A Novel Nucleolar Protein, NIFK, Interacts with the Forkhead Associated Domain of Ki-67 Antigen in Mitosis*

Received for publication, March 13, 2001
Published, JBC Papers in Press, May 7, 2001, DOI 10.1074/jbc.M102227200

Masatoshi Takagi, Mari Sueishi, Takuya Saiwaki, Ai Kametaka, and Yoshihiro Yoneda

The Journal of Biological Chemistry, Vol. 276, No. 27, pp. 25386–25391, 2001

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.

From the Department of Cell Biology and Neuroscience, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871 and the Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565-0871, Japan

In a previous study, we demonstrated that the forkhead associated (FHA) domain of pKi-67 interacts with the novel kinesin-like protein, Hklp2 (Sueishi, M., Takagi, M., and Yoneda, Y. (2000) J. Biol. Chem. 275, 28888–28892). In this study, we report on the identification of a putative RNA-binding protein of 293 residues as another binding partner of the FHA domain of pKi-67 (referred to as NIFK for nucleolar protein interacting with the FHA domain of pKi-67). Human NIFK (hNIFK) interacted with the FHA domain of pKi-67 (Ki-FHA) efficiently in vitro when hNIFK was derived from mitotically arrested cells. In addition, a moiety of hNIFK was co-localized with pKi-67 at the peripheral region of mitotic chromosomes. The hNIFK domain that interacts with Ki-FHA was mapped in the yeast two-hybrid system to a portion encompassed by residues 226–289. In a binding assay utilizing Xenopus egg extracts, it was found that the mitosis-specific environment and two threonine residues within this portion of hNIFK (Thr-234 and Thr-238) were crucial for the efficient interaction of hNIFK and Ki-FHA, suggesting that hNIFK interacts with Ki-FHA in a mitosis-specific and phosphorylation-dependent manner. These findings provide a new clue to our understanding of the cellular function of pKi-67.

The Ki-67 antigen (pKi-67), originally identified as the antigen for a monoclonal antibody raised against the nuclear extract from a Hodgkin’s lymphoma-derived cell line, was characterized as a class of proteins that localize around mitotic chromosomes (1). As a result, it is assumed that pKi-67 is involved in mitotic chromosome organization. pKi-67 is a convenient cell proliferation marker, since its expression is restricted to growing cells (2). Although the recent identification and characterization of a marsupial counterpart of pKi-67, restricted to growing cells (2). Although the recent identification and characterization of a marsupial counterpart of pKi-67, suggests that pKi-67 plays some type of role in the organization of higher order chromatin structure (3), the actual role of pKi-67 in the cell cycle progression remains unclear.

The N-terminal portion of pKi-67 is well conserved between human pKi-67 and chmadrin (62% identical) and contains a forkhead associated (FHA) domain. It was originally reported that the FHA domain constituted a region that has been conserved in a subset of forkhead-type transcription factors (4). The sequence profile has been reported for a variety of proteins with diverse functions (transcription, DNA repair, cell cycle progression, etc.). In several instances, the FHA domain preferentially recognizes partner proteins when they are present in the phosphorylated form (5–8). Moreover, the strong specificity of the FHA domain for phosphopeptides has been clearly demonstrated by binding assays with synthetic phosphopeptides (9). Therefore, it is currently thought that the FHA domain is a general phosphopeptide recognition motif that is involved in certain phosphopeptide-mediated signal transduction pathways (10). A search for the interaction partner(s) of the FHA domain of pKi-67, which could exist in the phosphorylated form, is an intriguing issue, since such interactions would constitute a significant component of the regulation of the cell cycle progression.

In order to investigate this issue further, a two-hybrid screening from a HeLa cDNA library was carried out using the N-terminal portion of pKi-67 as bait. As a result, two novel molecules that bind to the FHA domain of pKi-67 have been isolated. In our previous study, we revealed that one of them was a novel kinesin-like protein, Hklp2 (8). In this study, we report on the characterization of the other protein, which we refer to as NIFK (nucleolar protein interacting with the FHA domain of pKi-67). The interaction between human NIFK (hNIFK) and the FHA domain of pKi-67 occurs in a manner that is dependent on the mitosis-specific modification of hNIFK, which could include the phosphorylation of Thr-234 and Thr-238. Although the concrete function of hNIFK remains to be examined, the interaction found here provides some clues to our understanding of the relationship between the expression of pKi-67 and cell cycle progression. Moreover, the findings herein represent an example of a phosphopeptide recognition of the FHA domain and will contribute to a better understanding of the mode of action of the FHA domain.

