Communication

Mouse Cyclin-dependent Kinase (Cdk) 5 Is a Functional Homologue of a Yeast Cdk, Pho85 Kinase*

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Mouse cyclin-dependent kinase (Cdk) 5 and yeast Pho85 kinase share similarities in structure as well as in the regulation of their activity. We found that mouse Cdk5 kinase produced in pho85Δ mutant cells could suppress some of pho85Δ mutant phenotypes including failure to grow on nonfermentable carbon sources, morphological defects, and growth defect caused by Pho4 or Clb2 overproduction. We also demonstrated that Cdk5 coimmunoprecipitated with Pho85-cyclins including Pcl1, Pcl2, Pcl6, Pcl9, and Pho80, and that the immunocomplex could phosphorylate Pho4, a native substrate of Pho85 kinase. Thus mouse Cdk5 is a functional homologue of yeast Pho85 kinase.

Cyclin-dependent kinase (Cdk)1 plays a key regulatory role in the progression of the cell cycle. Vertebrate cells have various Cdks (Cdk1 to Cdk8) and cyclins (A, B, C, D, E, and H), and their different combinations are utilized at different stages of the cell cycle (1), whereas in budding yeast, a unique Cdk, Cdc28 kinase, functions by associating with distinct cyclins (2). Budding yeast also has a Cdk family whose members, including Pho85, Kin28, and Srb10 kinases, function in various cellular events (3–5).

Among mammalian Cdk family members, Cdk5 is not yet demonstrated to be involved in cell proliferation. Cdk5, activated by binding of p35 subunit (6), plays an important role in control of neurogenesis, including neurite outgrowth, axon guidance, and cell migration (7, 8). Cdk5 and yeast Pho85 kinases share 57% identity in the amino acid sequence (9, 10), and Pho85 has a pleiotropic function including response to nutrient conditions (11–13), PLC1-pathway (14), aminoglycoside sensitivity (15), and cell cycle regulation (16–19). Regulation of the two kinases appears similar: they are not further activated by Cdk-activating kinase (20–22), and substitutions of Ser-159 of Cdk5 and Ser-166 of Pho85 in the T-loop with alanine do not affect the kinase activities, whereas phosphorylation of Tyr-15 of Cdk5 and Tyr-18 of Pho85 appears to enhance binding of p35 and Pho80, respectively (23, 2). These similarities prompted us to test whether mouse Cdk5 kinase expressed in yeast cells can substitute Pho85 kinase and interact with Pho85-cyclins. Here we report that mouse Cdk5 kinase can suppress some of pho85Δ mutant phenotypes and can associate with Pho85-cyclins, including Pcl1, Pcl2, Pcl6, Pcl9, and Pho80, to phosphorylate Pho4. Thus mouse Cdk5 kinase is a functional homologue of yeast Pho85 kinase.

