Invited Review

Structure and function of cyclin-dependent Pho85 kinase of *Saccharomyces cerevisiae*

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Yeast *Saccharomyces cerevisiae* has five cyclin-dependent protein kinases (Cdks), Cdc28, Srb10, Kin28, Ctk1, and Pho85. Any of these Cdks requires a cyclin partner for its kinase activity and a Cdk/cyclin complex, thus produced, phosphorylates a set of specific substrate proteins to exert its function. The cyclin partners of Srb10, Kin28, and Ctk1 are Srb11, Ccl1, and Ctk2, respectively. In contrast to the fact that each of Srb10, Kin28, and Ctk1 has a single cyclin partner, Cdc28 and Pho85 are polygamous; Cdc28 has 9 cyclins and Pho85 has 10 cyclins. Among these Cdks, Kin28 and Cdc28 are essential Cdks and it is well known that Cdc28 kinase plays a major role in regulating cell cycle progression. Pho85 is a non-essential Cdk but its absence causes a broad spectrum of phenotypes such as constitutive expression of *PHO5*, inability to utilize non-fermentable carbon sources, defects in cell cycle progression, and so on. Pho85 homologues are expanding to higher eukaryotes. Pho85 is most closely related with Cdk5 in terms of the amino acid sequence. The functional analysis of the domains of Pho85 also supports the close relationship between Pho85 and Cdk5, in which it was shown that the method of regulation of these two kinases is similar. Furthermore, forced expression of the mammalian *CDK5* gene in a *pho85Δ* strain canceled a part of the *pho85* defects. In this review, we summarize the functions of both Pho85/cyclin kinase and emphasize yeast Pho85 as valuable model systems to elucidate the functions of their homologues in other organisms.

Key Words——Cdk; Cdk5; cyclin; Pcl; *PHO5*; phosphorylation; *Saccharomyces cerevisiae*

1. Cdks of Budding Yeast

Eukaryotic cells have various types of cyclin-dependent protein kinase (Cdk). Cdk activity requires binding of an activating subunit called cyclin. Cyclin was discovered as a protein oscillatory during cell cycle; however, subsequent work has revealed that some cyclin

maintains a constant level throughout the cell cycle. Therefore, the term cyclin now has a broader meaning: cyclin as a structurally related family of proteins which bind and activate Cdk.

In *Saccharomyces cerevisiae*, five Cdks are known: Cdc28, Srb10, Ctk1, Kin28, and Pho85. The cyclin partners for Srb10, Ctk1, and Kin28 are Srb11, Ctk2, and Ccl1, respectively. In contrast, Cdc28 has nine partner cyclins (Cln1, Cln2, Cln3, Clb1, Clb2, Clb3, Clb4, Clb5, and Clb6) and Pho85 has 10 partner cyclins (see below). By using these Cdks, cells perform cell cycle regulation and transcriptional regulation.

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Kin28/Ccl1 (Valey et al., 1993) has an essential role and is homologous to mammalian Cdk7/cyclin H with respect to the amino acid sequence. Kin28/Ccl1 complex, contained in the basal transcription factor TFIIF, phosphorylates the C-terminal domain of the largest subunit of RNA polymerase II but does not act as Cdk activating kinase (CAK) as Cdk7/cyclin H does in mammalian cells (Cismowski et al., 1995). Srb10/Srb11 (Liao et al., 1995), a non-essential kinase, is a component of the RNA polymerase II holoenzyme. This kinase also participates in phosphorylation of the C-terminal domain of the largest subunit of RNA polymerase II and is required for repression of gene expression, for example, in response to glucose. It was shown that longevity of Gcn4, a transcription factor for general amino acid control, is determined by Srb10/Srb11 kinase (Chi et al., 2001). Non-essential kinase Ctk1/Ctk2 kinase (Stern et al., 1995) also phosphorylates the C-terminal domain of the largest subunit of RNA polymerase II and seems to function in some stage of transcriptional regulation.

Cdc28/cyclin complexes are the main engine of yeast cell cycle progression (Nasmyth, 1993). A specific Cdc28/cyclin complex is required at different points in the cell cycle. Since the Cdc28 level is constant throughout the cell cycle, Cdc28 kinase activity is regulated by the level of partner cyclins (Morgan, 1995). At START in which cells decide to proceed the next round of cell cycle, Cdc28 is activated by a G1 cyclin (Cln1, Cln2, or Cln3) (Dirick et al., 1995; Stuart and Wittenberg, 1995). After START, Cdc28/B-type cyclin (Clb), Clb5 or Clb6, is required for the entry into S-phase (Epstein and Cross, 1992; Schwob and Nasmyth, 1993). Other B-type cyclins (Clb1–Clb4) accumulating during G2 are required for the progression of mitosis (Richardson et al., 1992; Surana et al., 1991). Thus the wave of cyclins determines the temporal activity of Cdc28/cyclin kinase. Yeast has only one Cdc28 as a cell cycle regulating Cdk, but higher eukaryotes have 8 cell cycle-regulating Cdks. In contrast to the fact that yeast cyclin associates with one protein Cdc28, cyclins in higher eukaryotes bind to multiple Cdks. For example, D-type cyclin associates with Cdk4 and Cdk6, both of which play pivotal roles in G1 progression.