EXPERIMENTAL PROCEDURES

Molecular Cloning of NIFK—One of the clones obtained by the two-hybrid screening using the FHA domain of pKi-67 as bait (8) contained a 293-residue open reading frame (ORF) of hNIFK. Extensive trials of 5′-rapid amplification of cDNA ends using SuperScript II (Life Technologies, Inc.) or BcaPLUS RTase (Takara) did not result in any additional upstream sequences. By using the amino acid sequence of hNIFK as a query, the mouse expressed sequence tag (EST) database was probed with the predicted amino acid sequence. A clone of mouse NIFK was isolated. In our previous study, we revealed that one of them was a novel kinesin-like protein, Hklp2 (8). In this study, we report on the characterization of the other protein, which we refer to as NIFK (nucleolar protein interacting with the FHA domain of pKi-67). The interaction between human NIFK (hNIFK) and the FHA domain of pKi-67 occurs in a manner that is dependent on the mitosis-specific modification of hNIFK, which could include the phosphorylation of Thr-234 and Thr-238. Although the concrete function of hNIFK remains to be examined, the interaction found here provides some clues to our understanding of the relationship between the expression of pKi-67 and cell cycle progression. Moreover, the findings herein represent an example of a phosphopeptide recognition of the FHA domain and will contribute to a better understanding of the mode of action of the FHA domain.

The abbreviations used are: FHA, forkhead-associated; ORF, open reading frame; EST, expressed sequence tag; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; mNIFK, mouse NIFK; CSF, cysotic factor.

1 The abbreviations used are: FHA, forkhead-associated; ORF, open reading frame; EST, expressed sequence tag; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; mNIFK, mouse NIFK; CSF, cysotic factor.

2 The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AB044971 and AB056870.
searched by TBLSTN, resulting in finding AA260128, AI048665, and AI851818 as probable cDNA fragments coding for the mouse NIFK (mNIFK). The mNIFK-specific primers P1 (5'- ATGGCTGGGTTAGCAGGCCC-3') and P2 (5'- TCACTGCTTGCTCTTCCTTTCTCGG-3') were designed with respect to these sequences. The cDNA of mNIFK was recovered by reverse transcriptase-polymerase chain reaction on polyadenylated RNA of mouse Ehrlich tumor cells (a gift of Dr. Takuya Shimamoto, Osaka University) using P1 and P2, subcloned directly into pGEM-T easy (Promega), and analyzed using an ABI310 Genetic Analyzer (Applied Biosystems, Inc.). The nucleotide sequence of mNIFK was identical to that of the clone 2310021G21 reported by the RIKEN group (11), except that the latter contained two additional nucleotides (cytosine and guanine after cytosine 914 and guanine 920 of the former, respectively). According to the RIKEN sequence, the amino acid similarity with hNIFK was lost at their C-terminal regions. Moreover, the same sequences as ours at the position of disagreement could be found from the mouse EST data base. The sequence described here was determined with great care, and we are certain that it is correct. Since the RIKEN clone was a cap-trapper selected cDNA (12), the sequence of the mNIFK contains a full-length ORF.

Recombinant Proteins—Plasmids coding for GST fusions of Ki-FHA (residues 1–168 of pKi-67) and the N-terminal half of hNIFK (residues 1–146) were created by cloning the appropriate inserts into pGEX (Amersham Pharmacia Biotech). A plasmid coding for the maltose-binding protein fused to hNIFK-(1–146) was created by cloning the appropriate insert into pMALc2 (New England Biolabs, Inc.). BL21 (DE3), transformed with these plasmids, was grown at 37 °C to a density of 0.6 (A600), cooled to 20 °C, and cultured in the presence of 0.1 mM isopropyl-b-D-thiogalactopyranoside for an additional 14 h at 20 °C. Proteins were purified from the bacteria according to protocols recommended by the manufacturer.

