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Review

Selection of antibodies from synthetic antibody libraries

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ABSTRACT

More than 2 dozen years had passed since the field of antibody engineering was established, with the first reports of bacterial [1–3] and mammalian cells [4] expression of recombinant antibody fragments, and in that time a lot of effort was dedicated to the development of efficient technological means, intended to assist in the creation of therapeutic monoclonal antibodies (mAbs). Research focus was given to two intertwined technological aspects: the selection platform and the recombinant antibody repertoires. In accordance with these areas of interest, it is the goal of this chapter to describe the various selection tools and antibody libraries existing, with emphasis on the later, and their applications. This chapter gives a far from exhaustive, subjective "historic account" of the field, describing the selection platforms, the different formats of antibody repertoires and the applications of both for selecting recombinant antibodies. Several excellent books provide detailed protocols for constructing antibody libraries and selecting antibodies from those libraries [5–13]. Such books may guide a newcomer to the field in the fine details of antibody engineering. We would like to offer advice to the novice: although seemingly simple, effective library construction and antibody isolation provide best benefits in the hands of professionals. It is an art as much as it is science.

Selection platforms for recombinant antibody repertoires

The numerous recombinant antibody display technologies available nowadays can be roughly divided into *in vitro* display technologies, including phage display which is the most common antibody display technique [14,15], ribosome display that is completely cell free [16,17], and into *in vivo* display platforms, such as bacterial, yeast and mammalian cell-surface display [18,19].

All these systems will be shortly described in this section, but first, for thematic reasons, it should be clearly stated that the purpose of antibody engineering is to mimic, direct, improve or even surpass the natural *in vivo* process of generating antibodies by the human immune system. Hence, as Bradbury and Marks [20] first pointed out, the selection platforms for recombinant antibody repertoires must enable four features and processes parallel to those occurring in nature: (1) *genotypic diversity* – the diversity *in vivo* which is primarily formed by gene rearrangement events that occurs during B-cell development through combinatorial shuffling of *V-J* or *V-DJ* gene segments of the variable domains of the light chain (V_L) and heavy chain (V_H), respectively and by non-templated nucleotide addition [14,21]. *In vitro*, this diversity is dependent on the nature of the recombinant antibody repertoire as well as on its construction method, as will be further discussed in the next section. (2) *Genotype–phenotype coupling* – *in vivo* and *in vitro* there is a physical linkage between the binding capability of a produced antibody (phenotype) and the DNA sequence encoding it. (3) *Selective pressure* and (4) *amplification* – antigen presence and its gradual reduction in blood concentration are the driving forces that promote *in vivo* generation of high affinity antigen-specific antibodies, whereas exposure of formed recombinant antibody libraries to an antigen of choice, under restricting conditions, followed by repetitive enrichment cycles and/or screening enable the isolation of suitable antibody candidates from these repertoires. Inevitably, careful design of the selection method must address the properties of the antibody repertoire, the technical means as well as their compatibility with the selection tool of interest.

To emphasize the interrelation between the display platform and the properties of the recombinant antibody repertoires, here are two examples of the possible consequences on the outcome of the selective process: (1) *in vitro* display platforms can be applied to very large antibody repertories (up to 10^{14} clones), whereas *in vivo* display platforms can only support modest sizes of antibody repertories (usually $\leqslant 10^9$), due to limitations in the transformation efficiency and of the screening tools [14,22,23]. The antibody repertoire size significantly influences the affinity of the antibody that can be isolated. Antibodies with affinity $\leqslant$100 pM can be isolated from libraries with more than 10^{10} clones, as opposed to antibodies with affinity $\leqslant$10 nM that can be isolated from libraries with 10^8 clones [24,25]. (2) As described by Wark

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and Hudson [26], selection of the same recombinant antibody libraries against the same antigen, using phage display and ribosome display, resulted in distinct antibodies that varied in their affinities and recognized epitopes. In fact, different antibodies can be isolated from a given library and a given display technology by altering the affinity selection protocol [27,28]. Meaning, the selection tool and the way it is applied play a role in the determination of selection outcome, even when the antibody repertoires are identical.

An equally important consequence derived from the interrelation between the display platform and the properties of the recombinant antibody repertoires concerns the feasible usage of each platform. Specifically, display platforms that can support large size antibody libraries are usually used for *de novo* antibody isolation, whereas display platforms that bear modest size libraries are used in most cases for the improvement of pre-existing antibodies. This topic will receive a detailed explanation in the third section of this chapter.

In vitro display platforms

Phage display

Filamentous bacteriophage (phage) was used in the early days of molecular biology for making single-stranded DNA for site-directed mutagenesis and for DNA sequencing [29,30]. Phage display, as a molecular diversity selection tool, was first demonstrated for selection of binders from peptide repertoires by George Smith from the University of Missouri [31]. Five years later, the first reports of the utilization of this technology for recombinant antibody repertoires were published [32–34]. To date, in spite of the time elapsed, the basic concept remained unchanged: phage clones, carrying a recombinant antibody fragment fused to a phage coat protein while the antibody-coding gene is fused at the DNA level to the gene encoding that phage coat protein are subjected to selective pressure. The selective pressure is mostly for binding to an antigen, referred to affinity selection or (bio)panning. Phages, that survive the repetitive cycles of selective pressure due to favorable binding capabilities, can be isolated from a given antibody repertoire and be further characterized and manipulated [25,35–37].

