The High Osmolarity Glycerol (HOG) MAP kinase pathway in the budding yeast *Saccharomyces cerevisiae* is one of the best characterized model signaling pathways. The pathway processes external signals of increased osmolarity into appropriate physiological responses within the yeast cell. Recent advances in microfluidic technology coupled with quantitative modeling, and techniques from reverse systems engineering have allowed yet further insight into this already well-understood pathway. These new techniques are essential for understanding the dynamical processes at play when cells process external stimuli into biological responses. They are widely applicable to other signaling pathways of interest. Here, we review the recent advances brought by these approaches in the context of understanding the dynamics of the HOG pathway signaling.

1. Introduction

Living organisms have evolved specialized biochemical pathways to cope with stressful, often changing environments. Even in simple cells such as yeast, thousands of specialized sets of sensing and signaling proteins form modules used to monitor and adapt to the environmental state and its variations. Such modules can be insulated or, on the contrary, connected to one another. Whereas insulation allows for robust and sensitive response, the interconnection of modules allows for higher-level behavior such as multiple input sensing and decision making through cross-talk [1]. For a given stimulus, the biochemical components of the different modules that play a role in the cellular response are usually well described in the literature. Their biological functions and interactions are known in detail, especially in model organisms such as the budding yeast. This knowledge comes from decades of complex, tedious, and elegant experiments. Genetic techniques such as gene deletion, mutation, and overexpression have been used to infer the connection patterns between proteins and the architectures of many modular functions. Biochemical assays provided crucial information on protein phosphorylation and kinase activity. Microarrays revealed the role of these modules in determining global gene expression.

Signaling pathways are naturally dynamic [2] in that cells must respond to external signals in a timely manner, and indeed, the cellular response is often affected by the temporal properties of the external signal. In addition, the internal dynamics and timing of events in the signaling pathway determine the cellular response. These internal dynamics determine the information flow, allowing cells to process and convey information from a sensory input to a specific protein in charge of orchestrating the cellular response [3]. Until recently, experimental techniques have been limited such that most studies have examined the response of a signaling pathway to a stationary stimulus. Accordingly, adaptation and cellular responses to environmental cues were usually studied only with respect to the magnitude of the stimulus without seriously taking into account dynamical aspects. Identification of the components of a signaling pathway through the techniques mentioned above, combined with studies of simple stationary stimuli, is not enough to
understand the dynamics or systems-level properties of a complex biological network.

With the emergence of systems biology, there has been an important paradigm shift, and it is becoming increasingly clear that the temporal variations of stimulatory inputs can be directly sensed by cells [5] and that studying cells in time-variable environments is a powerful way to determine signaling pathway architecture and to understand how they process information [6, 7]. Experimental microfluidics-based strategies have matured to allow for excellent control of the cellular environment both in time and space [8, 9]. This technology coupled with genetic engineering to fluorescently tagged proteins allows for real-time observation of the system's response using fluorescence microscopy. Finally, quantitative real-time measurements form the basis for the development of mathematical models and the use of signal analysis tools, such as reverse engineering, to model the dynamical aspects of signaling pathways [10]. These models in turn provide testable experimental predictions.

This review describes the recent strategies that have been developed to assess quantitatively the dynamics of the canonical HOG MAP kinase (MAPK) pathway in the yeast, *Saccharomyces cerevisiae*. We shall first briefly review the key characteristics of the organization of the HOG pathway. We then discuss the novel experimental and modeling tools [10, 11, 16–18] that are allowing new insights into the pathway’s dynamics and systems-level behavior.

## 2. MAPK Cascades in Yeast

Among signaling pathways, the Mitogen Activated Protein Kinases (MAPK) family has received considerable attention. MAPK pathways are very well conserved from yeasts to mammals [19–21] and several comprehensive reviews are available in the recent literature [22, 23]. MAP Kinase pathways are involved in many cellular processes such as stress response, the regulation of differentiation and proliferation. These pathways contain a canonical module of three protein kinases that act in series (Figure 1). Upon phosphorylation by an upstream protein, a MAP kinase kinase kinase (MAPKKK) phosphorylates a MAP kinase kinase (MAPKK) on conserved serine and threonine residues, which in turn phosphorylates a MAP kinase (MAPK) on a threonine (sometimes serine) and a tyrosine residue located adjacent to each other and separated by a single amino acid (Thr/Ser-X-Tyr). This dual phosphorylation site is located in the activation loop of the catalytic domain and its dual phosphorylation is needed for activation of the MAP kinase.

There are five MAPK modules in yeast (Table 1) [22].

The hyperosmotic glycerol (HOG) pathway is activated in response to a hyperosmotic stress [24–28]. The Cell Wall Integrity (CWI) module controls the cell wall integrity and is triggered in response to numerous stresses including cell wall deterioration, temperature shifts, and hypo osmotic shocks [29–31]. The pheromone pathway [32, 33] controls the mating response which involves an important morphological deformation of yeast cells. Finally, the filamentous growth pathway [33, 34] and the sporulation pathway [22] control the response to starvation for haploid and diploid cells although the sporulation pathway is not as well known as the other four MAPK pathways. Only its MAPK has been identified in diploid cells (Smk1p), and it is thought to drive the spore cell wall assembly [22]. Though they share numerous components, the five MAPK pathways of the yeast *Saccharomyces cerevisiae* are tightly regulated by crosstalk and mutual inhibition which permit faithful signaling, adaptation to their environment, and regulation of growth and morphogenesis [22]. Among these MAPK pathways, the HOG pathway (Figure 2) is particularly well suited to study signaling dynamics, since it can be reliably activated through increasing the osmolarity of the environment.

