COMMENTARY

Insulin signalling: putting the 'G-' in protein–protein interactions

Craig C. MALBON[1]

Department of Pharmacology, Diabetes & Metabolic Diseases Research Center, School of Medicine-HSC, State University of New York at Stony Brook, Stony Brook, NY 11794-8651, U.S.A.

Cell signalling via receptor tyrosine kinases, such as the insulin receptor, and via heterotrimeric G-proteins, such as $G\alpha_i$, $G\alpha_s$ and $G\alpha_q$ family members, constitute two of most avidly studied paradigms in cell biology. That elements of these two populous signalling pathways must cross-talk to achieve proper signalling in the regulation of cell proliferation, differentiation and metabolism has been anticipated, but the evolution of our thinking and the analysis of such cross-talk have lagged behind the ever-expanding troupe of players and the recognition of multivalency as the rule, rather than the exception, in signalling biology. New insights have been provided by Kreuzer et al. in this issue of the *Biochemical Journal*, in which insulin is shown to provoke recruitment of $G_{\alpha i}$-proteins to insulin-receptor-based complexes that can regulate the gain of insulin-receptor-catalysed autophosphorylation, a proximal point in the insulin-sensitive cascade of signalling. Understanding the convergence and cross-talk of signals from the receptor tyrosine kinases and G-protein-coupled receptor pathways in physical, spatial and temporal contexts will remain a major challenge of cell biology.

Key words: $G\alpha_{i2}$, insulin signalling, multivalent signalling, protein–protein interaction, receptor tyrosine kinase, Src homology 2 domain (SH2 domain).

RTKs (receptor tyrosine kinases) are a major subfamily of the 500+ protein kinases identified in the human genome, and include the well-known cell-surface receptors for EGF (epidermal growth factor), VEGF (vascular endothelial growth factor), PDGF (platelet-derived growth factor), IGF-I (insulin-like growth factor-I) and insulin. GPCRs (G-protein-coupled receptors) that transduce ligand activation to various effector molecules via heterotrimeric G-proteins constitute the most populous superfamily of cell-surface receptors, whose genes probably account for 5–10 % of the human genome. Since downstream effector pathways for both RTKs and GPCRs share common points of overlap [e.g. the MAPK (mitogen-activated protein kinase) cascades] that are essential to regulation of cell proliferation, differentiation and metabolic regulation, integration of signals from these two dominant pathways must exist. How does this integration of signalling occur? At what levels of each pathway/cascade is integration possible? What specific molecules (e.g. receptor and effector molecules, adaptors, and scaffolds) are involved in the integration? Each of these are fundamental questions of current research in cell signalling.

One of the best examples of integration of signalling can be observed at the convergence of two well-known pathways in metabolic regulation, i.e. insulin signalling representing the RTK family, and β_2AR (β_2-adrenergic receptor) signalling representing the GPCR superfamily. Catecholamines [e.g. the β-adrenergic agonist adrenalin (epinephrine)] are subject to counteraction by insulin at many levels of metabolic regulation, but only in the last 10 years have we obtained insights into how this regulation occurs. For counter-regulation of β_2AR by insulin, the first point of integration is at the most proximal of points in cell signalling, i.e. receptor-to-receptor, with this prototypic GPCR acting as a substrate for insulin-stimulated RTK-catalysed phosphorylation of the β_2AR at specific sites on the cytoplasmic C-terminus. Phosphorylation of β_2AR Tyr^{350} residue in response to insulin creates a phosphotyrosine SH2 (Src homology 2)-binding domain that effectively precludes the β_2AR from coupling to its cognate G-protein (G_s), while enabling the binding of the adaptor Grb2, the p85 regulatory subunit of PI3K (phosphoinositide 3-kinase) and the GTPase dynamin, all molecules involved in GPCR trafficking [1]. Similarly, with regard to insulin-stimulated activation of ERK (extracellular-signal-regulated kinase) 1/2 via the MAPK cascade, β_2ARs have been shown to amplify insulin signals by acting in a scaffold-like capacity [2]. Increasing the amount of cell-surface-expressed β_2AR in cells provoked an increase in the magnitude of the activation of ERK1/2 achieved in response to insulin and required insulin-stimulated phosphorylation of the β_2AR SH2-binding domain. Many other examples of cross-talk among receptors of the RTK and GPCR families are likely to be discovered.

Possible cross-talk between RTKs and G-proteins was first proposed in 1987, based upon the observation by Houslay and co-workers that experimentally induced diabetes leads to the loss of G_i-protein expression in liver [3]. Later, targeted elimination of $G\alpha_{i2}$ in liver, skeletal muscle and white adipose tissue of transgenic mice was found to induce frank insulin resistance [4], whereas expression of a constitutively active mutant ($Gln^{205} \rightarrow Leu$) of $G\alpha_{i2}$ in skeletal muscle, liver and adipose tissue markedly enhanced the glucose-tolerance of transgenic mice [5] and activated translocation of the insulin-sensitive GLUT4 glucose transporter to the cell surface [6]. In molecular terms, however, these provocative studies left unanswered the question of how G-proteins exert these influences on insulin signalling.

