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NOVEL ROLES FOR INSULIN RECEPTOR (IR) IN ADIPOCYTES AND SKELETAL MUSCLE CELLS VIA NEW AND UNEXPECTED SUBSTRATES

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Abstract

The insulin signaling pathway regulates whole-body glucose homeostasis by transducing extracellular signals from the insulin receptor (IR) to downstream intracellular targets, thus coordinating a multitude of biological functions. Dysregulation of IR or its signal transduction is associated with insulin resistance, which may culminate in type 2 diabetes (T2D). Following initial stimulation of IR, insulin signaling diverges into different pathways, activating multiple substrates which have roles in various metabolic and cellular processes. The integration of multiple pathways arising from IR activation continues to expand as new IR substrates are identified and characterized. Accordingly, our review will focus on roles for IR substrates as they pertain to three primary areas: Metabolism/glucose uptake, Mitogenesis/growth, and Aging/Longevity. While IR functions in a seemingly pleiotropic manner in many cell types, through these three main roles in fat and skeletal muscle cells, IR multi-tasks to regulate whole-body glucose homeostasis to impact healthspan and lifespan.

Keywords

insulin receptor; skeletal muscle; adipose tissue; insulin signaling; insulin receptor substrate; metabolism; mitogenesis; longevity

INTRODUCTION

Glucose homeostasis is regulated by the insulin signaling network, which transduces signals from extracellular stimuli such as insulin, to downstream intracellular effectors. Alteration of the insulin signaling pathway is often associated with insulin resistance, a major contributor to the development of type 2 diabetes (T2D). Insulin signaling initiates at one central start point – the insulin receptor (IR), which then proliferates, via several important focal points along multiple pathways. The insulin-induced signaling pathways regulating metabolic and mitogenic functions have been extensively studied in various cell types. And yet, despite established knowledge that the canonical insulin signaling cascade influences

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insulin sensitivity mechanisms, newly discovered IR substrates and interactions suggest that IR multitasks to orchestrate and achieve sensitivity through novel and unexpected means. Progress in the past two decades has uncovered novel substrates and roles for IR. It has also become apparent that insulin signaling and IR are linked to longevity, such that insulin resistance and diabetes is associated with shortened lifespan. Herein, we focus our attention on three major roles for IR in skeletal muscle cells and adipocytes, integrating findings of new and unexpected IR substrates in established signaling cascades of IR function in these specific cell types: Glucose Uptake, Growth/Mitogenesis, and Aging/Longevity.

Discovery of the Insulin Receptor

In 1971, Cuatrecas and Kono demonstrated insulin binding to cell surfaces and identified insulin receptor (IR) as a glycoprotein that was bound by insulin [1,2]. This was followed by the successful isolation of IR from rat liver and adipocyte membranes [3]. IR was revealed to have α - and β -subunits, with the α -subunit constituting a predicted extracellular ligand binding domain [4,5,6,7]. In the same year, IR was determined to be a protein kinase [8]. In adipocytes, hepatoma cells, and Chinese hamster ovary (CHO) cells, IR was demonstrated to undergo tyrosine phosphorylation following insulin stimulation [4,9,10,11], and the autophosphorylation of IR was found to be a critical event for the downstream transmission of the insulin signal [12,13,14,15,16]. In parallel with investigations into IR kinase activity, Human IR was cloned and sequenced simultaneously by two groups followed by determination of IR translation and its susceptibility to proteolytic cleavage [17], along with the structure of the IR gene promoter [18]. In 1994, the crystal structure of the tyrosine kinase domain of human IR was solved [19]. The IR gene was also found in lower eukaryotes like *Drosophila*, *C. elegans*, and hydra [20]. Followed by discovery of insulin receptor substrate (IRS) by White and colleagues, the search for additional IR substrates was exceedingly active in the 1990's, such that seven different downstream substrates for IR were identified within that decade [21,22,23]. These discoveries led to rapid progress in defining two main roles for IR in glucose homeostatic mechanisms of skeletal muscle and fat cells: 1) Metabolic, through glucose uptake and 2) Growth/Mitogenesis [24,25]. Through its ability to impact both metabolism and mitogenesis, more recent findings have established a third role for IR related to these cell types, in 3) aging and longevity.

Features of the Insulin Receptor

The IR gene contains 22 exons and 21 introns, spanning 150 kb of human chromosome 19 and mouse chromosome 8 [18,26,27,28]. As depicted in Fig. 1, IR is comprised of an extracellular α subunit and a transmembrane-spanning β subunit linked by disulfide bonds. Current models, based upon extensive three-dimensional mapping, depict one molecule of insulin binding to two sites located on each monomer of the α -subunits of IR heterotetramer in trans (named Site 1 and Site 2) [29,30,31]. The high-affinity ($K_D = 300$ pM) binding site created by two alpha subunits in trans [32] uses one face of insulin to interact with the L1 domain of Site 1 in one subunit and a second face of the same insulin molecule to interact with the alpha-CT region in the other α -subunit of IR [33]. This trans interaction induces a conformational change to the extracellular domain of IR, compacting it for concomitant signal transduction, further corroborated by crystallographic refinement [34], which supported prior functional mutagenesis studies of the Steiner group [32]. This two-alpha-subunit-in-trans feature of insulin binding to IR is highly conserved, dating back many millions of years [35].

Upon insulin binding, a conformational change to the intracellular portion of the IR protein occurs, resulting in activation of the β subunit's tyrosine kinase activity and autophosphorylation of the kinase regulatory domain, leading to phosphorylation of the juxtamembrane tyrosine residues that function as docking sites for a wide range of substrates

[14,36]. These events are followed by an additional conformational change within the β subunit, unmasking important substrate binding sites and, thus, stabilizing the receptor in an active conformation [37].

Insulin Receptor Trafficking

A single mRNA which encodes both α and β subunits of IR is synthesized in the endoplasmic reticulum, and subsequently translated into the pre-pro-receptor of 210 kD that undergoes both proteolytic cleavage and post-translational modifications to yield mature α and β subunits of 135 and 95 kD, respectively [5,17,38]. IR then translocates to the plasma membrane (PM), where it is found localized in the caveolae and clathrin-coated pits [39,40]. Following insulin stimulation IR is endocytosed, and once in the endosomes, insulin is released from IR via proteolytic degradation. IR is then recycled to the cell surface, while insulin is degraded in the lysosomes [41,42].

Insulin Receptor Gene Structure, Expression, and Localization

The highest expression of IR is found in adipocytes, while relative expression in skeletal muscle cells is 10-fold less [43]. Immunogold and immunofluorescence microscopy studies show that IR can localize to caveolar microdomains of the PM through interactions with the protein Caveolin, and recent studies using qualitative electron microscopy demonstrate IR to be predominately concentrated in the neck of the caveolae, which constitutes 17% of the adipocyte PM [44,45]. Caveolin proteins, which will be described in greater detail below, are important for propagating the IR signal to downstream signal transduction and glucose transport targets [46].

