63-65]. The yeast insulin-binding protein also differs from the putative insulin receptor from *Neurospora crassa* which migrated as a single band of 66 kDa on reducing SDS-PAGE but as two polypeptides of about 55 and 110 kDa on native PAGE [40,66,67]. The latter species has been suggested to represent the dimeric form of the functional, i.e. insulin-binding receptor which possesses no auto-phosphorylation activity or protein kinase activity towards exogenous substrates, and which contains no detectable phosphorylated amino acids. Conversely, in intact *Neurospora crassa* cells, the insulin-stimulated tyrosine phosphorylation of a 38-kDa protein has been detected that fails to bind insulin [66]. One may speculate that simple unicellular eukaryotes contain an insulin receptor tyrosine kinase consisting of distinct insulin-binding and tyrosine kinase subunits as realized invertebrates, which in case of *Saccharomyces cerevisiae* are non-covalently linked together the available data suggest that a signal transduction cascade linking the initial events of specific insulin-binding and phosphorylation of the corresponding insulin-binding protein to the metabolic end effector systems, such as GS, via modulation of the levels of relevant signaling molecules or second messengers and the activities of corresponding key regulatory enzymes, among them PP2A and PKA, has not evolved primarily for the typical (neuro) endocrine's system of multicellular invertebrates and vertebrates. Rather the insulin signaling cascade seems to be evolutionary much more ancient than has been thought and may originally constitute the regulatory component of metabolic, biosynthetic and proliferative pathways which are very characteristic for the physiology of yeast. In this sense, it may guarantee a more efficient storage of glycogen under conditions of limited glucose availability. Importantly, in former years *Saccharomyces cerevisiae* and *Tetrahymena thermophila* have been studied for a potential role of insulin in regulating cell proliferation in unicellular eukaryotes [68]. For this, an in vivo assay system was developed based on diluted Wickerham's medium containing 2% glycerol as the only carbon source in which the effects of insulin could be easily monitored. Addition of low concentrations of insulin significantly reduced the lag phase of growth of *Saccharomyces cerevisiae* [69]. Interestingly, activators of PKC, phorbol ester (PMA) or oleyl acetyl glycerol (DAG), in combination with bradykinin, a mammalian intercellular signaling molecule, which leads to a rise in intracellular Ca^{2+} caused a considerable shortening of the lag phase to a similar degree as insulin, whereas PMA and DAG alone were ineffective [69,70]. Furthermore, PMA and insulin-induced a synergistic effect, which enabled the cells to start proliferation within one hour of lag phase [69]. These results hinted to insulin stimulation of the transition of cells from the lag phase to proliferation via participation of a Ca^{2+} dependent PKC isoform. Altogether these findings suggest that an insulin-like signal transduction cascade is somehow involved in the regulation of metabolic and/or proliferative pathways in unicellular eukaryotes, such as yeast, which uses components homologous to those operating in the different branches of metabolic and mitogenic insulin signaling tissues, such as PP2A, PKA, and PKC. During evolution this ancient signaling pathway may have been adapted by multicellular [71] invertebrates and vertebrates predominantly for control of blood glucose homeostasis Strikingly, sequence-based searches gave no hints that budding yeast harbors members of the true protein tyrosine kinase family present in mammalian cells e.g. insulin receptor ß-subunit; for a review [72] Therefore, it may be anticipated that the predominant driving force for the evolution of protein tyrosine kinases was the requirement for signaling mechanisms devoted exclusively to cell-cell communication within multicellular organisms. However, based on sequence analysis, yeast despite the absence of true protein tyrosine kinases contains several so-called dedicated protein tyrosine kinases, so-called dedicated protein tyrosine kinases of both single specificity, such Swe1 [73] and dual specificity, such as Spk1 and Mps1 [74] with members of the MAPKK family among the latter. These dual-specify kinases have been demonstrated to (auto) phosphorylate serine, threonine and tyrosine residues in vitro, some of them within the typical TXY motif in the activation loop of the MAPK. Furthermore, several genes of *Saccharomyces cerevisiae*, among them YGR080W, are homologous to the sequence of the mammalian protein tyrosine kinase A6 [72]. However, the structure of this kinase is totally unrelated to that of the true protein tyrosine kinase family [75,76].

Conclusion

Taken together, the experimental data so far available provide some evidence for the existence of insulin-like and mimetic signal transduction cascades in unicellular eukaryotes like *Saccharomyces cerevisiae* which may represent the ancestors of the corresponding insulin signaling pathways in mammalian cells.