## 3. The HOG MAPK Signaling Pathway

Water homeostasis is fundamental for life. In nature, the environment can vary rapidly from isotonic to hyper or hypo osmotic conditions, and yeast cells have to adapt quickly [23, 47]. The first response after a hyperosmotic shock is the rapid loss in few seconds of cell volume due to water efflux and the activation of membrane sensory receptors followed by the activation of the HOG pathway which is completed after a few minutes (Figure 2) [11, 48]. Two distinct branches of the pathway detect changes in osmolarity and activate the
pathway. These branches converge at the level of the MAPKK Pbs2p. The first branch is referred to as the SHO1 branch [23, 49], while the second is referred to as the SLN1 branch [50, 51]. Sln1p negatively regulates the HOG signaling pathway and deletion of SLN1 is lethal due to pathway overactivation. This lethality is suppressed by knockout any of the downstream components SSK1, SSK2/SSK22, PBS2, or HOG1. Sln1p contains two transmembrane domains, a histidine kinase domain and a receiver sequence. Sln1p autophosphorylates on its histidine kinase domain. The phosphate group is then transferred to its receiver domain, which then phosphorylates Hog1p, Hog1PP translocates to the nucleus, and Hog1PP phosphorylates the MAPK Fus3/Kss1, Hog1, and Slt2 [65, 66]. Localization of Hog1p-GFP to the nucleus can be used as a reliable reporter of pathway activity.

Sho1p consists of four transmembrane domains and an SH3 domain. This domain permits the recruitment of molecular actors, notably the MAPKK Pbs2p, to the plasma membrane [54]. The upstream kinase Ste20p, the G-protein Cdc42p, and the MAPKK Ste11p needed for the activation of the protein Pbs2p are also recruited to the membrane [55]. Since it is a transmembrane protein, Sho1p has long been considered an osmosensor [56]. However, recent studies suggest that Sho1p is more an anchor protein than a sensor for osmolarity [55]. Hkr1p and Msb2p, two mucin-like [57–60] proteins that form heterooligomeric complexes with Sho1p [58, 59] have recently been proposed as osmosensors of the SHO1 branch. Components of the SHO1 branch also take part in pseudohyphal development and mating, indicating that Sho1p might not have a specific role in osmosensing but a more general role related to cell shape measurement [61].

MAPKKKs of these two initiating branches induce the phosphorylation of the MAP kinase kinase Pbs2p on the conserved residues Ser514 and Thr518 [62]. Pbs2p is a cytoplasmic protein essential for the activation of Hog1p by dual phosphorylation on the conserved Thr174 and Tyr176 [62]. PBS2 and HOG1 are essential for osmoadaptation as null mutations in both genes induce osmosensitivity [23, 63]. Pbs2p also plays the role of a scaffold for the SHO1 branch [49, 54, 56, 64] by anchoring the different components, promoting signal propagation between proper protein partners and preventing improper cross-talk between the Pheromone pathway and the HOG pathway. Once Pbs2p phosphorylates Hog1p, Hog1PP translocates to the nucleus in a manner that is dependent upon the karyopherin Nmd5p [65]. Localization of Hog1p-GFP to the nucleus can be used as a reliable reporter of pathway activity.

4. Sequential Response after a Hyperosmotic Shock

The activation of the Hog1p MAPK triggers several responses on different time-scales (Figure 3) [48]. A rapid non-transcriptional response in the cytoplasm corresponds to the closure of Fps1p [66] and the activation of several kinases (e.g., Rck2p [67], Pfk2p [68]). Fps1p belongs to the ubiquitous Major Intrinsic Protein (MIP) [69] family and is known to play a central role in yeast osmoadaptation by controlling both uptake and efflux of the osmolyte
5. Towards a Model of the HOG Pathway

Years of genetic and biochemical analysis have provided us with an extraordinarily precise description of the key players in the HOG pathway. What about the signaling dynamics of the pathway? How does the architecture determine the pathway’s signal processing ability? Classic molecular biology experiments were based on step shock experiments with an osmotic agent, such as NaCl or sorbitol at various concentrations. Phosphorylation states of key proteins have been measured at different time points after a step shock at the population level, showing a transient increase of phosphorylation (lasting several minutes) concomitant with nuclear enrichment of Hog1p [81]. Nuclear cytoplasmic shuttling of Hog1p was also observed qualitatively, indicating a fast deactivation of the pathway when cells are returned to an isotonic environment [81]. Levels of gene expression have been measured at different timepoints after an osmotic shock using microarrays [73]. Although done with a low resolution in time compared to biophysical experiments, these measurements give an idea of the dynamics of the activation of the pathway.

