1. The insulin receptor: Tyrosine-kinase activation and trafficking

Insulin elicits all of its known physiological effects by binding to the insulin receptor (IR) at the cell surface of target cells [1]. The mature IR is a heterotetramer composed of two extracellular α-subunits involved in insulin binding and two cytosolic-oriented β-subunits that contain the tyrosine kinase domain. Crystallographic studies have revealed that the extracellular domain adopts an inverse V shape in a folded-over, compact conformation [2,3]. Studies on the whole molecular complexes, and using the single-particle cryo-electron microscopy method, recently revealed an intramolecular mechanism of activation similar to the epidermal growth factor (EGF) receptor (EGFR) [5].

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The juxta-membrane region proximal to the kinase domain [4]. Studies on the whole molecular complexes, and using the single-particle cryo-electron microscopy method, recently revealed an intramolecular mechanism of activation similar to the epidermal growth factor (EGF) receptor (EGFR) [5].

2. The endosomal IR protein-proteins interaction network (PPIN) forms a T2D disease module

3. The endosomal T2D disease module connects signaling with trafficking and metabolism and shares functional similarities with the islets secretory pathway

4. Perspectives

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preparing for another cycle of insulin binding and kinase activation, or to a transport in late compartments for eventual late recycling or, ultimately, degradation of the active complexes within lysosomal compartments [6]. While the fate of internalized insulin has been well characterized, particularly in liver parenchyma, which is the main site of insulin clearance in physiological concentrations of circulating insulin [6,7], the molecular mechanisms underlying IR routing and signaling are relatively unknown when compared with the EGFR [6,8]. The original experimental repertoire including morphological analysis on fixed hepatocytes and in vitro assays has shown that the internalized IR-insulin complexes are distributed through prelysosomal sorting centers that are sensitive to acidotropic and microtubule-disrupting agents [9,10]. A slower recycling route originating from late compartments without apparent involvement of multivesicular bodies was also depicted in cultured hepatocytes [11]. Concomitant biochemical characterization of insulin and EGF revealed the presence of signaling molecules in mixed hepatic Golgi/endosome (G/E) fractions suggesting the presence of a signaling activity [6]. Studies mainly done on the EGFR in cell lines favored [12–16], or challenged [17–19], the concept of endosomal signaling. These different results are now explained by the diversity and plasticity of endosomes [20,21], which are also perceived as quantal signaling devices [22,23].

As for the EGFR, IR tyrosine kinase activity appears to be the crucial regulator selecting ligand-dependent movements [1,6]. A system in which each receptor-tyrosine kinase (RTK) is able to induce its own structure for internalization seems unrealistic, unless the different receptors share common elements. While the topology of the endosomal apparatus may be subjected to large variations between one experimental model and another, sorting is apparently achieved with large tubulovesicular compartments whose contents are continuously modified by the entry and exit of small 70–80 nm vesicles [24,25]. These sorting compartments enable the continuous transport of cargos and receptors separately resolving security problems inherent to complexity and energy. This is analogous to a cellular version of the Aldrin Marsycler (AMC), where large spaceships perpetually cycle back and forth between the orbits of Earth and Mars with only minor trajectory adjustments on each cycle, without requiring a significant amount of propellant. The spaceship does not stop when it flies by a planet. The astronauts have to board a small but speedy space taxi that catches up with the cycler. The system thus enables the transport of cargos and humans, separately resolving the costs inherent to security problems when cargo and astronauts are mixed, as experienced with the past shuttle program [26].

2. The endosomal IR-protein-proteins interaction network (PPIN) forms a T2D disease module

A PPIN (named GEN), constructed from a G/E fractions proteome, not only reproduced the general topology of endosomes, but also contained an IR subnetwork (named IRGEN) that is characterized by a marked enrichment in elements associated with T2D risk [27]. To date, genetic studies, including genome-wide association studies (GWAS), have identified a number of functionally heterogeneous common variants spread across the whole genome and explaining approximatively 10–20% of the T2D heritability [28–30]. The concept of network medicine based on the notion of preferential attachment inherent to scale-free networks implies that behind each cellular function there is a network consisting of genes, transcription factors, RNAs, proteins and metabolites. This understanding forces us to view diseases as the breakdown of a given module, that is also sensitive to societal factors (e.g., modern chronic overnutrition state), rather than a single or large group of genes [31]. It has rarely been possible, however, to translate such a massive amount of information on mutations and their associations with disease into primary mechanisms and therapeutic insights as well as the mechanisms underlying genotype-phenotype relationships [32]. For T2D, most of the common associated variants are located at enhancers in pancreatic islets [28], suggesting that this is a preferred location to search for a disease module. This marked enrichment in genes associated with T2D risk in IRGEN [27] suggested however that such a regulated network exists in the liver and that it is cofunctional in islets. These results prompted to ask to what extent new gene candidates linked to the known disease genes through the physical

