Science Signaling AAAS

www.sciencesignaling.org/cgi/content/full/8/359/ra5/DC1

Supplementary Materials for

MAPK feedback encodes a switch and timer for tunable stress adaptation in yeast

Justin G. English, James P. Shellhammer, Michael Malahe, Patrick C. McCarter, Timothy C. Elston, Henrik G. Dohlman*

*Corresponding author. E-mail: hdohlman@med.unc.edu

Published 13 January 2015, *Sci. Signal.* **8**, ra5 (2015)
DOI: 10.1126/scisignal.2005774

This PDF file includes:

Fig. S1. The antibodies generated against phosphorylated p38 recognize monophosphorylated and dually phosphorylated Hog1.
Fig. S2. Negative feedback only model fails to capture the Hog1 signaling profile.
Fig. S3. Hog1 signaling profile in the Ste50^{5A} strain.
Fig. S4. Hog1 signaling profile in *ssk1*Δ and *rga1*Δ yeast.
Fig. S5. Gating parameters for flow cytometry data.
Table S1. Transcription factor binding and interaction analysis.
Table S2. List of yeast strains used in this study.
Table S3. List of parameter values used in modeling.
Legends for database S1 and S2

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/8/359/ra5/DC1)

Database S1 (Microsoft Excel format). Annotated microarray data with GO analysis and organization.
Database S2 (Microsoft Excel format). Induction of 95 proteins from clusters 1 to 4 in response to 0, 350, or 650 mM KCl.

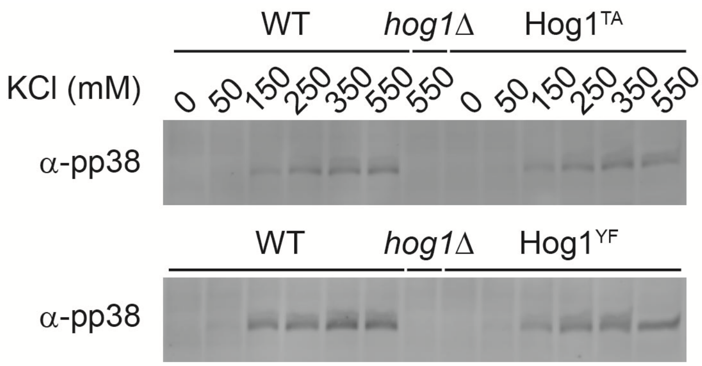

Fig. S1. The antibodies generated against phosphorylated p38 recognize monophosphorylated and dually phosphorylated Hog1. The indicated strains were treated with KCl for five minutes, collected, lysed, and analyzed by Western blotting using the phospho-p38 antibodies.

