Cloning and Characterization of the Murine Nek3 Protein Kinase, a Novel Member of the NIMA Family of Putative Cell Cycle Regulators*

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We have cloned and characterized murine Nek3 (NIMA-related kinase 3), a novel mammalian gene product structurally related to the cell cycle-regulatory kinase NIMA of Aspergillus nidulans. By RNase protection, low levels of Nek3 expression could be detected in all organs examined, regardless of proliferative index. In contrast to Nek1 and Nek2, Nek3 levels were not particularly elevated in either the male or the female germ line. Nek3 levels showed at most marginal variations through the cell cycle, but they were elevated in G2-arrested, quiescent fibroblasts. Furthermore, no cell cycle-dependent changes in Nek3 activity could be detected, and no effects upon cell cycle progression could be observed upon antibody microinjection or overexpression of either wild-type or catalytically inactive Nek3. Finally, Nek3 was found to be a predominantly cytoplasmic enzyme. These data indicate that Nek3 differs from previously characterized Neks with regard to all parameters investigated, including organ specificity of expression, cell cycle dependence of expression and activity, and subcellular localization. Hence, the structural similarity between mammalian Neks may not necessarily be indicative of a common function, and it is possible that some members of this kinase family may perform functions that are not directly related to cell cycle control.

Cyclin-dependent kinases (Cdks) are well established as key regulators of the eukaryotic cell cycle (1–3). In addition, genetic data demonstrate a cell cycle-regulatory role for several protein regulators of the eukaryotic cell cycle (1–3). In contrast to Nek1 and Nek2, Nek3 levels were not particularly elevated in either the male or the female germ line. Nek3 levels showed at most marginal variations through the cell cycle, but they were elevated in G2-arrested, quiescent fibroblasts. Furthermore, no cell cycle-dependent changes in Nek3 activity could be detected, and no effects upon cell cycle progression could be observed upon antibody microinjection or overexpression of either wild-type or catalytically inactive Nek3. Finally, Nek3 was found to be a predominantly cytoplasmic enzyme. These data indicate that Nek3 differs from previously characterized Neks with regard to all parameters investigated, including organ specificity of expression, cell cycle dependence of expression and activity, and subcellular localization. Hence, the structural similarity between mammalian Neks may not necessarily be indicative of a common function, and it is possible that some members of this kinase family may perform functions that are not directly related to cell cycle control.

The mammalian kinase most closely related to NIMA is Nek2, and accordingly, Nek2 has received most attention so far. Similar to NIMA, Nek2 is cell cycle-regulated in terms of both abundance and activity (17, 20). However, whereas NIMA has been implicated predominantly in chromatin condensation (11), recent studies indicate that one important function of Nek2 relates to the centromere cycle (21). Nek2 localizes to the centromere, and overexpression of active Nek2 profoundly affects centromere structure in cultured cells. Nek2 is highly expressed in testis (22–24), and the same is true for Nek1 (15). Interestingly, however, Nek1 and Nek2 display different expression patterns during spermatogenesis, suggesting that they may perform distinct functions.

We have previously isolated a partial cDNA for human Nek3 (17), but this cDNA lacked the coding information for the N-terminal end domain, and no biochemical studies on the Nek3 protein have been reported. Here, we describe the molecular cloning and biochemical characterization of a 56-kDa murine protein kinase, termed mNek3, that almost certainly represents a new kinase family member.
Cloning and Characterization of Murine Nek3 Protein Kinase

MATERIALS AND METHODS

cDNA Cloning and Mutagenesis—A mouse brain cDNA library in λZap II (Stratagene) was screened with a human Nek3 cDNA fragment (XhoI-XhoI, nucleotides 617–1555; Ref. 17), labeled with [α-35S]dCTP (NEN Life Science Products). Phages were converted into BlueScript (SK) by in vivo excision, and inserts were sequenced. One plasmid, termed pBS-mNek3, was found to contain the entire coding sequence of mouse Nek3. A PstII-XhoI fragment excised from pBS-mNek3 was inserted into the PstI site of a BlueScript-myc vector carrying the myc epitope tag (22). Plasmids pBS-myc-Nek3 and myc-tagged mNek3 were inserted into the HindIII-XhoI fragment and excised into the eukaryotic expression vector pRcCMV (Invitrogen Corp.), to generate pCMV-myc-Nek3. The affinity-purified anti-Nek2 antibody D5 (26), and the affinity-purified anti-Cdc2 antibody AR8 (27) from those determined previously for Nek2. Thus, despite their structural similarities, mammalian NIMA-related kinases may well perform widely different functions.

