

A REVIEW ON CONSTRUCTED GENETIC CASSETTES IN YEAST FOR RECOMBINANT PROTEIN PRODUCTION

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ABSTRACT. Conventional methods for covalent immobilization of proteins often result in denaturation due to chemical treatments. However, proteins immobilized at microbial cell surfaces by regular cellular processes could be bound covalently to the cell wall without being exposed to chemical treatment. Yeasts display systems provide several advantages over bacterial system. The secretory and post-translational pathway in yeast, are similar to those of higher eukaryotes which established them as better hosts for production of eukaryotic proteins. The expression of recombinant proteins immobilized at the cell surface of *Saccharomyces cerevisiae* has now been practiced for the last two decades. Although different surface display systems have been made for specific purposes, the system with broad applicability has not been developed so far. Most of the vectors constructed for surface display of recombinant proteins in yeast so far were created for single-use in particular case with ubiquitous laboratory plasmids that were not optimized for this purpose. Therefore, the construction of a new set of plasmids with optimized genetic cassette is still in demand. An optimized genetic cassette should allow easy and simple insertion of any gene of interest, with regulated and easily controlled expression level. In this review, we have tried to make a detailed study on all the genetic components used in successful yeast display systems till now in order to provide a good knowledge which will help the future researchers of this field to design an optimized genetic cassette which would be used for industrial scale application.

KEYWORDS: Yeast display system, yeast cell wall proteins, genetic cassette and recombinant protein.

INTRODUCTION

Yeasts application was initially intrinsic, but it became a choice of organism for scientific investigations with the development of science and technology. The structural components and properties of yeast have been the main reasons behind the immense success of it as a model microorganism. The cell wall of yeast is one of those vital components. The major components of fungal cell walls are polysaccharides and glycoproteins (Ruiz-Herrera 2016). In *Saccharomyces cerevisiae*, the cell wall contains $\beta(1\rightarrow3)$ -D-glucan, $\beta(1\rightarrow6)$ -D-glucan, chitin, and mannoprotein(s). Linked to the cell wall polysaccharides are a varied set of mannoproteins that collectively form yeast mannan, the electron-dense, fibrillar outer layer of the wall (Pagé et al. 2003). Among the cell wall proteins (covalently or non covalently bound) many are used as fusion protein; for instance Aga2p, α -agglutinin, Flo1p, Flo318, Cwp1, Cwp2, Ccw12, Pir1, Pir4 etc. are used in yeast surface display system.

The method of displaying recombinant proteins on the surface of *S. cerevisiae* via genetic fusion to an abundant cell wall protein can be defined as a technology known as yeast surface display (Gai & Wittrup 2007). Yeast surface display has become a leading platform for protein engineering as it has collective advantages, including: 1) a eukaryotic expression system that is capable of incorporating post-translational modifications, 2) low technical and time requirements, 3) inclusion

of epitope tags, that allows to normalize protein function (e.g., ligand binding) to surface expression and, thus, identification of proteins that both express at high levels and bind with high affinity to a target protein, and 4) being compatible with flow cytometric analysis, which allows quantitative measurements of equilibrium binding constants, dissociation kinetics, stability, and specificity of the displayed proteins without the laborious requirements of soluble protein expression and purification (Cherf & Cochran 2015). Although a number of potential biotechnological applications of microbial surface-displayed proteins have been reported in the last two decades, it is still challenging on how to improve the efficiency of display of protein complexes or cofactor containing enzymes or co-display of multiple enzymes and increase the quantity of protein displayed on the yeast surface. Besides, the industrial application of the yeast surface display system is still far away because most of the vectors constructed for surface display of recombinant proteins in yeast so far were created for single use in particular case with ubiquitous laboratory plasmids not optimized for this purpose. Therefore, the construction of new plasmids with optimized genetic cassette which will allow simple insertion of any gene of interest, with regulated and easily controlled expression level, is still needed. In this review work, we have tried to conduct studies regarding the components used in the major yeast surface display systems thus making a decision regarding the components which are best suited for designing optimal genetic cassettes for industrial application.

