

# Control of the cell cycle progression by the MAPK Hog1

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## Abstract

Eukaryotic cells coordinate various intracellular activities in response to environmental stresses, activating an adaptive program to maximize the probability of survival and proliferation. Cells transduce diverse cellular stimuli by multiple mitogen-activated protein kinase (MAPK) cascades. MAPK are key signal transduction kinases required to respond to stress. A prototypical member of the MAPK family is the yeast high osmolarity glycerol (Hog1). Activation of Hog1 results in the generation of a set of adaptive responses that leads to the modulation of several aspects of cell physiology that are essential for cell survival, such as gene expression, translation, and morphogenesis. This review focuses on the control of cell cycle progression by Hog1 which is critical for cell survival in response to stress conditions.

## The high osmolarity glycerol pathway

Cells are constantly exposed to stress situations such as changes in temperature, pH, radiation, availability of nutrients, access to oxygen and changes in osmotic pressure. Cells are able to coordinate intracellular activities in order to respond to such stresses. Mitogen-activated protein kinases (MAPKs) are a conserved protein family that sense and respond to extracellular environmental changes. MAPK Activation leads to the generation of a set of adaptive responses that involves the modulation of several physiological processes such as changes in gene transcription, protein regulation and control of cell cycle progression, which allow cells to adapt to the new environmental conditions.<sup>1,2</sup> When cells are subjected to hyperosmotic shock, they lose water in a passive diffusion process and a specific MAPK pathway is activated: the high osmolarity glycerol (HOG) pathway. To fight against the loss of water, cells have developed a battery of

mechanisms and the HOG pathway develops a central function in this process.<sup>3-5</sup>

Activation of the HOG pathway elicits a platform for cell osmoadaptation, which consists of the regulation at different levels of the transcription and translation of different genes allowing long-term adjustment (extensively reviewed in Saito and Posas).<sup>6</sup> Moreover, HOG activation also produces a short-term adaptation effect, such as glycerol accumulation or the reestablishment of ionic balance.<sup>7-9</sup>

The MAPK pathways are extremely conserved among eukaryotes and are composed of a tier of three consecutive levels of activated kinases. In the HOG pathway, these levels are composed of a layer of three MAPKKs (Ssk2, Ssk22, and Ste11) which are responsible for activating a unique MAPKK (Pbs2). After Pbs2 activation, Pbs2 phosphorylates and activates the Hog1 MAPK,<sup>10</sup> which is a homologue of the human p38 and c-Jun N-terminal. Once phosphorylated, the Hog1 is concentrated in the nucleus, where it can phosphorylate its protein targets. However, a portion of activated Hog1 is retained in the cytoplasm to regulate other cytosolic events.<sup>11</sup>

In this review, we present the latest studies on how Hog1 regulates the cell cycle progression, which is essential for cell survival preserving genomic integrity and cell viability in budding yeast.

## Regulation of the yeast cell cycle

The cell cycle, whereby one initial cell divides finally into two cells, basically consists of four phases: G1 phase (for Gap1, because apparently nothing happens, but in fact cells grow in volume and decide to divide), S phase (when DNA is synthesized), G2 phase (for Gap2, when cells continue growing), and M phase (for mitosis, when cells finally divide). After the M phase, cells again enter G1, therefore finishing a cycle.

In budding yeast, *Saccharomyces cerevisiae*, a highly regulated and complex network of proteins governs this process. Nevertheless, a single essential cyclin dependent kinase (CDK), Cdc28 (the functional homologue of Cdk1 in higher eukaryotes) controls cell cycle progression whose regulation is achieved mainly through the synthesis and degradation of cyclins and inhibitors, conferring its substrate specificity.<sup>12-14</sup>

At the beginning of the cell cycle, the nuclear concentration of Cln3 increases in relationship to the total cell mass<sup>15-18</sup> promoting the phosphorylation of Whi5 (an ortholog of Rb). This event allows the activation of the transcription factors SBF (a heterodimer of Swi4 and Swi6) and MBF (a heterodimer of

