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Application of modified yeast surface display technologies for non-Antibody protein engineering

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ABSTRACT

Yeast surface display (YSD) system has been widely used in protein engineering since it was established 20 years ago. Combined with fluorescence-activated cell sorting (FACS) technology and directed evolution, YSD has been proven of its extraordinary effectiveness for molecular engineering of various target proteins, especially for antibodies. Recently, a few remarkable efforts were exploited to modify the original Aga1-Aga2 YSD for the non-antibody protein engineering with successful outcomes, expanding its application on oxidase, Class II major histocompatibility complex (MHC-II), protease, sortase, lipoic acid ligase etc. Here, the methodologies of these optimized Aga1-Aga2 YSD technologies were introduced, and the recent progress of non-antibody protein engineering using these methods was summarized.

Contents

1. Introduction

The background of microbial surface display

The cell surface is a functional interface between the inside and outside of the cell. In biotechnology, the cell surface can be exploited by making use of known mechanisms of transporting heterologous proteins to the cell surface. However, lacking of effective

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platforms to present protein of interest on the cell surface largely hindered the development of surface display technology. In 1990, Scott and Smith discovered that short peptides could be displayed on the virion surface by fusing to the anchor protein of the filamentous phages without affecting their infection ability (Scott and Smith, 1990). Since then, various surface display platforms have been established and rapidly developed (Tanaka et al., 2012; Schuurmann et al., 2014; Domingo-Calap et al., 2016).

Surface display system has its major advantage that the target proteins can be displayed on the cell surface to make the manipulation of enzymatic reactions more feasible and easier. Three major microbial cell-surface display systems have been developed so far, including phage display, bacteria surface display, and yeast surface display (YSD) (Lipke and Kurjan, 1992; Little et al., 1993; Griffiths et al., 1994). The principle of phage display is displaying heterologous peptides or proteins through fusing them with coat protein of filamentous phages, which is widely used to isolate ligands, antigens, and antibodies (Hockney, 1994; Delhalle et al., 2012). Because of the phage's small bulk, the size and variety of the target peptides or proteins are highly restricted. In the bacteria surface display system, *e.g.* the *E.coli* OmpA system (Schreuder et al., 1993; Georgiou et al., 1997), heterologous proteins are normally inserted into the loop region of the cellular outer membrane protein, forming a protein complex, which is then co-displayed on the bacterial cell surface. However, inserting the foreign protein into the cellular outer-membrane protein frequently disrupts its functional structure, causing low surface display efficiency. These, together with the fact that many eukaryotic proteins are usually not well folded or misfolded in bacteria and phage, have put a request of developing a surface display system in eukaryotic cells.

Under this circumstance, YSD, which uses the protein anchored on the yeast cell wall as a surface carrier, was developed (Kondo and Ueda, 2004). Compared to phage and bacteria display systems, YSD has two extraordinary advantages. First, yeast is a unicellular eukaryote, favoring the expression and folding of the eukaryotic proteins with its post-translational system. This is important, as human antibody engineering, the most profitable protein engineering research field, has encountered massive difficulties in phage and bacteria display systems because of misfolding issues. Second, the heterologous protein in YSD can be alternatively fused at either the N- or C-terminal of the surface anchor protein without disrupting its core structure, thus keeping the structure of the surface anchor protein largely unchanged without undermining its surface displaying efficiency. In addition, other advantages also include high display efficiency and full exposure of target proteins out of the cells in YSD. Compared to phage and bacteria surface display systems, the mostly mentioned disadvantage of YSD is that yeast cells exhibit slower growth rate and lower foreign plasmid transformation efficiency. However, recent developments of yeast technology have already enhanced the yeast transformation efficiency to 10^8, making it feasible of generating variant libraries with sufficient information in laboratory scale (Kondo and Ueda, 2004; Benatuil et al., 2010).

