Regulation of Calcineurin Gene Expression in Schizosaccharomyces pombe

DEPENDENCE ON THE ste11 TRANSCRIPTION FACTOR*

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Calmodulin and its target enzymes are important regulators of numerous cellular processes, including reversible protein phosphorylation. The calmodulin-dependent protein phosphatase (calcineurin) has been suggested to play roles in activation of T cells and in the mating response of yeast. Recently, studies have shown it to be the target of immunosuppressant drugs such as cyclosporin and FK-506. In this study, we have cloned the gene for the catalytic subunit of calcineurin, CnA, from the yeast Schizosaccharomyces pombe. The gene (designated ppb1) has been mapped to chromosome II by analysis of the hybridization of a genomic DNA probe to an ordered library. The gene produces a single mRNA species of 2.5 kilobases, which varies during the cell cycle in exponentially growing cells. In addition, expression of ppb1 mRNA is induced by nitrogen starvation, a condition that favors mating in S. pombe. The ppb1 gene promoter contains a cis-acting element for the ste11 transcription factor, and we have shown that induction of the ppb1 mRNA during nitrogen starvation is dependent on the ste11 gene product. Together with earlier studies showing that disruption of the ppb1 gene in S. pombe results in sterility (Yoshida, T., Toda, T., and Yanagida, M. (1994) J. Cell Sci., 107, 1725-1735), our studies suggest that the ppb1 gene plays a role in the gene expression cascade that is essential for mating and sporulation in S. pombe.

Through the calcium-binding protein calmodulin (CaM), a large number of cellular processes are regulated (1). Presently, more than 30 different enzymes have been shown to be regulated in a Ca2+/CaM-dependent manner, making CaM an important mediator of intracellular signal transduction. Among the targets for CaM are enzymes involved in glycolgen metabolism, cyclic nucleotide metabolism, several protein kinases, and at least one protein phosphatase. Reversible protein phosphorylation is recognized as a fundamental regulatory mechanism in cells. The CaM-dependent protein phosphatase, calcineurin, has recently been suggested to play important roles in the control of cell growth and division (2, 3), regulation of gene expression (4, 5), and in response to mating pheromone in the yeast Saccharomyces cerevisiae (6, 7). The holoenzyme is composed of two subunits, the catalytic subunit (CnA), and a Ca2+/binding regulatory subunit (CnB) structurally related to CaM (8-10). While a great deal is known about calcineurin enzymology, it is only recently that inroads have been made into understanding specific roles of the enzyme in vivo.

Recently, it has been shown that calcineurin is a target for immunosuppressant drugs such as FK-506 and cyclosporin (11). In T cells, it appears to function in the activation of the transcriptional regulator NFAT (4, 5), a step requisite for T cell activation and inhibitable by immunosuppressants. In Aspergillus nidulans, a filamentous fungus related to yeast, we have shown that the CnA gene is essential for proliferation and that the mRNA is expressed in a cell cycle-dependent manner (2). In the yeast S. cerevisiae, multiple CnA genes exist, and while it appears they are not essential for vegetative growth, this enzyme appears to play a role in the mating pheromone response pathway (6, 7). Recently, a CnA homologue was isolated from Schizosaccharomyces pombe and gene disruption suggested that it is not strictly required for vegetative growth, although growth was temperature sensitive in a strain lacking the CnA gene (12). However, it was found that the lack of a CnA gene caused a sterile phenotype, suggesting that calcineurin is involved in the mating response in S. pombe.

In this study, we have also cloned the S. pombe CnA gene (designated ppb1). The gene we have isolated is identical to that observed in the previous study (12). Hybridization of a ppb1 probe to an ordered phage library allowed us to determine the location of the ppb1 gene in()S. pombe gene. In addition, we have examined the expression of ppb1 mRNA. We observed a moderate change during the cell cycle in exponentially growing cells. However, when cells are grown in nitrogen-free medium or allowed to reach saturation, CnA mRNA levels are markedly increased. The S. pombe CnA gene promoter contains a cis-acting element (TR element) previously shown to be responsive to the ste11 transcription factor (13). Our studies show that CnA expression during nitrogen starvation is directly dependent on ste11 and suggests that CnA may be an integral component of the signal transduction mechanism that functions during the mating response in yeast.

