The 5'-Flanking Region of the Human Calreticulin Gene Shares Homology with the Human GRP78, GRP94, and Protein Disulfide Isomerase Promoters*

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Calreticulin (CR) is a calcium binding protein that resides in the endoplasmic and sarcoplasmic reticulum and is reactive with human Ro/SS-A autoimmune sera. We have used human CR cDNA to isolate a human 6-kilobase genomic clone that contains 529 base pairs upstream of the presumed transcription start site, 9 exons, 8 introns, and several hundred base pairs 3' of a polyadenylation sequence. Analysis of the human CR promoter region reveals a number of potential regulatory sites also found in the human GRP78, GRP94, and protein disulfide isomerase promoters, including multiple Sp1 and CCAAT consensus sequences, an AP-2 recognition sequence (absent in protein disulfide isomerase), and multiple GC-rich areas. DNA footprinting and gel shift analysis on the CR 5'-flanking region demonstrates an area that is bound by protein found in human but not murine nuclear extracts. This sequence is homologous with previously determined regulatory sequences of the human GRP78 and GRP94 promoters. These data indicate that CR, GRP78, GRP94, and protein disulfide isomerase may in part have similar transcriptional regulation and suggest that their gene products, while structurally distinct may have similar functions or co-functions. These observations are of additional interest as all four of these genes encode acidic proteins that localize to the endoplasmic reticulum.

Patients with primary Sjogren's syndrome and lupus erythematosus frequently have autoantibodies that recognize the Ro/SS-A autoantigen (1). We have recently cloned a human Ro/SS-A autoantigen (1). We have demonstrated that this autoantigen is human calreticulin (CR), an acidic calcium binding protein that resides in the endoplasmic and sarcoplasmic reticulum (2-4). Its precise cellular function has not yet been determined. The human CR gene is not highly polymorphic, exists in a single copy on the short arm of chromosome 19, is highly conserved among animal species, and is expressed in a variety of different cell lines and tissues (1, 2). To better define the regulation of this gene's expression, a human genomic CR clone was isolated and sequenced, and DNA footprint studies were performed on the 5'-flanking region.

MATERIALS AND METHODS

Genomic Clone Isolation—A human genomic library derived from the RPMI 8402 T-cell line constructed in λ bacteriophage 2001 was obtained from Dr. Richard Baer, University of Texas, Southwestern Medical Center. This library was screened by the duplicate filter lift method with the 1.9-kb CR cDNA previously isolated in our laboratory (1). The cDNA was radiolabeled by the hexamer extension method with hexamer primers (Pharmacia LKB Biotechnology Inc.), [α-32P]-dCTP (Du Pont-New England Nuclear), and Escherichia coli DNA polymerase I (Klenow fragment, Promega Corp., Madison, WI) (5). Filters were prehybridized for 4 h and then hybridized overnight (along with 1 × 10^6 cpm of radiolabeled cDNA/ml of solution) in Hybridis (Oncor, Gaithersburg, MD). The filters were washed three times in 2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) and 0.1% sodium dodecyl sulfate at 37 °C for 15 min each, then once in 0.1 × SSC and 0.1% sodium dodecyl sulfate at 55 °C for 40 min. Filters were exposed to Kodak X-OMAT-AR film between intensifying screens for 20 h and then developed on a Konica XQ-60A film processor. Double positive areas were picked and subjected to plaque purification (5).

Southern Filter Hybridization—Six individual clones were digested with various restriction enzymes (Promega Corp.), and the restriction fragments were analyzed by Southern filter hybridization with radiolabeled portions of the 1.9-kb cDNA (5). A genomic clone that contained the 5'- and 3'-most portions of the 1.9-kb cDNA was identified.

Subcloning—This genomic insert was cut out of the λ phage arms with SacI (Promega Corp.), and the complete restriction digestion mixture was ligated into the SacI site of the pTZ18U plasmid (U. S. Biochemical Corp.) and T4 DNA ligase (Promega Corp.) (5). Bacterial cells transformed with the resulting pTZ18U genomic clone ligation mixture and plated on ampicillin-containing agar. Double filter lifts were made of the resulting colonies, and these filters were hybridized with radiolabeled 1.9-kb CR cDNA (5). Several positive colonies were picked, and their insert sizes were characterized by SacI restriction enzyme digestion and agarose gel electrophoresis (5). A colony containing an apparent full-length 6-kb DNA insert was utilized for plasmid amplification and purification (5).