Based on such measurements, several models have been proposed to describe mathematically the HOG signaling pathway and more generally osmoadaptation in yeast [82]. The most comprehensive and the first integrative one is due to Klipp et al. [81]. Their model takes not only the HOG signaling cascade into account (only the SLN1 branch), but...
Figure 3: Sequential sketch of yeast adaptation to a hyperosmotic shock. The evolution with time of the size, phosphorylation of Hog1p, and internal concentration of glycerol are schematically represented in the center of the picture. (1) After an increase of the external osmolarity (green), a first mechanical response corresponds to a rapid loss of water (blue arrow). It leads to a decrease of the cell size and a loss of turgor pressure. (2) HOG osmosensors (blue) activate the pathway and eventually lead to the phosphorylation of Hog1p. (3) Hog1PP induces several processes: (a) Inactivation of the glycerol channel Fps1p preventing glycerol leakage; (b) direct or indirect activation of cytoplasmic actors, for example, 6-phosphofructo-2-kinase (Pfk2p) involved in glycerol synthesis; (c) translocation in the nucleus. Note that there are other targets of Hog1p such as Sic1p, Hsl1p, Nha1p, and Tok1p. (4) Nuclear Hog1PP induces a large transcriptional response. In particular, the gene GPD1 leading to glycerol synthesis is upregulated. Negative feedbacks (glycerol production, phosphorylation of Sho1p, etc.) allow inhibition of the pathway activity. (5) Increase of the internal glycerol leads to water influx and progressive cell size recovery while Hog1p is exported from the nucleus. (6) Pathway is off, and turgor pressure and cell size are restored. The cell is adapted to its new environment.

also includes a description for the metabolic production of glycerol, as well as an elementary gene expression model for the enzymes involved in glycerol production. The model also includes the closure of the membrane glycerol transporter Fps1p and takes the dephosphorylation of nuclear Hog1p by Ptp2p into account. Most reactions in the model were described by the mass action rate law. The model consisted of 70 parameters, of which 24 had to be estimated. To estimate this number of parameters with the limited data available, the authors divided the model in modules and fitted them separately to data points. Their model reproduced accurately the transient response of the HOG pathway after a single hyperosmotic shock. This included the phosphorylation states of Hog1p and Pbs2p, as well as glycerol production and cell-size recovery. In addition, the model was able to correctly predict the effect of different mutations of proteins involved in the pathway. Mutants unable to produce glycerol (gpd1Δ, gpd2Δ) [83] or to close the Fps1p channel showed an increased duration of HOG activity. Mutants with an increased phosphatase Ptp2p activity showed a lower level of phosphorylated Hog1p but a similar period of HOG activity.

Although very promising, such an approach is still extremely difficult to fine tune since it relies on many unknown parameters. Comparison of the model outputs to experimental data is crucial. To further constrain and test complex models one needs quantitative, time-resolved experiments at the single-cell level in response to complex input signals.

As engineers do with electronic circuits and chips, a very powerful way to explore the dynamics of a given system is to observe its response to complex input signals. Such an approach lends itself to developing minimal models that
of the chemical environment of yeast cells [7]. Hersen et al. [11] designed a fast binary switch to repeatedly change the environment of single yeast cells between two chemical conditions as fast as every second (Figure 4(a)). They used a Y-shaped flow chamber, 50 μm high and 500 μm wide, with two inlets. One inlet was filled with an isotonic medium, and the other with the same culture medium complemented with sorbitol to increase its osmolarity. At such small scales, flows are laminar and fluids do not mix but rather simply flow side by side. The lateral position of the fluids interface is set by the relative hydrostatic pressure—or the relative flux—of the two inlets. Changing this pressure difference displaces the interface laterally in less than a second. Yeast cells, previously fixed in the channel through concanavalin-A coating were then repeatedly switched from an isotonic to a hyperosmotic environment. An interesting alternative developed by Eriksson et al. [12] consists of moving the cells with optical tweezers (Figure 4(b)) rather than moving fluids over fixed cells. This strategy removes the potential influence of cell adhesion on signaling dynamics related to morphological changes, but at the cost of technological complexity. Also, such a strategy is very time consuming. Holographic tweezers—a sophisticated version of optical tweezers—can help to increase the number of cells that can be observed in real time [86]. Another strategy was proposed by Charvin et al. [13, 87]. Yeast cells are fixed between a permeable dialysis membrane and a cover slip coated with a very thin layer of soft PDMS (Poly-Di-MethylSiloxane). A channel is placed on top of the membrane and allows flow of fresh media and exchange within a few minutes. Nutrients and other chemicals can freely diffuse through the membrane. With this device, environmental exchange happens more slowly, but cells can grow over several generations in a monolayer simplifying their observation through microscopy. Indeed, Charvin et al. used it to force periodic expression of cyclins in yeast growing exponentially up to 8–10 generations.

