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Review Article

Redundancy or specificity? The role of the CDK Pho85 in cell cycle control

Javier Jiménez, Natalia Ricco, Carmen Grijota-Martínez, Rut Fadó, Josep Clotet

Department of Basic Sciences, Faculty of Medicine and Health Sciences, International University of Catalonia, Barcelona, Catalonia

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Abstract: It is generally accepted that progression through the eukaryotic cell cycle is driven by cyclin-dependent kinases (CDKs), which are regulated by interaction with oscillatory expressed proteins called cyclins. CDKs may be separated into 2 categories: essential and non-essential. Understandably, more attention has been focused on essential CDKs because they are shown to control cell cycle progression to a greater degree. After clearly determining the basic and "core" mechanisms of essential CDKs, several questions arise. What role do non-essential CDKs play? Are these CDKs functionally redundant and do they serve as a mere backup? Or might they be responsible for some accessory tasks in cell cycle progression or control? In the present review we will try to answer these questions based on recent findings on the involvement of non-essential CDKs in cell cycle progression. We will analyse the most recent information with regard to these questions in the yeast *Saccharomyces cerevisiae,* a well-established eukaryotic model, and in its unique non-essential CDK involved in the cell cycle, Pho85. We will also briefly extend our discussion to higher eukaryotic systems.

Keywords: CDK (cyclin-dependent kinase), Pho85, cell cycle, *S. cerevisiae*

The other CDKs in cell cycle regulation

Most cell division control (CDC) genes were first identified in a visionary screening and described in a series of works by Leland H. Hartwell [1]. One of these genes encoding a kinase called Cdc28/cdc2 was soon identified as the master regulator of the cell cycle in eukaryotic cells [2, 3]. It became evident that this type of "master" kinases bind regulatory proteins called cyclins, so called because they are tightly regulated during each phase of the cell cycle [4], and thus the term cyclin-dependent kinase (CDK) was coined for these enzymes. Homology and later genome sequencing led to the description of many other CDKs. In the case of *Saccharomyces cerevisiae*, a nonessential CDK known as Pho85 was quickly identified [5]. In mammalian cells, many CDKs have been described but, strictly only 1, Cdk1, has been shown to play an essential role in cell cycle progression [6] see review on mammalian CDKs [7]. This scenario has opened an intriguing field of investigation and raised several questions: what roles do nonessential CDKs play in the mammalian cell cycle? Could these CDKs function as a backup measure to ensure some important process? Do these CDKs play a specific role under certain environmental conditions? Do these CDKs play secondary roles in cell cycle control?

The yeast model is made up of 2 CDKs involved in progression through the G_1 phase of the cell cycle: Cdc28, considered essential, and Pho85, the deletion of which is viable, however, it displays many traits directly related to cell cycle progression and control. Overexpression experiments suggest that, surprisingly, the functional homologue of Pho85 in mammalian cells is Cdk5 [8, 9]. Contrary to this finding, Cdk5 has not been shown to be involved in cell cycle progression control, but instead it plays a role in neuronal morphogenesis and migration in postmitotic neurons (see review by [10]). This divergence is not easily explained with the current data, but it suggests an even higher level of complexity in mammalian CDKs. The role of the Pho85 putative homolog appears to have

Table 1. Pho85 functions other than cell cycle regulation

	Phenotypic traits	References
Phosphate metabolism	Constitutive expression of phosphate starvation-dependent genes	[51-53] For a recent review.
Glycogen metabolism	Accumulation of glycogen in rich medium	[18]
Growth	Slow and/or poor growth in non-fermentable carbon sources	[18]
Morphogenesis and cell polarity	Irregular budding, actin depolymerisation and abnormal endocytosis	[54, 55]
Cell wall integrity	Increased sensitivity to tunicamycin, zymolyase and calcofluor white	[56]
Vacuole function	Increased sensitivity to cycloheximide, genticin (G-418), Hygromycin B and 4-nitroquinoline 1-oxide (4-NQO)	[56-60]
Aging	Short telomeres and extended chronological lifespan	[61, 62]

evolved into a more specific morphogenetic function while other CDKs, Cdk2 and Cdk6, have emerged as being responsible for supporting Cdk1 in driving the mammalian cell cycle, as discussed in this review.