Pho85 Cdk participates in multiple cellular functions by collaboration with partner cyclins. Pho85 is not essential but its absence causes various cellular abnormalities. Furthermore, *pho85*Δ strains show a synthetic

effect when combined with a mutation in a regulatory gene for cell cycle progression or morphology (Lenburg and O'Shea, 2001). These observations strongly suggest that Pho85 plays an important role to connect environmental cues with a mechanism controlling growth. The main issue of this review is to discuss how Pho85 kinase exerts its function by selecting one of 10 Pho85 cyclins (Table 1).

2. Identification of *PHO85* and Phenotypes Caused by *pho85* Mutations

The *PHO85* gene, and the *PHO80* gene as well, was identified as a negative regulator for the expression of the *PHO5* gene encoding repressible acid phosphatase; a loss of either Pho85 or Pho80 function resulted in constitutive expression of *PHO5* (Ueda et al., 1975). The mutants thereof were employed for screening responsible genes from the yeast genomic library by complementation. The nucleotide sequence of the *PHO85* gene revealed that the amino acid sequence of Pho85 is closely (51%) related with Cdc28 Cdk (Toh-e et al., 1988; Uesono et al., 1987). In spite of the high similarity between Pho85 and Cdc28, Pho85 cannot substitute for Cdc28 and vice versa. Pho80 was demonstrated to be a cyclin for Pho85, and it was shown that Pho85/Pho80 complex phosphorylates Pho4, a transcriptional activator for the expression of *PHO5* (Kaffman et al., 1994).

Disruptants of *PHO85* are viable but display various phenotypes, in addition to the constitutive synthesis of repressible acid phosphatase (Fujino et al., 1994; Huang, et al., 1996; Timblin et al., 1996). The *pho85*Δ strains grow slower on nutrient medium, do not utilize galactose, glycerol, or lactate as a carbon source, show a morphological change, and accumulate glycogen. Such a broad spectrum of phenotypes displayed by the *pho85*Δ cells may be ascribed to the presence of multiple cyclins, of which 10 cyclins were identified (see below). Interestingly, some of the *pho85* phenotypes, such as slow growth in nutrient medium and inability to utilize non-fermentable carbon sources, are cancelled by a *pho4* deletion.

The *PHO85* interacting genes were identified as mutants that require *PHO85* for viability (Lenburg and O'Shea, 2001). These genes were designated the *EFR* (pho Eighty Five Requiring) genes. So far, seven *EFR* genes, six of which are known genes, were identified but it is obvious that there are more *EFR* genes

since only one mutation corresponds to each gene. This observation implies a presence of a complex regulatory network in which Pho85 kinase plays a pivotal role.

3. Characteristic Domains of Pho85

The amino acid alignment of Cdk2s including Pho85 is shown in Fig. 1. Cdk has well conserved sequences at three regions; the phosphorylatable tyrosine site (Y19 of Cdc28 and Y15 of the fission yeast Cdc2), the PSTAIRE sequence, and the T-loop. From crystallographic analysis of human Cdk2, major features of Cdk2s can be assigned to the particular structure in the protein (De Bondt et al., 1993): the ATP binding site (glycine-rich loop) between $\beta 1$ and $\beta 2$, the PSTAIRE motif in the $\alpha 1$ helix, and the T-loop in L12. The inhibitory phosphorylation sites, T14 and Y15, are both in the middle of the glycine-rich loop that serves as a phosphate anchor in ATP binding. These residues are buried by the T-loop. Phosphorylation at one or both of these sites might inhibit kinase activity by interfering with protein substrate binding or by indirectly affecting ATP orientation by changing the conformation of the glycine-rich loop. The activating phosphorylation site, T160, is found at the apex of the T-loop blocking the protein substrate binding site. The PSTAIRE motif is contained in $\alpha 1$ helix and sits in the cleft between the two lobes, the N-terminal lobe containing $\beta 1$ and $\beta 2$ sheets and the C-terminal lobe.

The functions of the conserved sites were extensively studied by a genetic means. Substitution of the Y residue in the inhibitory phosphorylation site for F results in constitutive activation of the Cdk2s (Gould and Nurse, 1989). Phosphorylation of threonine in the T-loop (T169 of Cdc28 and T161 of Cdc2) by CAK is necessary for activation of the Cdk2 kinase (Gould et al., 1991; Morgan, 1995). The cyclin binding site was explored by the alanine scanning method, which revealed that the residues in the N-terminal lobe participate in the binding of cyclin (Ducommun et al., 1991). The initial step in Cdk2 activation is thought to be cyclin binding, which makes the phosphorylation site T160 accessible to the phosphorylation enzyme by removing the T-loop from its inhibitory position. T160 phosphorylation could stabilize the T-loop in active conformation.