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Mbpl and Swi6) inducing the transcription of a second wave of cyclins (*CLN1*, *CLN2*, *CLB5*, and *CLB6*). The activity of Cln1,2/Cdc28 stimulates bud formation however Clb5,6/Cdc28 remains inhibited by the presence of Sic1. When several residues in Sic1 are phosphorylated by Cln1,2/Cdc28, this leads to Sic1 recognition and ubiquitination by the SCF/Cdc4 complex;<sup>19</sup> and consequently targeted Sic1 destruction. When Sic1 is degraded, Clb5,6/Cdc28 activity rises, phosphorylating Sic1 at the same residues as those phosphorylated by Cln1,2/Cdc28. Thus, Sic1 degradation accelerates due to the positive feedback loop, resulting in an abrupt rise in Clb5,6/Cdc28 activity, which drives cells to S phase.

In *S. cerevisiae*, S phase is triggered fundamentally by two kinases, Clb5,6-Cdc28 and Dbf4-Cdc7, which phosphorylate specific proteins at the replication origins. DNA synthesis is instigated when Clb5,6/Cdc28 phosphorylates the replication proteins Sld2 and Sld3, which are components of the pre-initiation complex.<sup>20,21</sup> DNA synthesis begins from multiple origins that are distributed throughout the genome following a strict temporal program;<sup>22</sup> and the assembly of the protein complexes on the replication origins is a very fine-tuned process. Initially, Cdt1, Cdc6, MCMs and proteins form the pre-replication complexes. During G1, Cdc45, Sld2, Sld3, Dpb11 and a novel replication complex GINS (Go, Ichi, Nii, and San; five, one, two, and three in Japanese), are

loaded to constitute the pre-initiation complex. At this stage, the origins are already licensed to be activated by Clb5,6-Cdc28 and Dbf4-Cdc7. Those complexes phosphorylate specific target proteins on the pre-initiation complex to induce full activation of the replicative helicases and polymerases to start DNA replication from every single origin. Cell-cycle progression to the G2 phase mainly depends on another wave of cyclin production: the mitotic cyclins Clb1 and Clb2. The Mcm1/SFF complex is the transcription factor that regulates expression of *CLB1* and *CLB2*.<sup>23-25</sup> Entry into mitosis is driven mainly by the activity of Clb2-Cdc28, which is tightly regulated by Swe1 (the ortholog of higher eukaryotes Wee1). Swe1 controls the Clb1,2/Cdc28 activity by direct phosphorylation at a conserved tyrosine in Cdc28, which is reactivated by the phosphatase Mih1 (the ortholog of Cdc25). Swe1 degradation basically depends on two independent mechanisms; the phosphorylation by Clb2-Cdc28;<sup>26,27</sup> and its degradation by the Hsl1, Cdc5, Elm1 and Dma1.<sup>25,28-30</sup> When the septin ring is completed, Hsl1 is recruited. When bound to septins, Hsl1 tethers the adaptor protein Hsl7 to the bud neck, which is in turn required for Swe1 recruitment and is then targeted by Cdc5 leading to Swe1 destruction and release of Clb2-Cdc28 inhibition.<sup>31,32</sup> During vegetative growth, Swe1 does not appear to affect cell-cycle progression.<sup>33</sup> However, when bud formation is impaired, Swe1 remains active, inactivates the Clb1,2/Cdc28, and delays the cell cycle progression.<sup>34</sup> Thus, the tight regulation of Swe1 phosphorylation and its subsequent degradation is critical for the cell cycle progression in G2 phase.