CDKs control other processes

In addition to Cdc28 and Pho85, the genome of the budding yeast *S. cerevisiae* encodes 4 other CDKs: Kin28, Srb18/Cdk8, Sgv1/Bur1 and Ctk1 [11] which are mainly involved in transcriptional regulation [12] and will not be discussed in this review.

The role of Pho85 appears to go beyond the cell cycle. As we will see in this review, Pho85 has been shown to play a role in cell polarity, gene expression, phosphate and glycogen metabolism, and the signalling of environmental changes [13] (**Table 1**).

Pho85 as a non-essential CDK in *S. cerevisiae*: an introduction

Table 1 lists the functions of Pho85 that are controlled by 10 cyclins that may be grouped into 2 families according to cyclin box sequence similarity [14] (**Figure 1A**): (i) the Pho80 family (Pho80, Pcl6, Pcl7, Pcl8, and Pcl10) with prominent roles in regulating metabolism and sensing environmental changes [13], and (ii) the Pcl1,2 family (Pcl1, Pcl2, Pcl5, Pcl9 and Clg1). Pcl5 appears to be involved in nutrient sensing, like the Pho80 family [15]; see review by [16]. The rest of the members of this family show a tightly controlled cell cycle pattern of expression: Pcl9 is produced in late M and during early G_1, Pcl2 in late M until late G_1, and Pcl1 during late G_1 [14]. As thoroughly demonstrated in Cdc28 cyclins, there is a strong correlation between cyclin expression patterns and CDK function in a particular cell cycle phase (**Figure 1B**). Thus, many authors began to investigate the role played by Pho85 in cell cycle.

It is relevant to note that depletion of the different Pho85 cyclins renders the phenotypes listed above, demonstrating the essentiality of the interactions between Pho85 and its cyclins in its many functions besides the cell cycle regulation (**Table 1**) (For an outstanding review on Pho85 biology see [12]).

CDKs and the cell cycle

The current paradigm in cell cycle control and progression was established years ago in a series of works by L. H. Hartwell, T. Hunt and P. Nurse who were awarded the Nobel Prize in Physiology or Medicine in 2001. This pioneering work unveiled the cornerstone role of CDKs in driving cell cycle progression. In addition, these authors described the extraordinarily relevant role of the various cyclins, not only in the CDK activity itself, but also in its specificity and temporal regulation. However, recent findings by one of the laureates [17], who modified the normal expression and molecular characteristics of the CDK/cyclin complex with the aim of finding the minimal system necessary for cell cycle progression, suggest a certain degree of redundancy in the cyclins with regard to cell cycle progression that may be relevant in certain environmental situations. As previously mentioned in this review, the Barbacid group has demonstrated that despite the presence of several CDKs in mammalian cells, Cdk1 alone is sufficient to drive the cell cycle [6]. These 2 bodies of evidence have enabled us to compre-