EXPERIMENTAL PROCEDURES

Strains and Media—Yeast strains used were MYF115 (MATa leu2 ura3 trp1 ade1 his GAL1), MYF116 (MYF115 pho85Δ::LEU2), MYF121 (MATa leu2 ura3 trp1 ade1 his GAL1 pho85Δ::URA3), and MYF151 (MATa ade2–1 trp1–2 leu2–3, 112 his3–11, 15 ura3 GAL cln1::hisG cln2 METp-CLN2(TRY1) pho85Δ::LEU2) (19). MYF164 (pho85Δ) was derived from MYF121 by selecting a resistant to 5-fluoro-orotic acid (24) and was used to construct double mutants with pcl1Δ (MFY165), pcl2Δ (MFY175), pcl6Δ (MFY166), pcl9Δ (MFY167), or pho80Δ (MFY168). Yeast cells were grown in SD medium containing 0.67% Difco Yeast nitrogen Base, 2% glucose, and appropriate nutritional supplements (25); SGal medium where galactose replaces glucose in SD or SGLac medium where glycerol plus lactate replace glucose. Polymeric reaction Cloning and Construction of Plasmids—Cloning of PCL1, PCL2, CLB2, and PHO80 was described previously (19). DNA fragments encoding PCL6 or PCL9 were similarly cloned (19); the primers were synthesized to incorporate an NcoI site immediately downstream of the start codon of PCL6 (5′-AAATACGGGCGCCATGCTATCAGG) or an EcoRI site immediately downstream of the start codon of PCL9 (5′-CACAAAGATGATCTCTGACTACGAT) and a BglII site at the 3′ end of each ORF (5′-CATATTACGCATTTAGATCTGCCCGTAAC- TAG for PCL6 and 5′-GGGCGTATCTTAAGATCTTTGCTTGAAAA-ACG for PCL9). These fragments were incorporated into pmF906 (19), together with a TRP1 marker, to produce HA-cyclins under the control of the GAL10 promoter. To disrupt genomic loci of these cyclins, a TRP1 fragment was used to replace an EcoRV-SalI fragment of PCL1, an SspI-SspI fragment of PCL2, a BamHI-XhoI fragment of PCL6, and an NcoI-NcoI fragment of PCL9, and a LEU2 fragment was to replace a ClaI-XhoI fragment of PHO80. Successful disruption was confirmed by polymerase chain reaction. A cDNA clone encoding mouse Cdk5 kinase (26) and a URA3 marker were cloned into pmF906 plasmid to generate pmF1086. The CDNA fragment was also incorporated into pmF568 (URA3) to generate pmF1057 in which the kinase was produced under the control of the PFK1 promoter (27). Plasmid pmF1079 consists of a BamHI-SalI fragment containing the promoter and the ORF of PHO85 and a URA3 marker. To overproduce Pho4 and Clb2 proteins, DNA fragments encoding each protein were cloned into pmF906, together with a TRP1 or a LEU2 marker to generate pmF869 (GAL10-PH04-TRP1), pmF1084 (GAL10-PH04-LEU2), pmF922 (GAL10-CLB2-TRP1), and pmF1085 (GAL10-CLB2-LEU2).

Analysis of Mutant Phenotypes and Its Suppression—Utilization of nonfermentable carbon source, production of acid phosphatase, and accumulation of glycogen were assayed as described (11, 12, 28). Suppression of growth arrest of a cln1 cln2 pho85Δ triple mutant was tested with strain MYF151 as described (19). Morphological defects were microscopically observed with overnight culture, and cell number was counted with a hemocytometer. Suppression of growth defect caused by overproduction of Pho4 or Clb2 proteins was analyzed by streaking yeast transformants on SD and SGal media supplemented with nutrients but lacking uracil and leucine. For spotting cell suspension, overnight-cultured yeast cells were spotted on SD and SGal medium supplemented with 2% glucose or 2% galactose and incubated for 3 days.

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‡ The abbreviations used are: Cdk, cyclin-dependent kinase; GST, glutathione S-transferase; HA, hemagglutinin; Ab, antibody.

3 Nishizawa, M., Suzuki, K., Fujino, M., Oguchi, T., and Toh-e, A. (1999) Genes Cells, in press.

2 L.-H. Tsai, personal communication.
Suppression of pho85Δ Mutant Phenotypes by Cdk5—Among pleiotropic pho85Δ mutant phenotypes, we tested constitutive expression of acid phosphatase (11), growth arrest of a cln1 cln2 pho85 triple mutant (16, 17), accumulation of glycogen (12, 13), failure to grow on nonfermentable carbon sources (13, 28), abnormal morphology (29), and growth arrest caused by overproduction of Pho4 or Clb2 (this work). Overproduction of mouse Cdk5 kinase failed to suppress the first three phenotypes (Fig. 1) but could suppress the other mutant phenotypes (Figs. 2 and 3). Cdk5 could restore growth of pho85Δ cells on glycerol + lactate medium (Fig. 2A). Overproduction of Pho4 or Clb2 proteins directed by the GAL10 promoter caused a growth defect in the absence of Pho85 kinase, which was suppressed by overproduction of Cdk5 kinase (Fig. 2B). Overproduction of Cdk5 itself did not affect the growth of pho85Δ cells (Fig. 2B). pho85Δ mutant cells become large (29) and show an apparent defect in separation of daughter cells, resulting in multiple-budded cells (Fig. 3A). They did not separate after extensive sonication under which conditions the wild-type cells did. The ratio of cells with morphological defects to the total cells reached ~33% in pho85Δ cells, which was decreased to 1.8% by expression of PHO85 and to 0.8% by that of CDR5 (Fig. 3A). Thus Cdk5 could function in yeast to suppress some of pho85Δ mutant phenotypes.