Exit from mitosis after chromosome segregation is controlled by a signaling cascade termed the mitotic exit network (MEN). When all chromosomes are aligned, Cdc20/anaphase promoting complex (APC) is activated;<sup>35</sup> promoting the degradation of Clb2 protein.<sup>36</sup> This event represents the exit from mitosis and the start of a new cycle. MEN activation is initiated by the activation of Tem1, a G-protein.<sup>37</sup> When cells undergo anaphase, the spindle pole body enters the daughter cell where the Lte1 is located, promoting the activation of Tem1.<sup>38</sup> Tem1 activates the Cdc15 kinase, a critical component of MEN, which in turn activates the Cdc14 phosphatase. Cdc14 is tightly regulated by a competitive inhibitor, Net1, which holds Cdc14 in an inactive state during most of the cell cycle except during anaphase and telophase phases. Cdc14 is released by MEN or by the fourteen early anaphase release (FEAR) network, promoting exit from mitosis. Cdc14 activates the APC/Cdh1, which ubiquitinates and degrades the remaining Clb1 and Clb2. Cdc14 also directly dephosphorylates Clb/Cdc28 substrates such as Sic1;<sup>39</sup> which promotes Sic1 stabilization and consequently Clb/Cdc28 inhibition.

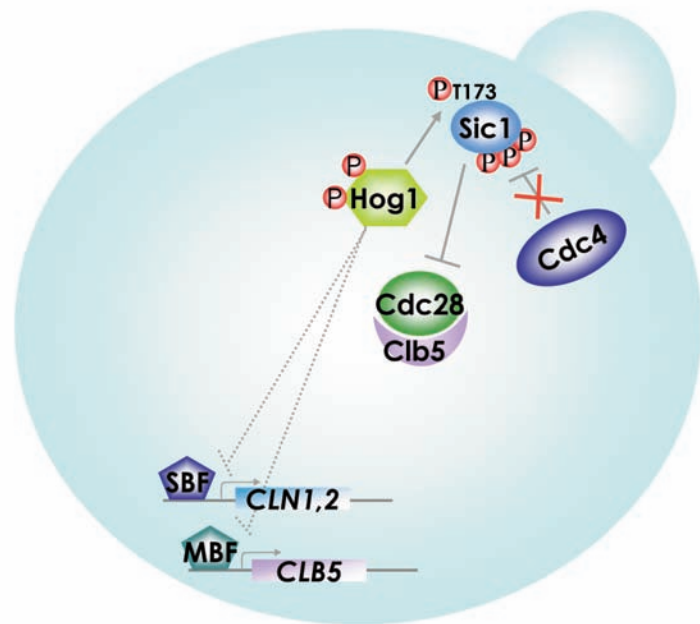
## Control of the G1/S transition by Hog1

When yeast cells are exposed to high osmolarity, the Hog1 MAPK is transiently activated and a corresponding cell-cycle delay in G1 is produced by regulating different levels of the basic cell-cycle machinery.<sup>15,40-43</sup> It can be demonstrated that this delay is caused by activated Hog1, and not by other effects of osmolarity, by using genetic means to activate Hog1 in the absence of osmolarity. In contrast, when Hog1 activation is sustained for a long time (by activation of the pathway using different alleles for *Sln1*, *Pbs2* or *Ssk2*), cells undergo a programmed cell death that requires the action of the proteinase Nma111 and SCF/CDC4.<sup>44</sup>

First, Hog1-mediated G1 arrest is partially mediated by down-regulation of the expression of the G1 cyclins *CLN1*, *CLN2* and of the S-cyclin *CLB5*<sup>15</sup> (Figure 1). The exact nature of the mechanism that represses the expression of SBF- and MBF-dependent genes under osmotic stress is still unknown. It is well described that *Clb1,2/Cdc28* activities are necessary to reach a threshold of Sic1 phosphorylation, with the subsequent ubiquitination by the SCF/Cdc4, and later degradation by the proteasome. Therefore, the down-regulation of *CLN1* and *CLN2* production might, at least in part, explain the delay in S-phase entry as a consequence of an increased accumulation of Sic1. The precise mechanism that represses the expression of the SBF- and MBF-dependent

genes under osmotic stress is unclear. One possibility might be that Hog1 was directly (or, by other downstream kinases, indirectly) inhibiting the activity of the transcription factors Swi4/Swi6 by phosphorylation. Another possibility could be that Hog1 directly phosphorylates Whi5, the ortholog of mammalian retinoblastoma (Rb). In any case, the exact mechanism by which Hog1 is able to repress G1 cyclin expression remains to be explored.