EXPERIMENTAL PROCEDURES

Culture Conditions—The following strains of S. pombe were used: wild type, cdc5 (h+; cdc25-22, leu1-32); and ste11 (h0, ste11, ura4). All yeast strains were cultured as described (14). Escherichia coli strain DH5α was cultured as described previously (15).

Isolation of the Calcineurin A Homologue of S. pombe—Two mixed oligonucleotides encoding all possible combinations encoding the pep-
tide sequences RGNHEC (5′-oligonucleotide) and MDVFTW (3′-oligo-nucleotide) were used to amplify a CnA homologue by polymerase chain reaction. The oligonucleotides are based on two regions that are completely conserved in all CnA homologues cloned to date. The expected product sizes were approximately 600 bp based on other CnA homologues. The PCR product was subcloned into pGEM3Zf(−) and several clones were sequenced to verify they were CnA homologues. One clone had a 45% identical, so one representative clone was used as a probe to screen an ordered P1 phage genomic library for S. pombe (16).

Nucleic Acid Methods—Total RNA was isolated from S. pombe as described previously (17). Following isolation, RNA was resuspended in sterile water and quantified by A260.

For isolation of genomic DNA, S. pombe cultures containing 3 × 10^7 cells per ml were used for each isolation. Genomic DNA was isolated as described previously (14).

Small-scale preparation of plasmid and cosmid DNA was performed by the alkaline lysis method as described previously using Wizard DNA Clean-Up resin (Promega) (15). For larger scale preparations, alkaline lysis followed by precipitation with polyethylene glycol/NaCl was used (15).

For radiolabeling of DNA hybridization probes, 200 ng of DNA was labeled with [32P]dCTP (Amersham) by the random primer method (15). Labeled DNA was separated from unincorporated nucleotides by G-50 Sephadex chromatography.

For analysis of isolated DNA by Southern blot, standard procedures were used (15). DNA was transferred to Magna Nylon transfer membrane by capillary blotting overnight using 20 × SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0) as the transfer buffer. After transfer, filters were air dried for 30 min, and the DNA cross-linked to the membrane by exposure to UV light (1200 J/cm^2) using a Stratagene Stratalink 2400 (Stratagene). Pre-hybridization and hybridization were as described previously (2).

For Northern blot analysis, previously described methods were used (2). After transfer to Magna Nylon membrane, the RNA was cross-linked to the membrane using UV light (1200 J/cm^2) using a Stratagene Stratalink 2400.

For slot-blot analysis, 10 µg of RNA in sterile water was mixed with an equal volume of 3 volumes of 20 SSC, 2 volumes of formaldehyde and denatured at 68 °C for 15 min. The sample was chilled in ice, and an equal volume of 15 × SSC was added. Blotting onto Magna Nylon transfer membrane was performed according to manufacturer’s specifications using a Mini-fold slot blot apparatus (Tyler Research). For quantitation of pbpl1 mRNA, 2-fold serial dilutions of total RNA samples were prepared, applied to transfer membrane, and hybridized as described above. The autoradiogram was scanned using an Apple Color One scanner set for 256 levels of gray and 300 dpi resolution. The hybridization signals were quantified using the program NIH Image (version 1.55). Determinations of relative mRNA levels were based on regions of the serial dilution series that showed a corresponding 2-fold difference in the calculated intensity, indicating that the signals were within the linear range of the system.

Following cross-linking, filters were prehybridized for 2 h at 42 °C in 50% deionized formamide, 5 × Denhardt’s, 6 × SSC, 0.5% SDS, 100 µg/ml denatured herring sperm DNA solution (2). Hybridization was carried out at the same temperature for 18 h in a fresh solution of the same composition as that used for prehybridization. Probe concentration was 5 × 10^5 cpm/ml. After hybridization, filters were washed five times in 2 × SSC, 0.1% SDS at room temperature, and twice for 15 min in 0.1 × SSC, 0.1% SDS at 55 °C. After washing, filters were covered with Saran Wrap and exposed to Kodak X-OMAT AR at −70 °C using intensifying screens.

