

Integrative Biology

Real-time optogenetic control of intracellular protein concentration in microbial cell cultures

Justin Melendez, Michael Patel, Benjamin Oakes, Ping Xu, Patrick Morton, Marcus Noyes and Megan McClean

Supplemental Figure 1	Use of the sampling and imaging system to quantify nuclear localization of the GEV transcription factor
Supplemental Figure 2	Single mRNAs in response to a single pulse of blue-light
Supplemental Figure 3	Lack of stress response induced by blue-light
Supplemental Figure 4	Diagram of the control software and hardware

Supplemental Figure Captions

Supplemental Figure 1: Use of the culturing and sampling system to measure subcellular localization of the GEV-GFP transcription factor. **(a)** Yeast strain yMM668 ((GAL10+pGAL1)::loxP, gal4Δ::LEU2 HAP1+ leu2Δ0::P_{ACT1}-GEV-GFP-KanMX) was grown to mid-log in the culturing apparatus. The apparatus was operated as a chemostat, with continuous addition of media and removal of effluent. At time T=2 hours, β-estradiol was added to a final concentration of 1μM causing localization of the GEV-GFP transcription factor to the nucleus. Nuclear localization was quantified as described in the using ImageJ and the top 5% of pixels via intensity was divided by the bottom 95% per cell in order to create a nuclear localization score. Over the course of the experiment (red line) β-estradiol dilutes out as new media is added to the culture and effluent is removed. However, the concentration of β-estradiol never falls below 100nM which is sufficient for GEV localization¹. Each blue dot represents the average nuclear localization at a given sampling timepoint. **(b)** Cells were imaged using automatic sampling and the microfluidic device. Cells were imaged every 3 minutes. Representative images are shown.

Supplemental Figure 2: Transcription in response to blue-light induction **(a)** In response to a single 5 min (100% intensity) pulse of blue light, mCherry transcripts in strain yMM1081 (MATα trpΔ63 leu2Δ1 ura3Δ52 P_{GAL1}-mCherry-caURA3 pGAL4AD-CIB1 pGAL4DBD-CRY2) accumulate after approximately 3 minutes and continue to accumulate for up to 20 minutes, indicating that there is a delay in deactivation of the blue-light induction system after the light is turned off. Here transcripts were counted using fluorescence *in situ* hybridization to visualize single mCherry transcripts as previously described². The average number of transcripts per cell is plotted. **(b)** Images of single mCherry mRNA molecules in single cells over the course of the experiment.

Supplemental Figure 3: Blue light does not induce a stress response. **(a)** Blue-light has previously been suggested to induce a stress response in *Saccharomyces cerevisiae* at high intensities³. To confirm that our blue-light induction was not inducing stress, we exposed a continuous steady-state culture of yMM389 (FY Mat a prototroph HAP1+) in phosphate-limited media to blue light as done for protein control experiments. We measured gene expression as previously described¹. A representative sample⁴ of stress-response genes are shown in **(a)**. No genes are induced more than 2-fold in response to blue light, and there is no consistent stress response. **(b)** We also monitored the localization of the general stress response transcription factor Msn2 tagged with mCherry in yeast strain yMM469 (FY prototroph HAP1+ MSN2-mCherry-HphMX). Blue-light over a range of intensities did not induce localization of Msn2, indicating that cells were not experiencing stress in response to blue-light. In contrast, heat shocking yMM469 at 45°C induces a clear Msn2 localization response.

Supplemental Figure 4: Diagram illustrating the relationship between different classes in the control software as well as integration with the microscope and the culturing apparatus. The control code is available upon request.

Supplemental Methods

Strains

Yeast strains used in this study are listed in **Supplemental Table 1**. All genetic manipulations were done using standard lithium-acetate transformation⁶.

Strain ID	Genotype	Reference
yMM389	FY Mata HAP1+	Gift of D. Botstein
yMM391	FY Mata/ α HAP1+	
yMM469	FY Mat a prototroph HAP1+ MSN2-mCherry-HphMX	<i>This Study</i>
yMM668	MAT α gal4 Δ ::LEU2 (P _{gal10+gal1}) Δ loxP leu2 Δ 0::P _{ACT1} -GEV-GFP-KanMX HAP1+	Mclsaac, <i>et al</i> 2011
yMM1079	MAT α , trp Δ 63, leu2 Δ 1, ura3 Δ 52, gal1 Δ mCherry-caURA3	<i>This Study</i>
yMM1081	MAT α , trp Δ 63, leu2 Δ 1, ura3 Δ 52, gal1 Δ mCherry-caURA3 pMM159 (pGal4AD-CIB1) pMM160 (pGal4BD-CRY2)	<i>This Study</i>
yMM1134	Mata α trp1 Δ 63 leu2 Δ 1 ura3-52 gal1 Δ mCitrine-KanMX	<i>This Study</i>
yMM1146	Mat α trp1 Δ 63 leu2 Δ 1 ura3-52	Gift of D. Botstein
yMM1158	Mat α trp1 Δ 63 leu2 Δ 1 ura3-52 gal1 Δ mCitrine-KanMX pMM281 (pVP16AD-CIB1) pMM160 (pGAL4DBD-CRY2)	<i>This Study</i>

Plasmids

Plasmids used in this study are listed in **Supplemental Table 2**.

