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news

Massive variability in a stable binding protein

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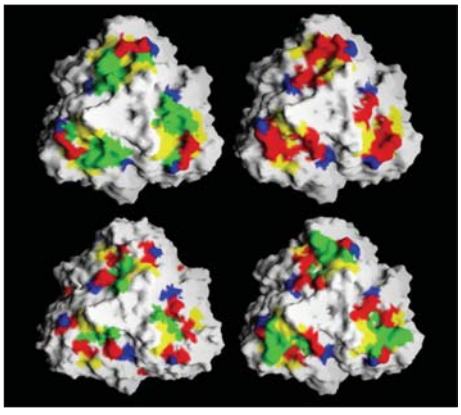
Bordetella is a nasty pathogen that causes whooping cough in humans, but this bacterium also plays host to a bacteriophage intruder. In order to gain entry to the bug, the bacteriophage is equipped with a massively variable protein, the major tropism determinant (Mtd). Now scientists at the University of California, San Diego, USA, have determined the structure of Mtd and its so-called C-type lectin (CLec) fold [1]. With 1013 different sequence possibilities, the protein's binding diversity gives antibodies a run for their money.

Structural variability

In order to infect *Bordetella*, the bacteriophage Mtd must bind to surface molecules that change depending on the bacteria's life stage. During the infective or Bvg+ stage, pertactin serves as the receptor, whereas in the environment Mtd must adapt to other expressed molecules.



Graphic representation of molecular structure of protein variants; colours represent different amino acids. Image courtesy of Jason Miller, UCSD.

Says Partho Ghosh, 'the basic biological problem for the phage is that it has chosen a moving target'. As a solution, the phage constantly and randomly produces variations of Mtd that might bind new receptor targets.

The structural variability of Mtd depends on a retroelement in the phage genome. When bits of RNA are re-transcribed, the mutations appear and are re-inserted back into the genome. The changes occur specifically at 12 adenine-encoded residues dispersed throughout the protein's C-terminal variable region. Each adenine is replaced by another nucleotide, often resulting in a different amino acid the next time the protein is translated.

Perhaps surprisingly, the overall structure of the protein remains static regardless of these changes. This is where the CLec fold differs dramatically from the variable loops of the immunoglobin fold used by antibodies. In that system, the overall protein backbone and conformation can also change dramatically. Ghosh explains, 'the antibodies are probably more variable, but there is a tradeoff'. Antibodies can assume 1014–1016 sequence variations. With the CLec fold, Mtd 'always folds the same, always looks the same, it's always stable. There's a different balance between diversity and stability'.

An *in vitro* diagnostic tool

That balance could lend therapeutic utility to the Mtd variability. Ghosh envisions a situation in which the protein may be used as an *in vitro* diagnostic tool. 'We think this protein can be used in applications where binding is important'. For example, if one wanted to use a protein as bait to find cancer cells, one could use this diversity to 'pan' for binding to cells expressing a particular marker. For this sort of use, the prokaryotic protein has advantages over antibodies, says Ghosh. 'Antibodies are tough to make, they tend to misfold.' Mtd, on the other hand, 'is made by bacteria anyway. We've never had a problem making it'. But the protein would not replace antibody therapies, mainly because of antigenicity problems. Kurt Drickamer of Imperial College London, UK, who was not associated with the work, also sees diagnostic potential in the CLec fold. 'There is hope that such sensors will vastly improve the speed, specificity and sensitivity of diagnostic tests for serum proteins and identification of bacteria, for example. How broadly useful the system turns out to be will be depend on how much the binding selectivity of the site can be changed, but by changing some of the 'invariant' residues, there is room for quite a bit of re-engineering of the site.'

Interestingly, the CLec fold is by no means unique to the *Bordetella* bacteriophage – it can be found in bacterial and eukaryotic proteins, including about 100 encoded by the human genome. Proteins containing the variable fold region run the gamut in terms of function – some of which remain to be seen. But the fold is 'used for binding functions of all kinds', says Ghosh. The bacteriophage CLec fold, though, appears to be a product of convergent evolution. 'One could argue that the entire family [of CLec-containing proteins] came from a common ancestor. But not this one.' And perhaps it's not surprising that nature would devise such a useful tool more than once.

Reference

1 McMahon, S.A. *et al.* (2005) The C-type lectin fold as an evolutionary solution for massive sequence variation. *Nat. Struct. Mol. Biol.* 12, 886–892

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