

Chapter 2

A Biological Overview of the Cell Cycle and its Response to Osmotic Stress and the α -Factor

2.1 Cell Cycle in Eukaryotes

The word “cell” originates from Latin *cella*, which means “small room”. It was applied for the first time by Hooke in his book *Micrographia* in September 1665. There are 10 to perhaps 100 million distinct life forms in the world [1, 32], many of which consist of various types of cells. Although different cells have disparate functions and shapes, they still have a similar basic chemistry [35].

Cells can be classified into (i) eukaryotic cells which contain a nucleus, and (ii) prokaryotic cells (such as bacteria) which lack any distinction between nucleus and cytoplasm [1, 32]. A cell can reproduce by growing and dividing into two daughter cells with the same genetic information [1, 32]. Single-celled organisms reproduce by cell division, whereas multicellular organisms need countless cell divisions to generate from founder cells (stem cells) diverse communities of cells that build the organs and tissues [1, 32].

Before each division all components and the “machinery” of a cell have to be duplicated, to allow the daughter cell to repeat the process. One crucial event of the cell cycle is the duplication of DNA to pass the genetic information to the next generation. Another crucial part of the cell cycle is the chromosome segregation, in which the sister chromatids are distributed to each daughter cell [1, 32]. The principle molecular machinery of the cell cycle that regulates DNA replication and chromosome segregation is preserved in eukaryotes.

2.1.1 Cell Cycle Phases

Cell cycle consists of four different phases: G1, S, G2 and M. In G1 phase cells increase in size and prepare for DNA replication. In S phase DNA replication occurs. From one double-stranded DNA molecule (chromosome) two identical double stranded DNA molecules (sister chromatids) are formed and held together

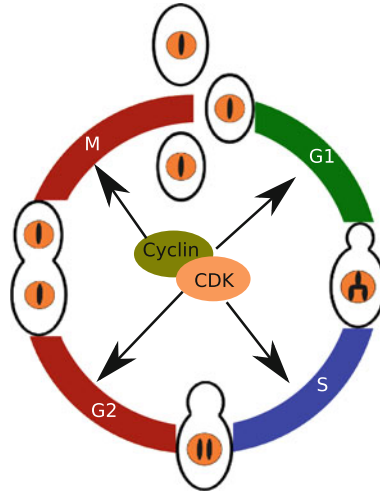


Fig. 2.1 Diagram of the eukaryotic cell cycle. G1 is the gap before DNA synthesis, during which the cell grows to a critical size. During S phase, DNA replication takes place. G2 phase is the gap between the S phase and the M phase. In G2 phase a set of control mechanism ensures that everything is ready to enter the M phase. Finally, during the M phase chromosome segregation takes place and the cell divides into two cells. G1, S and G2 phases are called *interphase*

with cohesin proteins. In G2 phase the accuracy of DNA replication and the morphology of the cell is checked. Finally, in M phase the cell divides into two daughter cells, each of which has a complete copy of the chromosome (see Fig. 2.1).

The details of the mechanism of the cell cycle are not identical in different types of cells [32]. Despite these differences, the main machinery of the cell cycle events – DNA replication; mitosis; cytokinesis and the presence of checkpoints – is highly conserved throughout all organisms.

2.1.2 DNA Replication

Before each cell division, a cell must duplicate its genome faithfully to avoid mutations. Also, DNA replication should happen once and only once per cell cycle. The synthesis and duplication of DNA is a complex process which fills a major fraction of the cell cycle. It starts in the late M phase and the early G1 phase, during which an initiator protein complex called pre-Replicative Complex (pre-RC), assembles at each origin of replication. This step is called licensing [1, 32]. The pre-RC complex consists of an Origin Recognition Complex (ORC), Mini-chromosome maintenance complex (Mcm) and licensing proteins. Once the pre-RC has mounted at the origins of replication in the late G1 phase, the origins are ready to fire [1, 32]. The S phase cyclins triggers the DNA synthesis at the onset of the S phase. The

S phase-dependent kinases (S-phase cyclins) are deactivated in the late M phase. The reformation of pre-RC is inhibited, while the S-phase cyclins are activated. This ensures the occurrence of the DNA replication only once per cell cycle [1, 32].

2.1.3 Mitosis and Cytokinesis

At the onset of the M phase, every cell has a tightly associated pair of chromosomes called sister chromatids. During the M phase, sister chromatids are segregated and distributed to each nascent cell [1, 32, 34]. This is followed by cytokinesis.

