The regulation of Net1/Cdc14 by the Hog1 MAPK upon osmostress unravels a new mechanism regulating mitosis

Javier Jiménez 1,2, Ethel Queralt 3, Francesc Posas 1,4, and Eulàlia de Nadal 1,4

1 Departament De Ciències Experimentalis I De La Salut, Universitat Pompeu Fabra (UPF), Barcelona, Spain; 2 Department of Ciències Bàsiques, Facultat De Medicina I Ciències De La Salut, Universitat Internacional De Catalunya, Barcelona, Spain; 3 Cell Cycle Group, Institut d’Investigacions Biomèdica De Bellvitge (IDIBELL), L’Hospitalet De Llobregat, Barcelona, Spain; 4 Institute for Research in Biomedicine (IRB Barcelona), the Barcelona Institute of Science and Technology, 08028 Barcelona, Spain

ABSTRACT
During evolution, cells have developed a plethora of mechanisms to optimize survival in a changing and unpredictable environment. In this regard, they have evolved networks that include environmental sensors, signaling transduction molecules and response mechanisms. Hog1 (yeast) and p38 (mammals) stress-activated protein kinases (SAPKs) are activated upon stress and they drive a full collection of cell adaptive responses aimed to maximize survival. SAPKs are extensively used to learn about the mechanisms through which cells adapt to changing environments. In addition to regulating gene expression and metabolism, SAPKs control cell cycle progression. In this review, we will discuss the latest findings related to the SAPK-driven regulation of mitosis upon osmostress in yeast.

Introduction
The budding yeast Saccharomyces cerevisiae has been used as a model to study environmental signal transduction pathways. Yeasts have the HOG (High Osmolarity Glycerol) pathway to sense, transduce and respond to an external increase in osmolarity. The cornerstone of the HOG pathway is the stress-activated protein kinase (SAPK) Hog1 (p38 in mammalian cells), which belongs to the mitogen-activated protein kinase (MAPK) family. Upon osmostress, the HOG pathway is rapidly activated to orchestrate a full set of actions to protect cells and ensure their fitness and survival. This response involves various aspects of cell biology, ranging from gene transcription regulation and metabolism control to cell cycle progression [1,2].

Beyond cyclin-dependent kinases (CDKs) and cyclins, the main proteins involved in cell cycle progression, cells have evolved to develop regulatory mechanisms aimed at ensuring their faithful duplication and, consequently, perpetuation. Safe and accurate cell duplication has many threads, both internal and external, and adaptation to a changing environment is a remarkable one. Osmostress in yeast cells has been used as a model to study the mechanisms used by cells to protect their progeny in a changing environment [3,4]. Among these mechanisms, the transient arrest of cell cycle progression has attracted the attention of several research groups, which have unveiled several mechanisms that govern G1, S and G2 phases. Recently, the molecular mechanism responsible for regulating cell cycle progression in mitosis has been reported. In this review, we will focus on the SAPK-dependent molecular mechanisms that regulate transient arrest of the cell cycle in response to stress, with a particular emphasis on mitosis, using osmostress as a prototypical case study.

1. The SAPK stress signaling pathway
The HOG pathway of the yeast S. cerevisiae is the paradigm of a SAPK signaling pathway. One of the five MAPK cascades present in this organism, the HOG pathway is the main signaling system responsible for cellular adaptation to osmostress [5]. Upon osmostress, this pathway is rapidly and

CONTACT Eulàlia de Nadal eulalia.nadal@irbbarcelona.org; Francesc Posas francesc.posas@irbbarcelona.org

