Two researchers from the University of Dundee, Scotland - Grahame Hardie and Dario Alessi - have jointly solved a mystery that each had been puzzling over for several years (see 'The bottom line' box for a summary of their work).

Hardie had been looking for the upstream kinase that regulates the metabolic regulator AMP-activated kinase (AMPK) (see the 'Background' box), while only a short walk away Alessi had been searching for the downstream target of the tumor suppressor and protein kinase LKB1. Like fitting two halves of a jigsaw together, the two researchers soon realized that each held the solution to the other's problem.

Hardie has studied AMPK for well over a decade. He knew that it was switched on in situations of metabolic stress, such as low glucose or hypoxia, and by exercise in muscle, and that this required it to be phosphorylated by an upstream kinase. His team had tried purifying this upstream kinase activity and had attempted to determine the amino-acid sequence associated with the activity. But the upstream kinase is not an abundant protein and their approach was not proving successful.

In an attempt to speed things up, Hardie’s group switched from human cells to Brewers’ yeast (Saccharomyces cerevisiae), an organism known to have a homologous kinase to AMPK called Snf1. With the help of two collaborators, Mike Stark in Dundee and Martin Schmidt in Pittsburgh, they could ‘mine’ the yeast genome sequence, which had been completed several years earlier, and use sophisticated analysis tools that are not yet as reliable for more complex mammalian systems. With this approach they
Background

- AMP-activated protein kinase (AMPK) is triggered in response to various forms of metabolic stress (such as exercise in muscle) that alter the relative concentrations of AMP and ATP within the cell.

- Having been activated by low ATP/high AMP, AMPK switches on catabolic pathways that generate ATP (such as glucose uptake and oxidation), while inhibiting ATP-consuming processes, such as biosynthesis and cell proliferation. AMPK thus plays a key role in regulating lipid and glucose metabolism and has been implicated in diabetes, obesity and cardiac diseases.

- Like many protein kinases, AMPK acts within a signaling cascade and is activated when it is phosphorylated by an upstream kinase.

- Peutz-Jeghers syndrome is a genetic condition that causes benign tumors and also puts people at greatly increased risk of developing more aggressive forms of cancer. LKB1, a protein kinase, is the product of the tumor suppressor gene LKB1 which is mutated in cells from patients with Peutz-Jeghers syndrome.

This wasn’t the first time that Grahame Hardie and I had discussed a possible connection between LKB1 and AMPK,” recounts Alessi. “Two years earlier we had thought about it and, although it was a very long shot at the time, had attempted a couple of experiments to see if my preparation of LKB1 would activate AMPK.” But the experiments showed no sign of success and the idea was rapidly forgotten. Anyway, the coincidence that two people working in adjacent labs might be studying two protein kinases that also happened to be adjacent to each other in a biological pathway seemed too good to be true.

But this year, things were different. In the intervening time, Alessi’s team (in collaboration with Hans Clevers’ group in The Netherlands) had characterized LKB1 more closely and found that it was only active when joined to two subunits, Ste20-related adaptor protein (STRAD) and mouse protein 25 (MO25). The earlier activation attempts had been carried out using LKB1 alone. Hardie visited Alessi’s lab late one afternoon, and by lunchtime the next day they had results from the first experiment. “We had hit the jackpot,” claims Alessi, and the results are published in this issue of Journal of Biology [2].

Working together, they had combined the Alessi lab’s human LKB1 and its subunits - which were expressed in cultures of human kidney cells (known as 293 cells, which readily take up DNA) - with AMPK that Hardie’s group had made by expression of the rat protein in bacteria. In this reconstituted cell-free system they found that LKB1 massively activated AMPK. “The great thing was that because Dario had been working on LKB1 for three or four years, although without knowing what lay immediately downstream of it, we were able to make progress very quickly. He had all the right reagents and tools available to test our ideas,” explains Hardie. Suddenly two areas of work joined together to form a whole that was much greater than the sum of its parts (see the ‘Behind the scenes’ box for more on the future of the work).

Some years earlier, Hardie’s group had shown that AMPK needed to be phosphorylated by an upstream kinase at a site (Thr172) in the ‘activation loop’ of the kinase domain. It was also known that, when activated, AMPK had a negative influence on cell growth, and that AMPK is activated by various kinds of stress, particularly stresses that cause depletion of ATP. “It switches off any non-essential processes in the cell, one of which would be cell division,” says Hardie. “If you are short of ATP you want to concentrate on surviving; you don’t want to think about dividing.”

