The serum from a patient with Sjögren's syndrome (RM serum) was used to screen a human testis cDNA expression library. A cDNA of 865 base pairs containing the entire coding sequence for a novel protein was isolated. The 14-kDa predicted protein contains an acidic domain (amino acids 6–80) with a high frequency of heptad repeats characteristic of a-helices that form dimeric coiled-coil structures and an alkaline carboxyl-terminal domain (amino acids 81–119). It seems to be widely expressed, but its expression level varies depending on tissues. A protein of apparent molecular mass of 14 kDa was immunoprecipitated from cell lysates by the autoimmune serum, and it was recognized by rabbit antibodies raised to a recombinant bacterial fusion protein generated from the cDNA clone. Conventional and confocal immunofluorescence microscopy on HeLa and 3T3 cells transiently transfected with a tagged form of the protein showed numerous punctate structures scattered throughout the nucleus. This novel protein has been termed NA14 for Nuclear Autoantigen of 14 kDa.

Human autoantibodies are valuable reagents for identification and study of novel cellular constituents since they usually recognize highly conserved components, which otherwise are difficult to detect due to their low immunogenicity. In addition, the autoantibodies usually react with the active sites of the antigen, and therefore, they are often capable of inhibiting its functional activity (1).

In a number of rheumatic diseases, the presence of the so-called antinuclear antibodies is a dominant feature. These diseases include systemic lupus erythematosus, mixed connective disease, polymyositis, scleroderma, and Sjögren's syndrome (2).

Human autoimmune sera have been used to characterize and identify several nuclear proteins including La antigen (3), centromere protein B (CENP-B) (4), Ku70 and Ku86 (5, 6), Sp-100 (7), PM-Scl (8), Mi-2 (9), and Ngp-1 (10).

RM serum, from a patient with Sjögren's syndrome, contains high titer autoantibodies to the Golgi apparatus and low titer autoantibodies to the nucleus (11). Western blotting and immunoprecipitation experiments demonstrated that this serum shows multiple autoreactivities and recognizes three major bands of 210, 130, and 45 kDa (p210, p130, and p45, respectively) in HeLa cell extracts. p210 was shown to be localized at the Golgi complex (11). We have recently cloned and characterized the cDNA encoding p210, now called GMAP-210, by immunoscreening of a HeLa cell cDNA library.1

In the present study, we used RM serum to screen a human testis oligo(dT) cDNA library, and we isolated a clone encoding a novel protein of 14 kDa, termed NA14, that localizes to the nucleus. The analysis of its amino acid sequence revealed the presence of a domain probably involved in the formation of a coiled-coil structure and sequence similarity with a number of proteins with coiled-coil motifs.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Lysis—**Jurkat cells were grown in RPMI 1640 medium (Bio-Whittaker) supplemented with 10% heat-inactivated FCS.2 HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FCS. Mouse NIH3T3 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum. 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin were included in all culture media. All cells were maintained in a 5% CO2 humidified atmosphere at 37 °C.

For cell lysis, cells were washed and harvested in phosphate-buffered saline. 2 × 107 to 108 cells per ml were lysed at 4 °C in Nonidet P-40 buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml pepstatin, leupeptin, and aprotinin) for 20 min. The extract was centrifuged at 20,000 × g for 20 min, and the supernatant was stored at −70 °C.

**Antibodies—**RM serum of the patient suffering from Sjögren’s syndrome (12, 13) was aliquoted, had sodium azide added, and then stored at −70 °C. IgG fraction (10 mg/ml final concentration) was purified from whole serum on protein A-Sepharose columns and stored in 50% glycerol at −70 °C. Specific antibodies from RM serum were affinity purified on nitrocellulose strips using immunoreactive proteins from total cell extracts as described (14).