Antibody Production—Antisera were prepared by immunizing two rabbits (kbs:JW) (purchased from Kitayama Labes Co., Ltd.) with purified GST-hNIFK-(1–146). The antibodies were affinity purified against maltose-binding protein fused to hNIFK-(1–146), which had been immobilized on a nitrocellulose membrane using a procedure described previously (13).

Cell Culture and Synchronization—HeLa cell extracts prepared from asynchronous (AS), aphidicolin-arrested (S), or nocodazole-arrested (M) cultures were analyzed by immunoblotting with antibodies specific for hNIFK, prior to (lanes 1–3) or after affinity purification with GST beads (lanes 1’–3’) or GST-Ki-FHA-(1–168) beads (lanes 1”–3”). Lanes 1–3 show 10% of the amount of the input. hNIFK was detected as a single band (band III) in asynchronous and aphidicolin-arrested cells. In mitotic cells, hNIFK was detected mainly as bands I and II. Asterisks indicate background signals due to a large amount of recombinant GST-Ki-FHA-(1–168). B, mitosis-specific modification of hNIFK consists of phosphorylation(s). Cell extracts of mitotic HeLa cells were prepared in the presence (lane 2) or absence (lanes 2 and 4) of phosphatase inhibitors (PI), denatured with SDS-containing sample buffer immediately (lanes 2 and 3) or after incubation at 30 °C for 30 min (lane 4), and analyzed by immunoblotting with antibodies specific for hNIFK.
and 100 μg/ml, respectively). Prometaphase-arrested HeLa cells were obtained as described previously (8). S-phase-arrested HeLa cells were obtained by treating ~75% confluent cultures with 1 μg/ml aphidicolin for 17 h.

Preparation of Cell Extracts and Pull-down Assay—Cell extracts were prepared from asynchronous or synchronized HeLa cells essentially as described previously (8) with minor modifications in the buffer composition. The extraction buffer (EB150) was supplemented with RNase A and okadaic acid at 50 μg/ml and 0.5 μM, respectively. For the preparation of the cell extracts shown in lanes 2 and 4 of Fig. 2B, phosphatase inhibitors (NaF, β-glycero phosphate, and okadaic acid) were excluded. Cell extracts were incubated with GST or GST-Ki-FHA-(1–168) coupled to glutathione-Sepharose beads (GS-4B; Amersham Pharmacia Biotech) for 1 h at 4 °C with occasional agitation, after which the beads were washed extensively with EB150 containing okadaic acid (0.5 μM). Associated proteins were obtained by boiling the sample buffer and separating in 10% acrylamide gel. hNIFK was detected by Western blotting using anti-hNIFK antibodies at 0.5 μg/ml. 

Immunofluorescence—HeLa cells grown on coverslips were washed once with ice-cold PBS(+); (137 mM NaCl, 3 mM KCl, 8 mM Na2HPO4, 1 mM KH2PO4; and 2 mM MgCl2), pre-extracted with 0.1% Triton X-100 in PBS(+) for 2 min on ice, and fixed with 4% formaldehyde in PBS(+) for 10 min at room temperature. In the experiment shown in Fig. 3A (d–f), cells were treated with 50 μg/ml RNase A in PBS(+) at 37 °C for 15 min before fixation. Procedures after fixation were the same as those described previously (8). Anti-hNIFK antibodies were used at a level of ~1 μg/ml. MIB-1 (Immunotech), the monoclonal antibody against pKi-67, was used at 1:100. The photographs shown in Fig. 3, A and B, were acquired with Axiophot2 (Zeiss) and an LSM510 confocal microscope (Zeiss), respectively.

Mapping and Dissection of the FHA Interaction Domain of hNIFK in Yeast—Full-length hNIFK and its deletion derivatives (depicted in Fig. 4A) were subcloned into pGAD GH (CLONTECH) using standard methods. Starting from pGAD GH-hNIFK-(226–269), T234A, T238A, T240A, and S247A mutants were prepared using the QuickChange site-directed mutagenesis system (CLONTECH) and were verified by sequencing. All proteins were tested for their interaction with the FHA domain of pKi-67 (residues 1–99), which was subcloned into pGAD424 (CLONTECH), in strain Y190 via the expression of reporter genes.