To examine the function of the conserved sequences of Pho85, mutations were introduced randomly or by PCR aided site-directed mutagenesis

Pho85	FKQLEKLCNGTYATVYKGLNKTGVYVALKEVKLD*SEEGTSPSTAIREISLMKEL
Pef1	YQR---GE---H---Q-RV---EI---VIRI-*AD---STAIREISLMKEL
PCTAIRE1	YIK-D---GE-----KS-L-DNL-----IR-E*H---A-C---V-L-L-D
C07G1.3	YEK-D---GE-----FR-RSIL-NKF-----IR-E*Q---A-C---CTAIREV-LRN
KIAA0834	YDK-D---GE-S-----KS-VN-KL-----IR-Q*E-----F---A-L-G
Dm PFTAIRE	YVK-P-GE-S-----FS-L-YQR-----IR-Q*E---A-F---A-L-L
ZC123.4	YEK-D---GE-----FR-RSIL-NKF-----IR-E*Q---A-C---V-L-LRN
CDK5	YEK---IGE---G-F--AKNRE-HEIV---R-R-DDDD--V--S-L--CL---
CDK2	-QKV---IGE---GVV--AR--L--E-V---KIR--TET--V-----L---
	$\beta 1$ $\beta 2$ $\beta 3$ $\alpha 1$
Pho85	KHENIVRLYDVHTENKLTLVFEFMDNDLKKYMDSRVTGNTPRGLELNLVKY
Pef1	R-P--MS-S--LQ-----M---Y-EK-----TYGNQGA***-PPSQ--N
PCTAIRE1	--A--T-H-I---KS-----YL-K---Q-L-DCGNI*****IMHN--L
C07G1.3	R-A-V-T-H-I---DRL-----VY-R---Q---CNNA*****MQM-NIRL
KIAA0834	--A--L-H-I---KET-----VYHT--CQ---KHFG*****HFDN--L
Dm PFTAIRE	--S--T-H-IV--RET--F---YC-T--SQ---EKHPG*****DHRVNRL
ZC123.4	R-A-V-T-H-I---DRL-----VY-R---Q---CNNA*****MQM-NIRL
CDK5	--K---H--L-SDK-----C-Q---F---CNGD*****DPEI--S
CDK2	N-PN--K-L-L-----Y---LHQDLKKFMDASALTG*****IP-PLIKS
	$\beta 4$ $\beta 5$ $\alpha 2$ $\alpha 3$
Pho85	FQWQLLQGLAFCHENKILHDLKPKQLNLLNKRGLKLGLDGLARAFIPVNTF
Pef1	TTQ---K-ISP---RV-----S---E---A-----SI-----
PCTAIRE1	--LF--R--Y--RQ-V-----E---E---A-----KSV--TK-Y
C07G1.3	--LY--R--Y--QRRV-----TAK-E---A-----KSV--TK-Y
KIAA0834	--LF--R--SYI--QRV-----SDT-E---A-----KSV--SE-Y
Dm PFTAIRE	--LF--R--SY--KRRV---V-----SDC-E---A-----KSV--SE-Y
ZC123.4	--LY--R--Y--QRRV-----TAK-E---A-----KSV--TK--
CDK5	--LF--K--G--SRNV-----RN-E---A-----RCY
CDK2	YLF-----SHRV-----TE-AI-A-----V-VRVY
	$\beta 6$ $\beta 7$ $\beta 8$ $\alpha 12$
Pho85	SSEVVTLWYRAPDVLMSRSTYSTSIDVSCGCLAEIMTG KLPFPGT*NDDE
Pef1	-N-----L--V-----M---A---R--A-S*-N-D
PCTAIRE1	-N-----P--I-L-TD--Q--M-GV---FY--A--R-----S*TV--
C07G1.3	-N-----P--L-L-TD--H--M-GV---F--A--RA---G*TPT-
KIAA0834	-N-----P--L-L-TE---CL-M-GV---FV---C--VAA---MKDIQD
Dm PFTAIRE	-H-----P--L-L-TE---L-M-GV---FV---V--M-T--IRDITYD
ZC123.4	-N-----P--L-L-TD--H--M-GV---F--AC--RA---G*TPT-
CDK5	-A-----P--F-AKL---M--A--F--LANAGR---N*DVDD
CDK2	TH-----EI-L-CXY--AV---LC--F--V-R-RA---D*SEID
	T-loop $\alpha 4$
Pho85	QLKLIFDIMGTFNLSWPSVTKLPKNPFIQRRPDRLRQVLQPHTEKP
Pef1	--LK--RL--T-QS--GISL--E-K-TETPIYKAQD-AYLFPPT****
PCTAIRE1	--HF--R-L--T-ET--GILSNEEFKTYNPKYRAEALLSHA-R****
C07G1.3	--G---RT--S-RPDRH-TICEK-TFY-YANRHYNP-PLCRQI-R****
KIAA0834	--ER--LVL--DT--GVHS--HFK-RRFTLYSSKNLRQAWNKLS*
Dm PFTAIRE	--DH--KLL--T-DT--G--HF-G-K-HKLGFR--KLGHNF-RLY**
ZC123.4	--G---RTL-S-RPDRH-TICEK-TFY-YANRHYNP-PLCRQI-R****
CDK5	--R--RL--T-EQ---MT--D-K-YPMY-ATTS-VNV-PK****
CDK2	--FR--RTL--D-VV--G--SM-D-K-SFPKWARQ-FSKV-P****
	$\alpha 5$ $\alpha 14$
Pho85	LDGNLMDFLHGLLQLNPDMLSAKQALHHPWF
Pef1	F-PLGL-L-RRM-R-Q-EL-TTGQD--Q-A--
PCTAIRE1	--SDGA-L-TK--FEGRN-I--ED-MK--F-
C07G1.3	I-AHGFEL-MKF--YEGKDRVSA-E-VKHPFL
KIAA0834	YVNHAEDLASK--CS-KN---QA-DS-EY-
Dm PFTAIRE	DIEGETIAN-F-----EQ--G-DD--Q--Y-
ZC123.4	I-AHGFEL-MKF--YEGKD-VSA-E-VK-PFL
CDK5	-NATGR-L-QN--KC--VQRI--EE--QH-Y-
CDK2	--EDGRSL-AQM-HYD-NK-I---AALA-PF-
	$\alpha 6$ $\alpha 7$