Architecture of yeast cell wall

The yeast cell wall is regarded as strong, but elastic, structure which is crucial not only for the maintenance of cellular shape and integrity, but also for progression throughout the cell cycle. During the time of growth and morphogenesis and in response to environmental changes, the cell wall is remodeled in a highly regulated and polarized manner; this is a process that is generally under the control of the cell wall integrity signaling pathway (Levin 2011). Cell wall is necessary for the survival of fungal cell. Digestion of cell walls in the absence of an osmotic protector may lead to cell lysis as there will be high internal turgor pressure. The cell wall also provides rigidity to the cell and determines the shape of fungal cells. For this reason cell wall formation can be used as a model for morphogenesis (Ruiz-Herrera 2016). The main components of fungal cell walls are polysaccharides and glycoproteins (Cabib et al. 1982). In *S. cerevisiae*, the cell wall makes up to 15-30% of the dry weight of the cell (Orlean 1997) and 25-50% of the volume based on calculations obtained from electron micrographs.

β -1,3-glucan

The β -1,3-glucan chains can be defined as polymerization of 1,500 glucose units which have a coiled spring-like structure that confers tensile strength and elasticity to the cell wall (Klis et al. 2002). β -1,3-glucan is found as a branched polymer with β -1,6 interchain links in cell wall extracts. β -1,3-glucan is generally covalently linked to the other wall components. β -1,3-glucan synthase is situated in the plasma membrane. Electron microscopy of regenerating spheroplasts indicates that the polysaccharide product is extracellular (Lipke & Ovalle 1998).

β -1,6-glucan

β -1,6-glucans are relatively small molecules. It links the components of each module together (Kollár et al. 1997). This polymer has a standard chain length of 350 glucose residues, with the β -1,6 backbone branched with β -1,6 side chains through 3,6substituted glucose residues on 15% of the residues (Magnelli et al. 2002).

Chitin

In the cell wall, about 40 to 50% of the chitin chains are linked to the non-reducing end of β -1,3-glucan via a β -1,4 bond engaging the reducing end of the chitin polymer (Kollár et al. 1995). Its

crystalline structure confers stretching resistance to the cell wall. Part of the chitin is placed in a ring at the base of the bud. On the other hand, some chitin is also deposited in a dispersed fashion in the lateral walls.

Yeast cell wall proteins

Linked to the cell wall polysaccharides there are a varied set of mannoproteins which collectively form yeast mannan which is the electron-dense, fibrillar outer layer of the wall (Osumi 1998). Yeast wall mannoproteins are highly glycosylated polypeptides, often 50-95% carbohydrate by weight, and therefore can be thought of as yeast proteoglycans (Van der Vaart 1995). Proteins that are covalently linked to cell wall glycan are referred to as cell wall protein (CWP) (Orlean 2012) and fall into the subgroups below -

GPI Proteins

Glycosylphosphatidylinositol (GPI) proteins are directed via the secretory pathway to the extracellular face of the plasma membrane through lipid anchors at their C termini. GPI proteins which are destined for the cell wall are isolated from the plasma membrane by cleavage of their anchors (Kollár et al. 1997). Lipidless GPI remnants of GPI-CWPs thus become attached to the external surface of the β -1,3-glucan network, this happens indirectly via β -1,6-glucan chains (Klis et al. 2006). Approximately, 70 GPI proteins have been recognized in the *S. cerevisiae* genome (Caro et al. 1997).

Pir Proteins

Another important class of CWPs is represented by five related polypeptides, Pir1-5 (Proteins with internal repeats) (Ecker et al. 2006). The Pir proteins are generally linked directly to β -1,3-glucan chains through a linkage. The glucan chain is linked to the protein via the G-carboxyl group of a Glu residue generally produced through a transglutaminase-type reaction, this converts the first Gln residue in the repeat sequence to Glu (Ecker et al. 2006).

Disulfide-linked proteins

From the walls of living cells, a variety of proteins can be released with sulfhydryl reagents. This indicates that they are directly attached via disulfides or retained behind a network of disulfide-linked proteins (Moukadiri & Zueco 2001).