1 These authors contributed equally to this work.

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transiently activated by two upstream branches: Sln1 and Sho1 [6,7]. The MAPK core module has three MAPKKKs (Ssk2, Ssk22 and Ste11) [8–11], which activate the MAPKK component (Pbs2) by phosphorylation. Subsequently, Pbs2 phosphorylates and activates the MAPK Hog1 (Figure 1) (reviewed in [2]. Upon activation, Hog1 rapidly translocates into the nucleus [12], where it exerts some of its main functions, such as reprogramming gene expression (reviewed in [1,13] and delaying cell cycle progression (focus of this review). p38 is the mammalian SAPK ortholog of Hog1. p38 responds to an increase in extracellular osmolarity and is essential for adaptation to osmostress [14]. However, it is activated and responds to other stimuli such as cytokines, DNA damage, oxidative and heat stress [15,16]. The core structure of the p38 pathway is similar to that of HOG in yeast [17], although the activation mechanism is not totally understood. In vivo replacement of components of the HOG pathway in S. cerevisiae by their mammalian counterparts demonstrated that there is a strong functional preservation of these MAPK pathways from yeast to mammals [14,18]. It is also worth mentioning the pleiotropic function of p38, which is also pivotal in regulating differentiation, proliferation, apoptosis, cell morphology and immune response [15,19,20].

2. Regulation of G1, S and G2 phases by SAPKs upon stress

Upon activation, Hog1 rapidly and transiently migrates into the nucleus, where it phosphorylates substrates to regulate cell cycle progression. This serves to prevent cells having to deal with cell cycle progression in stress conditions where successful accomplishment cannot be assured and thus viability is compromised. All the mechanisms presented below share the same strategy: arresting the cell cycle to provide cells with time to adapt to the osmotic change and resuming it only when the osmotic imbalance has been corrected and homeostasis restored. Research over the last 15 years has revealed several molecular mechanisms that occur during the different phases of the cell cycle.

The G1-S transition in budding yeast is driven by the complex of CDK and G1 cyclins or Clns (Cln1, Cln2 and Cln3), whose expression is controlled by the Swi4/6 cell cycle box (SBF) transcription factor [21,22]. SBF is kept inactive by the transcriptional repressor Whi5, the yeast functional ortholog of human RB. This repression is alleviated by phosphorylation of Whi5 by CDK, which leads to the expression of several proteins, including Clns, which are essential for cell cycle progression through G1. Additional proteins are involved in G1-specific transcription; Stb1 and Nrm1 regulate the activity of the Mlu1 cell cycle box (MBF) [23,24], while Msa1, able to interact with Stb1 and Nrm1, is a coactivator of both SBF and MBF [25]. In addition to factors involved in the expression of the CDK activators, there is an extra layer of regulation based on the CDKI (CDK Inhibitor) Sic1 [26]. Sic1 inhibits the CDK-Clb5 complex. When the levels of Clb5 surpass a certain threshold both by the firing of the Clb5 promoter and by the degradation of Sic1 (determined by its phosphorylation by CDK-Clns), cells abruptly enter S phase [27].

Activation of Hog1 by osmostress (or by genetic means using conditional hyperactive mutant alleles of upstream components of the MAPK pathway) yields a transient arrest in G1 via the stabilization of Sic1 and the down-regulation of G1 cyclins (Figure 2a) [28]. The stabilization of Sic1 and consequent Hog1-dependent transient G1 arrest is

![Figure 1. Schematic representation of the HOG and p38 SAPK pathways.](image-url)

In mammalian cells (left panel), the sensors are unclear. In budding yeast (right panel), two independent osmosensing mechanisms, the Sln1 and Sho1 branches, converge in the MAPK module. Activation of the sensors leads to the phosphorylation of the MAPK (p38 and Hog1) by specific MAPKKK and MAPKK, triggering the osmo-adaptive response by phosphorylation of multiple substrates.
essential for the adaptive response to osmostress, and cells lacking Sic1 or carrying the non-phosphorylatable allele sic1<sup>T73A</sup> show reduced viability under high osmolarity [29,30]. Additionally, Hog1 activation represses cyclins CLN2 and CLB5 expression through Whi5 and Msa1 phosphorylation to ensure coherent passage through G1/S. The phosphorylation of these two transcriptional regulators by Hog1 is essential for inhibiting G1-cyclin expression, regulating cell morphogenesis, and ensuring maximal cell survival upon stress [31]. Recently, it has been reported that the phosphatase calcineurin prolongs Hog1 activation and the extent of cell cycle arrest upon osmostress. The crosstalk between calcineurin and the MAPK contributes to the inactivation of multiple regulatory transcription factors of the cell cycle and the down-regulation of cell cycle-regulated genes [32].

