Micromanaging insulin secretion

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The human genome is peppered with genes for small microRNAs, whose functions are only beginning to come to light. One such microRNA is now implicated in the secretion of insulin from pancreatic beta cells.

Biological pathways critical to survival often rely on layers of fail-safe mechanisms. One such pathway is the secretion of insulin from pancreatic beta cells—failure to secrete insulin is lethal, and death by injection of insulin is a theme of fictional and real-life murder mysteries. Control of insulin transcription and secretion is set by potent stimulatory and inhibitory signals, finely tuned to the metabolic state with input from neuronal circuits and growth factors.

In the 11 November issue of *Nature*, Poy *et al.*[1] add a new layer of regulation to insulin secretion. They show that a species of microRNA (miR375) expressed selectively in beta cells targets myotrophin (also known as V-1), a cytoplasmic protien that promotes exocytosis of insulin granules. miR375 disrupts some aspect of translation or processing of myotrophin and possibly proteins with related function.

Much is already known about control of insulin. Stimulants of insulin transcription and secretion include circulating glucose and amino acids as well as peptides such as glucagon-like peptide 1 (GLP-1)[2,3], dubbed incretin peptides to signify that they control secretions from the pancreas. Negative regulators include somatostatins, which act on beta cells to prevent insulin release. Nutrient sensing in the brain regulates hormone secretion through nerve terminals in the pancreas, impinging on adrenergic receptors and other G-protein-coupled receptors on beta cells[4]. Superimposed on all this are input signals to beta cells from insulin itself and related growth factors[5].

First discovered in *Caenorhabditis elegans*[6,7], microRNA genes express short hairpin RNAs that are processed to form mature ~21-nucleotide RNA products (termed miRNAs). Interest in these tiny genes exploded with the observation that some miRNA genes are identical in human and worm, and that they use mechanisms related to RNA interference, a double-stranded RNA–mediated silencing pathway[8,9].

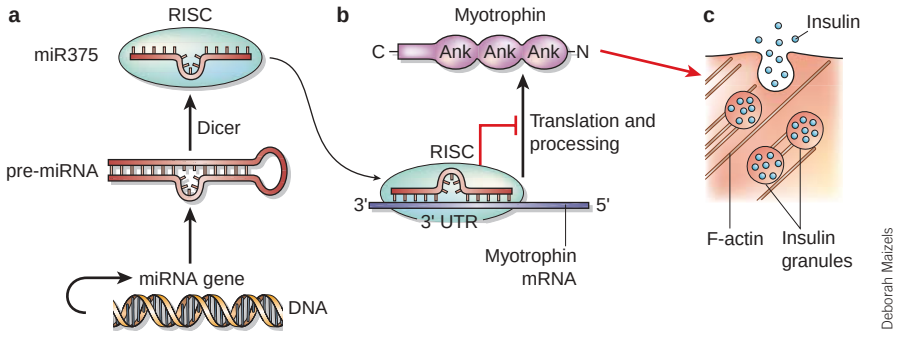

Figure 1 Insulin secretion and microRNA. (**a**) Genomic DNA encodes scores of small RNA strands that, upon transcription, assemble into hairpin structures (pre-microRNA). Next, processing by the ribonuclease Dicer results in the formation of the mature ~21-nucleotide microRNA. The duplex miRNA is unwound and the antisense strand (complementary to the myotrophin mRNA) is then assembled into the RNA-induced silencing complex (RISC). RISC facilitates the search for matching mRNA sequences which then base pair with the miRNA (the bulge indicates a short noncomplementary region often found at the center of miRNA-mRNA duplexes). (**b**) Myotrophin mRNA bound to the RISC complex is unable to be translated or otherwise processed to yield native myotrophin protein. (**c**) Poy *et al.* show that myotrophin promotes exocytosis at some unknown step, whereas its disruption by miR375 causes inhibition of insulin secretion.

At the heart of the RNA interference and miRNA mechanisms lies Dicer, a double-stranded RNA–processing enzyme that chops double-stranded RNA into 21–25-nucleotide RNA fragments (**Fig. 1**). The products of Dicer are loaded into a specialized silencing complex, RISC (RNA-induced silencing complex). RISC can function in two modalities. In one modality, the complex directs the cleavage of complementary mRNA sequences that have perfect base pairing in the central region of the duplex consisting of mRNA and guide RNA. A second mode—used by miR375—blocks the productive translation of imperfectly matched target mRNAs[1].

miR375 is one of hundreds of miRNA genes recently identified in molecular and computational screens of animal and plant genomes[7]. The question now is what do these genes do? To answer this question, it is necessary to identify the targets and to determine how elimination of specific miRNAs perturbs target mRNA expression. Nature has made finding the targets for miRNAs somewhat difficult by allowing imperfect base pairing at the center of the miRNA–target RNA duplex. Consequently, algorithms for predicting targets are error prone and must rely on tricks like comparing candidate target sites for phylogenetic conservation. Nonetheless, several recent studies have generated lists of candidate targets, and researchers have begun to validate these targets with reporter assays and functional studies.

The study by Poy *et al.*[1] represents an elegant piece of detective work along this line. MicroRNA was isolated from total RNA derived from the glucose-responsive pancreatic beta cell line MIN6, and miR375 was the most abundant of the new miRNA species identified that were conserved in other vertebrates.

