Published in final edited form as:

Science. 2013 January 25; 339(6118): 460–464. doi:10.1126/science.1227299.

Tunable signal processing through modular control of transcription factor translocation

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Abstract

Signaling pathways can induce different dynamics of transcription factor (TF) activation. We explored how TFs process signaling inputs to generate diverse dynamic responses. The budding yeast general stress responsive TF Msn2 acted as a tunable signal processor that could track, filter, or integrate signals in an input dependent manner. This tunable signal processing appears to originate from dual regulation of both nuclear import and export by phosphorylation, as mutants with one form of regulation sustained only one signal processing function. Versatile signal processing by Msn2 is crucial for generating distinct dynamic responses to different natural stresses. Our findings reveal how complex signal processing functions are integrated into a single molecule and provide a guide for the design of TFs with “programmable” signal processing functions.

Many transcription factors (TFs) display diverse activation dynamics in response to various external stimuli (1–4). To investigate how TFs process upstream signals, we studied the *S. cerevisiae* general stress responsive TF Msn2 (5). In the absence of stress, Msn2 is phosphorylated by Protein Kinase A (PKA) and localized to the cytoplasm; in response to stress, Msn2 is dephosphorylated and translocates to the nucleus where it induces gene expression (5).

Natural stresses elicit highly variable dynamics of Msn2 nuclear translocation (Fig. 1A) (6, 7), which are thought to result from oscillatory signaling inputs (presumably PKA activity) (8). To study how Msn2 processes oscillatory PKA inputs, we used an engineered yeast strain (6) carrying mutations in all three PKA isoforms that enable selective inhibition of PKA activity by a cell-permeable inhibitor, 1-NM-PP1 (9). We used this synthetic system and a microfluidics platform (10) mounted on a microscope to produce oscillatory inputs of PKA inhibition and monitored translocation of Msn2 to the nucleus. The input amplitude was chosen on the basis of the steady state amount of Msn2 nuclear localization in response to sustained inputs: high amplitude input (3 μ M 1-NM-PP1) led to maximal nuclear localization of Msn2; whereas low amplitude input (0.2 μ M 1-NM-PP1) induced an intermediate amount of nuclear localization (Fig. 1B, black circles). The pulse duration of oscillatory input was selected on the basis of duration of pulsatile Msn2 nuclear bursts in the physiological response to glucose limitation (6). With high amplitude oscillatory input, each input pulse induced a high amount of nuclear localization (Fig. 1C, left). In contrast,

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oscillatory input with low amplitude barely elicited any localization responses, although sustained input with the same amplitude led to a half-maximal amount of nuclear localization (Fig. 1C, right). Therefore, Msn2 filters temporal fluctuations of the input in an amplitude-dependent manner such that it tracks high amplitude inputs, but responds in a limited manner to low amplitude signals.

To understand how Msn2 translates signaling inputs into different translocation responses, we characterized Msn2 phosphorylation, which controls nuclear translocation (5, 11). We detected phosphorylation of eight PKA consensus sites, primarily located within the nuclear export signal (NES) and nuclear localization signal (NLS) domains (11) (Fig. S1). Two sites in the NES (S288, S304) and four sites in the NLS (S582, S620, S625, S633) were functionally important for regulation of nuclear transport (Fig. S2).

To intuitively understand the behaviors of the translocation system, we conducted a steady-state analysis that incorporated the separation of timescales for nuclear transport and phosphorylation. For simplicity, we represented regulation of nuclear export and import by phosphorylation of one site in the NES and a second site in the NLS, acting independently of each other (Fig. 2A). We assumed that the slowest timescales occur for nuclear import when the NLS is phosphorylated (k_{in}) and for nuclear export when the NES is unphosphorylated (k_{out}). In contrast, when the NLS is unphosphorylated or when the NES is phosphorylated, nuclear import and export, respectively, both were assumed to occur on faster timescales (k_{in}' , k_{out}') (12). This scheme gives four phosphoforms with distinct combinations of transport rates (Fig. 2B). Finally, we assumed that phosphorylation and dephosphorylation are fast relative to the translocation timescales (13, 14), so that we could treat transitions between the four phosphoforms, which are triggered by input of PKA inhibition, as effectively instantaneous. PKA and Msn2 phosphatases localize to both the cytoplasm and the nucleus in yeast (15–17), so Msn2 can be phosphorylated and dephosphorylated in both compartments.

