

● Antibody phage display

For more than 100 years, polyclonal antisera have been produced by immunisation of animals, against a multiplicity of epitopes (1). With the advent of Hybridoma technology, it was - for the first time - possible to produce a monoclonal antibody of a defined antigen specificity (2). However, despite a tremendous success story, hybridoma technology still has some limitations. The instability of some aneuploid hybridoma cell lines and



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the inability to produce antibodies against toxic, pathogenic, non-immunogenic or highly conserved antigens can be named here (3). To overcome the limitations of hybridoma technology, antibodies or antibody fragments can be generated by recombinant means (Fig.1). A major breakthrough in the field of antibody engineering was the generation of antibody fragments as recombinant proteins in the periplasmic space of *E. coli* (4-6). To circumvent the instability of hybridoma cell lines, the genes encoding the variable regions (V_H and V_L) of the monoclonal antibodies can be cloned into *E. coli* expression vectors in order to produce antibody fragments in the periplasmic space of *E. coli* which preserve the binding specificity of the parental hybridoma antibodies (7).

The production of mouse derived monoclonal antibody fragments in *E. coli* did not remove the major barrier for the broad application of antibodies in therapy as repeated administration of mouse derived antibodies causes a human anti-mouse antibody response (HAMA) (8). This problem can be overcome by two approaches: By humanisation of mouse antibodies (9) or by employing repertoires of human antibody genes. The second approach was achieved in two ways. First, human antibody gene repertoires were inserted into the genomes

of IgG-knockout-mice, allowing to generate hybridoma cell lines which produce human immunoglobulins (10). However,

this method still requires immunisation and is still not really available outside of commercial applications.



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Both restrictions do not apply for the more rational second approach: the complete *in vitro* generation of specific antibod-



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(13), puromycin display (14) or yeast surface display (15), phage display has become the most widely used method (Tab. 1, Fig. 1). The first antibody gene repertoires in phage were generated and screened by using the lytic phage Lambda (16,17) with limited success. The display method most commonly used today is based on the groundbreaking work of Smith on filamentous phage display (18). Here, the genotype and phenotype of peptides were linked by fusing their short gene fragments to the minor coat protein III gene of the filamentous bacteriophage M13, resulting in the expression of this peptide: pIII fusion protein on the surface of phage and allowed the affinity purification of the desired gene by peptide binding. In the same way antibodies fused to pIII can be presented on the surface of

phage M13 (19-23) (Fig. 2). Due to limitations of the *E. coli* folding machinery, complete IgG molecules could hardly be expressed in *E. coli* and displayed on the surface of phages. Therefore, two smaller antibody fragments are used for antibody phage display: the Fab fragment and the single chain Fv fragment (scFv). In a scFv, two polypep-

ptides constituting the antigen binding site (V_H and V_L) are covalently connected via a peptide linker. Two different genetic systems have been developed for the expression of

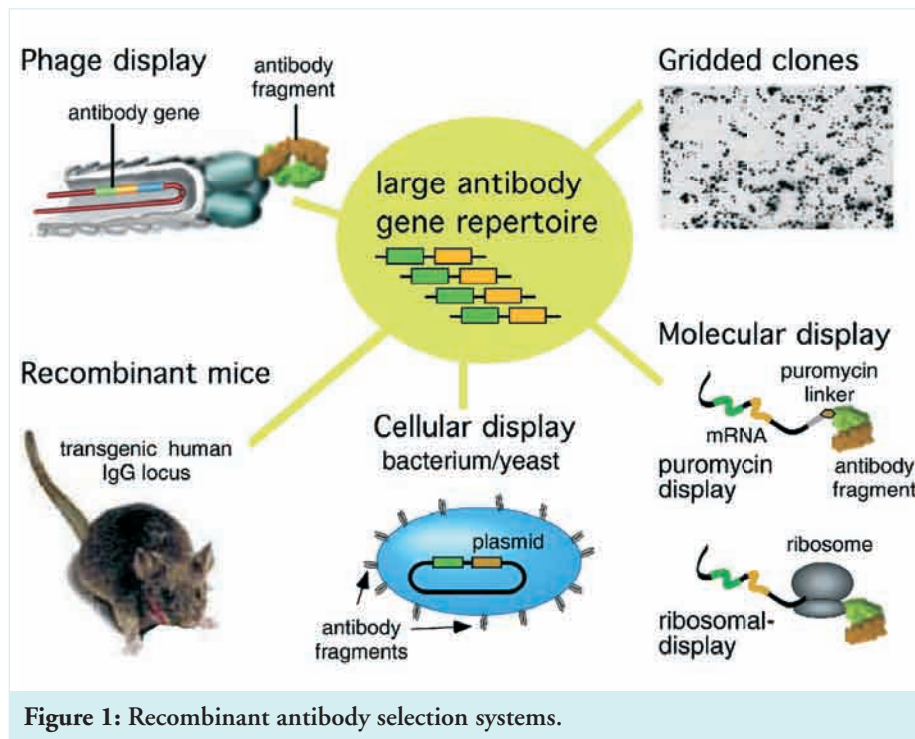


Figure 1: Recombinant antibody selection systems.

