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Emmanuel Van Obberghen Minkowski Award, 1985, Madrid

Emmanuel Van Obberghen was born in Belgium in 1945. In 1971 he obtained an M.D., and a Ph.D. in 1975 from the Vrije Universiteit Brussel. Dr. Van Obberghen was a research scientist in the Laboratory of Experimental Medicine, Vrije Universiteit Brussel from 1971 to 1975. From 1975 until 1980 Dr. Van Obberghen was Visiting Associate at the Diabetes Branch of the NIH in Bethesda, Md., USA. He was an INSERM Scientist at the INSERM Unit 145 in Nice, France from 1980 to 1985. Between 1985 and 1987 he was a Visiting Scientist at the Laboratory of Molecular Genetics of the NIH. Since 1987 Dr. Van Obberghen has been head of INSERM Unit 145 at Nice Medical School. Dr. Van Obberghen is Visiting Professor in Interdisciplinary Research at the Institute of Human and Nuclear Biology at the Medical School, Université Libre de Bruxelles, Brussels (1992–date); Vrije Universiteit Brussel, Master and Ph.D. Programme in Medical and Pharmaceutical Research (1993–1994); Ph.D. Programme in Molecular and Cellular Physiopathology, Medical School II, Naples, Italy (1993–1994).

Signalling through the insulin receptor and the insulin-like growth factor-I receptor

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Summary The insulin receptor and the insulin-like growth factor I receptor belong to the family of tyrosine kinase receptors. Both receptors appear as a disulphide-linked dimer; each half of the dimer consisting of a 130 k M_r α-subunit linked to a 90 k M_r β-subunit. Both halves of the dimer are linked together by disulphide bonds to form an $\alpha_2\beta_2$ structure. The insulin receptor functions as an allosteric enzyme in which the binding of the hormone to the α-subunit leads to a series of conformational changes resulting in activation of the β-subunit tyrosine kinase. Upon multisite autophosphorylation the latter becomes competent to phosphorylate cellular substrates resulting in the biological responses of insulin. Recent findings have recognized the mitogen activated protein kinase cascade as a central signalling circuitry linking cell surface receptors, such as the insulin receptor, to the nucleus, and playing a role in regulation of metabolism, growth and differentiation. [Diabetologia (1994) 37 [Suppl 2]: S125–S134]

Key words Phosphorylation/dephosphorylation, kinases, tyrosine kinase receptors.

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Abbreviations: EGF, Epidermal growth factor; MAP, mitogen activated protein kinase; ERK, extracellular signal related kinase; ISPK 1, insulin-stimulated protein kinase-1; IGF-I, insulin-like growth factor-I.

Insulin is a key actor in the regulation of metabolism with a major role in glucose homeostasis, and important effects on lipid and protein metabolism. Its most important target organs are the liver, skeletal muscle and fat tissue. Insulin induces its multiple effects by interaction with its cell surface receptors, which approximately 10 years ago were found to carry tyro-

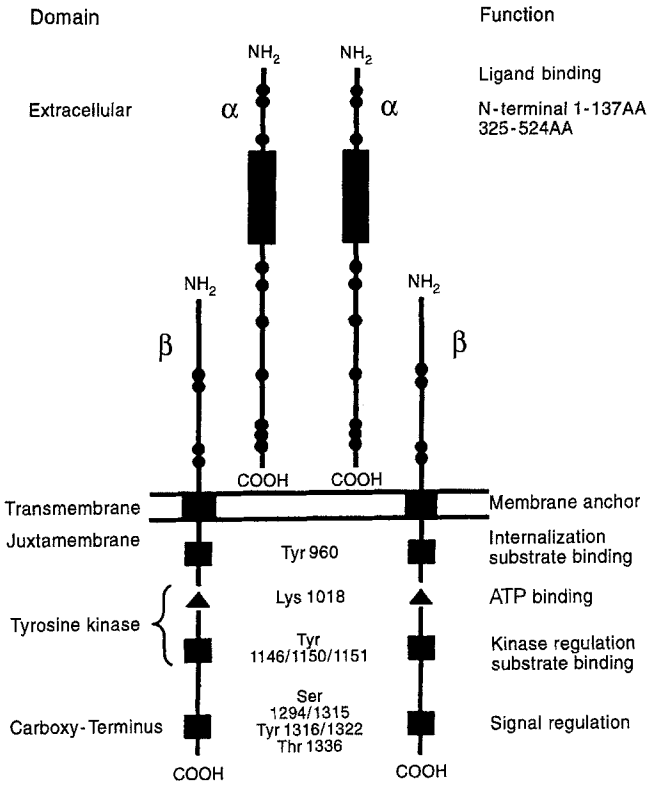

Fig. 1. Schematic representation of the insulin receptor. The black region in the α-subunit corresponds to a region rich in cysteine residues, black circles correspond to single cysteine residues. Adapted from Ullrich et al. [12]

