A Novel hsp110-related Gene, apg-1, That Is Abundantly Expressed in the Testis Responds to a Low Temperature Heat Shock Rather than the Traditional Elevated Temperatures*

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We isolated a novel hsp110-related gene, apg-1, from a testis cDNA library. The apg-1 transcripts were constitutively expressed in the testicular germ cells and, in some degree, most tissues examined. In a mouse TAMA26 Sertoli cell line, apg-1 transcripts were induced in 2 h by a temperature shift from 32 to 39 °C, but not by a shift from 37 to 42 °C, the traditional heat stress, or a shift from 32 to 42 °C. The heat response pattern of hsp110 expression was similar to that of apg-1. Although induction of a hsp70 transcript was observed in 2 h by a shift from 32 to 39 °C, the induction was more apparent by a shift from 37 to 42 °C or from 32 to 42 °C. Essentially similar differential response patterns were observed among these genes in NIH/3T3 fibroblasts as well. The nuclear run-on assay and the native gel mobility shift assay demonstrated that, by the 32 to 39 °C temperature shift, the apg-1 gene was transcriptionally activated, and heat shock factor 1 bound to the heat shock elements in the 5'-flanking region of the apg-1 gene. These results demonstrated that expressions of apg-1, hsp110, and hsp70 could be heat-induced at a temperature lower than the traditional elevated temperatures in somatic cells of both testis and nontestis origin and suggest that the mechanisms regulating the transcript levels of apg-1 and hsp110 are different from those of hsp70. Furthermore, the constitutive expression in germ cells suggests that APG-1 plays a specific role in spermatogenesis as well as in stress response.

Prokaryotic and eukaryotic organisms respond to elevated temperatures by synthesizing a distinct set of proteins termed heat shock proteins (HSPs) (1). Anoxia, ethanol, radiation, inflammation, and certain heavy metal ions also induce HSPs in the cells. An early and long-standing assumption regarding the heat shock response was that the HSPs protected cells from the toxic effects of heat and other stresses. Subsequently, a series of studies revealed that HSPs are also present in cells at normal temperatures. Now members of the HSP family are established as molecular chaperones, assisting in the folding and unfolding, assembly and disassembly, and transport of various proteins (2–5). HSPs are also shown to interact with mutant p53 and p60src (6–8), suggesting their involvement in cell cycle regulation.

Spermatogenesis begins at puberty and consists of three steps: the mitotic proliferation of the spermatogonia, meiosis at the spermatocyte stage, and the distinct cellular structural changes of the spermatids. Unlike somatic cells, the male germ cells are easily damaged at the body cavity temperature (9), indicating the presence of a differential heat sensitivity between somatic cells and germ cells. Sarge (10) recently reported that the temperature threshold for induction of HSP72 encoded by the hsp70 gene was lower in male germ cells than in somatic cells. To date, several HSPs have been found to be constitutively expressed in germ cells at specific stages of development. Two HSP70-related genes, hsp70.2 and hsc70r, are expressed in spermatocytes and spermatids, respectively (11, 12). HSP90 and HSP60 are expressed in spermatogonia and spermatocytes (13, 14). These findings suggest that HSPs play a role in normal germ cell development as well as in stress response.

In an attempt to identify genes involved in spermatogenesis, we previously used PCR and identified several protein tyrosine phosphatases expressed in differentiating germ cells (15). In the present study, we subtracted the testis cDNAs of the prepubertal mice from those of adult mice. One novel gene, apg-1, was isolated, the sequence of which showed homology to Chinese hamster hsp110 (16) and human hsp70RY (17). We analyzed the heat inducibility of apg-1 and hsp110 transcripts in the somatic testicular and nontesticular cells and found that optimal heat shock conditions for the inductions of the apg-1 and hsp110 transcripts were different from those of hsp70.