Immunoprecipitations and in Vitro Kinase Assays—In vitro translated mNek3 or whole cell lysates were diluted 10 times with NEB buffer, and subjected to immunoprecipitation using anti-Nek3 antibody (5F266) or nonspecific mouse IgG (Sigma), both at 20 μg/ml, and incubated with protein G-Sepharose beads (Amersham Pharmacia Biotech). Immunoprecipitates were washed three times with 0.5× NEB buffer and twice with 50 mM Hepes/KOH (pH 7.4), 5 mM MnCl2, and incubated for 30 min at 30 °C in a total volume of 30 μl of 50 mM Hepes/KOH (pH 7.4), 5 mM MnCl2, 5 mM MgCl2, 5 mM β-glycerophosphate, 5 mM NaF, 7.5 μg/ml heparin, 1 μM dithiothreitol, 0.5 μM ATP, 5 μg of [γ-32P]ATP (3000 Ci/mmol, Amersham Pharmacia Biotech), in the presence of casein as a substrate (1.4 mg/ml). Kinase reactions were stopped by addition of 0.5 volumes of gel sample buffer. Samples were boiled for 5 min, and proteins separated by SDS-PAGE.

RESULTS

Cloning and Sequencing of a Mouse Nek3 cDNA—To isolate the mouse homologue of human Nek3, a mouse brain cDNA library was screened with a human Nek3 cDNA fragment of two independent phages isolated from 108 plaques, one carried a cDNA insert that encompassed the other. Sequencing of the longer cDNA revealed a single open reading frame, coding for a 511-amino acid protein (GenBank™ accession number AF093416). As described below, this protein almost certainly represents the murine homologue of human Nek3, and, therefore, we refer to it as murine Nek3 (mNek3). Although the presumptive 5′-untranslated region of this cDNA lacks an in-frame stop codon, we believe that the first methionine represents the initiating methionine of mNek3, and that the isolated cDNA codes for the entire mNek3 protein. First, the nucleotide sequence surrounding this methionine conforms to the Kozak consensus sequence for initiation methionines (30). Second, and more importantly, the in vitro translated mNek3 protein was found to comigrate exactly with the endogenous mNek3 protein detectable by anti-Nek3 antibodies in Swiss 3T3 whole cell lysates (see Fig. 3A).

The mNek3 protein displays a typical serine/threonine protein kinase domain within its N-terminal half. Data base searches revealed that this domain shares greatest similarity with NIMA-related kinases, as expected. Within the C-terminal non-catalytic domain, however, there were no obvious similarities to any known proteins, except for human Nek3. As shown in Fig. 1, mNek3 shows 74% overall identity to human Nek3. The published human Nek3 sequence lacks the first two kinase subdomains, but over the remainder of the catalytic domain, human and murine Nek3 show 90% identity. In comparison, the catalytic domain of mNek3 shows significantly lower degrees of identity to murine Nek1 (55%), Nek2 (43%), or Stk2 (44%), and it shows 42% identity with A. nidulans NIMA. Concerning the non-catalytic C-terminal end domains of...
Identification of RNA (Fig. 2A) was obtained, although the differences between organs were quan-
titatively less pronounced (compare Fig. 2B with 2A); again, highest levels of Nek3 mRNA were seen in intestine, while tests and ovaries showed no particularly high levels of Nek3 expression. This latter result is in striking contrast to data reported previously for murine Nek1 and Nek2. Both of these kinases are in fact highly expressed in testis, although their precise expression patterns during spermatogenesis differ (15, 22–24).