the antibody::pIII fusion proteins. First, the antibody genes can be directly inserted into the phage genome fused to the wildtype pIII gene (19). Second, the genes encoding the antibody::pIII fusion proteins can be provided on a separate plasmid with an autonomous replication signal, a promoter, a resistance marker and a phage morphogenetic signal, allowing this „phagemid“ to be packaged into assembled phage particles. The insertion of antibody genes into the phage genome has some disadvantages. First, transformation efficiencies of phage genomes are much lower as of phagemids, thus hindering the construction of complex libraries. Second, replication and antibody expression is linked to the phage genome, which leads to a negative selection pressure. By using phagemids, replication and antibody production are independent from the phage genome. On the other hand, when using the phage genome for the insertion of antibody genes, all copies of pIII are fused to antibody fragments. This can be an advantage for the selection of low affinity binders from very complex antibody gene libraries, because it offers polyvalent antibody display and thence provides an avidity boost during initial selection step. But the infection rate can be reduced because pIII is responsible for the infection of F-pili expressing *E. coli*. As a result, phagemids are most widely used for antibody phage display. A large variety of phagemids for the display of scFvs or Fabs on filamentous phages have been constructed (for an overview see 24). Here, however, a helperphage, usually M13KO7 (25), is necessary for the production of the antibody phage to complement the phage genes

not encoded on the phagemid. Due to its mutated origin, the M13KO7 helperphage genome is not efficiently packaged during antibody phage assembly as the phagemid. The use of M13KO7 helperphage results in fractional and monovalent display of antibody fragments due to the competition of antibody::pIII fusion protein with wildtype pIII during phage assembly - the wildtype pIII will be incorporated into the phage coat preferentially. Mutant helperphage, e.g. hyperphage, are available which offer polyvalent antibody display by eliminating the supply of wildtype pIII during phage assembly (26).

● **Antibody libraries**

Four types of antibody phage display libraries can be discriminated. First, for the construction of immune libraries, the variable

region genes of Ig secreting plasma cells from immunized donors or from patients with an antibody titre against the desired antigen are used (22,27). Immune libraries are typically created and used in medical research to select an antibody present with high titers against one particular antigen, e.g. an infectious pathogen, and therefore would not be the source of choice for the isolation of antibodies with different specificities. The other three types of libraries can be considered as „single-pot“ libraries, as they are designed to provide antibody fragments binding to every possible antigen. Naive libraries are constructed from rearranged antibody genes from IgM producing B-cells of non-immunised donors. An example for this library type is the naive human Fab library constructed by de Haardt et al. (28). Semi-synthetic libraries are derived from unrearranged V-genes from pre-B cells (germline cells) or from a single antibody framework with at least one CDR region genetically randomized, such as the library described by Pini et al. (29). The fully synthetic antibody gene libraries consist of a human framework with randomly integrated CDR cassettes (30,31). To date, „single-pot“ antibody libraries with a theoretical diversity of up to 10¹¹ independent clones have been generated (32) to serve as a molecular repertoire for phage display selection procedures. An overview of antibody libraries and the comparison of their construction principles is given by Hust and Dübel (33).

● **Selection of Antibodies**

The technique for isolating specific antibodies by their binding activity *in vitro* is called „panning“, referring to the gold washers tool (34). Here, the antigen is immobilized on a solid surface, such as nitrocellulose (35), magnetic beads (36), a column matrix (21) or, most widely used, on plastic surfaces such

selection system		advantages	disadvantages
transgenic mice		somatic hypermutation	immunisation required, not freely available
cellular display	bacteria	N- and C-terminal and sandwich fusion, very large libraries	not matured, requires individual sorting
	yeast	display of larger proteins, N- and C-terminal and sandwich fusion	requires individual sorting
intracellular display	yeast two hybrid	screening library versus library possible	cytoplasm not optimal for antibody folding
molecular display	puromycin/ ribosomal	largest achievable library size	delicate method
phage display	filamentous	genomic	<i>in vitro</i> , robust, multivalent display
		phagemid	<i>in vitro</i> , robust
	T7	well suited for peptide display	no display of antibody fragments
arrays	gridded clones	robust, simple	small library sizes

Table 1: Comparison of recombinant antibody selection systems.