Mitosis is usually divided into four distinct stages in yeast: prophase, metaphase, anaphase and telophase. The prophase is the first stage during which chromosome condensation, centrosome separation and spindle assembly initiate. It is followed by the metaphase, during which the sisters are aligned at the centre of the spindle, anticipating the signal to separate in the anaphase. At the onset of the anaphase, the cohesin links between sister chromatids are abruptly removed and the separated sisters are dragged to the opposite poles of the spindle. Finally, the mitosis is completed in the telophase. During the telophase the chromosomes and other nuclear components are re-bundled into daughter nuclei [1, 32, 34]. Cytokinesis is the process of cell division, which happens at the end of mitosis. Its regulation depends on the progression through mitosis. The events of the mitosis are tightly controlled by the M phase cyclins. We will explain the control mechanism of the M phase for the model organism *Saccharomyces cerevisiae* in Sect. 2.1.5.

2.1.4 Checkpoints

There are several checkpoints in the cell cycle that control the cell cycle progression [25]. The main role of each checkpoint is to ensure whether certain conditions are satisfied before entering the next phase. If some irregularity is detected the cell is arrested until the problem is corrected. There are three main checkpoints operate at the end of G1, G2 and during M phase, respectively [18, 25]. These are example of conserved checkpoints among eukaryotes, but note that each species have several specific ones.

At the G1 checkpoints the size and also the DNA content of the cell are examined. If the cell is large enough and its DNA is intact it will proceed to the S phase. Otherwise it remains in the G1 phase [25]. At the G2 checkpoints the quality of the DNA synthesis, the size and the polarity of the cell are checked. If these conditions meet the required criteria, the cell will proceed to its M phase [25]. At the M phase checkpoint, which is referred to as metaphase checkpoint, the alignment of the chromosomes and the completeness of DNA replication are checked. Furthermore, the spindles need to be oriented towards the daughter cell. If all these conditions are met, the cell can divide [25].

2.1.5 Principles of the Cell Cycle Oscillation

The cell cycle is controlled by a complex molecular network which is responsible for the self-sustained oscillatory expression of a large set of genes. There are three main transitions in the cell cycle: the G1-to-S transition (START), the G2-to-M transition and the M-to-G1 transition (FINISH).

Cell cycle oscillations and transitions from one phase to the next are caused by the activation and inactivation of a complex that is composed of two main subunits: a catalytic subunit and a regulatory subunit (see Fig. 2.1). A catalytic subunit – Cyclin-dependent kinase (Cdk) – forms a dimer with a regulatory subunit cyclin [31]. These kinase complexes (Cdk-cyclins) control the cell cycle oscillation, as well as cell size and DNA replication. The activity of Cdk is regulated by the availability of its cyclin partners, binding to stoichiometric Cdk inhibitors, and inhibitory tyrosine phosphorylation [31].

The molecular machinery which regulates DNA replication and segregation is highly conserved, from unicellular eukaryotes, such as yeasts, to multicellular eukaryotes [35]. Therefore, simple eukaryotes, such as fission yeast and budding yeast, serve as model organisms to understand the cell cycle control mechanisms in Metazoa including humans. To understand such a complex system we have focused on the cell cycle machinery of budding yeast, *S. cerevisiae*, and its response to osmotic stress and the α -factor, and we have developed two mathematical models [39, 40], which are presented in Chaps. 3 and 4.

Our first model is based on Ordinary Differential Equation (ODE), which predicts the influence of osmotic stress in all stages of cell cycle. This model integrates the osmotic stress response network with the cell cycle machinery of budding yeast, *S. cerevisiae*. Since the cell cycle phases are interlinked, the effect of osmotic stress on the cell cycle cannot be predicted just by considering one single phase. We introduce a comprehensive mathematical model, which predicts how this elaborate system might work in the presence of osmotic stress. It also provides a workbench for further investigation of the molecular processes and the cell behaviour under various environmental conditions and experimental setups.

The second model is a Boolean representation of the cell cycle reactions to stresses, which investigates the dynamical properties of the cell cycle oscillation in the presence of multiple stresses; the osmotic stress and the α -factor. Since it is a discrete model, study of the entire finite set of the cell cycle states under various environmental conditions is achievable. This model shows that the cell cycle is robustly designed and it can recover from osmotic stress and the α -factor induced arrest.

The models are based on known interactions that control the yeast cell cycle and its response to osmotic stress and the α -factor, which we will review briefly in the next section.