Cdk5 Kinase Can Phosphorylate Pho4 in Vitro—Ten cyclin-like proteins are known to interact with Pho85, and among them Pho85 complexed with Pcl1, Pcl2, Pcl9, Pcl10, or Pho80 was shown to phosphorylate Pho4 in vitro (16–18, 30, 31). These facts led us to a hypothesis that Cdk5 may associate with Pho85-cyclin(s) to phosphorylate Pho4, resulting in suppression of the growth defect caused by Pho4 overproduction. To test this idea, we first immunoprecipitated Cdk5 from yeast extracts and assayed its kinase activity on Pho4, Sic1, or histone H1 as substrate. Immunoblotting of Cdk5 appeared most efficient (Fig. 4, lane 1) and growth defect caused by overproduction of Pho4 or Clb2 (B). In panel A, combinations of tested strains (MFY115 and MFY118) and plasmids expressing CDR5 (pMF1057), PHO85 (pMF1079), or vector (pMF558) are as indicated. In panel B, combinations of plasmids introduced into pho85Δ cells (MFY164) are similarly indicated. Pho4 (pMF1084), Clb2 (pMF1085), and Cdk5 (pMF1086) were overproduced under the control of the GAL10 promoter.

RESULTS AND DISCUSSION

Suppression of pho85Δ Mutant Phenotypes by Cdk5—Among pleiotropic pho85Δ mutant phenotypes, we tested constitutive expression of acid phosphatase (11), growth arrest of a cln1 cln2 pho85 triple mutant (16, 17), accumulation of glycogen (12, 13), failure to grow on nonfermentable carbon sources (13, 28), abnormal morphology (29), and growth arrest caused by overproduction of Pho4 or Clb2 (this work). Overproduction of mouse Cdk5 kinase failed to suppress the first three phenotypes (Fig. 1) but could suppress the other mutant phenotypes (Figs. 2 and 3). Cdk5 could restore growth of pho85Δ cells on glycerol + lactate medium (Fig. 2A). Overproduction of Pho4 or Clb2 proteins directed by the GAL10 promoter caused a growth defect in the absence of Pho85 kinase, which was suppressed by overproduction of Cdk5 kinase (Fig. 2B). Overproduction of Cdk5 itself did not affect the growth of pho85Δ cells (Fig. 2B). pho85Δ mutant cells become large (29) and show an apparent defect in separation of daughter cells, resulting in multiple-budded cells (Fig. 3A). They did not separate after extensive sonication under which conditions the wild-type cells did. The ratio of cells with morphological defects to the total cells reached ~33% in pho85Δ cells, which was decreased to 1.8% by expression of PHO85 and to 0.8% by that of CDR5 (Fig. 3A). Thus Cdk5 could function in yeast to suppress some of pho85Δ mutant phenotypes.

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We next studied an interaction of Cdk5 with Pho85-cyclins by coimmunoprecipitation from cell extracts prepared from pho85Δ cells overproducing Cdk5 and either HA-Pcl1, -Pcl2, -Pcl6, -Pcl9, or -Pho80. Any combination of the Pho85-cyclins and Cdk5 kinase could phosphorylate Pho4, and Pcl2-Cdk5 appeared most efficient (Fig. 4B, lane 10, top panel). The observation that Cdk5 was detected in the immunocomplexes
with Pcl1, Pcl2, Pcl6, Pcl9, or Pho80 suggests that the kinase can interact with these Pho85-cyclins in yeast cells (Fig. 4B, lanes 9–13, bottom panel). Western blotting analysis of the cell extracts demonstrated that there were no significant differences in the amount of HA-cyclins in the extracts (Fig. 4C).