Second, Hog1 arrests cells in G1 by an alternative mechanism: the direct phosphorylation of Sic1.<sup>40</sup> When Hog1 is activated, it interacts with Sic1 and phosphorylates the CDK inhibitor into a single residue (T173) at the carboxyl-terminal region of Sic1. This phosphorylation somehow interferes with the binding of Sic1 with the SCF/Cdc4, inhibiting Sic1 ubiquitination and consequently its degradation.<sup>40,45,46</sup> (Figure 1). Thus, Sic1 is stabilized, which implies an inhibition of Clb5,6/Cdc28 delaying the G1/S transition. The stabilization of Sic1 and the consequent Hog1-dependent G1 arrest is essential for the adaptive response to osmolarity since cells lacking Sic1 or carrying a non-phosphorylatable allele of Sic1 (T173A) display reduced viability in high osmolarity as a result of genomic instability.<sup>40</sup> Therefore, the complex and strict Hog1 control over the G1/S network clearly illustrates the need for cell adaptation to osmolarity prior to progressing to S phase. Mathematical modelling supported by quantitative *in vivo* experiments allowed defining and quantifying the temporal role and the direct contribution of the individual components of the G1/S transition



**Figure 1. Schematic diagram of the control of the cell cycle progression by Hog1 at the different phases of the cell cycle.**

controlled by Hog1.<sup>43</sup> Hog1-induced inhibition of the transcription of the gene encoding cyclin Clb5, rather than that of the gene encoding Cln2, prevented entry into S phase upon osmostress. By controlling the accumulation of specific cyclins, Hog1 delayed bud morphogenesis (through Clns) and delayed DNA replication (through Clb5). Hog1-mediated phosphorylation and degradation of Sic1 at Start prevented residual activity of the cyclin/CDK complex Clb5/Cdc28 from initiating DNA replication before adaptation to the stress.<sup>43</sup>

The triple targeting of *CLN1/2*, *CLB5* and Sic1 ensures a G1 transient arrest at any stage of G1 in response to osmostress. In this way, Hog1 inhibits cyclin expression in cells in early G1 phase, and later in G1, Hog1 inhibits Sic1 degradation. Of the three cyclin genes whose transcription is inhibited by Hog1, inhibition of *CLN1,2* expression delays bud morphogenesis and spindle pole body duplication, and inhibition of *CLB5* expression delays DNA replication and consequently entry into S phase. Later in G1, when these cyclins are already expressed, inhibition of cyclin expression can no longer prevent cell-cycle progression. Instead, Hog1-mediated phosphorylation and inhibition of the degradation of Sic1 prevents active Clb5/Cdc28 from initiating DNA replication. Therefore, Hog1 activation managed to control the basic processes that occur in G1 allowing replication only when cells were adapted to the new environmental conditions. Thus, these distinct mechanisms that operate at different time points ensure that no premature entry into S phase occurs under osmostress conditions. With regard to G1 entry, a role of Hog1 modulating re-entry into cell cycle from a resting state situation as G0 phase;<sup>47</sup> it has recently been described. Hog1 deficient cells show a delay in entering the mitotic cell cycle from the stationary phase. Moreover, when cells enter the mitotic cell cycle after being in the stationary phase, Hog1 is rapidly activated and concentrates in the nucleus where it modifies the expression of several genes. After discarding that these effects were not caused by an osmotic process, what the stimulus is that activates Hog1 at the entrance to cell cycle remains to be elucidated. Hog1 activation in this process may be by sensing the presence of glucose, nitrogen or phosphate. On the other hand, what are the targets of Hog1 to facilitate re-entry cycle? One possible target would be Rim15, which is a key element in establishing G0 phase. Nevertheless, further experiments are needed to prove this hypothesis.