Surface display of yeast

The cell surface can be defined as a functional interface between the inside and the outside of the cell. In biotechnology, surface of the cell can be exploited by making use of known mechanisms to transport proteins to the cell surface. The development of systems to display heterologous proteins on the cell surface of microorganisms is likely to be useful in the separation of produced polypeptides; the production of microbial biocatalysts, whole-cell adsorbents, and live vaccines; and the screening of modified or novel proteins. Utilizing the surface of living cells is also significant for many applications in molecular biology and microbiology (Ueda & Tanaka 2000). Yeast surface display (Figure 1) can be defined as a protein engineering technique which uses the expression of recombinant proteins incorporated into the yeast cell wall to isolate and engineer antibodies. Using it in bioindustrial processes applied to foods, alcoholic beverages, medicines, etc., is generally safe. As it has "Generally Regarded as Safe" status and can be used for food and pharmaceutical production, one of the most suitable microorganisms for practical purposes is the yeast *S. cerevisiae*. The display system in *S. cerevisiae* has applications in the development of cell-surface expression systems (Ueda & Tanaka 2000), application in cell-based selections, convenient screening of cDNA libraries,

protein engineering for improved production and stability, in the field of cell adhesion, protein affinity maturation, in binding or controlling assembly of a number of non-biological targets such as metal ion, in mapping of antibody epitope, cell-based selections etc. (Shusta et al. 2008).

Yeast display system in the last two decades

One of the first yeast surface display system was developed by Eric T. Boder and K. Dane Wittrup during 1997. They utilized yeast display system for screening combinatorial polypeptide libraries (Boder & Wittrup 1997). More precisely, in this particular work they utilized C-terminal fusion to the Aga2pp mating adhesion receptor of *S. cerevisiae* for selecting scFv antibody fragments with threefold reduced antigen dissociation rate via a randomly mutated library. It was also reported that the yeast surface display was used to select functional T cell receptor mutants. The yeast surface display of TCR was achieved only after the mutation of particular variable region residues (Kieckhefer et al 1997).

Spacer-mediated display of active lipase on the yeast cell surface was done in 2001 (Nakamura et al. 2001). A *S. cerevisiae* strain displaying an active lipase on the cell surface was developed by cell surface engineering. This was the first report of an active lipase displayed on the cell surface. Furthermore, it was recommended that inserting a linker peptide of the appropriate length as a spacer could be an improved method for effective displaying of enzymes, particularly those having the active region at the C-terminal portion, on the cell surface.

Fine epitope mapping of anti-epidermal growth factor receptor antibodies via random mutagenesis and yeast surface display was done in the year 2004 (Chao et al. 2004). In this research work, fine epitope mapping of therapeutically relevant monoclonal antibodies explicit for the epidermal growth factor receptor was achieved through random mutagenesis and yeast surface display.

Yeast surface display of a non-covalent MHC class II heterodimer complexed with antigenic peptide was achieved in 2005 (Boder et al. 2005). It was the first example of a non-covalent protein dimer which was displayed on yeast surface and of successful display of wild-type MHC class II. Results they found further point to the potential of using yeast surface display to engineer and analyze the antigen binding properties of MHC-II.

In 2006 researchers used yeast display system to construct and characterize a pseudo-immune human antibody library (Lee et al. 2006). The pseudo-immune library showed much higher affinity and specificity for the targeted antigen than those from the non-immune library. The results suggested that a pseudo-immune antibody library was very much efficient to isolate target-specific high affinity antibody from a relatively small sized library.

Isoflavone aglycones production from isoflavone glycosides by display of β -glucosidase from *Aspergillus oryzae* on yeast cell surface was achieved in 2008 (Kaya et al. 2008). The research work indicated that Sc-BGL1 assimilated the glucose before they inhibited the hydrolysis reaction, and efficient production of isoflavone aglycones can be achieved by engineered yeast cells displaying β -glucosidase.

Fine epitope mapping of monoclonal antibodies against hemagglutinin of a highly pathogenic H5N1 influenza virus using yeast surface display was performed in 2012 (Han et al. 2012). In this work a robust yeast display system for fine epitope mapping of viral surface hemagglutinin (HA)-specific antibodies was demonstrated. The results further augmented the tool chest for studying HA antigenicity, epitope diversity and accessibility in response to natural and experimental influenza infection and vaccines.