2.2 The Cell Cycle of *S. Cerevisiae*

2.2.1 Principles of the Budding Yeast Cell Division

In budding yeast, *S. cerevisiae*, the cell cycle is controlled by a robust molecular network, which consists of more than 800 genes [46]. Note that the G2 phase in *S. cerevisiae* is usually very short compared to the M phase, often not distinguished from the M phase and referred to as the G2/M phase. *S. cerevisiae* replicates by budding asymmetrically [24]. Daughter cells are smaller than mother cells, and hence the G1 phase of a daughter cell is longer than that of the mother cell [24, 32].

S. cerevisiae has five Cdks (Cdc28, Pho85, Kin28, Ssn3 and Ctk1). Cdc28 is the central coordinator of the major cell cycle events [31, 52]. The function of the other Cdks has not fully been understood yet [31]. In budding yeast Cdc28 constitutively expressed. The activity of Cdc28 is regulated by the availability of its cyclin partners, inhibitory tyrosine phosphorylation and binding to stoichiometric Cdk inhibitors (Sic1, Cdc6). Cdc28 has two types of associated cyclins: (i) three G1 cyclins (Cln1, Cln2, Cln3) and (ii) six B-type cyclins (Clb1 to Clb6) [31].

G1 cyclins regulate the events in the gap between mitosis and DNA replication, whereas B-type cyclins are expressed successively from START to FINISH. Among the G1 cyclins, Cln3 is responsible for cell growth and the activation of Cln1 and Cln2 [52]. G1 cyclins are essential for cell cycle progression. The cell cycle is arrested if the cell loses all Clns. However, if at least one of the G1 cyclins is expressed, the cell will still proceed to START [41]. As Cln1 and Cln2 have redundant functions [52], we will refer to both of them as Cln2. The activity of Cdc28-Cln2 is maximal at START (see Fig. 2.2) [17]. *cln1Δcln2Δ* cells initiate DNA synthesis with a delay and show slow growth [22].

The six B-type cyclins are divided into three distinct pairs of similar functions [31]:

(i) The major functions of Clb5 and Clb6, both represented by Clb5 from now on, are the initiation of the DNA synthesis, and the negative regulation of Cdc28-Cln2 activity [6]. *clb5Δclb6Δ* cells exhibit long delay in initiating the S phase; but the length of S phase is comparable to wild-type cells [6].

(ii) The mitotic cyclins Clb1 and Clb2 [28] are represented by Clb2 from now on. Clb2 is crucial for successful mitosis and *clb1Δclb2Δ* cells are not viable [48].

(iii) The remaining B-cyclins, Clb3 and Clb4, have redundant roles in S phase initiation and spindle formation [31].

2.2.2 The Molecular Mechanisms of the G1-to-S Transition

The G1-to-S phase transition – START – refers to the transcriptional cascade that triggers three main events: budding, DNA replication, and the duplication of spindle pole bodies [17]. The Cdc28-Cln3 complex catalyses START; it activates the

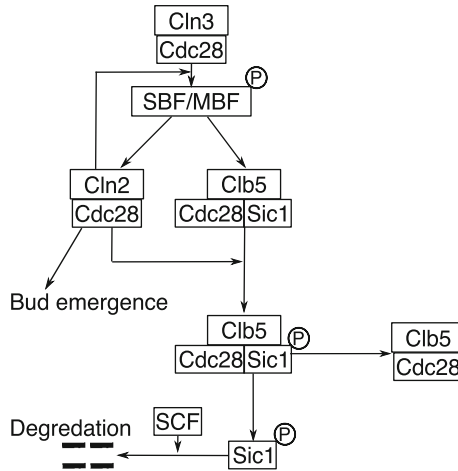


Fig. 2.2 G1-to-S transition: Cdc28-Cln3 complex activates the SBF and MBF transcription factors. These are transcription factors of *CLN2* and *CLB5* genes. Expression of *CLN2* and *CLB5* increases the levels of Cdc28-Cln2 and Cdc28-Clb5. The activation of Cdc28-Cln2 leads to the bud emergence. Moreover, there is a positive feedback loop from Cdc28-Cln2 to Cdc28-Cln3, which makes the G1-to-S transition irreversible. Sic1 maintains Cdc28-Clb5 in an inactive form during the G1 phase. To enable the G1-to-S transition, Sic1 needs to be phosphorylated and inactivated. Phosphorylated Sic1 is targeted for degradation by the SCF (Skp1-cullin-F-box) complex. The absence of Sic1 frees Cdc28-Clb5. Activity of Cdc28-Clb5 initiates DNA replication

transcription factor complexes: SBF and MBF (see Fig. 2.2). These, in turn, trigger the transcription of a set of more than 200 genes [27]; in particular *CLN2* and *CLB5*. The expression of *CLN2* and *CLB5* leads to the increased level of Cdc28-Cln2 and Cdc28-Clb5. The activation of Cdc28-Cln2 induces the bud formation. Moreover, there is a positive feedback loop from Cdc28-Cln2 to Cdc28-Cln3 (see Fig. 2.2), which makes this transition switch-like and irreversible [12, 17].