On the other hand, LKB1 was known to be mutated in cells from patients with Peutz-Jegher’s syndrome, and now there was the intriguing possibility that their disabled LKB1 leaves AMPK unable to perform its regulatory role, allowing cell proliferation to carry on unchecked. “This is a very important finding because it brings two
Verification
More work was clearly needed to establish whether the early results were an artifact observed only in a reconstituted cell-free system, or whether the finding would also hold in living cells. The first step was to look at LKB1 and AMPK in HeLa cells. Alessi and his team already knew that LKB1 is not expressed in these highly abnormal transformed human cancer cells, and Hardie’s team had previously discovered that HeLa cells produced AMPK, but that the kinase was not activated by treatments that usually activate AMPK. The answer seemed obvious - the lack of LKB1 explained the lack of activation of AMPK.

What’s more, Alessi and colleagues had already introduced genes into a line of HeLa cells that restored their ability to produce LKB1. Now Hardie tested these cells and found that AMPK was indeed activated in them. It was the first evidence that LKB1 could activate AMPK in intact cells. “When we initially tried to publish this work, however, the reviewers expressed caution,” notes Hardie. The issue was so big that they asked for a greater burden of proof. The problem was that HeLa cells, which are derived from a woman who died of cancer many years ago, have many other mutations as well as lacking LKB1. “The evidence showed that LKB1 was sufficient to activate AMPK, but because we could not rule out the possibility that other upstream kinases might also be missing in HeLa cells, we could not say that LKB1 was necessary,” explains Hardie.

As a consequence, Hardie and Alessi approached Tomi Mäkelä at the University of Helsinki, Finland, who was growing immortalized mouse embryo fibroblasts in which the LKB1 gene had been knocked out. “We were very fortunate that the Finnish group agreed to give us the cells so quickly,” says Hardie. Soon, the Hardie and Alessi labs showed that AMPK was activated normally in wild-type mouse embryo fibroblasts, but in the cells from the LKB1 knockout embryos it was not activated. This time the evidence seemed compelling. “Now LKB1 was both sufficient and necessary,” comments Hardie. “LKB1 is the upstream kinase in these cells, although we can’t say that this is true in all cell types. There could be other upstream kinases in tissues like muscle, or elsewhere, but at least in mouse embryo fibroblasts LKB1 seems to account for all of the upstream kinase activity acting on AMPK.”

The work also leaves open the possibility that LKB1 may influence many other downstream targets. “There are
suggestions that LKB1 is controlling cell polarity,” comments Pawson, adding, “one would imagine that this is through a different target - or is LKB1 only functioning as an AMPK kinase?” The degree of interest in this area is evident from the fact that while the Alessi and Hardie labs were carrying out these additional experiments, the groups of Marian Carlson and David Carling jointly published data pointing to the possibility that LKB1 might be the upstream kinase for AMPK [3].

Drugs, diabetes and cancer
At the same time as working on verifying LKB1 as the AMPK activating kinase, Hardie and Alessi had been testing their cells with two different drugs that stimulate AMPK in wild-type cells. One was phenformin, a close cousin of metformin, the active ingredient of the world’s best-selling product for combating type II diabetes (Glucofage). In muscle, phenformin and metformin activate AMPK and thus mimic the effect of exercise, stimulating the cells to increase the uptake and metabolism of glucose and fatty acids. In cells that have no LKB1 - either HeLa cells or those from LKB1 knockout mouse embryos - phenformin could not activate AMPK, but in modified HeLa cells containing the recombinant LKB1 gene, or in wild-type mouse fibroblasts, the drug worked.

Alessi and Hardie found a similar situation with AICA riboside, another AMPK-activating drug that laboratory tests had shown could be used as a treatment for diabetes in animals. The molecule is converted inside the cell into AICA riboside monophosphate (ZMP), an analog of AMP. A few years earlier, Hardie had shown that the levels of AMP within a cell are critical indicators of cell stress, and are used by the cell as the signal that switches on AMPK to regulate metabolism. They now discovered that treatment of cells with AICA riboside also had no effect in the absence of LKB1.

With the number of people who have type II diabetes increasing dramatically around the world (it is estimated that there will be 200 million by the end of this decade), many pharmaceutical companies are looking for new ways of combating the disease. The patent has just lapsed on metformin, and in any case it is not a very potent AMPK activator, so there is a keen interest in developing a second-generation drug that will directly target AMPK. But it will not be easy. Most drugs are inhibitors, but what is required here is an activator. Now, however, there is a second option, because it may be possible to achieve the same results by targeting LKB1.

As well as having an impact on diabetes, the work has an obvious potential spin-off for patients with Peutz-Jeghers syndrome. “Pharmaceutical companies have been working with AMPK for years, so they must have numerous compounds on their shelves that they could screen for efficacy against the disease,” says Woodgett. It may also be that studying LKB1 and AMPK holds the potential for yielding a new class of therapy for treatment of other more common types of cancer. One thing is certain - the two proteins are now hot property.

References
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