EXPERIMENTAL PROCEDURES

Cells and Heat Shock Treatment—The mouse WBB6F1 +/+–3T3–5 fibroblast, NIH/3T3 fibroblast, WEHI-3 myelomonocytic leukemia, P-815 mastocytoma, OTT6050 embryonal carcinoma, BMA1 bone marrow stromal cell, and TAMA26 Sertoli cell lines were used in the present experiments (18–20). They were cultured in appropriate media at 37 °C with 5% CO2 in air. To analyze the effects of heat stress, NIH/3T3 and TAMA26 cells were grown at 32 or 37 °C for 20 h prior to the heat shock. The culture media were replaced with the fresh media prewarmed to 37, 39, or 42 °C, and then the cells were incubated at the respective temperature for 2 h in a CO2 incubator. Whole testis tissues from W/Wv mutant mice were incubated in Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum at 32 °C for 30 min, and then the media were replaced with the fresh media prewarmed to 32 or 39 °C and incubated at the respective temperature for 2 h in a CO2 incubator.

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Animals—Sexually mature (4-month-old) and newborn (3-day- and 7-day-old) dd/y std mice and 4-month-old WBB/B1-F1/W-W/ mutants were purchased from Japan SLC Company (Hamamatsu, Japan). dd/y std is a strain of albino mice maintained in a closed colony (21). The adult WBB/B1-F1/W-W/ mice have no germ cells (22) and were obtained by mating a dominant 5C5/BB/aum-7r and a C57/Bl6 mutant (strain W-/+). RNA Extraction and Northern Blot Hybridization—Various mouse tissues and cells were homogenized in 0.1 M guanidinium thiocyanate solution, and the RNA was extracted as described (21). Thirty μg of total cellular RNA or 4 μg of poly(A) RNA was separated on 1% agarose-formaldehyde gels by electrophoresis and were blotted onto nylon filters (Hybond-N; Amersham Corp.). The filters were hybridized to [α-32P]dCTP-labeled, randomly primed cDNA fragments in a rapid hybridization buffer (Amersham) for 2 h. A 1873-bp EcoRI-DraII fragment of apg-1 cDNA, a 793-bp fragment of the mouse hsp70 cDNA, and a 1787-bp PstI fragment of the mouse hsp110 cDNA were used as probes. The probe for the mouse hsp70 was generated from NIH/3T3 fibroblast cDNA by PCR using primers 5'-GACCCTCAGCTCTGCTGACG- GACGT-3' (positions 1973–1992; Ref. 23) and 5'-TAGACCAACCGG- GAGAGCC-3' (positions 2746–2765). This probe potentially crossreacts with hsc70. For isolation of a full-length mouse hsp110 cDNA, a probe was generated from NIH/3T3 cDNA by PCR using degenerate primers 5'-ATIGA/GATG/CT/CT/AT/ATGAGA/CT/CTGA-3' (I, inosine) and 5'-TC/CT/CTC/ACC/AG/AT/TTT/CTTT/CTGG-3' (I, inosine) corresponding to the amino acids IECFMND and AKNAVEE, respectively, conserved among APG-1, Chinese hamster HSP110 (16), and human HSP70/RY (17). The PCR product was cloned into the pBluescript SK(-) vector (Stratagene, La Jolla, CA) and was used as a probe to screen a mouse brain cDNA library. The nucleotide sequence of the isolated clone was identical to the recently reported sequence of mouse hsp105a (24); data not shown). After hybridization, the filters were washed under stringent conditions (65°C for 30 min in a washing buffer composed of 0.1 M NaCl, 0.015 M sodium citrate, pH 7.0) and 0.1% SDS and then exposed to x-ray film at −80°C. The filters were finally stripped and rehybridized with a cDNA probe for the S26 ribosomal protein to correct for the amount of RNA loaded.

cDNA Subtraction and Screening of Libraries—cDNA libraries were constructed from the testes of 4-month-old and 3-day-old mice using a Time Saver cDNA synthesis kit (Pharmacia Biotech Inc.) according to the manufacturer's protocols. cDNAs from the 3-day-old mouse (competitor) were subtracted from cDNAs from the 4-month-old mouse (target) as described (25). After four cycles of subtraction, the remaining target cDNAs were cloned into the pBluescript SK(-) vector and sequenced. For isolation of a full-length cDNA clone, a cDNA library was made from adult mouse testis and ligated to an XZAPII phage vector (Stratagene). The mouse genomic library constructed from MboI-digested BALB/c mouse DNA was kindly provided by Dr. S.-I. Hayashi (Tottori University, Tottori, Japan). The cDNA and genomic libraries were screened with the isolated cDNA fragments and a 805-bp EcoRI fragment of apg-1 cDNA, respectively, under stringent conditions. The nucleotide sequences were determined after subcloning as described previously (21).