In Vitro Transcription-Translation and in Vitro Association Experiments—Full-length hNIFK was subcloned into pcDNA3.1(+) (Invitrogen) and pRSETc (Invitrogen) using standard methods to generate pcDNA-hNIFK and pRSETc-hNIFK, respectively. Starting from pRSETc-hNIFK, T234A and T238A mutants were prepared as described above. Cloned proteins (hNIFK, His-tagged hNIFK[T234A], and His-tagged hNIFK[T238A]) were transcribed and translated using the TNT T7 Quick Transcription/Translation System (Promega) in the presence of [35S]Met (PerkinElmer Life Sciences). A portion of each reactant (25 μl) was separated on the same gel after affinity purification with GST or GST-Ki-FHA-(1–168) beads. Labeled proteins were visualized by autoradiography. Xenopus egg extracts were prepared from unfertilized eggs as described previously (14), and their reliability was checked by the retardation of the electro-phoretic mobility of hamster CdC25C, expressed from pET3α-hamCdC25C (15) (a gift from Drs. Hideo Nishitani and Takeharu Nishimoto, Kyusyu University), after incubation, as described previously (16).

RESULTS
Cloning of Human and Mouse NIFK—By using the two-hybrid screening from HeLa cDNA library using the FHA domain of pKi-67 (named “Ki-FHA” in this report) as bait, we were able to obtain five positive clones. Two of these encoded certain portions of a novel kinesin-like protein (termed Hkhp2), and the interaction between pKi-67 and Hkhp2 occurred preferentially in a cell extract obtained from nocodazole-arrested cells (8). Here we analyzed the remainder of the positive clones. All clones contained overlapped nucleotide sequences, suggesting that they were derived from the same gene. The longest clone contained a 293-residue ORF (Fig. 1A) and the 3’- untranslated region of ~500 bases. The deduced protein had a calculated molecular mass of 34 kDa and contained a putative DNA binding domain at residues 42–122, which was characterized as comprising two ribonucleoprotein motifs (17) (Fig. 1, A and B). We refer to the protein as hNIFK (human nucleolar protein interacting with the FHA domain of Ki-67 antigen). By using the protein sequence of hNIFK as a query, a mouse EST data base was searched by TBLASTN. Several clones were likely to encode certain portions of the mouse counterpart, mNIFK. By linking the sequences of these clones, the plausible full-length sequence for mNIFK could be obtained. To confirm the existence and identity of this ORF, the cDNA was amplified from mouse Ehrlich tumor cells by reverse transcriptase-polymerase chain reaction and sequenced. The confirmed ORF in mouse codes for a 317-amino acid protein with a basic insertion at the residues 228–249. On the amino acid level, the NIFK of human and mouse showed 54 and 75% identity, respectively, in overall sequence and within the RNA binding domain (Fig. 1, B and C). Although a full-length mouse cDNA collection prepared by the RIKEN group (11) contains essentially the same cDNA, there are several minor conflicts resulting in differences in the amino acid sequences at their C-terminal portions (see “Experimental Procedures” for detail).