For purposes of illustration, we used a weak and a strong input to represent the amplitude of PKA inhibition (Fig. 2C). Because sites in the NLS are more preferred for PKA phosphorylation than those in the NES (11, 18) (Fig. S3), we assumed that weak input (partial inhibition of PKA) would lead to dephosphorylation of only the NES, and the NLS phosphorylated form (U_P^c) would then go to the nucleus with a slow import rate (k_{in}). In contrast, strong input would lead to dephosphorylation of both the NES and NLS and the fully unphosphorylated form (U_Uⁿ) would be transported into the nucleus with a fast rate (k_{in}'). Upon input removal, the NES and NLS are re-phosphorylated and the doubly-phosphorylated form (P_Pⁿ) is expected to be exported with a fast export rate (k_{out}') (Fig. 2C, 1st row). In accordance with this analysis, strong input (3 μ M 1-NM-PP1) led to rapid Msn2 translocation whereas weak input (0.2 μ M 1-NM-PP1) resulted in slower translocation, and export is rapid when PKA inhibition is removed (Fig. 3AB, WT).

We then analyzed how Msn2 might respond to oscillatory inputs. In response to a strong oscillatory input, Msn2 would go in and out of the nucleus with import and export rates (k_{in}' , k_{out}') that are fast relative to the input pulse duration and inter-pulse interval. Hence, Msn2 responded fully to each pulse and tracked the input dynamics (model: Fig. 2D, 1st row, left; data: Fig. 3C, WT). In response to a weak oscillatory input, Msn2 would enter the nucleus with a slow import rate (k_{in}) relative to the timescale of the input pulse, and therefore only a small amount of Msn2 entered the nucleus, effectively filtering out low amplitude signals (model: Fig. 2D, 1st row, middle; data: Fig. 3D, WT). In response to an input fluctuating between high and low amplitudes, because Msn2 would go out of the nucleus with a slow export rate (k_{out}) relative to the timescale of the inter-pulse intervals,

Msn2 was not fully exported during the interval and integrated the input fluctuations (model: Fig. 2D, 1st row, right; data: Fig. 3E, WT).

To further test the model and explore the influence of regulation of both import and export on signal processing, we studied cases in which only nuclear import or export, but not both, was regulated by phosphorylation because the functional phosphosites within the NES or NLS were mutated (Fig. 2C and D, Fig. 3).

In the case in which the NLS sites could not be phosphorylated (NLS 4A), Msn2 would enter the nucleus with a constitutively fast import rate (k_{in}') and go out of the nucleus with a fast export rate (k_{out}') upon input removal (Fig. 2C, 2nd row; data: Fig. 3A–B, NLS 4A). Hence, in response to oscillatory inputs with high amplitude, low amplitude, or fluctuating between high and low amplitude, Msn2 would have fast import and export rates and fully entered the nucleus during a pulse and exited the nucleus during inter-pulse intervals (model: Fig. 2D, 2nd row; data: Fig. 3C–E, NLS 4A).

If NLS sites were mutated to mimic constitutive phosphorylation (NLS 4E), Msn2 would enter the nucleus with a constitutively slow import rate (k_{in}) (Fig. 2D, 3rd row; data: Fig. 3A, NLS 4E). In response to oscillatory inputs, when the input duration is short relative to the timescale of the slow import rate, Msn2 went into the nucleus slowly and reached low concentrations (model: Fig. 2D, 3rd row; data: Fig. 3C–E, NLS 4E).

If NES sites cannot be phosphorylated (NES 2A), Msn2 would exit the nucleus with a constitutively slow export rate (k_{out}) (Fig. 2D, 4th row; data: Fig. 3B, NES 2A). In response to oscillatory inputs with high amplitude, low amplitude or fluctuating between high and low amplitude, when the interval is short relative to the time scale of the slow export rate, Msn2 would have a slow export rate, could not fully exit the nucleus during intervals, and therefore integrated responses to rapidly changing inputs (model: Fig. 2E, 4th row; data: Fig. 3C–E, NES 2A). In summary, NLS 4A, NLS 4E, or NES 2A “tracks”, “filters” or “integrates” the oscillatory inputs, respectively, whereas WT Msn2 exhibits a combination of all these processing behaviors.