Nuclear Run-on Assay—Isolation of nuclei and nuclear run-on assays were performed as described (26). The following plasmids labeled with nitrocellulose filters and hybridized to [α-32P]dUTP-labeled transcripts: pBluescript with no insert, pBluescript containing apg-1 cDNA, pBluescript containing hsp105a cDNA, and pBluescript containing S26 ribosomal protein cDNA.

Gel Mobility Shift Assay—The nuclear extracts were prepared from the heat-shocked cells as described by Schreiber et al. (27). Binding reactions were performed by adding 15 μg of the nuclear extract to a mixture containing 0.1 ng of [32P]labeled, double-stranded heat shock element (HSE) oligonucleotides found in the apg-1 genomic sequence (HSE-apg, 5'-CCCTTCTTCAGCTCTCTGACGCTGCACTCCCTCGCCG-3', corresponding to nucleotides 304–339, and its complementary oligonucleotide) or a self-complementary consensus HSE oligonucleotide, which contains four perfect inverted 5'-NGAAN-3' repeats after annealing (HSP-control, 5'-CTTAGAAGCTCTAGAGTCTCTAG-3') (10) in 25 μl of binding buffer (10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol) containing 0.1 μg of bovine serum albumin and 1 μCi of [32P]dATP (3000 Ci/mmol). For experiments involving antibodies, 0.5 μl of anti-heat shock factor 1 (HSF1) polyclonal antisera (Affinity BioReagents, Inc., Golden, CO) or preimmune antiserum was added to aliquots of extract and incubated for 30 min on ice before addition of the reaction mixture. Competition reaction mixtures contained a 100-fold molar excess of nonradioactive HSE oligonucleotide. The mixtures were incubated for 20 min at 25°C, and then free and bound DNAs were separated by electrophoresis in a nondenaturing 4% polyacrylamide gel. Gels were dried and exposed to film at −80°C with an intensifying screen.

RESULTS

Isolation of apg-1 from a Mouse Testis cDNA Library—After analyzing the subtracted cDNA library enriched with genes specifically expressed in the adult male testis, we isolated one clone (KH77), the expression of which was increased in the adult compared with newborn testis. The nucleotide sequence of the 776-bp insert of KH77 was novel with some homology to known hsp genes. To obtain complete cDNA, we screened a mouse testis cDNA library using KH77 as a probe. Of the 2 × 105 clones screened, more than 200 clones hybridized to the probe. Subsequent nucleotide sequence analysis of 20 clones containing cDNA fragments ranging from 1.5 to 2.7 kilobases revealed that all of these 20 clones contained partial sequences of the same gene, which we termed apg-1. Fig. 1 shows the nucleotide sequence and the predicted amino acid sequences of the longest clone. It contains 2782 nucleotides with a single open reading frame encoding 838 amino acids. Although there were no stop codons, a highly GC-rich sequence characteristic of a 5' untranslated region was present upstream of the first ATG codon. The predicted amino acid sequence of apg-1 indicated a product that lacked a leader sequence and an endoplasmic reticulum retention signal (28). The NH2-terminal region contained a single ATP binding motif, consisting of the conserved residues VVGIIDLG (positions 3–10), EKLK (positions 271–274), and IEIVGGATIKPAVE (positions 338–352) (29, 30). A homology search using the SWISSPROT data base revealed that APG-1 had homology in amino acid sequence to Chinese hamster HSP110 (16) and human HSP70/RY (17) (Fig. 2). The predicted amino acid sequence for APG-1 was longer than that of human HSP70/RY. Discounting this extended amino acid region present in the COOH terminus of APG-1, APG-1 was 64% identical to human HSP70/RY. The number of predicted amino acids in APG-1 was comparable to that of Chinese hamster HSP110, and their amino acid sequences were 57% identical.

