NEWS & VIEWS

DRUG DISCOVERY

Fresh target for cancer therapy

Raymond J. Deshaies

The Byzantine system for degrading proteins inside cells is already the target of a successful anticancer drug. A compound that inhibits another part of this system also shows promise in models of cancer in mice.

Drug discovery is hard. Even harder is drug discovery aimed at biological systems that haven't previously been tested as therapeutic targets. The extraordinary challenge is brought home with sobering clarity by numbers: in 2008, only one new compound was approved by the US Food and Drug Administration (FDA) for treating cancer. In the same year, the US pharmaceutical industry alone spent about $65 billion in the pursuit of new medicines[1], much of which went towards anticancer research — so the apparently slow progress in discovering new cancer medicines is certainly not due to lack of investment.

The identification of proteins that are 'druggable', and of molecules that inhibit them, are therefore unusual events that generate considerable excitement. On page 732 of this issue, Soucy *et al.*[2] report just such a finding. They have discovered a compound that inhibits the never-before-targeted NEDD8-activating enzyme (NAE), and show that this compound suppresses the growth of human lung-tumour tissue transplanted into mice.

In the early 1980s, it was found that protein degradation in cells involves a mechanism by which the small protein ubiquitin becomes covalently attached to the target protein, thereby generating a signal that is recognized by a large, protein-degrading enzyme complex called the proteasome[3]. The attachment process is mediated by a cascade of enzymes. Ubiquitin-activating enzyme (E1) primes ubiquitin for attachment to the protein target by adding an AMP molecule to ubiquitin's carboxy terminus. The activated ubiquitin is transferred to a ubiquitin-conjugating enzyme (E2) and then covalently grafted onto the target protein by a ubiquitin ligase enzyme (E3).

This curious mechanism was soon shown to control a broad range of processes, ranging from cell division to the turnover of damaged proteins. Twenty-five years on, we now know that the ubiquitin–proteasome system (UPS) is, as its name implies, a ubiquitous regulator of cell biology: in humans, the E3 family alone potentially consists of more than 650 enzymes. The extent of ubiquitin's impact on the regulation of proteins is rivalled only by the other big hitter of covalent modifications, phosphorylation.

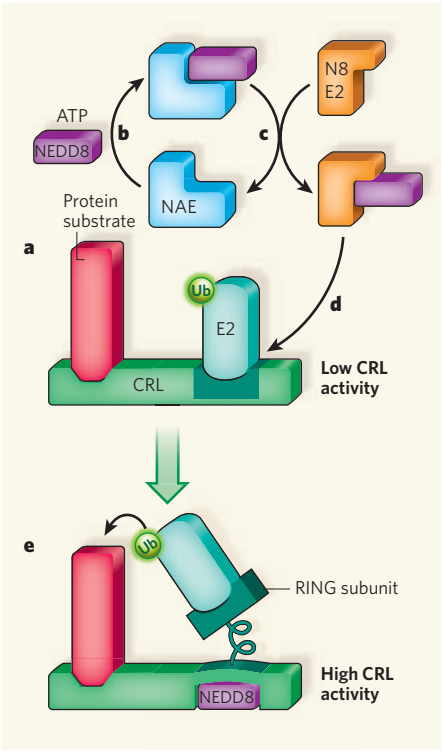

Figure 1 | Activation of CRL enzymes by the NEDD8 protein. When the ubiquitin (Ub) protein is covalently attached to other proteins, it often marks them for degradation. **a**, CRL enzymes mediate the final stage of ubiquitylation, in which Ub is transferred from an E2 enzyme to the protein substrate targeted for degradation. In the absence of NEDD8 (a ubiquitin-like protein), CRL activity is low. **b**, NEDD8 activation of CRLs begins when NEDD8 reacts with ATP and binds to the NEDD8-activating enzyme (NAE). **c**, **d**, NEDD8 is transferred to the NEDD8-conjugating enzyme (N8 E2), and then to the CRL. **e**, The attachment of NEDD8 to the CRL causes the CRL's RING subunit to spring up like a jack-in-the-box. This activates the CRL by bringing the Ub–E2 complex close to the target protein.