References

1. Jaldin-Fincati JR, Pavarotti M, Frendo-Cumbo S, et al. Update on GLUT4 vesicle traffic: A cornerstone of insulin action. Trends Endocrinol Metab. 2017;28:597-611.
2. Kubota T, Kubota N, Kadowaki T. Imbalanced insulin action in obesity and type 2 diabetes: Key mouse models of insulin signaling pathway. Cell Metab. 2017;25,797-810.
3. Tokarz VL, MacDonald PE, Klip A. The cell biology of systemic insulin function. J Cell Biol. 2018;217:2273-89.
4. Vitali V, Horn F, Catania F. Insulin-like signaling within and beyond metazoans. Biol Chem. 2018;399:851-57.
5. Kimura KD, Tissenbaum HA, Liu Y, et al. Daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science. 1997;277:942-946.
6. Uno M, Nishida E. Lifespan-regulating genes in *Caenorhabditis elegans*. Aging Mech Dis. 2016;2:16010.
7. Morris JZ, Tissenbaum HA, Ruvkun G. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. Nature. 1996;382:536-39.
8. Kenyon C, Chang J, Gensch E, et al. *C. elegans* mutant that lives twice as long as wild type. Nature. 1993;366:461-64.
9. Kenyon C. The first long-lived mutants: discovery of the insulin/IGF-1 pathway for ageing. Philos Trans R Soc Lond B Biol Sci. 2011;366:9-16.
10. Larsen PL, Albert PS, Riddle DL. Genes that regulate both development and longevity in *Caenorhabditis elegans*. Genetics. 1995;139:1567-83.
11. Avruch J. Insulin signal transduction through protein kinase cascades. Mol Cell Biochem. 1998;182:31-48.
12. Gottlieb S, Ruvkun G. daf-2, daf-16 and daf-23: genetically interacting genes controlling Dauer formation in *Caenorhabditis elegans*. Genetics. 1994;137:107-20.
13. Lin K, Dorman JB, Rodan A, et al. Daf-16: An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. Science, 1997;278:1319-22.
14. Ogg S, Paradis S, Gottlieb S, et al. The Fork head transcription factor daf-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature 1997;389:994-99.
15. Paradis S, Ruvkun G. *Caenorhabditis elegans* Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. Genes Dev. 1998;12:2488-98.
16. Riddle DL. *Caenorhabditis elegans* II, D Riddle, T Blumenthal, B Meyer, J Priess (Ed.) Cold spring harbor laboratory press, cold spring harbor NY. 1992b:791-813.
17. Voweis JJ, Thomas JH. Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. Genetics. 1992;130:105-13.
18. Thomas JH, Birnby DA, Voweis JJ. Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. Genetics. 1993;134:1105-10.
19. Thomas JH, Inoue T. Methuselah meets diabetes. Bioessays. 1998;20:113-15.
20. Dorman JB, Albinder B, Shroyer T, et al. The age-1 and daf-2 genes function in a common pathway to control the lifespan of *Caenorhabditis elegans*. Genetics. 1995;141: 1110-1399.
21. Gil EB, Link EM, Liu LX, et al. Regulation of the insulin-like developmental pathway of *Caenorhabditis elegans* by a homolog of the PTEN tumor suppressor gene. Proc Natl Acad USA. 1999;96:2925-30.
22. Friedman DB, Johnson TE. A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. Genetics. 1988;118:75-86.
23. Apfeld J, Kenyon C. Cell nonautonomy of *C. elegans* daf-2 function in the regulation of diapause and life span. Cell. 1998;95:199-210.
24. Wood WB. Aging of *C. elegans*: Mosaics and mechanisms. Cell. 1998;95:147-50.
25. Kaeberlein TL. Lifespan extension in *Caenorhabditis elegans* by complete removal of food. Aging Cell. 2006;5:487-94.
26. Uno M, Honjoh S, Matsuda M, et al. A fasting-responsive signaling pathway that extends life span in *C. Elegans.* Cell Rep. 2013;3:79-91.
27. Heimbucher T, Liu Z, Bossard C, et al. The deubiquitylase MATH-33 controls DAF-16 stability and function in metabolism and longevity. Cell Metab. 2015;22:151-63.
28. Legros F, Conrad V. Effects of insulin on oxygen uptake of *Acetabularia mediterranea*. Horm Res. 1973;4:107-13.
29. Legros F, Uytdenhoef P, Dumont I, et al. Specific binding of insulin to the unicellular alga *Acetabularia mediterranea*. Protoplasma. 1975;86:119-34.
30. Csaba G, Sudar F, Nagy SU, et al. Localization of hormone receptors in Tetrahymena. Protoplasma. 1977;91:179-89.
31. Christensen ST, Quie H, Kemp K, et al. Insulin produces a biphasic response in Tetrahymena thermophila by stimulating cell survival and activating proliferation in two separate concentration intervals. Cell Biol Internatl. 1996;20:437-44.
32. Csaba G, Lantos T. Effect of insulin on the glucose uptake of protozoa. Experientia. 1975;31:107-09
33. McKenzie MA, Fawell SE, Cha M, et al. Effects of mammalian insulin on metabolism, growth, and morphology of a wall-less strain of *Neurospora crassa*. Endocrinology. 1988;122:511-17.
34. Fawell SE, Lenard J. A specific insulin receptor and tyrosine kinase activity in the membranes of Neurospora crassa. Biochem Biophys Res Commun. 1988;155:59-65.