Sic1, the Cyclin Kinase Inhibitor (CKI) in the G1 phase, maintains Cdc28-Clb5 in an inactive form during most of START [42]. To enable the G1-to-S transition, Sic1 has to be inactivated. Cdc28 phosphorylates Sic1 [42] and targets it for degradation by the SCF (Skp1-Cullin-F-box) complex [33]. The absence of Sic1 triggers the activity of Cdc28-Clb5, which initiates the DNA replication and transfers the cell to the S phase (see Fig. 2.2).

2.2.3 The Molecular Mechanism of DNA Replication

DNA replication is a multi-step process which begins at the origins of replication (see Fig. 2.3). The multi-protein complex ORC (Origin Recognition Complex) remains bound to the origins of replication throughout the cell cycle. The first step of DNA

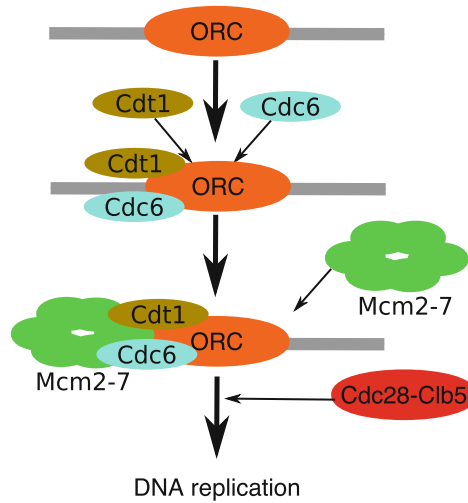


Fig. 2.3 DNA replication is a multi-step process which begins at the origins of replication. The multi-protein complex ORC remains bound to the origins of replication throughout the cell cycle. In the first step, the pre-replicative complex consisting of Cdc6, Cdt1 and Mcm complex binds to ORC. In the second step, DNA replication is triggered by Cdc28-Clb5

replication takes place during the late M phase and the early G1 phase. In this step, the pre-replicative complex, required for the initiation of DNA replication [16], binds to the ORC. The pre-replicative complex consists of the proteins Cdc6, Cdt1 and a complex of six proteins called Mcm (Mini-chromosome maintenance).

The second step occurs at the onset of the S phase (see Fig. 2.3). Activity of Cdc28-Clb5 first triggers DNA replication and later on disassembles the pre-replicative complex from the origins of replication. It also phosphorylates Cdc6, thereby promotes Cdc6 degradation and prevents *de novo* assembly of the pre-replicative complex during the G2/M phase. Therefore, Cdc28-Clb5 has a dual role: it enables the initiation of DNA replication; it also warrants that DNA replication happens once and only once before cell division [15].

2.2.4 The Molecular Mechanism of the G2-to-M Transition

The next cell cycle transition, G2-to-M, is mainly governed by the activity of Cdc28-Clb2 [48], which in turn is regulated by several mechanisms:

(i) The protein kinase Swe1 inhibits Cdc28-Clb2 activity by tyrosine phosphorylation of Cdc28 [43]. Swe1 is part of the morphogenesis checkpoint machinery, and it prevents the cell from entering into the G2 phase when aspects of bud formation are defective [43, 44]. Accumulation of Swe1 begins in the S phase [4] and it degrades quickly, when it is hyper-phosphorylated by the Hsl1-Hsl7 complex and

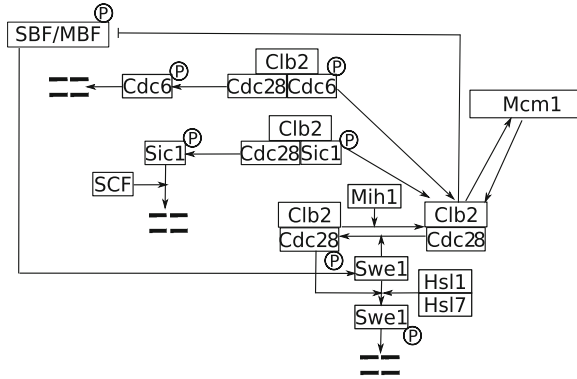


Fig. 2.4 G2-to-M transition: the activity of Cdc28-Clb2 controls the G2-to-M transition. The activity of this cyclin-Cdk complex is controlled via several mechanisms. Tyrosine phosphorylation of Cdc28 that is mediated by Swe1 inactivates Cdc28-Clb2. Moreover, Clb2 is transcriptionally controlled by Mcm1, which is activated by Cdc28-Clb2, thereby establishing a positive feedback loop. Active Cdc28-Clb2 also inactivates SBF/MBF transcription factors and promotes the G2-to-M transition

Cdc28-Clb2. It is worth mentioning that the phosphatase Mih1 reverses the tyrosine phosphorylation of Cdc28 [43].