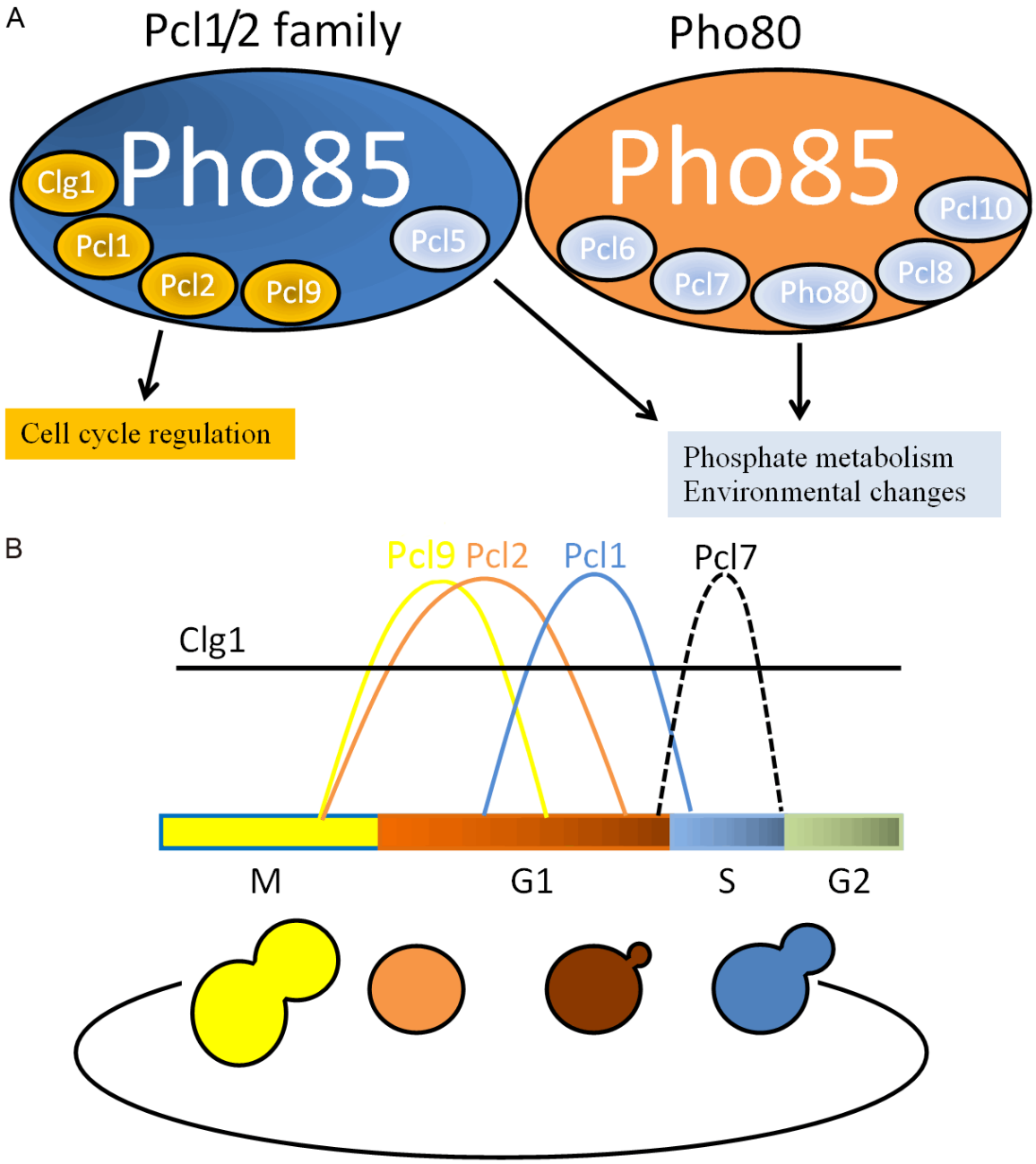

Figure 1. A: Schematic representation of the two families of Pho85 cyclins and their general functions. Note that every cyclin may interact with Pho85 independently from the others producing different active complexes. B: Representation of the temporal expression of the different Pho85 cyclins along the cell cycle. Data are coming from northern blot experiments in [14] and for Pcl7 in [35].

hend as never before the level of complexity involved in the regulation of cyclins and CDKs.

The present review is aimed at outlining how both mammalian and yeast cells bear several CDKs that at first glance appear to be redundant or accessories to cell cycle progression under normal conditions, but in fact should play some kind of role, according to the principle of cellular economy. To address this idea, we present a compilation of the different functions known to be carried out by Pho85, a non-essential CDK, in the eukaryotic model yeast *S. cerevisiae*. We will discuss the biological rele-

Table 2. Pho85 substrates related to the cell cycle

Substrate	Cyclin	Role of the phosphorylation	*In vitro/in vivo*	Reference
Sic1	Pcl1	Destabilization of the S-phase entry inhibitor	Yes/yes	[21]
Swi5	?	Exclusion of the Sic1 transcription factor from the nucleus		[22]
Whi5	Pcl9	Inhibition of SBF MBF transcriptional repression	Yes/yes	[12]
Pcl1	Pcl1	Cyclin ubiquitination and degradation	Yes/yes	[33]
Ssa1	Pcl2/Cgl2	Destabilization of its interacting cyclin Cln3	Yes/yes	[24]
Cln3	Pho80	Stabilization of the cyclin, larger amounts	Yes/yes	[25]
Clb6	?	Degradation of Clb6	Not tested/yes	[34]
Rim15	Pho80	Cytoplasmic sequestration, G_0 program inactive	Yes/yes	[63]
Ace2	Pcl1	Inhibition of the nuclear import of the transcription factor	Yes/yes	[45]
Rga2	Pcl1,2	Inhibition of the negative regulator of Cdc42 and polarity selection	Yes/yes	[48]
Cdc24	Pcl1/2	Release of Cdc24, the GEF of Cdc42		[27]
Bni4	Pcl1/2	Bud neck localization	Yes/yes	[50]

vance and implications of several CDKs controlling the same process in a living cell.