35. Fawell SE, McKenzie MA, Greenfield NJ, et al. Stimulation by mammalian insulin of glycogen metabolism in a wall-less strain of Neurospora crassa. Endocrinology. 1988;122:518-23.
36. Greenfield NJ, McKenzie MA. Metabolism of D-glucose in a wall-less mutant of *Neurospora crassa* examined by 13C and 31P nuclear magnetic resonances: effects of insulin. Biochemistry. 1988;27:8526-33.
37. Flawia MM, Torres HN. Activation of membrane-bound adenylate cyclase by glucagon in *Neurospora crassa*. Proc Natl Acad Sci USA. 1972;69:2870-74.
38. Flawia MM, Torres HN. Adenylate cyclase activity in *Neurospora crassa*. Modulation by glucagon and insulin. J Biol Chem. 1973;248:4517-23.
39. Muller G, Rouveyre N, Crecelius A, et al. Insulin signaling in the yeast *Saccharomyces cerevisiae* stimulation of glucose metabolism and Snf1 kinase by human insulin. Biochemistry. 1998;37:8683-95.
40. Clotet J, Posas F, Hu G-Z et al. Role of protein phosphatase 2A in the control of glycogen metabolism in yeast. Eur J Biochem. 1995;229:207-14.
41. Hardy TA, Huang D, Roach PJ. Interactions between cAMP-dependent and SNF1 protein kinases in the control of glycogen accumulation in *Saccharomyces cerevisiae*. J Biol Chem. 1984;269:27907-13.
42. Celenza JL, Carlson M. A yeast gene that is essential for release from glucose repression encodes a protein kinase. Science. 1986;233:1175-80.
43. Celenza JL, Carlson M. Mutational analysis of the *Saccharomyces cerevisiae* SNF1 protein kinase and evidence for functional interaction with the SNF4 protein. Mol Cell Biol. 1989;9:5034-44.
44. Muller G, Rouveyre N, Upshon C, et al. Insulin signaling in the yeast *Saccharomyces cerevisiae* induction of protein phosphorylation by human insulin. Biochemistry. 1998b;37:8705-13.
45. Muller G, Grey S, Jung C, et al. Insulin-like signaling in yeast: Modulation of protein phosphatase 2A, protein kinase A, cAMP-specific phosphodiesterase, and glycosyl-phosphatidylinositol-specific phospholipase C activities. Biochemistry. 2000;39:1475-88
46. Muller G, Rouveyre N, Upshon C, et al. Insulin signaling in the yeast *Saccharomyces cerevisiae* interaction of human insulin with a putative binding protein. Biochemistry. 1998a;37:8696-8704.
47. Hwang PK, Tugendreich S, Fletterick RJ. Molecular analysis of GPH1, the gene encoding glycogen phosphorylase in *Saccharomyces cerevisiae*. Mol Cell Biol. 1989;9:1659-66.
48. Farkas I, Hardy TA, Goebl MG, et al. Two glycogen synthase isoforms in *Saccharomyces cerevisiae* are coded by distinct genes that are differentially controlled. J Biol Chem. 1991;266:15602-607.
49. Rothman-Denes LB, Cabib E. Two forms of yeast glycogen synthetase and their role in glycogen accumulation. Proc Natl Acad Sci USA. 1971;66:967-74.
50. Rothman-Denes LB, Cabib E. Glucose 6-phosphate dependent and independent forms of yeast glycogen synthetase. Their properties and interconversions. Biochemistry. 1971;10:1236-42.
51. Huang KP, Cabib E. Separation of the glucose-6-phosphate independent and dependent forms of glycogen synthetase from yeast. Biochem Biophys Res Commun. 1972;49:1610-16
52. Becker JU, Wingender-Drissen R, Schiltz E. Purification and properties of phosphorylase from baker's yeast. Arch Biochem Biophys. 1983;225:667-678
53. Farkas I, Hardy TA, DePaoli-Roach AA, et al. Isolation of the GSY1 gene encoding yeast glycogen synthase and evidence for the existence of a second gene. J Biol Chem. 1990;265:20879-86.
54. Hardy TA, Roach PJ. Control of yeast glycogen synthase-2 by COOH-terminal phosphorylation. J Biol Chem. 1993;268:23799-805
55. Ingebritsen TS, Foulkes JG, Cohen P. The protein phosphatases involved in cellular regulation. Glycogen metabolism. Eur J Biochem. 1983;132:263-74
56. Peng ZY, Wang W, Wilson SE, et al. Identification of a glycogen synthase phosphatase from yeast *Saccharomyces cerevisiae* as protein phosphatase. J Biol Chem. 1991;266:10925-32
57. Sneddon AA, Cohen PTW, Stark MJR. Identification of a glycogen synthase phosphatase from yeast *Saccharomyces cerevisiae* as protein phosphatase 2A. EMBO J. 1990;9:4338-46.
58. Ronne HM, Carlberg M, Hu GZ, et al. Protein phosphatase 2A in *Saccharomyces cerevisiae*: Effects on cell growth and bud morphogenesis. Mol Cell Biol. 1991;11:4876-84.
59. Healy AM, Zolnierowicz S, Stapleton AE, et al. CDC55, a *Saccharomyces cerevisiae* gene involved in cellular morphogenesis: Identification, characterization, and homology to the B subunit of mammalian type 2A protein phosphatase. Mol Cell Biol. 1991;11:5767-80
60. Van Zyl W, Huang W, Sneddon AA, et al. Inactivation of the protein phosphatase 2A regulatory subunit: A results in morphological and transcriptional defects in *Saccharomyces cerevisiae.* Mol Cell Biol. 1992;12:4946-59
61. Posas F, Clotet J, Arino J. *Saccharomyces cerevisiae* gene SIT4 is involved in the control of glycogen metabolism. FEBS Lett. 1991;279:341-45
62. Toda T, Uno I, Ishikawa T, et al. In yeast, RAS proteins are controlling elements of adenylate cyclase. Cell. 1985;40:27-36
63. Lee AH, Glimcher L. Intersection of the unfolded protein response and hepatic lipid metabolism. Cell Mol Life Sci. 2009;66:2835-50