Predominant Expression of apg-1 in Male Germ Cells—Northern blot analysis of various tissues revealed that two apg-1 transcripts of 3 and 3.5 kilobases in length were abundantly expressed without heat shock in the testis (Fig. 3a). Lower levels of expression were seen in the brain, heart, and ovary. With longer exposures or reverse transcription-PCR, expression was detected in all tissues examined, including lung, spleen, liver, and kidney (data not shown).

To determine whether apg-1 was expressed in the germ cells and/or the somatic cells of the testis, testes from dominant spotting (W) mutant mice were examined. The W/W mutant mice lack germ cells in the adult, although the somatic cell elements are apparently normal (22). As shown in Fig. 3b, the expression level of apg-1 was low in testes of W/W mice, whereas it was high in testes of wild-type mice. This observation strongly suggests that the apg-1 transcripts were predominantly expressed in the germ cells.

To further characterize the cell type specificity of constitutive apg-1 expression, several murine cell lines were analyzed (Fig. 3c). The expression was detected, although at a lower level than that in the testis tissue, in all cell lines of various origins, suggesting that a high level expression was specific to male germ cells (Fig. 3c). Heat Induction of apg-1 Transcripts—To examine the heat inducibility of apg-1, we exposed the TAMAS26 Sertoli cells of testicular somatic cell origin to heat shock. As the temperature in the testis is lower than body cavity temperature (31), the temperature was shifted from 32 to 37, 32 to 39, or 32 to 42°C in addition to the traditional shift from 37 to 42°C. As shown in Fig. 4a, the apg-1 transcripts (top panel) were induced in
TAMA26 cells grown at 32°C for 20 h and then heat-shocked at 39°C for 2 h (lane 3). However, the apg-1 transcripts were not induced in 2 h by the shift from 37 to 42°C (Fig. 4a, lane 6) or the shift from 32 to 42°C (Fig. 4a, lane 4). The heat shock response pattern of hsp110 expression was similar to that of apg-1 (Fig. 4a, second panel). In contrast to apg-1 and hsp110, a strong induction of hsp70 (Fig. 4a, third panel) was observed by temperature shifts from 37 to 42°C (Fig. 4a, lane 6) and from 32 to 42°C (Fig. 4a, lane 4). A weaker induction was observed by the shift from 32 to 39°C (Fig. 4a, lane 3).

The heat shock response of testicular somatic cells was observed not only in cultured cells but also in testis tissues. We used whole testes excised from the germ cell-deficient W/W v mutant mice and incubated them at 39°C for 2 h. As shown in Fig. 4b, induction of apg-1, hsp110, and hsp70 expression was demonstrated.

We next examined the heat inducibility of apg-1 in nontesticular somatic cells (Fig. 5). The temperature shift from 32 to 39°C (Fig. 5, lane 3) but not from 32 to 42°C (Fig. 5, lane 4), induced the expression of apg-1 and hsp110 transcripts in NIH/3T3 fibroblasts. Hsp70 transcripts were also induced by the temperature shift from 32 to 39°C (Fig. 5, lane 3), but a stronger induction was observed by the shift from 32 to 42°C (Fig. 5, lane 4). These results demonstrated that apg-1, hsp110, and hsp70 transcripts were induced by a heat shock at temperatures lower than the traditional elevated temperatures, and that the optimal conditions of heat stress for induction of apg-1, hsp110, and hsp70 transcripts differed from those of hsp70.

To determine whether apg-1 and hsp110 transcripts were transcriptionally activated by the 32 to 39°C temperature shift, we performed nuclear run-on assays using the heat-shocked NIH/3T3 fibroblasts. As shown in Fig. 6, transcription of both apg-1 and hsp110 genes were increased 1 h after the temperature shift from 32°C to 39°C. These experiments were performed three times, and each study yielded similar results.
To analyze the regulatory mechanisms of apg-1 expression, we screened a mouse genomic library with apg-1 cDNA and obtained a clone containing a 20-kilobase fragment. The PstI-digested DNA fragments were then subcloned into plasmid vector pBluescript SK(+) and sequenced. By primer extension assay (32) and an oligo-capping method (33) using RNAs extracted from control and heat-shocked NIH/3T3 fibroblasts, a transcription start site was mapped to the C residue (Fig. 7, p) 198 bp upstream of the putative initiation codon of apg-1 (data not shown). As shown in Fig. 7, the 5'-flanking region of the transcription start site of apg-1 contained several Sp1 core sequences (34), suggesting that this region acts as the promoter of apg-1 expression. In addition to these Sp1 sequences, a putative HSE containing five pentamers was present (35–37).