Insulin Receptor Isoforms

In addition to multiple locales for IR, there are also multiple IR variants in fat and muscle cells. These IR variants exist due to alternative splicing of exon 11; exon 11 encodes 12 additional amino acids that are absent from the extracellular domain of isoform A (IR-A), but are present in isoform B (IR-B) [47,48]. The two IR isoforms have different tissue distributions, ligand specificities, kinetics and internalization rates, allowing for tissue-specific regulation. The 12 extra amino acids present in the B isoform act as a sorting signal to localize it to distinctive microdomains within the PM that may have specific regulatory functions [49]. Although ubiquitously expressed, IR-A is principally found in cancer cells and fetal tissues, while IR-B is predominantly expressed in insulin-sensitive tissues. Insulin and IGF, especially IGF-2, bind to IR-A with greater affinity than IR-B [50,51,52]. In regards to IR-A, an insulin stimulus initiates metabolic activity, whereas IGF-2 stimulation activates mitogenic signaling - the variation in activation being due to the differential recruitment and activation of substrates [53].

Inhibitors of Insulin Receptor

Down-regulation of IR signaling occurs by serine/threonine phosphorylation and tyrosine phosphatases [54,55]. De-phosphorylation of IR decreases its kinase activity and is important for IR regulation in insulin action. IR inhibitors include PC-1, pp63, GRB10 and protein tyrosine phosphatases like LAR, SHP-1 and PTPIB. PC-1 (plasma cell antigen-1) is a nucleotide pyrophosphatase, encoded by the ENPP1 gene, and a recent study suggests that variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes [56]. Further supporting this, earlier work had shown that PC-1 levels correlate inversely with insulin sensitivity in humans [57]. The gene for pp63, also known as AHSG and fetuin, is located on human chromosome 3q27, recently identified as a susceptibility locus for type 2 diabetes and the metabolic syndrome [58]. In AHSG knockout mice, IR phosphorylation levels and downstream signaling were increased,

correlating with increased insulin sensitivity [58]. GRB10 is an adapter protein which upon over expression inhibits IR-stimulated AKT and MAPK activity and causes insulin resistance [59]. Conversely, GRB10 knockout mice exhibited improved insulin signaling and peripheral insulin sensitivity, altogether supporting a role for GRB10 as a negative regulator of insulin signaling *in vivo* [60]. Lastly, protein tyrophosphate PTPIB knockout mice display improved insulin sensitivity, specifically in muscle, by maintaining IR in its tyrosine phosphorylated state; recent studies also demonstrate a neuronal role for PTPIB in regulation of leptin production, ultimately improving insulin sensitivity [61,62,63].

IR-RELATED PATHOLOGIES: FROM MOUSE TO HUMAN

IR pathologies are commonly linked to insulin resistance in skeletal muscle and/or adipocytes. Insulin resistance is a condition, in which the body produces insulin, yet the skeletal muscle and fat cells are refractory to insulin because IR exhibits a diminished capacity to respond. As a result, the ability of insulin to propagate an intracellular response signal to downstream messengers is diminished [64,65]. Failure in IR-related insulin signaling emanates from several aspects of IR function: insulin/ligand binding defects, tyrosine kinase activation defects, ligand-stimulated IR internalization and degradation, as well as inappropriate dissociation of insulin-IR complexes, leading to reduced availability of free receptors, all of which have been shown to contribute to T2D [66,67,68]. For example, in humans and animal models of peripheral insulin resistance, insulin target tissues have shown a significant ~50% decrease in IR kinase activity [69,70,71,72,73]. Moreover, T2D patients show reduced IR autophosphorylation in muscle biopsies obtained following an insulin infusion [74,75,76]. Obese individuals show decreased insulin binding in skeletal muscle, adipose tissue, and liver, due to reduced receptor abundance without an alteration in ligand-receptor binding affinity [68]. Similar to humans, obese mice also exhibit attenuated IR autophosphorylation compared to lean mice [72]. Decreased tyrosine phosphorylation of IR is likewise seen in cases of human gestational diabetes [57,77]. Chronic physical activity increases the signaling capacity of IR, and its function rapidly declines after decreases in physical activity [78,79]. As depicted in Table 1, human cases of either homozygous or heterozygous mutations in the α - or β -subunits of IR can ultimately lead to diseases such as Leprechaunism, Rabson-Mendenhall syndrome, or Type A insulin resistance [80,81]. Type A insulin resistance can also be linked to mutations in the IR proteolytic cleavage site [82]. Alternately, Type B insulin resistance is linked to the presence of IR autoantibodies, while myotonic dystrophy (MD1/MD2) results in insulin resistance and diabetes-like symptoms linked to an increased ratio of IR-A:IR-B expression [83,84,85].

IR Knockout Mouse Models

Three general categories of IR knockout mouse models have been generated since the advent of gene ablation technology in the 1980-90's: classic whole-body knockout, tissue-specific conditional knockout, and IR knock-in mice on the background of the classic knockout (Table 2). The classic knockout models were initiated in two independent laboratories, and both IR knockout (KO) mouse models displayed diabetic ketoacidosis [86,87]. Remarkably, the IR KO mice die within a few days after birth, which firmly established the requirement of IR for growth and development. However, IR heterozygous mice showed normal growth and glucose tolerance [87]. This phenomenon was similarly observed in humans carrying IR mutations: individuals with homozygous mutations of IR (Leprechaunism) have a high mortality, whereas patients with heterozygous mutations of IR (Type A) display normal growth (Table 1). In addition, IR heterozygous mice bred with IRS-1 or IRS-1/IRS-2 heterozygous mutant mice present with a more severe phenotype, including insulin resistance and diabetes [88,89].

More nuanced gene ablation came several years later with conditional knockout technologies, permitting selective ablation in skeletal muscle, white versus brown adipocytes, as well as a host of other tissues (which is beyond the scope of this review). This provided some surprising and unexpected findings, broadening our understanding of the relative role and requirement for IR in each cell type and in the context of whole body compensatory (or lack thereof) and cross-talk mechanisms. For example, skeletal muscle-specific IR knockout (MIRKO) mice did not develop insulin resistance or diabetes even though decreased IR kinase activity and decreased insulin-dependent glucose uptake in muscle were observed (Table-3 [90]), indicating that compensatory mechanisms including increased glucose uptake by adipose and suppressed gluconeogenesis in the liver were able to prevent the development of diabetes. In contrast, fat-specific IR knockout (FIRKO) mice have significant defects in insulin-stimulated glucose uptake, as expected based on years of work using isolated adipocytes, yet failed to develop insulin resistance or abnormal glucose tolerance, which was anticipated since the muscle is believed to account for 80% of glucose disposal [91,92,93]. Perhaps less expected however was that the FIRKO mice showed enhanced longevity (described later in *Role III: IR, Insulin Resistance and Aging*) with decreased fat mass and increased insulin sensitivity [94]. Brown adipocyte-specific IR knockout (BAIRKO) mice displayed glucose intolerance, yet displayed no insulin resistance, similar to that of the FIRKO mice [95]. GIRKO mice, which have IR simultaneously ablated in all GLUT4 expressing tissues (rather than in a single tissue), including skeletal muscle, white/brown adipose tissues and part of the CNS, do indeed develop insulin resistance and diabetes [96]. However, GIRKO mice also showed hepatic insulin resistance and beta cell dysfunction, and it was speculated that these defects may be resultant from impaired control of pancreas function, both of beta cells and other pancreatic cell types.