In general, M13 and fd-based display continue to be the most widespread antibody phage display selection tools [14,22] and with a few exceptions (pioneering work done at the Scripps Research Institute [34] where Fab was displayed on p8), all known antibody phage display repertoires are fused to the minor phage coat protein p3 [20], which is involved in the bacterial infection process through the F pilus and is present in 3–5 copies per phage [38] and not to the major phage coat protein p8 which was found to be less efficient, though more prevalent with 2700 copies per phage [39]. In addition, there are two available systems for antibody phage display: one is based on a phage vector that consists of a gene cassette encoding an antibody fragment-p3 fusion instead of the natural gene encoding p3. This system is known as the "3 system" and it enables polyvalent display of the recombinant antibody fragment, with 3–5 copies per phage. The other system, called "3 + 3 system", is based on two elements: the first is a phagemid, which consists the gene cassette encoding an antibody fragment-p3 fusion and a phage ss-DNA packaging signal, but lacks all other necessary components to make vital phages. The second element of the "3 + 3 system" is a helper phage carrying a defective packaging signal, while all other components necessary for replication, packaging and egress from the infected bacterium are intact. This display system results in the monovalent display of antibody-phage coat protein fusions, yet less than 10% of all phages display this chimera on their protein capsid [40]. Another key issue relates to the antibody formats used in phage display platforms: the leading formats, that were actually initially used in the pioneering studies performed by the groups of Sir Gregory Winter from MRC, Cambridge, UK [33] and Richard Lerner from the Scripps Research Institute, La-Jolla, California [41], are single-chain variable fragment (scFv)[1] and Fab [14,23]. Furthermore, in consideration with the all existing possibilities as for the phage type, display system, antibody format, etc. (reviewed in [15,20,35,42]), it is fair enough to say that phage display was and still is by far the most diverse selection tool for antibody repertoires. Phage display is illustrated schematically in Fig. 1.

Ribosome and mRNA display

Among display technologies, cell-free selection platforms (reviewed in [43]), ribosome display and mRNA display (reviewed in [44]) are considered to be very powerful molecular diversity selection tools for antibody repertoires [17], since they allow the screening of very large antibody libraries ranging from 10^{12} to 10^{14} individual clones and thus theoretically and practically enabling the isolation of antibodies with pM affinities [16,45–48]. These two methodologies share almost all features, including: the use of *in vitro* transcription and *in vitro* translation, in either coupled or uncoupled systems, in the presence of either rabbit reticulocyte lysate, wheat germ extract or an *Escherichia coli* S30 extract. Each of them can result in either monoribosome or in polysome display complexes and of course selection against antigen of interest and the use of reverse transcription polymerase chain reaction (RT-PCR) in order to amplify and preserve the affinity-selected clones for the following procedures [16,17,49,50]. However, the difference between the two resides in the displaying complex: in the case of ribosome display, the displaying entity is a stable ternary complex consisting of mRNA, nascent protein and halted ribosomes caused by the presence of antibiotics (such as chloramphenicol or cycloheximide for prokaryotic or eukaryotic ribosomes, respectively) or by the deletion of the stop codon from the translated mRNA [46,51–53]. Whereas, in the case of mRNA display, the ribosome-free complexes are made up of covalently linked mRNA and nascent protein, formed by the addition of the antibiotic agent puromycin that acts as a aminoacyl-tRNA mimetic [54,55]. It should be noted that scFv is the favorable format when using ribosome or mRNA display as the selection platforms, to ensure physical linkage between the variable domains [14,44]. Ribosome display is illustrated schematically in Fig. 2.

In vivo display platforms

Bacterial surface display

In 1993, George Georgiou's group from the University of Texas at Austin, in a proof of concept report demonstrated the use of bacteria as an antibody fragment display system [56]. In detail, they used an Lpp-OmpA' chimera [57] to display two specific scFvs on the outer membrane of the Gram negative bacterium *E. coli*. A few years later they realized this technology into a competent screening tool for antibody repertoires [58,59]. Another approach to display recombinant antibody repertoires on bacteria was developed by the same group several years later [60,61]. This system, which they named APEx (Anchored Periplasmic Expression) was based on the expression of antibody libraries, in a scFv format, in the periplasmic space anchored to the inner membrane of the *E. coli* bacterium. In both approaches, antigen-specific clones were isolated using flow cytometry and their DNA is obtained by PCR for ascending procedures. These two earlier bacterial surface display attempts suffered from the technological shortcoming of the FACS

[1] *Abbreviations used:* mAbs, monoclonal antibodies; scFv, single-chain variable fragment; PDGFR, platelet-derived growth factor receptor; EBV, Epstein-Barr virus; IRES, internal ribosome entry site; CDR, complementarity determining region; VEGF, vascular endothelial growth factor; BCR, B-cell-receptor; HAMA, human anti-mouse antibodies; APEx, Anchored Periplasmic Expression.