More complex devices have been proposed, though they require a high degree of expertise to fabricate and manipulate. Bennet et al. [88] developed an environmental switcher capable of generating sinusoidal inputs. Their multilayer device was composed of a microchemostat, with a depth of 4 μm to force yeast cells to grow in a monolayer, and a fluid mixer to generate complex time varying environmental signals for the cells in the chemostat chamber. They used this device, in a particularly elegant work, to revisit the wiring of the GAL system in yeast, by subjecting cells to sinusoidal inputs of carbon source over a range of frequencies. Taylor et al. [14] described a high throughput microfluidics single-cell imaging platform to study the dynamics of the pheromone response in yeast. They combined a fluidic multiplexer, an array of channels, and many sieve valves to trap cells and to control fluid delivery. They were able to perform simultaneous time lapse imaging of 256 chambers with 8 different genotypes with several dynamical inputs. Such a strategy, although very sophisticated, can enhance dramatically the quantity of data gathered to improve our knowledge and refine modeling of MAPK pathways in yeast [7].
7. New Insights from Coupling Complex Stimulus and Reverse Systems Engineering

Using such microfluidics strategies (Figure 4(a)), Hersen et al. studied the HOG pathway response to periodical osmotic stimulation over a range of frequencies. Interestingly, the HOG pathway acts as a low-pass filter, meaning that the output of the pathway (Hog1p nuclear localization) does not follow a fast varying input precisely, but rather integrates fast fluctuations over time. For wild-type strains, when the input signal varies slower than once every 200 s, Hog1p cytoplasmic—nuclear shuttling follows the input variations faithfully [11, 17]. However, when the input varies more rapidly than every 200 s, Hog1p nuclear translocation no longer follows the input faithfully, but instead integrates over the input fluctuations [11, 17]. This typical time is also the slowest time (or limiting step) of activation of the pathway although it was not possible from these experiments to point out which biochemical step was limiting. By genetic removal of one of the two branches, the contribution of each branch was also measured by Hersen et al., and it was found that the SHO1 branch is slower than the SLN1 branch by
almost a factor two. The SHO1 branch was actually unable to integrate the too fast variations of the input whereas the SLN1 branch, when taken alone, was displaying a similar behavior than wild-type cells [11]. Those investigations clearly evidenced that the pathway can be turned off very quickly and repeatedly, suggesting the existence of several feedback loops acting on different timescales.

An attempt to decipher the dynamical aspects of these feedback loops has been done by Mettetal et al. [10], who also examined the response of the Hog1p nuclear localization in response to an oscillating input. They constructed, based on these frequency experiments, a simple predictive model, which was not based on biological knowledge (Figure 5(a)). Subsequently, they identified the two variables of their model with the intercellular osmolyte concentration and the phosphorylation state of Hog1p and concluded that the pathway contains a Hog1-dependent and a Hog1-independent feedback mechanism. By underexpressing Pbs2p, thereby reducing the sensitivity of the Hog1-response to the input, they were able to isolate the Hog1-independent feedback from the Hog1-dependent feedback. Based on this they concluded that the Hog1-dependent feedback is required for fast pathway inactivation. By inhibiting translation, they showed indeed that the slow transcriptional response triggered by Hog1p is only necessary for the adaptation to multiple osmotic shocks, while for a single osmotic shock faster nontranscriptional feedback mechanisms dominate the response. Their conclusion is in perfect agreement with recent experimental investigations showing that even cells with Hog1p anchored to the membrane present an increase of glycerol production after a hyperosmotic shock [89]. Although the details are not known, Hog1p directly or indirectly activates the 6-Phosphofructo-2-kinase (PFK2) [68] which leads to an increase production of glycerol through Gpd1p activity.

Hao et al. also focused on rapid non-transcriptional feedback loops. First, they noticed that the response of the SHO1 branch is more transient than that of the SLN1 branch. Then, based on previous observations, they constructed three simple mathematical models, each describing another possible mechanism of HOG inactivation. One model was based on Hog1p mediating activation of a negative regulator (phosphatases), while the other two models focused on the negative control of a positive regulator. Analysis of the different models suggested a Hog1p-dependent feedback mechanism occurring early in the response. Their experimental analysis confirmed this and suggested that Hog1p acts negatively on Sho1p by phosphorylation, thereby implementing a direct negative feedback loop.

Muzzey et al. [18] followed a similar approach to study the feedback mechanisms within the pathway. They identified the transient activation of Hog1p with a feature called perfect adaptation, which states that the steady state output of the pathway does not depend on the strength of the osmotic shock. They argued that robust perfect adaptation requires at least one negative feedback loop containing an integrating component [90] and they analyzed the location of this integrator. They defined an integrating component as a dynamic variable whose rate of change does not depend on itself. They monitored multiple system quantities (cell volume, Hog1p, and glycerol) and used varied input waveforms to analyze the pathway. Similar to Hao et al. [16], they constructed different variants of a mathematical model, each with a different location of the integrating component. The authors found that the integral feedback property is Hog1p dependent and regulates glycerol uptake.

More recently, Zi et al. [15] analyzed the experimental frequency response of the HOG pathway done by Hersen et al. and Mettetal et al. They constructed a minimal model that can reproduce the response of the pathway to oscillating inputs (Figure 5(b)) [15]. They defined a signal response gain, which is defined as the ratio of the integrated change of the output of the pathway to the integrated input change and represents a measurement for the efficiency of signal transduction. They concluded that yeast cells have optimized this signal response gain with respect to certain durations and frequencies of osmotic variations.

These different analyses have shown that the HOG signaling cascade can be described in a very simple and modular way with several feedback loops operating to deactivate the pathway: two operating on short time scales through Hog1p activity (Sho1p deactivation and glycerol production increase), and one depending on transcriptional activation of GPD1. The dynamics of the pathway was also precisely measured and it was shown that it behaves as a low-pass filter with a cutoff frequency, probably set by protein concentration. Interestingly, the SHO1 branch which is known to be involved in other cellular processes was shown to be slower in activating the Hog1p MAPK than the SLN1 branch. Finally, those approaches have provided us with an easily tractable mathematical model of the HOG pathway that can be efficiently coupled to detailed mechanistic models to study in silico the behavior of this MAPK pathway. Taken together, the coupling between mathematical modeling and experimental frequency analysis of the HOG pathway has given very important insights into the HOG pathway dynamics and more generally its functioning, demonstrating the interest of developing such strategies for studying signaling pathways in yeast.