64. De Meyts P. Insulin/receptor binding: the last piece of the puzzle? What recent progress on the structure of the insulin/receptor complex tells us about negative cooperativity and activation. Bioessays. 2015;37:389-97

65. Belfiore A, Malaguarnera R, Vella V, et al. Insulin receptor isoforms in physiology and disease: An updated view. Endocr Rev. 2017;38:379-431

66. Kole HK, Lenard J. Insulin-induced stimulation of protein phosphorylation in *Neurospora crassa* cells. Faseb J. 1991;5:2728-34.

67. Kole HK, Muthukumar G, Lenard J. Purification and properties of a membrane-bound insulin binding protein, a putative receptor, from *Neurospora crassa*. Biochemistry. 1991;30:682-88

68. Christensen ST, Leick V, Rasmussen L, et al. Signaling in unicellular eukaryotes. Internatl Rev Cytol. 1988;177:181-253

69. Friis J, Szablewski L, Christensen ST, et al. Physiological studies on the effect of Ca^{2+} on the duration of the lag phase of *Saccharomyces cerevisiae*. FEMS Microbiol Lett. 1994;123:33-36

70. Overgaard AK, Friis J, Christensen L, et al. Effects of glucose, tetrapyrroles and protein kinase C activators on cell proliferation in cultures of *Saccharomyces cerevisiae*. FEMS Microbiol Lett. 1995;132:159-63.

71. Roberts RA, Gullick WJ. Bradykinin receptor number and sensitivity to ligand stimulation of mitogenesis is increased by expression of a mutant ras oncogene. J Cell Sci. 1989;94:527-35.

72. Hunter T, Plowman GD. The protein kinases of budding yeast: Six score and more. Trends Biochem Sci. 1997;22:18-22

73. Booher RN, Deshaies R, Kirschner MW. Properties of *Saccharomyces cerevisiae* wee1 and its differential regulation of p34CDC28 in response to G1 and G2 cyclins. EMBO J. 1993;12:3417-26.

74. Zheng P, Fay DS, Burton J, et al. SPK1 is an essential S-phase-specific gene of *Saccharomyces cerevisiae* that encodes a nuclear serine/threonine/tyrosine kinase. Mol Cell Biol. 1993;13:5829-42.

75. Flawia MM, Torres HN. Insulin inhibition of membrane-bound adenylate cyclase in *Neurospora crassa*. FEBS Lett. 1973;30:74-78

76. Riddle DL. *Caenorhabditis elegans* II, D Riddle, T Blumenthal, B Meyer, J Priess (Ed.) Cold spring harbor laboratory press, cold spring harbor NY, 1992a:739-68.

***Correspondence to**

Muller GA
Helmholtz Diabetes Center (HDC) at the Helmholtz Center Munchen, Institute for Diabetes and Obesity
Germany
Tel: +498931872048
E-mail:guenter.mueller@helmholtz-muenchen.de