Chapter 16

Optimizing *Saccharomyces cerevisiae* Induction Regimes

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Abstract

Recombinant membrane protein yields can be optimized in *Saccharomyces cerevisiae* by adjusting the induction time and temperature and/or by the addition of chemical chaperones. Here we describe a protocol for assessing the importance of these parameters.

Key words: Membrane protein, Overproduction, *Saccharomyces cerevisiae*

Abbreviations

GAL	Galactokinase
GFP	Green fluorescent protein
GPCRs	G protein-coupled receptors
TEF	Translation elongation factor 1α

1. Introduction

Tuning membrane protein production is an empirical process. To facilitate optimization we tag all membrane proteins with green fluorescent protein (GFP). This strategy has previously made it feasible to designate the subcellular localization of approximately 75% of the *Saccharomyces cerevisiae* proteome (1). There are also many examples of specific localization studies in yeast using GFP fusions in combination with assays of protein function (2–4). These support the view that fusion with GFP does not usually perturb function.

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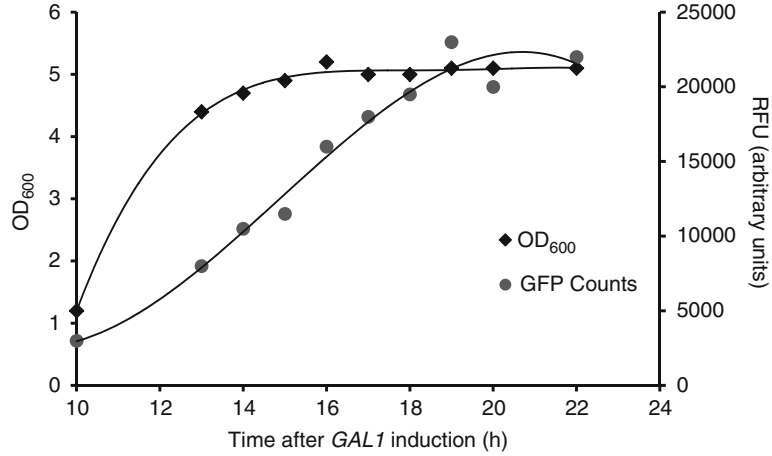

Fig. 1. Time course for the overexpression of a membrane protein–GFP fusion as monitored by GFP fluorescence (*circles*) and OD_{600} (*diamonds*) after induction with galactose in the FGY217 strain (9) under the control of the *GAL1* promoter (5).

We previously analyzed the recombinant yields of 20 yeast membrane protein–GFP fusions under the control of a constitutive *TEF2* and an inducible *GAL1* promoter (5). The majority of yeast membrane protein–GFP fusions exhibited higher yields under the control of the *GAL1* promoter compared with the *TEF* promoter (5). We were able to optimize induction times for optimal expression of membrane protein–GFP fusions in the former system using assays of fluorescence intensity. Most noticeably we found that the highest yields typically occur 12–16 h after the addition of galactose (Fig. 1).

Some studies have shown that addition of chemical chaperones such as dimethyl sulfoxide (DMSO), histidine, or glycerol, as well as lowering the cultivation temperature from 30°C to 20°C, can improve functional yields for a number of G protein-coupled receptors (GPCRs) in *Pichia pastoris* (6) as well as several other transporters in *S. cerevisiae* (7). We found that in most cases yields were enhanced by the addition of chemical chaperones (Fig. 2). We also compared induction times at both 30°C and 20°C and found that at least 36 h are required for growth at 20°C to achieve yields comparable with those at 30°C after 24 h. Using GFP fluorescence at different time points in the cultivation, both localization and monodispersity of the recombinant membrane protein–GFP fusions, could be assessed, information on which is a good indicator for the quality of the protein.

Recently, we tested an alternative induction scheme, whereby membrane protein production is induced in a nonselective rich medium such as yeast extract/peptone/dextrose (YPD). If protein production in a selective medium is nontoxic to the cells, i.e., if the culture can achieve a final OD_{600} greater than 7, then the plasmid

	A.t Ysl1	*H.s* Ctr1	*H.s* Sialin	Yea4p	Ctr3p	*A.t* Ysl2	Hut1p	Pho87p	*M.m* CMP-Sia-Tr	*C.e* Sqv7	MrH1p	Vrg4p	*H.s* Slc35 b1	*H.s* CMP-Sia-Tr	Azr1p	Isc1p	Kha1p	Hsp30p	Dur3p	Rft1p
Standard (mg/L)	0.0	0.1	0.1	0.4	0.4	0.6	0.7	1.0	1.0	1.2	1.4	1.7	1.7	1.7	2.1	2.2	3.1	3.3	3.3	3.5
Glycerol (mg/L)	0.0	0.0	0.0	0.1	0.2	0.2	0.1	0.3	0.7	0.3	0.7	0.9	0.8	1.9	3.2	2.6	3.7	0.7	3.1	4.3
DMSO (mg/L)	0.0	0.1	0.2	0.6	0.1	0.8	0.9	1.2	1.2	1.4	1.2	1.3	1.7	2.1	4.1	3.2	3.1	2.8	4.1	4.2
Histidine (mg/L)	0.0	0.1	0.1	0.2	0.4	0.6	0.9	1.2	1.0	1.3	1.4	2.0	1.7	1.9	2.5	3.5	3.1	3.0	4.0	3.2
20°C 36 hrs (mg/L)	0.3	0.1	0.1	0.5	0.2	0.4	0.4	1.0	0.7	0.7	1.9	1.8	0.3	1.3	2.4	1.8	3.0	2.7	2.7	3.0
20°C 24 hrs (mg/L)	0.1	0.0	0.0	0.2	0.5	0.3	0.2	0.4	0.4	1.1	0.1	0.8	0.1	0.8	3.7	1.0	1.7	1.3	1.6	3.1