Polymerase Chain Reaction Amplification and Nucleic Acid Sequencing—Synthetic oligonucleotides (oligos) 18–24 bases in length were used corresponding to both strands of the CR cDNA at approximately 300-base pair overlapping intervals. Each upstream 5'-oligo was paired with its downstream 3'-oligo. These oligo pairs were phosphorylated (5) and used in the polymerase chain reaction (PCR) with the DNA Amplification System (Perkin-Elmer) using 5 ng of the 6-kb genomic insert as a template. The resulting PCR amplification mixture was directly ligated into Smal-cut M13 mp19 plasmid after two ammonium acetate and one sodium acetate/ethanol precip-
labeled CR cDNA or with \( [\gamma-P]ATP \) end-labeled oligos. Single-stranded DNA was generated corresponding to both strands of DNA (7) and sequenced by the Sanger dideoxy method with \( [\gamma-P]dATP \) and modified \( T_7 \) DNA polymerase (Sequenase) according to the manufacturer's recommendations (U. S. Biochemical Corp.). Any discrepancies between the sequences of each strand were resolved by repeating the sequencing methods one or more times to determine the correct sequence. One or more PCR products were sequenced to determine intron-exon boundaries. Four different PCR products were sequenced to obtain the 5'-flanking (promoter) sequence.

Gel Mobility Shift Assay—Nuclear extracts were prepared as described by Dignam et al. (8). One microgram of each proteinase inhibitor (leupeptin, pepstatatin, and antipain) were used in the preparation of extracts. Gel retardation assays were performed as described previously (9). Approximately 0.02–0.05 µg of protein from nuclear extracts was incubated with 3 nM DNA probe in a total volume of 20 µl at room temperature. 10 µg of single-stranded calf thymus DNA and 1 µg of double-stranded calf thymus DNA were used as nonspecific competitors in each reaction. The CR promoter probe was the PstI-AccI fragment that was end-labeled by Klenow with \( [\alpha-P]dCTP \) (6). The immunoglobulin heavy chain promoter probe was prepared as described previously (9).

DNA Footprint Analysis—For DNA footprinting, the polyacrylamide gel was soaked in 200 µl of 50 mM Tris buffer (pH 8.0) after electrophoresis, and DNA footprinting was performed with copper-chromophore (copperphenanthroin) as described by Kuwabara and Sigman (10). Material from five lanes of retarded DNA probe was eluted, pooled, and separated in a 10% denaturing polyacrylamide gel (6). The G reaction of the CR promoter probe was performed by the Maxam and Gilbert method as described (11).

RESULTS

A human T-cell genomic library was screened with radiolabeled human CR cDNA. The initial screen produced six genomic clones, only one of which hybridized to the 5'-and 3'-most portions of the 1.9-kb CR cDNA. This genomic insert was excised from the λ phage arms in one piece with SacI and measured approximately 6 kb by agarose gel electrophoresis. Synthetic oligonucleotides corresponding to CR cDNA sequences of both strands approximately 300 base pairs apart were used to amplify the genomic insert. In this manner the various portions of the insert were amplified and subsequently sequenced. This allowed the construction of the genomic map shown in Fig. 1A.

The transcribed portion of the gene corresponding to its 1.9-kb cDNA sequence is contained within 4.5 kb of chromosomal DNA (1). There are 9 exons and 8 introns, the lengths of which are shown in Table I. Introns contribute about 2.4 kb to the gene. Four of the eight introns measure less than 100 bp. Like the vast majority of mammalian genes, most of the introns are type 0 or type I (Table I) (12). The exon–intron junctional sequences are highly homologous to the vertebrate exon–intron consensus sequence as shown in Table I (13).

The introns do not clearly fall between the previously predicted structural domains of the CR molecule (1, 4) as described in some proteins (12). However, intron 6 falls between two sequence replications, and intron 8 is located immediately proximal to the strongly charged carboxyl-terminal domain (Fig. 1, B and C) (1).