8. Future Directions

Although the structure and the dynamics of the HOG signaling pathway are now well understood, several key points remain to be elucidated, the most elusive one being the mechanistic functioning of the two osmosensors, Sln1p and the Sho1p complex. Another important aspect of a better understanding of the HOG pathway is to integrate its behavior with other cellular processes. In particular, in 2000, Gasch et al. [73] compiled genome expression profiles of S. cerevisiae yeast subjected to several stress conditions and discovered that genes normally induced after a hyperosmotic shock are downregulated in response to a hypo-osmotic shock and vice versa. The CWI pathway is activated by hypo-osmotic stimulation [29], its physiological role being to reinforce the cell wall and prevent the cell from bursting. HOG and CWI do not share direct components but were
seen to interact with each other [91, 92]. During cell growth both pathways may well be activated and deactivated within short intervals to balance between cell expansion and cell wall development. The Snl1p-dependent response regulator Skn7p [93, 94] could have a role in linking the cell-integrity pathway to the HOG pathway. Skn7p also interacts with Rho1p an upstream component of the CWI pathway. The evidence that Skn7p is apparently controlled by sensors of both the HOG pathway and the cell-integrity pathway makes Skn7p an excellent candidate for a regulator that coordinates osmoregulation and cell wall biogenesis [23, 93, 94]. More work is needed to better understand the putative role of Skn7p in coordinating different aspects of turgor pressure control and cell surface assembly. Using minimal models and fluctuating environments to activate periodically the CWI and/or the HOG pathway is one interesting way to explore their interactions. Similarly, it is known that the HOG pathway and the Pheromone pathway can interact [95–98]. For example, a hog1Δ strain will respond to a hyperosmotic shock by activating the response to pheromone pathway. Again, the dynamics of such cross-talk has not been intensely studied. Performing time varying inputs with both pheromone and hyperosmotic medium will provide invaluable experimental data to probe for the dynamical aspects of cross-talk between MAPK in yeast.

Since MAPKs pathways are highly conserved from yeast to mammalian cells, it would be interesting to test higher eukaryotic cells, in single cell experiments, for similar system level properties. Although more difficult to implement than for yeast cells, microfluidic technics can also be used to control the external environments of mammalian cells both in time and space. Transposing the approaches described here to mammalian cells will probably give further insights in their signaling pathways dynamics.

9. Conclusion

Since its initial discovery in 1993 [24], extensive molecular and genetic research has uncovered the molecular actors, interactions, and functions of the components in the HOG signaling pathway. However, these methods are limited in that one cannot predict the behavior of a complex system from the analysis of isolated components. Understanding of the entire system requires the use of novel techniques borrowed from engineering, physics, and mathematics. Microfluidic technologies combined with live-cell microscopy have allowed the use of temporally complex stimuli to interrogate pathway function. Kinetic information obtained through biochemistry combined with knowledge of the molecular components has allowed for complex quantitative models of the HOG pathway to be constructed. These models in turn provide experimentally testable predictions about pathway behavior and function. Simple “black-box” models designed to mimic only key components of the pathway have proven useful for understanding specific phenomena. Thus, genetic and biochemical data combined with novel experimental approaches and modeling have allowed for the prediction of the dynamics and systems-level properties of HOG pathway signaling processes. These techniques are easily extended to other signaling pathways of interests with the final goal being to understand the relationships between structure, kinetics, and dynamics at the systems-level in complex biological networks.

Acknowledgment

P. Hersen is supported by the ANR program of the French Government (ANR-JCh-DiSiP). A. Miermont and J. Uhlen-dorf are students of the Frontier in Life Sciences PhD program (Paris, France).

