

## Insulin receptor trafficking steers insulin action



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In this issue, Boothe and colleagues analyze insulin receptor trafficking in pancreatic beta cells to unravel the trafficking route of insulin receptors and provide novel insights into the potentially different roles of insulin receptor splice variants [1]. Glucose dependent insulin release from pancreatic beta cells is under the tight control of various nutritional, hormonal and nervous inputs. Among the various signaling cascades modulating beta cell function, autocrine activation of the insulin receptor regulates both insulin synthesis and release, and loss of the insulin receptor in pancreatic beta cells results in type 2 diabetes in mice [2]. However, analysis of insulin action in beta cells is complicated by continuous fluctuations in local insulin concentrations and the presence of proinsulin as a low affinity ligand. Binding of insulin to the insulin receptor induces signaling at the cell membrane and, upon internalization, at the endosome. The magnitude and kinetics of insulin receptor internalization depend on extracellular cofactors, as well as the amplitude of insulin receptor activation [3]. Insulin receptor internalization is required to turn off insulin signaling, but it induces endosome specific signal transduction, as insulin receptor signaling complexes at the endosome differ compared to those at the cell membrane [3]. Nevertheless, whether insulin action at the cell membrane or the endosome plays a beneficial or detrimental role for beta cell function and survival remains controversial [4].

To investigate insulin receptor internalization in beta cells, Boothe and colleagues developed overexpression constructs of insulin receptors A and B that were tagged with pH-resistant fluorescent proteins. In contrast to previous C-terminally tagged insulin receptor constructs, the authors introduced the fluorescence tags extracellularly, between the furin-cleavage site and the transmembrane domain. Compared to the cell surface localization of C-terminally tagged insulin receptors, the inter-domain tagged insulin receptors predominantly localized to intracellular vesicles, co-localizing with the endogenous insulin receptor in these cells. Hence, these data indicate that under regular culture conditions, the vast majority of insulin receptors are localized within intracellular vesicles. It will be interesting to see if this is specific to beta cells or if it can be also observed in other cells and how the localization in non-insulin producing cells changes upon hyper-insulinemia, insulin resistance and diabetes.

Further exploring this phenomenon, the authors demonstrate that insulin receptors undergo caveolin-1 mediated endocytosis with subsequent trafficking within flotillin-1 positive structures and degradation in lysosomes. Thus, the current study indicates that endocytosis of the insulin receptor bypasses the classical clathrin and Rab5a or Rab7 endosome pathway in pancreatic beta cells. These results are in line with previous data in primary adipocytes showing a localization of the insulin receptor to caveolae, caveolae-dependent endocytosis of the insulin receptor [5]. Nevertheless, the current study did not reveal differences in the localization of insulin receptor-A and insulin receptor-B and is in contrast to other studies analyzing insulin receptor internalization in hepatocytes or in the liver, showing receptor autophosphorylation followed by rapid internalization via clathrin-coated vesicles [6].

Boothe and colleagues also report that internalized insulin receptors activate Erk signaling in a caveolin-dependent manner, whereas Akt signaling remains unaffected. Interestingly, the insulin receptor R252C mutation in the human insulin receptor, which causes severe insulin resistance, links selective reduction in Erk but not Akt activation with reduced ligand binding affinity and receptor internalization [7]. Thus, these studies could suggest that the R252C mutation selectively reduces Shc mediated Erk activation at the endosome.

In the context of insulin action, Erk signaling is generally more associated with cell proliferation than signaling through Akt. Thus, it is tempting to speculate that, at least in part, the long sought-after differences in mitogenic versus metabolic function of insulin action could depend on differences in the spatial (cell membrane versus endosome) distribution of insulin receptor action and that this could be regulated by modulating ligand binding affinities. Evidence for the differential signaling outcome, depending on ligand availability and signaling intensity/duration, comes from Jimenez-Feltstrom and colleagues, who showed that low insulin concentrations have a stimulatory effect on insulin secretion, while high concentrations of insulin result in inhibition of insulin release from pancreatic beta cells [8]. Furthermore, we previously described the cell surface proteoglycan glypican-4 as an insulin receptor selective cell surface modulator of insulin binding affinity [9], suggesting that both the insulin receptor and IGF1R could be integrated into a network of regulatory cell surface proteins, modulating ligand binding affinity and thereby establishing cell type specific insulin receptor and IGF1R signaling and subcellular distribution.

Thus, it would be interesting to analyze differences or similarities between localization dependent IGF1R and insulin receptor signaling.