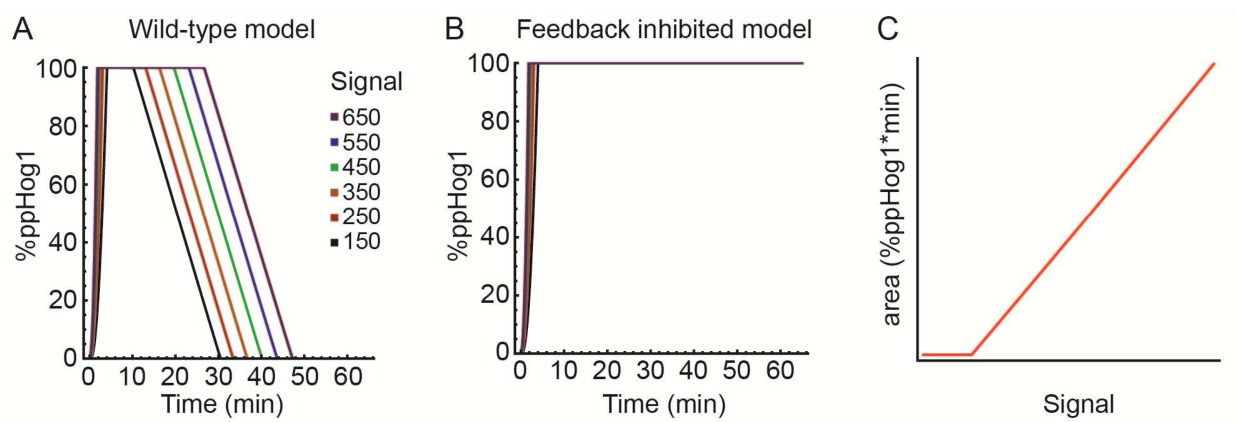

Fig. S2. Negative feedback only model fails to capture the Hog1 signaling profile. The model of Behar *et al*. (*23*) fails to explain catalytically-inactive Hog1 mutant experimental time-courses. (A) The model of the wild-type behavior. (B) The model when Hog1 feedback is inhibited model. (C) The total amount of ppHog1 from the model of wild-type cells.

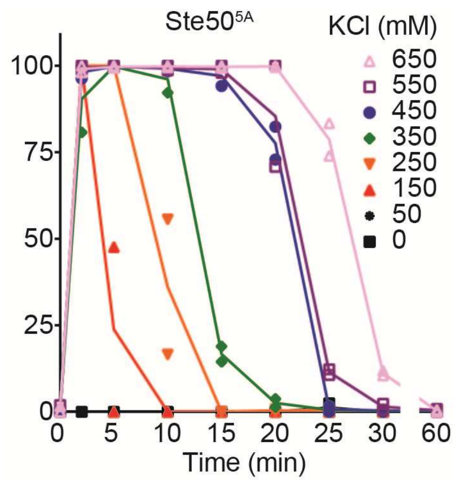

Fig. S3. Hog1 signaling profile in the Ste50^{5A} strain. Hog1 signaling profile in the Ste50^{5A} strain. Data represent two experiments.

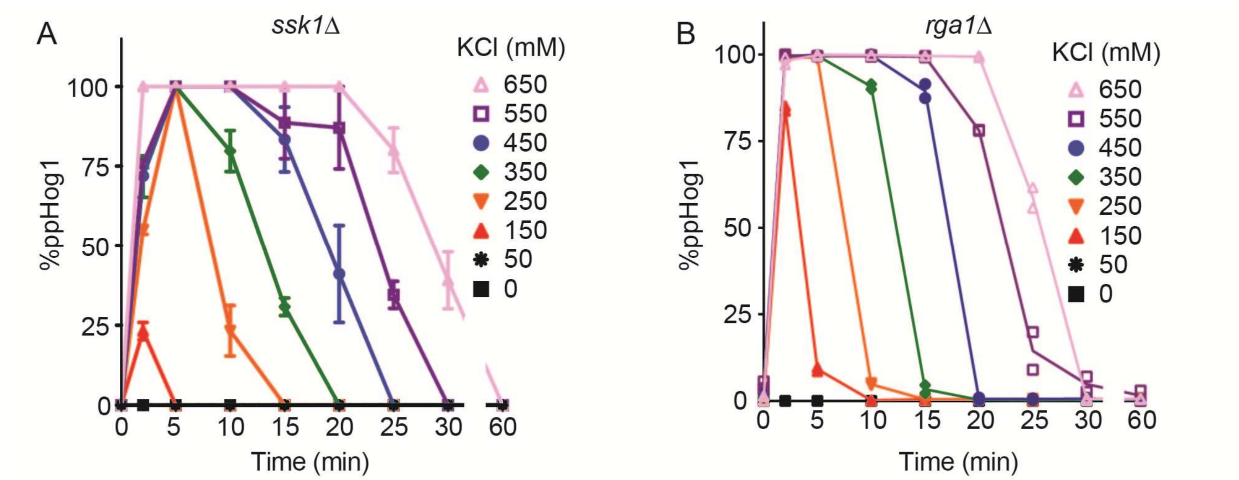

Fig. S4. Hog1 signaling profile in *ssk1*Δ and *rga1*Δ yeast. (A) Hog1 signaling profile in an *ssk1*Δ strain determined as described in Fig. 1. Data are means +/- SEM (n = 3). (B) Hog1 signaling profile in an *rga1*Δ strain, determined as described in Fig. 1. Data represent two experiments.