(ii) The expression of Clb2 is transcriptionally controlled. Cdc28-Clb2 activates Mcm1 [3], which in turn promotes the transcription of *CLB2*, thereby establishing a positive feedback loop (see Fig. 2.4). Active Cdc28-Clb2, therefore, mediates the G2-to-M transition, during which the replicated chromosomes are segregated and nuclear division takes place (see Fig. 2.6).

(iii) The degradation of Clb2 is required for the M-to-G1 transition. We will discuss this transition in the next section.

2.2.5 The Molecular Mechanism of the M-to-G1 Transition

The M-to-G1 transition – the exit from mitosis – is achieved mainly by the inactivation of Cdc28-Clb2 [47]. Cdc20 and Cdh1, two major APC (Anaphase Promoting Complex) components, inactivate Cdc28-Clb2 [7, 53]. First, Mcm1 mediates the activation of the Cdc20 during the anaphase; then, Cdc20 triggers the pathway that results in the activation of Cdh1 and Cdc14 [7, 47, 53]. Cdh1 degrades the remaining fraction of Clb2 during the exit from mitosis (see Fig. 2.5) [7]. Finally, the phosphatase Cdc14 promotes the transition to the G1 phase in two steps: (i) it activates Swi5, a transcription factor of Sic1 and Cdc6 [50]; (ii) it dephosphorylates Cdh1, Sic1 and Cdc6. Active Sic1 and Cdc6 bind directly to Cdc28-Clb2, resulting in its inactivation (see Fig. 2.5).

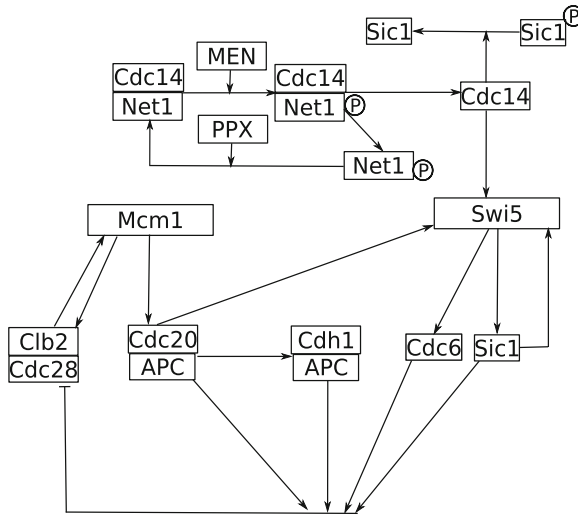


Fig. 2.5 M-to-G1 transition: this transition is achieved by the inactivation of Cdc28-Clb2. Cdc20 primarily degrades Clb2 and also mediates activation of Cdh1 and Cdc14. Then Cdh1 degrades the remaining fraction of Clb2 during the exit from mitosis. Cdc14 stimulates the activity of Sic1, Cdc6 and Cdh1 and therefore mediates the M-to-G1 transition

Figure 2.6 summarises the molecular mechanisms that control the cell cycle of *S. cerevisiae*. As explained above, the progression through the cell cycle occurs via constitutive activation of the corresponding cyclins in different cell cycle phases. The cell needs to pass through three main transitions successfully before dividing into two daughter cells. This succession of biochemical events comprising the cell cycle is distorted in the presence of stress. In order to study the response of the cell to different stresses it is important to understand: (i) the stress response signalling pathway, and (ii) the coupling of this pathway to the cell cycle control network. We have studied the cell cycle of *S. cerevisiae* under two different external signals; osmotic stress and the mating pheromone (α -factor). These signalling networks have been thoroughly studied in the past and found to affect the cell cycle progression. It has recently become clear that to study the response of the cell cycle to stress, the cell cycle and the stress signalling network have to be considered together. Recent studies have unveiled the key interactions between the cell cycle and the stress response networks [2, 8, 14, 19, 55]. To build a comprehensive model of the cell cycle response of *S. cerevisiae* to osmotic stress and the α -factor, we have considered the corresponding stress response signalling networks and their coupling with the cell cycle. In the following section we will give an overview of these two networks and their interactions with the cell cycle.