Activation of HSF1 for Binding to the HSE of apg-1—The levels and identities of proteins in heat-shocked NIH/3T3 cells that bind to the HSE-apg or the HSE-control containing four complete NGAAN repeats (10) were examined by the gel mobility shift assay (Fig. 8). Nuclear extracts were prepared from cells incubated at 37, 39, or 42 °C after culturing at 32 °C or from cells continued to be cultured at 32 °C, and the levels of HSE-binding proteins were determined. Extracts from cells maintained at 32 °C contained an activity that bound to HSE-apg (Fig. 8a, lane 1). With the nuclear extracts from cells heat-shocked at 39 °C, other shift bands with slower mobility were detected (Fig. 8a, lane 3). The level of the binding activity increased as the magnitude of the temperature shift increased (Fig. 8a, lanes 2–4). In the presence of excess unlabeled HSE oligonucleotides (competitors), the slower-migrating bands disappeared, but the faster-migrating band detected with both control and heat-shocked cell extracts did not (Fig. 8a, lane 5). The slower-migrating bands were supershifted in the presence of anti-HSF1 serum but not in the presence of preimmune serum (Fig. 8a, lanes 6 and 7). These results demonstrated that HSF1 was activated by both 32 to 39 °C and 32 to 42 °C heat stresses and bound to HSE-apg in vitro. Similarly, the binding activity of HSF1 to HSE-control was detected at 37 °C and
Both HSF1 and constitutive HSE binding factor are now upregulated in that of constitutive HSE binding factor are induced. The level of HSE binding activity of HSF1 and a concomitant decrease in that of constitutive HSE binding factor are induced. In addition to these transcription factors, another regulatory element or other elements including negative regulator(s) binding to HSE-apg, was also found to bind to HSF1 in heat-shocked cell extracts. Although HSF1 was activated to bind to HSE-apg by the presence of HSF1, should be considered.

DISCUSSION

HSPs are classified into families based on their approximate molecular masses and degrees of homology. The major classes of mammalian HSPs are HSP90s (83–99 kDa), HSP70s (68–80 kDa), HSP60s, and the smaller HSPs (25–28 kDa) (1). In addition, the presence of HSP110 has been known at the protein level (38), and recently, a cDNA for HSP110 was cloned from Chinese hamster (16) and mouse (named HSP105 in Ref. 24). APG-1 described herein and Chinese hamster HSP110 shared 57% homology in their amino acid sequences and together with Chinese hamster (16) and mouse (1) (38), and some other HSPs will constitute a distinct family. Despite this structural similarity, the tissue distributions of apg-1 and some other HSPs will constitute a distinct family. Without exogenous stress, HSP110 is ubiquitously expressed in various tissues (16, 24), whereas apg-1 transcripts were predominantly expressed in testicular germ cells. This expression pattern of apg-1 suggests an involvement of APG-1 in spermatogenesis. The fact that apg-1 transcripts were heat-induced in both testicular and nontesticular somatic cells suggests another role that APG-1 plays under heat stress conditions. Although the expressions of apg-1, hsp110, and hsp70 were inducible by the 32 to 39 °C temperature shift, the optimal heat stress conditions for the induction of apg-1 and hsp110 were different from those of hsp70. These results suggest that the functions of APG-1 and HSP110 in heat shock response are different from those of HSP70.

