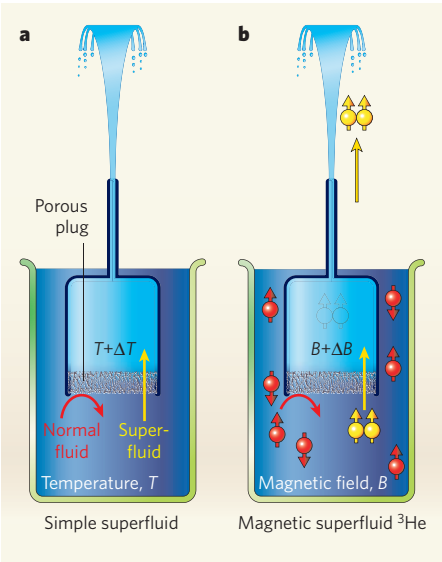

Figure 1 | The magnetic fountain effect. a, A simple bosonic superfluid (^{4}He, for example) comprises a condensed superfluid component mixed into the normal fluid state. When a temperature gradient ΔT is applied across a flow restriction such as a porous plug (a 'superleak'), the superfluid component flows up through the restriction to equalize the temperature, whereas the normal-fluid component is prevented from passing by its viscosity. The result is a 'fountain' of superfluid. **b**, In ^{3}He, the superfluid component consists of pairs of atoms (yellow), because the fermionic nature of ^{3}He atoms prevents them from condensing singly into a superfluid state. In the A_1 phase of ^{3}He studied by Yamaguchi *et al.*[2], the superfluid pairs are highly magnetized, with their spins aligned with a high magnetic field, whereas the unpaired atoms (red) are not magnetized. In this case, the magnetized spin pairs will move to equalize a magnetization difference generated by a magnetic field gradient ΔB, thus supplying the driving force for a magnetic fountain.

normal-fluid component. Because the superfluid condensate carries no thermal energy, the fluid downstream of the plug cools, whereas the fluid that has yet to pass through the plug warms up as the relative concentration of thermally excited normal fluid increases. This mechano-thermal effect, in which an applied pressure gradient results in a temperature gradient over the plug, is well known in superfluid ^{4}He. The fountain effect is the reverse effect, and occurs when a temperature gradient is applied across the superleak. This induces a flow as the superfluid moves to equalize the temperatures, and thus a pressure difference that, when directed upwards, produces a fountain[3] (Fig. 1a).

Yamaguchi and colleagues' magnetic analogy of the fountain effect involves the so-called A_1 phase of superfluid ^{3}He, which exists only in high magnetic fields. In the A_1 phase, all the atom pairs of the superfluid condensate align their spins along the field, whereas the unpaired atoms do not. The superleak allows only the highly magnetized superfluid component to pass, and thus acts as a spin filter: a mechanical flow produced by a pressure difference across the superleak will cause the fluid downstream to become more magnetized. Conversely, a difference in magnetization, generated by a field gradient, will induce a pressure difference across the superleak, because the superfluid flows to equalize magnetization (Fig. 1b).

The experiments[2] require sensitive measurements in very high magnetic fields and at extremely low temperatures. Having observed the effect, the authors went on to study magnetic relaxation processes using the device: a loss of magnetization will be reflected in a loss of fountain pressure, measured by the authors through the deflection of a diaphragm. Intriguingly, their findings suggest that the superfluid A_1 phase in liquid helium is not perfectly magnetized, as had been assumed. Rather, a small fraction of the condensate pairs seems to be orientated against the direction of the field. Consequently, the simplified concepts commonly used to characterize the A_1 phase might have to be revised.

Although the magnetic fountain effect by itself is a beautiful demonstration of a mechanical spin filter, it could also prove to be a useful tool for studying a wide range of magnetic phenomena, such as the relaxation of magnetization in high magnetic fields. There is also great potential for future technological applications based on this system or analogous electronic systems. Some exotic superconductors have structures similar to that of superfluid ^{3}He and might also exhibit a magnetic fountain effect. If such materials can be integrated into devices, then frictionless devices for manipulating spin that rely on externally applied quantities such as magnetic field can readily be envisaged. Such properties could well provide access to a new range of devices and micromachines in the rapidly growing field of spin-based electronics. ■

Shaun Fisher and George Pickett are in the Department of Physics, Lancaster University, Lancaster LA1 4YB, UK.

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DIABETES

Kicking off the insulin cascade

Catherine Jackson

Inhibition of the insulin-signalling pathway leads to insulin resistance, an early step in the development of type 2 diabetes. A novel family of protein activators seems to act near the pathway's inception.