**cDNA Cloning and Sequence Analysis**—RM serum diluted 1:1500 in TBST (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20) containing 5% non-fat dry milk was used for immunoscreening of 5 × 107 recombinants from a human testis oligo(dT)-primed cDNA library in λgt11. Positive clones were rescreened and plaque-purified. Two positive clones (A5 and A10) were isolated and subcloned into pTZ19R plasmid. The entire λ10 EcoRI insert was used as a probe to hybridize against a HeLa random primed cDNA AZAPII library. The clones obtained were isolated and subcloned in vivo into pBluescript plasmid using R408 helper plage as described in the manufacturer instructions (Stratagene). The nucleotide sequence was determined by using an automated sequencer from Pharmacia Biotech Inc.

Nucleic acids and protein sequences were analyzed by the University of California, San Francisco DNA Sequencing Facility. The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EMBL Data Bank with accession number(s) Z98932.

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2 The abbreviations used are: FCS, fetal calf serum; ECL, enhanced chemiluminescence; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; HA, hemagglutinin; PCK, protein kinase C; bp, base pair(s).
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Fig. 1. Cloning and sequencing of na14. A, map of na14 cDNA clones. Clones λ5 and λ10 were isolated from a Agt11 human testis cDNA library by immuno-screening with autoimmune RM serum. A λZAPII HeLa cDNA library was screened with λ10 yielding clones λZ2 and λZ20. The open reading frame is boxed. B, cDNA and predicted amino acid sequence of na14. A putative leucine zipper motif and the polyadenylation signal AATAAA are both underlined. Putative motifs predicted by PROSITE are indicated as follows: N-glycosylation site is underlined, casein kinase II phosphorylation sites are in bold, and PKC phosphorylation site is in bold and is italicized. The sequence is available from EMBL/GenBank™ under accession number Z96932. C, amino acid sequence alignment of human and mouse NA14. A mouse EST sequence (AA274144) homologous to human na14 was obtained by searching the GenBank™ data base with the FASTA program. A BESTFIT comparison between human NA14 and the predicted mouse amino acid sequence is shown. The percent of identity is 96.6.

PREPARATION OF GST FUSION PROTEINS AND RABBIT ANTI-AUTOANTIGEN FUSION PROTEIN ANTIBODIES—A fusion protein containing the complete EcoRI insert from λ10 was constructed at the carboxyl terminus of GST using the prokaryotic expression vector pGEX-4T-1. Plasmids were transformed in Escherichia coli strain DH5α. Expression of the GST fusion proteins was induced by the addition of 1 mM isopropyl-β-D-thiogalactoside, and the fusion proteins were isolated from bacterial lysates by affinity chromatography with glutathione-agarose beads (Sigma). NA14 was released from the fusion protein by treatment with 1% (w/v) thrombin in cleavage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2.5 mM CaCl₂) for 2 h at 25 °C. A polyclonal antibody was generated in rabbit using the entire GST-NA14 fusion protein. The antiserum obtained after the fourth immunization, named anti-NA14, was used in the experiments described.

NORTHERN BLOTTING—A poly(A)⁺ RNA blot from CLONTECH was hybridized with the ³²P-labeled cDNA insert from clone λ10 and washed as described in the manufacturer instructions.

ELECTROPHORESIS AND IMMUNOBLOT ANALYSES—Proteins were separated by SDS-PAGE on 15% acrylamide gels (21) and stained with Coomassie Brilliant Blue. Gels were electrophoretically transferred to nitrocellulose filters (22), and immunoblot analyses were carried out as described (11).

IMMUNOPRECIPITATION—HeLa and Jurkat cell lysates (1.5 × 10⁷ cells) were incubated with 5 μl of RM serum, normal human serum, or an irrelevant human autoimmune serum for 2 h at 4 °C, with agitation. 50 μl of a 50% solution of protein A-Sepharose was added, and incubation was continued for 1 h. Then the protein A-Sepharose beads were collected and washed five times with Nonidet P-40-buffer. Proteins were released from the beads by boiling in SDS-PAGE sample buffer and analyzed by immunoblotting.