Possible Cyclin Partner of Cdk5 in Yeast—It is believed that combination with different cyclins is responsible for distinct Pho85 function (31). For example, Pho80-Pho85 phosphorylates Pho4 to repress PHO5 expression (30); Pcl8, Pcl10-Pho85 acts on Gsy2 to regulate glycogen synthesis (31); and Pcl1-Pho85 phosphorylates Sic1 for its prompt degradation (19). Therefore, Cdk5 may associate with specific Pho85-cyclin(s) to suppress different pho85Δ mutant phenotypes. To answer this, we analyzed Cdk5 function in double mutant cells where pho85Δ was combined with pcl1Δ, pcl2Δ, pcl6Δ, pcl9Δ, or pho80Δ mutations.

With respect to morphological defects of the double mutants, Cdk5 kinase could reduce the ratio of cells with abnormal shape, but not as efficiently as in pho85Δ single mutant cells, and a deletion of PCL1 or PCLS appeared to result in less efficient suppression than did that of the other cyclin genes (Fig. 3B). Expression of Pho85 kinase in the double mutants gave similar effect. These results suggest that cyclin(s) other than those tested may be required for Pho85 and Cdk5 to affect cell morphology or that cyclins interacting with either kinase to regulate cell morphology are redundant although individual cyclin may contribute to the suppression to a different extent.

Mouse Cdk5 Kinase Can Function in Yeast

FIG. 4. Mouse Cdk5 kinase can phosphorylate Pho4 and form a complex with Pho85-cyclins. A, activities of Cdk5 and Pho85 kinases in vitro. Cdk5 and GST-Pho85 were immunoprecipitated from cell extracts of MY116 with anti-Cdk5 and GST Ab, respectively, and the immunoprecipitates were subjected to kinase assay. The bands corresponding to phosphorylated substrates are indicated. B, coimmunoprecipitation of Cdk5 with Pho85-cyclins and kinase activity of the immunocomplex on Pho4. Extracts were prepared from MY161 cells producing Cdk5 and individual HA-cyclin, and the epitope-tagged cyclin was precipitated with HAAb. The immunoprecipitates were then subjected to kinase assay with Pho4 as substrate and to immunoblotting with anti-Cdk5 Ab to detect the presence of Cdk5 in the immunocomplex. C, presence of individual HA-tagged Pho85-cyclins in the extracts used for the coimmunoprecipitation experiment was analyzed by loading ~40 μg of proteins onto SDS-polyacrylamide gel, followed by immunoblotting with HAm Ab. Positions of molecular weight marker proteins are designated at the left side of the panel.

Next we tested suppression of the growth defect caused by overproduction of Pho4 or Clb2 in the double mutants. As shown in Fig. 5, overproduction of these proteins caused the growth defect in the double mutants as well as in pho85Δ single mutant, and even in the wild-type cells (Fig. 5, panels B1 and B2). These single or double mutants could grow normally on galactose medium (panel B3). Overproduction of Cdk5 together with Pho4 could restore the growth of pho85Δ and the wild-type cells (Fig. 5, panel C1). The suppression by Cdk5 appeared dependent on a specific Pho85-cyclin: the absence of Pcl1, Pcl6, Pcl9, or Pho80 did not appear to affect the suppression efficiency, whereas that of Pcl2 resulted in almost no suppression by Cdk5 kinase (panel C1), suggesting that the Pcl2-Cdk5 complex may be most crucial to overcome the growth defect. To the contrary, Pho85 kinase did not show a specific requirement of cyclin to counteract the effect of Pho4 overproduction: the double and pho85Δ single mutants showed similar level of growth recovery by Pho85 (panel D1).

In the case of the growth defect caused by Clb2 overproduction, Cdk5 showed very weak suppression in the pho85Δ pcl6Δ mutant, whereas, in the other double mutants, it could suppress the defect as efficiently as in pho85Δ single mutant (panel C2), suggesting that Cdk5 may be highly dependent on Pcl6 to counteract the overproduction effect of Clb2. This is in clear contrast to the function of Pho85 where PCL6 was dispensable (panel D2), whereas the other Pho85-cyclins tested might individually contribute to the Pho85 function to a certain extent (panel D2). Overproduction of Cdk5 or Pho85 alone did not affect the growth of strains tested (panels C3 and D3). Taken together, these results suggest that the suppression by Cdk5 of the growth defect caused by Pho4 or Clb2 was dependent on specific Pho85-cyclin.