To extend the above expression studies to the protein level, an antibody (PepN3) was raised against a peptide that is highly conserved between mouse and human Nek3 (see Fig. 1). When tested by immunoblotting on total extracts from G0-arrested Swiss 3T3 cells, the PepN3 antibody recognized a single protein migrating at approximately 56 kDa (Fig. 3A). This band comigrated exactly with the protein migrating at approximately 56 kDa (Fig. 3A) and 2B, and was recognized by the PepN3 antibody (Fig. 3A, lanes 1 and 2), confirming that the coding sequence reported here almost certainly represents the complete mNek3 protein. The PepN3 immune reaction was completely abolished by pre-incubation of PepN3 with the immunogenic peptide, pepN3, but not by incubation with a control peptide, pepN2 (Fig. 3A, lanes 4 and 5, respectively). Finally, a single band migrating at the exact same position was also revealed when immunoblots were performed on Swiss 3T3 total cell lysates with the monoclonal anti-Nek3 antibody 5F2G6 (Fig. 3A, lane 6).

Fig. 3B shows the abundance of Nek3 protein in murine intestine and testis, respectively (central panel). For comparison, the left-hand panel shows the protein profiles of these samples, as revealed by Coomasie Blue staining, confirming equal loading. The right-hand panel shows the results of probing the same samples with antibodies against Nek1, one of the Neks shown previously to be expressed predominantly in testis (22–24). From these data it is clear that Nek3 is expressed at comparable levels in both testis and intestine, in striking contrast to Nek2. It is also noteworthy that the Nek3 expression seen at the protein level does not strictly parallel that seen at the mRNA level (compare Figs. 3B and 2A). This suggests that posttranscriptional mechanisms may contribute to control the
abundance of Nek3 protein in particular cell types.

**Nek3 Protein Expression during the Cell Cycle—**As NIMA-related kinases might be expected to play a role in cell cycle regulation, we examined the protein level of mNek3 throughout the cell cycle. To this end, extracts were prepared from synchronized Swiss 3T3 fibroblasts and analyzed by immunoblotting with the PepN3 antibody. Cells were synchronized in G₀ by serum starvation, in G₂ by release from the aphidicolin block, in M phase by nocodazole treatment, and in early G₁ phase by release from the nocodazole block. Exponentially growing cells were analyzed in parallel. Cell extracts were normalized for protein content, before the amounts of mNek3 translated mNek3 were used. Wild-type mNek3 and a mutant form of mNek3, carrying a point mutation in the putative ATP binding site (mNek3-D143A), were produced in a reticulocyte lysate, and samples were diluted into NBP buffer containing 0.1% deoxycholic acid. (For presently unknown reasons, the presence of this detergent was found to be critical for our ability to measure mNek3 kinase activity in vitro.) Following immunoprecipitation of the translation products, kinase assays were performed in the presence of β-casein, a protein previously shown to be a good substrate for several NIMA-related kinases (15, 20, 32, 33). Fig. 5 shows that both wild-type mNek3 and the catalytically inactive mNek3-D143A mutant were produced at similar levels (panel A), yet only wild-type mNek3 showed strong kinase activity toward β-casein, while mNek3-D143A was inactive (panel B). Likewise, only wild-type mNek3 displayed apparent autophosphorylation activity (panel B, lane 1). These data show that the above assay conditions allow a reliable measurement of mNek3 activity.