Plasmid ID	Alias	Genotype	Reference
pMM008	pRS416	URA3 CEN	Sikorski and Heiter, 1989
pMM040	pFA6a-link-yECitrine-KanMX	yECitrine KanMX	Sheff and Thorn, 2004
pMM066	pFA6-mCherry-caURA3	mCherry caURA3	Mclsaac, <i>et al</i> 2011
pMM145	pFA6-mCherry-pTEF-hph-tTEF	mCherry HphMX	McClellan Lab
pMM159	pSV40NLS-Gal4AD-CIB1	pSV40NLS-Gal4AD-CIB1 LEU2 2 μ	Kennedy, <i>et al</i> 2010
pMM160	pGal4BD-CRY2	pGal4BD-CRY2 TRP1 CEN	Kennedy, <i>et al</i> 2010
pMM223	pFA6a-link-yEVenus-SpHIS5 (pKT90)	yEVENUS SpHIS5	Sheff and Thorn, 2004
pMM281	pSV40NLS-VP16-CIB1	pSV40NLS-VP16-CIB1 LEU2 2 μ	<i>This Study</i>
pMM301	pGal1-Venus	pGal1-Venus scURA3 CEN	<i>This Study</i>

Oligos

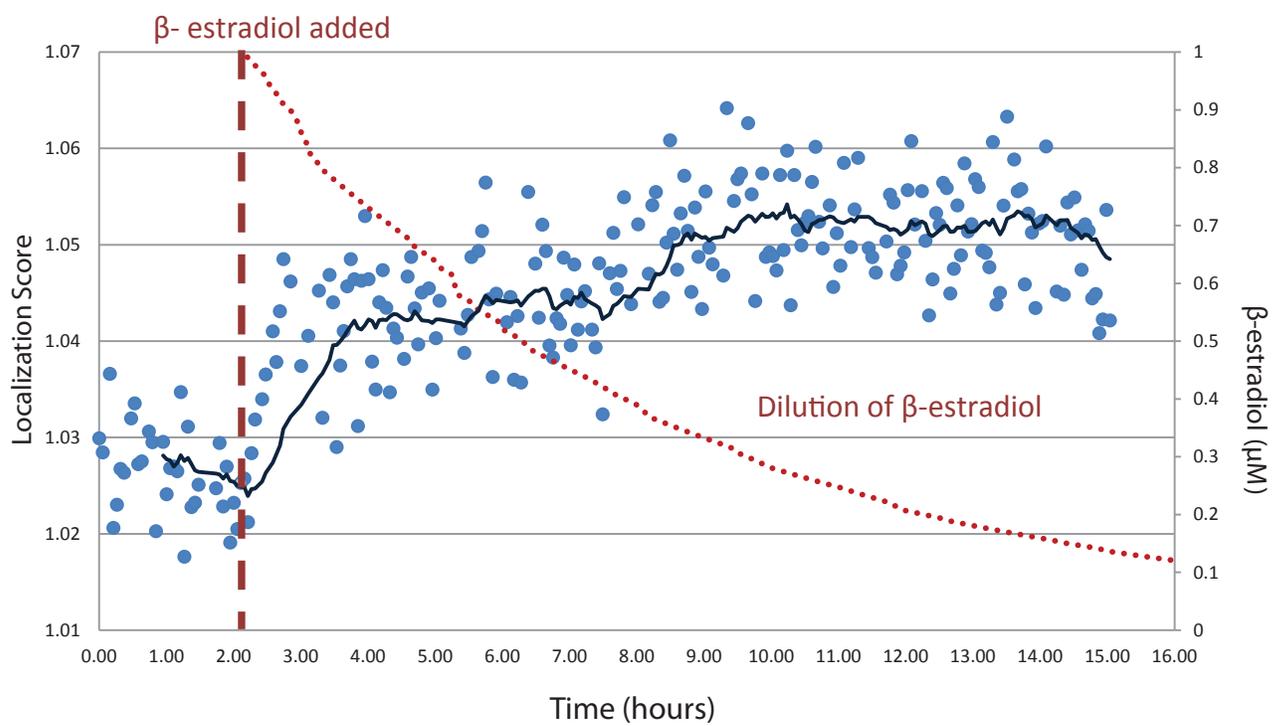
All oligos used in this study were ordered from IDT (www.idtdna.com) with standard desalting.

