Developmentally Dictated Expression of Heat Shock Factors: Exclusive Expression of HSF4 in the Postnatal Lens and Its Specific Interaction with αB-crystallin Heat Shock Promoter*

Received for publication, May 25, 2004, and in revised form, July 26, 2004
Published, JBC Papers in Press, August 12, 2004, DOI 10.1074/jbc.M405813200

T. Somasundaram‡ and Suraj P. Bhat¶¶
From the ‡Jules Stein Eye Institute, ¶Brain Research Institute, and ¶¶Molecular Biology Institute, University of California, Los Angeles, California 90095-7000

The molecular cascade of stress response in higher eukaryotes commences in the cytoplasm with the trimerization of the heat shock factor 1 (HSF1), followed by its transport to the nucleus, where it binds to the heat shock element leading to the activation of transcription from the down-stream gene(s). This well-established paradigm has been mostly studied in cultured cells. The developmental and tissue-specific control of the heat shock transcription factors (HSFs) and their interactions with heat shock promoters remain unexplored. We report here that in the rat lens, among the three mammalian HSFs, expression of HSF1 and HSF2 is largely fetal, whereas the expression of HSF4 is predominantly postnatal. Similar pattern of expression of HSF1 and HSF4 is seen in fetal and adult human lenses. This stage-specific inverse relationship between the expression of HSF1/2 and HSF4 suggests tissue-specific management of stress depending on the presence or absence of specific HSF(s). In addition to real-time PCR and immunoblotting, gel mobility shift assays, coupled with specific antibodies and HSE probes, derived from three different heat shock promoters, establish that there is no HSF1 or HSF2 binding activity in the postnatal lens nuclear extracts. Using this unique, developmentally modulated in vivo system, we demonstrate 1) specific patterns of HSF4 binding to heat shock elements derived from αB-crystallin, Hsp70, and Hsp82 promoters and 2) that it is HSF4 and not HSF1 or HSF2 that interacts with the canonical heat shock element of the αB-crystallin gene.

Induced transcription from heat shock promoters is mediated by the activation of transacting HSFs1 (1, 2). There are four known HSFs (HSF1, HSF2, HSF3, and HSF4). HSF3 is an avian HSF (3, 4). Although yeast and Drosophila melanogaster have a single gene that encodes an HSF, higher eukaryotes, animals, and plants have multiple genes that code for HSFs (4–6). HSF1 and HSF2 transcription factors have almost identical gene structures (4). The heat shock response starts with the cytoplasmic HSF and its trimerization and transport to the nucleus, where it binds to the heat shock element (HSE) in the heat shock promoter, activating transcription of the down stream heat shock gene(s) (1, 4). Both HSF1 and HSF2 contain three hydrophobic repeats, HR-A, -B, and -C. HR-A and -B are involved in trimerization upon reception of the stress signal. HR-C, located at the carboxyl terminus, has been suggested to inhibit trimerization in the uninduced state. HSF4, on the other hand, does not contain the HR-C domain; it therefore exists as a trimeric unit and binds to the DNA constitutively (for review, see Ref. 4).

HSF1 is considered to be the universal HSF and mediates expression of heat shock genes upon reception of a stress signal such as high temperature, whereas HSF2 is associated with developmental control. Although it has not been experimentally established, the assumption in this generalization is that all tissues and cells contain HSF1 as a pre-existing HSF in the cytoplasm to enable a cell or a tissue to mount a response to heat shock or stress. Furthermore, it is not yet clear whether each HSF activates a distinct set of target genes. The mechanism of the activation of heat shock promoter in response to stress, mostly studied in cultured cells, has been well elucidated; however, the developmental control of the heat shock promoter is not yet understood. It is clear, however, that the heat shock factors (7) and heat shock proteins do have developmental roles (8–11). The only heat shock factor in the fission yeast is known to be required for growth at normal temperatures as well (12).

