YEAST-BASED LIGAND ASSAY SYSTEM FOR DETECTING G PROTEIN-COUPLED RECEPTOR ACTIVATION IN WATER-IN-OIL DROPLETS

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ABSTRACT

We present a yeast-based ligand assay system to detect activation of G protein-coupled receptors (GPCRs) in water-in-oil (W/O) droplets. A microfluidic device is used to encapsulate template DNA encoding peptides, a cell-free coupled transcription–translation system, and budding yeast cells expressing GPCR into W/O droplets. The yeast cells are genetically engineered to express green fluorescent protein (GFP) via their intracellular signaling pathway in response to ligand stimulation of GPCR. We successfully demonstrated that the synthesized peptide ligand activated the cognate GPCR in W/O droplets. This will be a new platform for identifying novel peptide agonists for mammalian GPCRs.

KEYWORDS

GPCR, Ligand assay, Droplet, Yeast, Cell-free protein synthesis

INTRODUCTION

GPCRs are transmembrane receptors that transduce an extracellular signal into an intracellular signal, and are targets of many therapeutic drugs. Thus, screening many thousands of samples for each target GPCR is routine practice to identify novel ligands and drugs in pharmacological research. The screening of GPCR ligands has generally been based on a mammalian cell-based assay system that monitors downstream events of signal cascades. However, mammalian cell-based assays have the disadvantage that endogenous receptors on host cells respond to their ligands, resulting in false positive signals. Simple assay systems are needed that can pair biologically active ligands with their cognate receptors. The budding yeast is a familiar host cell for the study of GPCRs because it possesses the uncompetitive and monopolistic G-protein signaling pathway, to which a variety of mammalian GPCRs have been coupled. We accordingly developed a yeast-based ligand assay system to detect GPCR activation in W/O droplets.

EXPERIMENT

Figure 1A shows a schematic representation of our GPCR ligand assay system. This system is based on linking genotype and phenotype by *in vitro* compartmentalization using W/O droplets [1]. A DNA library encoding peptides, a cell-free coupled transcription–translation system, and budding yeast cells expressing the target GPCR are co-encapsulated into W/O droplets using a microfluidic device. Yeast cells are genetically engineered to express green fluorescent protein (GFP) via their intracellular signaling pathway in response to ligand stimulation of GPCR [2] (Figure 1B). Thus, the droplets will fluoresce when synthesized peptides stimulate GPCRs on yeast cells, thereby enabling easy identification of possible ligand candidates. We demonstrated that a synthetic peptide ligand activated the cognate GPCR in W/O droplets.

A microfluidic device with a cross-junction microchannel was used to generate W/O droplets. The device was fabricated from polydimethylsiloxane (PDMS) using standard soft-lithography and mold-replica techniques. Both the aqueous and oil solutions were injected into the channel by air pressure controlled with electro-pneumatic transducing regulators. The aqueous solution contained a reconstituted cell-free coupled transcription–translation system (PURExpress; New England BioLabs), PCR products encoding GFP or peptides, yeast cells harboring the reporter plasmid, and 2% (w/v) glucose. The oil solution consisted of mineral oil with 4.5% (v/v) Span 80, 0.45% (v/v) Tween 80, and 0.05% (v/v) Triton X-100. The generated droplets (approximately 23 μm in diameter) were collected into a PCR tube and incubated for 4 hours at 30°C to allow gene translation and ligand stimulation. The yeast strain and plasmid used in this study are shown in Table 1.

***Table 1**: Genotype of yeast strain and plasmid description*

Strain/plasmid	Genotype/description	Reference
Strain		
MI-7	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 far1Δ sst2::kanMX4*	[3]
Plasmid		
pMHG-FIG1	Multicopy reporter plasmid containing *FIG1* promoter, GFP reporter gene, 2*μ* origin, and *HIS3* marker	[4]

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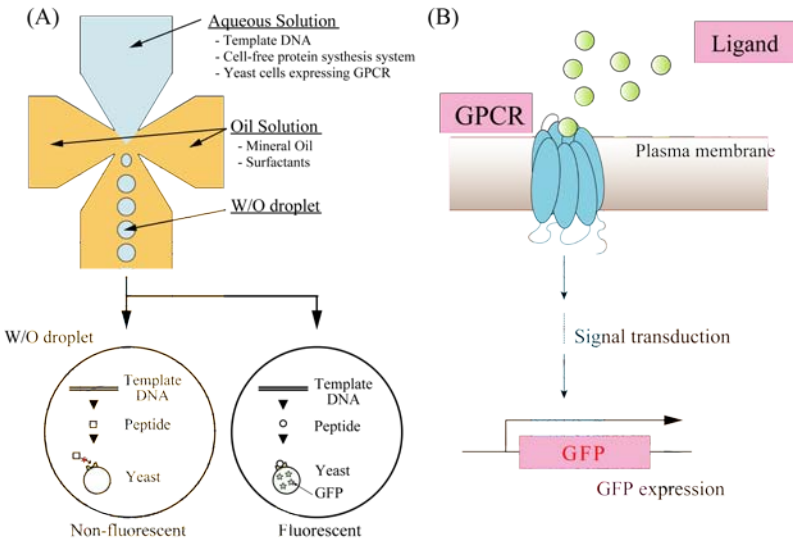

***Figure 1**: (A) Schematic of yeast-based ligand assay system for detecting GPCR activation in W/O droplets. (B) Schematic showing the yeast cell genetically engineered to express GFP in response to ligand stimulation.*

RESULTS AND DISCUSSION

We first tested cell-free GFP synthesis in the presence of yeast cells in W/O droplets. GFP expression was observed in the droplets, indicating that the presence of yeast cells did not significantly inhibit protein expression (Figure 2).