Many of the cellular processes that are vital for human health are regulated by the insulin/insulin-like growth factor signalling pathway, including glucose homeostasis, fat metabolism, cell growth and differentiation, and ageing[1,2]. Defects in this pathway are central to the development of obesity and related diseases such as diabetes, in cancer and in the process of ageing. In this issue, Hafner *et al.* (page 941)[3] and Fuss *et al.* (page 945)[4] report the exciting discovery of a group of factors involved in the insulin-signalling pathway — the ARNO/cytohesin family of Arf activators.

The Arf proteins are molecular switches that control the assembly of multiprotein complexes onto membranes to regulate protein trafficking throughout the cell and membrane-associated signalling events[5]. Arf proteins are inactive when bound to guanine nucleotide diphosphate (GDP) and are active when bound to guanine nucleotide triphosphate (GTP). For their activation, Arf proteins rely on guanine nucleotide exchange factors (GEFs), which catalyse the replacement of GDP with GTP (ref. 5). The ARNO/cytohesin proteins are Arf GEFs that have a characteristic catalytic domain called a Sec7 domain. They are part of a larger family of Sec7-domain proteins that have key roles in membrane trafficking and signal-transduction pathways throughout the cell[5,6]. There are four ARNO/cytohesin family members in humans: cytohesin-1, ARNO/cytohesin-2, GRP1/cytohesin-3 and cytohesin-4 (ref. 6).

There is only one known membrane-permeable, small-molecule inhibitor of the Arf GEFs, the fungal toxin brefeldin A, which is highly specific and targets only three of the Arf GEFs (refs 5, 7). The ARNO/cytohesin proteins, however, have only a very weak affinity for brefeldin A, and their activity is not inhibited by this drug[6,7].

Previously, Michael Famulok and colleagues reported[8] the discovery of an RNA aptamer — a small RNA molecule that binds specifically to a particular protein — that inhibited the guanine nucleotide exchange activity of the ARNO/cytohesin proteins, but not the three GEFs targeted by brefeldin A. Now the same group (Hafner *et al.*[3]) reports an innovative method for 'converting' an RNA aptamer into a membrane-permeable molecule, and the consequent discovery of the first small-molecule inhibitor

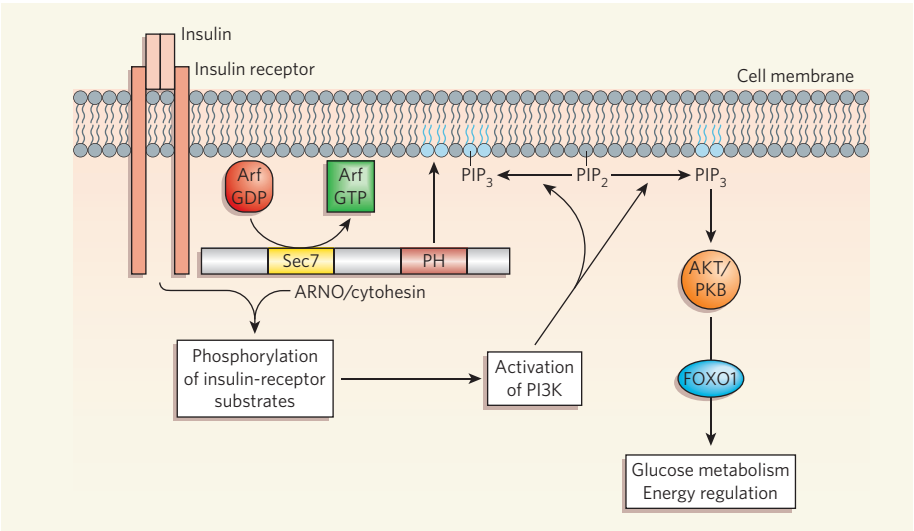

Figure 1 | The insulin-signalling cascade. The binding of insulin to its receptor on the cell surface leads to a cascade of events that eventually activates the FOXO1 gene regulatory factor, which controls the expression of genes involved in glucose metabolism and energy regulation. Hafner *et al.*[3] and Fuss *et al.*[4] show that the ARNO/cytohesin family of proteins is involved in the pathway, possibly near its start. These proteins are guanine nucleotide exchange factors, using their Sec7 domain to catalyse the exchange of GDP for GTP to activate the Arf proteins that mediate the formation of protein complexes on cellular membranes. One of the steps in insulin signalling is the conversion of the lipid molecule PIP_2 to PIP_3, a lipid that is specifically recognized by protein domains called pleckstrin homology (PH) domains.

specific for the ARNO/cytohesin family of Arf GEFs. Their approach involves attaching a fluorescent tag to the RNA aptamer, then screening a large library of small molecules to find those that could displace the fluorescent RNA molecule from its protein target. The authors went on to show that the candidate molecule identified by this screen, SecinH3, does indeed bind to the ARNO and cytohesin proteins with high affinity and inhibits their exchange activity on Arf. By contrast, it binds much less efficiently to other Sec7-domain proteins, and fails to inhibit their exchange activity.