Mathematical modeling and quantitative analysis of G1 progression upon osmostress has allowed evaluation of the contribution of the different Hog1-dependent mechanisms. Whereas inhibition of CLN2 expression and Sic1 stabilization are important to prevent S phase entry in response to stress occurring close to Start, the inhibition of CLB5 expression is critical in the response to osmostress occurring at any stage of G1 [33–35].

Although the cell cycle machinery is more complex in mammals from a molecular perspective, the same principles described above in yeast govern cell cycle progression. CDK activity is regulated by two families of CDKIs: the INK and the
Cip/Kip, which include p21CIP1, p27KIP1 and p57KIP2 [36]. In response to osmooestress (and other stresses), p38 delays G1 progression by directly targeting p57KIP2, by phosphorylating its Thr143, thereby increasing its affinity toward the CDK and, as a result, reducing its activity and causing a transient G1 arrest [37,38] (Figure 2a). Moreover, p38 induces p21 mRNA stabilization, without affecting its transcription or the stability of the protein. Inhibition of p38 impairs p21 accumulation and, as a result, the ability of cells to arrest in G1 in response to gamma radiation [39]. p38-activated signaling leads to p27 stabilization [40,41]. Additionally, p38 phosphorylates the Retinoblastoma (RB; Whi5 ortholog) tumor suppressor in the N-terminal region, at Ser249 and Thr252, thereby revealing a new interaction surface between RB and E2F transcription factor. This interaction leads to an increase in RB affinity for E2F and, in turn, to a downregulation of E2F-dependent gene expression (e.g. CycA2) and reduction of cell proliferation (Figure 2a). Remarkably, the p38-dependent phosphorylation of RB is dominant over the effect of CDKs, yielding RB insensitive to CDK inactivation, which is typical of cancer cells. Moreover, a p38 phosphomimetic RB mutant blocks cyclin expression, prevents cell proliferation in cancer cell lines, and leads to reduced tumor size in a mouse xenograft model. These observations thus suggest that phosphorylated RB acts as a super-repressor that prevents cancer cell proliferation [17,42–44].

Hog1 also plays a key role in S phase by transiently delaying DNA replication in response to osmooestress [45]. S phase progression in yeast is driven mainly by CDK-Clb5/Clb6 activity, which phosphorylates substrates at the early and late replication origins. Cells dispose a specific S phase checkpoint pathway mediated by Rad53 to cope with genotoxic agents or stresses that endanger correct progression of DNA replication [46,47]. Interestingly, Hog1-dependent arrest in S phase upon osmooestress is independent of the Rad53-dependent checkpoint. Hog1 interacts with and phosphorylates Mrc1, a component of the replication complex, at the N-terminal Thr169, Ser215 and Ser229 sites [48], (Figure 2b). Mrc1 phosphorylation by Hog1 delays early and late origin firing by preventing Cdc45 loading, as well as slowing down replication-complex progression [49,50]. This mechanism is especially relevant because it allows cells to circumvent conflicts between DNA replication and transcription during a burst in transcription, which takes place as a crucial response for adaptation [51]. The N-terminal phosphorylation of Mrc1 blocks replication and prevents transcription-associated recombination (TAR) and genomic instability during stress-induced gene expression in S phase. Interestingly, cells adapt to sudden increases in transcription caused by factors other than osmooestress while replicating by the same Mrc1-dependent mechanism, although signaling and kinases other than Hog1 are involved. Thus, Mrc1 integrates multiple signals, thereby defining a general safeguard mechanism to protect genomic integrity upon transcriptional outbursts [52].