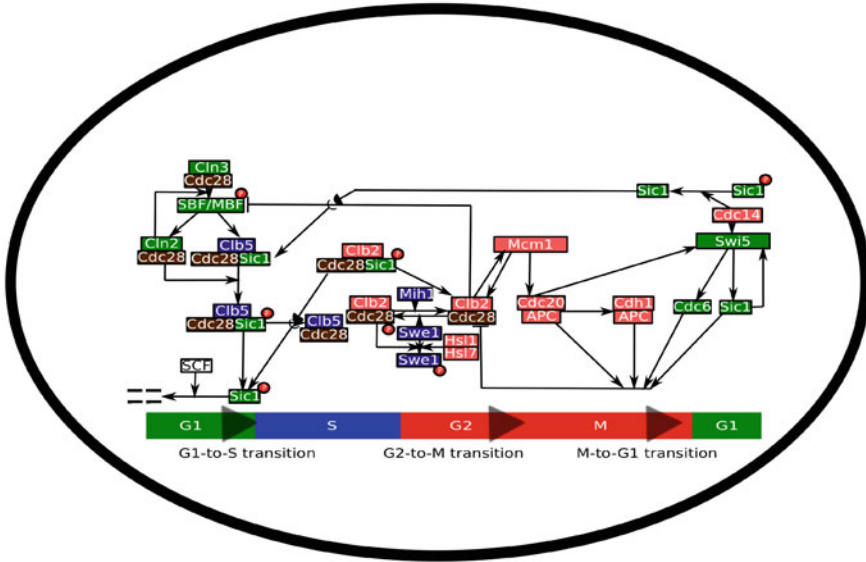


Fig. 2.6 Simplified schematic of budding yeast's cell cycle control network. As soon as cyclins are synthesised, they bind to Cdc28. As this tethering is fast, the cyclins alone have not been shown in the figure. Cell cycle control components are coloured based on the phase of activity, namely, green for G1 phase, blue for S phase and pink for G2/M phase. For details of the interactions see text

2.3 Osmotic Stress Response

Osmoregulation is a homeostatic process which controls: internal turgor pressure, the water content, and the volume of the cell. Various receptors sense osmotic stress and cause the activation of the High-Osmolarity Glycerol (HOG) MAPK signalling [9]. The HOG MAP kinase pathway, like any other MAP kinase pathway, consists of a cascade of three kinases: a MAP kinase (MAPK), a MAP kinase kinase (MAPKK), and a MAP kinase kinase kinase (MAPKKK) [21, 26].

For *S. cerevisiae* Sln1, Msb2, Hkr1 and Sho1 are osmosensors and upstream of two branches (see Fig. 2.7), which monitor changes in the turgor pressure and independently regulate three MAPKKKs (Ste11, Ssk2 and Ssk22) [26, 36, 38, 49, 54]. Consequently, MAPKKKs phosphorylate and activate the MAPKK (Pbs2) [26]. Activation of Pbs2 results in the activation of Hog1 via phosphorylation [26]. Dually phosphorylated Hog1 (Hog1PP) then accumulates in the nucleus and regulates the response of the cell to osmotic stress; (i) it mediates the production of glycerol to compensate for the turgor pressure loss [21]. (ii) Hog1PP interacts with cell cycle regulated components and block progression through the G1-to-S and the G2-to-M transitions [2, 8, 14, 19, 55]. Phosphorylated Hog1 is the main player in the interactions between the osmotic stress response and the cell cycle.

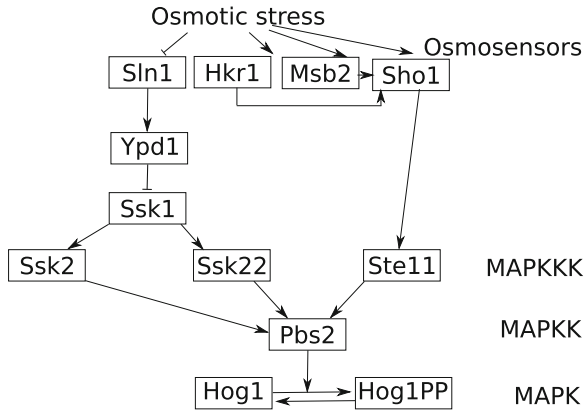


Fig. 2.7 Osmotic stress response network of *S. cerevisiae*; Sln1, Sho1 and Msb2/Hkr2 sense osmotic stress. These sensors are upstream of two branches which monitor changes of the turgor pressure and, independently, regulate three MAPKKKs (Ste11, Ssk2 and Ssk22). Subsequently, MAPKKKs phosphorylate and activate MAPKK (Pbs2). Activation of Pbs2 results in the activation of Hog1 via phosphorylation. Phosphorylated Hog1 then accumulates in the nucleus and activates gene expression of proteins involved in glycerol production. Thereby, glycerol is produced to compensate for the loss of turgor pressure