To determine the activity of endogenous mNek3 through the cell cycle, mNek3 activity was measured in immunoprecipitates prepared from Swiss 3T3 fibroblasts synchronized at different stages of the cell cycle (Fig. 5D, lanes labeled a). For each sample, the recovery of mNek3 was monitored by immunoblotting (Fig. 5C, lanes labeled a). As a control for specificity, parallel analyses were performed on immunoprecipitates prepared from each sample using control mouse IgG (Fig. 5, C and D, lanes labeled c). As shown in Fig. 5C, roughly similar...
amounts of mNek3 were recovered from exponentially growing cells (Expo) and from cells synchronized at particular cell cycle stages (as indicated), although a slightly higher amount of mNek3 was precipitated from G0-arrested cells, consistent with the data shown in Fig. 4A. As is evident from Fig. 5D, mNek3 activity roughly paralleled the amount of mNek3 protein present in each sample, and thus showed little variation through the cell cycle. These data also show clearly that mNek3 is active in lysates prepared from G0-arrested cells. Attesting to the specificity of these immunoprecipitations and activity measurements, no mNek3 protein and virtually no β-casein kinase activity were detectable in the control samples (Fig. 5, C and D, lanes labeled c). Taken together, these data strongly indicate that mNek3 activity is determined largely, if not exclusively, at the level of protein expression, and they provide no evidence for posttranslational regulation of mNek3 kinase activity during the cell cycle. Most importantly, both mNek3 protein and activity levels were largely constant throughout the cell cycle, but increased in quiescent cells.

**Subcellular Localization of mNek3**—We have attempted to localize endogenous mNek3 protein by indirect immunofluorescence microscopy, both in cultured cells and on cryostat sections prepared from mouse intestine. However, although a variety of standard fixation procedures were used, we have so far been unable to detect a signal that could reliably be attributed to mNek3. It is possible that this reflects the low abundance (Fig. 2) and rather uniform distribution (see below) of mNek3. Alternatively, we cannot exclude the possibility that the epitopes recognized by presently available antibodies may be masked in vivo. To overcome this difficulty and obtain some information about the subcellular localization of mNek3, we have taken two approaches. First, indirect immunofluorescence microscopy was used to study the localization of myc epitope tagged mNek3 after ectopic expression; and second, the partitioning of endogenous mNek3 was determined after biochemical fractionation of cultured cells.

In the first type of experiment, cDNAs encoding myc epitope-tagged wild-type or catalytically inactive mNek3 were introduced by transient transfection into human U2OS osteosarcoma cells, and the corresponding proteins detected with a monoclonal antibody against the myc epitope. Both myc-mNek3 (Fig. 6A) and myc-mNek3-D143A (data not shown) were found to be diffusely distributed throughout the cytoplasm. As the related kinase Nek2 has recently been shown to localize to centrosomes (21), we have carefully examined
whether a similar localization might be discernible for mNek3. However, co-staining with centrosomal markers provided no evidence for an association of mNek3 with centrosomes (data not shown).

To corroborate these findings and extend them to endogenous mNek3, subcellular fractionation experiments were performed. When Swiss 3T3 fibroblasts were subjected to a fractionation procedure that allows the separation of nuclear and cytoplasmic proteins (29), mNek3 was found predominantly in the cytoplasmic fraction, although minor amounts could also be seen in the nuclear fraction (Fig. 6B, top panel). Reprobing of the same fractions with an antibody against an small nuclear ribonucleoprotein (26) revealed a predominantly nuclear localization, as expected, while α-tubulin could be detected in both fractions (Fig. 6B, middle and bottom panels). These results confirm that mNek3 is a predominantly cytoplasmic protein, although a quantitatively minor population may also be present in the nucleus. Furthermore, our data do not exclude the possibility that a fraction of mNek3 might associate with cellular membranes.

**Antibody Injection and Kinase Overexpression Experiments**—To directly address a possible function of mNek3 in cell cycle regulation, two types of experiments were performed. First, we have microinjected polyclonal anti-Nek3 antibodies into Swiss 3T3 cells, and scored the injected cells for their ability to undergo one or two successive cell divisions. Anti-Nek3 antibody-injected cells were found to divide as efficiently and with the same kinetics as cells injected with control rabbit IgG (data not shown). Thus, this assay provided no evidence to suggest a requirement for Nek3 during cell cycle progression. We cannot exclude that the antibodies used in these experiments were unable to neutralize Nek3 function, but we note that a very similar approach has readily allowed us to demonstrate a requirement for another kinase, Polo-like kinase 1, during cell division (34). In a second series of experiments, we have overexpressed myc epitope-tagged versions of either wild-type or catalytically inactive mutant Nek3 in U2OS cells. After 22 h, the ability of transfected cells to undergo DNA replication was determined by a 1-h pulse labeling with bromodeoxyuridine (BrdUrd). As a positive control, the Cdk inhibitor p27Kip1 was overexpressed in parallel. When monitoring BrdUrd-incorporation in cells overexpressing wild-type Nek3 or catalytically inactive Nek3, the percentage of BrdUrd-positive cells was found to be very similar to that in surrounding cells (data not shown). In contrast, BrdUrd incorporation was almost completely suppressed in cells in which a G1 block had been imposed by overexpression of p27Kip1, as expected (data not shown). These results do not rigorously exclude a cell cycle function for Nek3, but they certainly lend no support for such a function.