Accili and colleagues also generated knock-in mice, which express a kinase-defective insulin receptor mutation (a K1030M substitution) on the genetic background of the classic IR^{+/-} knockout mouse (IR^{+/-}:K1030M). Given that this mutant is known to inhibit the function of dimeric IR, when it was “knocked-in” specifically to skeletal muscle [97] and fat cells [98] it resulted in impaired glucose intolerance. As such, the GIRKO mice exhibit a more severe phenotype than either the MIRKO or FIRKO mouse models, likely because the IR^{+/-}:K1030M disrupts IR function simultaneously in both skeletal muscle and adipose tissues, rather than each tissue independently. Another such IR knock-in mouse model, IR^{P1195L/P1195L} homozygous mice (a P1195L substitution in the kinase domain of the β -subunit), yielded an even more severe phenotype, diabetic ketoacidosis, while IR^{P1195L/Wt} heterozygous mice grew normally but were hyperinsulinemic and insulin resistant [99] (Table 2). In a third IR knock-in model, IR was re-expressed in GLUT4 expressing tissues (GIRKI) on the classic IR^{-/-} knockout background, but failed to rescue the diabetic phenotype of the IR KO [100], suggestive of a tremendous level of cross-talk between tissue systems dependent on IR and its signaling.

INSULIN RECEPTOR ROLES AND SUBSTRATES IN FAT AND SKELETAL MUSCLE CELLS

IR utilizes several distinct substrates to fulfill its functions in the three main roles discussed in detail within this review: *I. Metabolism; II. Growth/Mitogenesis, III. Aging/Longevity*. These substrates are subdivided into categories, corresponding to their respective roles, as grouped in Fig. 2:

Role I – IRS-1/2, Caveolin-1 (Cav-1), Caveolin-3 (Cav-3), APS/CAP/Cbl, Munc18c

Role II – Shc, IRS-1, Gab-1, STAT-5, Jak-1, aP2

Role III – Aspects of substrates used in both metabolism and mitogenesis.

Role I: Metabolism – Glucose Uptake and the Maintenance of Glucose Homeostasis

Glucose homeostasis is maintained by the coordinated efforts of insulin release from the pancreatic islet beta cell in response to elevated blood glucose levels, and the response to that insulin by the peripheral insulin responsive tissues (skeletal muscle and adipose) to clear the excess glucose, thereby restoring glucose homeostasis. To this end, IR signals to multiple substrates in these tissues: IRS proteins, APS/Cbl/CAP complexes, and most recently, Munc18c, a non-classical signaling component of the SNARE (soluble N-ethylmaleimide sensitive factor attachment receptor) machinery. IR signaling through these substrates is coordinated in multiple cascades to evoke the main event required for glucose uptake by these cell types: the mobilization of insulin-responsive GLUT4-laden vesicles from intracellular storage pools to the cell surface [101,102,103]. Once at the surface, GLUT4 proteins are incorporated into the PM to facilitate entry of glucose into the cells and out of circulation. To cause this, insulin-activated IR binds and phosphorylates IRS proteins (Fig. 3). The IRS proteins signal downstream to elicit a PI3 kinase-dependent signaling cascade, whilst APS/CAP/Cbl-signaling is purportedly an IRS and PI3 kinase-independent signaling cascade [104]. Both pathways converge to evoke GLUT4 vesicle mobilization. In contrast, Munc18c, a member of the SNARE machinery required to dock and fuse GLUT4 vesicles at the PM, is not a traditional signaling cascade element but is directly phosphorylated by IR. By orchestrating these three substrate activation events, IR can propagate the insulin signal within minutes to coordinate both the mobilization and docking/fusion of GLUT4 vesicles to achieve a 3 to 5-fold increase in cellular glucose uptake [105,106,107,108]. Each substrate and downstream effector pathway is described in detail below, with the metabolic phenotypes of corresponding IR substrate knockout mice listed in Table 3.

IRS Signaling Through the PI3K-Dependent Pathway

Insulin Receptor Substrate (IRS-1): IRS-1 initializes the classical PI3 kinase-dependent pathway downstream of IR in the metabolic signaling cascade. IRS-1 contains a pleckstrin homology (PH) and phosphotyrosine binding (PTB) domain which accounts for its intrinsic affinity for IR; specifically, IRS-1 binds via its PTB domain to Tyr960 in IR. The center and C-terminus of IRS proteins act as a hub for scaffolding molecules downstream of IRS. Although IRS-1 is the primary substrate used in skeletal muscle/adipose insulin signaling, six different IRS proteins serve different cellular functions, owing to their differences in tissue distribution and intrinsic activity [109]. For example, both IRS-1 and IRS-2 are expressed in skeletal muscle and adipocytes, yet IRS-1 is principally used in muscle, as knockdown or knockout of IRS-2 in muscle did not affect glucose transport (Table-3 and [110,111]). However, brown adipocytes lacking IRS-2 do show impairment of glucose uptake [110,111], suggesting that some mechanistic departures between fat type (brown versus white) and/or muscle exist. Moreover, even though IRS-3 expression appears to be adipocyte specific, it is not considered a primary IR substrate [112]. Furthermore, while IRS-4 is undetectable in muscle and adipose [113], IRS-5 is ubiquitously expressed and IRS-6 is abundant in skeletal muscle, though their roles in insulin signaling are still unclear. IRS-1, as the primary functional IRS in fat and muscle cells, binds to its main downstream effector, PI3K, through its C-terminus, activating PI3K and propagating the next step of the IR signaling cascade [114]. Importantly, decreased content of IRS-1 is associated with some cases of insulin resistance in animals and humans [115].

PI3K: PI3K is a dimer comprised of an 85 kDa adapter subunit and a 110 kDa catalytic subunit. PI3K contains SH2 domains that bind to specific motifs within IRS [114,116]. PI3K catalyzes the addition of a phosphate at the third position of the inositol ring of

phosphoinositol to generate PIP3. p70S6 kinase, Akt, and PKC are all downstream effectors of PI3K. Once phosphorylated, these substrates serve as docking molecules which bind and activate other cellular kinases, initiating divergent signaling pathways that mediate cellular insulin action. Two forms of Akt, Akt1 and Akt2, expressed in skeletal muscle and adipose cells, are localized near the PM and are phosphorylated by PDK-1 to exert downstream effects in both the cytoplasm and nucleus [117,118]. Akt1 knockout mice have defects in both survival and growth whereas, mice deficient in Akt2 show defective adipose tissue glucose uptake in response to insulin, supportive of a critical role for Akt2 in adipocyte insulin signaling [119,120]. Akt2 signals downstream to AS160 (a Rab GTPase activating protein), targeting several Rabs, such as Rab2a, Rab8a, Rab10, and Rab14, to trigger GLUT4-vesicle trafficking/mobilization, although the identity of the precise target Rab is still unknown [121,122].