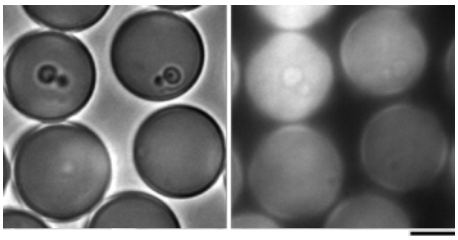

***Figure 2**: Cell-free GFP synthesis in the absence and presence of yeast cells in W/O droplets. The bright-field image (left) shows yeast cells encapsulated in droplets. The fluorescent image (right) shows GFP fluorescence. Two upper droplets contain yeast cells, while two lower droplets do not. Scale bar represents 10 μm.*

We next encapsulated the α-factor gene and yeast cells endogenously expressing Ste2p in the droplets (Figure 3). α-factor, a yeast peptide mating pheromone, binds Ste2p and induces GFP expression via the pheromone signaling pathway. After incubation, GFP-expressing cells were clearly observed in response to α-factor stimulation (Figure 3A). In contrast, a noncognate peptide for Ste2p (Pou-Helix1) and the absence of template DNA did not induce GFP expression (Figure 3B and C).

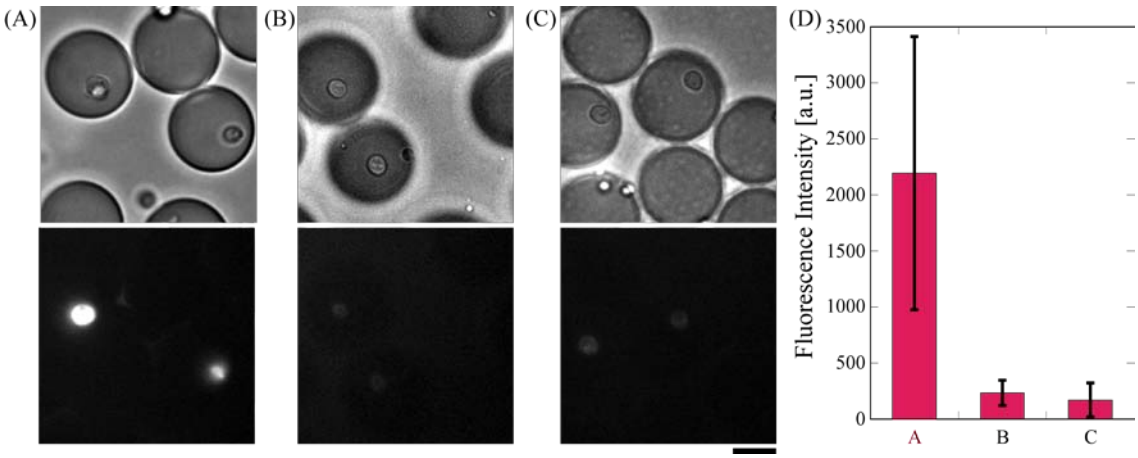

***Figure 3**: Ligand synthesis and ligand stimulation in W/O droplets. (A-C) Bright-field images (top) show yeast cells encapsulated in droplets. Fluorescence images (bottom) show GFP fluorescence or endogenous autofluorescence. (A) In the presence of template DNA encoding α-factor (approximately 25000 molecules/droplet). (B) In the presence of the template DNA encoding noncognate peptide for Ste2p (Pou-Helix1) (approximately 25000 molecules/droplet). (C) In the absence of template DNA. Scale bar represents 10 μm. (D) Average fluorescence intensity of yeast cells in W/O droplets. Error bars represent standard deviation (A, 136 cells; B, 112 cells; C, 107 cells).*

We also performed the same experiments using small amounts of template DNA. In the presence of the five-molecule template DNA per droplet, most yeast cells showed no fluorescence, but some cells showed discrete intensity levels (Figure 4). This result indicated that the α-factor synthesized in the droplets successfully induced GFP expression.

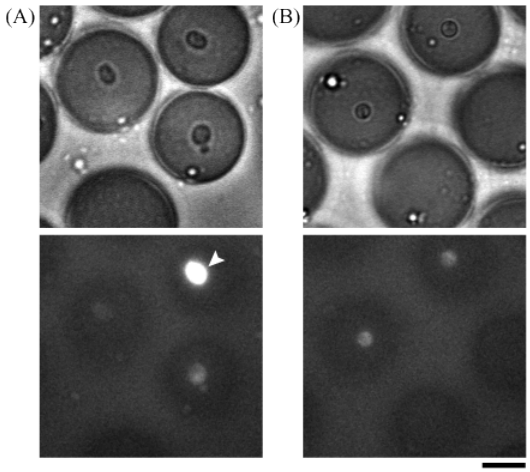

Figure 4*: α-factor synthesis from the five-molecule template DNA and ligand stimulation in W/O droplets. Bright-field images (top) show yeast cells encapsulated in droplets. Fluorescence images (bottom) show GFP fluorescence or endogenous autofluorescence. (A) In the presence of five-molecule template DNA. White arrowhead shows a fluorescent yeast cell in response to α-factor stimulation. (B) In the absence of DNA template. Scale bar represents 10 μm.*

CONCLUSION

We established a yeast-based ligand assay system to detect GPCR activation using droplet-based microfluidics. Yeast cells are known to be suitable hosts for mammalian G-protein coupled receptor ligand screening. Our system will provide an effective approach for the high-throughput screening of peptide ligands targeted at mammalian GPCRs by evolutionary molecular engineering.

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