This commentary refers to "Inter-domain tagging implicates caveolin-1 in insulin receptor trafficking and Erk signaling bias in pancreatic beta-cells by Tobias Boothe et al.", http://dx.doi.org/10.1016/j.molmet.2016.01.009.

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## Commentary

Boothe and colleagues report no differences between insulin receptor and IGF1R trafficking in beta cells. However, a final conclusion would require a more detailed analysis, as the IGF1R forms heterodimers with both insulin receptor isoforms, though with different affinities for insulin, complicating the current analysis. Hence, using a combination of transgenic mouse lines and these new insulin receptor and IGF1R imaging tools will allow for dissecting the spatial and temporal distribution of insulin receptor/IGF1R action.

The study by Boothe et al. offers novel insights into the regulation of insulin receptor trafficking in beta cells opening up various new research opportunities. Recently, clathrin-dependent internalization of the epidermal growth factor receptor (EGFR) was shown to result in recycling of the receptor, whereas a clathrin-independent endocytosis pathway leads to degradation of EGFR [10]. Since the current study reports caveolin-dependent trafficking of the insulin receptor, it would be interesting to see if there is indeed no recycling of the insulin receptor back to the plasma membrane, in beta cells, as described for the clathrin-independent endocytosis of the EGFR in Hela cells.

Future studies will need to integrate the role of cell type specific insulin receptor trafficking and its associated signaling to further understand the complex regulation of insulin action. Moreover, alterations in ligand binding affinity could not only influence signal transduction at the cell surface, but alter receptor internalization and thereby specific signaling from the endosome, potentially contributing to impaired beta cell function, and promoting diabetes pathogenesis.

## REFERENCES

[1] Booth, T., Gareth, E., Lim, G.E., Cen, H., Skovsø, S., Piske, M., et al., 2016. Inter-domain tagging implicates caveolin-1 in insulin receptor trafficking and Erk signalling bias in pancreatic beta-cells. Molecular Metabolism [in press].

- [2] Otani, K., Kulkarni, R.N., Baldwin, A.C., Krutzfeldt, J., Ueki, K., Stoffel, M., et al., 2004. Reduced beta-cell mass and altered glucose sensing impair insulin-secretory function in betalRKO mice. American Journal of Physiology, Endocrinology and Metabolism 286:E41-E49.
- [3] Morcavallo, A., Stefanello, M., Iozzo, R.V., Belfiore, A., Morrione, A., 2014. Ligand-mediated endocytosis and trafficking of the insulin-like growth factor receptor I and insulin receptor modulate receptor function. Frontiers in Endocrinology (Lausanne) 5:220.
- [4] Szabat, M., Page, M.M., Panzhinskiy, E., Skovso, S., Mojibian, M., Fernandez-Tajes, J., et al., 2016. Reduced insulin production relieves endoplasmic reticulum stress and induces beta cell proliferation. Cell Metabolism 23: 179–193.
- [5] Fagerholm, S., Ortegren, U., Karlsson, M., Ruishalme, I., Stralfors, P., 2009. Rapid insulin-dependent endocytosis of the insulin receptor by caveolae in primary adipocytes. PLoS One 4:e5985.
- [6] Jose, M., Biosca, J.A., Trujillo, R., Itarte, E., 1993. Characterization of the hepatic insulin receptor undergoing internalization through clathrin-coated vesicles and endosomes. FEBS Letters 334:286–288.
- [7] Hamer, I., Foti, M., Emkey, R., Cordier-Bussat, M., Philippe, J., De Meyts, P., et al., 2002. An arginine to cysteine(252) mutation in insulin receptors from a patient with severe insulin resistance inhibits receptor internalisation but preserves signalling events. Diabetologia 45:657–667.
- [8] Jimenez-Feltstrom, J., Lundquist, I., Obermuller, S., Salehi, A., 2004. Insulin feedback actions: complex effects involving isoforms of islet nitric oxide synthase. Regulatory Peptides 122:109–118.
- [9] Ussar, S., Bezy, O., Bluher, M., Kahn, C.R., 2012. Glypican-4 enhances insulin signaling via interaction with the insulin receptor and serves as a novel adipokine. Diabetes 61:2289–2298.
- [10] Sigismund, S., Argenzio, E., Tosoni, D., Cavallaro, E., Polo, S., Di Fiore, P.P., 2008. Clathrin-mediated internalization is essential for sustained EGFR signaling but dispensable for degradation. Developmental Cell 15:209–219.