2.4 Interaction Between the Cell Cycle Network and the Osmotic Stress Pathway

In the last decade different interactions between Hog1PP and cell cycle regulated proteins leading to cell cycle arrest have been experimentally identified [2, 8, 14, 19, 55]. Crucially, the cell cycle phase during which the osmotic stress is applied determines the mechanisms of the interaction between the osmotic stress response and the cell cycle machinery.

2.4.1 Osmotic Stress Blocks the G1-to-S Transition

In untreated cells, activity of Cdc28-G1-cyclin complexes triggers the G1-to-S transition by targeting Sic1 (CKI) for degradation; thereby freeing the S phase cyclin to initiate DNA replication (see Fig. 2.2) [42]. In *S. cerevisiae*, if osmotic stress is applied during the G1 phase, activation of Hog1 results in G1 arrest by a dual mechanism (see Figs. 2.8 and 2.9) [8, 19]:

(i) Hog1PP downregulates the transcription of the G1 cyclins, leading to Sic1 accumulation [8, 19].

(ii) Hog1PP stabilises Sic1; Phosphorylation of Sic1 by Hog1PP, reduces the binding of Sic1 to the SCF complex to less than 30%. This complex formation is

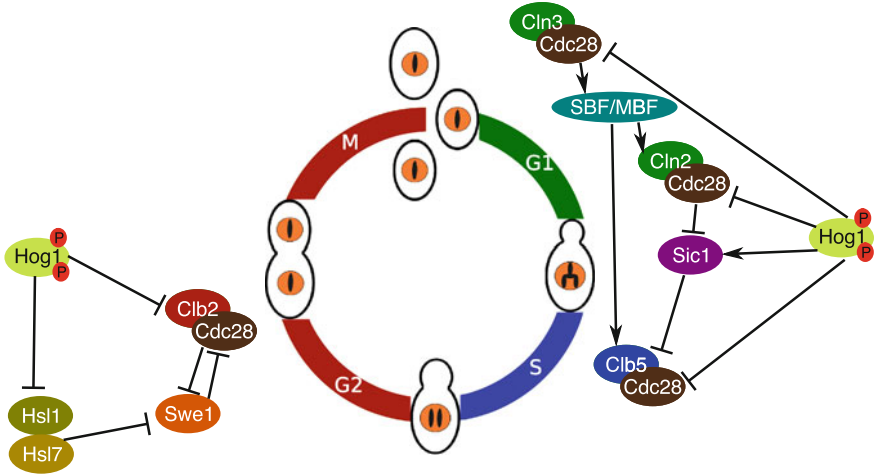


Fig. 2.8 Summary of the interactions of Hog1PP with cell cycle components. Hog1PP blocks the G1-to-S transition by phosphorylation and accumulation of Sic1; and transcriptional downregulation of G1 cyclins. Also, Hog1PP blocks the G2-to-M transition by indirect accumulation of Swe1; and transcriptional downregulation of G2/M cyclins. For details of interactions see text

required to initiate the ubiquitin-mediated proteolysis pathway responsible for Sic1 degradation [45]. Therefore, the presence of Hog1PP stabilises Sic1 [19].

Hence, the G1 arrest as a response to osmotic stress is a consequence of the direct phosphorylation of Sic1 and the downregulation of the G1 cyclins by Hog1PP.

2.4.2 Osmotic Stress Blocks the G2-to-M Transition

The progression through the S and G2/M phases are also delayed by osmotic stress due to three main mechanisms: (i) direct downregulation of *CLB5* transcription by Hog1PP [55], (ii) accumulation of Swe1 and (iii) downregulation of *CLB2* transcription (see Figs. 2.8 and 2.9).

As mentioned before, Swe1 is a part of the morphogenesis checkpoint and halts the cell cycle progression if bud formation is defective. In non-stress conditions, Swe1 is absent in G1 and accumulates during late G1 and S phases before disappearing rapidly in the G2/M phase [29, 44]. The Hsl1-Hsl7 complex and active Cdc28-Clb2 mediate the rapid degradation of Swe1 [30, 44]. Hsl1 is stable in the G1 phase, and accumulates during the S phase to peak in G2/M phase; its degradation is synchronised with the nuclear division [30]. Hsl1 changes its location between the nucleus and the bud neck, depending on the cell cycle phase. In contrast, Hsl7 shuttles between the spindle pole body and the bud neck and its total levels do not change significantly during the cell cycle [13, 30].