Consensus sequences termed HSEs are located upstream of the promoter of hsp genes and are required for the proper induction by stress and/or other elements (34, 35, 39, 40). Two transcription factors, HSF1 and HSF2, have been shown to interact with the HSE in mammalian cells (41–43). HSF1 mediates the induction of hsp gene expression in response to elevated temperatures (42, 43), whereas HSF2 is believed to regulate the expression of hsp genes under nonstress conditions (44). In addition to these transcription factors, another protein, constitutive HSE binding factor, has been shown to bind to the HSE (45). With heat shock, a rapid increase in the level of HSE binding activity of HSF1 and a concomitant decrease in that of constitutive HSE binding factor are induced. Both HSF1 and constitutive HSE binding factor are now supposed to be involved in the regulation of heat shock gene expression, the former as a positive regulator and the latter as a negative regulator (46). We demonstrated that the increase in the level of HSE binding activity of HSF1 in NIH/3T3 cells paralleled the intensity of hsp70 expression induced by temperature shift from 32 to 37 °C and above (Figs. 5 and 8). These results are consistent with the hypothesis that HSF1 is activated in response to the magnitude of temperature upshift rather than the absolute high temperatures (26). The putative HSE elements in the 5′-flanking region of the apg-1 gene, HSE-apg, was also found to bind to HSF1 in heat-shocked cell extracts. Although HSF1 was activated to bind to HSE-apg by both the 32 to 39 °C and 32 to 42 °C temperature shifts, induction of apg-1 expression was observed under the former, but not the latter, conditions. Thus, the regulation of heat induction of apg-1 transcripts cannot be explained by the HSF1 activation alone. Some other mechanisms are responsible for the differential induction of hsp70 and apg-1 transcripts. Several possibilities including negative regulator(s) binding to HSE-apg and/or other elements in vivo and an alteration of chromatin accessibility to HSF1, should be considered.

The temperature of the testis is tightly maintained at 30 °C (31), and male germ cells are easily damaged at the body cavity temperature (9). Recently, the temperature thresholds for activation of HSF1 and induction of HSP72 have been demonstrated to be lower in the germ cells than in somatic cells (10). In the present study, we demonstrated that hsp70 as well as apg-1 and hsp110 transcripts were induced by temperature shift from 32 to 39 °C in two somatic cells and tissues. Nuclear run-on assays showed an increase in transcription rates. These results revealed the presence of a heat shock response operating at temperatures lower than the previously described ones.
in testicular and nontesticular somatic cells. Since this type of heat stress is expected to occur even in body cavity organs under certain conditions, such as during recovery from transient brain ischemia (47), the low temperature heat shock response described herein may have broad implications in pathological and physiological conditions. Whether HSPs interact with different or the same proteins under the traditional and low temperature heat shock conditions is an interesting question and may help elucidate the biological significance of these heat shock responses.