In this paper we demonstrated that mouse Cdk5 kinase could suppress some of pho85Δ mutant phenotypes. This limited function could stem from defective interaction with some
Pho85-cyclins and/or from failure to phosphorylate appropriate substrates to a sufficient level. We could detect, by coimmunoprecipitation, an interaction of Cdk5 with Pcl1, Pcl2, Pcl6, Pcl9, or Pho80 (Fig. 4B), but Cdk5 function in vivo appeared to be dependent on specific Pho85-cyclins, as observed in the suppression of the growth defect caused by Pho4 or Cdc28 overproduction (Fig. 5). Thus it is formally possible that Cdk5 fails to interact with specific cyclin(s) in vivo, resulting in failure to suppress certain pho85Δ mutant phenotypes. Alternatively, the affinity of Cdk5 for certain Pho85-cyclins may not be strong enough to form a complex of full activity, and/or complexes of Cdk5 and Pho85-cyclins may exhibit altered or loosened substrate specificity compared with that exerted by a combination of Pho85-cyclins and its native kinase. Either of these could result in weak kinase activity unable to phosphorylate appropriate substrates to a sufficient level. This idea can explain why Cdk5 failed to suppress growth arrest of a cln1 cln2 pho85 mutant (Fig. 1). We previously demonstrated that Pcl1-Pho85 can phosphorylate Sic1, targeting it to degradation, which is a major role of Pho85 kinase in the absence of Cln1, 2-Cdc28 kinase activity for cells to proceed through G1 (19). Although Cdk5 could associate with Pcl1, it failed to phosphorylate Sic1 to a detectable level (Fig. 4, A and B). This idea can also be applied to the argument why Cdk5 that could phosphorylate Pho4 failed to suppress constitutive expression of PHO5 (Fig. 1). Phosphorylation of Pho4 regulates both its export from and import into the nucleus (32, 33), and a recent report demonstrated that the phosphorylation status affects the efficiency of translocation of the transcription factor (34). We imagine that phosphorylation of Pho4 by Cdk5 may decrease the level of the transcription factor in the nucleus sufficient to inactivate transcription of genes responsible for the growth arrest but not to the level enough to repress PHO5 expression. In other words, Pho4-responsive genes may have a different threshold of the transcription factor in the nucleus.

Since the discovery that yeast Cdc28 kinase is a functional homologue of mammalian Cdc2 and other Cdks functioning in the cell cycle, the yeast system has been providing a convenient tool to study the regulation mechanism of the cell cycle. In this paper, we demonstrated that the mammalian Cdk family has another functional homologue of the yeast Cdks. This discovery will lead to further understanding of the function of Cdk5 and Pho85 kinases. The yeast system will provide a tool to identify yet unknown factors that associate with p35-Cdk5 to regulate events involved in neuronal developments. Conversely, Cdk5 kinase can be used to search for yeast proteins interacting with cyclin-Pho85 complex to regulate cell-cycle progression and cell morphology. These studies will provide more insights into regulatory mechanisms of Cdks functioning in the events both related and unrelated to the cell cycle.