Fig. 1. Homology of the amino acid sequence of Pho85 and Cdk2s related to it.

The indicated amino acid sequences were aligned using the Pho85 sequence (7–296) as reference. A few N-terminal and C-terminal amino acids were removed from the comparison, because these regions are unique. - indicates the same amino acid as that of Pho85. * indicates a space. The sources of the Pho85 homologues are as follows. Pef1, *Schizosaccharomyces pombe*; PCTAIRE1, human; C07G1.3, *Caenorhabditis elegans*; KIAA0834, human; Dm PFTAIRE, *Drosophila melanogaster*; ZC123.4, *C. elegans*. The Cdk2 sequence is also included as a representative of the Cdc2 family. The structural features of Cdk2 are shown at the bottom of the alignment. α indicates an α helix and β a β sheet.

(Nishizawa et al., 1998b; Santos et al., 1995). It is known that dominant negative mutations can occur in three positions in Cdc28, which are conserved in Pho85. Santos et al. (1995) introduced mutations into these sites by mimicking the Cdc28 mutants by site-directed mutagenesis. They found that none of them were dominant negative, implying that the regulation of Pho85 kinase is different from that of Cdc28.

Phosphorylation of Y15 is inhibitory to Cdc2 kinase activity of fission yeast (Gould and Nurse, 1989); however, phosphorylation of the corresponding Y residue of Pho85 does not necessarily cause an inhibitory effect. Rather, a mutation *pho85Y18F* inactivated Pho85/Pho80 kinase activity (Fujino et al., 1994; Nishizawa et al., 1998b). Nishizawa et al. (1998b) showed that bacterially produced Pho85 should be phosphorylated prior to adding Pho80 cyclin to activate kinase activity. Under these conditions, Pho85 was phosphorylated at a Y residue and only the phosphorylated form of Pho85 could bind with Pho80. The fact that the Pho85Y18F mutant protein was not phosphorylated suggests that the Y18 residue can be phosphorylated in vivo. If this is the case, phosphorylation at the Y18 residue of Pho85 is not inhibitory for the Pho85 kinase activity but, on the contrary, has a stimulatory role. However, when Pcl1 was used as a Pho85 cyclin, phosphorylation of Pho85 prior to mixing with Pcl1 was not necessary. The Pho85/Pcl10 complex was formed without prior phosphorylation of Pho85 as well (Wilson et al., 1999). These observations strongly suggest that the Y18 residue, or the glycine-rich region of Pho85, serves as a specificity-determinant of cyclin selection.

Cdk2, Cdc28 of budding yeast and Cdc2 of fission yeast are phosphorylated at the T residue in the T-loop to be activated (Gould et al., 1991; Morgan, 1995). The amino acid residue corresponding to the T167 residue of Cdk2 is S166 in Pho85. Santos et al. (1995) reported that substitution of this residue to the A residue inactivated Pho85 kinase activity, by which they claimed that the S166 residue functions as an activation phosphorylation site as in the case of Cdk2. This result is quite contrary to the results presented by others. Nishizawa et al. (1998b) substituted the corresponding residue to alanine and found no change in the phenotype of such a mutant. Wilson et al. (1999) reported that no phosphorylated residue was found in their active Pho85/Pcl10 kinase by electrospraying experiments using trypsinized Pho85. Pho85 does not undergo phosphorylation by CAK (Espinoza et al.,

1998) or Cdk-activating kinase, and is active in the absence of CAK activity (Sutton and Freiman, 1997). Furthermore, removal of the putative phosphorylation sites in the T-loop did not affect enzyme activity (Nishizawa et al., 1998b). These results indicate that Pho85 regulation is quite different from that in Cdk2/Cdc2. These regulatory features of Pho85 kinase are similar to those operating in the regulation of mammalian Cdk5 in that (i) Pho85 and Cdk5 are not further activated by CAK (Poon et al., 1997; Sutton and Freiman, 1997), though the S residue in the T-loop of Cdk5 is phosphorylated by an unknown kinase to activate Cdk5 (Sharma et al., 1999) and (ii) phosphorylation at the Y residue in the glycine-rich region (Y15 in Cdk5 and Y18 in Pho85) enhances binding of p35 to Cdk5 (Zuckerberg et al., 2000) and of Pho80 to Pho85 (Nishizawa et al., 1998b).