Entry into mitosis is driven by the activity of the CDK-Clb2 complex, which is negatively regulated by Swe1 to ensure that cells have the required size to accomplish cell division [53,54]. Cells remain in G2 until Swe1 is degraded by two independent mechanisms, namely phosphorylation by CDK-Clb2 [55], and degradation by the Hsl1 and Cdc5 kinases when targeted to the septin ring by Hsl7 [56]. Hog1 activation stabilizes Swe1 and down-regulates the cyclin CLB2, triggering a transient arrest in G2 phase (Figure 2c) [57–59]. Upon osmooestress, Hog1 phosphorylates the Hsl1 kinase in Thr169, which delocalizes Hsl7 from the septin ring and impairs Swe1 recruitment to the bud neck. This prevents Swe1 degradation, leading a transient G2 arrest and, thus, a delay in progression into M phase, thereby allowing adaptation to osmooestress. It should be noted that the same mechanism has been proposed in Schizosaccharomyces pombe [60], together with alternative mechanisms where the SAPK sty1 regulates cdc25, the phosphatase involved in inhibiting the mitosis repressor wee1 [61]. Similar to the fission yeast, activated p38 is required for a G2/M checkpoint involving Cdc25B and Cdc25C, which regulate the activity of the CDK-cyclin B1 complex during mitosis. Upon DNA damage, the downstream kinase MK2 phosphorylates Cdc25, which creates a docking site for 14–3–3 proteins that will retain Cdc25 in the cell cytoplasm, thus preventing Cdc2-CyclinB dephosphorylation and activation [62–64].
3. Mitosis

Mitosis ensures the accurate inheritance of genetic information. The genome, which is packed into chromosomes, is distributed between the two daughter cells during mitosis. Before mitosis, sister chromatids are tightly interlinked via the intertwining of their DNA (DNA catenation) and by specialized protein complexes called cohesins. In early mitosis, the connected sister chromatids are prepared for separation under the influence of a sophisticated regulatory system based on mitotic CDK-cyclin complexes. First, during prophase, the chromosomes are condensed into flexible rods, which are easily moved by the mitotic spindle. Entry into mitosis also leads to the separation of the two centrosomes (spindle pole bodies in yeast). In metaphase, the sister chromatids are aligned at the center of the spindle (the metaphase plate). At the onset of anaphase, the cohesin links between the sister chromatids are abruptly dissolved, and the separated sister chromatids are pulled to opposite poles of the spindle, a process called anaphase A. In anaphase B, the spindle poles move apart, completing the segregation of the sister chromatids into the two opposing halves of the dividing cell. Mitosis is completed in telophase, when the chromosomes and other nuclear components are repackaged into identical daughter nuclei and the mitotic spindle is disassembled. In vertebrate cells, the nuclear envelope breaks down in early mitosis. By contrast, yeasts do not dismantle their nuclear envelope, and the mitotic spindle forms inside the nucleus (referred to as closed mitosis).

Mitotic CDKs trigger entry into mitosis, promoting nuclear envelope breakdown, spindle assembly and organization, chromosome condensation, and Golgi fragmentation, and contribute to APC/C regulation [65–70]. When chromosomes are correctly attached and aligned and the bipolar tension forces are present, the spindle assembly checkpoint (SAC) is satisfied and the anaphase-promoting complex (APC/C or cyclosome) is triggered by its co-activator Cdc20, promoting the metaphase to anaphase transition. Upon activation, the APC/C-Cdc20 complex ubiquitinates several proteins, promoting their degradation by the proteasome. The most important APC/C-Cdc20 targets are securin and B-type cyclins [71,72].