**DISCUSSION**

In *A. nidulans*, NIMA is an essential gene, and the NIMA kinase appears to be a key regulator of the G2 to M transition (reviewed in Refs. 11, 35, and 36). The precise molecular function of NIMA remains uncertain, but the kinase has been implicated in mediating chromatin condensation (37), DNA structure checkpoint signaling (38), and, most recently, the nuclear localization of the Cdc2/cyclin B complex (39). To what extent these proposed roles reflect a single underlying function of NIMA remains to be determined. With Nek1, a functional homologue of NIMA has been described in *N. crassa* (18). Kinases structurally related to NIMA have also been identified in budding yeast and fission yeast, but these kinases, termed Kin3p/Npk1p (12, 13) and fin1 (14), respectively, are non-essential. The function of Kin3p/Npk1p is largely unknown, but fin1 has been proposed to play a role in chromatin condensation (14), in line with early studies on NIMA.

Whether bona fide functional homologues of NIMA exist in mammals remains unclear, but several NIMA-related kinases (Neks) have been identified. Sequencing of cDNA fragments indicates that the mammalian genome harbors at least six distinct Neks (35). It is an important task, therefore, to determine whether these kinases perform unique, related, or redundant functions. On the one hand, it is possible that different mammalian Neks may all perform NIMA-related functions, but that, during evolution, these kinases may have assumed specialized tasks during the cell cycle, or be expressed specifically in particular organs or at particular developmental stages. Alternatively, it would be premature to exclude the possibility that Neks might perform fundamentally distinct functions. Some Neks might functionally resemble NIMA, but others might regulate entirely different processes, not necessarily related to cell cycle progression altogether. Precedents for both scenarios exist in the Cdk family. In line with the first viewpoint, a single major Cdk regulates cell cycle progression in yeast, whereas multiple Cdks cooperate in mammals. In line with the second viewpoint, most Cdks control cell cycle progression, but some appear to function primarily in transcription (reviewed in Refs. 3, 40, and 41).

In this study, we have characterized mNek3, a novel member of the mammalian Nek family. mNek3 resembles the other known mammalian Neks in that it carries the catalytic domain at the N terminus. In the C-terminal non-catalytic domain, however, mNek3 differs from the other Neks (as well as from NIMA and the yeast NIMA family members Kin3p/Npk1p and fin1) in that it lacks predicted coiled-coil regions. In fact, the C-terminal end domain of mNek3 shows no detectable similarity to any other NIMA family member, except for the putative human Nek3 homologue (17).

RNase protection experiments suggest that mNek3 is expressed at rather low levels (in the order of 10 copies of mRNA/cell) in all organs analyzed. When data were calibrated on a per cell basis (i.e. standardized for DNA equivalents; Ref. 31), highest expression of mNek3 was seen in the small intestine; when calibrated for equivalent amounts of RNA, relatively high expression was seen in small intestine, thymus, lung, and spleen. Regardless of the precise mode of data analysis, however, no particularly high expression of mNek3 could be seen in either the male or the female germ line. This result was confirmed also by comparing the amounts of mNek3 protein present in intestine and testis. It is clear, therefore, that the organ specificity of mNek3 expression differs very markedly from that of Nek1 and Nek2, both of which were recently shown to be expressed to far higher levels in the germ lines than in any other mammalian tissue (15, 22–24).