APS/CAP/Cbl Signaling Through the PI3K-Independent Pathway—Use of PI3K inhibitors has implicated an IR-dependent but PI3K-independent signaling pathway. One well-described PI3K independent pathway involves the APS/Cbl/CAP complex [123], which provides a second signal cascade that functions in parallel with PI3K to evoke GLUT4 translocation and glucose uptake in response to the insulin-IR signal. As depicted in Fig. 3, this pathway is initiated by recruiting Cbl (a proto-onco protein) to the activated IR via adapter proteins CAP (c-Cbl associated protein) and APS (Adapter protein with PH and SH2 domain) [124,125]. APS is phosphorylated by IR, which in turn phosphorylates Cbl, leading to Cbl association with CAP and CrkII [126]. This complex then translocates to the caveolar-rich region of the PM with the help of flotillin, a lipid raft protein [127,128,129], and along with guanine nucleotide exchange factor C3G, activates TC10 in lipid rafts [130,131]. TC10 binds to a number of downstream effectors such as CIP4, Exocyst, N-Wasp and Arp-2/3, which have roles in actin dynamics [132]. Actin cytoskeleton re-organization is vital in insulin sensitive tissues to form a mesh which is critical for GLUT4 vesicle tracking and translocation to the PM. Actin depolymerization with latrunculin prevents GLUT4 translocation, suggesting a requirement for intact F-actin structure in insulin-stimulated GLUT4 translocation [133,134]. Studies using jasplakinolide, an F-actin nucleating and stabilizing agent, inhibits GLUT4 translocation, further substantiating the role for actin re-organization in glucose transport [135,136]. The various proteins involved in the PI3K-independent pathway are discussed in detail below.

APS: APS was identified in a yeast two hybrid adipocyte library screen using human IR as bait [137,138]. APS binds to IR with more specificity than other members of the adapter family proteins, Lnk and SH2B [139]. APS binds to IR through its SH2 domain and does so within the activation loop of IR [137]. After IR activation, APS is rapidly phosphorylated at Tyr618 (within 5 minutes), followed by rapid de-phosphorylation (within 10 minutes); mutation of Tyr618 ablates IR-mediated phosphorylation of APS [137]. APS expression is highest in skeletal muscle tissue, with adipose showing the second highest levels amongst all tissues examined [137,138]. Despite this, APS deficient mice show no defect in GLUT4 translocation (Table 3). It has been speculated that this may be explained by compensatory actions from other family members such as SH2B, since SH2B has been noted to undergo IR-mediated phosphorylation [139,140]. APS also has a role in actin cytoskeletal organization through the protein Enigma [141]. Enigma and APS co-localize with F-actin in small ruffling structures, and co-localization further increases under insulin-stimulated conditions. As such, Enigma links APS to cytoskeletal organization, although how Enigma fits in with the CAP-Cbl signaling pathway remains to be determined.

Caveolin-1/3: Caveolin proteins are membrane-localized by virtue of their single and centrally located transmembrane domain, as well as their inherent affinity for cholesterol

[142]. Caveolin-1 is required for caveolar biogenesis in adipocytes, the cholesterol-rich lipid raft domains that have been linked to the IR-dependent but PI3K-independent signaling activity [143]. Caveolin-1 is richly expressed in adipocytes and serves as an IR substrate, wherein IR catalyzes phosphorylation of Tyr14 in mature adipocytes [144,145,146]. Residues 82-101 in the scaffolding domain of Caveolin-1 bind to a specific motif within the kinase domain of IR. Caveolin-1 is thought to be a key player in the PI3K-independent pathway, since it recruits IR to the caveolae to activate the APS/CAP/Cbl pathway [44,147]. Caveolin-1 deficient mice exhibited increased degradation of IR, resulting in decreased insulin action and insulin resistance particularly in adipose tissue (Table 3, [147]). Distinct from Caveolin-1, Caveolin-3 is found only in skeletal muscle [148]. Similar to Caveolin-1, Caveolin-3 binds directly to IR in skeletal muscle and acts as a scaffolding molecule when activated by phosphorylation and is similarly sufficient for the formation of caveolae in muscle [149]. Caveolin-3 is also required for TC10 localization in myocytes [150]. Distinct, however, is that Caveolin-3 is necessary for PI3K and Akt activation, as evidenced by attenuated PI3K activation in Caveolin-3 null myotubes [151]. Caveolin-3 is further necessary for skeletal muscle GLUT4 translocation, as substantiated by diminished insulin-stimulated glucose uptake in Caveolin-3 knockout mice (Table 3 and [149,150]).

IR-dependent Munc18c Signaling to the SNARE complexes—Munc18c was recently reported as a novel direct substrate of IR [105]. Munc18c is an essential regulator in SNARE mediated exocytosis, by virtue of its role and requirement as a binding partner for Syntaxin 4, the only active Syntaxin t-SNARE protein in skeletal muscle and fat compulsory for GLUT4 vesicle translocation/exocytosis (Table 3 and [152,153]). It had been presumed for years, based upon analogy from yeast orthologs of Munc18 and Syntaxin association/dissociation dynamic mechanisms, that downstream of AS160, a Rab-type protein would serve to dissociate Munc18c from Syntaxin 4 at the PM. This was predicted to facilitate the opening and accessibility of Syntaxin 4 to incoming VAMP2 (Vesicle Associated Membrane Protein 2)-laden GLUT4 vesicles. However, no such Rab protein has ever been definitively identified. Emerging evidence regarding a putative role for Munc18c phosphorylation in this mechanism led to investigations on kinase(s) that might circumvent the need for the absent/unidentified Rab-like protein, and indeed, IR was found to carry out this role [105]. As IRS-1 failed to bind Munc18c and no binding differences were seen in the presence of a PI3K inhibitor, it was concluded that IR signaling to Munc18c was independent of IR/IRS/PI3K signaling and that IR signaling to Munc18c occurred in parallel to that of the PI3K pathway in the mechanism of insulin-stimulated glucose uptake [105]. IR was found to require Munc18c residue Tyr521 for binding and phosphorylation, although a second residue, Tyr219, was also found to be essential in insulin-stimulated Munc18c phosphorylation and GLUT4 vesicle translocation in 3T3L1 adipocytes, perhaps as a sequential phosphorylation event following Tyr521 modification [105]. Given this, it is conceivable that the function of a Rab-like protein downstream of AS160 to facilitate SNARE pairing may *not* be required in the process of GLUT4 vesicle translocation. A working schematic model has been re-drawn in this regard (Fig. 3), to show insulin receptor bifurcating into two simultaneous functions: 1) triggering the classic signaling pathway downstream to PI3K to induce vesicle mobilization towards the cell surface, and 2) triggering Munc18c phosphorylation-dissociation from Syntaxin 4, allowing Syntaxin 4 to participate in SNARE complexes with incoming GLUT4 vesicles [105,154].