Sister chromatid separation at the onset of anaphase is triggered upon cleavage of the Scc1 subunit of cohesin by the protease separase [73]. Before anaphase, separase (Esp1 in budding yeast) is maintained inactive by the binding of securin [74]. To prevent the early segregation of sister chromatids, separase must be kept inactive until the chromosomes are aligned and attached to the microtubules. At the metaphase to anaphase transition, APC/C-Cdc20 ubiquitinates securin, targeting it for proteasomal degradation and thereby activating separase. Active separase cleaves the Scc1 subunit of the cohesin complex upon its phosphorylation by Cdc5, promoting the separation of the sister chromatids [73–75]. The APC/C-Cdc20 complex also targets cyclins B for degradation, promoting the first wave of Cdk1 inactivation [76,77]. However, destruction of cyclins B by APC/C-Cdc20 is not sufficient to nullify all Cdk1 activity, which is essential for mitotic exit. Activation of the mitotic phosphatase Cdc14 is therefore essential to counteract Cdk1 activity. Cdc14 contributes to Cdk1 inactivation by promoting the accumulation of the Cdk1 inhibitor Sic1, the dephosphorylation and activation of the second APC/C co-activator Cdh1, and the dephosphorylation of Cdk1 substrates [78,79].

Cdc14 belongs to a family of highly conserved dual-specificity phosphatases (DUSPs) that is conserved from yeast to humans (reviewed in [80,81]) and tightly regulated by changes in its subcellular localization. During most of the cell cycle, Cdc14 is kept sequestered at the nucleolus by its binding to the nucleolar protein Net1 (also called Cfi) [82]. The dimer Cdc14-Net1, together with Sir2 and Fob1, form the RENT (regulator of nucleolar silencing and telophase) complex, which regulates ribosomal DNA (rDNA) silencing and segregation [83–87]. In anaphase, Cdc14 is dissociated from the RENT complex and released from the nucleolus, allowing its translocation throughout the cell, active as a phosphatase [83,85]. Different localization of Cdc14 phosphatase allows the targeting of distinct substrates during anaphase progression. In addition, the net balance of Cdk1 and Cdc14 activities toward their substrates also regulates their phosphorylation status, thereby contributing to the order of substrate dephosphorylation and progression through mitosis [88].
The release of Cdc14 from the nucleolus depends on two regulatory networks: the Cdc-Fourteen Early Anaphase Release (FEAR) network (reviewed in [80,89]), which initiates Cdc14 release, and the Mitotic Exit Network (MEN), a G-protein signaling cascade that completes Cdc14 activation and release to the cytoplasm (reviewed in [90,91]) (Figure 3). FEAR acts in early anaphase, when Cdk1 activity is high and promotes the first wave of Cdc14 release from the nucleolus to the nucleus. Numerous proteins, including Slk19, Fob1, Spo12, Clb2, Cdc5, Zds1, PP2A-Cdc55, separase (Esp1) and Hit1, have been implicated in FEAR since their mutants exhibit delayed release of Cdc14 from the nucleolus during early anaphase [80,92–99]. FEAR-dependent Cdc14 release requires Net1 phosphorylation at Cdk1 consensus sites [95,96]. In metaphase, Net1 is maintained in an under-phosphorylated state by the phosphatase PP2A-Cdc55. At anaphase onset, separase, together with Zds1/2 proteins, promotes the PP2A-Cdc55 inactivation via the Cdk1-dependent phosphorylation of the Cdc55 regulatory subunit, allowing the accumulation of phosphorylated Net1 isoforms [96,98,100,101]. Increase levels of phosphorylated Net1 by Cdk1-Clb2, with the contribution of the polo-like kinase Cdc5, stimulates Cdc14 release from the nucleolus since the phosphorylated form of Net1 has lower affinity toward Cdc14 [93–96,102]. The early activated Cdc14 is required for anaphase progression. It leads to spindle stabilization and elongation in anaphase [103,104], positioning of the anaphase nucleus [105], segregation of repetitive DNA regions such as rDNA and telomeres [86,106,107], recruitment of condensin to rDNA [87,99], and full Cdc14 activation by a positive feedback loop activating MEN by Cdc15 dephosphorylation [92,108].