4. Cyclin Partners of Pho85 Cdk

Disruption of the *PHO85* gene results in a pleiotropic phenotype including constitutive expression of *PHO5*. *PHO80* was identified as a gene whose mutation caused a constitutive phenotype of the *PHO* expression. *pho80Δ* and *pho85Δ* showed the same phenotype with respect to *PHO* regulation but a *pho80Δ* mutant represents a part of the phenotypes shown by the *pho85Δ* mutants. Through studies on G1 cyclins, two new cyclin genes, *HCS26* (Espinoza et al., 1994; Ogas et al., 1991) and *ORFD* (Measday et al., 1994), were identified. Later, the Cdk partner for these cyclins was found to be Pho85. Hcs26 and ORFD were renamed as Pcl1 and Pcl2 (Pho85 cyclin), respectively. *PHO85* is non-essential but *cln1Δ cln2Δ pho85Δ* was found to be inviable. Furthermore, *cln1Δ cln2Δ pcl1Δ pcl2Δ* was also inviable, indicating that Pho85/Pcl1,2 kinase has some role in G1 progression. *pcl1Δ* or *pcl2Δ* or *pcl1Δ pcl2Δ* cells are normal in the regulation of the *PHO* genes. When the phenotypes displayed by the *pho85Δ* cells are compared with those caused by the disruption of its cyclin partner, the latter comprise a part of the former, implying that there are more cyclin partners for Pho85 kinase. This turned out to be true. Measday et al. (1997) attempted to screen Pho85-interacting proteins by the two-hybrid method using *PHO85* as bait and identified *CLG1*, *PCL6*, 7, 8, and 10. By searching DATA base of yeast genome, Pcl5 and Pcl9 were identified as members of Pcl. In total, ten Pho85 cyclins are present in *S. cerevisiae*. Phylogenetic

analysis of the cyclin box regions of Pho85 cyclins revealed that the Pcl family can be divided into two sub-families; Pcl6, 7, 8, 10, and Pho80 belong to one group (Pho80 subfamily) and Pcl1, Pcl2, Pcl9, Pcl5, and Clg1 to the other (Pcl1, 2 subfamily) (Fig. 2).

5. Functions of Pho85/Cyclin Kinases

Pho85/Pho80 kinase. Among the functions in which Pho85/cyclin kinases participate, regulation of *PHO5* expression has been studied most extensively (Kaffman et al., 1994, 1998a, b; Komeili and O'Shea, 1999; O'Neill et al., 1996; Schneider et al., 1994) (Fig. 3). One of the physiological substrates of this kinase was shown to be Pho4, a transcription factor whose non-phosphorylated form, in association with Pho2, binds to the promoter of the *PHO5* gene to activate its transcription. Pho80 is also phosphorylated by Pho85/Pho80 kinase, but the biological significance of this phenomenon remains unknown (Fujino et al., 1994). Pho85/Pho80 kinase in cells grown in the presence of a sufficient amount of inorganic phosphate in medium localizes to the nucleus, and phosphorylates Pho4 at the five SP dipeptide sites (Komeilli and O'shea, 1999; O'Neill et al., 1996), thereby excluding Pho4 from the nucleus (Kaffman et al., 1998a). When the inorganic phosphate concentration becomes limiting, Pho81, a Cdk inhibitor, binds to Pho85/Pho80 kinase to inhibit its kinase activity (Schneider et al., 1994). Then, under-phosphorylated Pho4 in the cytoplasm is transported into nucleus to activate transcription of the *PHO5* gene (O'Neill et al., 1996). Mutations in the *PHO80* gene display some distinctive phenotypes in addition to constitutive expression of *PHO5*, such as hypersensitivity to aminoglycoside antibiotic (Wickert et al., 1998) and ability to take up dTMP (Bisson and Thorner, 1982), indicating that Pho85/Pho80 kinase regulates some other phenomena than *PHO5* regulation (Oshima, 1997).

Pho85/Pcl8, 10 kinase. In glycogen biosynthesis in yeast cells, two glycogen synthase encoded by *GSY1* and *GSY2* play a key role. *GSY2* encodes the major nutritionally regulated form of glycogen synthase whose activity is inhibited by phosphorylation. Huang et al. (1996) demonstrated that Pho85 is responsible for in vivo phosphorylation of Gsy2 by genetic and biochemical methods. In the absence of Pho85 kinase, Gsy2 keeps the level of phosphorylation low and production of glycogen is stimulated. Huang et al. (1998)

Fig. 2. Phylogenetic relationship between Pho85 cyclins. The cyclin box of each cyclin was used for comparison (Measday et al., 1997).

