be possible, or at least not so straightforward, to contrive for the majority of GPCR receptors in a mammalian cell system.

[0006] Presently, there is an absence of an efficient and robust method for identifying functional antibodies, particularly the agonist antibodies, to GPCRs. Accordingly, there is a need to develop a fast, reliable, and low cost method that overcomes the disadvantages described above.

SUMMARY

[0006a] In one aspect, there is provided a method for identifying a functional antibody or a fragment thereof that is capable of binding to, and stimulating the activity of, a target transmembrane protein comprising:

a) providing yeast cells transformed with yeast expression vectors encoding a library of antibodies or fragments thereof, wherein said yeast cells express said target transmembrane protein;

b) expressing said library of antibodies or fragments thereof in the yeast cells, wherein said expressed antibodies or fragments thereof are secreted into the periplasmic space of said yeast cells and retained in the periplasmic space with free movement;

c) incubating the yeast cells in or on a selective medium or under a restrictive temperature, or a combination thereof; and

d) detecting a predetermined phenotype in the yeast cells, wherein manifestation of the predetermined phenotype is indicative of binding of the functional antibody or a fragment thereof to said target transmembrane protein and stimulation of the activity of said target transmembrane protein by the functional antibody or a fragment thereof.

[0007] In one example, there is provided a method for identifying a functional antibody or antigen-binding protein or a fragment thereof that is capable of binding to, and stimulating the activity of, a target transmembrane protein comprising:

a) providing yeast cells transformed with yeast expression vectors encoding a library of antibodies or antigen-binding proteins or fragments thereof, wherein said yeast cells express said target transmembrane protein;

b) expressing said library of antibodies or antigen-binding proteins or fragments thereof in the yeast cells, wherein said expressed antibodies or antigen-binding proteins or fragments thereof are secreted into the periplasmic space of said yeast cells;

c) incubating the yeast cells in or on a selective medium or under a restrictive temperature, or a combination thereof; and

d) detecting a predetermined phenotype in the yeast cells, wherein manifestation of the predetermined phenotype is indicative of binding of the functional antibody or antigen-binding protein or a fragment thereof to said target transmembrane protein and stimulation of the activity of said target transmembrane protein.

[0008] In one example, there is provided an antibody or antigen-binding protein or a fragment thereof that is the agonist of a target transmembrane protein identified according to the method as disclosed herein, wherein the target transmembrane protein is the human G-protein coupled receptor (GPCR) GLP-1R, or a yeast GPCR, the α-factor receptor Ste2p.

[0009] In one example, there is provided an antibody or antigen-binding protein or a fragment thereof identified by the method as disclosed herein comprising (i) a heavy chain variable domain comprising a VHCDR1 having the amino acid sequence GYSFTSYW (SEQ ID NO: 1); a VHCDR2 having the amino acid sequence IYPGDSDT (SEQ ID NO: 2); and a VHCDR3 having the amino acid sequence CARLGYYDSSGYYSEDY (SEQ ID NO: 3); and (ii) a light chain variable domain comprising a VLCDR1 having the amino acid sequence SGRSGFA (SEQ ID NO: 4), a VLCDR2 having the amino acid sequence VNSDGSH (SEQ ID NO: 5), and a VLCDR3 having the amino acid sequence QTWGTGIWV (SEQ ID NO: 6).

DEFINITIONS

[0010] The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for a better understanding of the following description.

[0011] The phrase “antibodies or antigen-binding proteins or fragments thereof” as used herein refers to protein molecules encompassing natural and engineered antibodies, antibody fragments and proteins not related to antibodies and their fragments, such as protein domains and oligo-peptides, that are capable of binding to an epitope of a target antigen *in vivo* and *in vitro* in a selective manner.

[0012] The term “antigen” as used herein refers to proteins of interest that are capable of being recognized and reacted to by antibodies *in vivo* and *in vitro* in a selective manner. Preferred antigens encompass proteins that reside in the plasma membrane of a cell and act as

sensors, receptors, or transporters for specific substances in the extracellular space. Further preferred antigens are the family of transmembrane proteins known as G-protein coupled receptors.