We have shown previously that the increased expression of the small heat shock protein gene αB-crystallin in the postnatal (PN) rat ocular lens coincides with the appearance of a trimeric HSF-HSE complex that is formed between HSFs and the heat shock element (HSE-αB) present in the heat shock promoter of the αB-crystallin gene (13, 14). This trimeric complex appears in a developmentally (temporally) controlled fashion with highest efficiency around PN day 10, complementing the increased αB-crystallin expression at this stage. It is noteworthy that HSE-αB is a canonical heat shock element (2, 13), yet the appearance of the trimeric complex, as assessed by gel mobility shift assays, is developmentally dictated (absent in the fetal lens and appearing only in the postnatal lens) and tissue-specific (13). To understand the developmental control of the heat shock promoter of the αB-crystallin gene and identify the HSF that interacts with this promoter, we studied the expression of three members of the mammalian HSFs, HSF1, HSF2, and HSF4, in the rat lens. We show that there is very little HSF1 or HSF2 in the postnatal adult lens and that HSF4 predominates. Further, gel mobility binding assays done with [32P]HSE-αB and nuclear extracts of the fetal as well as postnatal lens tissues reveal that the heat shock promoter of the αB-crystallin gene selectively binds to HSF4 and not to HSF1.
or HSF2. We further compared HSF4 binding activity in the PN day 10 nuclear extracts using three heat shock elements derived from three different heat shock promoters. These data revealed promoter specific HSF4 binding characteristics.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Antibodies for HSF1 and HSF2 were purchased from Chemicon International (Temecula, CA). These are rat anti-HSF1 and anti-HSF2 monoclonal antibodies raised against recombinant mouse full-length HSF1 and HSF2. Previously used (13) anti-HSF1 antibody (Affinity Bioreagents Inc., Golden, CO) cross-reacts with HSF4 (data not shown). Antibodies for HSF4 (total) and for HSF4b (specific for HSF4b containing the DNA binding domain) were purchased from Affinity Bioreagents Inc. (Golden, CO). Antibodies were characterized by immunoblotting and competition with the authentic original peptide and a non-specific peptide.

**Primers and Probes**—Oligonucleotides of various lengths were synthesized on a DNA synthesizer (Applied Biosystems, Foster City, CA) or purchased from commercial sources (Invitrogen Inc. Carlsbad CA). The oligonucleotide pairs used for PCR amplification are as follows: HSF1, 5′-AGCCCTGAGGAGTGGAC-3′ (41–60) and 5′-TTTGACTGCTC- CAGA-3′ (389–350) (GenBankTM accession number X53084); HSF2, 5′-GGCTAAAATGAGAAGCAG-3′ (22–41) and 5′-AATCTTG- GACACTCTCTTCA-3′ (521–530) (GenBankTM accession number NM-031694). HSF4, 5′-AAGGGTGTGAGCAGATGAG-3′ (248–267) and 5′-AGACACTGTTAGCCTTGGC-3′ (803–818) (GenBankTM accession AB029349). The number in parentheses after the sequence of each primer indicates the nucleotide positions. The oligonucleotides used for RNA extraction, RT-PCR, and real-time PCR—Rat tissues (200–300 mg) were homogenized using the TRIzol reagent (Invitrogen), and RNA was isolated by following the manufacturer’s protocol. The quality of the RNA was checked on 0.8% E-gel (Invitrogen). RT-PCR was performed using the RT-PCR core kit (Applied Biosystems) using random hexamer primers. In general, 1 μg of total RNA was reverse-transcribed in a 20-μl reaction followed by PCR in a 50-μl solution containing 2.5 μl of the reverse transcription reaction (RT) mix, 0.2 μM concentrations of each primer, 1.5 units of platinum TaqDNA polymerase (Invitrogen) in the reverse transcription buffer. Optimal concentrations of MgCl2 used in each of these reactions were determined for each primer set.

The real time PCRs were performed using Finzymes-Dynamo SYBR green qPCR kit and Opticon 2 (MJ Research, Boston, MA) in a 20-μl reaction containing 1 μl of the RT mix, 0.25 μM concentrations of primer, and 10 μl of the 2× qPCR reaction mix. The annealing temperature for the experimental samples was set at 58 °C; for β-actin reactions, it was set at 55 °C. The fluorescence was measured at 80 °C. The melting curve analysis was performed at the end of the reaction (after 40 cycles) between 50 °C and 90 °C to assess the quality of the final PCR products. The threshold cycles, C(t) values were calculated by fixing the basal fluorescence at 0.05 units. Five replicate reactions were performed for each sample and the average C(t) was calculated. ΔC(t) values were calculated as C(t) sample − C(t) β-actin. The N-fold increase or decrease in expression was calculated by ΔΔC(t) method with the fetal C(t) value as the reference point. N-fold difference was determined as 2−ΔΔC(t) (sample − ΔΔC(t) fetal) (16).