Considering the role of NIMA in cell cycle regulation, it was of considerable interest to determine the expression and activity of mNek3 at various stages of the cell cycle. Immuno-blotting experiments, performed on lysates prepared from synchronized Swiss 3T3 fibroblasts, revealed that mNek3 levels vary at most marginally during the cell cycle, and no evidence could be obtained for posttranslational modifications that would result in altered gel electrophoretic mobility. Interestingly, however, we found that mNek3 protein levels were 2–3-fold higher in G2-arrested, quiescent cells than in proliferating cells. This somewhat surprising result contrasts with data reported for other NIMA family members. In particular, both NIMA and Nek2 levels vary drastically through the cell cycle (20, 32).

Using β-casein as an exogenous substrate, we have also measured mNek3 activity during the cell cycle in Swiss 3T3 fibroblasts. We found that mNek3 activity essentially paral-
leled the amounts of mNek3 protein, suggesting that mNek3 may be regulated primarily, and perhaps exclusively, at the level of protein expression. It remains possible, of course, that accessory proteins and/or posttranslational modifications may modulate mNek3 activity in vivo, but that such hypothetical regulators or modifications may have been lost during cell solubilization and/or immunoprecipitation.

Finally, we have examined the subcellular localization of mNek3. While we have been unable to reliably detect endogenous mNek3 by immunofluorescence microscopy, a myc epitope-tagged mNek3 was found to localize predominantly to the cytoplasm. Furthermore, a predominantly cytoplasmic localization was observed for endogenous mNek3, when subcellular fractionation was combined with immunoblotting. Our data do not exclude the possibility that mNek3 may also associate with membranes, or that minor amounts of this kinase may be present in the nucleus. They clearly indicate, however, that mNek3 is not associated with centrosomes. This is interesting in view of our recent finding that mammalian Nek2 localizes to centrosomes and functions in relation to the centrosome cycle (21).

In conclusion, we have shown that murine Nek3 differs in virtually all aspects examined from both NIMA and Nek2, the mammalian kinase most closely related to NIMA. In particular, Nek3 displays no major changes in either abundance or activity during the cell cycle, and antibody microinjection as well as overexpression experiments provide no evidence for a cell cycle-related function. While the precise molecular function has not yet been determined for any of the NIMA family members, our present study, in conjunction with previous work on Nek1 and Nek2 (15, 17, 20, 21), clearly indicates that the mammalian Nek family members display widely different properties. Taken at face value, the available evidence is difficult to reconcile with the idea that all mammalian Neks functionally resemble each other and/or Aspergillus NIMA. Instead, the emerging picture suggests that kinases structurally related to NIMA may have assumed rather disparate functions during evolution. To positively identify these functions remains a major challenge for the future.