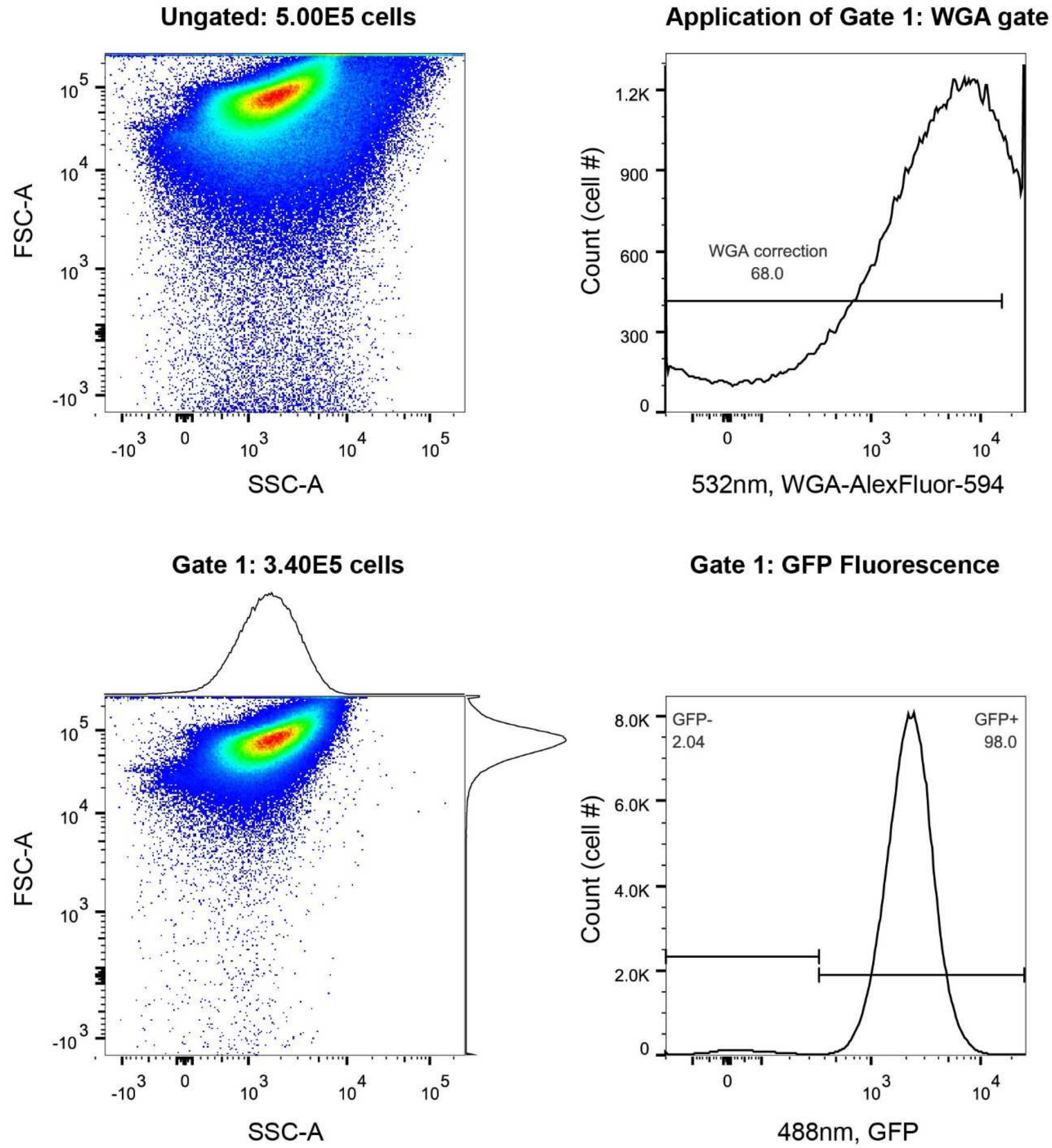

Fig. S5. Gating parameters for flow cytometry data. Gate 1 establishes a cut-off for auto-fluorescent debris and dead cells determined by staining with wheat germ-agglutinin (WGA) conjugated to AlexFluor-594. Fluorescence minus one (FMO) experiments were performed to compensate for bleed-through on line. Data are compensated fluorescence values. FSC-A, forward scatter area; SSC-A, side scatter area.

