DIABETES

# GPR40 no longer an orphan

Until now, the G-protein-coupled receptor GPR40 was classed as an orphan receptor, that is, its ligands were unidentified. In the 13th March issue of *Nature*, Hinuma and colleagues from Takeda Chemical Industries Ltd, Japan, show that GPR40, which is abundantly expressed in the pancreas, functions as a receptor for long-chain free fatty acids (FFAs).

Type 2 insulin-resistant diabetes accounts for 90–95% of all diabetes — the increasing incidence largely driven by the dramatic rise in obesity in Western societies. A complex network of signalling pathways are activated by stimulation of the insulin receptor, but in patients who suffer from type 2 diabetes, those receptors on cells in tissues such as muscle, fat and liver become less responsive, or resistant, to insulin. Various factors modify the way that insulin is secreted from pancreatic β cells in response to raised glucose levels in the blood plasma. FFAs provide an important energy source as nutrients, and act as signalling molecules in a variety of cellular processes, including insulin secretion. Although FFAs are thought to promote insulin secretion, this mechanism is not clearly understood.

By looking for changes in intracellular calcium ion flux, the authors identified GPR40 ligands as saturated FFAs of C12 to C16 length and unsaturated FFAs of C18 to C22. Then, they performed experiments to specifically inhibit the expression of GPR40 in pancreatic β-cells using small interfering RNA (siRNA) technology, monitoring glucose-stimulated insulin secretion from the cells in the presence of FFAs. The results showed that long-chain FFAs amplify the insulin secretion from pancreatic β-cells by activating GPR40.

These results shed light on the role of FFAs in glucose metabolism and indicate that they play a direct role in stimulating production of insulin. GPCRs are well characterized as drug targets, and development of agonists or antagonists of GPR40 could have potential as new anti-diabetic drugs.

*Melanie Brazil*


**References and links**

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BIOTECHNOLOGY

# Fingers in the genome pie

The goal of creating artificial transcription factors (TFs) to regulate the expression of target genes has been pursued most vigourously by using zinc fingers (ZFs) — protein domains that bind to three-base-pair sequences of DNA — to guide the effector domains of TFs to the appropriate regulatory elements in the genome. Unlike RNA interference and antisense approaches, artificial TFs are able to upregulate, as well as silence, gene expression, which opens new avenues for therapeutic interventions. Two papers published this month in *Nature Biotechnology* report new *in vivo* strategies for identifying ZF proteins for the construction of artificial TFs.

Kim *et al.* mined the human genome for sequences encoding ZF domains, and then analysed the binding specificities of these domains individually (by attaching the domains to a ZF protein scaffold), which generated 56 ZFs with distinct DNA-binding specificities for three-nucleotide sequences. These domains were then analysed in combinations of three to determine their nine-base-pair specificity. From these ZF domains, TFs were designed to target the promoter of the human vascular enothelial growth factor (*VEGF*) gene, and these bound with the expected specificities and activated the expression of *VEGF*.

Barbas and colleagues have developed a genome-wide approach for identifying TFs that activate or silence a target gene, or produce a desired phenotype. In this method, a library of randomly generated ZFPs was delivered to a population of human cells, which was then analysed for the ectopic expression of cell-surface proteins. As such, only those ZFPs that are able to bind to DNA sequences in their native chromatin-bound state, and activate gene expression, are identified by this technique.

Both of these papers report the *in vivo* selection of ZFs with specific DNA-binding properties, which obviates the problems of translating results from an *in vitro* set up to the *in vivo* siutation, which can impose additional constraints on the functioning of ZFs. For example, the ZF domains identified by Kim *et al.* might be less immunogenic than *de novo* engineered ZF domains, and those identified by Barbas *et al.*, having been selected for in a human cell line, are immediately known to be able to bind to the right DNA sequences in their natural chromatin-bound state. Together, these papers add to the methodological armamentarium available to TF engineers.

*Daniel Jones*


**References and links**

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