TRANSIENT TRANSFECTION OF HEBA AND 3T3 CELLS—The full-length open reading frame of na14 (EcoRI insert from λ10) was cloned in-frame with the HA epitope into the eukaryotic expression vector pECE (24) to obtain an HA epitope-tagged NA14. The resulting plasmid, pECE-NA14, was purified with a plasmid kit from Qiagen followed by phenol extraction with chloroform.

of Wisconsin Genetics Computer Group sequence analysis software package version 8.1 for UNIX computers (15). Comparisons to known sequences were performed by BLAST (16) on the Internet server. Secondary structure analysis was conducted with the software programs Coils (17), and PeptideStructure (18–20).
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RESULTS AND DISCUSSION

Cloning of na14 cDNA—When serum RM was used to screen 5 × 10^9 plaques from a human testis λgt11 oligo(dT)-primed cDNA expression library, two clones (λA5 and λA10) were isolated. The cDNA inserts were subcloned into pTZ19R and sequenced. The two clones contained the poly(A) tail as expected and gave identical sequences in the overlapping regions, clone λA5 being 14 bp longer than λA10 and containing an 865-bp insert. Analysis of the sequence showed that it was not part of GMAP-210 cDNA. In addition, affinity purified antibodies from p130 and p45 did not show staining of the phage plaques. From these data, we concluded that the clones obtained did not correspond to any of the three major proteins recognized by the autoimmune RM serum (11). To obtain an additional sequence, a Clontechgt11 oligo(dT)-primed cDNA library was screened with a human testis λgt11 oligo(dT)-primed cDNA library containing sequences similar to the 5′-untranslated region, and a clone was found. The identity between the human and the murine mRNA is present in both fetal and adult normal tissues, as well as in cancer tissues and transformed cell lines.

Northern analysis using the entire λA10 insert as a probe showed a single band that fits well with the size of the cDNA contained in λA5 (Fig. 2, see below).

Sequence Analysis—As shown in Fig. 1B, the entire sequence comprises 865 bp and contains an open reading frame of 360 bp. The first ATG, at positions 47–49, is in excellent agreement with Kozak’s sequence for eukaryotic translation initiation (25). The open reading frame is terminated by a stop codon at positions 404–406. A polyadenylation signal ATTAA (26) is found at positions 837–842 in the 3′-untranslated region, and a poly(A) tail is present at the end of the sequence. These data confirm that the complete coding sequence was present in λA5 and λA10.

The predicted protein sequence consists of 119 amino acids and has a calculated molecular mass of 13,656 daltons and a pI of 5.18. This protein was named NA14.

The PROSITE Dictionary of Protein Sites and Patterns revealed consensus motifs for N-glycosylation, casein kinase II phosphorylation, and PKC phosphorylation. These domains have also been noted in many other autoantigens, including the nuclear mitotic antigen (NuMA) (27), the nuclear autoantigenic sperm protein (NASP) (28), lamin B (29), myosin heavy chain (30), SS-A/Ro (31), and Ku86 (6). These domains have also been described in a number of other prokaryotic and eukaryotic proteins. A sequence motif similar to the leucine zipper pattern of eukaryotic transcription factors is also found between amino acids 8 and 22 (Fig. 1B). This motif promotes dimerization through α-helical coiled-coil formation. Certain proteins that contain this motif are able to bind to DNA and are thought to be regulatory in their functions (32); however, these structures can also mediate dimerization or oligomerization of proteins that do not bind to DNA (33–36). These observations suggest that NA14 could participate in protein-protein interactions.

The cDNA sequence was used to search the EMBL and GenBank™ data bases for homologous sequences. No overall homology was found with known genes. When the deduced amino acid sequence for NA14 was used to search the SwissProt data bank, 20–28% identity was found with myosin heavy chain and other proteins that contain coiled-coil domains.

Interestingly, several homologous mouse EST sequences, some of which contained complete coding sequence for na14, were found. The identity between the human and the murine cDNA sequences was higher than 85%. The high degree of conservation at the amino acid level between the human and the predicted mouse NA14 protein should be noted. Both proteins are 96.6% identical (Fig. 1C).

A search on the TIGR Human Gene Index using the BLAST program revealed many human EST sequences similar to na14 cDNA. Table I lists those being more than 95% identical to na14 cDNA, indicating the region of na14 to which homology was shared, the percent of identity, and the tissues or cell strains used to prepare the cDNA libraries from which the sequences were cloned. na14 mRNA is present in both fetal and adult normal tissues, as well as in cancer tissues and transformed cell lines.