Fig. 3. Regulation of *PHO5* expression by Pho85/Pho80 kinase.

searched for a cyclin partner by a two-hybrid screening using *GSY2* as bait and identified Pcl10 as the cyclin partner of Pho85 in this phenomenon. Disruption of

PCL10 did not cause accumulation of glycogen, implying the presence of functionally redundant cyclin. Since Pcl8 is the most closely related with Pcl10 in terms of the amino acid sequence, Huang et al. (1998) constructed a disruptant of the *PCL8* gene. Disruption of either one of the two *PCL* genes did not display a significant phenotypic change; however, disruption of both *PCL* genes resulted in hyper-accumulation of glycogen without changing the total level of glycogen synthase. The double disruptant is otherwise normal, such as in *PHO* regulation, in cell cycle progression, in morphology, and in carbon source utilization. Pcl8 and Pcl10 belong to the Pho80 subfamily of the Pcl cyclins.

Pho85/Pcl6, Pcl7 kinase. Pcl6 and Pcl7 belong to the Pho80 subfamily of Pcl's, and are most closely related with Pho80 with respect to the amino acid sequence in the cyclin box (Measday et al., 1997; Fig. 2). The amino acid sequence similarity between Pcl6 and Pcl7 is extended beyond that of the cyclin box. Consistent with the previous report that Pcl7 physically interacts with Pho85, Pho85/Pcl7 immunoprecipitates made by anti-Pcl7 antibody showed protein kinase activity against Pho4 but not histone H1 or β -casein. In contrast to the fact that Pho80 interacts with Pho4 by two-hybrid assay, Pcl7 did not do so. Lee et al. (1998) felt disinclined to assume that Pho4 is an *in vivo* substrate for Pho85/Pcl7 kinase. However, since the phenotypes shown by the defect of Pho85/Pcl7 kinase were suppressed by introducing a *pho4* mutation, it is still possible that Pho4 is an *in vivo* target of Pho85/Pcl7 kinase. This possibility should be pursued in future.

Lee et al. (2000) found that Pcl7 interacts with Pho81, a phosphate-sensitive Pho85/Pho80 kinase inhibitor. This finding suggests that Pho81 acts an inhibitor of Pho85/Pcl7 kinase, and this was found to be the case. Furthermore, it was shown that the inhibition of Pho85/Pcl7 kinase by Pho81 was also phosphate-dependent.

PCL7 expression is cell cycle-dependent and peaks at early-to-mid-S phase. Consistent with this, Pho85/Pcl7 kinase activity is also cell cycle regulated, peaking in S phase. In contrast, Pcl6, closely related Pcl7, does not show cell cycle-dependent expression. Both *pcl6* Δ and *pcl7* Δ cells were unable to use galactose, lactate, or ethanol as a carbon source and proline as a nitrogen source, and grew slower than the wild type cells in nutrient medium. A *pcl6* Δ *pcl7* Δ double mutant strain showed the same phenotype exhib-

ited by a single mutant for the above phenotypes. In contrast to the report that *pho85* Δ strain hyper-accumulates glycogen (Huang et al., 1996; Timblin et al., 1996), a *pcl6* Δ *pcl7* Δ strain hypo-accumulated glycogen in their strain background. Thus, the phenotype of glycogen accumulation appears to be variable depending on the strain. For example, a W303 *pho85* Δ does not accumulate glycogen.

Pho85/Pcl1, 2 kinase. Pcl1, Pcl2, Pcl9, Clg1, and Pcl5 comprise a subfamily of the Pho85 cyclins designated the Pcl1,2 subfamily. Expression of *PCL1* and *PCL2* is regulated by the transcription factor SBF like G1 cyclin genes, *CLN1* and *CLN2*, whereas *PCL9* and *PCL2* are regulated by the transcription factor Swi5 that activates transcription of a set of genes at late M phase. *PCL2* is under dual regulation. The rest of the members of the Pcl1, 2 subfamily are not regulated by cell cycle factors. A quintuple disruptant is still viable but shows morphological abnormalities as a *pho85* disruptant does (Lee et al., 1998). In contrast to the fact that neither a *pcl1* Δ *pcl2* Δ strain nor a *cln1* Δ *cln2* Δ strain shows cell cycle arrest, a *cln1* Δ *cln2* Δ *pcl1* Δ *pcl2* Δ strain, and a *cln1* Δ *cln2* Δ *pho85* Δ strain as well, was found to arrest at the late G1 phase (Espinoza et al., 1994), indicating that Pho85/Pcl1,2 kinase exerts some influence at the G1-S transition. It is well known that Cdc28/Clb kinase inhibitor Sic1 should be degraded during the G1-S transition to activate Cdc28/Cln2 kinase. Cdc28/Cln2 phosphorylates Sic1 *in vitro* (Schwob and Nasmyth, 1993), and phosphorylation of Sic1 is necessary for its ubiquitinylation to be targeted to degradation by the 26S proteasome (Verma et al., 1997). We showed that Pho85/Pcl1 phosphorylated Sic1 at one of the phosphorylation sites and that this phosphorylation is necessary for prompt degradation of Sic1 during G1-S transition (Nishizawa et al., 1998a). In contrast to the fact that *cln1* Δ *cln2* Δ *cln3* Δ *sic1* Δ cells are viable, *cln1* Δ *cln2* Δ *pho85* Δ *sic1* Δ were not able to grow to colonies. However, the latter strain did increase in cell number during incubation but, at the same time, it lost viability rapidly. Another difference between a *cln1* Δ *cln2* Δ *cln3* Δ strain and a *cln1* Δ *cln2* Δ *pcl1* Δ *pcl2* Δ is that the latter strain can be suppressed by high osmotic pressure in the medium but that of the former can not (Lenburg and O'Shea, 2001). They also found that a mutation in various genes governing morphology showed a synthetic effect with *pcl1* Δ or *pcl1* Δ *pcl2* Δ , indicating that Pho85/Pcl1,2 participates in maintenance of cell wall