The insert includes 529 bp 5' to the approximate transcription start site that we arbitrarily reference as position 1, which is the 5'-most base in the CR cDNA clone. Within this 5'-flanking sequence are several putative regulatory sequences (see Table II). These include a TATA box (−28 to −22), four CCAAT sequences (−93 to −89, −124 to −120, −194 to −190, and −207 to −211), and several GC-rich areas including four putative Sp1 binding sites (−12 to −7, −74 to −69, −312 to −307, and −362 to −357) (Fig. 2). These sequences are typical for the promoter elements of genes transcribed by RNA polymerase II (14, 15). The promoter element also contains the AP-2 sequence CCCAGGC (−521 to −515) found in SV40 and bovine papilloma virus enhancers, the human c-myc and growth hormone regulatory regions, and in the histocompatibility H-2Kb genes (16). The human histone H4TF-1 recognition sequence GATTTT is present at positions −183 to −188 (17). The sequence GGGNNGGG, where N is any base, occurs seven times in the CR promoter region (Fig. 2). Two of these are inclusive of Sp1 binding sites. There are also a number of other poly G-rich sequences including nine GGG, three GGGNGGG, one GGGNNNGGG, one GGGNNNGGGNGGG, and one GGGNNNGGGNNNGGG sequences. The palindromes TGGTCGAACCA and CACGTG begin at positions −223 and −372, respectively (Fig. 2).

At the 3'-end of the insert is a putative polyadenylation signal sequence ATTTAA (18). There is a GT-rich area 65–92 nucleotides downstream from the polyadenylation site.
Multiple CCAAT sequences in the promoter region are somewhat unusual, and although it is uncertain how many of these are functional, their multiplicity does suggest a more complex system of transcriptional control. Interestingly, multiple CCAAT sequences and two putative Sp1 binding sites are present in the human glucose-regulated protein (GRP78)

**DISCUSSION**

Using our previously isolated 1.9-kb human CR cDNA (1), we have isolated the human genomic form of this gene that measures approximately 5 kb from its RNA polymerase II promoter region to its downstream GT-rich processing element. The presence of multiple GC-rich areas in the promoter portion of this gene, including four putative Sp1 binding sites, suggests that its protein product may have a housekeeping function (14).

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**FIG. 2. CR promoter region.** The numbers reference nucleotide positions relative to the putative messenger RNA transcription initiation site. The methionine ATG initiation codon is in **boldface.** The TATA box, four CCAAT sequences, and four putative Sp1 binding sites are **underlined.** Seven GGNNNGGG sequences (CCCCNNCC in complementary strand) are **overlined** with **asterisks.** All GGG (CCC) triplications are in lower case. Two palindromes are **overlined** with **asterisks.** The sequence corresponding to the synthetic oligonucleotide used in the gel shift inhibition assay is **overlined.**

Where 26 of 28 nucleotides are either guanine or thymine (data not shown). Such downstream GT-rich areas are thought to be important in the cutting of the transcript to the polyadenylation signal prior to the addition of the poly(A) tail (19).

In order to better understand the transcriptional regulation of the CR gene, gel mobility shift analysis and DNA footprinting were performed. A PsI-AclI DNA fragment, containing the region from -219 to +14, was used as a binding probe. This region contains many of the possible promoter cis-elements: CCAAT (-89 to -93, -124 to -120, -194 to -190, -207 to -211), Sp1 (-12 to -7, -74 to -69), and TATA (-28 to -22) sites for basal level transcription by RNA polymerase II (14, 15). Nuclear extracts from Wil-2, an Epstein-Barr virus-transformed human B-cell line, and BCL1, a murine lymphoma B-cell line, were used in the analysis. The results are shown in Fig. 3.

The mobility shift pattern of the Wil-2 nuclear extract with the CR promoter probe showed a doublet of retarded bands (Fig. 3). A mixture of the BCL1 extract and CR probe showed no gel retardation (Fig. 3). These results indicate the presence of trans-factors in the Wil-2 extract which form DNA-protein complexes with the CR promoter that are not in the murine BCL1 cell. Identical retarded bands were seen in the same assay when human HeLa cell nuclear extract was used in the analysis (data not shown).

Both Wil-2 and BCL1 nuclear extracts had an octamer transcription factor-1 (OTF-1) complex when incubated with an immunoglobulin heavy chain promoter probe (Fig. 3). This result indicates that the failure of the BCL1 extract to form DNA-protein complexes with the CR promoter was not caused by a general degradation of the BCL1 extract.