Table 1: Application of yeast cell wall proteins in yeast surface display system

Name	Application
Aga2p	<p>Screening of combinatorial polypeptide libraries through yeast surface display (Boder & Wittrup 1997).</p> <p>Yeast surface display and flow cytometry for fine affinity discrimination (Van Antwerp & Wittrup 2000).</p> <p>Yeast surface display and random mutagenesis for fine epitope mapping of anti-epidermal growth factor receptor antibodies (Colby et al. 2004).</p> <p>Conformation of an immunoglobulin domain and post-translational regulation of expression in yeast surface display (Parthasarathy et al. 2006).</p> <p>Yeast surface display is used for fine and domain-level epitope mapping of botulinum neurotoxin type a neutralizing antibodies (Levy et al. 2007).</p> <p>Yeast surface display for high-resolution profiling of homing endonuclease binding and catalytic specificity (Jarjour et al. 2009).</p> <p>Biosensor detection systems: engineering stable, high-affinity bioreceptors by yeast surface display (Richman et al. 2009).</p>
α -agglutinin	<p>Yeast surface display for the development of novel whole-cell immunoadsorbents of the IgG-binding domain (Nakamura et al. 2001).</p> <p>Production of isoflavone aglycones from isoflavone glycosides by display of β-glucosidase on yeast cell surface of <i>Aspergillus oryzae</i> (Kaya et al. 2008).</p>
Flo1	Based on the Flo1p flocculation functional domain, construction of yeast strains with high cell surface lipase activity by using novel display systems (Matsumoto et al. 2002).
Flo318	Improvement of novel yeast cell surface display system for homo-oligomeric protein by co expression of native and anchored subunits (Furukawa et al. 2006).
YICwp1	Formation of a new plasmid for surface display on cells of <i>Yarrowia lipolytica</i> (Yue et al. 2008).
Cwp2	The heterodimeric interaction of two coiled-coil adapters through yeast surface display of antibodies (Li et al. 2009).
Pir1	Formation of two <i>Pichia pastoris</i> display vectors, EGFP-Pir1-a with C terminus non-truncated Pir1 and EGFP-Pir1-b with C terminus-truncated Pir1 (Yang et al. 2014).
Pir4	As a fusion partner; use of the cell wall protein Pir4 for the expression of Bacillus sp. BP-7 xylanaseA in <i>S.cerevisia</i> . (Andrés et al. 2005).
Ccw12	Surface display of heterogenous proteins in the yeast cell wall and their applications in Biotechnology (Hossain 2018).

Table 2: Application of various promoters in yeast surface display system

Name	Application
<i>GALI0</i>	Directed evolution of protein expression, affinity, and stability through yeast surface display (Boder & Wittrup 1997). Yeast surface display for the engineering of antibody affinity (Colby et al. 2004). Non-covalent MHC class II heterodimer complexed with antigenic peptide by yeast surface display (Boder et al. 2005). Simultaneous saccharification and fermentation of cellulose to ethanol by yeast surface display of tri functional mini cellulosomes (Colby et al. 2004).
<i>GAPDH</i>	Cell surface engineering for construction of a starch utilizing yeast (Murai et al. 1997). Development of novel whole-cell immune adsorbents through yeast surface display of the IgG-binding domain (Nakamura et al. 2001).
<i>UPR-ICL</i>	Using novel display systems based on the Flo1p flocculation functional domain, formation of yeast strains with high cell surface lipase activity (Matsumoto et al. 2002). Co-expression of native and anchored subunits, development of novel yeast cell surface display system for homo-oligomeric protein by co-expression of native and anchored subunits (Furukawa et al. 2006).
<i>IPTG</i>	Formation of a human light chain variable domain (VL) intracellular antibody specific for the amino terminus of huntingtin through yeast surface display (Parthasarathy et al. 2006).
<i>TEF1</i>	Yeast surface display for fine and domain-level epitope mapping of botulinum neurotoxin type neutralizing antibody was done (Colby et al. 2004).
<i>SEDI</i>	Isoflavone aglycones production from isoflavone glycosides by display of β -glucosidase from <i>Aspergillus oryzae</i> on yeast cell surface. (Kaya et al. 2008).
<i>GPD</i>	A common strategy for the evolution of bond-forming enzymes using yeast display (Chen et al. 2011).
<i>T7</i>	Yeast surface functional display of complex cellulosomes via adaptive assembly (Tsai et al. 2012).
<i>TDH3</i>	On the cell surface of <i>S.cerevisiae</i> , display of cellulases for high yield ethanol production from high-solid lignocellulosic biomass (Matano et al. 2012).
<i>PHO5</i>	Surface display of heterogenous proteins in the yeast cell wall and their applications in Biotechnology (Hossain 2018).