Role II: IR Substrates in Mitogenesis and Growth

In addition to metabolism, skeletal muscle and fat cells require IR for proliferation and growth. IR is a well-known mitogenic signal initiator, through its usage of substrates like Shc, IRS-1, Gab-1, STAT-5b, Jak-1, and aP2 (Fig. 2). Moreover, IR can form heterodimers with IGF1R (Insulin like growth factor receptor) and transmit mitogenic signals from the

growth factor IGF (Insulin like growth factor). This dimerization of IR with IGFR, as well as IR's interaction with each main substrate, is discussed below, with description of the associated downstream signaling pathways depicted in Fig. 4.

Shc (Src Homology 2 and Collagen like)—Shc is phosphorylated in response to insulin in skeletal muscle and adipose cells and plays a critical role in p21^{Ras} (Ras)-dependent mitogenic signaling [155,156,157]. Shc contains SH2, PH, and PTB domains; IR interacts with Shc through Shc's PTB domain [158]. Shc binds to IR's juxtamembrane region at Tyr960, competing for occupancy with IRS-1 due to presence of SAIN (SHC and IRS-1 NPXY-binding) domains contained in both Shc and IRS proteins [159,160,161]. Upon insulin stimulation, Shc is phosphorylated by IR at Tyr317 which then provides a docking site for Grb2 (which is constitutively bound to SOS (Son of Sevenless)), causing the translocation of Shc-Grb2-SOS complexes from the cytoplasm to the PM, where they function to activate Ras [162]. Downstream of Ras stimulation, Raf becomes activated, which in turn phosphorylates MEK to trigger ERK regulation. ERK subsequently translocates from the cytoplasm to the nucleus to activate transcription factors such as Elk-1, which induce cell cycle progression to support mitogenesis [163].

IRS-1—IRS-1 plays a dual role in mitogenesis and metabolism. IR-phosphorylated IRS-1 also can bind to a limited pool of Grb2 to induce Ras activation, akin to Shc's action, but Shc is the predominant activator of Ras for two major reasons: 1) binding affinity of Grb2-Shc is 3-13 fold higher than that of IRS-1 binding to Grb2, and 2) IRS-1 is phosphorylated more rapidly in comparison to Shc, hence SOS-Grb2 complexes, which initially bind with IRS-1, will switch and bind Shc to activate Ras [164].

Gab-1 (Grb-2 Associated Binder-1)—Gab-1 functions as an adapter protein to dock other SH2 domain containing proteins to IR [165]. Hence, Gab-1 binds to IR directly, but with low affinity. In adipocytes, Gab-1 was further identified by use of quantitative proteomic screens for IR substrates [166,167]. The protein domain structure of Gab-1 shows a high degree of homology with IRS-1, although Gab-1 lacks a PTB domain [165]. Gab-1 does contain a PH domain through which it binds to phospholipids to ultimately assist in its recruitment to IR. Gab-1 acts as an adapter protein for Grb2 and competes with SOS for binding to Grb2, but its role in downstream Ras/MAPK signaling is still not clearly elucidated [168]. Over expression of Gab-1 enhances cell growth initiated by receptor tyrosine kinases [165], although this remains to be tested in fat or skeletal muscle cell types in response to insulin.

Signal Transducer and Activator of Transcription-5b (STAT-5b)—STAT-5b belongs to a family of DNA binding proteins that contain SH2 domains and was discovered as an IR substrate in a yeast two hybrid screen followed by studies in adipose and skeletal muscle [169,170,171]. STAT-5b is the only known transcription factor to be phosphorylated by IR. STAT-5b binds to IR via Tyr960, the same IR residue required for binding to IRS-1 [169]. Under basal conditions, STAT-5b is found in the cytoplasm, but upon phosphorylation it homodimerizes through SH2 domains and translocates to the nucleus. Upon translocation to the nucleus, phosphorylated STAT-5b induces gene transcription, although which genes it activates specifically in response to IR activation remains to be determined [172,173]. One mitogenic action by IR is STAT-5's mediation of adipogenesis/adipocyte differentiation via regulating expression of the peroxisome proliferator activated receptor- γ [174]. Moreover, STAT-5b knockout mice exhibit a loss of sexual dimorphic growth, supporting the concept that STAT-5b is critical in growth [175]. STAT-5b also acts as a transcriptional activator for SOCS-3 [171,176]. Once activated, SOCS-3 inhibits STAT-5b by competing for binding to IR at the same residue. In this manner SOCS-3

negatively regulates insulin signaling by inhibiting STAT-5b function. This finding correlates with the finding that SOCS-3 mRNA levels are significantly increased in the skeletal muscle of type 2 diabetic patients compared with control subjects [177].

Janus Kinase (Jak)-1—Another substrate of IR is Jak-1, belonging to a family of cytoplasmic signal transduction molecules: Jak-1, Jak-2, Jak-3 and Tyk-2 [178,179,180,181]. Both Jak-1 and Jak-2 are tyrosine phosphorylated in response to insulin in fat and skeletal muscle cells, yet Jak-1 is phosphorylated more rapidly in comparison with Jak-2 [182,183]. Jak-1 contains multiple Janus homology (JH) domains; three of which bind to IR (JH-1 domain in the C-terminus, and JH6-JH-7 in the N-terminus of Jak-1) [184]. Once phosphorylated by IR, Jak-1 associates with the SH3 domain of Grb2, distinct from the manner by which IRS-1 binds to Grb2 [182], yet elicits similar downstream activation of Ras/Raf/MEK/ERK. Jak-1 can phosphorylate STAT-5b in vitro [182], which would be expected to induce STAT-5b translocation into the nucleus, although this event remains to be tested in fat or skeletal muscle cell types in response to insulin. Jak-1 also phosphorylates IRS-1, yet at a different site than that targeted by IR [184]. It remains to be determined whether Jak-1 phosphorylation of IRS-1 yields a differential functional response, mitogenic or otherwise, than does the direct IR-IRS-1 interaction.

Adipose protein (aP2)—Adipose protein-2 (aP2) was the first adipose-localized fatty acid binding protein found to serve as an IR substrate [185,186]. Tyrosine phosphorylation of aP2 is dependent upon its ability to bind fatty acid [185]. More specifically, fatty acid binding causes a conformational change in aP2 which exposes Tyr19, leading to its phosphorylation. Thus, aP2 loaded with fatty acid is highly susceptible to phosphorylation, and this in turn reduces further fatty acid transport, leading to fatty acid accumulation to inhibit lipolysis [185]. In this manner, it has been proposed that IR's phosphorylation of aP2 may provide a clue toward explain the anti-lipolytic function of aP2, and aP2 may be considered to function as a growth-promoting factor [185].