2.5 Mating-Pheromone Response

S. cerevisiae cells can exist as either diploid (cells have two homologous copies of each chromosome) or haploid (cells have one copy of each chromosome). The mating of yeast only occurs between two types of haploid yeast; *MAT_a* and *MAT_α* cells. Opposite mating type yeasts (*MAT_a* and *MAT_α*) mate by secreting pheromone (e.g., α -factor); presence of pheromone triggers a cascade of events to prepare the cell for mating, such as: (i) changes in the expression of about 200 genes [5]; (ii) and arrest in the G1 phase of the cell cycle [5].

S. cerevisiae senses the presence of extracellular pheromone by a signal transduction network called mating pathway [5]. The mating pathway is a MAPK signalling network and one of the best understood signalling pathways in eukaryotes [5]. Upstream of this pathway there are cell-surface receptors (Ste2, Ste3) to which the mating pheromone binds (see Fig. 2.10) [10, 23]. For the mating pathway of *S. cerevisiae*, the MAPKKK is Ste11; the MAPKK is Ste7; and there are two MAPKs, Kss1 and Fus3 [5].

External pheromone is sensed by receptors. This leads to the regulation of Ste11. Consequently, Ste11 phosphorylates and activates Ste7 (see Fig. 2.10). Activation of Ste7 results in the activation of Kss1 and Fus3 via phosphorylation. Activation of Fus3 regulates pheromone-induced gene expression and phosphorylates Far1, which

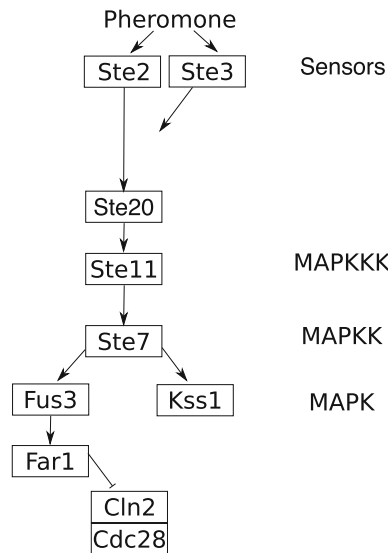


Fig. 2.10 Pheromone stress response network of *S. cerevisiae*. An external pheromone is sensed by Ste2 and Ste3. They are upstream of the MAPKKK (Ste11). Consequently, MAPKKKs phosphorylate and activate MAPKK (Ste7). Activation of Ste7 results in the activation of Kss1 and Fus3 via phosphorylation. Phosphorylated Fus3 then phosphorylates Far1, which induces cell cycle arrest

regulates the mating process in various ways. For example, Far1 mediates the cell cycle arrest [11, 51].

2.5.1 Pheromone Arrests the Cell Before START

As explained above, Cdc28 is the main regulator of the cell cycle of *S. cerevisiae*. Far1 is one of the inhibitors (CKIs) of Cdc28 and can inactivate Cdc28-Cln2 (see Fig. 2.10); Therefore, it blocks the cell cycle progression before start [11, 37].

Since the pheromone arrests cells in the G1 phase, it is frequently used to synchronise a population of cells at the START. This method of synchronisation is called α -factor synchronisation and is one of the “block-and-release” synchronisation methods [20].

Despite all these known interactions between the stress signalling pathway and the cell cycle network (see Figs. 2.9 and 2.10), many more aspects of the cell cycle response to stresses are still unknown. For example: (i) the response of cells in the S and M phases at the onset of stress; (ii) the influence of various levels of stress on the cell cycle; (iii) the main interactions in response to osmotic stress; (iv) the response of different mutated cells to osmotic stress; (v) the dynamics of the cell cycle regulation in the presence of stress; and (vi) the impact of “block-and-release” synchronisation on cell cycle dynamics, are unknown.

As we argued in this Chapter, the complexity of cell cycle machinery and stress response network (see Fig. 2.9) defies intuition and requires a systems biology approach. Hence, to study the regulation of all cell cycle events, which are controlled by an interconnected network, in the presence of extracellular signals, we present two comprehensive and complementary mathematical models [39, 40]. These models include all known interactions between the cell cycle and these two extracellular signals and answer these questions, for the first time.

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