Mitotically Modified hNIFK Interacts Efficiently with the FHA Domain of pKi-67—The FHA domain appears to be a phosphopeptide recognition domain (10). In our previous report, we revealed that the FHA domain of pKi-67 (Ki-FHA) actually recognized mitotically modified, probably phosphorylated, Hkhp2 (8). To examine the issue of whether Ki-FHA also recognizes phosphorylated hNIFK, we first prepared specific antibodies against hNIFK. The antibodies recognized a protein
that had an apparent molecular mass of 38 kDa (band III) from the cell extracts of asynchronous and S-phase-arrested HeLa cells (Fig. 2A, lanes 1 and 2, and Fig. 2B, lane 1). In contrast, the antibodies mainly recognized 44- and 40-kDa proteins (bands I and II) in mitotic HeLa cells (Fig. 2A, lane 3 and Fig. 2B, lane 3). When mitotic HeLa extracts were prepared in the absence of phosphatase inhibitors, the band I disappeared and was replaced by a smeared signal between bands II and III (Fig. 2B, lane 2). After incubation of the phosphatase inhibitor-free extract for 30 min at 30 °C, the band III became prominent (Fig. 2B, lane 4). These observations suggest that hNIFK is differently modified (having at least two variations) in mitosis and that the modifications include phosphorylation(s) that is the origin of the mobility shifts in SDS-PAGE. The phosphorylation(s) of hNIFK was counteracted by a cellular phosphatase(s). From the cell extracts of differently synchronized HeLa cells, proteins that interacted with Ki-FHA were pulled down with a GST-Ki-FHA fusion protein and analyzed by Western blotting with the anti-NIFK antibodies. As shown in Fig. 2A, the mitotic forms of hNIFK (bands I and II) were efficiently pulled down (lane 3). A small quantity of ~39-kDa protein was pulled down from the asynchronous and S-phase HeLa extracts (lanes 1’ and 2’). This band is not identical to band II or band III in its electrophoretic mobility and, therefore, is unlikely to be derived from the mitotic cells that existed somewhat in the asynchronous and S-phase-arrested cultures.

**Comparison of Cellular Behaviors of hNIFK and pKi-67 during the Cell Cycle**—The cellular localization of hNIFK was examined in HeLa cells using the affinity-purified antibodies against hNIFK. hNIFK was found mainly in nucleoli but also in other (nucleoplasmic and cytoplasmic) spaces (Fig. 3A, b). Essentially the same observation was obtained as for the localization of GFP fusion protein of hNIFK (not shown). The staining of hNIFK was greatly reduced by treatment of cells with RNase A prior to fixation (Fig. 3A, c), supporting the conclusion that hNIFK is an authentic RNA-binding protein, as deduced from its primary structure. The remaining population of hNIFK, after RNase extraction, was found at the center of nucleoli (Fig. 3A, e), whereas pKi-67 was mainly localized at the outer region of nucleoli (possibly the region known as “dense fibrillar component”) before and after RNase extraction (Fig. 3A, c and f). In mitosis, hNIFK would be likely to co-localize with pKi-67 at the surface of mitotic chromosomes (Fig. 3B). In most cases, an hNIFK moiety was preferentially associated with certain regions of the mitotic chromosomes (Fig. 3B, arrowheads). The issue of which regions of the chromosomes are preferred by hNIFK remains to be examined.

**The Residues 226–269 of hNIFK Are Sufficient for Its Interaction with the FHA Domain of pKi-67 in Yeast**—To map the FHA domain-binding region of hNIFK, a deletion analysis of hNIFK was performed using the yeast two-hybrid system. Various deletion mutants (Fig. 4A), expressed as fusion proteins with the GAL4 activation domain (denoted GAL4 AD), were tested for interaction with the FHA domain of pKi-67 fused to the DNA binding domain (DBD) of GAL4 in yeast. As summarized in Fig. 4A, the region corresponding to the residues 226–269 of hNIFK was found to be sufficient for interaction with the FHA domain of pKi-67. Alignment of this region with the FHA domains is thought to interact directly with phosphorylated targets (10), it is likely that some of these residues represent targets of phosphorylation and subsequent binding of the FHA domain of pKi-67. We therefore constructed hNIFK mutants that contained point mutations at the above four residues and tested their interaction with Ki-FHA in the yeast two-hybrid system (Fig. 4C). A substitution of Thr-240 or Ser-247 with alanine had no effect on the interaction. In contrast, a single alanine point mutation at either Thr-234 or