DNA sequencing the dye-exchange-mediated chain termination method was applied as described (18) using a Sequenase version 2.0 kit (U. S. Biochemical Corp.) and 32P-dATP (Amersham). Following electrophoresis, the gel was fixed for 10 min in 10% methanol, 10% glacial acetic acid and dried under the vacuum at 80 °C for 45 min. The dried gel was exposed to Kodak X-OMAT AR film at room temperature overnight.

Synchronization of S. pombe—cdc25–22 cells were first grown at the permissive temperature (25 °C) until they reached a density of 5 × 10^7 cells/ml. To synchronize cells, the culture was shifted to the restrictive temperature (35 °C) for 4.25 h resulting in G1 arrest. To release cells, the culture was returned to permissive conditions for 5 h, and cells were harvested from the culture at 20-min intervals. 1-ml samples were fixed with 3.7% formaldehyde and stained with 4',6'-diamidino-2-phenylindole dihydrochloride (Sigma) as described (17) to examine binucleate index as indicators of cell cycle synchrony. For each time point, at least 300 cells were scored. For RNA isolation, 5 × 10^7 cells were washed with sterile water and then frozen in liquid nitrogen.

Nutritional Shifts—S. pombe strain YEG-17 (h+) or a ste11– strain was used to examine pbpl1 mRNA levels under different nutritional conditions. Cells were initially grown to a density of 8 × 10^6 cells/ml and then washed and inoculated into new culture medium at 5 × 10^6 cells/ml. Media used were normal minimal medium as before, low glucose minimal, or nitrogen-free minimal medium (14). For stationary phase cultures, cells were placed in regular minimal medium and allowed to grow to saturation. For the other media, samples were taken at various times, and RNA was isolated and analyzed by slot-blot analysis.

Assay of β-Galactosidase Activity—β-Galactosidase activity was detected in cells containing lacZ reporter constructs as follows. A single colony selected from an EMM (17) agar plate was streaked onto a Whatman filter 1 filter (75-mm circle) overlaid on the appropriate nutrient agar plate. EMM + adenine + leucine was used to look at β-galactosi-dase activity in growing cells while malt extract (17) agar was used to test for expression under conditions that induce mating. After overnight growth, the filter was removed from the plate, immersed in liquid nitrogen to permeabilize the cells, and then laid on top of a second filter saturated with 2 buffer containing X-Gal (150 µM sodium phosphate, pH 7.5, 10 mM KCl, 1 mM MgSO4, 4 mM 2-mercaptoethanol, 0.33 mg/ml X-Gal). The filters were then incubated at 30 °C to allow color development. The filters were then air-dried and imaged by scanning as described above.

RESULTS

Previous studies have suggested that CnA plays important roles in eukaryotic cells, being required for T cell activation and essential for cell division in A. nidulans (2, 4). We were therefore interested in cloning the CnA gene of S. pombe as a prelude to studies examining the role of this enzyme in yeast development. To obtain a hybridization probe for screening genomic DNA libraries, we designed oligonucleotides to use in PCR amplification of a portion of an S. pombe CnA homologue (as described under “Experimental Procedures”). Following PCR amplification, a fragment of the expected size was obtained, which was then subcloned and sequenced. The sequence contained a predicted peptide 75% identical to human and A. nidulans CnA, indicating that we had obtained a portion of the S. pombe gene by PCR.

