CHEMICAL BIOLOGY

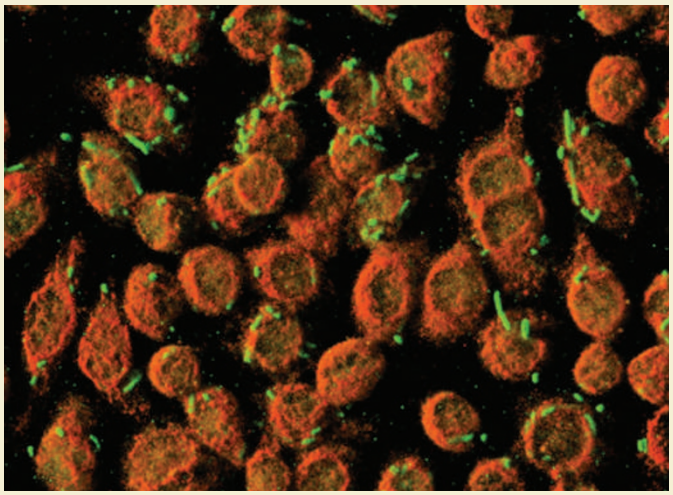
Protein picker

Reporting in *Nature Chemical Biology*, Ngo *et al*. describe a system for cell-selective protein labelling in mixtures of cells (J. T. Ngo *et al. Nature Chem. Biol.* doi:10.1038/nchembio.200; 2009). Their work is based on the principle that proteins can be tracked in experiments if they are engineered so that some of their constituent amino acids contain a tag, such as a radioactive label; tagged amino acids can be added to cells in culture, whereupon the cells incorporate them into newly formed proteins. The general problem with this approach is that all the cells in the culture become labelled, whereas it might be that only certain types of cell need to be tagged.

Ngo and colleagues' solution to that problem involves non-naturally occurring amino acids that contain azide (N_3) groups in their side chains. Azides don't react with biological molecules, but under certain conditions they do react quickly with synthetic molecules that contain alkyne groups (which have carbon–carbon triple bonds). Once incorporated into proteins, azide-containing amino-acid residues will thus react with alkyne-containing fluorescent dyes or affinity reagents, so tagging the proteins for imaging, detection or separation.

Non-natural amino acids are already used for protein labelling, but Ngo *et al*. take the idea further. They exploit an azide-containing amino acid that is incorporated into proteins only by cells that express a mutant of the methionyl-tRNA synthetase enzyme (which is involved in protein synthesis). When the authors added this amino acid to a co-culture of normal and mutant *Escherichia coli* cells, only the mutants were subsequently tagged with an alkyne-containing affinity reagent or fluorescent dye.

To show that their technique could be applied to mixtures of bacterial and mammalian cells, Ngo *et al*.



infected mouse macrophages with *E. coli* cells that express the mutant enzyme, in the presence of the azide-containing amino acid. They then treated the macrophages with a green alkyne-containing dye, which tagged only the bacterial cells (pictured; macrophages are stained orange, and are about 15 micrometres in diameter). The authors were also able to specifically tag newly synthesized bacterial proteins with an affinity reagent, which was then used as a handle to isolate those proteins from the culture.

The authors' approach allows the cellular origins of proteins in complex multicellular systems to be determined. It could therefore be handy for isolating proteins from pathogens in studies of infections, for example, or for identifying the complement of proteins of a single bacterial species living in a community of many other microbial organisms.

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proliferation. The missense *CBL* mutations found in MPNs introduce amino-acid substitutions that disable ubiquitin-ligase activity.

Consistent with a previous report[10], Sanada *et al*.[2] show that mice lacking the *Cbl* gene produce increased numbers of immature blood cells. They also find that *Cbl* inactivation promotes the development of leukaemia in mice engineered to express the pro-leukaemic *BCR–ABL* gene. Mutant Cbl proteins inhibit ubiquitination of growth-factor receptors in blood-cell lines, even in cells that retain a normal copy of the *CBL* gene[2,7], and the authors demonstrate that this inhibition is associated with prolonged receptor activation and an enhanced proliferative response to cytokine growth factors. Although these studies provide evidence that *CBL* is a tumour-suppressor gene, Sanada and colleagues' data[2] also suggest a more complex role for *CBL* in leukaemogenesis. For example, if mutation of a single *CBL* allele is sufficient to disrupt normal ubiquitin-ligase activity, it is unclear why MPN cells also inactivate the normal copy of the gene through aUPD. The authors' findings that the effects of the mutant Cbl proteins are more pronounced in cells that lack a normal *CBL* gene suggest that other biochemical properties contribute to tumour growth. This idea is consistent with the observation that mice lacking *Cbl* do not spontaneously develop MPNs, as would be expected if the gene acted purely to suppress tumour formation. Finally, when the authors over-expressed mutant *CBL* in fibroblasts, these cells showed cancerous properties[2].

So what are we to make of *CBL*? Is it a tumour suppressor or an oncogene, or, as Shakespeare might have put it, "more than kin and less than kind"? On the one hand, there is strong selective pressure to delete the normal *CBL* allele in tumour cells, resulting in loss of ligase activity that restrains the output of activated growth-factor receptors. These are impeccable credentials for a tumour-suppressor protein. However, the blood cells of patients with MPNs invariably retain at least one gene that encodes a functional, albeit mutant, Cbl protein. Furthermore, mutated Cbl proteins seem to acquire unexpected growth-promoting functions[2] — this gain-of-function characteristic is not seen in blood stem cells that lack *CBL*. Such features implicate *CBL* as a bona fide oncogene.

Tumour suppressor or oncogene? Perhaps a solution to this conundrum is that this multi-domain protein fine-tunes the growth of blood stem cells and progenitor cells by simultaneously promoting and restraining growth through distinct protein–protein interactions. Recent studies offer intriguing clues about the potential growth-promoting biochemical properties of mutant Cbl proteins. First, Sanada *et al*.[2] find that expression of the mutant Cbl proteins is associated with aberrant phosphorylation of STAT5, an activator of gene transcription. Aberrant phosphorylation is a biochemical feature of some types of MPN[11], and although in this report[2] it may be due to loss of Cbl-mediated ubiquitination of cytokine receptors that activate STAT5, other mechanisms are possible. Second, *CBL* mutations and mutations in the oncogene *NRAS* were mutually exclusive in the adult MPN patients studied by the authors. *CBL* mutations were also found only in specimens from children with juvenile myelomonocytic leukaemia (a type of MPN) without mutations in *NRAS* or *KRAS*[8]. As this childhood leukaemia is an MPN in which a hyperactive form of the cell-signalling molecule Ras has a central role, there is likely to be a connection between mutant Cbl proteins and Ras signalling. Data suggesting that Cbl regulates Ras trafficking in cells are intriguing in this respect[12]. The elegant functional studies of Sanada *et al*.[2] thus raise fascinating questions about the nature of oncogenic Cbl-mediated interactions in MPNs and how such interactions might be targeted to treat these disorders. ■

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