## Regulation of the S phase by high osmolarity glycerol 1

The Hog1 SAPK is not only important for regulating the G1/S transition but it also plays

a crucial role once the cells are already in S-phase in delaying DNA replication in response to osmostress.<sup>48</sup> During S phase, the genome replicates, which is a highly ordered process involving many proteins. Cells have evolved a specific S-phase checkpoint to cope with multiple genotoxic agents that endanger the proper progression and completion of DNA replication. The S-phase checkpoint is mediated by Rad53 which safeguards DNA replication and preserves genomic integrity. In the presence of DNA damage or replication stresses, the Rad53-dependent checkpoint pathway delays S phase avoiding late origin firing.<sup>49-52</sup> Strikingly, Hog1-dependent arrest in S phase upon osmostress is independent of the known Rad53-dependent checkpoint pathway, suggesting that there must be a novel S-phase checkpoint pathway that delays DNA replication in the absence of DNA damage or replication stress.<sup>48</sup>

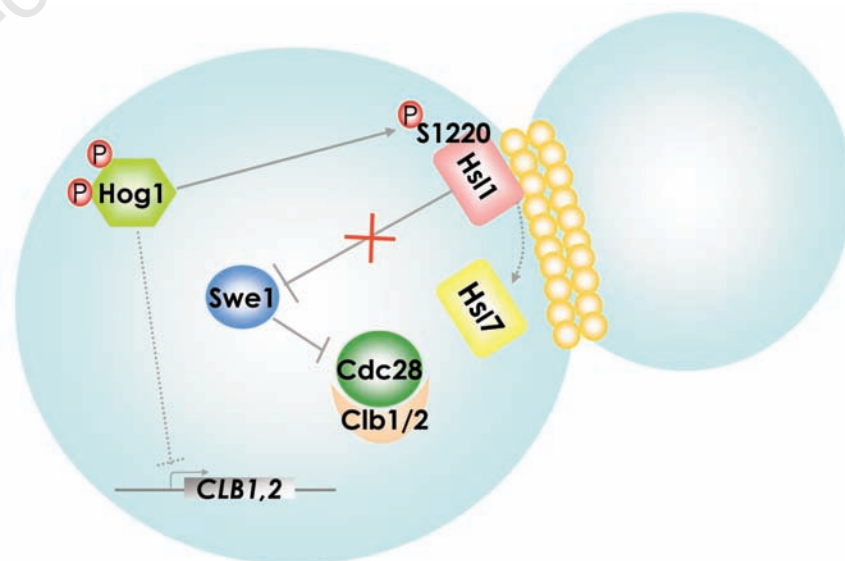
When cells are stressed in early S phase, Hog1 controls the S phase progression by delaying the expression of the S-phase cyclins *CLB5*. If cells are stressed later in S phase, Hog1 physically interacts with several components of the replication complex, as well as delaying phosphorylation of the Dpb2 subunit of the DNA polymerase.<sup>48</sup> Although the molecular mechanism by which Hog1 delays DNA replication remains unclear, this function is clearly independent of the SAPK cell cycle direct targets Sic1 and Hsl1 and the S-phase DNA Rad53 checkpoint.

In response to osmostress, Hog1 orchestrates a fast and transient activation of transcription of hundreds of stress-responsive

genes essential for adaptation to stress.<sup>2</sup> Adaptive responses to osmostress require the induction of the expression of a very large number of genes. It is therefore conceivable that initiating or ongoing replication might occur on the genes that are being transcribed for adaptation. It is easy to see that if the large replication complex and the transcription complex attempted to occupy the same space, they would interfere with each other's function. In fact, it has been shown that collision between RNA Pol II and DNA polymerase induces transcription-associated recombination.<sup>53-55</sup> Thus, delaying replication in response to osmostress must be important both to provide proper adaptive gene expression and to prevent genomic instability.