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**Fig. 1.** A module-based approach to identify type 2 diabetes-relevant diagnostic and therapeutic candidate genes that tend to co-localize in the endosomal interactome. Disease-associated genes tend to co-localize in the human physical-protein interaction network (PPIN), forming a proto-module (blue oval). The proto-module expands physically in the IR-containing endosomes PPIN (IREN, 88% of the nodes associated with the type 2 diabetes genetic risk). Proteins having at least three interactions with seeds are considered as high candidates (blue nodes) and are validated by experimental methods (adapted from Boutchueng et al. PLoS One 2018;13:e0205180).
association of their products can be dynamically identified by diffusion [31], in the more specific environment of IR-containing endosomes. To accomplish this task, IR-containing vesicles were captured from the same insulin-stimulated G/E fractions and a local physical PPIN covering early and later events of IR routing at a 50% insulin saturating dose was constructed. By integrating a highly confident T2D protomodule (OMIM and GWAS), a T2D disease module was thus identified (named IREN) by diffusion. Proteins physically linked to “deseased” proteins were validated as candidates on the basis of at least three interactions (instead of a filter of one interaction), intracellular colocalisation, coexpression and localisation on genomic risk areas [33] (Fig. 1). The obtained hypothesis-free IREN topology is remarkably robust and organized around a few major central hubs, including the cell cycle kinase Cdk2, the IR itself (an internal control), PTPLAD1, Rab5c, and the V-ATPase components (Fig. 2). Of interest, the T2D-protomodule functional specialisation expands in IREN (to include cell cycle, trafficking, signalling components, reactive oxygen species components) [33]. “Hub bottleneck” (also named centrality) is also thought to dictate essentiality and constitutes the dynamic component of a regulated network [34,35]. This is particularly the case for IREN, given its responsibility to acute insulin stimulation. Cdk2, which displays the highest centrality and is a high-confidence candidate associated with T2D genetic risk, indeed readily associates with key elements including the IR, PTPLAD1, Rab5c, tubulin and actin cytoskeletons all containing appropriate phosphorylation sites. In such a network, PTPLAD1, in the same incoherent input, controls IR tyrosine phosphorylation and other key interactions [33] (Fig. 2). Insulin-dependent Cdk2/cyclinE complexes were previously reported, and they were functionally related with a capacity to decrease vesicle fusion events in vitro [36]. Cdk2 was also mechanistically linked with candidate proteins controlling insulin clearance, including CEACAM1, SHP-1 (PTPN6) and β-catenin [37,38]. On the other hand, targeted Cdk2 deletion in the pancreas was reported as inducing glucose intolerance primarily by affecting glucose-stimulated insulin secretion [39]. Similar to the secretory pathway, the endosomal apparatus consists of multiple compartments linked via anterograde and retrograde transport [40,41]. The endocytic and secretory pathways thus share regulatory elements of the cell division machinery either in a pause to decide strategy (similar to checkpoints of the cell division cycle) for appropriate routing or, alternatively, for regulated relief of the continuous braking action of Cdk2 in fusion events.

PTPLAD1 itself is particularly sensitive to insulin as it translocates readily from a yet-to-be-fully characterized intracellular compartment to the plasma membrane following insulin stimulation, and then it internalizes along with the IR [27]. Insulin-regulated PTPLAD1 also has an interesting consequence for signaling, as formation of ligand-receptor complexes, reactive oxygen species components) [33]. “Hub bottleneck” (also named centrality) is also thought to dictate essentiality and constitutes the dynamic component of a regulated network [34,35]. This is particularly the case for IREN, given its responsibility to acute insulin stimulation. Cdk2, which displays the highest centrality and is a high-confidence candidate associated with T2D genetic risk, indeed readily associates with key elements including the IR, PTPLAD1, Rab5c, tubulin and actin cytoskeletons all containing appropriate phosphorylation sites. In such a network, PTPLAD1, in the same incoherent input, controls IR tyrosine phosphorylation and other key interactions [33] (Fig. 2). Insulin-dependent Cdk2/cyclinE complexes were previously reported, and they were functionally related with a capacity to decrease vesicle fusion events in vitro [36]. Cdk2 was also mechanistically linked with candidate proteins controlling insulin clearance, including CEACAM1, SHP-1 (PTPN6) and β-catenin [37,38]. On the other hand, targeted Cdk2 deletion in the pancreas was reported as inducing glucose intolerance primarily by affecting glucose-stimulated insulin secretion [39]. Similar to the secretory pathway, the endosomal apparatus consists of multiple compartments linked via anterograde and retrograde transport [40,41]. The endocytic and secretory pathways thus share regulatory elements of the cell division machinery either in a pause to decide strategy (similar to checkpoints of the cell division cycle) for appropriate routing or, alternatively, for regulated relief of the continuous braking action of Cdk2 in fusion events.

