Protein surgery

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Studies of human tumours and the immune system have revealed that cutting and pasting of proteins can generate new peptide variants. This startling finding has implications for both proteomics and immunity.

Question: how many different peptides composed of nine amino acids can a cell produce from a protein made up of 500 amino acids? Answer: 482 — the first peptide consists of amino acids 1 to 9 of the protein; the second contains amino acids 2 to 10; and so on to the last peptide, which comprises amino acids 482 to 500.

At least, that's what we thought. But the implication of the paper on page 252 of this issue, by Hanada and colleagues[1], is that protein splicing allows for a much higher number. In other words, by slicing a protein into pieces, stitching different portions together, and then cutting out strings of nine sequential amino acids from the melded pieces, cells can manufacture entirely new sets of peptides from the original protein.

Hanada and colleagues' work centres on the use of peptides by the immune system. Every cell in the body is covered with peptides composed of eight to ten amino acids, glued to receptors known as major histocompatibility complex (MHC) class I molecules. The peptides represent every protein that is being made in the cell, and are crucial in allowing the immune system to detect and eliminate intruders. If, for instance, a virus has infiltrated a cell, then the evidence of its presence will be displayed on the cell surface. The immune system's 'killer' T cells will detect that tell-tale sign and take steps to destroy the infected cell.

Previously, Hanada and colleagues had cloned a human killer T cell that they discovered infiltrating a patient's kidney cancer[2]. They found that the T cells recognized a peptide derived from a particular cellular protein, fibroblast growth factor-5 (FGF-5), that is overproduced in the tumour. In this case, the peptide was presented on a type of MHC class I molecule called HLA-A*03, which is known to display nine-amino-acid peptides with a tyrosine residue at position 3 and a lysine or arginine at position 9.

But what is the precise FGF-5-derived peptide that is recognized by the killer T cells — in other words, what is the T-cell 'epitope'? To find out, Hanada et al.[1] prepared target cells expressing truncated FGF-5 genes. The aim was to make it easier to identify the peptide by narrowing down the region of FGF-5 to search. They discovered that cells expressing a 60-amino-acid chunk of FGF-5, amino acids 161–220, were recognized by the T

cells. Knowing the requirements of the MHC molecule, it should then have been a matter of routine to identify the T-cell epitope. But the T cells recognized none of the possible strings of eight, nine or ten sequential amino acids from the 60-amino-acid fragment.

A clue to the mystery came from the authors' finding that (accidentally?) omitting several amino acids from within this 60-amino-acid fragment did not prevent T cells from recognizing their targets. But cells expressing fragments that were shortened at either end were not recognized. This serendipity prompted the authors to analyse a series of more extended deletions. The smallest construct that allowed T-cell recognition consisted of amino acids 172–176 and 199–220 of FGF-5 — suggesting that what the peptide recognized was actually a patchwork, containing bits from two separate parts of the protein. Indeed, Hanada et al. found that a peptide consisting of five residues from one end of this construct and four from the other was the T-cell epitope in question.

How could a cell make such a cut-and-paste peptide? The authors first wondered whether RNA splicing was the answer. This is a process in which, after a gene has been copied into messenger RNA but before that mRNA is translated into protein, segments of the RNA are excised and the remaining fragments are spliced together. But Hanada et al. judged this to be unlikely, as none of the known 'signatures' of RNA splicing are present in the gene sequence. Could the answer instead be sloppy translation, with the protein-making machinery skipping stretches of mRNA and starting again later? Experiments ruled this possibility out. So a post-translational mechanism seemed likely.