The PCR-derived clone was then used as a probe to screen an ordered P1 phage library (obtained from Dr. Elmar Maer, Imperial Cancer Research Fund, London) (16). Hybridization of the probe to the filter containing P1 phage DNA resulted in 10 positive signals (Fig. 1). The location of the signals was mapped by a computer-based analysis system at ICRF. From this, a cosmid identified on the P1 phage hybridization pattern. The pbpl1 gene was localized to cosmid 32h9, which maps to chromosome II, between cdc10 and top2 on the S. pombe genetic map (16). Southern blot analysis of genomic DNA using the PCR probe showed that a 2.1-kb EcoRI fragment contained at least part of the CnA gene and that a hybridizing species of identical size was contained in the 32h9 cosmid. This 2.1-kb EcoRI fragment was subcloned and used to further map the cosmid. Our mapping results showed that the pbpl1 gene was entirely contained within two adjacent HindIII fragments in the 32h9 cosmid (Fig. 2). The sequence of the pbpl1 gene from A676 through the 1st exon is shown in Fig. 3.

While this work was in progress, we were in contact with Prof. M. Yanagida (Kyoto University) who informed us that they had also cloned a CnA homologue from S. pombe (12). Comparison of our sequence with theirs showed perfect agreement, indicating that we had cloned the same gene. In that study, it was found that disruption of the CnA gene in S. pombe was not lethal, as we have also observed (data not shown). However, other interesting phenotypes were observed. Overexpression caused a variety of cytological defects in interphase cells, while a pbpl1 null strain was sterile, suggesting a defect in the mating response pathway (12). Because of these effects, we were interested in determining whether expression of the
ppb1 mRNA levels were examined by Northern blot analysis. In addition, the percentage of binucleate cells was monitored to assess the degree of synchrony and the position of S phase, which occurs coincident with the maximum of binucleated cells (14). The results show that ppb1 mRNA levels vary slightly during the cell cycle with maximum levels observed coincident with each S phase (Fig. 4).

Next, we tested whether ppb1 mRNA levels vary during changes in the growth conditions of cells. Since previous studies suggested that ppb1 might be important in responses to mating pheromone in S. cerevisiae (6, 7) and that S. pombe lacking calcineurin is sterile (12), we were interested in determining if ppb1 mRNA expression could be induced by conditions that favor transition to the sexual cycle. Parallel cultures of exponentially growing cells were shifted to nitrogen-free medium, glucose-free medium, or allowed to grow to saturation. RNA was isolated, and ppb1 mRNA levels were determined by slot-blot hybridization of serially diluted total RNA samples. The results indicate that ppb1 mRNA levels are significantly increased when cells are deprived of nitrogen or allowed to grow to saturation as compared to histone H2A mRNA (Fig. 5). Scanning densitometry was used to quantify CnA mRNA levels using these serially diluted RNA samples. The results indicate that ppb1 mRNA levels are increased 8-fold in response to nitrogen starvation. Examination of the time course of induction showed that levels increase as soon as 2 h after shifting to nitrogen-free medium, with maximal levels of expression observed within 8 h after the shift (Fig. 6A). Previous studies have shown that the ste11 transcription factor regulates the expression of some genes in response to nitrogen starvation. The expression of ste11 is inhibited by cAMP via activation of cAMP-dependent protein kinase (13). Regulation of ste11-dependent genes occurs...
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First, the binding to the element (13). Inspection of the cis-acting element (TR element) in the 5'-untranslated region of these genes (13). Inspection of the five upstream sequence revealed a potential TR element starting at position -137 relative to the initiation ATG codon (the TR consensus is TTCTTTGTTY). The putative element in the ppb1 gene promoter matches at 9/10 positions and contains a conserved G residue at position 7 previously shown to be essential for stem binding to the element (13).

To determine if the induction of ppb1 expression during nitrogen starvation is dependent on stem1, we examined ppb1 mRNA levels under two different sets of conditions. First, ppb1 mRNA levels were examined in cells cultured in the presence of cAMP and caffeine. This treatment raises intracellular cAMP levels, which would be expected to inhibit stem1 expression and in turn the expression of stem1-dependent genes. The results show that cells treated in this way fail to increase ppb1 mRNA levels upon nitrogen starvation, suggesting that ppb1 gene expression requires expression of the stem1 transcription factor (Fig. 6B). Next, we examined ppb1 mRNA levels in a stem1 null strain. Using the same experimental protocol as above, we observed that ppb1 mRNA levels did not increase in the stem1 strain upon nitrogen starvation, suggesting that the TR element present in the ppb1 gene promoter is functional and regulates ppb1 expression in vivo (Fig. 6C).