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REFERENCES
1. Norbury, C., and Nurse, P. (1992) *Ann. Rev. Biochem.* **61**, 441–470
2. Nigg, E. A. (1995) *Bioessays* **17**, 471–480
3. Morgan, D. O. (1997) *Annu. Rev. Cell Dev. Biol.* **13**, 261–291
4. Osmani, S. A., Pu, R. T., and Morris, N. R. (1998) *Cell* **53**, 237–244
5. Osmani, A. H., McGuire, S. L., and Osmani, S. A. (1991) *Cell* **67**, 283–291
6. Oakley, B. R., and Morris, N. R. (1983) *J. Cell Biol.* **96**, 1155–1158
7. Bergen, L. G., Upshall, A., and Morris, N. R. (1984) *J. Bacteriol.* **159**, 114–119
8. Ye, X. S., Xu, G., Pu, R. T., Fincher, B. R., McGuire, S. L., Osmani, A. H., and Osmani, S. A. (1995) *EMBO J.* **14**, 986–994
9. O’Connell, M. J., Norbury, C., and Nurse, P. (1994) *EMBO J.* **13**, 4926–4937
10. Lu, K. P., and Hunter, T. (1995) *Cell* **81**, 415–424
11. Osmani, S. A., and Ye, X. S. (1996) *Biochem. J.* **317**, 633–641
12. Schweitzer, B., and Philippsen, P. (1992) *Mol. Gen. Genet.* **234**, 164–167
13. Jones, D. G., and Rosamond, J. (1990) *Gene (Amst.)* **90**, 87–92
14. Krien, M., Bugge, S., Palatsides, M., Assouline, G., Morimyo, M., and Connell, M. (1998) *J. Cell Sci.* **111**, 967–976
15. Letwin, K., Mizzen, L., Motro, B., Ben-David, Y., Bernstein, A., and Parvinen, M. (1992) *EMBO J.* **11**, 3521–3531
16. Levedakou, E. N., He, M., Baptist, E. W., Craven, R. J., Caner, W. G., Welch, P. L., Simmons, A., Naylor, S. L., Leach, R. J., Lewis, T. B., Bowcock, A., and Liu, R. T. (1994) *Oncogene* **9**, 1977–1988
17. Schultz, S. J., Fry, A. M., Sutterlin, C., Ried, T., and Nigg, E. A. (1994) *Cell Growth Differ.* **5**, 625–635
18. Pu, R. T., Xu, G., Wu, L., Vierula, J., O’Donnell, K., Ye, X. S., and Osmani, S. A. (1995) *J. Biol. Chem.* **270**, 18110–18116
19. Fry, A. M., and Nigg, E. A. (1995) *Methods Enzymol.* **283**, 270–282
20. Fry, A. M., Schultz, S. J., Bartek, J., and Nigg, E. A. (1995) *J. Biol. Chem.* **270**, 12989–12995
21. Fry, A. M., Meraldi, P., and Nigg, E. A. (1998) *EMBO J.* **17**, 470–481
22. Rhee, K., and Wolgemuth, D. J. (1997) *Development* **124**, 2167–2177
23. Tanaka, K., Pavlichenin, M., and Nigg, E. A. (1997) *Exp. Cell Res.* **237**, 264–274
24. Arama, E., Yanai, A., Klifon, G., Bernstein, A., and Motro, B. (1998) *Oncogene* **16**, 1813–1823
25. Schmidt-Zachmann, M. S., and Nigg, E. A. (1993) *J. Cell Sci.* **105**, 799–806
26. Reuter, R., Lehner, C. F., Nigg, E. A., and Luftman, R. (1986) *FEBS Lett.* **201**, 25–30
27. Krek, W., and Nigg, E. A. (1991) *EMBO J.* **10**, 305–316
28. Krek, W., and Nigg, E. A. (1991) *EMBO J.* **10**, 3331–3341
29. Grayson, J., Williams, R. S., Yu, Y. T., and Bassel-Duby, R. (1995) *Mol. Cell. Biol.* **15**, 1870–1878
30. Kozak, M. (1996) *Mamm. Genome.* **7**, 563–574
31. Schmidt, E. E., and Schibler, U. (1995) *J. Cell Biol.* **128**, 467–483
32. Osmani, A. H., O’Donnell, K., Pu, R. T., and Osmani, S. A. (1993) *EMBO J.* **10**, 2669–2679
33. Lu, K. P., Osmani, S. A., and Meas, A. R. (1993) *J. Biol. Chem.* **268**, 8769–8776
34. Lane, H. A., and Nigg, E. A. (1996) *J. Cell Biol.* **135**, 1701–1713
35. Lu, K. P., and Hunter, T. (1995) *Prog. Cell Cycle Res.* **1**, 187–205
36. Fry, A. M., and Nigg, E. A. (1995) *Curr. Biol.* **5**, 1122–1125
37. Osmani, A. H., Kogle, D. B., Doonan, J. H., and Morris, N. R. (1988) *Cell* **52**, 241–251
38. Ye, X. S., and Osmani, S. A. (1997) *Prog. Cell Cycle Res.* **3**, 221–232
39. Wu, L., Osmani, S. A., and Mirabito, P. M. (1998) *J. Cell Biol.* **141**, 1575–1587
40. Dynlacht, B. D. (1997) *Nature* **389**, 149–152
41. Nigg, E. A. (1996) *Curr. Opin. Cell Biol.* **8**, 312–317
42. Hanks, S. K., Quinn, A. M., and Hunter, T. (1988) *Science* **241**, 42–52