**Fig. 4. Mapping of the FHA domain binding region in hNIFK.** A, identification of the FHA domain of hNIFK that interacts with the FHA domain of pKi-67 (Ki-FHA). Various hNIFK constructs in pGAD GH were tested for interaction with Ki-FHA using two-hybrid system in yeast. +, positive interaction; −, lack of interaction (failure of cells to grow on selective medium and activation of the β-galactosidase gene); −, lack of interaction (failure of cells to grow on selective medium). B, alignment of the FHA domain binding region of human NIFK with the corresponding region (amino acids 248–291) of mouse NIFK. C, identification of the residues of hNIFK crucial for its binding with Ki-FHA. Various hNIFK mutants in which each conserved threonine residue was mutated to alanine were tested for the interaction with the Ki-FHA using two-hybrid system in yeast. +, positive interaction; −, lack of interaction.
Fig. 5. Interaction of hNIFK with Ki-FHA in a mitotic environment in a manner dependent on Thr-234 and Thr-238 of hNIFK. A, pull-down of in vitro translated hNIFK with GST-Ki-FHA (1–168). hNIFK was translated in vitro in the presence of [35S]methionine, incubated with (lanes 2–2') or without (lanes 1–1') the CSF extracts, and analyzed by SDS-PAGE and autoradiography. Lanes 1 and 2 correspond to 24% of the inputs. B, analysis of the mitotic modification of in vitro translated hNIFK. Wild-type (lanes 1 and 1'), T234A (lanes 2 and 2'), and T238A (lanes 3 and 3') hNIFK were translated in vitro in the presence of [35S]methionine, incubated with (lanes 1'–3') or without (lanes 1–3) the CSF extracts, and analyzed by SDS-PAGE and autoradiography. Note that T234A and T238 hNIFK, but not wild-type hNIFK, are His-tagged. C, pull-down of hNIFK mutants with GST-Ki-FHA (1–168). Wild-type (lanes 1–1'), T234A (lanes 2–2'), and T238A (lanes 3–3') hNIFK were translated in vitro in the presence of [35S]methionine, incubated with the CSF extracts, and analyzed by SDS-PAGE and autoradiography prior to (lanes 1–3) or after affinity purification with GST beads (lanes 1’–3') or GST-Ki-FHA (1–168) beads (lanes 1'–3'). Lanes 1–3 correspond to 24% of the inputs.

Thr-238 abolished the interaction.

Threonine 234 and 238 of hNIFK and Their Phosphorylations Appear to Be Crucial for Interaction with the FHA Domain of pKi-67—To test whether hNIFK binding to pKi-67 is dependent on the mitotic phosphorylation of hNIFK at Thr-234 and Thr-238, we applied an experimental system, originally developed by Kirschner and co-workers (18), to identify mitotic phosphoproteins systematically. In our experiment, in vitro translated hNIFK and its mutants (T234A and T238A) were incubated with mitotic extracts of Xenopus eggs (CSF extracts) and then separated on SDS-PAGE directly or after affinity purification with GST-Ki-FHA beads. After incubation with the CSF extract, certain population of in vitro translated hNIFK migrated with a reduced mobility (Fig. 5A, lane 2), the origin of which was phosphorylation(s) since the shifts were abolished by treatment with alkaline phosphatase (not shown). hNIFK was pulled down efficiently with GST-Ki-FHA beads, not with GST beads, only after incubation with the CSF extract (Fig. 5A). In contrast, T234A and T238A mutants of hNIFK, which had been treated with the CSF extract, were pulled down less efficiently with GST-Ki-FHA beads (Fig. 5C). These observations indicate that hNIFK binds specifically to Ki-FHA in a mitosis-specific manner and is dependent on the presence of Thr-234 and Thr-238 in hNIFK, suggesting that Thr-234 and/or Thr-238 of hNIFK are phosphorylated and recognized by Ki-FHA in a mitosis-specific manner.

The FHA domain has generated considerable interest, not only because of its nature as a signaling module, which functions through interactions with phosphorylated target molecules (10), but because of a link with cancer; genes that are mutated in the Nijmegen breakage syndrome (NBS1) and the Li-Fraumeni syndrome (CHK2) encode FHA domain-containing proteins (24–27). Recent progress around the FHA domain, that is the screening of optimal phosphopeptide sequences recognized by various FHA domains (28–30) and the determination of the three-dimensional structures of the Rad53p FHA domains (28–31), has enhanced our knowledge of the mode of phosphopeptide recognition of FHA domains. Although the issue of whether this knowledge fits situations that actually occur in cells requires further testing, the identification of
actual ligands of FHA domains and the mapping of biologically relevant FHA domain binding sites on these ligands have been accomplished only in a limited number of cases (5–8). From this viewpoint, our findings should be of some use as a new example. Further analysis of the interactions between Ki-FHA and NIFK, along with past studies (10), might result in a much more general understanding of the mode of phosphopeptide recognition of FHA domains and thereby provide the basis for the development of novel classes of therapeutic agents that target FHA-mediated signal transduction pathways.