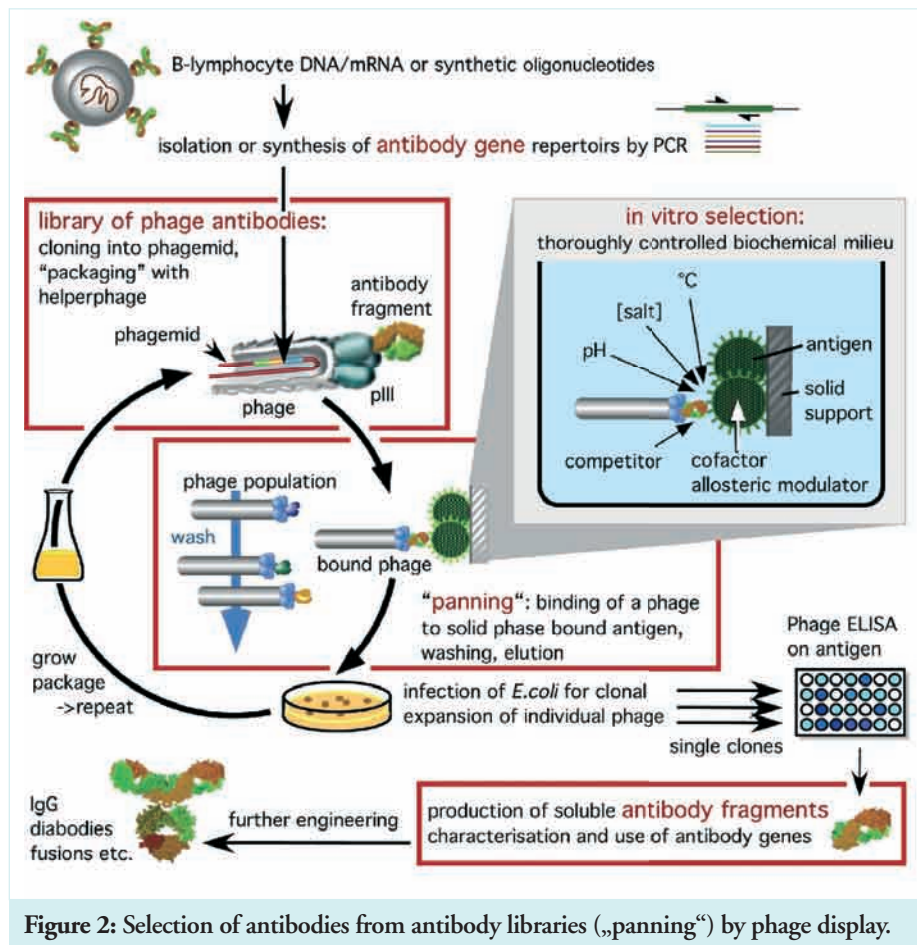


Figure 2: Selection of antibodies from antibody libraries („panning“) by phage display.

as polystyrene tubes (37) or 96-well polystyrene microtiter plates (20). For the selection procedure, parallelised/automated screening approaches are used more and more (38). The antibody phages are incubated with the immobilized antigen, followed by thorough washing to remove the vast excess of non-binding antibody phages. The bound antibody phages can subsequently be eluted and reamplified by infection of *E. coli* (Fig.2). This amplification allows detection of a single molecular interaction during panning, as a single phage, by its resistance marker, can give rise to a bacterial colony after elution. This explains the tremendous sensitivity of the method. This selection cycle can be repeated by infection of the resulting *E. coli* colonies with a helper phage to produce new antibody phages, which then can be used for further rounds of panning. The number of antigen specific antibodies should increase with every panning round. Usually 2-6 panning rounds are necessary to select specifically binding antibody phages. The enrichment of specific antibody clones can be controlled by polyclonal ELISAs. Monoclonal ELISAs are used for the identification of individual antibody clones. Various methods for the selection of antibodies from antibody phage display libraries are described by Kontermann and Dübel (39).

● Applications

After the completion and refinement of the human genome, the characterization of individual gene products in respect of their functions, their modifications, their cellular localization and their regulation in both space and time has generated an increased demand for antibodies for their analysis. Taking into account that the human genome contains ~ 25,000 genes, and that their products are found in different splice variants and also with post-translational modifications, it can be estimated that at least 100,000 different protein products have to be investigated to gain a complete picture of what is going on in the proteome of a cell. Antibody phage display combined with parallel screening approaches (33,40,41) offers a chance to decipher the post-genomic enigma by generating antibodies against thousands of targets.

Recombinant antibodies can be used for the diagnosis of pathogens, toxins and diseases. Here, antibody phage display offers the possibility of generating antibodies against targets which can not be used for conventional immunisation. For the last few decades, antibodies have been anticipated as molecules well suited for various therapeutic approaches. Today, the majority of antibodies used for therapy or being tested in clinical trials are chimeric or humanised IgG

antibodies, which were derived from well characterised mouse hybridoma antibodies. Therefore, these antibodies still contain murine sequences that can evoke a HAMA response. Antibody phage display offers the possibility of isolating fully human antibody fragments from recombinant human antibody gene libraries, and the first antibody generated in this way has recently been approved (42). Recombinant antibody fragments can easily be fused to other human antibody fragments to create bispecific antibodies, to human constant regions to generate fully human IgG molecules or to a range of other molecules such as drugs, toxins and enzymes. These homologous and heterologous antibody fusion proteins will provide novel approaches for the therapy of various diseases, such as cancer and infectious diseases (for review see 43).

With most of these therapeutic concepts still to enter clinical studies and parallelised methods for the selection of research antibodies (44), antibody phage display will continue to provide the nucleus of major projects in research, diagnostic and therapy.

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