References

[1] L. H. Hartwell, J. J. Hopfield, S. Leibler, and A. W. Murray, “From molecular to modular cell biology,” Nature, vol. 402, no. 6761, pp. C47–C52, 1999.
[2] P. Nurse, “Life, logic and information,” Nature, vol. 454, no. 7203, pp. 424–426, 2008.
[3] R. Brent, “Cell signaling: what is the signal and what information does it carry?” FEBS Letters, vol. 583, no. 24, pp. 4019–4024, 2009.
[4] N. L. Novèrè, M. Hucka, H. Mi et al., “The systems biology graphical notation,” Nature Biotechnology, vol. 27, no. 8, pp. 735–741, 2009.
[5] A. Jovic, B. Howell, and S. Takayama, “Timing is everything: using fluidics to understand the role of temporal dynamics in cellular systems,” Microfluidics and Nanofluidics, vol. 6, no. 6, pp. 717–729, 2009.
[6] O. Lipan and W. H. Wong, “The use of oscillatory signals in the study of genetic networks,” Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 20, pp. 7063–7068, 2005.
[7] S. Paliwal, J. Wang, and A. Levchenko, “Pulsing cells: how fast is too fast?” HFSP Journal, vol. 2, no. 5, pp. 251–256, 2008.
[8] G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang, and D. E. Ingber, “Soft lithography in biology and biochemistry,” Annual Review of Biomedical Engineering, vol. 3, pp. 335–373, 2001.
[9] M. R. Bennett and J. Hasty, “Microfluidic devices for measuring gene network dynamics in single cells,” Nature Reviews Genetics, vol. 10, no. 9, pp. 628–638, 2009.
[10] J. T. Mettetal, D. Muzzey, C. Gómez-Uribe, and A. van Oudenaarden, “The frequency dependence of osmo-adaptation in Saccharomyces cerevisiae,” Science, vol. 319, no. 5862, pp. 482–484, 2008.
[11] P. Hersen, M. N. McClean, L. Mahadevan, and S. Ramanathan, “Signal processing by the HOG MAP kinase pathway,” Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 20, pp. 7165–7170, 2008.
[12] E. Eriksson, J. Enger, B. Nordlander et al., “A microfluidic system in combination with optical tweezers for analyzing rapid and reversible cytological alterations in single cells upon environmental changes,” Lab on a Chip, vol. 7, no. 1, pp. 71–76, 2007.
[13] G. Charvin, F. R. Cross, and E. D. Siggia, “A microfluidic device for temporally controlled gene expression and long-term fluorescent imaging in unperturbed dividing yeast cells,” PLoS One, vol. 3, no. 1, Article ID e1468, 2008.
[14] R. J. Taylor, D. Falconnet, A. Niemistö et al., “Dynamic analysis of MAPK signaling using a high-throughput microfluidic
single-cell imaging platform,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 10, pp. 3758–3763, 2009.

[15] Z. Zi, W. Liebermeister, and E. Klipp, “A quantitative study of the Hog1 MAPK Response to fluctuating osmotic stress in *Saccharomyces cerevisiae*,” *PLoS One*, vol. 5, no. 3, Article ID e9522, 2010.

[16] N. Hao, M. Behar, S. C. Parnell et al., “A systems-biology analysis of feedback inhibition in the Sho1 osmotic-stress-response pathway,” *Current Biology*, vol. 17, no. 8, pp. 659–667, 2007.

[17] M. N. McLean, P. Hersen, and S. Ramanathan, “In vivo measurement of signaling cascade dynamics,” *Cell Cycle*, vol. 8, no. 3, pp. 373–376, 2009.

[18] D. Muzzey, C. A. Gómez-Uribe, J. T. Mettetal, and A. van Oudenaarden, “A systems-level analysis of perfect adaptation in yeast osmoregulation,” *Cell*, vol. 138, no. 1, pp. 160–171, 2009.

[19] C. Widmann, S. Gibson, M. B. Jarpe, and G. L. Johnson, “Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human,” *Physiological Reviews*, vol. 79, no. 1, pp. 143–180, 1999.

[20] D. Sheikh-Hamad and M. C. Gustin, “MAP kinases and the adaptive response to hypertonicity: functional preservation from yeast to mammals,” *American Journal of Physiology*, vol. 287, no. 6, pp. F1102–F1110, 2004.

[21] R. E. Chen and J. Thorner, “Function and regulation in MAPK signaling pathways: lessons learned from the yeast *Saccharomyces cerevisiae*,” *Biochimica et Biophysica Acta*, vol. 1775, no. 8, pp. 1311–1340, 2007.

[22] M. C. Gustin, J. Albertyn, M. Alexander, and K. Davenport, “Map kinase pathways in the yeast *Saccharomyces cerevisiae*,” *Microbiology and Molecular Biology Reviews*, vol. 62, no. 4, pp. 1264–1300, 1998.

[23] S. Hohmann, “Osmotic stress signaling and osmoadaptation in yeasts,” *Microbiology and Molecular Biology Reviews*, vol. 66, no. 2, pp. 300–372, 2002.

[24] J. L. Brewster, T. De Valoir, N. D. Dwyer, E. Winter, and M. C. Gustin, “An osmosensing signal transduction pathway in yeast,” *Science*, vol. 259, no. 5102, pp. 1760–1763, 1999.

[25] S. M. O’Rourke, I. Herskovitz, and E. K. O’Shea, “Yeast go the whole HOG for the hyperosmotic response,” *Trends in Genetics*, vol. 18, no. 6, pp. 405–412, 2002.

[26] P. J. Westfall, D. R. Ballon, and J. Thorner, “When the stress of your environment makes you go HOG wild,” *Science*, vol. 306, no. 5701, pp. 1511–1512, 2004.

[27] S. Hohmann, “Control of high osmolarity signalling in the yeast *Saccharomyces cerevisiae*,” *FEBS Letters*, vol. 583, no. 24, pp. 4025–4029, 2009.

[28] S. Hohmann, M. Krantz, and B. Nordlander, “Yeast osmoregulation,” *Methods in Enzymology*, vol. 428, pp. 29–45, 2007.

[29] K. R. Davenport, M. Sohaskey, Y. Kamada, D. E. Levin, and M. C. Gustin, “A second osmosensing signal transduction pathway in yeast: hypotonic shock activates the PKC1 protein kinase-regulated cell integrity pathway,” *Journal of Biological Chemistry*, vol. 270, no. 50, pp. 30157–30161, 1995.