After the first wave of FEAR-Cdc14 release, when the Cdk1 mitotic kinase activity declines, the MEN kinases sustain Net1 phosphorylation and Cdc14 activation. The MEN pathway is GTPase-driven and is closely related to the mammalian Hippo pathway, which is involved in mitotic exit regulation, spindle orientation checkpoints (SPOC) and cytokinesis. The core of the MEN cascade consists of two serine/threonine kinases, Cdc15 (the Pak-like kinase) and Dbf2-Mob1 (NDR/LATS-related MEN kinases). The upstream effector of the MEN is Tem1, a small Ras-like GTPase that is localized at the centrosome (or spindle pole body, SPBs in yeast) [108]. Tem1 activity is negatively regulated by the two-component GTPase-activating protein (GAP) Bfa1-Bub2 [109,110] and positively regulated by the Lte1 protein [111–113]. In metaphase, PP2A-Cdc55 keeps Bfa1 dephosphorylated, thereby contributing to the activation of Bfa1-Bub2 [114]. When cells reach anaphase with a correct aligned mitotic spindle, Cdc5 phosphorylates Bfa1 and inactivates Bfa1-Bub2 GAP [115,116]. In addition, Cdk1-dependent PP2A-Cdc55 inhibition at early anaphase [96,101] also promotes the accumulation of phosphorylated Cdc5-dependent Bfa1. Active Tem1 interacts with and activates the Pak-like kinase Cdc15 [117,118]. In addition, the released FEAR-Cdc14 dephosphorylates Cdc15, facilitating Cdc14 activation in a positive feedback loop [92,119]. Once active, Cdc15 recruits the LATS-related Dbf2-Mob1 complex to the SPB and

Figure 3. Molecular mechanisms for Cdc14 activation and cell cycle progression in mitosis. For mitosis to progress, substrates phosphorylated by CDK must be dephosphorylated. This action is carried out by the phosphatase Cdc14, which is sequestered in the nucleolus and kept inactive by its interaction with Net1 during all phases of the cell cycle except mitosis. There are two mechanisms devoted to releasing Cdc14, namely FEAR, which is shown on the left of the figure, and MEN, on the right. PP2A-Cdc55 phosphatase keeps Net1 dephosphorylated (and as a consequence Cdc14 sequestered) throughout the cell cycle until securin is degraded, a process that activates separase and causes chromosome separation. The inhibition of PP2A-Cdc55 along with the phosphorylation of Net1 by CDK and polo (Cdc5) allow Cdc14 release from the nucleolus to the nucleus, where it becomes active, dephosphorylating its nuclear substrates. Full release of Cdc14 to the cytoplasm requires the action of MEN, which is activated in coordination with SPB migration to the daughter cell. Fully cytoplasmic released Cdc14 triggers the end of mitosis by resetting all CDK substrates and it determines the morphogenetic mechanisms for septum formation and cell separation.
phosphorylates Dbf2 [120,121]. Moreover, Cdc14 and PP2A-Cdc55 dephosphorylate Mob1, thereby alleviating its Cdk1 inhibitory phosphorylation [114,122,123]. The Dbf2-Mob1 kinase mediates Cdc14 release from the nucleus, thus maintaining Net1 phosphorylated [124–126] and retaining Cdc14 in the cytoplasm by phosphorylation at sites adjacent to its nuclear localization signal (NLS) [125]. Cytoplasmatic Cdc14 directly promotes mitotic exit via dephosphorylation of several Cdk1 targets, the second APC/C activator Cdh1, the transcription factor Swi5, and the Cdk1 inhibitor Sic1 [78,127–129]. Cytoplasmatic Cdc14 also regulates cytokinesis, ensuring timely septum disruption after cytokinesis (reviewed in [130–132]).