Acknowledgments—We are grateful to Dr. Takuya Shimamoto (Osaka University, Japan) for providing us with polyadenylated RNA of mouse Ehrlich tumor cells and Drs. Hideo Nishitani and Takeharu Nishimoto (Kyusyu University, Japan) for the hamster Cdc25C clone.

REFERENCES

1. Gerdes, J., Schwab, U., Lemke, H., and Stein, H. (1983) Int. J. Cancer 31, 13–20
2. Gerdes, J., Lemke, H., Baich, H., Waquer, H. H., Schwab, U., and Stein, H. (1984) J. Immunol. 133, 1710–1715
3. Takagi, M., Matsuoka, Y., Kurihara, T., and Yoneda, Y. (1999) J. Cell Sci. 112, 2463–2472
4. Hofmann, K., and Bucher, P. (1995) Trends Biochem. Sci. 20, 347–349
5. Stone, J. M., Collinge, M. A., Smith, R. D., Horn, M. A., and Walker, J. C. (1994) Science 266, 793–795
6. Sun, Z., Hsiao, J., Fay, D. S., and Stern, D. F. (1998) J. Biol. Chem. 273, 25413–25419
7. Sueshi, M., Takagi, M., and Yoneda, Y. (2000) J. Biol. Chem. 275, 28886–28892
8. Durocher, D., Henckel, J., Fersht, A. R., and Jackson, S. P. (1999) Mol. Cell 4, 387–394
9. Li, J., Lee, G., Van Doren, S. R., and Walker, J. C. (2000) J. Cell Sci. 113, 4143–4149
10. Li, J., Lee, G., Van Doren, S. R., and Walker, J. C. (2000) J. Cell Sci. 114, 4143–4149
11. Kawaji, H., Shinyagawa, A., Shibata, K., Yoshino, M., Itoh, M., Ishii, Y., Arakawa, T., Hara, A., Fukunishi, Y., Komuro, H., Adachi, J., Fukuda, S., Aizawa, K., Iwasa, M., Nishiyama, J., Saito, T., Okazaki, Y., Gojobori, T., Bono, H., Kasukawa, T., Saito, R., Kadota, K., Matsuoka, H. A., Ashburner, M., Batalov, S., Casavant, T., Fleischmann, W., Gaasterland, T., Gissi, C., King, B., Koichiwa, K., Kuehl, P., Lewis, S., Matsuo, Y., Nikaido, I., Pandi, G., Quackenbush, J., Schriml, L. M., Staubli, F., Suzuki, R., Tomita, M., Wagner, L., Washio, T., Sakai, K., Okido, T., Furuno, M., Anno, H., Baldarelli, R., Barsh, G., Blake, J., Boffelli, D., Bojunga, N., Caru, P., de Bonaldo, M. F., Brownstein, M. J., Bult, C., Fletcher, C., Fujita, M., Garibaldi, M., Gustinich, S., Hill, D., Hofmann, M., Hume, D. A., Kani, M., Lee, N. H., Lyons, P., Marchionni, L., Mashima, J., Mazzei, J., Mombaerts, P., Nagane, P., Ring, B., Ringwald, M., Rodríguez, I., Sakamoto, N., Sasaki, H., Sato, K., Schonbach, C., Saya, T., Shibata, Y., Storey, K. F., Suzuki, H., Toyo-oka, K., Wang, K. H., Weitz, C., Whitaker, C., Wilm, L., Wynshaw-Boris, A., Yoshida, K., Hasegawa, Y., Kawai, J., Kohtsuki, S., and Hayashi, Y. (2001) Nature 409, 685–690
12. Carninci, P., Shibata, Y., Hayatsu, N., Sugahara, Y., Shibata, K., Itoh, M., Konno, H., Okazaki, Y., Muramatsu, M., and Hayashizaki, Y. (2000) Genome Res. 10, 1617–1630
13. Adachi, Y., and Yanagida, M. (1989) J. Cell Biol. 108, 1195–1207
14. Murray, A. (1991) Methods Cell Biol. 36, 581–605