The complexity and biological importance of the UPS fuelled speculation about its potential as a target for drug discovery. Proof that it is indeed a worthy target was provided in 2003, when the FDA granted Millennium Pharmaceuticals approval to market a proteasome inhibitor, bortezomib, to treat relapsed refractory multiple myeloma[4] (a cancer of immune cells called plasma cells). The drug arose from pioneering research on proteasome inhibitors[5,6] and was the first new compound approved for treating multiple myeloma since the 1970s. Bortezomib was first synthesized within just seven years of the discovery of the proteasome itself, and gained FDA approval in near-record time (about 8 years; once synthesized, most compounds take about 15 years to gain FDA approval). It thus serves as a remarkable testament to the way in which basic research can drive rapid advances in molecular medicine.

Bortezomib's target isn't very substrate-specific — it degrades all ubiquitin-tagged proteins that are destined for elimination. Such lack of substrate specificity is generally regarded as a disadvantage for drug discovery, as it can cause unwanted side effects. Despite this, bortezomib is a great success, both clinically and commercially: in 2009 it is expected to achieve worldwide sales of $US1 billion. This impressive performance has fanned interest in the potential of drugs that target substrate-specific factors of the UPS, such as the E3 enzymes. But the details of how these enzymes work are poorly defined, and so there is great uncertainty about how to target them and whether such efforts will be rewarded by the development of a successful drug.

Soucy *et al.*[2] (another team from Millennium) now report a clever way around these problems. They realized that, instead of targeting E3 enzymes directly, it might be possible to inhibit other enzymes that activate them. One such enzyme is NAE, which is required to activate E3 enzymes known as cullin-RING ubiquitin ligases (CRLs)[7]. Human cells potentially express more than 350 CRLs. The cullin subunit forms a scaffold on which CRLs assemble, and is reversibly switched on and off when the ubiquitin-like protein NEDD8 is attached and removed.

As is the case for the ubiquitylation of proteins, the attachment of NEDD8 to cullins relies on the activity of a dedicated set of E1, E2 and E3 enzymes[8,9] (Fig. 1). In the first step of

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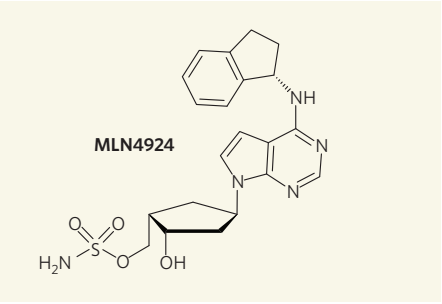

Figure 2 | Structure of an NAE inhibitor. Soucy *et al.*[2] have discovered MLN4924, an inhibitor of CRL activity that blocks the first step of NEDD8 activation.

'neddylation', NAE catalyses the reaction of NEDD8 with ATP (a fuel molecule). The active site of NAE that catalyses this process has been well defined by enzymological studies and X-ray crystallography[10], providing invaluable information on targeting the enzyme for drug discovery. Reasoning that this active site might be a prime target for small, drug-like molecules, Soucy *et al.* carried out a high-throughput screen of a chemical library for NAE inhibitors. This identified an AMP analogue as a good starting point for a medicinal-chemistry programme, from which the authors eventually obtained MLN4924 (Fig. 2) as a highly potent and selective inhibitor of NAE.

With MLN4924 in hand, Soucy *et al.*[2] set out to characterize the dynamics of neddylation–deneddylation cycles and the consequences of abrupt inactivation of NAE. They found that CRLs account for a substantial fraction (about 20%) of all proteasome-dependent protein degradation. More startling was their finding that the NEDD8 cycle is extraordinarily fast — MLN4924 induces nearly complete loss of neddylated cullins within five minutes in cells grown in culture. The consequent inactivation of CRLs leads to a large build-up of CRL substrates, as well as over-replication of and damage to DNA. These results demonstrate that MLN4924 will be an awesome tool for cell-biological investigations on the functions of CRLs and their regulation by NEDD8.