To study the processing of natural stress signals, we monitored WT and mutant Msn2 translocation in response to different stresses (Fig. 4A–C and S5). We also monitored the dynamics of WT Msn2-mCherry and mutant Msn2-YFP expressed together in the same cells – this allowed us to directly compare the responses of WT and mutant Msn2 to the same stochastic input signals triggered by natural stress (Fig. 4D and S6). Glucose limitation induced sporadic pulses of rapid nuclear localization of WT Msn2 with frequency regulated by stress intensity; osmotic stress elicited a single pulse of nuclear accumulation; and oxidative stress led to sustained nuclear localization (Fig. 4A–C, and S5, WT). NLS 4A, which tracks the inputs, was more responsive to inputs and exhibited a high frequency of small rapid bursts of nuclear translocation (Fig. 4D, 1st row), and thus produced similar response frequency to low and high levels of glucose limitation (Fig. 4A–C, 2nd row, panels marked with blue asterisks; Fig. S5D, left, blue circles). By contrast, NLS 4E filtered out the sporadic translocation bursts in response to glucose limitation (Fig. 4D, 2nd row) and therefore exhibits similar dynamics to both glucose limitation and osmotic stress (Fig. 4A–C, 3rd row, panels marked with green asterisks). NES 2A integrated the sporadic bursts in response to strong glucose limitation (Fig. 4D, 3rd row) and exhibited prolonged nuclear accumulation, similar to that of oxidative stress responses (Fig. 4A–C, 4th row, panels marked with red asterisks). Consistent with the analysis of artificial inputs, WT Msn2 and the mutants differed in how they processed signaling inputs triggered by natural stresses and therefore generate different responses. WT Msn2 with dual regulation of nuclear import and export generates distinct translocation responses to different stress conditions, whereas the

mutants that have only one mode of nuclear transport regulation fail to fully differentiate the different stresses into distinct translocation outputs. Cells may use these diverse TF translocation patterns to induce distinct gene expression programs (6), or to elicit different levels of noise in single cell responses, both of which might be beneficial for survival under stress conditions.

Nucleocytoplasmic translocation of many mammalian TFs, such as NFATs, STATs, and Smads (19–21), is controlled by regulation of both their nuclear localization and nuclear export signals. Hence, the proposed dual regulation mechanism may represent a general mechanism for shaping the dynamic behaviors of these TFs. Complex signal processing behaviors can be achieved by signaling circuits comprised of multiple molecules (22–29). We reveal a single TF molecule can also mediate sophisticated signal processing functions by assembling independent functional modules. These functions are “tunable” by phosphorylation at multiple sites in each module and “programmable” by mutating or reassembling functional modules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank A. Murray, P. Cluzel, S. Ramanathan, B. Stern, and M. Rust for comments on the manuscript, and S. Mukherji, X. Zhou, A. S. Hansen and other members of the O’Shea lab for helpful discussions. J.G. is supported by NIH R01 GM081578. E.K.O. is an Investigator of the Howard Hughes Medical Institute.

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Fig. 1. Tunable signal processing behaviors of Msn2

(A) Illustration of the distinct single cell dynamic responses of Msn2 to various stresses. (B) Steady state abundance of Msn2 in the nucleus in response to various concentrations of 1-NM-PP1. In response to each concentration of 1-NM-PP1, Msn2 exhibited uniform and stable nuclear localization in single cells and did not exhibit stochastic fluctuations as observed in natural stress responses. Open circles: responses to different concentrations of 1-NM-PP1; closed circles: responses to 3 μM and 0.2 μM 1-NM-PP1, which are used as high and low amplitude inputs, respectively, for the following analysis. AU – arbitrary unit. (C) Averaged single-cell time traces of Msn2 nuclear translocation (Lower panels; n: ~50 cells; error bar: single-cell variances) in response to oscillatory inputs with high and low amplitudes (Upper panels). Left: high-amplitude input produced by 3 μM 1-NM-PP1; right: low amplitude input produced by 0.2 μM 1-NM-PP1. Pulse duration - 3 min; pulse interval - 2 min. To emphasize the fact that 3 μM 1-NM-PP1 elicits a steady state response that is approximately twice the response elicited by 0.2 μM 1-NM-PP1, the y-axes of the upper panels are not presented on a linear scale.

