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Infection Prevention 2018-Recent advances in phage display technology- Sharad Kumar Yadav- Veterinary University-

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Emergence of prokaryotic and eukaryotic expression system for the use of antibody production in the beginning years of 1980s was a surprise entry for the new era of 'IMMUNOTECHNOLOGY' [a branch of biotechnology that employs sets to bio-technique for the production of immunobiologicals]. Entry of phage display system during the years of 1990s for the antibody fragment expression has been created a bigger achievement and turned into a milestone, which concludes the method of phage display technology for the production of small fragments of antibodies that must possess the quality and the characteristic of binding to the antigens specifically which are popularly and most commonly known as Nanobodies. Phage display technology is a simpler, less time consuming and more efficient approach than the conventional methodology of the antibody production, which contains a number of component for the production of small fragment specific antibodies that includes the target or the antigen in anticipation to the ligand or binder or the antibody which are meant to be produced, next to this it concludes a Phage Display Library, A Phage system, Appropriate Selection or screening process, Appropriate Expression and purification system. The most common bacteriophages used are M13, and FD filamentous phage, though T4, T7 and λ Phage have also been used in some cases.
Introduction: Over the past decade, several library-based methods have been developed to discover ligands with strong binding affinities for their targets. These methods mimic the natural evolution for screening and identifying ligand-target interactions with specific functional properties. Phage display technology is a well-established method that has been applied to many technological challenges including novel drug discovery.

Areas covered: This review describes the recent advances in the use of phage display technology for discovering novel bioactive compounds. Furthermore, it discusses the application of this technology to produce proteins and peptides as well as minimize the use of antibodies, such as antigen-binding fragment, single-chain fragment variable or single-domain antibody fragments like VHHs.

Expert opinion: Advances in screening, manufacturing and humanization technologies demonstrate that phage display derived products can play a significant role in the diagnosis and treatment of disease. The effects of this technology are inevitable in the development pipeline for bringing therapeutics into the market, and this number is expected to rise significantly in the future as new advances continue to take place in display methods. Furthermore, a widespread application of this methodology is predicted in different medical technological areas, including biosensing, monitoring, molecular imaging, gene therapy, vaccine development and nanotechnology. Phage display technology was first introduced in 1985 by George Smith. It was used as an expression vector, capable of presenting a foreign amino acid sequence accessible to binding an antibody. Since then, a large number of phage displayed peptide and protein libraries have been constructed (Bass et al. 1990, McCafferty et al. 1990, Barbas et al. 1991, Smith 1991, Smith and Scott 1993, Hoogenboom 2002, Szardenings 2003), leading to various techniques for screening such libraries. This technology has had a major influence on the work and discoveries done in the fields of immunology, cell biology, pharmacology and drug discovery.

Phage display allows the presentation of large peptide and protein libraries on the surface of filamentous phage, which leads to the selection of peptides and proteins, including antibodies, with high affinity and specificity to almost any target. The technology involves the introduction of exogenous peptide sequences into a location in the genome of the phage capsid proteins. The encoded peptides are expressed or "displayed" on the phage surface as a fusion product with one of the phage coat proteins. This way, instead of having to genetically engineer different proteins or peptides one at a time and then express, purify, and analyze each variant, phage display libraries containing up to 1010 variants can be constructed simultaneously. Phage particles withstand very harsh conditions, such as low pH and low temperatures, without losing bacterial infectivity. Thus, protocols using low pH and high concentration urea have been used to dissociate bound phage from a target. In addition, bound phage does not need to be eluted from a microtiter well or animal tissue before bacterial infection. Instead, infection can proceed after addition of bacteria directly into the well or to the homogenized organ or tissue.

The strength of phage technology is its ability to identify interactive regions of proteins and other molecules without preexisting notions about the nature of the interaction. The past decade has seen considerable progress in the applications of phage display technology. Different screening methods have allowed isolation and characterization of peptides binding to several molecules in vitro, in the context of living cells, in animals and in humans (Arap 2002b). Here we review the applications, as well as recent innovations and future directions of phage display technology.
Fd particles are able to infect a variety of Gram-negative bacteria, including E. coli, using pili (F pilus in E. coli) as receptors. Filamentous phage infection does not produce lytic infection in E coli., but rather induces a state in which the infected bacteria produce and secrete phage particles into the growing medium. Infection begins by the attachment of phage pIII to the F pilus of a male E. coli. The circular single-stranded DNA enters the bacteria where it is converted by the host DNA replication machinery into double-stranded plasmid replicative form. By rolling circle replication, the replicative form makes single-stranded DNA and the templates for expression of proteins pIII and pVIII are formed. Phage descendants are assembled by packaging of the single-stranded DNA into protein coats and extruded through the bacterial membrane (Russel 1991).

Most of the currently used phage display vectors use the N-terminus of pIII protein or pVIII protein to display the foreign peptide or protein (Smith and Scott 1993). The pIII libraries display 3-5 copies of each individual peptide (Scott and Smith 1990), whereas pVIII libraries can display up to 2700 copies of small (up to six amino acids) peptides (Greenwood et al. 1991). The pIII and pVIII proteins can display peptides of various lengths and cysteine residues can be introduced to the fusion peptide to create conformational constraints by the formation of "loops" between disulfide bridged cysteine residues. Furthermore, the exogenous peptides are well exposed, facilitating the insert-target interactions. Large peptide inserts of up to 38 amino acids can be introduced into the amino terminus of pIII protein without the loss of phage infectivity or particle assembly.

Working with phage display technology

Detailed description of the materials, methods and space needed for those who want to start phage work may be found in specialized textbooks (Barbas et al. 2000, Pasqualini and Arap 2002). The basic protocols used with phage studies can also be obtained in these books, whereas its variations are found in published studies. Initial investment is relatively small, as most of the fundamental materials are common laboratory devices, such as

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Petri dishes, Falcon tubes and centrifuges. However, one cannot forget the most important tool for phage work: k91kan E. coli and peptide or antibody phage libraries. The construction of a peptide phage library involves a detailed protocol and, for those who are not experienced with phage work, one way to obtain reliable phage libraries and start a "bio-panning" is to establish collaboration with a more experienced laboratory. This is a very important step, as the panning results depend directly on the quality of the library. After the "bio-panning" is started, the most expensive step is to sequence the clones obtained after a few rounds of selection, as hundreds of clones are usually sequenced in each panning.

Biography

Sharad Kumar Yadav has 28 years of teaching and research experience and has served to various senior positions of the University including Registrar of the DUVASU University. He is currently Professor, Head of Department of Veterinary Microbiology, and Director at Cow Research Institute at DUVASU, Mathura India. He has published number of papers in reputed International & National journals and has a vast experience in the arena of BHV-I virus.

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