Soucy *et al.* took their studies a remarkable step further by asking two key questions. Can neddylation be inhibited so as to cause accumulation of CRL substrates in human tumours that have been transplanted into mice? And if so, does this affect the growth of the tumours? They had good reason to expect such effects, because they had observed that MLN4924 induces cell suicide in proliferating cancer cells *in vitro*, possibly as a result of the deregulation of DNA synthesis. Happily, the answer to both questions is a resounding 'yes'. Most impressively, the authors report nearly complete regression of transplanted human lung-tumour tissue in MLN4924-treated mice, with no obvious side effects. It remains unclear, however, why a drug that inactivates so many different CRLs (presumably also those in healthy cells) should kill only cancer cells.

These are exciting findings[2], but it is prudent to remember that many promising drug candidates have been shown to cure cancer in mice, only to fail spectacularly in humans. Although MLN4924 is sufficiently promising that Millennium is conducting clinical trials in humans, it remains to be seen whether it will become the second marketed drug that deliberately targets components of the ubiquitin system.

What is clear is that research on the UPS — and more specifically, on CRLs and the NEDD8 pathway — has led to a thorough description of neddylation, the identification of NEDD8's cullin targets, and an exploration of the effects of neddylation on the structure and function of CRLs[11,12], all in the short span of about 10 years. This has culminated in the discovery of an exciting drug candidate[2], currently in clinical trials as an anticancer therapy. And it is worth remembering that NAE genes were first uncovered in the mustard weed *Arabidopsis* in a screen for mutants resistant to the plant hormone auxin[13]. Perhaps the seeds of the next breakthrough in cancer therapy will also sprout from some unlikely place. ■

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ASTROPHYSICS

Hidden Universe uncovered

Ian Smail

An experiment flying on a balloon at the edge of the atmosphere offers the deepest far-infrared view of the sky yet achieved, revealing previously unidentified, dust-obscured, star-forming galaxies in the early Universe.

To the human eye, the night sky is a canopy of stars. With the aid of a small telescope it is possible to see our nearest Galactic neighbours, and using sensitive charge-coupled detectors and the world's largest optical telescopes we can go beyond this and peer into the farthest reaches of the Universe. However, even the most sensitive of these visible-light surveys miss much of the light emitted by galaxies over the history of the Universe. This missing light comes from the youngest stars, which are still cocooned in their natal dust clouds. Dust clouds absorb starlight and re-emit it at far-infrared wavelengths. Surveys of the sky in the far-infrared and the longer-wavelength submillimetre wavebands are thus essential if we are to obtain a complete picture of the star-formation history of galaxies, and hence to identify more precisely the epoch at which galaxies such as our own formed.

On page 737 of this issue, Devlin and colleagues[1] present results from an experiment that identifies for the first time the sources of the bulk of this far-infrared and submillimetre emission in the Universe: a population of dust-obscured, star-forming galaxies seen in the first 5 billion years after the Big Bang. The implication of these observations is that the active growth phase of most galaxies that are seen today is well behind them — they are declining into their equivalent of middle age.

For more than a decade, astronomers have known that the birth of many of the stars that formed in young galaxies in the early Universe is hidden from direct view by dust and finally emerges at far-infrared wavelengths. Because of the subsequent expansion of the Universe, this far-infrared radiation is redshifted (its wavelength is stretched to longer wavelengths), and appears in the submillimetre waveband today. Unfortunately, Earth's atmosphere is relatively opaque to submillimetre wavelengths. Hence, to identify these young, dust-obscured galaxies, astronomers need to get their experiments above the atmosphere, either by sending them into space or by flying them at high altitude.

As its name suggests, the Balloon-borne Large-Aperture Submillimeter Telescope (BLAST) uses a high-altitude balloon to fly a telescope with a 2-metre mirror and a sensitive submillimetre detector at altitudes of up to 40 kilometres to undertake surveys of the cosmos (Fig. 1). Devlin *et al.*[1] report the results of BLAST's most recent flight, an 11-day voyage

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