Table 3: Application of various genetic tags in yeast surface display system

Name	Application
HA epitope tag	<p>Screening combinatorial polypeptide libraries through yeast surface display (Boder & Wittrup 1997).</p> <p>Yeast surface display and flow cytometry for fine affinity discrimination (Van Antwerp & Wittrup 2000).</p> <p>Non-covalent MHC Class II heterodimer complexed with antigenic peptide by yeast surface display (Boder et al. 2005).</p>
C-myc epitope tag	<p>A nonimmune <i>S. cerevisiae</i> surface display library for flow-cytometric isolation of human antibodies (Feldhaus et al. 2003).</p> <p>Yeast surface display for construction and characterization of a pseudo-immune human antibody library (Lee et al. 2006).</p> <p>Yeast surface display for high-resolution profiling of homing endonuclease binding and catalytic specificity (Jarjour et al. 2009).</p> <p>On the yeast surface, functional display of complex cellulosomes via adaptive assembly (Tsai et al. 2012).</p>
His tag	<p>Yeast surface display for the development of a human light chain variable domain (VL) intracellular antibody specific for the amino terminus of huntingtin (Colby et al. 2004).</p>
SV5 epitope-tag	<p>Yeast surface display for fine and domain-level epitope mapping of botulinum neurotoxin type a neutralizing antibodies (Levy et al. 2007).</p>

Table 4: Plasmids used in various yeast surface display system

Name	Application
pCT202	<p>Isolation of anti-T cell receptor scFv mutants by yeast surface display. (Kieke et al. 1997).</p> <p>A yeast surface display system for the discovery of ligands that trigger cell activation. (Cho et al. 1998).</p>
pCT302	<p>Rapid method for measuring scfv thermal stability by yeast surface display (Orr et al. 2003).</p> <p>Fine epitope mapping of anti-epidermal growth factor receptor antibodies through random mutagenesis and yeast surface display. (Chao et al. 2004)</p> <p>Biosensor detection systems: engineering stable, high-affinity bioreceptors by yeast surface display (Richman et al. 2009).</p>
pGA11	<p>Construction of a starch-utilizing yeast by cell surface engineering (Murai et al. 1997).</p>

pMWIZ1	Development of novel whole-cell immunoadsorbents by yeast surface display of the IgG-binding domain (Nakamura et al. 2001).
pWIFSPROROL	Construction of yeast strains with high cell surface lipase activity by using novel display systems based on the Flo1p flocculation functional domain (Matsumoto et al. 2002).
pWRSL14	Spacer-mediated display of active lipase on the yeast cell surface (Nakamura et al. 2001).
pPNL6	Flow-cytometric isolation of human antibodies from a nonimmune <i>S.cerevisiae</i> surface display library (Feldhaus et al. 2003).
pGEX4T	Development of a human light chain variable domain (VL) intracellular antibody specific for the amino terminus of huntingtin via yeast surface display (Colby et al. 2004).
pCT-DR4 β	Yeast surface display of a non-covalent MHC class II heterodimer complexed with antigenic peptide (Boder et al. 2005).
pIAP45	Post-translational regulation of expression and conformation of an immunoglobulin domain in yeast surface display (Parthasarathy et al. 2006).
pWI3RSA	Development of novel yeast cell surface display system for homo-oligomeric protein by co-expression of native and anchored subunits (Furukawa et al. 2006).
pCTCON	Selection of horseradish peroxidase variants with enhanced enantioselectivity by yeast surface display (Levy et al. 2007).
pYD2	Fine and domain-level epitope mapping of botulinum neurotoxin type A neutralizing antibodies done by yeast surface display (Richman et al. 2009).
pINA1317	Construction of a new plasmid for surface display on cells of <i>Yarrowia lipolytica</i> (Yue et al. 2008).
pK113-BGL	Isoflavoneaglycones production from isoflavone glycosides by display of β -glucosidase from <i>Aspergillus oryzae</i> on yeast cell surface (Kaya et al. 2008).
pMAT12	Yeast surface display of antibodies via the heterodimeric interaction of two coiled-coil adapters (Wang et al. 2010).
pLCHOOP	A hybrid biocathode: surface display of O ₂ -reducing enzymes for microbial fuel cell applications (Szczipak et al. 2012).