Pho85 substrates involved in the cell cycle

Historically, the best strategy to determine the function of a protein was to look at the cell phenotype when mutated or deleted. The initial phenotype data could then be complemented by genetic and biochemical evidence. In this section we will follow this structure in order to outline the various processes and functions in which Pho85 is involved and, more importantly, analyse the nature of these functions in terms of how they might interact with essential CDK functions in the processes in question.

As mentioned before, the oscillating cyclins described in Pho85 are mainly present around the G_1 phase of the cell cycle. This fact, together with the G_1 delay shown in *pho85*Δ [18], clearly suggest that the involvement of Pho85 in cell cycle regulation takes place mainly in G_1. Searching for the cause of G_1 delay, the Toh-e group described the accumulation of Sic1, a well-known inhibitor of G_1-S transition through Cdc28/Clb5-complex inactivation in *pho85*Δ cells [19, 20]. These authors demonstrated that Pho85/Pcl1 is able to phosphorylate Sic1 *in vitro* and, more importantly, that a Sic1 mutant in the 3 phosphorylation consensus sites for Pho85 is stabilized, mimicking the *pho85*Δ phenotype and providing evidence of an *in vivo* function of this phosphorylation. The destabilization of Sic1 by Pho85/Pcl1 appears to be of special relevance in situations where the DNA-damage G_1 checkpoint is active and cell cycle progression must be resumed [21]. Pho85 has also been shown to phosphorylate and exclude Swi5 from the nucleus. Swi5 is the transcription factor responsible for the expression of Sic1 in the late M phase [22]. It should be noted that under normal conditions Sic1 is also targeted for degradation by Cdc28/Cln phosphorylation [23], allowing Cdc28/Clb5 activity and entry and progression in the S phase. In contrast, in special situations where Cdc28/Cln activity is very low (e.g. DNA damage, nutrient starvation, pheromone presence), Pho85 is the CDK in charge of phosphorylating Sic1 and allows the cell cycle to resume. This role is evidenced by the findings that *pho85*Δ cells are hypersensitive to α-factor treatment [24] and DNA damage agents [21], and show an important G_1 delay during phosphate starvation [25].

With this phenotypic and biochemical evidence in mind, a specific function in the action of both essential Cdc28 and non-essential Pho85 starts to emerge: Cdc28 drives the cell cycle under normal growing conditions and only under very special circumstances, when its activity is down-regulated, Pho85 takes control of driving the cell cycle through G_1.

However, other pieces of genetic and phenotypic evidence have shown a degree of redundancy in the function of these 2 CDKs: (i) *pho85*Δ, *cln1*Δ, and *cln2*Δ cells are not viable, demonstrating that at least one of the CDKs must be active for progression from G_1 to S [26] and (ii) *pcl1*Δ, *pcl2*Δ, *cln1*Δ, and *cln2*Δ cells are able to move from G_1 to S, but they show cata-

strophic morphogenetic aberrancies [27], suggesting that both CDKs play a redundant role in the essential process of morphogenesis.

Beyond the phenotype approach, other, more complex strategies were used to identify Pho85 substrates. The following is a summary of some of these approaches, along with a description of the more relevant substrates (**Table 2**).

A synthetic dosage lethality screen carried out in the Andrews lab demonstrated that the overexpression of *WHI5* in a *pho85*Δ strain produces serious growth impairment, suggesting that Whi5 could be a Pho85 substrate [12]. Whi5 is a G_1-specific transcription repressor that binds to the SBF and MBF transcription factors essential for the G_1 transcription program, which includes the expression of the cyclins Cln1, Cln2, Pcl1 and Pcl2 [28, 29]. Cdc28/Cln3 and Cdc28/Cln2 complexes are able to phosphorylate Whi5, leading to eviction from G_1 gene promoters and nuclear exclusion of the repressor. According to the de Bruin and Constanzo, Pho85/Pcl9, and probably also Pcl1, is able to phosphorylate Whi5 and determine the activation of the G_1 promoters, but not nuclear exclusion. It is not known whether Cdc28 and Pho85 phosphorylate Whi5 at the same residue, but these authors suggest that in this particular case both kinases collaborate to control Whi5 and that they perform this task using distinct mechanisms. These findings have revived the debate about redundancy versus specificity in the cell cycle control functions of these 2 CDKs.