**Gel Mobility Shift Assay**—Gel mobility shift assays were performed as described previously (13). Nuclear or cytoplasmic extracts (~20–30 μg of protein) and approximately 20–30 fmol of [32P]-labeled oligonucleotides (double-stranded) were used in a typical assay. The reactions were carried out at 30 °C for 15 min. For super-shift assays, 1 μl of the antisera used showed nonspecific binding with crystallins, present in very high concentrations in the lens extracts. These are not shown in the immunoblots; in most cases, the gels were run longer to exclude them from immunoblotting.

**RESULTS**

HSF1 and HSF2 Are Mostly Expressed in the Fetal Lens—Fig. 1 shows that HSF 1 and HSF2 are predominantly expressed in the fetal lens in the rat. The RT-PCR gel analyses (Fig. 1a) show a gradual decrease in the level of HSF1 as well as HSF2 RNA transcripts from the fetal to postnatal stages. The real-time PCR (Fig. 1b) shows a large dramatic decrease from fetal to PN day 20 in the content of these transcripts. Whereas HSF1 shows a 25-fold decrease in transcripts, the change in HSF2 transcripts is even larger, a 175-fold decrease over the same period (Fig. 2b). The data in the Fig. 1b are complemented by the immunoblotting data (Fig. 1c). Although it is difficult to be quantitative on these immunoblots, between the fetal and the PN day 3 stages, a significant decrease in seen in both HSF 1 and HSF2 protein levels. Beyond the PN day 3, neither of these proteins can be detected at appreciable levels (Fig. 1c).

HSF4 Is the Predominant HSF of the Postnatal Lens—The pattern of HSF1 and HSF2 presence stands in stark contrast to that seen with HSF4 (Fig. 2). The RT-PCR gel analyses (Fig. 2). The RT-PCR gel analyses (Fig. 2).
HSF4 is predominantly expressed in the postnatal rat lens. a, RT-PCR analyses of HSF4 in the rat at different ages. Total lens RNAs was used. The arrow indicates the PCR product. b, real-time PCR analyses as in Fig. 1. c, immunoblot analyses for HSF1 and HSF2 proteins (arrows) in the nuclear (N) and cytoplasmic (C) lens extracts using specific antibodies. The numbers on the left side of the immunoblots indicate the molecular mass (KDa) markers. Lanes marked 3, 5, 10, and 20 refer to age in postnatal days. F, fetal; A, adult.

2a) suggests only a gradual increase in the intensity of the stained HSF4 band from the fetal to postnatal stages; however, the data obtained with real-time PCR (Fig. 2b) indicate that there is a substantial (about 25-fold) increase in HSF4 transcripts from the fetal stage to PN day 10. Again, this observation is complemented by the immunoblotting data presented in Fig. 2c, which was done using two different polyclonal antibodies specific for two different isoforms of HSF4. These two isoforms (HSF4a and HSF4b) are produced by alternative splicing involving exons 8 and 9 of the HSF4 gene (17). HSF4b is known to constitutively bind to DNA and activate transcription.

HSF4a, on the other hand, has been suggested to repress transcription (17, 18). In the data presented in Fig. 2c, there is very little difference in the mobility of the HSF4 protein band detected by anti HSF4 (total) (an antibody that will detect all HSF4 polypeptides that contain an unaltered C terminus) and the mobility of the protein detected by HSF4b-specific antibody, suggesting that the predominant reactive band on these immunoblots represents HSF4b (Fig. 2c). HSF4 is of smaller molecular mass and is made in very low amounts (17) and is thus undetectable in these immunoblots.

Presence of HSF4 in the Human Lens Extracts—The data presented in Fig. 2 clearly establish that HSF4 expression is minimal in the fetal lens and predominant in the postnatal lens. Because of the reported association between HSF4 mutations and juvenile cataractogenesis (19), we sought to examine the status of HSF4 in the human lens (Fig. 3). Although these data are derived from experiments done with single lens extracts for each age, the pattern of postnatal expression of HSF4 seems to be more or less recapitulated in the human lens. HSF1 is seen mostly in the fetal lens extracts (Fig. 3a), whereas HSF4 detectability is retained even in lens extracts made from older human lenses (62 years was the oldest lens examined; Fig. 3b).