Fig. 2. A theoretical analysis of transcription factor translocation

(A) Nomenclature used to define the status of phosphorylation and localization of the TF: the 1st “P/U” - the NES is phosphorylated (P) or unphosphorylated (U); the 2nd “P/U” - the NLS is phosphorylated (P) or unphosphorylated (U); “c/n” - in the cytoplasm (c) or nucleus (n). (B) Phosphorylation states determine the rate constants of nucleocytoplasmic transport. Unphosphorylated (U) or phosphorylated (P) NES has slow (dashed line) or fast (solid line) nuclear export rates (k_{out} , k_{out}'), respectively; unphosphorylated (U) or phosphorylated (P) NLS has fast (solid line) or slow (dashed line) nuclear import rates (k_{in}' , k_{in}), respectively. Thus, each phosphoform has a specific combination of nuclear import and export rates. (C) Schematic of the translocation model. Left: schematic of WT and phosphosite mutants; right: model structures and reaction flows (grey arrows) in response to strong or weak inputs and input removal. 1st row: WT; 2nd row: NLS 4A - S582A, S620A, S625A, S633A; 3rd row: NLS 4E - S582E, S620E, S625E, S633E; 4th row: NES 2A - S288A, S304A. We did not specifically study the case in which the NES sites are constitutively phosphorylated because Ser-to-Glu mutants of the NES sites behaved similarly to Ser-to-Ala mutants, suggesting that Glu cannot mimic phosphorylation on NES sites (Fig. S2A). (D) Predicted responses to various dynamic inputs: 1st column: oscillatory high-amplitude input; 2nd column: oscillatory input with varied amplitudes; 3rd column: input fluctuating between high and low amplitudes. Black: responses of WT; blue: NLS 4A; green: NLS 4E; red: NES 2A. The ranges of input timescales necessary to generate the predicted responses are determined

by the fast and slow timescales of transport rates and are listed above each column. Model output was generated by a steady-state analysis of the translocation system (Supplementary Materials).

Fig. 3. Distinct signal processing by WT, NLS and NES phosphosite mutants of Msn2
 (A) Averaged single-cell time traces of Msn2 nuclear translocation in response to sustained inputs with low ($0.2 \mu\text{M}$ 1-NM-PP1, solid triangles) or high ($3 \mu\text{M}$ 1-NM-PP1, solid circles) amplitudes. Inputs were applied at time point zero. (B) Averaged single-cell time traces of Msn2 nuclear translocation in response to removal of high-amplitude input ($3 \mu\text{M}$ 1-NM-PP1). (C) Averaged single-cell time traces of Msn2 nuclear translocation in response to oscillatory high-amplitude ($3 \mu\text{M}$ 1-NM-PP1) inputs. Pulse duration – 3 min; pulse interval – 4 min. (D) Time traces of Msn2 nuclear translocation in response to oscillatory inputs with a mixture of low ($0.2 \mu\text{M}$ 1-NM-PP1) and high ($3 \mu\text{M}$ 1-NM-PP1) amplitude pulses. (E) Time traces of Msn2 nuclear translocation in response to input fluctuating between high ($3 \mu\text{M}$ 1-NM-PP1) and low ($0.2 \mu\text{M}$ 1-NM-PP1) amplitudes. For (A)–(E), data points are average single-cell time traces (n : ~50 cells; error bar: single-cell variances). The simple model in Fig. 2 has been fit to the time trace data in this figure and the solid lines in (A)–(E) are model fitting results (see Supplemental Material - “Model parameters are constrained by experimental data” for details). The dependence of the responses on the timescales of input and transport rates is presented in Supplemental Material - “The relationship between timescales of input and timescales of transport rates”.

Fig. 4. Distinct responses of WT, NLS, and NES phosphosite mutants of Msn2 to natural stresses Single-cell responses of WT, NLS 4A, NLS 4E, and NES 2A to glucose limitation (A), osmotic stress (B), and oxidative stress (C) (n: ~50 cells, each stress condition). Representative single-cell time traces of Msn2 nuclear translocation are shown. Asterisks emphasize the conditions under which the mutants fail to distinguish two different stresses. Quantification of the time traces is presented in Fig. S4. (D) Time traces of WT Msn2-mCherry and mutant Msn2-YFP, monitored in the same cells, in response to glucose limitation (black – WT, blue – NLS 4A, green – NLS 4E, red – NES 2A). More single-cell traces are shown in Fig. S5.