Beyond antibodies – lessons from bacterial 'immunity'

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Isolation and production of highly specific protein-based binding molecules are crucial to the ever expanding diagnostics, therapeutics and protein array fields. Traditionally, such reagents have been sourced from vertebrate immune systems, where antibodies have evolved over millennia into highly effective molecules of immune surveillance capable of targeting a huge range of targets in response to infection and disease. Now, a growing number of alternative protein scaffolds are being investigated as specific binding molecules incorporating a diverse and powerful range of binding and recognition interfaces [1]. These are being sourced from human proteins, from alternative immune molecules present in evolutionarily old vertebrates, and from highly evolved binding proteins in prokaryotic systems.

Work in our laboratory has focused on developing two such scaffold proteins – the unique IgNAR antibody isotype found in sharks [2], and the Im7 immunity protein from *E. coli* [3]. Im7 is synthesised in *E. coli* carrying the *colE7* plasmid, and its function is to bind and neutralize the co-expressed bacterial toxin ColE7, a potent non-specific nuclease [4]. The ColE7-Im7 complex is then exported from the bacterium and targets neighbouring susceptible bacteria, whereupon the complex dissociates and the ColE7 DNase domain is translocated into the cytoplasm where it mediates chromosomal degradation. Im7- ColE7 binding represents one of the tightest known non-covalent protein-protein interactions ($K_D \sim 10^{-14}$ M).

Structurally, the Im7 protein and its homologues Im9, Im8 and Im2 are composed of four anti-parallel α-helices wrapped around a central hydrophobic core, which stabilise folding of the soluble protein [5]. Sequences of these related immunity proteins show ~50% identity at the amino acid level; however, they bind with high affinity only to their cognate DNase domains, with sequence variability located within the solvent exposed variable loop 1 (predominantly) and loop 2 (to a lesser extent), accounting for the discrimination between binding partners. Different immunity proteins can readily form loop hybrids, reflecting their clear divergence from an ancestral protein. In contrast, the underlying framework is well conserved (Figure 1a), in a manner reminiscent of the underlying immunoglobulin framework supporting six complementarity-determining region loops in antibody variable domains (Figure 1b).

Advantages of Im7 as a scaffold protein include its small size, high stability conferred by the conserved four anti-parallel α-helical framework, and the presence of the two variable surface-exposed loops.

We have already demonstrated that heterologous loop grafts onto the Im7 scaffold retain stability, and the next step is design and construction of large molecular libraries based on randomisation of these Im7 regions, introducing diversity through both sequence and loop-length variability. The development over the last decade of powerful laboratory-based display and selection technologies such as phage- and ribosome- display means that these libraries can be readily screened against target antigens in *vitro*. This mimics *in vivo* antibody selection following animal immunisation; additional laboratory-based techniques can then be applied to similarly mimic affinity maturation processes.

Another example of diversity creation in the prokaryotic world occurs in the ongoing battle of infection and resistance between *Bordetella* bacteriophage and their hosts. *Bordetella* species have separate environmental and pathogenic stages, thus the cell surface proteins

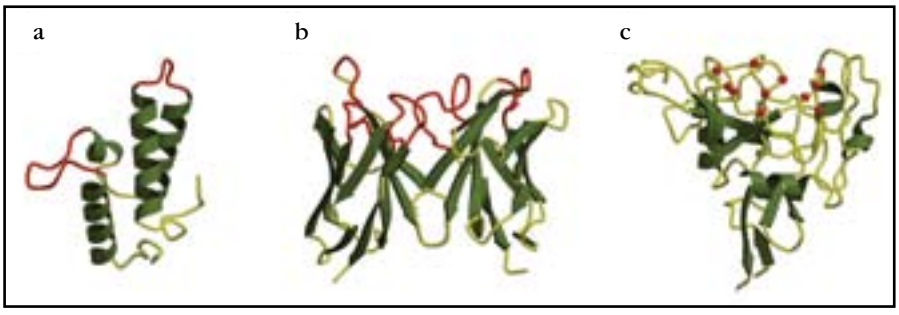

Figure 1. Target binding scaffolds.
(a) Im7 immunity protein from *E. coli* (PDB:1CEI).
(b) Antibody Fv fragment consisting of variable heavy and variable light domains (PDB:1MOE).
(c) C-type lectin domain from *Bordetella* bacteriophage receptor (PDB:1YU1).
Underlying scaffolds and their secondary structures are in green and yellow, and variable surface loops or the Ca atoms of residues implicated in target recognition are indicated in red. Pictures were generated using Molscript and Raster3D.

targeted as phage receptors change both by differential gene expression dependant on phase of life cycle and by conventional mutagenesis, leading to host resistance.

To compensate, *Bordetella* bacteriophage have evolved a novel mechanism of diversity generation where massive sequence variation ($>10^{13}$) is precisely introduced into the target-binding face of the C-type lectin phage receptor [6]. The process is retroelement-mediated and, in a novel twist, sequence diversity is generated only in codons containing an adenine nucleotide, providing an elegant mechanism for confining variation to topographically close but linearly distinct residues on a single face of the protein (Figure 1c).

This mutagenesis strategy provides an example of a bacterial predator protein (phage C-lectin) targeting its prey (bacterial receptor) in a manner similar to the antibody (predator) – antigen (prey) interaction. Similar levels of diversity are generated in both systems and, interestingly both achieve enhanced functional affinity (avidity) by multimerisation, that is through dimerisation in the mature antibody molecule and by trimerisation in the phage.

Thus, while the paradigm for molecular recognition, for us, remains the antibody-antigen system, study of the world of prokaryotes and the continuous battle for survival observed in bacterial and phage populations demonstrates the powerful selection pressures operating on such binding molecules and their targets. Features of the binding and diversity so well characterised for antibodies are also present in these systems, but with novel features and mechanisms that point the way to development of the biotechnological molecular recognition molecules of the future.

References

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It is important that members provide information on potential topics and speakers for symposia to the appropriate Divisional Chairs. Symposia are organised by NSAC and are divided into four main divisions. Each division has a Chairman who oversees the organisation of 10 themed symposia, each with three speakers. Each division also represents a number of SIGs.

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