DISCUSSION

Optimized genetic cassettes should consist of host promoters, signal sequences, regions with suitable restriction sites, cell wall anchoring sequences and genetic tags. Therefore, to make decisions regarding which components would be the best for developing an optimized genetic cassette for industrial scale application we conducted studies regarding these components thus making decision; which components are the best suited for designing optimized genetic cassette.

Promoter is one of the most vital components of a genetic cassette. The rate of transcription of a particular gene depends upon promoter. We have found that various promoters used in various display systems. In Table 1, we have listed those promoters and the yeast display systems they have been used. *GALI*, *GALI0*, *GAPDH*, *UPR-ICL*, *IPTG*, *TEF1*, *SEDI*, *GPD*, *T7*, *TDH3* and *PHO-5* are

the major promoters found in the major yeast surface display system. Among these promoters *GALI* and *GAL10* are clearly the most popular ones. The *GALI* promoter has been used in yeast surface display for screening combinatorial polypeptide libraries (Boder & Wittrup 2000), isolation of anti-T cell receptor scFv mutants by yeast surface display (Colby et al. 2004), yeast surface display system for the discovery of ligands that trigger cell activation (Lee et al. 2006), yeast surface display for post-translational regulation of expression and conformation of an immunoglobulin domain (Levy et al. 2007) etc. The *GAL10* promoter has been used in various yeast surface display systems such as yeast surface display for directed evolution of protein expression, affinity, and stability, flow-cytometric isolation of human antibodies from a nonimmune *S. cerevisiae* surface display library (Feldhaus et al. 2003), engineering antibody affinity by yeast surface display (Colby et al. 2004), yeast surface display of a non-covalent MHC class II heterodimer complexed with antigenic peptide (Boder et al. 2005), yeast surface display of Trifunctional Minicellulosomes for simultaneous saccharification and fermentation of cellulose to ethanol (Colby et al. 2004). The *GALI* and *GAL10* both these promoters are inducible promoters which is very important for industrial application as its more convenient to use inducible promoter. The reason is, through using inducible promoter the synthesis of a particular gene can be controlled easily. *GALI* and *GAL10* both these promoters are induced in the presence of galactose but having said that there is certain limitations as the performance rate of these promoters cannot be controlled as the activity of these promoters are depended on the presence of galactose not on the quantity of galactose present. Therefore, these promoters performance rate cannot be increased or decreased according to the demand which why it is a major lacking in these promoters particularly for their application in the industrial scale. However according to the past yeast surface display systems the efficiency of these promoters is very high (Boder & Wittrup 2000). *PHO-5* is another inducible promoter which is still not very popular in yeast surface display systems but it has potential for being used in the optimized genetic cassette for industrial scale application (Hossain 2018). Currently, the *PHO-5* promoter's performance rate can be increased and decreased accordingly with the amount of inorganic phosphate being used. Therefore, the expression rate of the gene of interest can be controlled eventually through *PHO-5* promoter. So in the case of promoters selection *PHO-5*, *GALI* and *GAL10* are our suggestions to be used in the optimized genetic cassette for industrial scale application of yeast surface display.