sine kinase activity in the receptor β-subunit [1–3]. Nearly 40 years ago the pioneering work of Edwin Krebs and Edmond Fischer implicated protein phosphorylation as a means of regulating glycogen phosphorylase activity (4). Protein phosphorylation/dephosphorylation is now recognized as a critical mechanism in signal transduction used by numerous growth factors and hormones, including insulin [4, 5]. Until recently the link between the insulin-stimulated (serine/threonine) phosphorylation/dephosphorylation of proteins and the insulin-stimulated receptor tyrosine kinase has remained elusive. During the last couple of years, an explosion of new information has allowed the unravelling of the components of the network going from the cell surface tyrosine kinase receptors to intracellular events and modification of gene expression.

Structure of the insulin receptor

The insulin receptor and the related IGF-I receptor belong to the family of receptors with tyrosine kinase activity, which have common structural and functional properties. Schematically, they have an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic domain displaying the tyrosine kinase activity [6, 7]. For all the receptors belonging to this family tyrosine kinase activity is necessary for their signalling. With the exception of insulin and IGF-I receptors, which have a dimeric structure, the other family members exist as a monomeric structure, in absence of their ligand. However, the general rule for monomeric tyrosine kinase receptors is that the functionally active receptor has a dimeric structure induced by ligand interaction. Hence, tyrosine kinase receptors in their activated form have a structure similar to that of the insulin receptor [6, 7].

The insulin receptor gene encodes a polypeptide of M_r 155 k [8]. This proreceptor undergoes several post-translational processing steps including proteolytic cleavage into α- and β-subunits, glycosylation, and a fatty acid acylation. The mature insulin receptor has a M_r of 300–350 k and, as already mentioned, appears as a disulphide-linked dimer. Each half of the dimer consists of an α-subunit, M_r 130 k, linked to a β-subunit, M_r 90 k [8–10]. The two halves of the dimer are linked together by disulphide bonds between the two α-subunits to form an $\alpha_2\beta_2$ structure. Note that given its subunit composition the insulin receptor can also be viewed as a heterotetramer, but this makes the comparison with single chain tyrosine kinase receptors more difficult. Both subunits are glycoproteins, the α-subunits are entirely extracellular, while the β-subunits are transmembrane glycoproteins [6, 11–13]. Two isoforms of the insulin receptor exist, due to alternative splicing of the receptor transcript to include, or not, exon 11 [4, 8]. Expression of exon 11 leads to the presence of 12 additional amino acids situated at the carboxyterminus of the insulin receptor α-subunit. Although the precise physiological role of the two isoforms of the receptor remains to be defined, the protein containing the residues encoded by exon 11 has reduced binding affinity[14] and receptor downregulation [15], but increased tyrosine kinase activity [16].

Schematically one can distinguish the following major functional domains in the insulin receptor [8–10, 13] (Fig. 1):

(i) in the α-subunit:
a hormone binding domain within the extreme N-terminal region comprising amino acids 1–137, and a second comprising amino acids 325–524 [17, 18];
(ii) in the β-subunit:
1. the juxtamembrane region involved in receptor internalization and substrate recognition [10];
2. the tyrosine kinase domain with the ATP binding site in which lysine 1018 plays a major role, and three regulatory autophosphorylation sites tyrosine 1046, 1050, and 1051 (numbering system published by Ullrich et al. [13]).
3. the carboxy-terminus containing two autophosphorylation sites, tyrosine 1316 and tyrosine 1322, the role of which is not precisely known. In addition

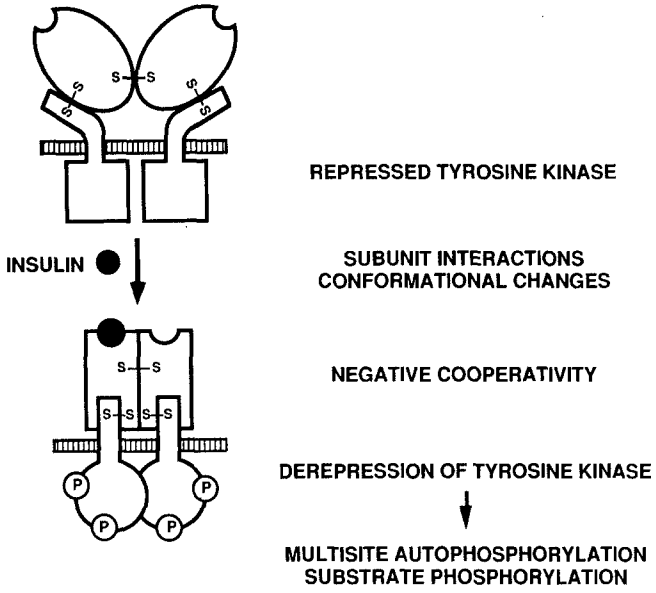

Fig. 2. Schematic representation of the insulin receptor activation

the carboxy-terminus also carries several threonine/serine phosphorylation sites, which are thought to participate in negative regulation of the receptor tyrosine kinase activity.