Table S1. Transcription factor binding and interaction analysis. Percent value represents number of query set gene promoters associated with the transcription factor (TF). p-value calculations and cut-offs were determined as outlined on YEASTRACT. Significance of gene set (left) to TF (top) association indicated as highly significant (**, $p < 1E^{-5}$), significant (*, $p < 1E^{-5}$), or nonsignificant ($p > 0.01$). 67 random genes were selected from the yeast genome as a negative control.

	Msn2	**Msn4**	**Sko1**	**Hot1**	**Hog1**
random (67)	51.56% (0.245)	37.50% (0.346)	21.88% (0.016)	1.56% (0.152)	9.38% (0.003)
All microarray (252)	95.63% (0**)	87.30% (0**)	46.83% (0**)	17.86% (0**)	10.71% (1.2E-09**)
C1 (107)	98.10% (0**)	94.39% (0**)	57.94% (0**)	28.97% (0**)	13.08% (5.4E-07**)
C2 (111)	96.40% (0**)	87.39% (0**)	37.84% (6.3E-12**)	11.71% (8.3E-12)**	10.81% (2.4E-05**)
C3&4 (34)	85.29% (1E-06**)	64.71% (1.8E-4*)	41.18% (8.0E-08**)	2.94% (0.052)	2.94% (0.27)
flow cytometry (67)	94.03 (0**)	82.09% (1E-15**)	49.25% (9.3E-14**)	14.93% (9.24E-11**)	13.43 (2.55E-05**)

Table S2. List of yeast strains used in this study.

Strain	Genotype	Background	Reference
BY4741	*MATa*, *his3Δ1*, *leu2Δ*, *met15Δ*, *ura3Δ*	BY4743	(*73*)
JGE001	*hog1*^{T100A}	BY4741	This study
JGE002	*ssk1Δ::KanMX4*	BY4741	This study
JGE003	*ssk2Δ::KanMX4*	BY4741	This study
JGE004	*ssk22Δ::KanMX4*	BY4741	This study
JGE005	*pbs2Δ::KanMX4*	BY4741	This study
JGE006	*msb2Δ::KanMX4*	BY4741	This study
JGE007	*hkr1Δ::KanMX4*	BY4741	This study
JGE008	*rga1Δ::KanMX4*	BY4741	This study
JGE009	*rga2Δ::KanMX4*	BY4741	This study
JGE010	*bem3Δ::KanMX4*	BY4741	This study
JGE011	*ste20Δ::KanMX4*	BY4741	This study
JGE012	*ste11Δ::KanMX4*	BY4741	This study
JGE013	*opy2Δ::KanMX4*	BY4741	This study
JGE014	*ste50Δ::KanMX4*	BY4741	This study
JGE015	*hog1Δ::KanMX4*	BY4741	This study
JGE016	*ptc1Δ::KanMX4*	BY4741	This study
JGE017	*ptc2Δ::KanMX4*	BY4741	This study
JGE018	*ptp2Δ::KanMX4 ptp3Δ::LEU2*	BY4741	This study
JGE019	*ptp2Δ::KanMX4*	BY4741	This study
JGE020	*ptp3Δ::LEU2*	BY4741	This study
JGE021	*hog1*^{T174A}	BY4741	This study
JGE022	*hog1*^{Y176F}	BY4741	This study
JGE023	*ste50*^{5A} (S155A, S196A, S202A, S248A, Thr341A)	BY4741	(*18*)

Table S3. List of parameter values used in modeling.

Parameter	Value
k1	0.303763
k1m	0.675469
k2	7.73342
k2m	2.14647
a	3.77649
k3	0.000935217
k3m	0.550563
k4	0.0039341
k4m	0.987791
k5	71.749
k5m	122.966
k6	0.675903
k6m	8.02219
kn	0.000118967
kc	0.000102928
k8	0.0168266
k8m	0.678538
k9	0.000133456
k9m	0.585395
k10	0.000116113
k12	0.000173807

Database S1. Annotated microarray data with GO analysis and organization. C1, cluster 1; C2, cluster 2; C3 & 4; clusters 3 and 4. Data were classified by "biological process."

Database S2. Induction of 95 proteins from clusters 1 to 4 in response to 0, 350, or 650 mM KCl.