The signal and anchoring sequence of the cell wall protein that is to be used as fusion protein is also a significant component of the optimized genetic cassette. For this, we have conducted study regarding the cell wall protein used as fusion protein in major yeast surface display systems over the year. In Table 2, we have listed a list of cell wall proteins. Aga2pp, α -agglutinin, Flo1p, Flo318, native mammalian GPCR, YICwp1, Cwp2 Pir1 and Pir4 proteins have been used as fusion proteins. In this review, we found that Aga2p is the most popular cell wall protein to be used as fusion protein. It has been used in various yeast surface display systems such as for screening combinatorial polypeptide libraries (Boder & Wittrup 1997), yeast surface display for directed evolution of protein expression, affinity, and stability (Boder & Wittrup 2000), isolation of anti-T cell receptor scFv mutants by yeast surface display (Colby et al. 2004), fine epitope mapping of anti-epidermal growth factor receptor antibodies through random mutagenesis and yeast surface display, development of a human light chain variable domain (VL) intracellular antibody specific for the amino terminus of huntingtin via yeast surface display (Chao et al. 2004), post-translational regulation of expression and conformation of an immunoglobulin domain in yeast surface display (Parthasarathy et al. 2006). α -agglutinin is another popular cell wall protein which has been used in various yeast surface display as fusion such as, development of novel whole-cell immune adsorbents by yeast surface display of the IgG-binding domain (Nakamura et al. 2001), Isoflavoneaglycones production from isoflavone glycosides by display of β -glucosidase from *Aspergillus oryzae* on yeast cell surface (Kaya et al. 2008) etc. Although the Aga2pp and α -agglutinin proteins are the most popular proteins used in different yeast surface display, their potential to be used as a fusion protein for industrial scale application still arises some questions as these proteins are not commonly used in recent or the most

latest yeast surface display systems. The main reason behind this is there are more cell wall proteins like Pir4 and ccw12 which have shown better results in certain yeast surface display system intended for industrial scale application (Wang et al. 2010).

Genetic tags are also a vital component of the genetic cassette for yeast surface display systems. Selection of proper genetic tags is an important task to develop an optimized genetic cassette for industrial scale application of the yeast surface display system. Now gene tags are mainly important in identifying or confirming the proper attachment of the protein of interest on yeast surface. Since the beginning of yeast surface display systems various genetic tags have been used. So to make proper deduction regarding which genetic tag should be used for the industrial scale application of yeast surface display, we conducted a comparative study regarding various genetic tags used in major yeast surface display systems. In Table 3, we listed those genetic tags, such as HA epitope tag, c-myc epitope tag, His tag, SV5 epitope-tag. Among these tags; HA epitope tag is one of the most popular ones. It has been used in various surface displays for instance, yeast surface display for screening combinatorial polypeptide libraries (Boder & Wittrup 1997), yeast surface display for directed evolution of protein expression, affinity, and stability (Boder & Wittrup 2000), engineering antibody affinity by yeast surface display and yeast surface display of a non-covalent MHC class II heterodimer complexed with antigenic peptide (Boder et al. 2005). C-myc epitope tag is another popular genetic tags used in various yeast surface displays such as, fine affinity discrimination by yeast surface display and flow cytometry (Van Antwerp & Wittrup 2000), a yeast surface display system for the discovery of ligands that trigger cell activation (Lee et al. 2006), rapid method for measuring ScFv thermal stability by yeast surface display (Orr et al. 2003), flow-cytometric isolation of human antibodies from a nonimmune *S. cerevisiae* surface display library (Feldhaus et al. 2003) etc. After the comparative study we came to know that the HA epitope tag has been used in diverse type of yeast display systems. That is why using this tag as a component of the optimized genetic cassette for industrial implication of yeast surface display systems is most likely to provide positive outcomes.

CONCLUSION

The absence of optimized genetic cassettes is a major barrier in connecting the dots between yeast surface display and its industrial application. That is why development of optimized genetic cassette for industrial application of highly potential technique is the demand of this time. For this purpose we conducted comparative studies regarding major components which were used in various yeast surface display systems over the years. Finally on the basis of these studies we made certain deduction regarding most suitable components for developing the optimized genetic for industrial application of yeast surface display systems. We hope that our findings would be helpful in connecting the dots between yeast surface display system and its industrial application.