Mitotic exit is precisely and tightly coordinated to ensure that cell division occurs only after chromosomes are properly replicated and equally segregated between the two new daughter cells. Problems during mitotic exit can lead to genomic instability, genetic diseases and neurodegenerative disorders [133,134]. Various surveillance mechanisms or checkpoints delay mitotic progression to guarantee faithful inheritance of the genetic material. In budding yeast, the main mitotic checkpoints are the DNA damage checkpoint (DDC), the spindle assembly checkpoint (SAC) and the spindle position checkpoint (SPOC) (reviewed in [135]). The DDC delays the metaphase to anaphase transition in response to DNA lesions to give the cell time to repair the DNA damage [115,136,137]. In the presence of DNA damage, Pds1 is phosphorylated by the DDC-effector kinase Chk1, thereby preventing its ubiquitination and degradation by the proteasome [71,138]. Rad53, the other DDC-effector kinase, contributes to Pds1 stability by preventing Pds1 and Cdc20 interaction [138] and halts elongation of the mitotic spindle and MEN activation by Cdc5 inhibitory phosphorylation [136,139].

The SAC responds to unattached kinetochores by arresting the cells in metaphase and inhibiting MEN activity (reviewed in [140,141]). The molecular mechanisms leading to MEN inhibition upon SAC activation remain unclear. At metaphase, SAC proteins inhibit the APC/C-Cdc20 complex in the presence of unattached microtubules [142,143]. In addition, Aurora B (Ipl1 in S. cerevisiae) and Shugosin (Sgo1) are also important to sense the lack of tension upon incorrect kinetochore-microtubule attachments and to promote bi-orientation of the chromosomes [144–147].

The SPOC inhibits MEN to prevent mitotic exit until the spindle is properly positioned. Upon spindle misalignment, the main SPOC kinase effector Kin4 regulates the activity and localization of the Bfa1-Bub2 complex, thereby preventing Bfa1 activation by Cdc5 phosphorylation [148–152]. Although the DDC, SAC and SPOC are induced in response to distinct stimuli, there is crosstalk between them, the polo-like kinase Cdc5 being the central hub that coordinates mitotic progression with the main cell cycle check-points [136,150,153–155].

4. Regulation of mitosis by the Hog1 SAPK upon stress

Mitosis is another phase in which a molecular mechanism for cell cycle control in response to osmotic stress has been proposed. In a MEN mutant background, enhanced activation of Cdc14 leads to accelerated mitosis upon osmotic stress in a Hog1-dependent manner [156]. Indeed, mathematical model predictions supported the notion that cells stressed at the late G2/M phase display accelerated exit from mitosis and arrest in the next cell cycle [35]. However, the same model predicted the contribution of mechanisms other than the hyperactivation of Cdc14 to mitosis regulation upon osmotic stress [35] Along these lines, a novel molecular mechanism by which Hog1 activation regulates the M phase in response to stress has recently been described [157] (Figure 4).

There are several lines of evidence demonstrating that cells arrest in M phase in response to osmotic stress. Biochemical (Clb2 destruction) and cellular (DNA content) readouts revealed that cells synchronized in early M phase by Cdc20 depletion or other methods delay the next cell cycle G1 entry by around 60 min in response to 0.4 M NaCl. Moreover, genetic activation of Hog1 using a temperature-sensitive SLN1 (sln1ts) mutant allele in early metaphase showed a similar delay,
thereby suggesting a direct role of the HOG pathway in this transient cell cycle arrest. Activation of Hog1 resulted in cells progressing normally through anaphase, timely elongated spindles, and apparently separated nuclei, but with a delay in spindle disassembly and physical separation [157]. Accordingly, nucleolar release of Cdc14, is delayed upon Hog1 activation. Of note, Net1, which is responsible for the timely release of Cdc14, is targeted by Hog1. In vitro and in vivo experiments showed that Hog1 phosphorylates Net1 at Thr62 and Ser385, which, in fact, are distinct residues to those targeted by CDK to release Cdc14 in unperturbed cells. Net1 phosphorylation by Hog1 does not prevent Clb2-CDK phosphorylation on Net1, but stabilizes the interaction between Net1 and Cdc14, even in the presence of the Clb2-CDK complex, thereby impairing Cdc14 release. Nucleolar retention of Cdc14 upon HOG pathway activation is abolished when genomic net1T62A, S385A mutations are present. Thus, Hog1 phosphorylates Net1, altering its affinity for the phosphatase Cdc14, whose activity is essential for mitotic exit and completion of the cell cycle.