Tissue Distribution of NA14—To investigate the expression of na14, Northern blot analysis of mRNAs from different human tissues was performed with λA10 insert as a probe. As seen in Fig. 2, the highest level of expression is observed in testes although na14 can be detected in all the tissues assayed. This
result agrees with the data summarized in Table I.

Polycional Antiserum Production and Analysis of Expression of NA14 by Western Blotting and Immunoprecipitation—The entire insert from A10 clone was subcloned into a pGEX-4T-1 bacterial expression vector. A GST fusion protein of the expected molecular mass of 41 kDa was produced. This protein was recognized by RM autoimmune serum (Fig. 3A, left panel).

To show the specificity of the recognition, the fusion protein was cut with thrombin to generate GST and NA14. RM serum only recognized the NA14 moiety (Fig. 3A, left panel).

A polyclonal antiserum was raised in rabbit against the GST fusion protein. The resulting immune rabbit serum (anti-NA14) reacted strongly with the fusion protein and with both GST and NA14 thrombin products by immunoblotting (Fig. 3A, right panel).

The expression of NA14 was assessed in three laboratory cell lines by Western blotting with the rabbit polyclonal serum. Nonidet P-40 lysates from HeLa (human epithelial cells), Jurkat (human T lymphocytes), and NIH3T3 (murine fibroblasts) were resolved by SDS-PAGE, transferred to a nitrocellulose filter, and revealed with the polyclonal serum anti-NA14. The endogenous NA14 was not observed in 3T3 or HeLa cell lysates, but it could be detected in lysates from Jurkat cells after long blot exposures, suggesting that NA14 is present at low levels in these cells (Fig. 3B, left panel). The band of 66 kDa observed in all lanes was revealed by the preimmune rabbit serum (not shown).

HeLa and Jurkat cell lysates were also incubated with RM serum or other irrelevant sera, and complexes were precipitated with protein A-Sepharose. Immunoprecipitates were then analyzed by Western blotting with anti-NA14 polyclonal serum (Fig. 3B, right panel). NA14 was detected in RM immunoprecipitates from Jurkat cells but not HeLa cells or when a normal human serum or another autoimmune serum was used. These results show that (i) NA14 is an autoantigen specifically recognized and immunoprecipitated by RM autoimmune serum, (ii) NA14 seems to be more expressed in Jurkat than in

| Accession | Region in na14 | Percent identity | Library |
|-----------|---------------|-----------------|---------|
| U46389    | 22–375        | 98.0            | Pancreatic cancer cell |
| EST98372  | 79–297        | 98.6            | Thyroid |
| EST55796  | 107–354       | 98.8            | HSC172 cells I |
| EST96976  | 116–286       | 98.2            | T-cell lymphoma |
| EST64848  | 116–359       | 96.3            | LNCAP cells I |
| EST66254  | 116–362       | 99.6            | LNCAP cells I |
| EST60596  | 116–461       | 97.1            | LNCAP cells I |
| EST9615   | 208–522       | 95.3            | Synovial sarcoma |
| EST28170  | 292–653       | 98.4            | Cerebellum II |
| H81770    | 458–836       | 97.4            | Melanocyte 2NhHM, Soares |
| EST96842  | 486–854       | 96.5            | Testis I |
| N94604    | 501–854       | 97.8            | Senescent fibroblasts |
| EST9013   | 575–855       | 97.9            | NIH3T3, Soares |
| H29471    | 621–863       | 97.0            | Infant brain 1NIH, Soares |
| F18425    | 632–843       | 99.5            | Skeletal muscle HM1 |
| EST68368  | 697–854       | 97.4            | Fetal lung II |
| N57963    | 727–861       | 97.8            | Fetal liver + spleen |

HeLa cells although this protein should also be present in this cell line since two clones were isolated from a HeLa cDNA library (Fig. 1), and (iii) the size of the endogenous protein (Fig. 3B) fits well with the size of the cloned protein as observed after thrombin cleavage of GST-NA14 (Fig. 3A), confirming that the entire cDNA for NA14 was cloned.