In this issue of the *Biochemical Journal*, an article from the Kreiger-Brauer group provides an exciting new dimension to our understanding of the role of the G-protein $G\alpha_{i2}$ in insulin signalling [7]. Using insulin receptors isolated from plasma membranes of human fat cells, these investigators show that activation of the insulin receptor autophosphorylation by insulin was sensitive to activators (e.g. GTPγS) as well as to inhibitors (e.g. GDPβS and pertussis toxin) of G-protein function. Even more revealing, when plasma membranes are pre-treated with nanomolar concentrations of insulin, the amount of $G\alpha_{i2}$ that

[1] To whom correspondence should be addressed (e-mail craig@pharm.sunysb.edu).

associates with the insulin receptor is increased. The ability of insulin to recruit $G\alpha_{i2}$ to the insulin receptor signalling complex represents a novel form of cross-talk between RTK- and GPCR-mediated pathways, and other examples of G-proteins that modulate RTK signalling cascades have been reported [7,8]. For example, $G\alpha_{i2}$ has been shown to interact also with the IGF-I receptor in response to stimulation by IGF-I [8], but the functional consequences of that protein–protein interaction on RTK signalling remains to be established.

Although the concept of modular protein–protein interactions providing the basis for many cell signalling pathways has been evolving over the last 20 years [9], only recently has this multivalent, dynamic, combinatorial perspective on convergence of RTK and GPCR signalling come into focus. When we consider the physical nature of supramolecular complexes that are involved in protein synthesis, protein trafficking and other cellular functions, one must be struck by the unintentional negative impact that the Singer–Nicolson 'fluid mosaic' model of membrane proteins [10] appears to have had on our ability to transcend from the *petite* to the *grande* vision for cell signalling. The possible explanations offered to explain the ability of insulin to recruit $G\alpha_{i2}$ to insulin receptor complexes are probably only a few of the possibilities for protein–protein modules that span major RTK/GPCR signalling pathways. State-of-the-art proteomics, *in silico* biocomputational modelling in search of new motifs created by protein–protein interactions and sensitive read-outs capable of deciphering the effects of subtle mutations of those new, bipartite surfaces will be essential to test these provocative possibilities as well as to test our abilities to think of cell signalling on the truly *grande* scale.

Received 14 April 2004/16 April 2004; accepted 19 April 2004
Published on the Internet 8 June 2004, DOI 10.1042/BJ20040619

I express gratitude to the National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, and to the American Cancer Society for their generous support.

REFERENCES

1 Baltensperger, K., Karoor, V., Paul, H., Ruoho, A., Czech, M. P. and Malbon, C. C. (1996) The β-adrenergic receptor is a substrate for the insulin receptor tyrosine kinase. J. Biol. Chem. **271**, 1061–1064
2 Wang, H., Doronin, S. and Malbon, C. C. (2000) Insulin activation of mitogen-activated protein kinases Erk1,2 is amplified via β-adrenergic receptor expression and requires the integrity of the Tyr^{350} of the receptor. J. Biol. Chem. **275**, 36086–36093
3 Gawler, D., Milligan, G., Spiegel, A. M., Unson, C. H. and Houslay, M. D. (1987) Abolition of the expression of inhibitory guanine nucleotide regulatory protein G_i activity in diabetes. Nature (London) **327**, 229–232
4 Moxham, C. M. and Malbon, C. C. (1996) Insulin action impaired by deficiency of the G-protein subunit $G_{i\alpha 2}$. Nature (London) **379**, 840–844
5 Chen, J. F., Guo, J. H., Moxham, C. M., Wang, H. Y. and Malbon, C. C. (1997) Conditional, tissue-specific expression of Q205L $G\alpha_{i2}$ *in vivo* mimics insulin action. J. Mol. Med. **75**, 283–289
6 Song, X., Zheng, X., Malbon, C. C. and Wang, H. (2001) $G_{\alpha i2}$ enhances *in vivo* activation of and insulin signaling to GLUT4. J. Biol. Chem. **276**, 34651–34658
7 Kreuzer, J., Nurnberg, B. and Krieger-Brauer, H. I. (2004) Ligand-dependent autophosphorylation of the insulin receptor is positively regulated by G_i-proteins. Biochem. J. **380**, 831–836
8 Dalle, S., Ricketts, W., Imamura, T., Vollenweider, P. and Olefsky, J. M. (2001) Insulin and insulin-like growth factor I receptors utilize different G protein signaling components. J. Biol. Chem. **276**, 15688–15695
9 Pawson, T. and Nash, P. (2003) Assembly of cell regulatory systems through protein interaction domains. Science **300**, 445–452
10 Singer, S. J. and Nicolson, G. L. (1972) The fluid mosaic model of the structure of cell membranes. Science **175**, 720–731