Meanwhile, Fuss *et al.*[4] were studying fruitflies that carry mutations in the single gene (called *steppke*) encoding ARNO/cytohesin in this organism, and they discovered that the mutant flies were smaller and developed more slowly than their normal counterparts[4]. These characteristics can arise from defects in insulin signalling, and indeed, the authors found a profile of gene regulation in these mutant flies consistent with a defect in the insulin cascade. Moreover, when they treated the flies with SecinH3, they found nearly identical traits to those caused by mutations in the *steppke* gene.

These results prompted Famulok's group to test the effect of SecinH3 on insulin signalling in mammalian cells. Insulin and insulin-related growth factor bind to a family of receptors. These receptors are protein kinases; that is, they are able to attach phosphate chemical groups to target proteins — a common means of passing information from one protein to the next along a cell-signalling pathway. And so begins the complex cascade of insulin signalling[2] (Fig. 1). Binding of insulin to its receptor stimulates phosphorylation of a set of 'insulin receptor substrate' proteins, which in turn recruit and activate a lipid kinase, phosphatidylinositol 3-kinase (PI3K). PI3K converts the phosphoinositide PIP_2 to PIP_3, a lipid species that is specifically recognized by proteins with pleckstrin-homology (PH) domains, notably the protein kinase AKT/PKB (ref. 2). AKT/PKB phosphorylates a number of targets including FOXO1, a factor that regulates the expression of insulin-sensitive genes.

In cultured liver cells (HepG2), treatment with SecinH3 produced a gene-expression profile remarkably similar to that of cells with defects in the insulin receptor. Moreover, mice fed SecinH3 had traits similar to mice genetically engineered to have no insulin receptor in the liver tissue[3]. In particular, the SecinH3-fed mice showed signs of insulin resistance, one of the first steps in the development of type 2 diabetes and a cause of 'metabolic syndrome', which is a growing global health problem.

Where in the insulin-signalling pathway does ARNO/cytohesin act? In fruitflies, Fuss *et al.*[4] show genetically that the cytohesin encoded by *steppke* acts upstream of PI3K. In mammalian cells, Hafner *et al.*[3] found that SecinH3 inhibited phosphorylation of insulin receptor substrate 1 by the insulin receptor. Furthermore, co-immunoprecipitation experiments showed that in cells the insulin receptor is physically associated with ARNO and cytohesin-3, as well as one of their substrates, Arf6, whose activation is inhibited by SecinH3. These results indicate that the ARNO/cytohesin proteins act very early in the insulin cascade.

The ARNO/cytohesin proteins all have a very similar modular structure, with an amino-terminal coiled-coil domain, the central Sec7 domain, and a PH domain at the carboxy terminus that specifically binds to PIP_2 and/or PIP_3 (Fig. 1)[6]. Remarkably, variants of the cytohesins in mammalian cells that differ by only a single glycine residue in their PH domains have different capacities to bind PIP_2 versus PIP_3 (ref. 9). In fruitflies, all cytohesin variants seem to have the PH domain that binds with much greater affinity to PIP_3 than to PIP_2. The ARNO/cytohesin proteins, as well as Arf1 and Arf6, are recruited to the cell membrane by the insulin-stimulated production of PIP_3 (refs 9–11). Cells have no PIP_3 before stimulation with insulin or other growth factors. So, this raises the interesting possibility that production of PIP_3 by the insulin-signalling cascade creates a positive-feedback loop that leads to recruitment of additional ARNO/cytohesin molecules to the PIP_3-enriched regions of the membrane.

Metabolic syndrome develops as a consequence of obesity and insulin resistance, and has grown to epidemic proportions in recent years with the rapid escalation in obesity throughout the world[1]. The discovery of a role for ARNO/cytohesin proteins in insulin signalling provides a new target for the development of drugs to combat this growing health problem. The method developed by Famulok and colleagues provides a powerful way of identifying small-molecule inhibitors of therapeutic value. An aptamer library has much more variability than a standard drug library, so it can be used in an initial screening with a much higher probability of success. The method presented can then be used to easily and rapidly screen multiple small-molecule libraries to find drug-like compounds with similar inhibitory potential to that of the original aptamer.

The results presented by Hafner, Fuss and colleagues[3,4] open up a fresh area for investigation, with many questions to be answered. What are the Arf substrates that ARNO/cytohesin GEFs act on in the insulin-signalling pathway? How does ARNO/cytohesin regulate the protein kinase activity of the insulin receptor on its substrate proteins? Are defects in ARNO/cytohesin proteins the basis for insulin resistance in type 2 diabetes? Given how important this signalling pathway is to human health, we can look forward to answers appearing quickly. ■

Catherine Jackson is in the Laboratory of Enzymology and Structural Biochemistry, Bat 34, CNRS, 91198 Gif sur Yvette, France.
e-mail: catherine.jackson@lebs.cnrs-gif.fr

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