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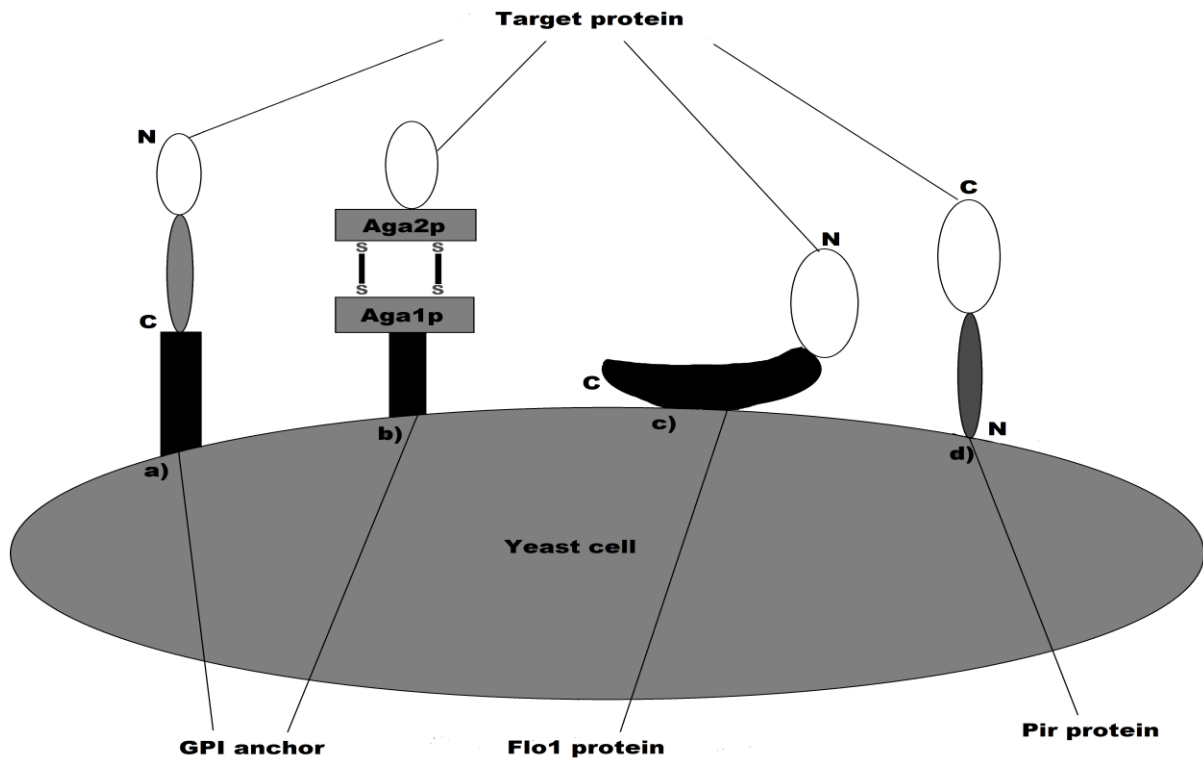


Figure 1 : Cell surface display system in *S. cerevisiae* can be classified into three categories depending on the binding approaches of anchor protein to the cell surface: (a) GPI-anchored system shows C-terminal fusion of the anchor protein with the target protein. GPI proteins are linked to the β -1,6-glucan of the cell wall by the remnant of C-terminal GPI-anchor and can provide only N-terminal display of heterologous proteins (b) α -agglutinin binds to β -1,6-glucan (Kapteyn *et al.*, 1996), so the foreign protein fused to the N-terminus of α -agglutinin are anchored covalently at the cell surface (c) Flo1p display system uses a lectin like cell wall protein Flo1 as anchor. The Flo1 contains the flocculation functional domain near the N-terminus. d) Pir display system uses Pir1-4 proteins which provide N-terminal binding of the recombinant protein on the cell surface.