HSF4 Is Maximally Expressed in the Ocular Lens—Considering that HSF4 is the predominant HSF in the postnatal lens, it was of interest to assess the distribution of HSF4 in different tissues in the rat. The data presented in Fig. 4 demonstrate that among the tissues examined in the PN day 10 rat, the ocular lens shows the highest expression of HSF4, both at the RNA (Fig. 4a) and protein (Fig. 4b) levels. Although appreciable amounts of RNA transcripts are found in the lung, muscle, and small intestines (Fig. 4a), very little protein was seen in these tissues (Fig. 4b).

HSF4, Not HSF1 or HSF2, Interacts with HSE-aB—The pattern of maximal postnatal expression of HSF4 around PN day 10–15 (Fig. 2c) is temporally consistent with the high expression of the aB-crystallin gene in the rat lens at around PN day 10 (there is a 10-fold difference in the number of aB-crystallin transcripts between the fetal and the PN day 10 stages 2 and the appearance of the HSE-aB/HSF trimeric complex (13). But this only provides circumstantial evidence that the HSF in the HSE-aB/HSF complex is probably HSF4. We identified the HSF in the HSE-aB/HSF complex by super-shift

2 M. Sapru, J. Horowitz, D. Bok, and S. Bhat, unpublished observations.
analyses using specific antibodies such as anti-HSF1, anti-HSF2, and anti-HSF4, the same antibodies that were used for immunoblotting in Figs. 1 and 2. In these experiments, we used nuclear extracts rather than whole-cell extracts, as was done previously (13). In so doing, we can detect trimetric complexes between \[^{32}\text{P}]\text{HSE-Hsp70}\) and the HSFs even in the fetal lens extracts, albeit at very low levels (see Fig. 5a, complex III). Fig. 5a shows that even when there is very little trimeric complex (Fig. 5a, control lane), only anti-HSF4 results in the super-shift of the complex III. Fig. 5b shows that data obtained from experiments in which PN day 10 lens nuclear extracts were used. In these experiments, no HSF1- or HSF2-related activity was detected in the trimeric complexes as ascertained by lack of super-shifted complexes. Super-shift was seen only when an anti-HSF4 antibody was used (Fig. 5b).

HSF4 Interacts with HSE-Hsp70 and HSE-Hsp82—The experiments done with HSE-\(\alpha\)B (Fig. 5) were repeated with heat shock promoter sequences derived from rat Hsp70 (20) and \(D.\ melanogaster\) Hsp82 (21). Fig. 6, A and B, show that in the PN day 10 nuclear extracts, complexes obtained with \[^{32}\text{P}]\text{HSE-Hsp70}\) and \[^{32}\text{P}]\text{HSE-Hsp82}\) only contained HSF4, as indicated by super-shift with anti-HSF4 (Fig. 6, A and B). In the fetal lens nuclear extracts, again it is anti-HSF4 that pro-
shows that only anti-HSF4 results in a super-shift of the complex formed by [32P]HSE-Hsp70, whereas with [32P]HSE-Hsp82. The data (Figs. 1 and 2).

and HSF2 proteins in these extracts as also indicated by immunoblotting that no super-shifted complexes are obtained with anti-HSF1 and anti-HSF2 in fetal lens nuclear extracts, super-shifted complexes, although present in Fig. 6, C and D. It must be noted that in the fetal lens nuclear extracts, super-shifted complexes, although much weaker in intensity, were also obtained with anti-HSF1 (Fig. 6, C and D). It was not clear from these experiments with fetal lens nuclear extracts whether anti-HSF2 produced any super-shifted complexes.