[30] H. Martín, J. M. Rodriguez-Pachón, C. Ruiz, C. Nombela, and M. Molina, “Regulatory mechanisms for modulation of signaling through the cell integrity Slt2-mediated pathway in *Saccharomyces cerevisiae*,” *Journal of Biological Chemistry*, vol. 275, no. 2, pp. 1511–1519, 2000.

[31] J. C. Harrison, E. S. G. Bardes, Y. Ohya, and D. J. Lew, “A role for the Pkc1p/Mpk1p kinase cascade in the morphogenesis checkpoint,” *Nature Cell Biology*, vol. 3, no. 4, pp. 417–420, 2001.

[32] E. A. Elion, “Pheromone response, mating and cell biology,” *Current Opinion in Microbiology*, vol. 3, no. 6, pp. 573–581, 2000.

[33] K. Tedford, S. Kim, D. Sa, K. Stevens, and M. Tyers, “Regulation of the mating pheromone and invasive growth responses in yeast by two MAP kinase substrates,” *Current Biology*, vol. 7, no. 4, pp. 228–238, 1997.

[34] F. Banuett, “Signaling in the yeasts: an informational cascade with links to the filamentous fungi,” *Microbiology and Molecular Biology Reviews*, vol. 62, no. 2, pp. 249–274, 1998.

[35] B. Ren, F. Robert, J. J. Wyrick et al., “Genome-wide location and function of DNA binding proteins,” *Science*, vol. 290, no. 5500, pp. 2306–2309, 2000.

[36] V. Gavrias, A. Andrianopoulos, C. J. Gimeno, and W. E. Timberlake, “*Saccharomyces cerevisiae* TEC1 is required for pseudohyphal growth,” *Molecular Microbiology*, vol. 19, no. 6, pp. 1255–1263, 1996.

[37] M. Proft, A. Pascual-Ahuir, E. de Nadal, J. Arío, R. Serrano, and F. Posas, “Regulation of the Sko1 transcriptional repressor by the Hog1 MAP kinase in response to osmotic stress,” *The EMBO Journal*, vol. 20, no. 5, pp. 1123–1133, 2001.

[38] P. M. Alepuz, E. de Nadal, M. Zapater, G. Ammerer, and F. Posas, “Osmostress-induced transcription by Hot1 depends on a Hog1-mediated recruitment of the RNA Pol II,” *The EMBO Journal*, vol. 22, no. 10, pp. 2433–2442, 2003.

[39] P. Shore and A. D. Sharrock, “The MADS-box family of transcription factors,” *European Journal of Biochemistry*, vol. 229, no. 1, pp. 1–13, 1995.

[40] K. Madden, Y. J. Sheu, K. Baetz, B. Andrews, and M. Snyder, “SBF cell cycle regulator as a target of the yeast PKC-MAP kinase pathway,” *Science*, vol. 275, no. 5307, pp. 1781–1784, 1997.

[41] K. Doi, A. Gartner, G. Ammerer et al., “MSG5, a novel protein phosphatase promotes adaptation to pheromone response in *S. cerevisiae*,” *The EMBO Journal*, vol. 13, no. 1, pp. 61–70, 1994.

[42] X. L. Zhan, R. J. Deschenes, and K. L. Guan, “Differential regulation of Fus3 map kinase by tyrosine-specific phosphatases PTP2/PTP3 and dual-specificity phosphatase MSG5 in *Saccharomyces cerevisiae*,” *Genes & Development*, vol. 11, no. 13, pp. 1690–1702, 1997.

[43] C. P. Mattison and I. M. Ota, “Two protein tyrosine phosphatases, Ptp2 and Ptp3, modulate the subcellular localization of the Hog1 MAP kinase in yeast,” *Genes & Development*, vol. 14, no. 10, pp. 1229–1235, 2000.

[44] J. Warnka, J. Hanneman, J. Lee, D. Amin, and I. Ota, “Ptc1, a type 2C Ser/Thr phosphatase, inactivates the HOG pathway by dephosphorylating the mitogen-activated protein kinase Hog1,” *Molecular and Cellular Biology*, vol. 21, no. 1, pp. 51–60, 2001.

[45] T. Maeda, A. Y. M. Tsai, and H. Saito, “Mutations in a protein tyrosine phosphatase gene (PTP2) and a protein serine/threonine phosphatase gene (PTC1) cause a synthetic growth defect in *Saccharomyces cerevisiae*,” *Molecular and Cellular Biology*, vol. 13, no. 9, pp. 5408–5417, 1993.

[46] H. Martin, M. Flández, C. Nombela, and M. Molina, “Protein phosphatases in MAPK signalling: we keep learning from yeast,” *Molecular Microbiology*, vol. 58, no. 1, pp. 6–16, 2005.