Following insulin stimulation, the proteins ATIC, PTPLAD1 and AMPK associate within seconds with the tyrosine kinase-activated IR as well as control its tyrosine kinase activity and traffic [27]. ATIC is a key rate-limiting metabolic enzyme involved in the de novo production of purines, which are building blocks of DNA and RNA (biomass) but are also found in ATP, GTP, ADP, AMP, cyclic AMP, NADP, SAM and coenzyme A and thus are not only a source of energy for living cells but also the cofactors for numerous metabolic and signaling enzymes. AMPK is the energy sensor (adenylates) engaging catabolic versus anabolic processes in response to decreases in the ATP/AMP ratio [47]. AMPK activation also results in the phosphorylation of the cargo-binding kinases light chain, KLC2, of Ki67 and in the disruption of Ki67 association with PI3K and Akt [48] which would be necessary for early and late endocytosis events [49]. Ki67 is involved in the Rab5-dependent movement of vesicles towards either the plus or the minus end of microtubules [50]. Hence, an insulin-sensitive module formed minimally by the apparently unrelated proteins ATIC and AMPK is aware of the state of activation of the IR and acts locally in a concerted manner with PTPLAD1 (IR tyrosine dephosphorylation), Rab5 and kinases (trafficking) [27]. A concrete problem for the cell concerns its energy sources, and it seems that the cell has found an efficient way to link in a safe and economic manner the continuous local energy demand to a manufacturing center. ATP is the source of energy for the cell, and its level is controlled in part by ATIC (synthesis). The fact that the ATIC substrate, and antidiabetic, AICAR can activate AMPK [51] emphasizes the idea that all the conditions are present to autoregulate the IR module in concert with insulin inputs. This ATIC circuitry linking signaling with metabolism (Fig. 3) suggests the presence of a morphoeic mechanism [52] whereby ATIC monomers, in equilibrium with dimers [53], interact physically in the node, and ATIC dimers support the two last steps of the de novo purine biosynthetic metabolic pathway. Such morphoeic activity linking signaling with metabolism was already reported for the embryonic isofrom rate-limiting glycolytic enzyme PKM2 [54].

The ATP-consuming proton pumping activity, mediated by V-ATPase, is key for appropriate insulin clearance in physiological concentrations of insulin [6,7]. By contrast with ligands such as EGF and prolactin, insulin readily dissociates from the IR in the acidic luminal pH environment and is subsequently degraded by a luminal protease activity that is now more related to the acidic cathepsin D [6] than to the neutral insulin-degrading enzyme (IDE) [55]. Amylin, a peptide which is co-secreted with insulin was, however, reported as a good substrate for IDE [56]. V-ATPase was found connected with ATIC and AMPK [33] and the widely used anti-diabetic drug metformin, targeting the mitochondrial production of ATP [51], was recently shown to act also on the endolysosomal system through V-ATPase and AMPK [57,58], indicating the presence of connections between the T2D disease module and drug therapy. Additional layers of feedback loops are anticipated as a robust

Fig. 2. A representation of the T2D disease module and associated layers of feedback loops in endosomes. The action of insulin occurs via a receptor tyrosine kinase (IR) located at the surface of target cells. Following insulin binding there is also, within seconds, internalization of the tyrosine kinase-activated complexes into the endosomal apparatus. Shown is the physical protein interaction network (PPIN) forming the disease module (IREN). The general topology of IREN is based on few major hubs, with the kinase Cdk2 displaying the highest centrality. Candidates (yellow and blue colors and black characters) and DAGs (diabetes associated genes, pink color and black characters) form a single-connected disease module of 88% of nodes) covering 92% of interactions (1070 out of 1147 I.E. interactions). The functional groups are represented according to the colors of the borders indicated in the legends.

3. The endosomal T2D disease module connects signaling with trafficking and metabolism and shares functional similarities with the islets secretory pathway
phosphorylation signal that was readily abolished by V-ATPase inhibition was reconstituted in endosomes, suggesting the presence of an endosomal homeostatic pathway, informing the cell that the lumenal acidification process is optimized [33].

4. Perspectives

We now know that hyperglycemia can be caused by a combination of genetic and environmental factors that affect circulating insulin
The liver is a major organ that controls insulin action on metabolic homeostasis. Circulating glucose availability is regulated through the insulin dependent reversible storage of glucose and glycogen as well as increased lipogenesis with canonical insulin signaling pathways [60]. Since approximately 50% of the insulin secreted by the pancreas is removed after its first pass by the liver before reaching the peripheral circulation [7], hepatic extraction through insulin-mediated endocytosis is also viewed as an adaptive mechanism that could relieve the stress on pancreatic β-cells imposed by insulin resistance [61–64]. Alternatively or in addition, a moderate chronic hyperinsulinemia due to a reduction in insulin clearance may be the primary mechanism resulting in insulin resistance [65,66]. How the identification of a T2D disease module hidden in the close neighborhood of the internalized IR can be of importance for a better understanding of the primary mechanism of the disease, precision medicine and new drug therapy? A priori, hepatic endosomes might not be the best place to study therapy of complex diseases [68,69]. Insulin resistance might be also an essential endosomal response that limit anabolic processes to nutrient oversupply.