**Comparative Binding Profiles of HSF4 with Three Different HSEs**—The data in Fig. 6 indicated that HSF4 in PN day 10 lens nuclear extracts binds with the HSEs in the αB-crystallin promoter, Hsp70 promoter, and Hsp82 promoter. It was of interest to assess whether HSF4 bound to all the HSE sequences with similar or differential efficiencies. The data presented in Fig. 7 show a plot of the relative binding of HSF4 to [32P]HSE-αB, [32P]HSE-Hsp70, and [32P]HSE-Hsp82 as a function of the concentration of the protein in the nuclear extracts made from PN day 10 lens. Although it seems that the binding efficiency is much higher with HSE-Hsp70, the binding of HSE-αB sequence stands out in that it gets saturated very early at lower concentrations; the binding of HSE-Hsp70 and HSE-Hsp82 does not show saturation at lower concentrations. The novel binding characteristics of HSE-αB clearly stand out, particularly the early saturation and inhibition at higher concentrations. This distinct pattern of interaction between HSF4 and HSE-αB compared with HSE-Hsp70 and HSE-Hsp82 is further supported by the data obtained in competition studies (Fig. 8). These assays show that the binding of [32P]HSE-Hsp70 to HSF4 is equally well competed with by its homologous competitor, HSE-Hsp70, as well as by HSE-Hsp82, but not as efficiently by HSE-αB (Fig. 8).

**DISCUSSION**

This investigation reports on the exclusive expression of HSF4 in the postnatal ocular lens and its specific interaction with the heat shock promoter of the αB-crystallin gene. Using real-time PCR, immunoblotting, and gel mobility shift assays coupled with the use of specific antibodies, we showed that there are no HSF1 and HSF2 binding activities in the postnatal ocular lens. The data presented in this article support two important observations: 1) all tissues do not contain all the HSFs and tissues such as the postnatal ocular lens express only one, HSF4, and 2) heat shock promoters, although canonical, show differential binding to HSF4. The observation of the singular presence of HSF4 in the postnatal ocular lens affects a number of perceptions about the stress response and its universality. Our data point to a developmental stage- and tissue-specific response to stress based on the differential presence or absence of specific heat shock factor(s) and their specific interactions with heat shock promoters. This is well exemplified by the situation in the ocular lens, wherein the small heat shock protein αB-crystallin gene is highly expressed (22). The heat shock promoter of the αB-crystallin gene has been shown to respond to heat and chemical stress in cells in culture (23–25). However, in experiments in which the intact rat lens, in organ culture, was exposed to heat stress, no induction of the αB-crystallin gene was observed (26). Considering that the response of the HSF4 to stress activation is poorer compared with that seen with HSF1 (17), the present data (indicating HSF4 as the predominant HSF of the postnatal ocular lens), may explain why lenses, when exposed to heat stress, do not show appreciable change (induction) in the concentration of αB-crystallin (26, 27).

Three important corollaries follow from the observation of the inverse relationship of the expression of HSF1/2 and HSF4 in fetal and postnatal stages, respectively (Figs. 1–3). One, the simultaneous presence of HSF1 as well as HSF2 in the fetal stages may compensate for the absence of either one of these HSFs during early developmental stages. This may be the reason why no ocular lens abnormalities have been reported in mice null for HSF1 or HSF2 (28–30). Considering that in the fetal nuclear extracts there is almost non-existent or very weak binding of HSF1 or HSF2 to various HSEs (Figs. 5 and 6), it is also possible that these transcription factors may be inactive at this stage. Second, mutations in HSF4 have been recently reported to be associated with the most prevalent form of early childhood cataracts (lamellar cataracts) (19). The almost exclusive expression of HSF4 in the postnatal ocular lens reported here corresponds remarkably to the timing of the appearance of this disease phenotype (juvenile cataractogenesis). Coupled with the demonstration that HSF4 and not HSF1 can be detected in the adult human lens extracts (Fig. 4), these data also provide a molecular basis for the association of late-onset cataract, such as Marner’s cataract, with a mutation in the HSF4 DNA binding domain (19). How these mutations alter HSF4 DNA binding abilities remains to be investigated. Third, a knockout of the HSF4 gene would have no repercussions on the development of the lens but might have severe physiological consequences to the postnatal lens.