15. Seki, T., Kamihasa, K., Nishitani, H., Takagi, T., Russell, P., and Nishimoto, T. (1992) Mol. Cell Biol. 2, 1373–1388
16. Nishijima, H., Nishitan, H., Seki, T., and Nishimoto, T. (1997) J. Cell Biol. 138, 1105–1116
17. Burd, C. G., and Dreyfuss, G. (1994) Science 265, 615–621
18. Stukenberg, P. T., Lastig, K. D., McGarry, T. J., King, R. W., Kuang, J., and Kirschner, M. W. (1997) Curr. Biol. 7, 338–348
19. Winzer, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Booke, J. D., Russey, H., Chiu, A. M., Connelly, C., Davis, K., Dietrich, F., Dow, S. W., El Bakkoury, M., Foury, F., Friend, S. H., Genten, I., Giese, G., Hegemann, J. H., Jones, T., Lahb, M., Liu, H., Davis, R. W., and et al. (1999) Science 285, 901–906
20. Goezly, P., Echeverri, G., Ogeria, K., Coulon, A., Jones, S. J., Copley, R. R., Duperon, J., Gege, J., Brehm, M., Cassin, E., Hannak, E., Kirkham, M., Pichler, S., Fehr, K., Gossens, A., Leidel, A., Alleaume, A. M., Martin, C., Olu, N., Bork, P., and Hyman, A. A. (2000) Nature 48, 331–336
21. Kuhn, A., Vente, A., Doree, M., and Grummt, I. (1998) J. Mol. Biol. 284, 1–5
22. Heix, J., Vente, A., Reit, R., Budde, A., Michaeli, T. M., and Grummt, I. (1998) EMBO J. 17, 3981–3987
23. Sirri, V., Roussel, P., and Hernandez-Verdun, D. (2000) J. Cell Biol. 148, 259–270
24. Bell, D. W., Varley, J. M., Sztaylo, T. E., Kang, D. H., Wahrer, D. C., Shannon, K. E., Lubratoch, M., Veselka, S. J., Isetticher, B. J., Fraumeni, J. F., Birch, J. M., Li, F., Garber, J. E., and Haber, D. A. (1999) Science 282, 2528–2531
25. Carney, J. P., Maser, R. S., Oliveares, H., Davis, E. M., Le Beau, M., Yates, J. R., Hays, L., Marog, W. F., and Peterlin, J. H. (1998) Cell 93, 477–486
26. Matsuura, S., Tauchi, H., Nakamura, A., Kondo, N., Sakamoto, S., Sone, S., Smeets, D., Solder, B., Belchardo, B. Y., Der Kaloustian, V. M., Oshiruwa, M., Nakamura, Y., and Komatsu, K. (1998) Nat. Genet. 19, 179–181
27. Varon, R., Vissinga, C., Platzer, M., Ceresaletti, K. M., Chrzanowksa, K. H., Saar, K., Beckmann, G., Seemanov, E., Cooper, P. R., Nowak, N. J., Stumm, M., Weemaes, C. M., Gatti, R. A., Wilsen, R. K., Digweed, M., Rosenthal, A., Sperling, K., Fuccoppon, P., and Res, A. (1998) Cell 93, 467–476
28. Durocher, D., Taylor, I. A., Sabatowski, H., Daire, L. F., Westcott, S. L., Jackson, S. P., Smerdon, S. J., and Yaffe, M. B. (2000) Mol. Cell 6, 1169–1182
29. Liao, H., Byeon, I. J., and Tsai, M. D. (1999) Mol. Biol. Cell 10, 281, 285–2892
30. Wang, P., Byeon, I. J., Liao, H., Beebe, K. D., Yongkiettrakul, S., Pei, D., and Tsai, M. D. (2000) J. Mol. Biol. 302, 927–940
31. Liao, H., Byeon, I. J., and Tsai, M. D. (1999) J. Mol. Biol. 294, 1041–1049

Nucleolar Protein Binding to the FHA Domain of pKi-67 25391