## Regulation of G2 phase by high osmolarity glycerol 1

Activation of Hog1 induces a transient cell-cycle delay in G2 progression by the decrease in Clb2/Cdc28 activity as a consequence of indirect Swe1 accumulation and the down-regulation of *CLB2* transcription<sup>56,57</sup> (Figure 2). The mechanism required to decrease Clb2/Cdc28 activity is well understood: Hog1 acts on the machinery of the morphogenetic checkpoint that controls the CDK inhibitor levels of Swe1. Activated Hog1 interacts and directly phosphorylates Hsl1 in a single residue (S1220) within the Hsl7-docking site, which promotes the delocalization of Hsl7 from the bud neck that results in Swe1 accumula-



**Figure 2. Schematic diagram of the control of the cell cycle progression by Hog1 at the G2 phase.**



tion.<sup>57</sup> Upon Hog1 activation, cells containing a non-phosphorylatable allele of Hsl1 (S1220A) are unable to promote Hsl7 delocalization. These mutants cannot accumulate enough Swe1 inhibitor and fail to arrest in G2 and render cells osmosensitive as a consequence of premature progress into M phase. Interestingly, whereas the morphogenetic checkpoint monitors the absence of a septin ring, which implies the delocalization of Hsl1, Hsl7, and Swe1 at the bud neck, Hog1 activation specifically delocalizes Hsl7, and consequently Swe1, without affecting the Hsl1 or the subcellular localization of septins.<sup>57</sup> Thus, the tight regulation of Swe1 localization, phosphorylation and its subsequent degradation are critical for the timely activation of the Clb2/Cdc28 complex and ensure the proper assembly of the septin ring throughout G2 progression. In contrast, the mechanisms that Hog1 employs to down-regulate *CLB2* transcription levels are not known, but this could be a secondary effect based on the decrease in Cdc28/Clb2 activity. Furthermore, the combined absence of the CDK inhibitors *SIC1* and *SWE1* results in a synergistic phenotype of osmosensitivity;<sup>57</sup> highlighting the relevance of the cell cycle control by Hog1 at different stages of the cell cycle. In any case, Hog1 activation affects not only the activity of Cdc28/Clb2 but also the Cln2 levels by a down-regulation of *CLB2* transcription<sup>56,57</sup> (Figure

2). The mechanism that leads to the repression of *CLB2* is unknown, but it is tempting to speculate that the transcriptional activators and Fkh1 Fkh2 will be involved. Moreover, it is necessary to study the possible effect of Hog1 on the Cdc28 complexes with the cyclins Clb3 Clb4.

## Exit from mitosis

Exit from mitosis could also be regulated by the Hog1 MAPK under osmotic stress. In response to osmotic stress, MEN mutants exit from mitosis in a manner that is dependent on Hog1. In such MEN mutants, the HOG pathway seems to regulate exit from mitosis by promoting the function of the FEAR network that activates Cdc14, although the exact mechanism remains unclear<sup>58</sup> (Figure 3). What is well demonstrated, in a situation of osmotic stress, is that M phase synchronized cells circulate more rapidly during anaphase to telophase transition (our unpublished observations, 2005). This suggests that Hog1 activation at this point of the cell cycle could be sped up by M. (which is contrary to the observed transitions of G1, S and G2). Nevertheless, it will be necessary to perform further experiments to find the possible target/s of Hog1 at this point.

Another important area to explore the role of

Hog1 is what happens at the end of the cell cycle, at cytokinesis, which is a point in the cell cycle when cells are especially sensitive to osmotic stress. In our opinion, after an osmotic shock, Hog1 must avoid premature entry into cytokinesis. Nevertheless, again further experiments are needed to validate these speculations.

## Conclusions

The evidence presented in this review presents Hog1 as a guardian to protect the cell from external stresses at any stage of the cycle in which it is found. Indeed, several well-known protein targets of Hog1 covering the entire cell cycle (Figure 3). Clearly the mitosis phase remains to be explored, but it is tempting to speculate that Hog1 must be regulating some processes at such a critical moment for cell viability.

In any case, in the cell cycle phases in which Hog1 acts as a main regulator, the MAP kinase blocks the cell cycle progression with a delay of 20-30 minutes, which is the time required to activate various metabolic enzymes, such as Pfk2;<sup>59</sup> Gpd1<sup>60</sup> or Gph1;<sup>61</sup> which leads to an accumulation of glycerol in the cell.

Nevertheless, this evidence gives rise to other important questions:

After cells are acclimatized to the new environmental conditions and have therefore increased the inner levels of glycerol, which are the mechanisms that can revoke the Hog1 delay? In other words, what are the different protein phosphatases that dephosphorylate the Hog1 targets, and consequently allow cells to continue in the cell cycle progression?