Activation of the insulin receptor

The insulin receptor as an allosteric enzyme. It is now generally accepted that insulin receptor tyrosine kinase activity is essential for all known early and late cellular responses evoked by insulin. In this context it is important to understand the molecular mechanism by which the hormone activates the kinase. Our data and those from several other laboratories indicate that the insulin receptor behaves as an allosteric enzyme, where, in the disulphide-linked dimeric receptor, hormone binding to the α-subunit activates the cytoplasmic tyrosine kinase of the β-subunit through conformational changes (see article by De Meyts in this issue). The first suggestion in favour of ligand-induced receptor changes was provided by Donner and Yonkers [19] and Pilch and Czech [20], who showed that insulin binding alters the conformation of the α-subunit and modifies the relative position of the two α-subunits in the $\alpha_2\beta_2$ receptor. Later, Herrera and Rosen [21] reported that receptor autophosphorylation is associated with a conformational change in the vicinity of the major autophosphorylation sites of the receptor. To delineate these conformational changes and to establish whether the ligand-induced changes were different from those induced by phosphorylation, we developed a radioimmunoassay of the receptor using metabolically labelled receptors (native receptors and kinase deficient receptors) and antipeptide antibodies directed against defined receptor domains. Specifically, antipeptide antibodies to the following sequences of the receptor β-subunit were used: (i) positions 962–972 in the juxtamembrane domain, (ii) positions 1247–1261 at the end of the kinase domain, and (iii) positions 1294–1317, and (iv) positions 1309–1326, both in the receptor C-terminus. We made the following key observations: (i) insulin binding to the α-subunit leads to a conformational change in the receptor β-subunit, which appears to be limited to the C-terminus; (ii) phosphorylation of the receptor results in different conformational changes, which can be detected in at least three regions, the juxtamembrane domain, the kinase domain, and the C-terminus [22, 23].

Based on our observations [1–3] and on data from other laboratories [19–21, 24, 25] we proposed the following schematic model for the insulin receptor activation [23]. Insulin binding to the receptor α-subunit induces a conformational change in the extracellular domain [19, 20], and a modification in the interaction between the two receptor halves [25]. These changes are transmitted to the β-subunit down to its C-terminus, leading to a short-lived, pre-activated receptor that becomes competent to bind ATP. The ensuing receptor autophosphorylation induces a second conformational change, distinct from the first, which affects the major part of the cytoplasmic domain. Although the precise role of these phenomena remains to be determined, we would like to suggest that they could lead to unmasking of the receptor catalytic domain and/or of binding sites for cellular proteins, allowing enzyme-substrate interactions (Fig. 2) [23].

The physiological relevance of the hormone-induced conformational change in the receptor was first suggested by our observation that it also took place in intact cells [23]. More important, our recent work on a naturally occurring receptor mutant provides compelling evidence in favour of the physiological role of conformational changes in insulin receptor kinase activation [26]. In brief, a mutation substituting a valine for phenylalanine at residue 382 in the insulin receptor α-subunit has been found in two sisters with a genetic form of extreme insulin resistance. This receptor mutation impairs the ability of the hormone to activate autophosphorylation of solubilized receptors and phosphorylation of substrates. Hence, it was thought that a defect in the insulin-induced conformational change might explain the functional defect of the mutant receptor. This appears to be the case, since we were able to demonstrate that the mutant receptor is locked in its inactive configuration. However, we found two monoclonal antibodies directed to the extracellular domain which are capable of restoring the mutant receptor kinase activity. Accordingly, activation of the mutant receptor with these antibodies was accompanied by restora-

tion of conformational changes in the β-subunit C-terminus [26].

From these data, we have drawn the two following conclusions: (i) a causal link exists between receptor kinase activation and the occurrence of conformational changes; (ii) ligands other than insulin, such as antibodies, which perturb the extracellular domain, can serve as alternative ways to restore the mutant insulin receptor kinase. This might have promising implications for the design of new drugs to activate the insulin receptor kinase.

Intermolecular insulin receptor phosphorylation. Is activation of the $\alpha_2\beta_2$ insulin receptor due to an intramolecular mechanism or an intermolecular one? This is rather difficult to answer since the autophosphorylating receptor is both substrate and enzyme at the same time. Classically the order of the phosphorylation reaction has been assumed to be indicative of the intramolecular (first order) versus intermolecular (non-first order) nature of the mechanism involved, but such analysis is not without potential artifacts. Therefore, we have used a different approach which allows us also to investigate the intramolecular versus intermolecular nature of receptor phosphorylation in intact cells and with purified receptors.