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REFERENCES

1. Lindquist, S., and Craig, E. A. (1988) Annu. Rev. Genet. 22, 631–677
2. Craig, E. A., Weissman, J. S., and Horwich, A. L. (1994) Cell 76, 365–372
3. Hendrick, J. P., and Hartl, F.-U. (1995) Annu. Rev. Biochem. 64, 349–384
4. Pfanner, N., Rassow, J., van der Klei, I. J., and Neupert, W. (1992) Cell 68, 999–1002
5. Glick, B. S., Beaseley, E. M., and Schatz, G. (1992) Trends Biochem. Sci. 17, 453–459
6. Pinhashi-Kimhi, O., Michalovitz, D., Ben-Zeev, A., and Oren, M. (1986) Nature 320, 182–185
7. Hainaut, P., and Milner, J. (1992) EMBO J. 11, 3513–3520
8. Lindquist, S., and Xu, Y. (1993) Proc. Natl. Acad. Sci. U. S. A. 78, 7074–7078
9. Moore, C. R., and Chase, D. (1923) Anat. Rec. 28, 344–345
10. Sarge K. D. (1995) J. Biol. Chem. 270, 18745–18748
11. Zakeri, Z. F., Wolgemuth, D. J., and Hunt, C. R. (1988) Mol. Cell. Biol. 8, 2925–2932
12. Matsumoto, M., and Fujimoto, H. (1990) Biochem. Biophys. Res. Commun. 166, 43–49
13. Ohak, S., Bunick, D., and Hayashi, Y. (1995) J. Histochem. Cytochem. 43, 67–76
14. Meinhardt, A., Parvinen, M., Bacher, M., Aumuller, G., Hakovirta, H., Yagi, A., and Seitz, J. (1995) Biol. Reprod. 52, 798–807
15. Kaneo, Y., Takano, S., Okamura, K., Takenawa, J., Higashitsuji, H., Nakayama, H., and Fujita, J. (1993) Biochem. Biophys. Res. Commun. 197, 625–631
16. Lee-Yoon, D., Easton D., Murawski, M., Burd, R., and Subjeck, J. R. (1995) J. Biol. Chem. 270, 15725–15733
17. Fathallah, D. M., Cherif, D., Dellagi, K., and Arnaout, M. A. (1993) J. Immuno- nol. 151, 810–813
18. Fujita, J., Osumi, Y., Ebi, Y., Nakayama, H., and Kanakura, Y. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2888–2891
19. Hay, R., Caputo, J., Chen, T. R., Macy, M., MacClintock, P., and Reid, Y. (eds) (1992) ATCC Cell Lines & Hybridomas, 7th Ed., American Type Culture Collection, Rockville, MD
20. Fujita, J., Yoshida, O., Miyamoto, T., and Mori, K. J. (1983) Gann 74, 334–337
21. Kaneo, Y., Nonoguchi, K., Fukuyma, H., Takeno, S., Higashitsuji, H., Nakayama, H., Takenawa, J., Nakayama, H., and Fujita, J. (1995) Oncogene 10, 945–952
22. Russel, E. S. (1979) Adv. Genet. 20, 357–459
23. Hunt, C., and Calderwood, S. B. (1990) Gene (Amst.) 87, 199–204
24. Yasuda, K., Nakai, A., Hatayama, T., and Nagata, K. (1985) J. Biol. Chem. 270, 29718–29723
25. Higuchi T., Kanzaki, H., Nakayama, H., Fujimoto, M., Hayashita, K., Kijima, K., Iwai, M., Mori, T., and Fujita, J. (1995) Endocrinology 136, 4973–4981
26. Abrao, K., Phillips, R., and Morimoto, R. I. (1991) Genes Dev. 5, 2117–2127
27. Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419
28. Jackson, M. R., Nilson, T., and Peterson, P. A. (1990) EMBO J. 9, 3153–3162
29. Bork, P., Sander, C., and Valencia, A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7290–7294
30. Flaherty, K. M., McKay, D. B., Kaseh, W., and Holmes, K. C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5041–5045
31. Harrison, R. G., and Weiner, J. S. (1948) J. Physiol. (Lond.) 107, 48P
32. Mc Knight, S. L., and Kingsbury, R. (1982) Science 217, 316–324
33. Maruyama, K., and Sugano, S. (1994) Gene (Amst.) 138, 171–174
34. Briggs, M. R., Kadowaga, J. T., Bell, S. P., and Tjian, R. (1986) Science 234, 47–52
35. Lindquist, S. (1986) Annu. Rev. Biochem. 55, 1151–1191
36. Pelham, H. (1985) Trends Genet. 1, 31–35
37. Perisic, O., Xiao, H., and Lin, J. T. (1989) Cell 59, 797–806
38. Subjeck, J. R., Shyy, T., Shen, J., and Johnson, R. (1983) J. Cell Biol. 97, 1389–1395
39. Cohen, R. S., and Moseelson, M. (1985) Cell 43, 737–746
40. Kothary, R., Clapoff, S., Darling, S., Perry, M. D., Moran, L. A., and Rossant, J. (1989) Development (Camb.) 105, 707–714
41. Shuetz, T. J., Gallo, G. J., Sheldon, L, Tempst, P., and Kingston, R. E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6911–6925
42. Robert, S. K., Giorgi, G., Clos, J., and Wu, C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9605–9610
43. Sarge, R. D., Zimarino, V., Holm, K., Wu, C., and Morimoto, R. I. (1991) Genes Dev. 5, 909–911
44. Sistonen, L., Serge, K. D., Phillips, B., and Morimoto, R. I. (1992) Mol. Cell. Biol. 1, 4104–4111
45. Liu, K. W., Kim, D., Yang, S.-H., and Li, G. C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3078–3082
46. Kim, D., Outang, H., and Li, G. C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2126–2130
47. Buso, R., Dietrich, W. D., Globus, M. Y.-T., Valdes, I., Scheinberg, P., Ginsberg, M. D. (1987) J. Cereb. Blood Flow Metab. 7, 729–738