

Chapter 3

Application of Phage Biotechnology in Nanobiotechnology

Rana Singleton

Alabama State University, USA

Carrie Sanders

Alabama State University, USA

Alain B. Waffo

Alabama State University, USA

ABSTRACT

To date, the phage display system has enabled the discovery of material binding peptides. Diversity and functionality of these peptides could be improved using RNA-based display systems instead of the conventional DNA-based ones. RNA phage replication systems possess unique features that make them a versatile tool for any combinatorial approach and evolutionary application. Phage display was used to monitor the chemical surface properties and to initiate nanoparticle assembly. Novel bio-panning was recently used in RNA-based display to screen new functionality without acidic elution used in other conventional DNA phage display systems. Therefore, Hybrid RNA phages would be a perfect platform for attachment and exploration of nanoparticles. In this chapter, the authors present an overview on research conducted on these cross fields and areas. They not only focus on the novel selection and amplification process but also on the importance of RNA phage and its peptide display as tools for preventing nanoparticle aggregation.

INTRODUCTION AND HISTORY OF PHAGE DISPLAY

Introduction

The main purpose of recombinant biotechnology is to mimic and improve natural biomolecule production *in vitro*. This production, coupled with

genetic engineering, is making biomolecules more appropriate for *in vitro* study or *in vivo* application. Recombinant and engineering technologies involving either DNA or protein can produce only one entity at a time while phage display is the only technology linking DNA and protein. Hybrid phage recombinant and/or engineered contains at the same time the DNA and its protein meaning

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linkage of genotype to phenotype (Smith, 1993). Purification and preparation of recombinant protein is one of the most difficult tasks in molecular biology. Phage has been assimilated into a plasmid and can be easily purified through the sucrose or cesium chloride gradient (Vidaver et al., 1973). Moreover, the amplification of phages is easily done through rounds of infection of the host. This unique functional features of wild type and hybrid phages attest to their value as tools for the development of a platform for nanotechnology application. Nowadays phage display is used to select important peptide(s) binding to any fixed (immobilized) target. Hybrid phage becomes a platform while the target can be assimilated to nanoparticles. Peptides have the potential to initiate the synthesis, assembly and orientation of nanomaterials (Merzlyak & Lee, 2006). For the past 3 decades this technology has been done using DNA phages (Jespers et al., 1996; Parmley & Smith, 1988). Typically DNA phage has a head and tail with a double-stranded DNA block. DNA-based genotype replicates with the typical proof reading activity of its putative polymerase (Tran et al., 1997). The main goal of a phage display library is to apply molecular evolutionary to biotechnology, leading to the creation of biomolecules with great variety of functionalities. The diversified library is obtained by a combinatorial approach which is diminished by the correction of the DNA polymerase. This can be overcome by using the promiscuity features of RNA polymerase and/or an RNA-based system. Nothing is known about display system based on RNA phage. RNA phages have been purified from animal and human sewages (Abedon, 2008) and can be used to increase the diversity of such library. RNA-based replication system would generate diverse peptides as molecular linkers and synthesizers and assemblers better for nanoparticles which aggregate during high-throughput synthesis than any other system. In this review we not only focus the application of RNA phage in nanobiotechnology, but also on the concepts for the technical implementation of evolutionary strategy to this novel important field.

History and Evolution of Phage Display

As previously mentioned, the purpose of phage display is to create a peptide or antibody screening library. This may seem like a vague definition, but we will revisit this definition at the end of this section, where you will have a better understanding of it. But, for now, take it at face value. The first peptide phage display library was published in 1990, followed by the completion of the first antibody phage display library in 1991 (Hoogenboom et al., 1991). Phage peptide libraries allow us to screen a large amount of proteins against a target molecule (protein) to determine interaction. One could also screen a particular phage-peptide recombinant against a DNA library specific for a cell or an entire organism in order to determine the function of that particular peptide in that cell or organism (Brown, 2000). The creation of antibody phage display has allowed us to better understand our own immune system through interactions as well as help revolutionize antibodies for use in human therapy - usually as anti-tumor/cancer drugs or anti-inflammatory agents (Azzazy & Highsmith, 2002).

One such success story would be the creation of Humira. Humira is an anti-inflammatory drug used to treat several inflammatory autoimmune diseases and conditions such as arthritis, psoriasis, and Crohn's disease. Humira was developed as the result of phage display and an antibody library that developed from it. Many inflammatory conditions and diseases are caused by TNF (Tumor Necrosis Factor). TNF is a superfamily of cytokines that initiate a cascade of inflammatory responses in the body that lead to the symptoms associated with the above-mentioned autoimmune diseases. Humira is an anti-TNF- α protein or a TNF- α antibody that blocks the binding of TNF- α to receptors, thus hindering the inflammatory cascade response it would normally cause. Thus, this made TNF- α , an attractive target for an antibody phage display library. Once the development of the antibody phage library was completed, it al-

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