In brief, mouse NIH 3T3 fibroblasts were transfected with two cDNA constructs, the first one coding for a kinase deficient human insulin receptor (kinase deficient due to a mutation of the ATP-binding site, i.e. lysine 1018 mutated to alanine), and a second one coding for a chimeric receptor with the extracellular and transmembrane domains corresponding to the human epidermal growth factor (EGF)-receptor, and the intracellular domain to the human insulin receptor [27]. The kinase deficient insulin receptor has an $\alpha_2\beta_2$ structure, while the EGF-insulin chimera shows a monomeric structure in the absence of ligand. These receptors are structurally different and can be immunopurified with antibodies to their distinct human extracellular domain. Using this model system we were able to show that addition of EGF will lead to activation of the chimeric receptor, but more importantly, will also lead to tyrosine phosphorylation of both the kinase deficient insulin receptors and peptides corresponding to the "normally" occurring autophosphorylation sites of the insulin receptor. Using highly purified receptor preparations the same phenomena were observed, demonstrating a direct transphosphorylation of kinase deficient insulin receptors by the insulin receptor cytoplasmic domain of the chimeric receptors. From these data we concluded that the autophosphorylation of the insulin receptor occurs through an intermolecular model rather than an intramolecular one. Recent studies show that also within an $\alpha_2\beta_2$ insulin receptor a *trans* mechanism occurs, in which hormone binding to the α-subunit of one of the α-β dimers appears to lead to the phosphorylation of the β-subunit of the other α-β dimer i.e. situated in *trans* [28]. Note that intermolecular phosphorylation appears to be the rule, since it has been found for all the tyrosine kinase receptors examined so far [7, 29, 30]. Concerning the biological implications of such an intermolecular transphosphorylation/transactivation model involving insulin receptors we have suggested that it could underlie the phenomenon of spare receptors seen in insulin action [27]. Indeed, the fact that the maximal effect of insulin for various bioresponses is observed at low level of receptor occupancy (e.g. 5–10 % occupancy of receptors is needed to see the maximal effect of insulin on glucose transport in adipocytes) could be due to ligand-occupied receptors transphosphorylating and by doing so, transactivating unoccupied receptors resulting in an amplification step at the receptor level [27].

We next hypothesised that transphosphorylation/transactivation could also occur between heterologous but related receptors, such as insulin receptors and IGF-I receptors. Our rationale was as follows: Insulin and insulin-like growth factor-I (IGF-I) are structurally related polypeptides that elicit a similar pattern of biological effects after binding to their respective cell surface receptors [31]. Like their ligands, insulin receptors and IGF-I receptors are highly homologous. They are oligomeric glycoproteins composed of two extracellular α-subunits and two transmembrane β-subunits, which have a high degree of homology in most domains. Both receptors bind insulin and IGF-I, but each receptor binds its cognate ligand with a 100–1000-fold higher affinity than the cross-reacting polypeptide. For both receptors interaction of the ligand with the α-subunit stimulates the β-subunit tyrosine kinase activity leading to autophosphorylation of the latter and tyrosine phosphorylation of intracellular substrates. Despite their homologous structures and their overlapping biological effects, the insulin and the IGF-I receptors seem to play significantly different physiological roles. The main function of insulin consists in the regulation of metabolism, while IGF-I is considered to be involved chiefly in cellular proliferation and differentiation [32]. Generally speaking, the effects on cell growth seen with high concentrations of insulin can be accounted for by cross-binding of insulin to the IGF-I receptor. However, in some cell lines, which express the two receptors (i.e. human skin fibroblasts and CHO-K1 cells), insulin seems to be mitogenic through its own receptor [33, 34]. Additionally, in fibroblasts overexpressing the human insulin receptors [35, 36] and in a murine lymphoid T-cell leukaemia cell-line totally devoid of IGF-I receptors, it has been observed that insulin leads to mitogenesis through its own receptor [37, 38]. Based on the prevailing idea discussed earlier that intermolecular transphosphorylation occurs among homologous tyr-

osine kinase receptors, we thought that it might also take place among heterologous, but related receptor tyrosine kinases. Hence, we investigated whether the activated insulin receptor kinase could phosphorylate and transactivate the IGF-I receptor. Using a cell-free phosphorylation assay we were able to demonstrate that hormone-stimulated insulin receptors can indeed transphosphorylate, and more importantly, transactivate IGF-I receptors [39].

Further, recent studies have identified the existence of insulin/IGF-I receptor heterodimers in various tissues and cell lines, composed of an insulin receptor $\alpha\beta$ half-dimer and an IGF-I receptor $\alpha\beta$ half-dimer [40, 41]. Based on these observations it seems reasonable to imagine that transphosphorylation/transactivation reactions between insulin and IGF-I receptor β-subunits also exist within normally occurring insulin/IGF-I receptor hybrids. While the precise physiological significance of transphosphorylation and transactivation involving insulin and IGF-I receptors remains to be determined, the demonstration of such receptor interactions provides us with a novel insight into the intricate biological effects evoked by the polypeptides binding to these receptors. We proposed that such phenomena may play a role in mediating growth promoting responses of insulin, and as such explain, at least in part, the pleiotropism of insulin and related growth factors [39].