The idea that osmotic stress causes a cycle arrest to facilitate adaptation is transportable to other stresses: desiccation, oxidative, heat, etc. In *S. cerevisiae*, it seems that Hog1 only responds to osmotic stress, but in *S. pombe* and in mammalian cells the Hog1 orthologs are activated by oxidative stress also indicating that different stimuli lead to the same protective effects.

Are these mechanisms evolutionarily preserved? It is legitimate to speculate that these processes are indispensable, and consequently, different organisms, from yeast to human, must respond in a similar manner to these injuries.

In fact, it has been reported that the mammal-Hog1-ortholog p38 phosphorylates the CDK inhibitor p21<sup>62</sup> and down-regulates the cyclin D1 expression<sup>63</sup> which implies a clear parallelism with what is happening in yeast.

In which situations may be relevant this mechanism? i) yeast cells live on grape skin, consequently yeast cells are submitted to continuously changing conditions of dedication

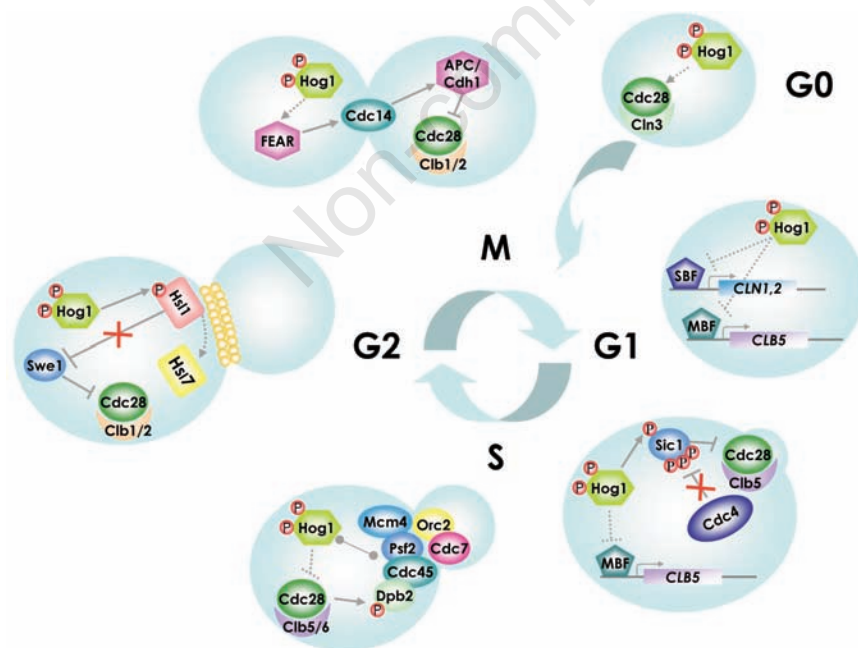


Figure 3. Schematic diagram of the control of the cell cycle progression by Hog1 at the different phases of the cell cycle.

and humidity gain; ii) another situation is when the physiological grape breaks, releasing the juice which implies that yeast cells suffer an osmotic shock.

Are these mechanisms of osmoadaptation in mammals really relevant? Regarding mammals, their cells are constantly subjected to osmotic changes. Perhaps the most obvious occurs in the lung epithelium cells (which can be found in the sudden humidity changes) or cells from the lining of the bladder where it has been shown that p38 is activated and stops the G2 cycle. In addition, in a very recent study, osmotic stress has been reported to promote the phosphorylation of the CDK inhibitor p57 by p38, facilitating the inhibitor accumulation and, consequently, delaying the G1 transition.<sup>64</sup>

In summary, we can conclude that proper adaptation to osmotic stress seems to be a prerequisite for advancing to the following cell cycle phase, and thus, it seems rational to think that the same MAPK, Hog1, is able to control the progression for the different cell cycle phases. This regulation might be critical to certify that cells at any stage of the cell cycle are competent to progress to the next phase with the appropriate adaptive responses.

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