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REFERENCES

1. Nigg, E. A. (1995) Bioessays 17, 471–480
2. Nasmyth, K. (1993) Curr. Opin. Cell Biol. 5, 166–179
3. Toh-e, A., Tanaka, K., Uesono, Y., and Wickner, R. B. (1988) Mol. Gen. Genet. 214, 162–164
4. Ciszewski, M. J., Laff, G. M., Solomon, M. J., and Reed, S. I. (1995) Mol. Cell. Biol. 15, 2983–2992
5. Liao, S.-M., Zhang, J., Jeffery, D. A., Koleske, A. J., Thompson, C. M., Chao, D. M., Viljoen, M., van Vuuren, H. J. J., and Young, R. A. (1995) Nature 374, 193–196
6. Tsai, L.-H., Delalle, I., Caveness, V., Chae, T., and Harlow, E. (1994) Nature 371, 419–423
7. Nikolic, M., Dudek, H., Kwon, Y., Ramos, Y., and Tsai, L.-H. (1996) Genes Dev. 7, 816–825
8. Nikolic, M., Chou, M. M., Lu, W., Mayer, B. J., and Tsai, L.-H. (1998) Nature 395, 194–198
9. Meyerson, M., Enders, G., Wu, C., Su, L., Gorka, C., Nelson, C., Harlow, E., and Tsai, L. (1992) EMBO J. 11, 2909–2917
10. Uesono, Y., Tanaka, K., and Toh-e, A. (1987) Nucleic Acids Res. 15, 10299–10309
11. Toh-e, A., Ueda, Y., Kakimoto, S., and Oshima, Y. (1973) J. Bacteriol. 115, 727–738
12. Huang, D., Farkas, I., and Roach, P. J. (1996) Mol. Cell. Biol. 16, 4357–4365
13. Timblin, B., Tatchell, K., and Bergman, L. (1996) Genetics 143, 57–66
14. Flick, J., and Thormer, J. (1998) Genetics 148, 33–47
15. Wickertz, S., Finck, M., Herz, B., and Ernst, J. F. (1998) J. Biol. Chem. 180, 1887–1894
16. Espinoza, F. H., Ogas, J., Herskowitz, I., and Morgan, D. O. (1994) Science 266, 1388–1391
17. Meadmay, V., Moore, L., Ogas, J., Tyers, M., and Andrews, B. (1994) Science 266, 1391–1395
18. Auer, B. L., Johnson, A. L., Toyn, J. H., and Johnston, L. H. (1998) Mol. Biol. Cell 9, 945–956
19. Nishizawa, M., Kawasumi, M., Fujino, M., and Toh-e, A. (1998) Mol. Cell. Biol. 19, 2393–2405
20. Poon, R., Lew, J., and Hunter, T. (1997) J. Biol. Chem. 272, 5703–5708
21. Sutton, A., and Preimann, R. (1997) Genetics 147, 57–71
22. Espinoza, F. H., Farrell, A., Nourse, J. L., Chamberlin, H. M., Gileadi, O., and Morgan, D. O. (1998) Mol. Cell. Biol. 18, 6365–6373
23. Lazar, J.-B., Kitzmann, M., Cavardere, J.-C., Muller, Y., Clos, J., Fernandez, A., and Lamb, M. (1996) Neuron. Lett. 218, 21–24
24. Boeke, J. D., LaCroute, F., and Fink, G. R. (1984) Mol. Gen. Genet. 197, 345–346
25. Rose, M. D., Winston, F., and Hieter, P. (1990) Methods in Yeast Genetics: A Laboratory Course Manual, pp. 177–178, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
26. Tsai, L.-H., Takahashi, T., Caviness, V., and Harlow, E. (1993) Development 119, 1029–1040
27. Nishizawa, M., Taga, S., and Matsubara, A. (1994) Mol. Gen. Genet. 245, 301–312
28. Fujino, M., Nishizawa, M., Yoon, S.-J., Oguchi, T., and Toh-e, A. (1994) in Phosphate in Microorganisms: Cellular and Molecular Biology (Torriani-Gorini, A., Silver, S., and Yagi, E., eds), pp. 70–75, American Society for Microbiology, Washington, D. C.
29. Meadmay, V., Moore, L., Retnakaran, R., Lee, J., Donoviel, M., Neiman, A. M., and Andrews, B. (1997) Mol. Cell. Biol. 17, 1212–1223
30. Kaffman, A., Herskowitz, I., Tjian, R., and O'Shea, E. K. (1994) Science 263, 1153–1156
31. Huang, D., Moffat, J., Wilson, W., Moore, L., Cheng, C., Roach, P., and Andrews, B. (1998) Mol. Cell. Biol. 18, 3289–3299
32. Kaffman, A., Rank, N., and O'Shea, E. (1998) Genes Dev. 12, 2673–2683
33. Kaffman, A., Rank, N., O'Neill, E., Huang, L., and O'Shea, E. (1998) Nature 396, 482–486
34. Komell, A., and O'Shea, E. K. (1999) Science 284, 977–980