Table 1. Summary of functions of the Pho85 subfamily of Cdk.

Cdk	Cyclin	Cki*	In vivo substrate	Function	Reference
Pho85 (budding yeast)	Pho80	Pho81	Pho4	PHO regulation Aminoglycoside sensitivity	(1–3) (4)
	Pcl6 Pcl7	? Pho81	? ?	Utilization of galactose, lactate, ethanol, proline Slow growth, glycogen hypo-accumulation	(5)
	Pcl8 Pcl10	?	Gsy2	Glycogen accumulation	(6, 7)
	Pcl1 Pcl2	? ?	Sic1	Proteolysis of Sic1 G1-S transition Morphogenesis	(8) (9)
	Pcl9 Pcl5	? ?	? ?	Random budding Overlapping with Pcl1 subfamily cyclins	(9) (9)
	Clg1	?	?		
	Pac1	?	?	G1-S transition	(10)
Pef1 (fission yeast)					
Cdk5 (human)	p35	?	tau	Neuronal development	(11)
PHOA (<i>Aspergillus nidulans</i>)	?	?	?	Sexual development	(12)

* Cdk inhibitor.

(1) Toh-e et al., 1988; (2) Kaffman et al., 1994; (3) Schneider et al., 1994; (4) Wickert et al., 1998; (5) Lee et al., 2000; (6) Huang et al., 1996; (7) Huang et al., 1998; (8) Nishizawa et al., 1998a; (9) Lee et al., 1998; (10) Tanaka and Okayama, 2000; (11) Tsai et al., 1994; (12) Bussink and Osmani, 1998.

integrity or in morphological events. The phenomenon that *cln1Δ cln2Δ pho85Δ* cells arrest in G1 whereas *cln1Δ cln2Δ pho85Δ sic1Δ* cells died quickly can be interpreted by assuming that Pho85/Pcl1,2 kinase exerts two functions; one is phosphorylation of Sic1 to target Sic1 for ubiquitinylation and the other is morphological preparation for division. The *sic1* disruption forced the *cln1Δ cln2Δ pho85Δ* cells to proceed to S phase without morphological preparation for cell cycle progression, thereby killing the cells for an unbalanced growth.

The functional linkage between Pho85/Pcl2 kinase and cytoskeleton was implicated from the observation by a two-hybrid screening showing that Pcl2 interacted with Rvs167, inactivation of which causes a broad defect in actin-related phenomena (Lee et al., 1998). Protein complex formation between Pcl2 and Rvs167 in

vivo was confirmed by the immunoprecipitation method and by the in vitro binding assay using GST-Rvs167 and in vitro-made Pcl2. Such interaction is consistent with the fact that the phenotypes due to the deletion of *pho85* or *pcl1,2* subfamily overlap with those of *rvs167Δ*, suggesting that Pho85/Pcl1,2 kinase and Rvs167 have a common role in actin cytoskeleton organization. Furthermore, Rvs167 is phosphorylated and some isoforms of phospho-Rvs167 are produced in a Pho85-dependent manner, suggesting that Pho85 kinase possibly regulates cytoskeleton via phosphorylation of Rvs167.

From a search for genes having a target site of the transcription factor Swi5 that is a zinc finger motif transcription factor and is expressed under the cell cycle control during G2 and M phase, Aerne et al. (1998)

found *PCL2* and *PCL9*. These two cyclin genes are expressed in late M phase, although the main peak of *PCL2* expression is in G1. The observation of the peak of Pho85/Pcl9 kinase activity at late M phase suggests that Pho85 kinase has a certain role in this time point. Relationship between Pho85 kinase and Swi5 is further substantiated by the observation that either Pcl2 or Pho80 interacts with Swi5 in a binding assay in vitro (Measday et al., 2000). Swi5 was isolated as a Pcl9-binding partner by a two-hybrid screening. Measday et al. (2000) showed that Swi5 is phosphorylated by Pho85/Pcl2 and Pho85/Pho80 kinase in vitro. These results strongly suggest that Swi5 is phosphorylated by Pho85 kinase and that Swi5 regulation is altered in the absence of Pho85. This was shown to be the case since Swi5 seems to be hyper-activated in the absence of Pho85 (Measday et al., 2000).