Transfection and Subcellular Localization—Subcellular localization of endogenous NA14 was examined in several cell lines by indirect immunofluorescence with anti-NA14 serum, but no staining pattern could be easily recognized (not shown), consistent with the low level of the protein shown by immunoblot analysis (Fig. 3). This prompted us to investigate the subcellular location of NA14 in transfected cells. An HA-tagged form of NA14 was subcloned into the eukaryotic expression vector pECE. Plasmid pECE-HA-NA14 was introduced into human HeLa cells and mouse 3T3 cells by electroporation. Expression of the protein was assessed by Western blotting (Fig. 4, a and f). HeLa and 3T3 cells transiently transfected with HA-NA14 were examined by indirect immunofluorescence microscopy with anti-HA antibodies, anti-NA14 polyclonal an-
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NA14 is a nuclear protein. pECE-HA-NA14 was transfected by electroporation into HeLa cells (A-E) and NIH3T3 cells (F-J). The expression of the tagged protein was assessed by Western blotting with anti-NA14 rabbit serum, autoimmune RM serum, and anti-HA monoclonal antibodies (A and F). The only band detected corresponds to HA-NA14. Exposure time after ECL incubation was 1 min. The localization of the transfected protein was analyzed by immunofluorescence microscopy by staining with rhodamine-linked anti-HA antibodies (C, E, H, J) and with fluorescein-linked anti-rabbit antibodies, respectively. A speckled nuclear pattern is observed.

Although no nuclear localization signal was found in the sequence, immunofluorescence studies of HA-NA14 transfected cells demonstrated that this protein is restricted to the nucleus. The remarkable small size of this protein (the smallest coiled-coil protein described to our knowledge) could allow its free diffusion through the nuclear pore. This fact together with the ability to dimerize through the α-helical domain suggests that the interaction with other nuclear proteins may be responsible for its nuclear localization despite the absence of a nuclear localization signal. Consistent with this, NA14 was absent from Jurkat nuclear extracts prepared by addition of a high-salt buffer to isolated nuclei, to release soluble proteins without lysing the nuclei (not shown). The well defined pattern of NA14 distribution additionally support that it could be integrated in a complex with other nuclear proteins, probably through hydrophobic interactions since the protein is extracted by detergents but not by high salt concentrations.

Autoantibodies directed against nuclear components are frequently found in the sera of patients with autoimmune diseases. In this study, we have used the antibodies from a patient with Sjögren’s syndrome, a disease characterized by lymphocytic infiltrates and fibrosis of exocrine glands, to clone and characterize a novel nuclear autoantigen.

Interestingly, one of the EST sequences identical to na14 cDNA (Table I, accession number U46389) was identified as one of many distinct sequences that are differentially expressed in pancreatic cancer (37). This raises the possibility that na14 could be a potential oncogene or a cancer-associated marker. These data, together with the different levels of expression observed in different tissues or cell lines, suggest that the expression of NA14 might be regulated in normal or pathological conditions. Further experiments will be necessary to ascertain these views and to find the role of this new nuclear protein.

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REFERENCES

1. Tan, E. M. (1991) Cell 67, 841–842
2. van Venrooij, W. J., and Pruijn, G. J. M. (1995) Curr. Opin. Immunol. 7, 819–824
3. Chambers, J. C., and Keene, J. D. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2115–2119
4. Earnshaw, W. C., Sullivan, K. F., Machlin, P. S., Cooke, C. A., Kaiser, D. A., Pollard, T. D., Rothfield, N. F., and Cleveland, D. W. (1987) J. Cell. Biol. 104, 817–829
5. Reeves, W. H., and Stoege, Z. M. (1989) J. Biol. Chem. 264, 5047–5052
6. Yaneva, M., Wen, J., Ayala, A., and Cook, R. (1989) J. Biol. Chem. 264, 13407–13411
7. Szostekc, C., Guldner, H. H., Netter, H. J., and Will, H. (1990) J. Immunol. 145, 4338–4347
8. Ge, Q., Frank, M. B., O’Brien, C., and Targoff, I. N. (1992) J. Clin. Invest. 90,
Characterization of Nuclear Autoantigen NA14

