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Review Article

Cells and cell lysates: A direct approach for engineering antibodies against membrane proteins using yeast surface display

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ABSTRACT

Membrane proteins (MPs) are often desirable targets for antibody engineering. However, the majority of antibody engineering platforms depend implicitly on aqueous solubility of the target antigen which is often problematic for MPs. Recombinant, soluble forms of MPs have been successfully employed as antigen sources for antibody engineering, but heterologous expression and purification of soluble MP fragments remains a challenging and time-consuming process. Here we present a more direct approach to aid in the engineering of antibodies to MPs. By combining yeast surface display technology directly with whole cells or detergent-solubilized whole-cell lysates, antibody libraries can be screened against MP antigens in their near-native conformations. We also describe how the platform can be adapted for antibody characterization and antigen identification. This collection of compatible methods serves as a basis for antibody engineering against MPs and it is predicted that these methods will mature in parallel with developments in membrane protein biochemistry and solubilization technology.

1. Introduction

Membrane resident receptors comprise the majority of all proteins targeted by FDA-approved drugs and experimental therapeutics entering clinical trials [1]. A current trend in drug development is a push toward biopharmaceuticals, notably fully human monoclonal antibodies (MAbs) [2–4], that can be used to target such MPs. Generation of lead MAbs is most often based on platform technologies, including the immunization of transgenic animals or the screening of large, recombinant antibody libraries [5]. These platforms oftentimes rely on one critical element for generating and characterizing MAbs: a soluble form of the protein target [6,7]. The "drug" effect of an MAb consists of binding to an epitope on its protein target. This binding can lead to a desired compromise in the target's activity (often influencing downstream cellular and/or systemic events) [8,9], or can initiate localized delivery of a drug payload [10,11]. It is therefore paramount that antibodies bind target protein epitopes in their native conformations [12,13]. These requirements for soluble and native-like MPs are particularly limiting given the nature of membrane proteins. Full-length membrane proteins are poorly soluble without the combination hydrophobic–hydrophilic interactions presented by lipids, and once introduced to a purely aqueous environment, membrane proteins are prone to misfolding, aggregation, and denaturation [14]. Soluble, recombinant peptides (usually comprised of extracellular domains or fragments) produced in microbial or mammalian hosts may lack the proper post-translational modifications, are time-consuming to produce and purify, and may ultimately present a different target than what exists *in vivo* [15]. Whole cells, and detergent-soluble cell lysates are a direct, powerful solution to this problem, provided that they can be integrated into popular antibody discovery platforms. Indeed, as antibody engineering technology has matured, many examples have emerged with whole cells playing the role of antigen.

XenoMouse technology and phage display, two widely used platforms for antibody discovery, incorporate whole cells as a means of generating antibodies against membrane proteins. The research and development leading to panitumimab (Vectibix) [16,17] provides an instructional review of the XenoMouse platform where the engineered animals were immunized by direct injection of antigen-expressing cells. Additional flexibility was enabled through the development of HEK293 expression vectors, capable of accepting a variety of membrane proteins [18]. Phage display, being an *in vitro* platform, is highly adaptable to the use of whole cells. Studies have succeeded in identifying reactive

Abbreviations: MAb, monoclonal antibody; scFv, single chain variable fragment; MP, membrane protein; OD, optical density; YSD, yeast surface display; PM, plasma membrane; FACS, fluorescence-activated cell sorting; MACS, magnetic-activated cell sorting; YDIP, yeast display immunoprecipitation; SA–PE, streptavidin–phycoerythrin; TfR, transferrin receptor.

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peptides and antibodies to many cells and tissues including: brain and kidney [19], lung [20], heart [21], and breast tissue [22]. Recent years have also seen the first *in vivo* phage selection performed in humans [23]. These results briefly highlight the use of whole cells in the prevailing antibody discovery platforms. The technology used for antibody discovery and production, however, has progressed dramatically, leading to alternative cell-surface display technologies [24]. One of these, yeast surface display (YSD), has gained in popularity among academic researchers and has recently been commercialized [25]. Here we describe powerful YSD methods using whole cells (yeast biopanning) or detergent-solubilized cell lysates as sources of MPs for antibody engineering.

Yeast-display is among the many cell-surface display systems for protein engineering, and as will be described in this review, possesses advantages for antibody engineering against membrane proteins [24,26]. Much like phage display, yeast are engineered to express peptides or antibody fragments on their surface while harboring the genetic information via a plasmid inside the cell (Fig. 1A). Being eukaryotes, yeast also have an endoplasmic reticulum equipped with specific enzymes and chaperones that lead to high fidelity folding and expression of mammalian antibody fragments. Enhanced protein folding, when combined with the ability to generate very large ($\sim 10^{10}$ clones) libraries [27] (Fig. 1A-ii) leads to a powerful platform for the identification of novel antibodies [28]. Importantly, yeast display libraries can be screened using fluorescence-activated cell sorting (FACS) (Fig. 1C-iv) which affords an impressive combination of quantitative screening and throughput. Modern FACS instruments support rates in excess of 25,000 events per second, allowing even large libraries to be screened quickly and precisely. In the typical embodiment however, YSD requires the use of a soluble antigen (Fig. 1C-ii). Two methods have recently been developed to address this limitation. First, our lab demonstrated a yeast "biopanning" method where yeast-displayed single chain variable fragments (scFv) were selected by successive rounds of incubation on mammalian cell monolayers [29] (Fig. 1A and B). Yeast biopanning was later used to isolate a number of unique scFv that bind plasma membrane (PM) proteins of a rat brain endothelial cell line (RBE4), and in some instances, internalized into the RBE4 cells [30]. A second YSD-based method using whole cell contacting approaches incorporated lymphoid-derived cells to screen a library of T-cell receptors against native peptide-MHC ligands [31]. Enrichment of high affinity pMHC binders was aided by separation of yeast-lymphoid cell complexes by density gradient centrifugation. Although incorporation of whole cells overcame the need for a soluble antigen, neither approach took advantage of FACS for high-throughput, quantitative screening as mentioned earlier. Our laboratory overcame this limitation through the use of detergent solubilized whole cell lysates and demonstrated YSD-based screening using the cell lysates as a soluble antigen source [32] (Fig. 1A and C). This method allows the high-throughput screening of YSD libraries against MP antigens, while avoiding the problem of MP aggregation and eliminating the need for heterologous production of truncated MPs. We have also demonstrated the use of lysate-based YSD to identify and characterize targeted membrane proteins [33], often one of the most challenging aspects of validating new antibody-antigen combinations. Below, we detail these methods for YSD-based selection and characterization of antibodies against antigens located in the cell membrane, and highlight the various advantages and challenges of such approaches.