A massive screen for substrates of many of the yeast kinases carried out in the Snyder lab [30] identified a phosphopeptide from Pcl1 during the search for Pho85/Pcl1 substrates. This finding suggested that Pho85 cyclin turnover is controlled by phosphorylation by its own CDK, as occurs in Cdc28 [31, 32]. In our lab we have demonstrated that Pcl1 is phosphorylated by the Pho85/Pcl1 complex *in vitro* and *in vivo*, resulting in ubiquitination and proteasome destruction by the Dma1 E3 ligase system [33]. It is important to note that this ubiquitination mechanism is different than that used to degrade Clns (Cdc28 cyclins), shedding light on the fact that both cyclin families are not exactly concomitant in the cell cycle [33]. This shift in the presence of cyclins implies that the activities of these CDKs take place at slightly different temporal frames, further supporting the idea of a non-redundant function.

The Kron group, when looking for specific interactors of the chaperon Ssa1, found that Pho85/Pcl2 and Pho85/Clg1 are able to phosphorylate Ssa1 at Thr36 and demonstrated that this phosphorylation is important for destabilizing Cln3 in nitrogen starvation or pheromone presence [24], which determines the G_1 cell cycle arrest described under these environmental conditions. Interestingly, Cdc28 also targets Ssa1 at Thr36, however, as the Kron group elegantly demonstrate, the phosphorylation processes carried out by both CDKs occur under different conditions and at different moments of the cell cycle. These authors propose that the phosphorylation of Ssa1 by Pho85/Pcl2, Clg1 is mainly involved in G_1 arrest during nitrogen scarcity or pheromone presence, while the function of Ssa1 phosphorylation by Cdc28 is to limit the presence of Cln3 to the G_1 phase of the cycle. This work also makes a very interesting connection between cell cycle progression and nutrient availability, providing long-awaited molecular clues to this crosstalk.

After consolidating these fragmentary results, what emerges is that despite of the redundancies observed in CDK functions; Pho85 appears to play a specific, multi-layered role in G_1 progression alongside Cdc28. Under some conditions (e.g. acting on Whi5) Pho85 promotes G_1 progression, but mainly it acts as an integrator of signals such as nutrient availability, DNA damage or stress.

Pho85 involvement beyond G_1 regulation

Despite the findings in *pho85*Δ phenotype studies and the large body of evidence showing Pho85 involvement in G_1, other data suggest that this CDK may be involved in other phases of the cell cycle.

The Haase group showed that degradation of the S phase cyclin Clb6 is clearly dependent on both Cdc28 and Pho85: Clb6 is stabilized only when both CDKs are inactive (*cdc28-AS* plus the inhibitor 1-NM-PP1 and *pho85*Δ) [34]. Interestingly, this stabilization also occurs when all 3 consensus phosphorylation sites are mutated to Ala, indicating that Cdc28 and Pho85 may phosphorylate different residues in Clb6. These findings need further experimental

support to determine which site is used by each kinase, but in any case they clearly suggest biochemical specificity in the selection of phospho-acceptor residues.

When discussing the possible role of Pho85 in S phase progression, it is worth mentioning that the expression of the cyclin Pcl7 is slightly cell cycle-regulated, although present all around the cell cycle is peaking in the S phase indicating that this cyclin might play a role in this phase [35]. Although regulated by the cell cycle, Pcl7 is a member of the Pho80 family of cyclins and is involved in nutrient sensing. How these 2 characteristics fit together has yet to be discovered.