6. Pho85 in Other Organisms

In the phylogenetic analysis performed by Liu and Kipreos (2000), it was shown that the Pho85 clade expands to metazoan. The major metazoan sub-branch in the Pho85 clade are, the Cdk5 branch, the PFTAIRe branch, and the PCTAIRe branch. Cdk5 is expressed in post-mitotic neuronal cells and is required for the central nervous system and its development (Chae et al., 1997; Ohshima et al., 1996). The murin PFTAIRe is expressed in brains, testes, and embryos. The function of PCTAIRe is presently unknown. A PHO85 homologue was found in fission yeast (Liu and Kipreos, 2000; Tanaka and Okayama, 2000) and in *Aspergillus nidulans* (Bussink and Osmani, 1998). The fission yeast Pho85 homologue, Pef1 (pombe *PHO* eighty-five), forms a complex with Pcl-like cyclin, Pac1. Pef1/Pac1 complex activates the transcription factor Res1-Cdc10-Rep during G1-S transition but does not participate in phosphate metabolism (Tanaka and Okayama, 2000). PhoA, *A. nidulans* Pho85 homologue, responds to phosphate concentration but does not regulate phosphatase. PhoA helps to integrate environmental cues with the developmental fate. A Pcl-like cyclin is not known in *A. nidulans*.

Functions of Pho85 family Cdk are summarized in Table 1.

7. Pho85 as a Model of Mammalian Cdk5

Among Cdk5, mammalian Cdk5 is the closest homo-

logue of Pho85, sharing 57% identity in the amino acid sequence. Regulation of the two kinases also seems similar in that (1) they are not further activated by CAK (Espinoza et al., 1998; Poon et al., 1997; Sutton and Freiman, 1997), (2) substitutions of Ser159 of Cdk5 and Ser166 of Pho85 in the T-loop with alanine do not affect the kinase activities (Nishizawa et al., 1999), and (3) phosphorylation of Tyr15 of Cdk5 and Tyr18 of Pho85 appears to enhance binding of p35 and Pho80, respectively (Lazaro et al., 1996; Nishizawa et al., 1998b). Consistent with these similarities between Pho85 and Cdk5, expression of mouse Cdk5 in a *pho85Δ* strain complemented some of the defects shown by the *pho85Δ* (Huang et al., 1999; Nishizawa et al., 1999). These authors also demonstrated that Cdk5 could bind Pho85 cyclins to phosphorylate one of the in vivo targets of Pho85 kinase, Pho4. Furthermore, Huang et al. (1999) reported that Pho85 introduced in COS7 cells was activated by p35, or p25 (or both). Altogether, studies of Pho85 in yeast will provide new insights into Cdk5 function.

8. Perspective

Single cell organisms such as yeast survive by regulating their growth; they stop growing under adverse conditions and start growing when environment becomes favorable. To keep growing under unfavorable conditions is hazardous for such cells. Therefore, the key regulators of cell growth, such as Cdc28 kinase of budding yeast, are of obvious importance, but regulators that connect environmental cues to the cell growth are necessary for their long survival. The latter regulators may be non-essential under favorable conditions for the cell growth. Pho85 kinase of budding yeast is such an example of factors delivering environmental cues to the main engine of the cell cycle progression, which is consistent with the fact that the *pho85* disruptant is viable but shows a broad spectrum of phenotypes as mentioned above. How many genes are under the control of Pho85? The recent study by Nishizawa et al. (2001) is a promising approach to elucidate genes under the control of Pho85 in a genome-wide view. Since Pho85 kinase, by selecting one of 10 Pho85 cyclins as a cyclin partner, acts in various facets of cell growth control, it is of interest to unveil a group of genes under the control of each Pho85/cyclin. To this end, it is necessary to construct cells expressing a particular Pho85/cyclin. A study along these lines

is under way.

How is Pho85/cyclin kinase activity regulated? Except for Pho85/Pho80, it is not known how the Pho85/cyclin complex is regulated. Since *PHO85* is expressed constantly throughout the cell cycle, Pho85 kinase activity should be regulated by a cyclin partner and/or by a Cdk inhibitor. Among Pho85 cyclins, production of Pcl1, 2, 9 and Pcl7 is known to be regulated in a cell cycle-dependent manner. However, the biochemical mechanism of their oscillation remains unknown. As for Cdk inhibitors, only Pho81 is known as an inhibitor of Pho85/Pho80 kinase (Schneider et al., 1994) and Pho85/Pcl7 kinase (Lee et al., 2000). Furthermore, how the environmental cues affect Pho85 regulators should be addressed in future.

The search for the presence of Pho85 homologues is expanding to other organisms. Among them, structural and functional homologies between Pho85 and mammalian Cdk5 are of interest. It is surprising that Pho85 makes a complex with p35 and p25, cyclins of mouse Cdk5, to stimulate the kinase activity (Huang et al., 1999), and that Cdk5 binds with some of the Pho85 cyclins to produce an active kinase (Huang et al., 1999; Nishizawa et al., 1999). Such a functional similarity between yeast Pho85 and its homologue in other organisms makes the yeast a valuable model system.

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