[47] E. de Nadal, P. M. Alepuz, and F. Posas, “Dealing with osmostress through MAP kinase activation,” *EMBO Reports*, vol. 3, no. 8, pp. 735–740, 2002.
A. Zarrinpar, R. P. Bhattacharyya, M. P. Nittler, and W. A. Lim, M. Krantz, D. Ahmadpour, L. G. Ottosson et al., "Robustness S. J. Mansour, K. A. Resing, J. M. Candi et al., "Mitogen- T. Y abe, T. Y amada-Okabe, S. Kasahara et al., "HKR1 encodes T. K a tebaya s h i , K . Ta n a k a , H. Y. Ya n g et a l . , " Tr a n s m em br a n e K. Ta tebaya shi, K. T a n a k a , H. Y. Ya n g et a l . , " Tr a n s m em br a n e T. Maeda, M. T akekawa, and H. Saito, " Activation of yeast V. Reiser, D. C. Raitt, and H. Saito, "Y east osmosensor Sln1 and F. P osa s , S. M . W urg l e r - M u r p h y , T . M a e d a , E . A . W i t t e n , T . T. Maeda, S. M. Wurgler-Murphy, and H. Saito, " A two- H. Saito and K. T atebayashi, "Regulation of the osmoregula- H. D iha zi , R . K e ss l e r , a n d K . E s c h r i c h , " H i g ho s m o l a r i t y M. Rep, M. Proft, F. Remize et al., "The E. de Nadal, L. Casadome, and F. Posas, "Targeting the MEF2-
is essential for growth under osmotic stress in *Saccharomyces cerevisiae*, and its expression is regulated by the high-osmolarity glycerol response pathway, "Molecular and Cellular Biology", vol. 14, no. 6, pp. 4135–4144, 1994.

[80] C. Ferreira, F. Van Voorst, A. Martins et al., "A member of the sugar transporter family, Stl1p is the glycerol/H+ symporter in *Saccharomyces cerevisiae*," Molecular Biology of the Cell, vol. 16, no. 4, pp. 2068–2076, 2005.

[81] E. Klipp, B. Nordlander, R. Krüger, P. Gennemark, and S. Hohmann, "Integrative model of the response of yeast to osmotic shock, " Nature Biotechnology, vol. 23, no. 8, pp. 975–982, 2005.

[82] P. Gennemark, B. Nordlander, S. Hohmann, and D. Wedelin, "A simple mathematical model of adaptation to high osmolarity in yeast," In Silico Biology, vol. 6, no. 3, pp. 193–214, 2006.

[83] S. Karlsgren, N. Pettersson, B. Nordlander et al., "Conditional osmotic stress in yeast: a system to study transport through aquaglyceroporins and osmostress signaling," Journal of Biological Chemistry, vol. 280, no. 8, pp. 7186–7193, 2005.

[84] P. Wilding, J. Pfahler, H. H. Bau, J. N. Zemel, and L. J. Kricka, "Manipulation and flow of biological fluids in straight channels micromachined in silicon," Clinical Chemistry, vol. 40, no. 1, pp. 43–47, 1994.

[85] Y. Xia and G. M. Whitesides, "Soft lithography," Annual Review of Materials Science, vol. 28, no. 1, pp. 153–184, 1998.

[86] A. Lafong, W. J. Hossack, J. Arlt, T. J. Nowakowski, and N. D. Read, "Time-Multiplexed Laguerre-Gaussian holographic optical tweezers for biological applications," Optics Express, vol. 14, no. 7, pp. 3065–3072, 2006.

[87] G. Charvin, F. R. Cross, and E. D. Siggia, "Forced periodic expression of G cyclins phase-locks the budding yeast cell cycle," Proceedings of the National Academy of Sciences of the United States of America, vol. 106, no. 16, pp. 6632–6637, 2009.

[88] M. R. Bennett, W. L. Pang, N. A. Ostroff et al., "Metabolic gene regulation in a dynamically changing environment," Nature, vol. 454, no. 7208, pp. 1119–1122, 2008.

[89] P. J. Westfall, J. C. Patterson, R. E. Chen, and J. Thorner, "Stress resistance and signal fidelity independent of nuclear MAPK function," Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 34, pp. 12212–12217, 2008.

[90] T. M. Yi, Y. Huang, M. I. Simon, and J. Doyle, "Robust perfect adaptation in bacterial chemotaxis through integral feedback control," Proceedings of the National Academy of Sciences of the United States of America, vol. 97, no. 9, pp. 4649–4653, 2000.

[91] R. Alonso-Monge, E. Real, I. Wojda, J. P. Bebelman, W. H. Mager, and M. Siderius, "Hyperosmotic stress response and regulation of cell wall integrity in Saccharomyces cerevisiae share common functional aspects," Molecular Microbiology, vol. 41, no. 3, pp. 717–730, 2001.

[92] R. García, J. M. Rodríguez-Peña, C. Bermejo, C. Nombela, and J. Arroyo, "The high osmotic response and cell wall integrity pathways cooperate to regulate transcriptional responses to zymolase-induced cell wall stress in *Saccharomyces cerevisiae*," Journal of Biological Chemistry, vol. 284, no. 16, pp. 10901–10911, 2009.

[93] J. L. Brown, H. Bussey, and R. C. Stewart, "Yeast Skn7p functions in a eukaryotic two-component regulatory pathway," The EMBO Journal, vol. 13, no. 21, pp. 5186–5194, 1994.

[94] J. L. Brown, S. North, and H. Bussey, "SKN7, a yeast multicopy suppressor of a mutation affecting cell wall β-glucan assembly, encodes a product with domains homologous to prokaryotic two-component regulators and to heat shock transcription factors," Journal of Bacteriology, vol. 175, no. 21, pp. 6908–6915, 1993.
Submit your manuscripts at http://www.hindawi.com