IGFR—In tissues expressing both IR and IGFR, one α and β subunit pair each of IR and IGFR heterodimerize to form IR-IGFR hybrids (HR, depicted in Fig. 4)[187]. HR binds to IGF with the same affinity as does an IGFR homodimer, and binds to insulin with a lower affinity [187]. As a ligand for HR dimers, IGF-1 stimulation yields mitogenic rather than metabolic downstream signaling cascades [188]. This is most relevant to the newly recognized association between occurrences of diabetes and cancer, given that in malignant cells, IR and IGFR are over-expressed, with the more mitogenic IR-A isoform having the highest expression [188,189]. Furthermore, HR dimers composed of the IR-A isoform are found in abundance in malignant cancerous cells, and have been noted to exert a stronger mitogenic effect with IGF stimulation, leading to excessive cell growth and resulting in cancer [53,190,191].

Role III: Insulin Receptor (IR), Insulin Resistance and Aging

Aging is a process that is controlled, at least in part, by IR, by virtue of IR's prominent roles in metabolism and in growth/mitogenesis. The most effective method to increase lifespan in model systems and non-human primates is via caloric restriction, as caloric restriction results in lower fat mass, lower serum insulin and IGF-1 levels, and increased insulin sensitivity [192]. That insulin and IGF-1 levels are reduced in models of lifespan extension reflects upon the role played by IR and HR dimers in both metabolism and growth; over-stimulation of these receptors by excessive loads of their ligands exerts a negative effect upon lifespan. Indeed, it has been suggested that signaling pathways, such as insulin/IR/PI3K, which play key roles during development, may also be implicated in pro-aging [193]. Thus, mutations that inactivate such pathways are predicted to result in slowed aging to

increase lifespan. However, given that IR is clearly essential for life, as exemplified by the early lethality exhibited by the classic whole-body IR knockout mice and high morbidity and mortality rates observed in human patients lacking IR (Table 1), there is clearly a ‘sweet spot’ for IR signaling to be considered a positive and not a negative event.

IR Mutations in Aging—The inverse relationship between decreased insulin signaling and increased lifespan is consistent with studies from nematodes and flies, whereby any genetic defect in IR/insulin signaling pathway increases lifespan [192,194,195]. For example, in *Drosophila*, mutation of insulin-like receptor, or loss of an insulin receptor substrate protein (CHICO), leads to significant extension of longevity [196,197]. Similarly, in *C. elegans*, a mutation of the IR homolog DAF-2 results in the doubling of the worm life span [198,199]. The Ruvkun group further revealed linkage between longevity and fat storage regulation, such that lipid hydrolysis in fat storage tissue extended life span, while reduced lipid hydrolysis partially suppressed longevity in daf-2 mutants [200,201]. Consistent with this, the linkage between fat and longevity in mammals became evident, from data showing that fat-specific IR knockout mice (FIRKO) exhibited an 18% increase in lifespan [94], coordinate with lower fat mass, body weight, and increased insulin sensitivity [91]. However, this lifespan extension was not universally observed with all tissue-specific IR knockout strains; it was unique to the FIRKO mice. Ablation of the IR gene in skeletal muscle, liver, β -cell, brown adipose, and brain does not appear to expand lifespan [90,95,202,203,204]. As such, this unique feature of the FIRKO mice suggests that over-stimulation of IR in the adipocyte is related to decreased longevity. Indeed, several studies reveal key roles for IR and/or IGFR signaling in modulating fat mass and longevity. One such study demonstrated that body fat accumulation positively correlates with age-related decreases in insulin sensitivity [205]. In line with this, centenarians, referring to humans living 100+ years, reportedly have reduced body fat mass compared to younger individuals [206,207]. Caloric restriction and reduced fat mass also reverse age-related insulin resistance in humans [208]. These studies suggest that improved insulin sensitivity leads to increased longevity in animal models, as well as in humans.

IR Signaling and Lifespan – FOXO, Sirtuins, and mTOR—IR signaling to impact the expression of longevity genes incorporates features from the IR signaling cascades which impact metabolism and growth, although involves proteins such as forkhead box transcription factor (FOXO) and sirtuins, which have not yet been discussed in this review. For example, IRS-1 is believed to serve as the IR substrate towards impacting aging gene expression, signaling downstream to PI3K and PDK-1 to Akt (Fig. 5 left). Substantiating this, whole body IRS-1 knockout female mice show an 18% extension of lifespan, concurrent with lower body fat without reduction in food consumption [209]. Interestingly, the same study showed IRS-1 knockout male mice to have no gain in longevity, suggestive of gender specific IRS-1 action in extending lifespan. In worms (*C. elegans*), IR signaling moves downstream of DAF-2 (Fig. 5 right), the worm IR homolog, showing remarkable conservation of function given the evolutionary distance [210]. DAF-2 then signals downstream to IST-1 (an IRS homolog), AGE-1 (a PI3K homolog), PDK-1 and on to Akt [211]. In addition, Akt signals downstream to block FOXO in mammals, and also to DAF-16, the worm FOXO homolog [212]. Akt can also signal downstream to the mammalian Target of Rapamycin (mTOR, serine/threonine kinase), with mTOR known to be a modulator of the protein translation machinery, to relay the initial signal from insulin/IR or caloric restriction [213].

FOXO: Akt-mediated phosphorylation of FOXO results in the sequestration of FOXO in the cytosol, keeping it from the nucleus where it would otherwise act to promote the expression of longevity genes [193]. It is through this sequestration of FOXO that insulin

signaling through IR to Akt activation is negatively correlated with longevity, and a reduced ratio of phospho-FOXO: total FOXO considered optimal for longer lifespan [214]. Conversely, increased FOXO expression correlates with increased longevity [214]. In humans, the gene variants FOXO1 and FOXO3A are specifically linked to longevity [215,216,217]. In worms and flies, homologs of FOXO1 have been shown to extend lifespan through insulin/IGF-1 in a similar fashion, whereby again, FOXO expression levels positively correlate with extended lifespan [198,214].

Sirtuins: Increased Sirtuin expression is demonstrated to extend lifespan in yeast, worms, and flies [218,219,220,221]. Sirtuins are NAD⁺-dependent protein deacetylases which modulate FOXO activity via nuclear retention [222]; nuclear-localized FOXO is linked to activation of longevity genes and deactivation of pro-aging genes [193]. In worms, Sir2.1 (homolog of SirT1) directly regulates DAF-16 (worm homolog of FOXO1) via its deacetylation, suggesting that sirtuins promote longevity through FOXO [223]. In mammals, seven sirtuins are expressed (SirT1-7), although early data showed that it is chiefly SirT1 that links NAD metabolism to IR-mediated signaling to exert control over glucose homeostasis [224]. Subsequently, caloric restriction has been shown to activate SirT1 and coordinately enhances skeletal muscle insulin sensitivity as well as lifespan [225]. Resveratrol, an activator of sirtuins, has been shown to extend the lifespan of high-fat diet fed mice [226]. A recent study shows that SirT1 expression is decreased in muscle from type 2 diabetic subjects [227]. Supporting this linkage further, SirT1 depletion from 3T3L1 adipocytes was found to inhibit insulin-stimulated glucose uptake and GLUT4 translocation [228].