Could the explanation be protein splicing? This would involve the cells in cutting the protein into pieces and sticking them back together in a different order. Similar processes of protein surgery have been observed before, in single-celled organisms and some plants. Hanada and colleagues — two of whom are, coincidentally, from a surgery department — find that it also occurs here. They show that cells can use a synthetic 49-amino-acid fragment, in which
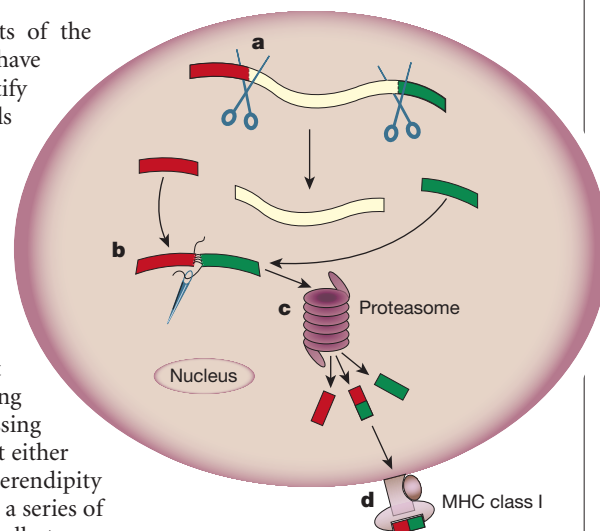
Figure 1 **Protein splicing and the immune system. Killer T cells scrutinize short peptides displayed on MHC class I molecules on the surface of other cells. Hanada et al.[1] have discovered that the process of peptide generation from larger proteins can occur by protein splicing. a, A protein is cleaved by an (as yet unidentified) endopeptidase enzyme. b, Two of the pieces are sewn together (also by an unknown mechanism). c, The stitched-together intermediate is probably then sliced into shorter pieces by the proteasome. d, After further processing, nine-amino-acid peptides are glued to MHC class I molecules, to be displayed on the cell surface. Details such as cellular compartments, peptide transport and trimming 'exopeptidases' have been omitted for clarity.**

the two ends of the T-cell epitope are separated by 40 amino acids, to construct the intact nine-amino-acid epitope. This could only happen if the cells were to cut the protein fragment into pieces, join two of the bits with a peptide bond, and funnel the new piece into the normal epitope-generating pathway (Fig. 1).

So far, two categories of natural protein splicing have been described. In single-celled organisms, controlled protein splicing is an important means of generating functional proteins. It is regulated by 'inteins' (intervening sequences) that catalyse their own excision out of a protein; the flanking fragments, or 'exteins', are then ligated[3,4]. Inteins must have a particular structural domain if they are to catalyse this reaction, and the smallest

intein that can have such a domain consists of 134 amino acids[5,6]. So it is unlikely that the events described by Hanada *et al.* are regulated in the same way. More similar is the process, seen in jack beans, of enzyme-mediated protein splicing — the ligation of polypeptide stretches to result in a functional protein[7]. In addition, protease enzymes, which generally slice up proteins, have been engineered to work in reverse[8]. Whatever the mechanism, this is, to my knowledge, the first time that protein splicing in human cells has been reported.

What are the implications of these findings? First, the potential number of different proteins and protein derivatives produced from our 30,000 genes increases enormously. Processes such as DNA recombination and RNA splicing were already known to increase this number; the discovery of protein splicing adds to the toolkit. Second, although we do not yet know how frequently protein splicing occurs, we have to consider the possibility that cells can produce non-continuous T-cell epitopes not only from tumour proteins but also from infectious agents and our own normal proteins — with implications for vaccine development and autoimmune diseases, as well as for cancer research.

Do mammalian cells use protein splicing to produce functional proteins or peptides that have any physiological role apart from the one leading to its discovery? We have no idea. The enzymes involved in protein splicing are also unknown. Those responsible for cutting might be conventional 'endopeptidases', including the cell's garbage-disposal unit, the proteasome. But what entity can join two protein fragments together? Can proteases work in reverse naturally, as well as being engineered[8] to do so? Could the whole process of cutting and ligation happen inside the proteasome? Also, do sequence motifs dictate where within a protein splicing occurs? Here at least we might know the answer: given that the T-cell epitope described by Hanada *et al.*[1] is identical in every one of the kidney-tumour cells — and in other cells that are engineered to overproduce FGF-5 — this must be the case.

More questions asked than answered? Take it as an indication of an unexpected discovery. ∎

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1. Hanada, K.-i., Yewdell, J. W. & Young, J. C. *Nature* **427**, 252–256 (2004).
2. Hanada, K.-i. *et al. Cancer Res.* **61**, 5511–5516 (2001).
3. Noren, C. J., Wang, J. & Perler, F. B. *Angew. Chem. Int. Edn* **39**, 450–466 (2000).
4. Perler, F. B. *et al. Nucleic Acids Res.* **22**, 1125–1127 (1994).
5. Perler, F. B. *Nucleic Acids Res.* **30**, 383–384 (2002).
6. Perler, F. B. *Cell* **92**, 1–4 (1998).
7. Carrington, D. M., Auffret, A. & Hanke, D. E. *Nature* **313**, 64–67 (1985).
8. Jackson, D. Y. *et al. Science* **266**, 243–247 (1994).