Signalling by insulin and IGF receptors

IRS-I and Pt Ins-3 kinase. After the discovery that the insulin receptor is a tyrosine kinase and knowing that several proteins are modified on serine/threonine residues (phosphorylated or dephosphorylated), it was suggested that some or all of the effects of insulin were due to a phosphorylation/dephosphorylation cascade with a switch kinase at some level that converts the phosphotyrosine signal into phosphoserine/phosphothreonine signals. According to this scenario it was anticipated that the first, direct receptor substrate ought to be phosphorylated on tyrosine residues [9, 42].

Several laboratories, including our own, reported on endogenous tyrosine phosphorylated substrates with M_r values ranging from 15–220 k [43–45]. A major breakthrough was achieved by White et al. [45] who identified, and later on cloned and sequenced [46] what they have called IRS-I, insulin receptor substrate-I. In brief, IRS-I or p185 is a cytoplasmic protein containing 34 tyrosines, 14 included in consensus sequences YMXM or YXXM, which represent recognition motives for specific target proteins containing SH2 domains [10, 46]. SH2 domains (SH=Src homology) are non-catalytic regions of approximately 100 amino acids able to bind tyrosine-phosphorylated polypeptides. These domains have been shown to have an important role in activation of signal transduction pathways by formation of multimeric protein complexes [47, 48]. Among the proteins containing SH2 domains and which have been found to directly interact with several tyrosine kinase receptors is Ptd Ins-3-kinase. Ptd Ins-3-kinase is a heterodimeric protein composed of an 85-kDa subunit (p85) and of a 110 kDa subunit endowed with Ptd Ins-kinase activity. The p85 subunit contains one SH3 and two SH2 domains and is the regulatory subunit of the kinase [47, 48]. This enzyme phosphorylates the D-3 position of the inositol ring of the phosphatidylinositol to produce newly identified phosphatidylinositol phosphates: PtdIns*3P*, PtdIns(3,4)P_2 and PtdIns(3,4,5) P_3. These phosphoinositiol lipids are thought to participate in a recently disclosed pathway for transmission of cellular signals including those induced by insulin. However, previous work had shown an insulin-stimulating effect on Ptd Ins-3-kinase only in fibroblasts [49] or CHO cells [50], which are not canonical examples of physiological target tissues for insulin. Therefore, to study the biological relevance of Ptd Ins-3-kinase in mediation of insulin metabolic responses, it was critical to show the occurrence and regulation of this enzyme in rat adipocytes and skeletal muscle, which are major insulin targets for glucose transport and utilisation. We were able to show that in rat adipocytes [51], 3T3L1 adipocytes [52] and skeletal muscle [53] Ptd Ins-3-kinase is efficiently and rapidly stimulated by insulin, and that this activation appears to be coupled to a recruitment of the Ptd Ins-3-kinase to the plasma membrane [51]. We would like to suggest that the shift of the Ptd Ins-3-kinase to the membrane could represent the necessary event that allows the enzyme to encounter and phosphorylate its natural substrates, i.e. the inositol phosphates. More recently, using in vitro reconstitution experiments, we have shown that Ptd Ins-3-kinase associates directly with IRS-I obtained from insulin or – IGF-I stimulated fibroblasts. Importantly, the interaction between Ptd Ins-3-kinase and tyrosine phosphorylated IRS-I appears to be sufficient to activate the Ptd Ins-3-kinase [54]. Our data also emphasize the crucial role of IRS-I in the mechanism of Ptd Ins-3 kinase activation by both insulin and IGF-I receptors. We did not detect major differences in the way the insulin receptor compared to the IGF-I receptor leads to tyrosine phosphorylation of IRS-I and thereafter interacts with Ptd Ins-3-kinase [54]. This could suggest that the specificity of the cellular programme elicited by the two receptors is not likely to be mediated at this level. While Ptd Ins-3-kinase has been implicated in cell growth, the precise role of the inositol 3-phosphates remains to be determined. Our work showing an insulin-regulation of Ptd-Ins-3 kinase in major insulin target tissues (adipocytes and muscle) favours the idea that Pt-Ins-3-kinase function is not strictly

limited to growth promotion, but rather could also operate at the level of metabolic control. In this context the 110 k Ptd Ins-3-kinase subunit presents homology with the yeast protein Vps34p, which plays a role in the targetting of proteins to the yeast vacuole [55]. By analogy it is tempting to speculate that the Ptd Ins-3-kinase is involved in the insulin-induced glucose transporter translocation in adipocytes and skeletal muscle [53].

