© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

Full-length antibodies on display

Sachdev S Sidhu

Display of IgG libraries on bacteria promises to streamline antibody engineering.

The transformation of antibodies over the last decade from research tools into the most successful class of targeted therapeutics could not have occurred without key advances in antibody engineering in microorganisms[1]. But the complex structure of full-length antibodies has necessitated a cumbersome 'divide and conquer' approach—antibodies are first dissected into fragments that can be engineered in microorganisms and then the optimized fragments are stitched back into the full-length form for production in mammalian cells. In this issue, Georgiou and colleagues[2] present an approach that circumvents these limitations by allowing libraries of full-length antibodies to be screened in *Escherichia coli*.

The heterotetrameric IgG molecule (**Fig. 1a**) is a remarkable example of intramolecular synergy. The antigen-binding fragment (Fab) domain mediates antigen recognition, and the central fragment (Fc) domain controls effector functions. Each arm of the Y-shaped molecule bears a unique Fab formed by a combination of light and heavy chains. These arms are joined to a glycosylated Fc, which is common to all IgG molecules and mediates *in vivo* interactions that extend half-life and activate effector functions in response to antigen recognition. Although some of these interactions depend on correct glycosylation of the Fc[3], the effects of many approved therapeutic antibodies do not involve immune cell recruitment.

Antibody engineering has relied on three major technological advances. The first is hybridoma technology, which converts B cells from immunized mice into cell lines that can proliferate and produce monoclonal antibodies (mAbs) indefinitely[4]. Although this 'take what you can get' strategy has enabled many applications for which mAbs have become indispensable, it has significant limitations: rodent antibodies are immunogenic to humans, the sequences of mAbs in hybridoma cell lines are unknown and protein engineering using mammalian cells is far from straightforward. Furthermore, as antibodies are raised in animals, there is no possibility of controlling the selection process or introducing flexibility into antibody design.

The second major advance in antibody engineering was the development of methods for expressing antibodies in bacteria. Although IgG molecules are extremely stable once folded, their complex heterotetrameric structure makes expression in microorganisms exceedingly difficult. This limitation was solved by expressing fragments that lack the Fc region but retain the antigen-binding site. Robust methods for the microbial expression of either Fabs[5] or single-chain variable fragments (scFvs)[6] have enabled powerful site-directed mutagenesis for precision molecular engineering. These methods allow antibodies to be cloned, expressed and optimized with respect to properties such as affinity, specificity and stability.

The most notable demonstration of precision antibody engineering was the development of 'humanization' methods. Site-directed mutagenesis is used to transfer antigen-binding sites from rodent antibodies to human frameworks[7], thereby retaining antibody function while reducing the potential for immunogenicity. Improved understanding of antibody structure and folding has enabled expression systems that permit assembly of full-length antibodies in bacteria[3]. Simmons *et al.*[3] used two cistrons to independently express and secrete light and heavy chains, and it was concluded that ability to individually tailor the translational levels of the two chains was instrumental for high level IgG production from bacteria.

The third important development was the so-called 'display' technologies, which combine the best aspects of the hybridoma and recombinant approaches in the form of combinatorial repertoires of antibody fragments that can be manipulated in microorganisms[8,9]. Antibody fragments are displayed on the surfaces of bacteriophage, yeast or bacteria, which also contain the encoding DNA. Display technologies resemble hybridoma technologies in that they provide access to diverse antibody repertoires, and they resemble recombinant technologies insofar as they permit facile molecular engineering. Unlike animal immunization, *in vitro* display approaches permit precise control over selection conditions and thus fine-tuning of structure and function. The sequences of selected clones can be rapidly deduced from the encoding DNA, and recombinant antibody fragments can be produced directly in bacteria. However, full-length antibodies cannot be produced without a cumbersome transfer from bacteria to mammalian expression systems.

With the development of "*E*-clonal" antibodies, Georgiou and colleagues give us the whole shebang—a bacterial system that combines expression of full-length IgG molecules and surface-displayed libraries. In a clever adaptation of their previously described anchored periplasmic expression (APEx) technology[10] (**Fig. 1b**), they have designed a system in which full-length antibodies are secreted into the bacterial periplasm, where they are captured by an inner membrane protein that binds the Fc domain (**Fig. 1c**). After permeabilization of the outer membrane, the displayed antibodies can interact with fluorescently labeled antigen, and binding clones can be selected by flow cytometry. As the anchoring protein is not encoded by the plasmid carrying the antibody genes, soluble, full-length, aglycosylated IgG molecules can subsequently be produced by transformation of a bacterial strain devoid of the Fc-binding anchor protein. By providing a complete, bacterial system for library selection and IgG production, the

Sachdev S. Sidhu is in the Department of Protein Engineering, Genentech, Inc., 1 DNA Way, S. San Francisco, California 94080, USA.
e-mail: sidhu@gene.com