The data presented in Fig. 5 lead to the conclusion that αB-crystallin is downstream of HSF4. We already know that mutations in αB-crystallin lead to cataractogenesis in the human lens (31). αB-crystallin has chaperone-like activities (22,
Thus, a malfunctioning HSF4 could result in the lack of appropriate concentrations of the \( \beta \)-crystallin gene product, which could impair important physiological activities. Depending on the expression of HSF4 gene, this could have both temporal and spatial consequences in the generation and maintenance of the transparent phenotype of the ocular lens, as seen in the lamellar and Marner's cataracts. In addition to \( \beta \)-crystallin, HSF4 may activate a number of genes in the postnatal and the adult lens, as suggested by the presence of HSF4 in the adult human lens extracts (Fig. 4; see also Figs. 6 and 7). The observation that the heat shock element of the \( \beta \)-crystallin heat shock promoter selectively binds to HSF4 (Fig. 5) suggests that different HSFs may activate different downstream targets, leading to differential gene activity. HSF1 and HSF2 have been recently reported to activate various heat shock genes differentially (33). Differential binding of HSFs to HSEs in vitro has been reported (34, 35).

The data presented in Figs. 5 and 6 demonstrate that there are no HSF1 or HSF2 binding activities in the PN day 10 lens nuclear extracts. This presents a unique in vivo system that contains only one HSF, HSF4. The binding profiles of HSF4 to various HSEs are presented in Fig. 7. Although binding with HSE-Hsp70 seems more efficient (Fig. 7), it is very similar to binding by HSE-Hsp82 in its initial pattern. Both HSE-Hsp70 and HSE-Hsp82 show sustained binding for HSF4 over a long range of protein concentrations. On the other hand, the interaction with HSE-\( \beta \)-crystallin is very different; saturation of the binding is reached at much lower protein concentrations. The fact that the binding of HSF4 with HSE-\( \beta \)-crystallin is inhibited at higher concentrations (compare binding with HSE-Hsp70 and HSE-Hsp82; see also inset, Fig. 7) may suggest the presence of a sequence-specific inhibitor in the lens nuclear extracts, but that remains a speculation at this time. The uniqueness of the binding of HSE-\( \beta \)-crystallin to HSF4 in the day 10 nuclear extracts is further supported by the competition experiment indicating that HSE-\( \beta \)-crystallin is not an efficient competitor for HSE-Hsp70 (Fig. 8). A clear insight into the significance of these binding profiles must await characterization of the expression profiles of HSP70 in the ocular lens.

The data presented in this manuscript indicate that differential activation of downstream genes (e.g. \( \beta \)-crystallin) may be brought about both by the timing of the expression of the HSFs and the specificity of the promoter-HSF interactions. Further studies will illuminate whether the specific patterns of binding affinity (Figs. 5–8), at various developmental stages
are sequence dictated (genetic) or epigenetic, involving protein modifications (36).

Acknowledgments—We thank Helen Tam and Michael Katz for technical help and Dr. Kumar for reading the manuscript. We are grateful to Travis lab for the use of the Optican 2 (MJ Research Inc., Boston, MA) for real-time PCR analyses and to the Horwitz laboratory for the human lens homogenates.