mTOR (mammalian Target of Rapamycin): Chronic activation of mTOR signaling (by nutrients or prolonged insulin stimulation) correlates with insulin resistance in vivo, and specifically in fat and muscle cells [229,230,231]. TOR is a large protein kinase that forms two structurally and functionally different complexes that are highly conserved from yeast to mammals, named TOR complex 1 (TORC1, raptor) and complex 2 (TORC2, rictor); TORC1 signaling occurs downstream of Akt, whereas TORC2 signals upstream of Akt [232]. As such, TORC2 has been implicated in Akt activation, such that when in complex with its associated protein Rictor to form the TORC2 complex, it directly phosphorylates Akt on Ser473, which facilitates Thr308 phosphorylation by PDK1 to promote insulin signaling [233]. Inhibition of this signaling step in *C. elegans* TORC2 mutants resulted in extended lifespan [234]. Treatment of mice with Rapamycin, an inhibitor of mTOR, has been shown to extend their median and maximal lifespan [235]. Rapamycin blunts the normal progression of signaling from mTOR to p70S6 kinase (specifically S6K1), ultimately reducing the phosphorylation of S6K1 in visceral adipose tissue [235]. Indeed, S6K1 female knockout mice show a 19% extension of lifespan coupled with improved insulin sensitivity, similar to the effects of caloric restriction in mice [236]. However, akin to the IRS-1 knockout mice, the S6K1 males show no extension of lifespan. While rapamycin to this point could be touted as a veritable “magic pill”, some data would suggest otherwise. For example, chronic rapamycin treatment, used as an immunosuppressant in human transplant recipients, paradoxically promoted glucose intolerance and caused diabetes-like symptoms [237,238]. Moreover, adipocytes from type 2 diabetic patients showed marked reductions in mTOR and S6K proteins [239], correlating with recent evidence from human adipocytes whereby inhibition of mTOR/S6K1 resulted in impaired glucose uptake [240,241]. This may be complicated by the virtue of crosstalk between S6K and IRS-1, since it has been shown that S6K activation can negatively feedback to inhibit IRS-1 signaling [242].

PERSPECTIVES FOR THE FUTURE

In this review, we have summarized the various IR substrates known to function in skeletal muscle and fat cells, focusing on their interaction and roles in metabolic and mitogenic cellular processes, in vitro and in vivo. Although progress in the last decade has provided a better understanding of the molecular mechanisms involved in insulin action, much work is needed to understand how a single extracellular insulin signal is transmitted to achieve such a diverse series of intracellular events. The coordination by IR of the vast variety of events is mind-boggling. For example, the ability of IR to orchestrate both GLUT4 vesicle mobilization while at the same time readying the SNARE machinery – events occurring in different subcellular compartments – demonstrates the ultimate level of multitasking by IR. In this case such orchestration by IR was discovered by identification of an already characterized factor, Munc18c, as a novel IR substrate. New linkages with known factors are critical given that insulin resistance is a complex phenomenon and is not attributable to a single defect.

In addition, more connections such as this can be anticipated as proteomic databases are mined and new substrates discovered and tested in vivo to reveal various new IR signaling nodes. Indeed, the function of nearly 1/5th of the IR substrates found in protein-protein interactions screens are currently unknown [243]. An example of a new signaling node for IR is exemplified by its use of the substrate PDZKII, a PDZ-domain containing Ca^{2+} ATPase that also binds to the calcium binding protein SERCA2 [166]. Such an interaction could implicate IR in calcium signaling, and could provide for a putative role for calcium in insulin action in fat and skeletal muscle cells. In another example, low density lipoprotein receptor-related protein (LRP-6), a member of the WNT/ β -catenin pathway, was identified as an IR substrate in a proteomic screen [166], and could potentially link insulin and Wnt signaling upon further study. Thus, it will be important to move beyond the initial screening if we are to truly understand how such novel and unexpected IR substrates interplay with the classic roles for IR to impart the complexities of insulin resistance and diabetes.

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ABBREVIATIONS

Akt	Protein kinase B
aP2	Adipocyte protein 2
APS	Adapter protein with Plekstrin homology and Src homology domain
Arp2/3	Actin related protein 2/3
AS160	Akt substrate of 160 kDa
BAIRKO	Brown adipocyte specific insulin receptor knockout
CAP	c-Cbl-associated protein
Cav-1	Caveolin-1
Cav-3	Caveolin-3

CHO	Chinese hamster ovary cells
CIP4	Cdc42 interacting protein
CrkII	CT10-related kinase
Erk1/2	Extracellular signal regulated kinases 1 or 2
FIRKO	Fat specific muscle insulin receptor knockout
FOXO	Forkhead box protein O-1
Gab-1	Grb2 associated binding protein
GIRKI	GLUT4 expressing tissues insulin receptor knock-in mouse
GIRKO	GLUT4 expressing tissues insulin receptor knockout
GLUT4	Glucose transporter 4
Grb2	Growth factor receptor bound 2
Grb10	Growth factor receptor bound 10
HR	Hybrid receptor
IGF	Insulin like growth factor
IGFR	Insulin like growth factor receptor
IR	Insulin receptor
IRS-1/2	Insulin receptor substrate-1 or 2
Jak-1	Janus kinase-1
L1	Ligand Binding domain 1
L2	Ligand binding domain 2
LAR	Leukocyte antigen related
LRP	Lipoprotein receptor related protein
MAPK	Mitogen activated protein kinase
MIRKO	Muscle specific muscle insulin receptor knockout
MD1/2	Myotonic dystrophy
mTOR	Mammalian target of rapamycin
N-Wasp	Neuronal Wiskott Aldrich Syndrome protein
PC-1	Plasma cell membrane glycoprotein-1
PDK-1	Phosphoinositide dependent protein kinase 1
PH	Plekstrin homology
PI3K	Phosphatidylinositol-3-kinase
PIP3	Phosphatidylinositol (3, 4, 5) – triphosphate
PKC	Protein kinase C
PM	plasma membrane
PP63	Phosphoprotein of 63 kDa
PTB	Phosphotyrosine binding domain

PTP1B	Protein tyrosine phosphatase 1B
p21^{Ras}	Rat sarcoma protein (Ras)
SAIN	Shc and IRS NPXY binding domain
SERCA	Sarcoplasmic /endoplasmic reticulum calcium ATPase
SH2	Src homology 2
Shc	Src homology containing protein
SHP	SH2 containing tyrosine phosphatase
SirT	Sirtuins
SNARE	Soluble N- ethylmaleimide sensitive factor attachment receptor
SOCS-3	Suppressor of cytokine signaling 3
SOS	Son of Sevenless
STAT	Signal transducer and activator of transcription
T2D	Type 2 Diabetes
t-SNARE	target membrane SNARE protein
Tyk-2	Tyrosine kinase-2
VAMP2	Vesicle associated membrane SNARE protein-2

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Figure 1. Schematic Representation of the Major Features of IR

IR is a heteromeric structure comprised of two extracellular α subunits that bind insulin, and two intracellular transmembrane β subunits. The intracellular domains contain the tyrosine kinase (TK) activity. Insulin binding to the extracellular domain of IR induces a conformational change to the intracellular domain, such that the receptor undergoes autophosphorylation.