[0013] The term “antibody” is used herein in the broadest sense to refer to various forms of immunoglobulin molecules and their immunologically active fragments, natural or engineered, including monoclonal antibodies, recombinant antibodies, polyclonal antibodies, chimeric antibodies, humanized antibodies, bispecific antibodies, heteroconjugate antibodies, chimeric antigen receptors (CAR), a single variable domain, a domain antibody, antigen-binding fragments, immunologically effective fragments, diabodies, multispecific antibodies, human antibodies, camelid antibodies, single-chain variable fragments (scFv), disulfide-linked variable fragments (sdFv), Fab fragments, F (ab') fragments, and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention) and epitope-binding fragments of any of the above. The immunologically active antibody fragments may also be fused to any polypeptide fusion partner including the Fc region or a fragment thereof. Examples of Fc variant-fusions include scFv-Fc fusions, variable region (e.g., VL and VH)-

Claims

1. A method for identifying a functional antibody or a fragment thereof that is capable of binding to, and stimulating the activity of, a target transmembrane protein comprising:

a) providing yeast cells transformed with yeast expression vectors encoding a library of antibodies or fragments thereof, wherein said yeast cells express said target transmembrane protein;

b) expressing said library of antibodies or fragments thereof in the yeast cells, wherein said expressed antibodies or fragments thereof are secreted into the periplasmic space of said yeast cells and retained in the periplasmic space with free movement;

c) incubating the yeast cells in or on a selective medium or under a restrictive temperature, or a combination thereof; and

d) detecting a predetermined phenotype in the yeast cells, wherein manifestation of the predetermined phenotype is indicative of binding of the functional antibody or a fragment thereof to said target transmembrane protein and stimulation of the activity of said target transmembrane protein by the functional antibody or a fragment thereof.

2. The method of claim 1, wherein the target transmembrane protein is a G-protein coupled receptor, or GPCR.

3. The method of claim 1, wherein the functional antibody or a fragment thereof is an agonist of GPCR.

4. The method of any one of claims 1 to 3, wherein the library of antibodies or fragments thereof is a single-chain variable fragment (scFv), or a Fab.

5. The method of claim 1, wherein the yeast cells in step a) comprise one or more reporter genes, wherein expression of the one or more reporter genes is inducible by activation of a GPCR-mediated signal transduction pathway.

6. The method of claim 5, wherein the GPCR-mediated signal transduction pathway is based on the yeast mating pheromone signaling pathway.

7. The method of claim 5, wherein the one or more reporter genes is an antibiotic resistance gene, a temperature-sensitivity complementing gene, or a fluorescent protein gene, or combinations thereof.

8. The method of claim 7, wherein the antibiotic resistance gene is the G418-resistance gene or the zeocin-resistance gene, or both.

9. The method of claim 7, wherein the temperature-sensitivity complementing gene is CDC26.
10. The method of claim 7, wherein the fluorescent protein gene is the green fluorescent protein gene GFP or the red fluorescent protein gene RFP or the yellow fluorescent protein gene YFP or the blue fluorescent protein gene BFP, or the cyan fluorescent protein gene CFP.
11. The method of any one of claims 1 to 10, wherein the predetermined phenotype is cell growth in the presence of one or more antibiotics, or cell growth at a restrictive temperature, or generation of fluorescence due to expression of a reporter fluorescent protein gene, or combinations thereof.
12. The method of any one of claims 1 to 11, wherein the target transmembrane protein is expressed on the plasma membrane of the yeast cells.
13. The method of any one of claims 1 to 12, wherein the yeast is *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*.
14. The method of any one of claims 1 to 13, wherein a nucleic acid sequence encoding the antibodies or fragments thereof is operably linked to a yeast promoter.
15. The method of claim 14, wherein the nucleic acid sequence encoding the antibodies or fragments thereof further comprises a secretion signal sequence, which facilitates the localization of the said antibodies or antigen-binding proteins or fragments thereof to the periplasmic space of the yeast cells.
16. The method of claim 15, wherein the secretion signal sequence is encoded by the *FLO1* gene or the yeast mating pheromone gene MFα1.
17. The method of any one of claims 1 to 16, wherein the selective medium comprises one or more antibiotics.
18. The method of any one of claims 1 to 17, wherein the selective medium is used at a temperature range of 30 to 40°C.
19. The method of any one of claims 1 to 18, wherein the functional antibody or a fragment thereof is a monoclonal, recombinant, polyclonal, chimeric, humanised, bispecific and heteroconjugate antibodies, a single variable domain, a domain antibody, antigen-binding fragments, immunologically effective fragments, single-chain variable fragments, a single chain antibody, a univalent antibody lacking a hinge region, a minibody, or a diabody.

20. The method of any one of claims 2 to 19, wherein the GPCR is the yeast α-factor receptor Ste2p.