REFERENCES
1. Morimoto, R. I., Kroeger, P. E., and Cotto, J. J. (1996) in Stress-inducible Cellular Responses (Feige, U., Morimoto, R. I., Yahara, I., and Polla, B., eds) Birkhauser Verlag, Basel, Switzerland
2. Wu, C. (1995) Annu. Rev. Cell Dev. Biol. 11, 441–469
3. Morimoto, R. I. (1998) Genes Dev. 12, 3788–3796
4. Pirkkala, L., Nykanen, P., and Sistonen, L. (2001) EMBO J. 15, 1118–1131
5. Sorger, P. K., and Pelham, H. R. (1988) Cell 54, 855–864
6. Wiederrecht, G., Seto, D., and Parker, C. S. (1988) Cell 54, 841–853
7. Jedlicka, P., Mortin, M. A., and Wu, C. (1997) EMBO J. 16, 2452–2462
8. Heikkila, J. J. (1993) Dev. Genet. 14, 1–5
9. Nagineni, C. N., and Bhat, S. P. (1992) Exp. Eye Res. 54, 193–200
10. Walsh, D., Grantham, J., Zhu, X. O., Wei Lin, J., van Oosterum, M., Taylor, R., and Edwards, M. (1999) Environ. Med. 43, 79–87
11. Arrigo, A. P. (1995) Neurobiol. Appl. Neurobiol. 21, 488–491
12. Gallo, G. J., Prentice, H., and Kingston, R. E. (1993) Mol. Cell. Biol. 13, 749–761
13. Somasundaram, T., and Bhat, S. P. (2000) J. Biol. Chem. 275, 17154–17159
14. Srivivasan, A. N., and Bhat, S. P. (1984) DNA Cell Biol. 13, 651–661
15. Bhat, S. P., Horwitz, J., Srivivasan, A., and Ding, L. (1991) Eur. J. Biochem. 202, 775–781
16. Zhao, W. L., Daneshpouy, M. E., Mounier, N., Briere, J., Leboeuf, C., Plouin, P., Turpin, E., Cayuela, J. M., Ameisen, J. C., Gisselbrecht, C., and Janin, A. (2004) Blood 103, 695–697
17. Tanabe, M., Sasaki, N., Nagata, K., Liu, X. D., Liu, P. C., Thiele, D. J., and Nakai, A. (1999) J. Biol. Chem. 274, 27845–27856
18. Freitag, W., Zhang, Y., Dai, R., Anderson, M. G., and Mivechi, N. F. (2001) J. Biol. Chem. 276, 14685–14694
19. Bu, L., Jin, Y., Shi, Y., Chu, R., Ban, A., Ebergh, H., Andres, L., Jiang, H., Zheng, G., Qian, M., Cui, B., Xia, Y., Liu, J., Hu, L., Zhao, G., Hayden, M. R., and Kong, X. (2002) Nat. Genet. 31, 276–278
20. Mestril, R., Chi, S. H., Sayen, M. R., and Dillmann, W. H. (1994) Biochem. J. 298 Pt 3, 561–569
21. Zimario, V., Tsai, C., and Wu, C. (1990) Mol. Cell. Biol. 10, 752–759
22. Bhat, S. P. (2003) Prog. Drug Res. 60, 205–262
23. Sadamitsu, C., Nagano, T., Fukumaki, Y., and Iwaki, A. (2001) J. Biochem. (Tokyo) 129, 813–820
24. Klena, R., Frohli, E., Steiger, R. H., Schafer, R., and Anyama, A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3652–3656
25. Head, M. W., Hurwitz, L., and Goldman, J. E. (1996) J. Cell Sci. 109 (Pt 5), 1029–1039
26. de Jong, W. W., Hoekman, W. A., Mulders, J. W., and Bloemendal, H. (1986) J. Cell Biol. 102, 104–111
27. Collier, N. C., and Schlesinger, M. J. (1986) Exp. Eye Res. 43, 103–117
28. Kallio, M., Chang, Y., Manuel, M., Alastalo, T. P., Rallu, M., Grütz, Y., Pirkkala, L., Loones, M. T., Paslaru, L., Larney, S., Hiard, S., Morange, M., Sistonen, L., and Mezer, V. (2002) EMBO J. 21, 2591–2601
29. McMillan, D. R., Xiao, X., Shao, L., Graves, K., and Benjamin, I. J. (1998) J. Biol. Chem. 273, 7523–7528
30. McMillan, D. R., Christians, E., Forster, M., Xiao, O., Connell, P., Plummer, J. C., Zuo, X., Richardson, J., Morgan, S., and Benjamin, I. J. (2002) Mol. Cell. Biol. 22, 8005–8014
31. Berry, V., Francis, P., Reddy, M. A., Collyer, D., Vithana, E., MacKay, I., Dawson, G., Carey, A. H., Moore, A., Bhattacharya, S. S., and Quinlan, R. A. (2001) Am. J. Hum. Genet. 69, 1141–1145
32. Horwitz, J. (2003) Exp. Eye Res. 76, 145–153
33. Trinklein, N. D., Chen, W. C., Kingstone, R. E., and Myers, R. M. (2004) Cell Stress Chaperones 9, 21–28
34. Manuel, M., Rallu, M., Leones, M. T., Zimario, V., Mezer, V., and Morange, M. (2002) Eur. J. Biochem. 269, 2527–2537
35. Kroeger, P. E., Sarge, K. D., and Morimoto, R. I. (1993) Mol. Cell. Biol. 13, 3370–3383
36. Hashikawa, N., and Sakurai, H. (2004) Mol. Cell. Biol. 24, 3648–3659