In addition to the relationship with Whi5 mentioned before, Pcl9 is expressed late in the M phase [36]. Analysis of the *pcl9*Δ cell phenotype revealed a defect in polarity selection, a finding that is enhanced in a background defective for the rest of the Pcl1/2 cyclins, phenocopying the *pho85*Δ polarity defects. So far targets of Pho85 in M-phase have not been described. Nevertheless, in a search for Pho85 partners some interesting proteins have emerged. To give some examples, in a dosage lethality screen [37], Cdc15 and Cdh1/Hct1 were revealed. Cdc15 is a protein kinase involved in the mitosis exit network (MEN) responsible for the full release of Cdc14 from the nucleolus, mitosis completion and cell cycle progression to G_1 (see review by [38]). Cdh1/Hct1 is the adaptor protein for directing anaphase-promoting complex (APC/C) to degrade substrates such as Clb2 (see classical review by [39]). Clb2, the most relevant cyclin of Cdc28 in G_2/M progression, also appeared in a screening for Pho85 physical interacting proteins [40]. In a phosphopeptide search peptides coming from Pds1, Cdc26 and Cdc27 were shown to be phosphorylated by Pho85 [30]. Pds1 is the so-called securin involved in the inhibition of the separase (Esp1) and the cleavage of cohesins, permitting chromosome segregation in the M phase (see review by [41]). Cdc26 and Cdc27 are part of the APC/C. Although further elucidation is needed, the evidence thus far indicates that Pho85 may play a role in the M phase of the cell cycle.

G_0 regulation by Pho85

Yeast cells enter into G_0 under adverse environmental conditions such as nutrient scarcity [42]. In this scenario it is plausible that Pho85, a sensor for phosphate availability, is also involved in this aspect of cell cycle control [43]. Under phosphate-rich conditions, Pho85/Pho80 inactivates Rim15 by phosphorylation at Thr 1075, and this substrate is effectively sequestered in the cytoplasm via association with the 14-3-3 proteins Bmh1 and Bmh2. As a result, the G_0 program remains inactive [43].

Our group has demonstrated that Pho85/Pho80 is able to phosphorylate *in vitro* the Cln3 cyclin, protecting it from proteasome degradation *in vivo.* During phosphate scarcity, the Pho85/Pho80 complex becomes inactive, leading to Cln3 hypo-phosphorylation showing a shorter half-life and, consequently, cells arrest in G_1 and remain in G_0 until environmental conditions can ensure a fruitful cell cycle progression [25]. It is worth noting that: (i) this is the first insight, at the molecular level, into the ancient topic of how the absence of phosphate is able to directly control cell cycle progression and (ii) for the first time, we describe the crosstalk between 2 CDKs in which a non-essential CDK controls the function of the essential CDK. As discussed in the G_1, the role of Pho85 G_0 is specific because its presence is essential to resuming the cell cycle after a period of nutrient scarcity, a function that Cdc28 does not carry out. This mechanism is quite relevant; the cell's ability to arrest and resume growing when in nature, exposed to a changing environment of benignity or adversity, is reflective of its ecological and evolutionary success.

Pho85 involvement in polarity

It is important to mention morphogenesis and polarity regulation when discussing cell cycle progression because both topics are closely coordinated and sometimes even co-regulated. In this section we will discuss the involvement of Pho85 in processes that determine the polarity and morphology of a yeast cell and expand on the initial findings of the O'Shea group [44].

The first independent action of a newly born yeast cell, following the septation process, is most likely the degradation of the septum by a series of chitinases and glucanases. These proteins are produced specifically by the daughter cell by means of the transcription factor Ace2. Ace2 is imported to the nucleus by activation of

the so-called RAM pathway, only when the septum has to be digested, and is sequestered in the cytoplasm for the remainder of the cell cycle (see review by [38]). Two mechanisms enforce Ace2 cytoplasmic sequestration: (i) phosphorylation of CDK consensus sites in Ace2 by both Pho85 and Cdc28, and (ii) an unknown mechanism mediated by Pho85 that functions independently from its kinase activity, according to [45]. These authors describe how phosphorylation by both Pho85 and Cdc28 is needed for Ace2 cytoplasmic retention since the inhibition of each kinase is not sufficient to deregulate Ace2 localization, suggesting they have a parallel and cooperative role rather than a redundant function. Furthermore, the authors propose an additional exclusive role for Pho85, most likely through a yet-undefined component that fully explains Ace2 cytoplasmic retention after nuclear exclusion in early G_1.