Condensed-matter physics

Supersolid helium

John Beamish

Superfluids flow without resistance. It's hard to imagine, but quantum mechanically possible, that solids should do the same at low enough temperatures. Helium-4 might be the first known 'supersolid'.

At temperatures below 2.176 K, helium-4 enters a superfluid state and flows without friction. This 'perpetual motion' makes superfluidity — perhaps even more than its electronic counterpart, superconductivity — the most dramatic manifestation of quantum mechanics on a macroscopic scale. Despite its appeal, and despite many searches for superfluidity in other systems, it remains an uncommon phenomenon. From 1938, when superfluidity was discovered[1,2], helium-4 was the only known example until 1972 when the phenomenon was seen[3], at much lower temperatures, in helium-3. That temperature difference between the two isotopes' behaviour reflects the intimate connection of superfluidity to Bose–Einstein condensation — the transition that occurs when 'bosons' collect in a single quantum mechanical state. Atoms of helium-4 are bosons, but helium-3 atoms are 'fermions' and must pair up before they can condense into a single state.

In 1995, advances in laser-cooling and magnetic-trapping techniques led to the achievement[4] of Bose–Einstein condensation in rubidium vapour, adding to the list of superfluid systems. That list now includes other gases, such as spin-polarized hydrogen gas[5], and, most recently, molecular gases of paired fermions[6,7]. On page 225 of this issue, Kim and Chan[8] claim the first observation of superfluid behaviour in a solid. A sample of solid helium-4, confined in the nanoscale pores of Vycor glass and rotated in a torsional oscillator, underwent a transition below about 175 mK that indicated the onset of 'supersolid' behaviour (Fig. 1). If it can be confirmed that superflow is occurring in the solid helium, this is a remarkable result indeed.

Despite their rarity, superfluids are fundamental to, for example, statistical mechanics and fluid dynamics, and they are a valuable test bed in fields as diverse as turbulence and cosmology. So it is not surprising that superfluidity has been sought in new systems. Solids, with their atoms localized on a periodic lattice, are certainly the most unexpected phase of matter in which to find superfluidity. However, the low atomic mass and the weak interatomic forces in solid helium make it very different from conventional solids. A quantum mechanical effect known as 'zero-point motion' dominates its properties, to the extent that it does not freeze at all unless external pressure (of at least 25 bar) is applied. At higher pressures helium does crystallize, but zero-point motion remains important and produces a very compressible low-density solid. In such a 'quantum solid', defects such as vacancies on the atomic lattice are easily created and can be very mobile. They might even exist in finite concentrations at absolute zero. Although such zero-point vacancies have not been directly observed, they would be expected to condense into a coherent state at low temperatures. And because mass flow accompanies the movement of vacancies, such a state could exhibit superfluid flow[9,10].

Kim and Chan's results[8] suggest supersolid behaviour in helium that is confined in the pores of Vycor. Vycor is a glass with a disordered network of nanoscale pores, and it has been widely used to study confinement effects in both liquid and solid helium. Confinement tends to suppress freezing, and substantially higher pressures are required to solidify helium in Vycor than when it is not confined. The authors suggest that Vycor's small pores should also enhance the concentration of vacancies in the solid helium, which might explain why they were successful when similar measurements with bulk samples of helium-4 did not detect any supersolid.

If this discovery of a supersolid is confirmed, it is a major advance for the field, because much of our understanding of superfluids is based on the translational invariance that is absent in a periodic solid. There are many open questions, for example the vacancy concentration required for supersolidity and its relationship to fundamental quantities such as the fraction of a sample that is superfluid and the critical velocities therein. There is also an interesting connection to recent work[11] on Bose-condensed gases confined in a periodic optical lattice of laser beams, which showed a transition from superfluid to insulator as the lattice potential was increased.

Kim and Chan's claim is sure to generate interest — and some controversy. Their torsional-oscillator technique is based on the classic experiment by Andronikashvili[12], in which a stack of oscillating disks immersed in liquid helium was used to determine how much of the helium was being viscously dragged by the disks (the normal fraction) and how much had decoupled from the disks (the superfluid fraction). Kim and Chan