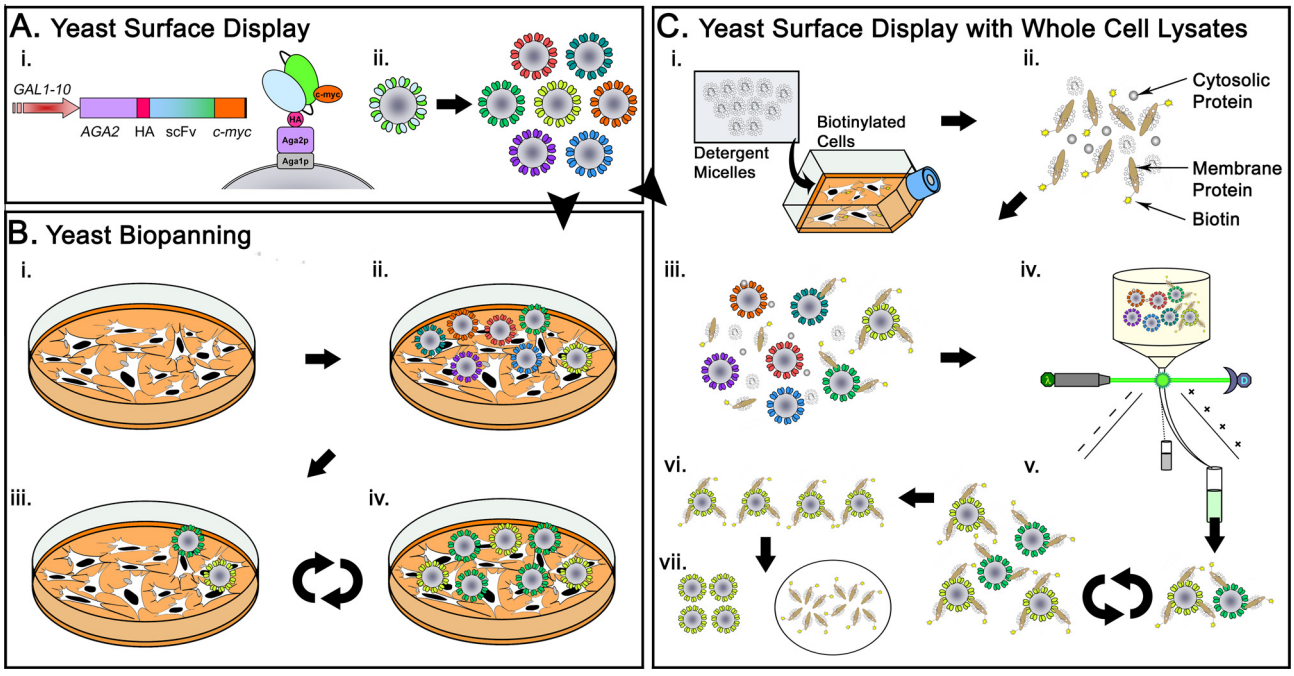

Fig. 1. The two components of any yeast surface display screen are yeast-displayed antibody (A-scFv) and an antigen (B-cells or C-cell lysates). The yeast surface display vector directs galactose-inducible display of the antibody construct as well as HA and c-*myc* expression level tags, with high valency on the yeast surface (A-i). Recombinant libraries (non-immune, immune, mutagenic or a parental clone) are cultured and surface expression is induced (A-ii). In yeast biopanning, monolayers of whole cells act as the antigen (B-i). Induced yeast are incubated on the monolayers (B-ii) and non-specifically bound yeast are removed by washing (B-iii). A library may be enriched for binding yeast through repeated application of this process (B-iv). Cell lysates act as a soluble source of antigen, allowing yeast antibody libraries to be sorted by flow cytometry. Cells are lysed and membrane proteins are solubilized by the addition of a detergent-based lysis buffer (C-i). The membrane proteins may be specifically biotinylated prior to lysis as an aid in identifying MP-binding yeast clones. Detergent-solubilized MPs in the form of cell lysate (C-ii) are mixed with scFv-displaying yeast (C-iii), and the resulting mixture is sorted using FACS (C-iv). Repeating this procedure leads to the enrichment of yeast clones that bind a desired antigen (C-v). Membrane proteins may be eluted from the yeast surface in a process termed yeast-display immunoprecipitation (YDIP, C-vi). Separation of the eluted proteins by SDS–PAGE, followed by Western blotting or mass spectrometry comprises a direct method of antigen characterization (C-vii).