Figure 2. Substrates of IR

Diversity of IR functional roles can be explained by the different substrates which bind and become activated by IR. Direct substrates of IR present in skeletal muscle and adipose cells are grouped into two categories, Metabolic and Mitogenic.

Figure 3. Metabolic Role of IR

Three signaling pathways are required for the GLUT4 vesicle translocation in muscle and fat: 1) Phosphorylated IR binds and phosphorylates IRS-1 which activates PI3K. This further leads to PDK1 activation and subsequently Akt1/2 phosphorylation. Akt activates AS160, and AS160 which through, as of yet, undefined steps results in GLUT4 translocation, facilitates glucose uptake. 2) IR also phosphorylates APS which in turn phosphorylates Cbl. Cbl constitutively interacts with CAP, which associates with flotillin to stabilize the recruitment of this complex in caveolae. Phosphorylated Cbl in the caveolar lipid raft recruits CrkII, and CrkII constitutively binds to the nucleotide exchange factor C3G. C3G catalyzes the exchange of GTP for GDP on the lipid raft-associated protein TC10, a downstream effector important in GLUT4 translocation. 3) IR phosphorylates Munc18c which relays signal to the SNARE machinery to coordinate SNARE complex formation and vesicle fusion.

Figure 4. Mitogenic Role of IR

IR has major effects on cell growth and proliferation. Binding of insulin to its receptor leads to autophosphorylation of the β subunits and tyrosine phosphorylation of IRS. Other targets are Shc, Jak-1 and Gab-1. Shc is phosphorylated and binds to the complexes between exchange factor Sos and Grb-2, leading to Ras and subsequently Raf activation and downstream to MEK. MEK further activates ERK which translocates to the nucleus to activate the transcription factor Elk-1 to impact gene expression to promote cell growth and proliferation. IRS-1, Jak-1 and Gab-1 also bind to SOS-Grb2 complex to promote cell growth. Jak-1 can also activate Irs-1 to bind to Grb2 and STAT5b to promote cell growth. Receptor dimers are depicted as IR:IR bound by insulin (green), IR:IGFR heterodimers (HR) and IGFR:IGFR bound by IGF-1 (yellow).

Figure 5. Roles of Insulin/IR Signaling in Aging and Longevity

Left panel: In mammals, three ligands (insulin, IGF-1, and IGF-2) bind to the insulin receptor isoforms A or B (IR-A or -B) or the IGF-1 receptor (IGF-1R) and activate the downstream signaling cascade: IRS-1/2 activates PI3K, leading to PDK-1 activation and Akt1/2 (also known as protein kinase B, PKB) phosphorylation. In turn, Akt phosphorylates FOXO family transcription factors (FOXO1a, 3a, 4, 6) to retain them in the cytosol, preventing their nuclear localization and activation of longevity genes and suppression of pro-aging gene expression. *Right panel:* In *C.elegans*, insulin binds to a single receptor (DAF-2) and activates the insulin receptor substrate homolog protein-1 (IST-1) to trigger activation of the phosphatidylinositol 3-kinase homolog AGE-1 (ageing alteration-1/ AAP-1), leading to activation of PDK1 and Akt. Akt then phosphorylates DAF-16, a homolog of mammalian FOXO1 transcription factor to block its downstream transcriptional activities. Inhibition of insulin signaling via PTEN (DAF-18 in worms) or SirT1 (Sir2.1 in worms) allows FOXO to increase the expression of longevity genes and negatively regulate the expression of pro-aging genes.

Table 1

Clinical Phenotypes of IR Mutations in Human Subjects

Type	Genetic Defect	Clinical Phenotype	Refs.
Leprechaunism	Homozygous IR mutation	Severe congenital insulin resistance, growth retardation, high mortality (2 years old) Onset: congenital	[81,244]
Rabson–Mendenhall syndrome	Heterozygous IR mutation	Severe insulin resistance, acanthosis nigricans, growth retardation, abnormal dentition Onset: congenital	[80,244]
Type A Insulin resistance	Heterozygous mutation of IR	Extreme insulin resistance, acanthosis nigricans, hirsutism, polycystic ovarian disease, usually non-obese, normal growth Onset: adolescence	[80]
Type B Insulin resistance	Presence of autoantibody against IR	Insulin resistance Onset: adult	[80]
MD1/MD2 (myotonic dystrophy)	Increased IR-A/IR-B ratio	Glucose intolerance, insulin resistance Onset: adult and congenital	[83]

Table 2

Muscle or Adipocyte-Specific IR KO and Transgenic Mice

Tissue	Phenotype	Glucose uptake?	Refs.
Whole-body:			
Constitutive IR KO	Diabetic ketoacidosis	ND	[86,87]
<i>IR</i>^{+/-}; <i>IRS-1</i>^{+/-}	Diabetes, insulin resistance	ND	[88]
<i>IR</i>^{+/-}; <i>IRS-1</i>^{+/-}/<i>IRS-2</i>^{+/-}	Insulin resistance	ND	[89]
Tissue-specific Knockout:			
MIRKO	Dyslipidemic without diabetes, insulin sensitive adipocytes	Decreased	[90,245]
FIRKO	Protection against obesity, enhanced longevity	Decreased	[91,94]
BAIRKO	Glucose intolerance without insulin resistance	ND	[95]
GIRKO	Insulin resistance and diabetes	Decreased	[96]
Tissue-specific Knock-in:			
<i>IR</i>^{+/-}; <i>K1030M</i>	Impaired glucose tolerance	ND	[98]
<i>IR</i>^{P1195L/P1195L}	Diabetic ketoacidosis	ND	[99]
<i>IR</i>^{P1195L/Wt}	Insulin resistance, hyperinsulinemia	ND	[99]
GIRKI	Diabetes	ND	[100]

ND, not determined; KO, knockout

Table 3

Knockout Mouse Models of IR Substrates for Metabolic Functions in Muscle and Adipose

IR substrate KO mice	Phenotype	Refs.
<i>Cav-1</i> ^{-/-}	Insulin resistance	[147]
<i>Cav-3</i> ^{-/-}	Insulin resistance and decreased insulin-stimulated glucose uptake in skeletal muscle	[246]
<i>APS</i> ^{-/-}	Increased insulin sensitivity; no effect on insulin-stimulated glucose uptake	[247]
<i>IRS-1</i> ^{-/-}	Decreased insulin-stimulated GLUT4 translocation and glucose uptake in skeletal muscle and adipocytes	[248,249]
<i>IRS-2</i> ^{-/-}	Decreased insulin-stimulated GLUT4 translocation and glucose uptake in adipocytes	[111]
<i>Munc18c</i> ^{+/-}	Decreased insulin-stimulated GLUT4 translocation in skeletal muscle	[250]