Because these data suggest that stem1 regulates ppb1 expression, we decided to directly test whether the TR element present in the ppb1 gene promoter was functional in vivo or whether ppb1 expression was indirectly regulated by stem1. Two different reporter constructs were constructed as follows. Fragments with 5'-end points either at -157 bp or -127 bp relative to the start of translation and identical 3'-end points (-1) were synthesized by PCR. Each fragment was first subdivided into pGEM3Zf(-) and sequenced to ensure no polymerase-induced mutations had been introduced. The first fragment (-157) contains the TR element, while the second (-127) starts just after the TR element. Each fragment was then ligated 5' to the E. coli lacZ gene, which encodes β-galactosidase. These plasmids also contain an ars sequence, to permit replication in S. pombe, and the S. cerevisiae URA3 gene, to permit selection of cells containing the reporter plasmid.

Each of the reporter constructs was transformed into S. pombe, and ura prototrophs were selected. Cells were then streaked onto filters overlaid on plates containing normal growth medium (EMM) or medium that induces mating (malt extract agar). The filters were then removed, and the presence of β-galactosidase was assayed as described under “Experimental Procedures.” As can be seen (Fig. 7), both constructs direct...
the expression of low levels of β-galactosidase when cells are grown on EMM. On medium that induces mating, the −157 construct produced higher levels of β-galactosidase, as indicated by the darker staining. This result is consistent with the previously observed induction of ppb1 mRNA expression upon nitrogen starvation. In contrast, the levels of β-galactosidase in cells containing the −127 construct were unchanged. These data directly demonstrate that the TR element in the ppb1 gene is functional in vivo and that the ppb1 gene is directly regulated by the ste11 transcription factor during the mating response.

**DISCUSSION**

In this study, we have cloned the S. pombe homologue of the calmodulin-dependent protein phosphatase catalytic subunit (calcineurin A) by screening an ordered P1 phage library with a genomic fragment produced by PCR. Mapping of P1 phage containing the ppb1 gene allowed mapping of the ppb1 gene to chromosome II between the cdc10 and top1 genes. In the earlier study, the ppb1 gene was said to be localized to chromosome I, 200 kb away from the ste11 gene (12). The reason for the discrepancy with our mapping is unclear. Our sequence exactly matches that of the one from Prof. Yanagida’s group (12), so it is not another calcineurin gene, and the cosmids that contains our ppb1 gene was obtained from ICRF after they had interpreted the P1 phage hybridization pattern. Thus, it would seem that one of the ordered libraries is not correct.

The ppb1 gene product is highly conserved relative to other calcineurin homologues, being over 70% identical to S. cerevisiae (6), human (19), and Neurospora crassa (20) calcineurin homologues within the conserved catalytic domain. Unlike S. cerevisiae, we have no evidence of other CnA homologues in S. pombe based on low stringency hybridization or during our original PCR reactions, where we consistently obtained a single product during amplification when using degenerate oligonucleotides. However, ppb1 gene disruption was shown not to be lethal (12). This might suggest that there are redundant CnA genes in S. pombe or that this protein phosphatase is not essential for vegetative growth. This would be in contrast to our previous studies in A. nidulans, where we observed that CnA is an essential gene (2), but consistent with studies in the yeast S. cerevisiae (6).

Examination of ppb1 mRNA levels during the cell cycle showed moderate change, similar to what has been observed in A. nidulans, where CnA mRNA levels increased prior to S phase (2). Based on this, it is not clear why overexpression of ppb1 would have such drastic cytological effects as previously reported (12). We have observed that expression of a truncated, CaM-independent form of mouse CnA in S. pombe has no effects on cells. It is possible, therefore, that overexpression of the full-length protein might have other effects due to binding to CaM or other target proteins. In the previous study, the authors apparently used the normal version of the thiamine-regulated nmt1 promoter present in the plasmid pREP1, and so it is possible that the exceptionally high levels of expression obtained using this promoter might have nonspecific effects. Further, more detailed studies should be able to resolve this issue.