The delayed Cdc14 release from the nucleolus upon activation of Hog1 is coupled to a defect in rDNA and telomere segregation, and it eventually delays cell division. Of note, the mutant net1T62A, S385A, which cannot be phosphorylated by Hog1, displays reduced viability upon osmostress. This observation thus indicates that Hog1 contributes to maximizing cell survival upon stress by regulating mitotic exit. As a summary, the model emerging from these lines of evidence can be presented as follows; in an unperturbed cell cycle, Clb2-CDK complex phosphorylates Net1 and determines Cdc14 release, which in turn promotes the dephosphorylation of several targets to promote cell division. However, when the HOG pathway is activated at early M phase, Hog1 phosphorylates Net1, thus increasing Net1 affinity for Cdc14, and, consequently, making the complex more resistant to dissociation by CDK activity. The nucleolar retention of Cdc14 determines the transient arrest in the progression to G1. Of note, in mammalian cells, p38 is required for mitosis progression and is essential for the timely stable attachment of all kinetochores to spindle microtubules, but not for the fidelity of mitosis [158,159]. However, other evidence shows that loss of p38y results in multipolar spindle formation and chromosome misalignment, which induce a transient M phase arrest [160], indicating also a role in regulation of mitosis.

**Figure 4. Hog1 regulation of mitosis upon osmostress.** Increased levels of phosphorylated Net1 by the Cdk1-Clb2 complex, with the contribution of the polo-like kinase Cdc5, promotes the release of Cdc14 from the nucleolus. Activated Hog1 phosphorylates Net1 at Thr62 and Ser385, altering its affinity for the phosphatase Cdc14 and rendering the Net1-Cdc14 complex more resistant to CDK activity. Consequently, Cdc14 is kept sequestered at the nucleolus and mitosis progression is delayed.
**Concluding remarks**

During the last two decades, a large body of evidence has demonstrated that cell cycle progression is delayed in a context of stress, understood as changes in the environment. In parallel with other adaptive responses at the transcriptional and metabolic level, cells stop their cell cycle and growth to provide a time window for adaptation. Only when homeostasis has been restored, does the cell cycle resume and can all the delicate processes leading to the accurate division of the genome be achieved. Several molecular mechanisms devoted to providing cells with this invaluable time for adaptation have been described in detail. Despite some idiosyncratic differences, these mechanisms are reasonably well-conserved among eukaryotes. More interestingly, these mechanisms are present in all phases of the cell cycle, in contrast to other control mechanisms that are focused on one specific phase of the cell cycle. These observations thus indicate the importance of regulation of the whole cell cycle in response to external or environmental conditions. Recently, we have added a new piece of the puzzle by describing a molecular mechanism responsible for arresting the cell cycle in its final phase, namely mitosis. This mechanism gives coherence to the remaining arresting mechanisms in G1, S and G2 phases. Additional mechanisms of cell cycle progression upon stress will appear, some of them by using strategies that are qualitatively different to targeting particular activators or inhibitors and that rather modulate more general machineries. The use of cutting-edge technologies and approaches are starting to reveal mechanisms such as the regulation exerted by the HOG pathway on the antisense RNA of the CDK Cdc28 upon osmstress [161,162]. Taken together, the new knowledge gained will provide a comprehensive view of cell cycle regulation by osmostress and more generally by external modulators.

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**ORCID**

Javier Jiménez [http://orcid.org/0000-0002-0402-4427](http://orcid.org/0000-0002-0402-4427)
Ethel Queralt [http://orcid.org/0000-0003-0045-0039](http://orcid.org/0000-0003-0045-0039)
Francesc Posas [http://orcid.org/0000-0002-4164-7076](http://orcid.org/0000-0002-4164-7076)
Eulàlia de Nadal [http://orcid.org/0000-0003-0039-5607](http://orcid.org/0000-0003-0039-5607)

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