559–570
9. Seelig, H. P., Monshbrugger, I., Ehrfeld, H., Fink, T., Renz, M., and Genth, E. (1995) *Arthritis Rheum.* 38, 1389–1399
10. Racevskis, J., Dill, A., Stocker, R., and Fineberg, S. A. (1996) *Cell Growth. Differ.* 7, 271–280
11. Riss, R. M., Tassin, A. M., Celati, C., Antony, C., Boissier, M. C., Homberg, J. C., and Bornens, M. (1994) *J. Cell Biol.* 125, 997–1013
12. Rodríguez, J. L., Gelpi, C., Thompson, M., Real, F. J., and Fernández, J. (1982) *Cyt. Exp. Immunol.* 49, 579–586
13. Blascheck, M. A., Pennec, Y. L., Simitzis, A. M., Le Goff, P., Lamour, A., Kerdraon, G., Jouquan, J., and Youinou, P. (1988) *Scand. J. Rheumatol.* 17, 291–296
14. Krohne, G., Stick, R., Kleinschmidt, J. A., Moll, R., Franke, W. W., and Hausen, P. (1982) *J. Cell Biol.* 94, 749–754
15. Devereux, J., Haeberli, P., and Smithies, O. (1984) *Nucleic Acids Res.* 12, 387–395
16. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* 215, 403–410
17. Lupas, A., Van Dyke, M., and Stock, J. (1991) *Science* 252, 1162–1164
18. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* 157, 105–132
19. Chou, P. Y., and Fasman, G. D. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol.* 47, 45–148
20. Garnier, J., Osguthorpe, D. J., and Robson, B. (1978) *J. Mol. Biol.* 120, 97–120
21. Towbin, H., Staehlin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* 76, 4350–4354
22. Wilson, I. A., Niman, H. L., Houghten, R. A., Cherenson, A. R., Connolly, M. L., and Lerner, R. A. (1984) *Cell* 37, 767–778
23. Ellis, L., Cluaser, E., Morgen, D. O., Edery, M., Roth, R. A., and Rutter, W. J. (1986) *Cell* 45, 721–732
24. Kozak, M. (1987) *Nucleic Acids Res.* 15, 8125–8148
25. Wickens, M. (1990) *Trends Biochem. Sci.* 15, 277–324
26. Yang, C. H., Lambie, E. J., and Snyder, M. (1992) *Dev. Biol.* 154, 57–44
27. Pollard, K. M., Chan, E. K. L., Grant, B. J., Sullivan, K. F., Tan, E. M., and Glass, C. A. (1990) *Mol. Cell. Biol.* 10, 2164–2175
28. Cunningham, M. W., Antone, S. M., Gulizia, J. M., McManus, B. A., and Gauntt, C. J. (1995) *Cyt. Immunol. Immunopathol.* 68, 118–123
29. Chan, E. K. L., Hamel, J. C., Buyon, J. P., and Tan, E. M. (1991) *J. Clin. Invest.* 87, 68–76
30. Abel, T., and Maniatis, T. (1989) *Nature* 341, 24–25
31. Landschulz, W. H., Johnson, P. F., Adashi, E., Graves, B. J., and McKnight, S. L. (1988) *Genes Dev.* 2, 786–800
32. Buckland, R., Malvoisin, E., Beauverger, P., and Wild, F. (1992) *J. Gen. Virol.* 73, 1703–1707
33. Roe, J. L., Durée, T., Zupan, J. R., Repetti, P. P., McLean, B. G., and Zambryski, P. C. (1997) *J. Biol. Chem.* 272, 5838–5845
34. Surette, M. G., and Stock, J. B. (1996) *J. Biol. Chem.* 271, 17066–17073
35. Gress, T. M., Muller-Pillasch, P., Geng, M., Zimmerhackl, P., Zebetner, G., Friess, H., Büchler, M., Adler, G., and Lehrach, H. (1996) *Oncogene* 13, 1819–1830