We have found that the ppb1 mRNA is induced in response to nitrogen starvation, which is a primary signal in regulating the mating response in yeast (21). Several genes are known to be induced by nitrogen starvation, of which a subset has been shown to be dependent on the ste11 transcription factor, itself a nitrogen starvation-induced gene (13, 22–24). The ste11 gene product regulates expression by a 10-bp cis-acting element (TTCTTTGTTY) known as a TR element. Inspection of the ppb1 gene promoter revealed that it contains a putative ste11 regulatory element, matching at 9/10 positions. Our observation that nitrogen starvation does not induce ppb1 mRNA expression in a strain lacking a functional ste11 gene supports the conclusion that the ppb1 gene does contain a functional TR element and is regulated during nitrogen starvation by the ste11 transcription factor. Lack of this element results in a loss of the nitrogen starvation-dependent increase in expression, clearly showing that nitrogen starvation-induced ppb1 expression is directly dependent on the ste11 transcription factor.

Significantly, a protein phosphatase in S. cerevisiae homologous to the vaccinia virus VH-1 gene product and S. pombe cdc25 protein phosphatase, has also been shown to be induced by nitrogen starvation (25). Calcineurin levels also increase in response to treatment of S. cerevisiae cells with the α-factor-mating pheromone (7). The precise role either for the VH-1 phosphatase homologue or the calcineurin gene in the S. pombe mating response is unknown. It has been shown that mating and sporulation are inhibited by at least two protein kinases. The S. pombe cAMP-dependent protein kinase (the pka1 gene of S. pombe) has been previously shown to inhibit ste11 gene expression, in turn inhibiting the expression of ste11-dependent genes required for mating (13). A second protein kinase, pat1/ran1, also inhibits mating in S. pombe (26). In this case the effect is mediated via the mei2 gene, which is dispensable for the mating response. Thus, the role of protein phosphatases may be to antagonize protein kinases, which might otherwise inhibit the mating response. It will be important to determine if substrates either for pka1 or pat1 kinases are dephosphorylated by calcineurin.

In S. cerevisiae, it has been suggested that CnA antagonizes the mating pheromone response pathway. This conclusion is based on the demonstration that cells lacking the cna1 and cna2 genes are unable to recover from α-factor-induced arrest (6). This apparent disparity in roles for CnA in the two yeast may be a consequence of the different modes of mating. In S. pombe, mating is induced by starvation, while in S. cerevisiae it occurs in rich medium (21, 27). In addition, the expression of the CnA homologues has not been examined in S. cerevisiae, so it is unknown if CnA expression increases during the mating response as we have shown in S. pombe.

It is possible that the role of CnA is as part of a gene expression cascade involved in regulating the expression of genes specific to the sexual cycle. It has been shown in T cells that CnA activity is required for T cell activation (4). In this system, the role of CnA is to dephosphorylate the factor NFAT, which is an upstream regulator of interleukin gene expression.

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2. D. Plochocka-Zulinska, G. Rasmussen, and C. Rasmussen, unpublished results.
(4, 5). It is possible, therefore, that CnA is a regulator of gene expression, downstream of ste11, and responsible for controlling other genes essential for the mating response.

Consistent with this is the observation that ppb1 mRNA levels remain elevated during nitrogen starvation, maintaining peak levels 24 h after the switch to nitrogen-poor media. Not all nitrogen starvation-induced genes have this response. The res2 gene is induced within 6 h by nitrogen starvation, but levels decline by 12 h (23). In addition, ppb1 mRNA is increased in haploid cells, suggesting that CnA may have a role early in the mating response pathway, prior to formation of the zygote. Finally, it has been shown that the loss of the calcineurin gene causes sterility in S. pombe (12). This suggests that calcineurin plays an essential role in the sexual cycle of this organism. The observation that ppb1 mRNA levels are induced by nitrogen starvation and remain elevated for a prolonged period suggests that CnA may be required throughout mating, meiosis, and sporulation. Future studies will examine the specific role of the ppb1 gene in each of these processes.