2. Brief theory introduction to YSD

In the YSD system, heterologous proteins expressed in the yeast are fused to surface anchor protein, forming a protein complex. This protein complex was then displayed on the yeast cell surface, leading to feasible reproduction of the *in vitro* reaction system for the displayed biocatalysts, and easy detection of the products from catalyst. Nowadays, a matured high-throughput screening approach for protein engineering has been established when YSD is combined with fluorescence-activated cell sorting (FACS) technology and directed evolution (Fig. 1). As an easy manipulation platform,

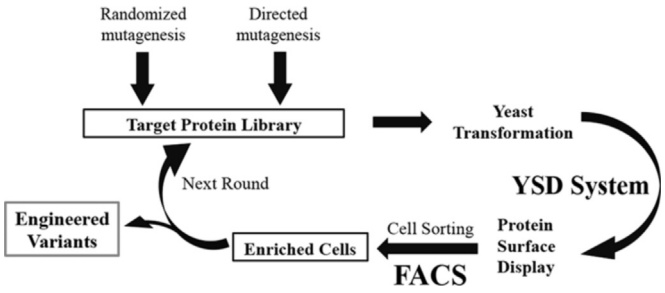

Fig. 1. Schematic summary of YSD platform for protein engineering.

the YSD system appears unique advantages for protein engineering comparing to the bacteria and phage display systems, especially for the eukaryotic functional proteins requiring post-translational modifications. In most YSD systems, glycosylphosphatidylinositol (GPI)-anchored proteins were typically used for displaying heterologous proteins, including agglutinin (α-agglutinin and a-agglutinin), flocculin Flo1p, Cwp1p, Cwp2p, Sed1p, Tip1p, YCR89w, and Tir1 (Lipke et al., 1989; Lipke and Kurjan, 1992; Kuroda and Ueda, 2014). Through the yeast endoplasmic reticulum (ER)-Golgi secretory pathway, the GPI-anchored proteins are transported to the yeast cell surface, forming a β-1, 6-glucan bridge with the mannoprotein layer of the cell wall (Scott and Smith, 1990; Lu et al., 1995; Ueda and Tanaka, 2000; Dudgeon et al., 2012). GPI-anchored proteins have been demonstrated to mediate the display of a range of heterologous proteins upon protein fusion. In 1993, α-galactosidase from *Cyamopsis tetragonoloba* became the first heterologous protein that was displayed on the yeast cell surface after being fused to the C-terminal of the protein (Schreuder et al., 1993). Since then, many other proteins, including Class II major histocompatibility complex (MHC-II) (Boder et al., 2005), epidermal growth factor receptor (EGFR) fragments (Chao et al., 2004), lipase from *Rhizopus oryzae* (ROL) (Tanino et al., 2006), single-chain variable fragment (scFv) (Feldhaus et al., 2003), and other enzymes were successfully displayed on the yeast cell surface for further engineering. Moreover, the combination with other newly developed technologies has greatly strengthen the capability of YSD, expanding its application in engineering a number of functional proteins (Wen et al., 2011; Bagriantsev et al., 2014; Maute et al., 2015; Frago et al., 2016), as well as catalytic enzymes (Matsuura et al., 2013; Jin et al., 2014), antibodies (Doerner et al., 2014; Rhiel et al., 2014; Van Deventer and Wittrup, 2014), and combinatorial protein libraries (Ueda, 2009). Recently, the YSD system was also developed to display redox active enzymes for microbial fuel cell applications (Szczupak et al., 2012), actinidain for fast characterization of food allergens (Popovic et al., 2015), xylose reductase along with other enzymes for xylitol production (Chen et al., 2016), and α-amylase and glucoamylase simultaneously for maximum ethanol production (Inokuma et al., 2015).

Among the GPI-anchored proteins for surface display, Flo-anchoring protein displays its C-terminal end associated protein on the yeast cell surface through non-covalent interactions between its flocculation functional domain and the cell-wall α-mannan (Van Mulders et al., 2009). Besides the Flo protein, other mannose proteins, such as Cwp1p, Cwp2p, Sed1p, Tip1p, Tir1p and YCR89W (Table 1), are also developed as anchor as well as carrier proteins for immobilizing the target protein on the cell wall of *S. cerevisiae* or *Pichia pastoris* (Tokuhiro et al., 2008; Wasilenko et al., 2010; Liu et al., 2014b), among which Cwp2p was identified to be the only GPI-anchored cell wall mannoprotein in the interior of the cells (Van der Vaart et al., 1997). Different from these cell surface proteins that function as both the surface anchor and heterologous protein carrier, the Aga1-Aga2 YSD system is composed of