The genetic architectures of human disorders are typically classified into two main categories: complex traits, such as T2D, displaying a polygenic architecture arising from numerous low-effect common variants, and rare traits that tend to have high-effect monogenic variants [70,71]. To date, genetic studies have identified a number of functionally heterogeneous T2D common variants [28–30]. The presence of rare T2D variants with high causalities [72], has not been well established by GWAS yet [30,73], except in an homogeneous cohort of Latino patients [74], and therefore there is still a necessity to understand the complexity of GWAS data better, for example by stratifying more lean, prediabetic and obese patients [75–77]. Variants frequently influence multiple phenotypes, often in unexpected ways [78]. The notion of inter-connected diseases also implies a knowledge of the comorbidities that exist between complex and Mendelian diseases [31]. As such, genes that are disrupted in Mendelian disorders are dysregulated by noncoding variants in complex traits [78,79] as exemplified by betathalassemia and T2D [78]. The comorbidity between T2D and other complex diseases such as cancer, neuropathies [31] and Mendelian diseases [78] can be accurately examined with regard to the T2D disease module. The hypothesis of phenotype-specific enrichment of Mendelian disorders around GWAS variants should also allow a greater resolution in identifying gene–phenotype relationships and achieve the goals of precision medicine [78,79]. It finds a particular echo in T2D, where a majority of variants are found in non-coding regions [73], and

Fig. 3. A representation of the IR/ATIC/PTPLAD1/AMPK circuitry. Insulin inputs in a double incoherent mode: IR autophosphorylation plus PTPLAD1 recruitment at the cell surface (1, blue color) are converted into robust oscillations in output through the overlay of two positive feedback systems driven by the metabolic enzyme ATIC (2, red color), a local interacting loop counteracting the action of PTPLAD1. (3, red color), adenylates production. Variations in ATIC levels or in adenylates synthesis or a decline in ATP levels independent of de novo purine biosynthesis increase the AMPK activity and IR endocytosis (adapted from Bouchueng et al. Mol Cell Proteomics 2015;14:1079–92).
furthermore many variants identified in coding regions have been reclassified as false leads [30].

Diabetes is presently classified into two main categories, type 1 and type 2 diabetes, but type 2 diabetes is particularly heterogeneous in terms of genetics, clinical presentation and outcomes. An important goal for clinicians and researchers is to classify subtypes of diabetes for lean and obese patients in order to more accurately select therapies and predict clinical complications [80–82]. Given the complexity of such a task, it would make sense to start from a class of well-defined interactions, and this is exactly what the combination of hypothesis-free methods with hypothesis-driven approaches offers with the description and validation of a T2D disease module. Recently, a soft clustering of genetic loci associated with T2D allowed the identification of two groups related with insulin deficiency, and three related with insulin resistance [83]. A data-driven cluster analysis based on six additional variables (glutamate decarboxylase antibodies, age at diagnosis, BMI, HbA1c, β-cell function and insulin resistance) allowed the identification of five overlapping clusters of patients within a cohort with different disease progression and risk of complications, thus pointing out an avenue for precision medicine [82]. Clustering can be refined with the integration of a T2D disease module that helps to link to a primary mechanism for each group of patients (insulin signaling response, clearance and production). It would be then possible by using a T2D disease module panel, containing minimally genes of the protomodule (e.g., HNF4A, IR, IGF1, IGF1R) and highly confident candidates (e.g., Cd2k, ATIC, Rab5C, PTPLAD1, ATPSV1A) (Fig. 1) [33], to subclassify smaller cohorts (50–100) of patients to help diabetologists in their day-to-day practice. Moreover, the T2D disease module can be used to explain how, in the presence of high glucose, alteration of endosomal response is functional to avoid excessive nutrient accumulation inside the cells.

Finally, a better knowledge of the T2D disease module will facilitate the appropriate use of existing antidiabetic therapies and enable the development of new drugs. Removing nodes would be a too drastic strategy to study and rewire a network positively [84]. Instead, the use of small surface interactors (edgetic approach) [85,86] seems relevant because gene essentiality in humans is more based on complexity and haploinsufficiency as exemplified for components of the cell cycle [87], PTPLAD1, which is physically connected to a Golgi essentialome can be positively rewired with glucose tolerance [90], supporting the idea that the T2D disease module –

Declaration of Competing Interest

There is no conflicts of interest to declare.

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