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REFERENCES

1. Means, A. R., VanBerkum, M. F. A., Bagchi, I., Lu, K. P., and Rasmussen, C. D. (1991) Pharmacol. Ther. 50, 255–270
2. Rasmussen, C., Garen, C., Brining, S., Kincaid, R. L., Means, R. L., and Means, A. R. (1994) EMBO J. 13, 3917–3924
3. Cunningham, K. W., and Fink, G. R. (1992) J. Cell Biol. 124, 351–363
4. O'Keefe, S. J., Tamura, J., Kincaid, R. L., Tod, M. J., and Oneill, E. A. (1992) Nature 357, 692–694
5. Woodrow, M., Clipstone, N. A., and Cantrell, D. (1993) J. Exp. Med. 178, 1517–1522
6. Cyert, M. S., Kunisawa, R., Kain, D., and Thorner, J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7376–7380
7. Cyert, M. S., and Thorner, J. (1992) Mol. Cell. Biol. 12, 3460–3469
8. Stewart, A. A., Ingebritsen, T. S., Manalan, A., Klee, C. B., and Cohen, P. (1982) FEBS Lett. 137, 80–84
9. Stewart, A. A., Ingebritsen, T. S., and Cohen, P. (1983) Eur. J. Biochem. 132, 289–295
10. Klee, C. B., Draetta, G. F., and Hubbard, M. J. (1988) in Advances in Enzymology and Related Areas of Molecular Biology (Meister, A., ed) Vol. 61, pp. 149–209. John Wiley & Sons, New York
11. Liu, J., Farmer, J. D., Jr., Lane, W. S., Friedman, J., Weissman, I., and Schreiber, S. L. (1991) Cell 66, 807–815
12. Yoshida, T., Toda, T., and Yanagida, M. (1994) J. Cell Biol. 107, 1725–1735
13. Sugimoto, A., Iino, Y., Maeda, T., Watanebe, Y., and Yamamoto, M. (1991) Genes & Dev. 5, 1990–1999
14. Alfa, C., Fantes, P., Hyams, J., McLeod, M., and Warbrick, E. (1992) Experiments with Fission Yeast: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
15. Maniatis, T., Fritsch, E., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
16. Hohiels, J. D., Maier, E., Mott, R., McCarthy, L., Grigoriev, A. V., Schalkwyk, L. C., Nizetic, D., Francis, F., and Lehrbach, H. (1993) Curr. Biol. 3, 109–120
17. Moreno, S., Klar, A., and Nurse, P. (1991) Methods Enzymol. 194, 795–823
18. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
19. Guerin, D., and Klee, C. B. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9183–9187
20. Higuchi, S., Tamura, J., Giri, P. R., Polli, J. W., and Kincaid, R. L. (1991) J. Biol. Chem. 266, 18104–18112
21. Egel, R. (1989) in Molecular Biology of Fission Yeast (Nasim, A., Young, P., and Johnson, B. F., eds) pp. 31–73, Academic Press, Inc., New York
22. Okazaki, N., Okazaki, K., Tanaka, K., and Okuyama, H. (1991) Nucleic Acids Res. 19, 7043–7047
23. Miyamoto, M., Tanaka, K., and Okuyama, H. (1994) EMBO J. 13, 1873–1880
24. Sugiyama, A., Tanaka, K., Okazaki, K., Nogawa, H., and Okuyama, H. (1994) EMBO J. 13, 1881–1887
25. Guran, K., Hakes, D. J., Wang, Y., Park, H.-D., Cooper, T. G., and Dixon, J. E. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 12175–12179
26. Egel, R., Nilsson, O., and Welgyn, D. (1990) Trends Genet. 6, 369–373
27. Thorner, J. (1981) in The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) pp. 143–180, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
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