Poy *et al.*[1] provide good evidence that miR375 targets and attenuates expression of myotrophin, a protein containing ankyrin repeats, which mediate protein-protein interactions in other proteins. The authors show that downregulating myotrophin expression in turn depresses insulin secretion. Because total insulin content is not affected, the secretory pathway or its regulation seem to be likely targets of myotrophin action. Antisense-mediated inactivation of miR375 enhanced myotrophin expression and insulin secretion in cultured MIN6 beta cells, whereas depletion of myotrophin by either

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high expression of miR375 or siRNA-based gene silencing reduced insulin secretion. Furthermore, miR375-mediated depression of myotrophin expression appeared to be dependent on the segment of complementary sequence in the 3′ untranslated region of myotrophin mRNA.

Poy *et al.* next began to address how myotrophin promotes exocytosis of insulin granules. The authors[1] conclude that it does so at a late stage of exocytosis because miR375 expression had no effect on glucose-induced intracellular calcium oscillations, a major mediator of exocytosis. Also, even when calcium was artificially raised inside the beta cell, miR375 expression still attentuated the exocytosis of granules containing insulin. Thus myotrophin must act distal to the ATP-sensitive potassium channel, which is the target of current antidiabetic drugs such as sulfonylureas and the 'glinides.'

One possible site of action that the authors dismiss is cortical F-actin, which is thought to impede insulin secretion[10,11]. Using microscopic techniques, they actually found higher numbers of insulin granules near the plasma membrane in beta cells overexpressing miR375, but could not detect any diminution of actin fibers. But the door is still slightly ajar on this question. Expression of myotrophin in transgenic animals leads to reduced expression of cytoplasmic β-actin in the heart[12]. Moreover, it is possible that increased turnover of polymerized actin without much change in steady-state F-actin could promote secretion[13].

There are several other potential sites of action for myotrophin or other targets of miR375. For example, myotrophin might regulate interactions between L-type calcium channel proteins and a special pool of insulin granules[14]. Other targets could include downstream effectors of calcium and cAMP signaling, which mediates the effects of incretins. In addition to turning on protein kinase A, cAMP activates guanine nucleotide exhange factors that modulate Ras-like GTP-binding proteins[15]—and disruption of one such exchange factor diminishes insulin secretion in beta cells. The complex machinery related to this pathway is yet to be worked out, but it offers deep potential for acute regulation of exocytosis.

Alternatively, myotrophin could regulate longer-term mechanisms that control beta cell function. For example, glucose and incretins both activate the transcription factor CREB, which promotes beta cell viability. TORC2, a coactivator of CREB, is synergistically dephosphorylated in response to calcium and cAMP, causing TORC2 to enter the nucleus[16]. Thus, beta cell regulation through CREB and other transcription factors offers multiple sites for miRNAs to operate.

Finally, the study by Poy *et al.*[1] raises an important question. Is the expression of miR375 or other functional miRNAs in beta cells regulated by glucose or other boosters of insulin release? Curiously, no data on this point are provided. Experiments to test this are straightforward. It is possible that miR375 is simply a constitutive brake on insulin secretion, which is in itself interesting. But dramatic swings of miR375 levels in response to nutrients or incretins could place this micro RNA at the center of a snarl of pathways that modulate insulin levels. If that is the case, an exiting new era of beta cell biology—and possibly therapy—is about to dawn.

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Cancer drug discovery: the wisdom of imprecision

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Drug discovery is either an exact business that is based on detailed knowledge of target structure or it is a fishing expedition that uncovers new drugs through screening of random compounds for their biological effect on target function. Isolation of a new p53 activator with anticancer properties strengthens the reputation of this second approach (pages 1321–1328).

Most human cancers inactivate the function of the tumor suppressor p53 during their progression. There is good reason: p53 blocks proliferation of or eliminates cells that have experienced DNA stress or have deregulated proto-oncogenes. The restoration of function of this protein in tumors has therefore been a focus of intensive drug development efforts.

In this issue, Issaeva *et al.*[1] provide the newest entry in this effort. They report the isolation of a small molecule that rescues impaired p53 function by blocking p53's interaction with its natural inhibitor, HDM-2. The molecule, RITA (reactivation of p53 and induction of tumor cell apoptosis), selectively kills tumor cells of various origins, which happen to retain wild-type p53. RITA is remarkable not only for its potential as an anticancer therapy but also because of how it was discovered. The work flies in the face of two entrenched concepts in drug discovery: that compounds that inhibit protein-protein interactions are poor drug candidates, and that 'rational' drug design based on clean structure-function studies yield the best outcome.

Although half human cancers have mutated p53, most of the rest retain wild-type but nonfunctional p53. This situation presents a therapeutic advantage: the cell expressing wild-type p53 is loaded with a potentially deadly weapon waiting to be awakened in order to kill the tumor or block tumor-cell proliferation. Two types of tumor-specific mechanisms prevent the function of wild-type p53: depletion of an essential cofactor and overexpression of an inhibitor. Loss of Arf is an example of the first type and the overexpression of the natural mediator of proteasomal degradation of p53, HDM-2, represents the second scenario.

Although technically both mechanisms lead to the same result (inactivation of the

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