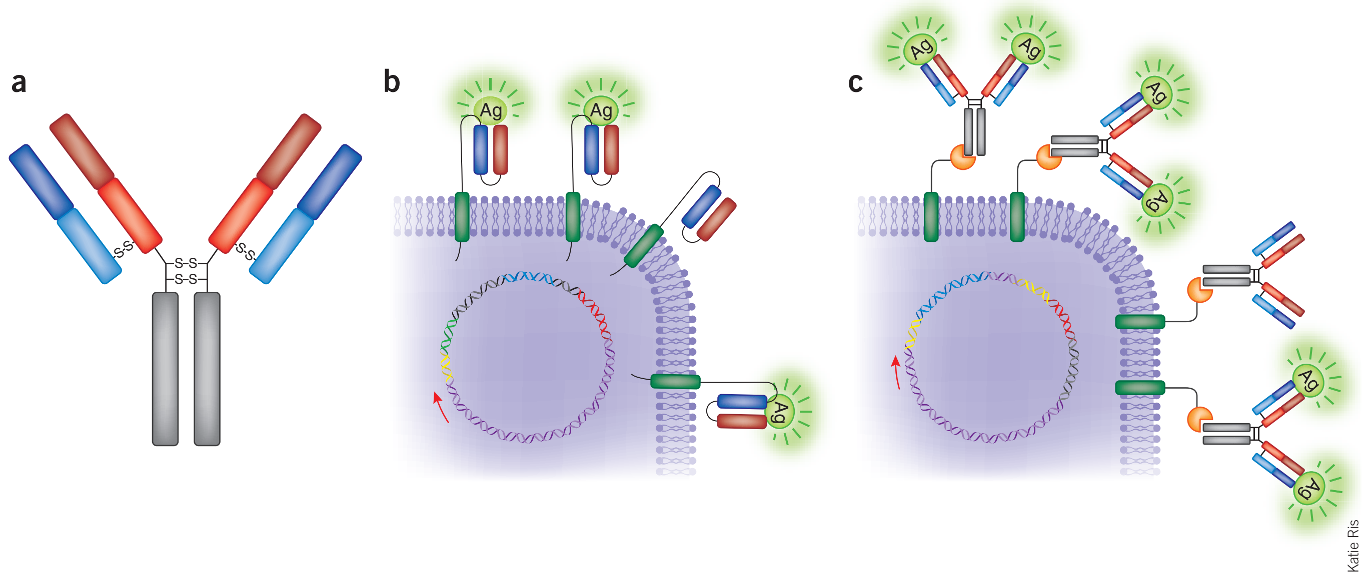

Figure 1 Development of *E*-clonal antibody display. (**a**) The IgG molecule is a heterotetramer of two light chains (blue) and two heavy chains (red and gray) held together by disulfide bonds (-S-S-). The Fab (red and blue) contains the antigen-binding site, which is formed by hypervariable loops at the tips of the Fv domains (dark red and dark blue). The Fc (gray) is common to all IgG molecules and mediates effector functions. (**b**) The APEx technology[10] enables the display of scFv molecules on the surfaces of bacteria by direct fusion with an anchor domain (dark green) inserted in the inner membrane. The APEx plasmid contains a promoter (arrow) that drives the expression of a single open reading frame, which encodes the fusion protein that is targeted to the periplasm by a secretion sequence (yellow). Each cell displays several thousand fusion proteins, and the use of fluorescently labeled antigen (Ag) enables the selection of binding clones by fluorescence-activated cell sorting. (**c**) The *E*-clonal antibody system builds on the APEx concept and enables the display of full-length IgG molecules. The plasmid contains a single promoter that drives the expression of both light and heavy chains, which assemble to form full-length IgG molecules. Secreted IgG is captured by an Fc-binding protein (orange) that is anchored in the membrane using the APEx method. Cells displaying antigen-binding IgG are selected by flow cytometry, and free IgG protein can be purified from a bacterial strain lacking the membrane anchor and Fc-binding protein.

© 2007 Nature Publishing Group http://www.nature.com/naturebiotechnology

E-clonal approach should simplify many areas of antibody research.

Surprisingly, the *E*-clonal system uses a single, bicistronic system for expression of the light and heavy chains, which differs from the expression system of Simmons *et al.*[3], and it will be interesting to see whether a combination of the two approaches may improve the display system even further. It will also be interesting to see how this new technology compares with established display methods. Phage display, which has progressed to the point of using libraries of synthetic antibodies with man-made antigen-binding sites, does not rely on the natural immune response[9]. In contrast, the examples provided by Georgiou and colleagues still depend on the transfer of natural repertoires from immunized mice and are thus subject to the limitations associated with animal immunization. However, the *E*-clonal approach is not restricted to natural repertoires, and in principle, most—if not all—of the antibody design strategies developed for phage display should be applicable. Finally, established display technologies have not yet achieved fully human glycosylation, and this issue has not been addressed by Georgiou and colleagues. Although it is likely that complementary bacterial and mammalian expression systems will still be needed for some time, the *E*-clonal technology promises to ease the path to therapeutic antibody development.

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

1. Imai, K. & Takaoka, A.C. *Nat. Rev. Cancer* **6**, 714–727 (2006).
2. Mazor, Y. *et al. Nat. Biotechnol.* **25**, 563–565 (2007).
3. Simmons, L.C. *et al. J. Immunol. Methods* **263**, 133–147 (2002).
4. Kohler, G. & Milstein, C. *Nature* **256**, 495–497 (1975).
5. Better, M., Chang, C.P., Robinson, R.R. & Horowitz, A.H. *Science* **240**, (1988).
6. Skerra, A. & Pluckthun, A. *Science* **240**, 1038–1041 (1988).
7. Carter, P.J. *Nat. Rev. Immunol.* **6**, 343–357 (2006).
8. Hoogenboom, H.R. *Nat. Biotechnol.* **23**, 1105–1116 (2005).
9. Sidhu, S.S. & Fellouse, F.A. *Nat. Chem. Biol.* **2**, 682–688 (2006).
10. Harvey, B.R. *et al. Proc. Natl. Acad. Sci. USA* **101**, 9193–9198 (2004).