Fig. 2. Mean yields (mg/L) from cultures induced under various conditions: standard growth medium lacking uracil; addition of glycerol (10% w/v); addition of DMSO (2.5% w/v); addition of histidine (0.04 mg/mL); growth at 20°C for 36 h or growth at 20°C for 24 h. For each membrane protein–GFP fusion, the best-yielding condition is shaded and is at least 0.2 mg/L (5).

is likely to be retained even in a nonselective medium. In these cases a higher biomass, in the region of $OD_{600} = 15$, is achievable while retaining the same yields per OD_{600} unit. Thus, it is possible to improve yields by more than a factor of three. Using this scheme we have produced the human glucose transporter, GLUT-1, at yields of more than 7 mg/L (8).

2. Materials

- Materials for yeast transformation and growth media are found in Chapter 4.
- DMSO (Sigma).
- Histidine (Sigma).
- Glycerol (Sigma).
- 20% galactose (w/v; Sigma).
- YSB (yeast suspension buffer): 50 mM Tris–HCl (pH 7.6), 5 mM EDTA, 10% glycerol, 1× complete protease inhibitor cocktail tablets (Roche).
- 96-well black optical-bottom plates (Nunc).
- SpectraMax M2e microplate reader (Molecular Devices).
- Confocal microscope (TCS SP2 upright confocal microscope; Leica).

3. Methods

1. Membrane protein–GFP fusions that are produced well under standard culture conditions are good candidates for further optimization. Thus, inoculate 10 mL medium lacking uracil with 2% glucose with an appropriate yeast colony (see Chapter 8) in 50-mL aerated capped tubes.

2. Dilute the overnight culture to an OD_{600} of 0.1–0.12 into six 50-mL aerated capped tubes, each containing 10 mL of medium lacking uracil with 0.1% glucose. Label duplicate tubes as follows: no chaperone; + DMSO; + histidine. Incubate at 30°C, 280 rpm.
3. Monitor the OD_{600} of the cultures. At $OD_{600} = 0.6$ (after approximately 7 h) induce with galactose to a final concentration of 2% w/v. Add DMSO (2.5% w/v) or histidine (0.04 mg/mL) to the correspondingly labelled tubes (see Note 1).
4. 22 h after induction, measure the final OD_{600}, centrifuge the cells at $3{,}000 \times g$ for 5 min, remove the supernatant and resuspend the cell pellet in 200 μL YSB.
5. Transfer 200 μL cell suspension to a black Nunc 96-well optical-bottom plate (see Note 2).
6. Measure the GFP fluorescence emission at 512 nm following excitation at 488 nm in a microtitre plate spectrofluorometer. For plate readers with a bottom-read option, choose this setting. Estimate membrane protein yield (in mg/L) from the yeast whole-cell fluorescence reading by applying the methodology detailed in Table 1 in Chapter 8.
7. Repeat the culture condition that gave the highest yield (steps 1–6) at a post-induction (after addition of galactose) temperature of both 30°C and 20°C. For the latter, induce cells for 36 h (see Note 3).
8. After induction for 22 h at 30°C or 36 h at 20°C, harvest the cells as in step 4 and resuspend in medium lacking uracil with 50% glycerol.
9. Add 1 μL cell suspension to a microscope slide and place a cover slip on top. Focus on the sample using Köhler illumination at 10× magnification. Set the focal plane to 0.
10. Add a drop of lens oil and change to a higher magnification, oil-immersion lens. Turn off the bright field lamp. Turn on the blue light to check the total number of fluorescent cells. Turn off the blue light. Use the Argon laser emitting at 488 nm to image the cells.
11. Judge the optimal induction regime based on the conditions that give the highest yield of correctly targeted membrane protein–GFP fusion (see Note 4).

4. Notes

1. For initial optimization, we recommend the addition of DMSO or histidine as we find that most often these give an average yield improvement of 30%.

2. Because yeast cells settle to the bottom of the plate, proceed to the next step within 5 min of transfer to ensure accurate measurements.
3. Cells grow more slowly at 20°C, thus a longer incubation time is needed to increase biomass to the same levels as cultures grown at 30°C.
4. In addition to localization by confocal microscopy, the quality of the recombinant material can also be assessed by fluorescence-detection size exclusion chromatography (FSEC; Chapter 8). Functional assays should always be carried out where possible.

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