The MAP-kinase cascade. The mechanism by which cellular signals generated at the plasma membrane as modifications in tyrosine phosphorylation result in changes in the level of serine/threonine phosphorylation has been a major focus during the last years in the research area relating to cell metabolism and mitogenesis in general, and not only in the area of insulin action.

In the mid 1980's we painstakingly started to purify tyrosine phosphorylated enzymes with serine/threonine kinase activity, which were stimulated by insulin [56]. In 1987 Ray and Sturgill [57] identified a mitogen-activated protein kinase [named MAP-kinase, or also more recently, extracellular signal regulated kinase (ERK)], a serine/threonine kinase that phosphorylated and partially reactivated dephosphorylated 90 k S6 protein kinase II (referred to as the pp 90^{rsk} or RSK for ribosomal S6 kinase). More important, in 1990 the same authors showed that MAP kinases have to be tyrosine and serine/threonine phosphorylated to be active [58]. Another major breakthrough came in 1991 when two MAP-kinases $p42^{mapk}$ (ERK-2) and $p44^{mapk}$ (ERK-1) were cloned and sequenced, and found to belong to the family of kinases which are involved in regulating the cell cycle in eukaryotes as diverse as man and yeast [59]. Taking advantage of the published amino acid sequences of ERK1 we produced an antipeptide antibody to ERK1 and showed that the insulin-stimulated serine/threonine kinase activity that we were attempting to isolate earlier corresponded, at least in part, to the MAP-kinase, ERK1 [60]. We were able to demonstrate that ERK1 is a dual kinase as far as its autophosphorylation is concerned, although the exact role of the phenomenon in activation of the enzyme remains to be determined [60]. Assuming that ERK1 was part of a phosphorylation/dephosphoryation cascade, we decided to use the antipeptides to ERK1 to isolate upstream and/or downstream components of the cascade. In doing so, we were able to isolate a functional complex consisting in ERK1 and the 90 k S6 kinase II, the formation of which was favoured by insulin (in fibroblasts) or nerve growth factor (in PC12 cells) [61, 62]. The essence of the whole concept of a kinase cascade triggered by a ligand-activated receptor kinase such as the insulin receptor – and involving MAP kinase and S6 kinase was based for the major part on clever reconstitution experiments. Our data showing the existence of ligand-regulated functional enzymic complexes in intact cells provided a solid argument in favour of an important physiological role of these two kinases in the signalling pathway used by growth factors and hormones. While the precise role of the RSK in S6 phosphorylation remains to be determined, data from Dent et al. [63] and Gomez et al. [64] puts the MAP-kinase cascade in a hot spot of insulin action. Indeed, the authors demonstrated that ISPK1 (insulin stimulated protein kinase-1), is closely related, if not identical, to the frog S6 kinase II, and plays a major role in glycogen metabolism since it phosphorylates the G subunit of phosphatase 1, that in turn leads to (i) dephosphorylation and activation of glycogen synthase and, (ii) dephosphorylation and inactivation of phosphorylase kinase. Together these data demonstrate that, besides its potential effect on protein synthesis via the S6 kinase II/S6 protein pathway, MAP kinase appears as a key kinase in the regulation of major metabolic responses such as glycogen synthesis. In addition, preliminary data from our laboratory indicate that MAP-kinase might be involved in regulation of glucose transport [65]. In fact, the MAP-kinases are likely to subserve a broad range of cellular functions as a growing list of substrates is appearing [for review see 66, 67]. In brief, at least the following cytoplasmic substrates, have been identified; $p90^{rsk}$, ISPK, MAPKAP-kinase 2 (MAPK-activated protein kinase 2), and phospholipase A2. Further, a key nuclear function is suspected, given the fact that a series of transcription factors are substrates such as c-Myc, c-Myb, $p62^{TCF}$, and SRF. Finally, tyrosine kinase receptors such as the EGF receptor also appear to be substrates for the MAP-kinases.

During the last couple of years an amazing flurry of reports have appeared addressing the sequence of events which link cell-surface receptor tyrosine kinases to gene expression. Together these reports enable us to build a kinase cascade going from the cell-surface to the nucleus – as shown in Figures 3 and 4. It is impossible in such a short review to cite all the scientists who have contributed to this research area, but the work of the two pioneers E. Fischer and E. Krebs, 1992 Nobel Prize Laureates in Physiology or Medicine, has had a decisive impact in the conceptualisation of a phosphorylation/dephosphorylation cascade. While it is clear that the depicted pathway is an over-simplified model, it clearly reflects the impressive progress which has been made in understanding signalling by tyrosine kinase receptors such as the insulin and IGF-I receptor. The complete signalling circuitry is certainly not linear, but very likely extremely complex and intricate as the different kinases involved have several substrates, and evidence already exists for negative feedback loops. One extremely urgent issue to be addressed relates to the speci-