Other critical morphological events occur concomitantly with G_1 progression, including polarity, site selection, bud emergence and spindle duplication. The role of CDKs in morphological events has been demonstrated by the fact that a cell lacking all G_1 cyclins (Cln1, Cln2, Pcl1 and Pcl2) is perfectly proficient in the progression through G_1 but displays a catastrophic morphology [27]. Furthermore, Pcl1 and Pcl2 have been localised at sites of polarized growth, suggesting that these cyclins play a role in such processes [27]. Polarized growth in G_1 is directed by Cdc42, a Rho family GTPase, which when absent renders round, unbudded cells (see review by [46]). Cdc42 is positively regulated by the guanine nucleotide exchange factor (GEF) Cdc24, which is only available at the cytoplasm when its nuclear kidnapper Far1 is phosphorylated by Cdc28/Cln2 [47]. Cdc42, like all the GPTases, is negatively regulated by a GTPase activating protein (GAP) known as Rga2. Rga2 was discovered in a screening for synthetic dosage lethality (Rga2 overexpression is lethal in a *pho85*Δ strain) and has been shown to be regulated by both Cdc28 and Pho85 phosphorylation by [48]. These authors proposed a model in which Cdc28/Cln2 induce Cdc42 activation by releasing the activator Cdc24 while at the same time Cdc28 and Pho85 phosphorylate and inhibit the function of the Cdc42 negative regulator Rga2. Phosphorylation sites in Rga2 were shown to be very complex, mainly due to the 18 consensus sites and the difficultly in identifying which sites are used by each CDK. Nevertheless, the absence of either of these CDKs has been shown to be lethal when Rga2 is overproduced, suggesting that Cdc28 and Pho85 are redundant in Rga2 function.

Bni4 is an adaptor protein responsible for targeting many proteins to the bud neck during polarized growth [49]. Bni4 is phosphorylated *in vivo* by Pho85/Pcl1, Pcl2, not by Cdc28, the phosphorylation of which is essential for Bni4 bud neck localisation [50]. To our knowledge, Bni4 is the first described protein selectively targeted by Pho85 and not by Cdc28. DeMarini made this discovery by means of an interesting method to select proteins that are phosphorylated by only 1 of the CDKs. The rationale behind this model is that 2 proteins showing synthetic lethality are most likely to follow parallel pathways, just as 2 proteins showing synthetic dosage lethality are most likely to be involved in the same pathway. Thus, a synthetic lethality in a *cln1*Δ, *cln2*Δ background that is also showing synthetic dosage lethality in a *pcl1*Δ, *pcl2*Δ background is likely to be a specific target for Pho85 [50].

Are Pho85 and Cdc28 redundant in cell cycle regulation?

Cdc28 and Pho85 share many substrates involved in cell cycle control. Unlike Cdc28, Pho85 is not an essential protein, suggesting a redundant function in the CDKs, although a prominent role must be assigned to Cdc28. In this scenario, Pho85 may be acting as a mere "backup" in the control of a process of special importance and therefore is tightly regulated. However, the fact that under normal growing conditions the absence of Pho85 leads to a large variety of phenotypic traits has pointed to the idea that a more complex involvement between these kinases is actually taking place.

Non-essential CDKs may have a function under special growing conditions such as nutrient scarcity or the presence of mating pheromones. In these scenarios, Pho85 has been shown to play an "essential" role; *pho85*Δ cells that underwent phosphate starvation for 7 hours or pheromone arrested or when DNA damage occurs show a partially or totally impaired cell cycle restart, and thus Cdc28 is not proficient in driving cell cycle progression under these conditions and alternative mechanisms are in charge of this function.

This review has outlined several pieces of evidence demonstrating that even in the case of a shared substrate, the actions of essential and non-essential CDKs can be different, whether because the phospho-acceptor sites are different or the biological effect is different, or both.

To conclude, we wish to stress the importance and necessity of robust mechanisms that integrate and respond with reliability and flexibility in cells living in a changing and fickle environment, survival being the ultimate reward. We propose that it is precisely the variety of CDKs that allow cells to successfully react to too many different situations and produce a specific response to each of them.

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Address correspondence to: Dr. Javier Jiménez, Department of Basic Sciences, Faculty of Medicine and Health Sciences, International University of Catalonia, Barcelona, Catalonia. E-mail: jjimenez@uic.es

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