Oligo	Sequence
oMM032	gtcgcaacacatcaagactcataaaaaacatggagacattGGTCGACGGATCCCCGGG
oMM033	ttatgaagaagatctatcgaataaaaaaatgggtctaTCGATGAATTCGAGCTCG
oMM306	ATATACCTCTATACTTTAACGTCAAGGAGAAAAACTATAggtgacggtgctggtta
oMM307	AATCGAAATCTCTTACATTGAAAACATTATCATAACAATCAtcgatgaattcgagctcg
oMM400	tccaaaaagaagagaaaggcgaattgggtaccgccTCGGAGCTCCACTTAGACGG
oMM401	cgctagcttcggcctcgcctatagtgagtcgtattaaaCCCACCGTACTCGTCAATTC

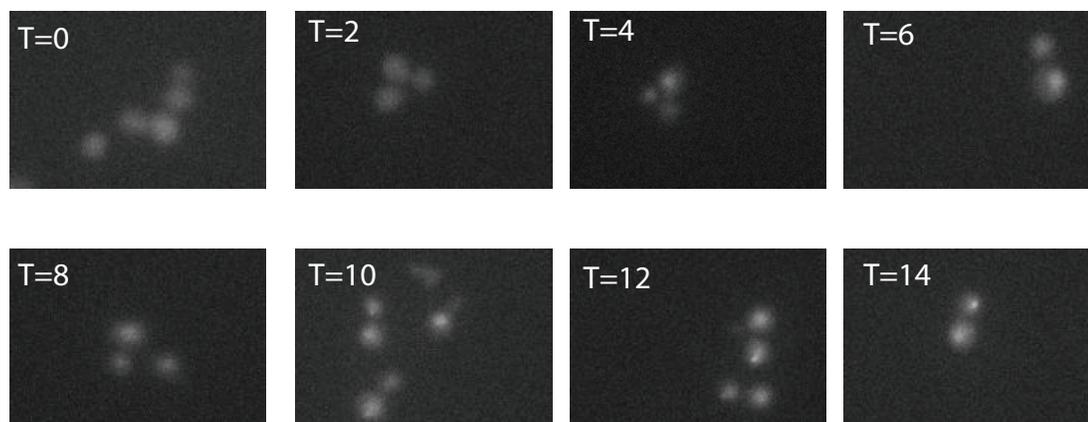
Supplemental References

- 1 R. S. Mclsaac, S. J. Silverman, M. N. McClean, P. A. Gibney, J. Macinskas, M. J. Hickman, A. A. Petti and D. Botstein, *Mol Biol Cell*, 2011, **22**, 4447–4459.
- 2 R. S. Mclsaac, S. J. Silverman, L. Parsons, P. Xu, R. Briehof, M. N. McClean and D. Botstein, *J Vis Exp*, 2013, e50382.
- 3 K. Logg, K. Bodvard, A. Blomberg and M. Kall, *FEMS Yeast Research*, 2009, **9**, 875–884.
- 4 A. Gasch, P. Spellman, C. kao, O. Carmel-Harel, M. Eisen, G. Storz, D. Botstein and P. Brown, *Molecular Biology of the Cell*, 2000, **11**, 4241–4257.
- 5 M. Brauer, C. Huttenhower, E. Airoidi, R. Rosenstein, J. matese, D. Gresham, V. Boer, O. Troyanskaya and D. Botstein, *Molecular Biology of the Cell*, 2008, **19**, 352–367.
- 6 R. D. Gietz and R. H. Schiestl, *Nat Protoc*, 2007, **2**, 35–37.

a

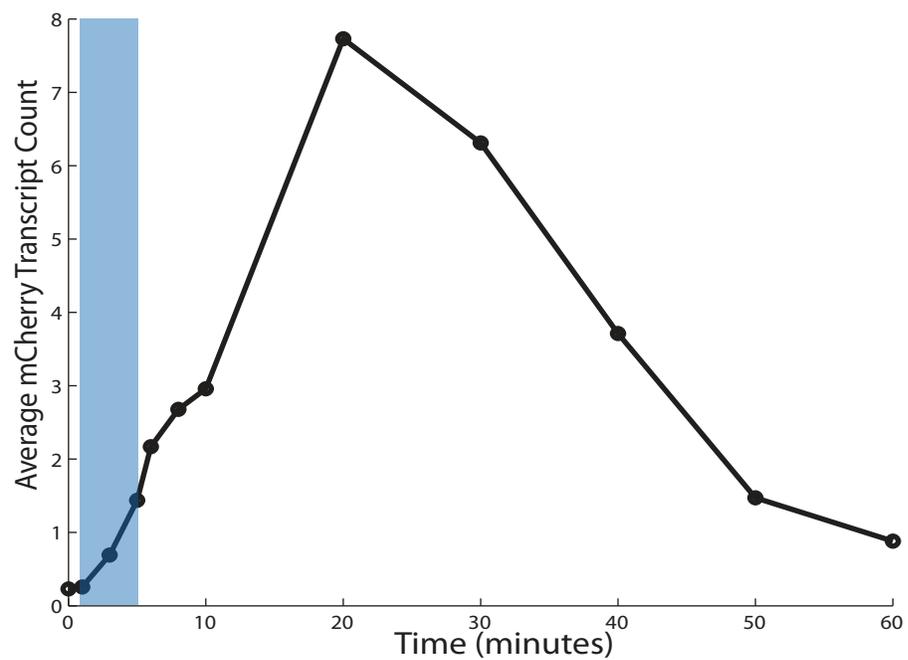


b



Melendez, et al **Supplemental Figure 2**

a



b