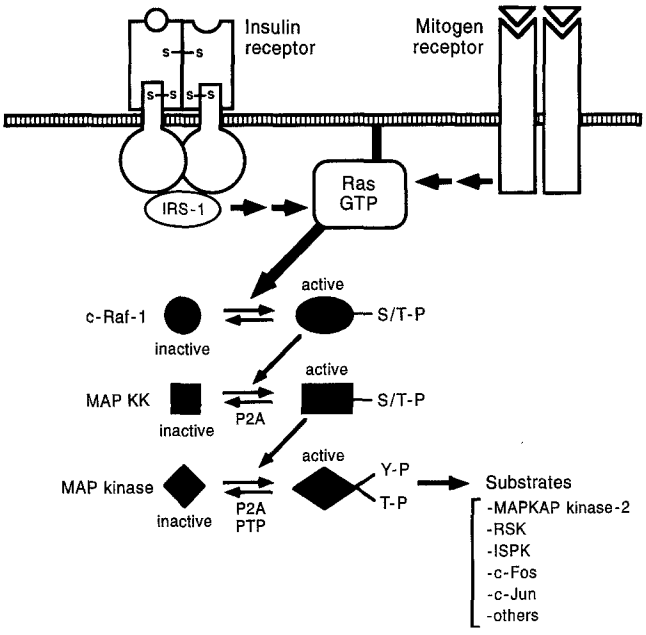

Fig. 3. Insulin/mitogen activation of the MAP-kinase cascade

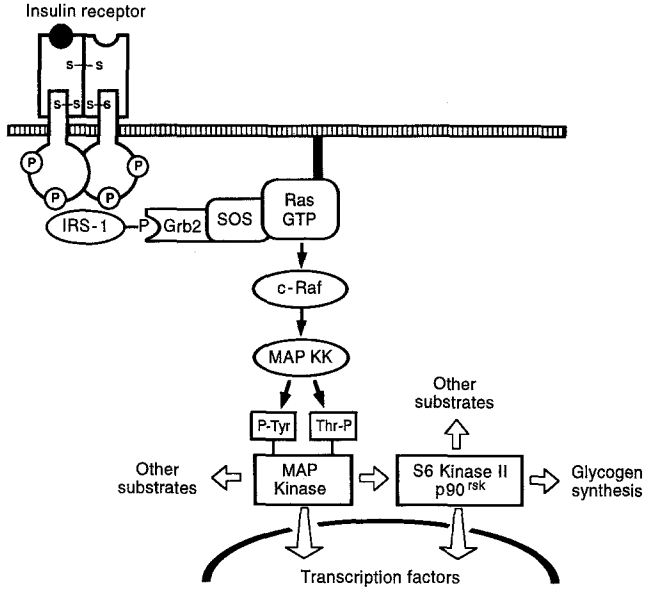

Fig. 4. Insulin and the MAP-kinase. IRS-I: Insulin receptor substrate-I; Grb-2: growth factor receptor bound-2; SOS: Son of Sevenless: guanine nucleotide exchange factor

ficity of signalling as this MAP-kinase cascade is triggered not only by several tyrosine kinase receptors, but also by G protein coupled receptors; all of these receptors having clearly distinct final effects on cell metabolism and destiny. Classically it is believed that different tyrosine kinase receptors generate their specific cellular programme by interacting with a particular signalling pathway or with a particular combination of signalling molecules. However, we have gathered data indicating that – at least in some cell systems – specificity might also be generated by the

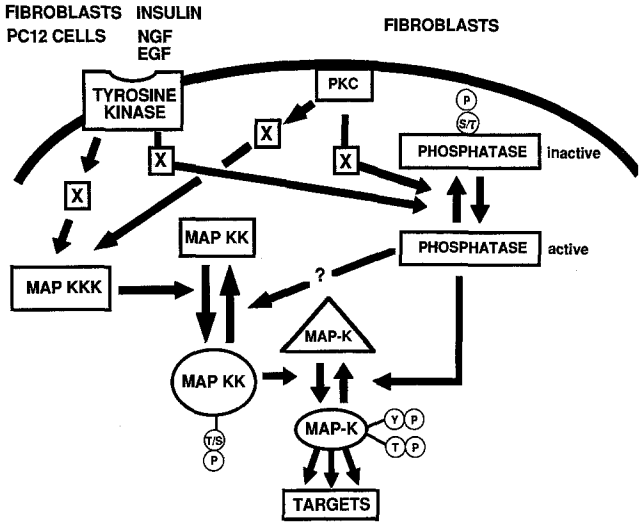

Fig. 5. Role of phosphatases in the MAP-kinase cascade

way receptor tyrosine kinases "talk" to signalling molecules [62, 69]. Thus, we would like to suggest that a particular cellular programme can result from the same signalling events, but which have a vigor and duration specific for a tyrosine kinase receptor chiefly involved in regulation of metabolism, or for a receptor mainly participating in the control of cell growth and differentiation.

Another important factor which could create specificity relates to the cellular localization of the signalling molecules involved. For example, the MAP-kinase, ERK1, will translocate to the nucleus under certain conditions [62, 67]. It is clear that the potential substrates in cytoplasmic versus nuclear compartments are different, and hence the occurrence or absence of the nuclear translocation event will participate in generation of specific signals.

Finally, phosphatase activities leading to modulation or termination of the signalling molecules are also potentially important actors in the generation of biological specificity (Fig. 5). Again, limiting ourselves to the MAP kinase cascade we have evidence showing that phosphatase activities dephosphorylating MAP kinase are regulated differently depending on the agent used to stimulate the kinase cascade, and this certainly could participate in fine tuning of the signalling, and hence of the final bioresponses [70, 71].

Conclusion

While the progress made during the last couple of years in our understanding of the mode of action of tyrosine kinase receptors, including the insulin and IGF-I receptor, is impressive, our current view is certainly fragmented. In this context the MAP kinase signal transduction cascade appears as an important

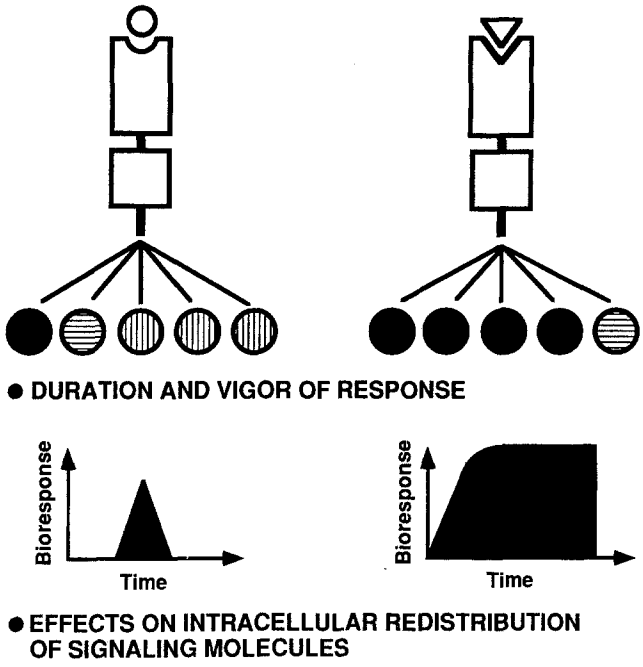

Fig. 6. Specificity of biological programmes induced by tyrosine kinase receptors: receptor specific effects on the same signalling molecules

pathway used by several cell activators, but it is unlikely to be the only one triggered by insulin. Given the explosion of new information, which we have witnessed recently, one can be confident that rapid progress will continue to be achieved. Already the recently discovered molecules participating in insulin action may represent possible new avenues of research for prevention and treatment of insulin resistance and diabetes, which is our ultimate goal.

Acknowledgements. This review has been written on the occasion of a very exciting event, the Minkowski Award Winners Symposium, and I have therefore focused its content – as much as possible – on my research activities since 1985, the year I received the award in Madrid. I would like to thank firstly the scientists, who have introduced me to the fascinating world of the insulin receptor during my stay at the Diabetes Branch of the National Institutes of Health (Bethesda, USA) from 1975 to 1980: Pierre De Meyts, Philip Gorden, Ronald Kahn, David Neville and Jesse Roth. I am very grateful to Pierre Freychet who attracted me to Nice in 1980. It is my great pleasure to thank talented collaborators who were or still are in my laboratory: Robert Ballotti, Véronique Baron, Mireille Cormont, Anne Debant, Morten Fröden, Sophie Giorgetti, Pierre Hainaut, Stanley Heydrick, Dominique Jullien, Perla Kaliman, Aline Kowalski, Christine Lebrun, Isabelle Mothe, Tien Nguyen, Pascal Peraldi, Jean-Claude Scimeca, Jean-François Tanti and Sophie Tartare. Special thanks to Max Fehlmann and Yannick Le Marchand-Brustel who have been precious friends and scientific companions for many years. Steen Gammeltoft has been a critical partner in some of our earlier work; Axel Ullrich provided us with essential tools for receptor studies. Joseph Schlessinger, a loyal friend for almost 20 years, has been a most stimulating collaborator, but more important an advisor for scientific and personal matters. I have been honoured by the invaluable support of Edwin Krebs. Finally, I would like to thank the organizations which made this research possible at the financial level: INSERM, ARC, (Association pour la Recherche contre le Cancer), La Ligue Française contre le Cancer, AFM (Association Française contre la Myopathie), Université de Nice-Sophia Antipolis, Bayer-Pharma, Servier, and Alfediam-Lilly.

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