**TABLE II**

| Sequence            | Position(s) | Designation | Function                       |
|---------------------|-------------|-------------|-------------------------------|
| TATAAAA             | -28         | TATA box    | Transcription initiation      |
| CCAAT               | -93, -124, -194, -207 | CCAAT box | RNA polymerase II TRE*        |
| CCGGGG              | -12, -74, -312, -362 | Sp1        | RNA polymerase II TRE         |
| CCCAGGC             | -521        | AP-2        | SV40 enhancer                 |
| GATTTC              | -183        | H4TF-1      | Histone TRE                   |
| TGCTGACCA           | -213        | Palindrome  | ?                              |
| CAGCTG              | -366        | Palindrome  | ?                              |
| GGNNNGGG            | -62, -75, -250, -393, -436, -512, -519 | GGNNNGGG | ?                              |

*TRE, transcription regulatory element.

**FIG. 3. Gel mobility shift analysis of Wil-2 and BCL1 extracts.** Lanes 1–3 represent extracts mixed with the CR promoter probe, and lanes 4–6 were extracts mixed with the immunoglobulin heavy chain promoter probe. The arrowheads mark the free probe. Lanes 1 and 4 are probe alone control; lanes 2 and 5 are BCL1 extract; lanes 3 and 6 are Wil-2 extract. The octamer transcription factor-1 (OTF-1) complex is marked.

**REFERENCES**

1. Calreticulin, GRP78, and GRP94 Have Similar Promoter Elements
and GRP94) and protein disulfide isomerase promoters (20-22). GRP78 and GRP94 have greater than a 45% sequence homology with heat shock protein (HSP) 70 and HSP90/83, respectively (23, 24). Like HSP70 and HSP90, the GRP proteins and protein disulfide isomerase are thought to play a role in protein transport, folding, and assembly (23,25).

Previous studies have demonstrated that GRP78 (also known as BiP) may assist with the linkage of immunoglobulin heavy and light chains in B-lymphocytes (26-28). GRP gene expression, like GRP gene expression, is inducible by glucose deprivation, and light chains in B-lymphocytes (26-28). GRP gene expression, like GRP gene expression, is inducible by glucose deprivation, and light chains in B-lymphocytes (26-28).

The specific retarded bands seen in the CR gel mobility shift assay (Fig. 3) are not likely to be the DNA-protein complexes. DNA fragments were treated with copper-phenanthroline in the gel matrix for 3-4 min after electrophoresis as shown in Fig. 3. The free DNA probe and retarded DNA probe were recovered and subsequently loaded onto a 10% denaturing polyacrylamide gel. Lane 1 is a G reaction of the CR promoter probe; lane 2 is the DNA footprint pattern of free probe; lanes 3 and 4 are DNA fragments from retarded complexes with HeLa extract and Wil-2 extract, respectively. Arrows mark the enhanced cleavage sites on the DNA fragment within the complexes. The protected areas are half-bracketed.

Fig. 4. DNA footprint analysis of retarded DNA-protein complexes. DNA fragments were treated with copper-phenanthroline in the gel matrix for 3-4 min after electrophoresis as shown in Fig. 3. The free DNA probe and retarded DNA probe were recovered and subsequently loaded onto a 10% denaturing polyacrylamide gel. Lane 1 is a G reaction of the CR promoter probe; lane 2 is the DNA footprint pattern of free probe; lanes 3 and 4 are DNA fragments from retarded complexes with HeLa extract and Wil-2 extract, respectively. Arrows mark the enhanced cleavage sites on the DNA fragment within the complexes. The protected areas are half-bracketed.

Fig. 5. A, CCAAT and CCAAT-like sequences in the promoter regions of GRP94, GRP78, CR, and protein disulfide isomerase. ~ indicates the complementary strand. The top-most sequence of GRP94, GRP78, and CR contains a DNA footprint-protected sequence that is overlined. For ease of sequence comparisons, nucleotide doublings are in boldface, and higher base repetitions are in lower case. B, two different sequence alignments of the protected regions with greatest sequence homology. Upper case letters in consensus sequence represent a 3 out of 3 base match, and lower case letters represent a 2 out of 3 base match. The numbers reference nucleotide positions relative to the putative messenger RNA transcription initiation site. CCAAT and CCAAT-like sequences are underlined. Gaps are represented by - and nucleotide matches by :.

Consensus CCAAT-AGGAG gGCAC  C T gGGCAT GgCCAG

Fig. 5. A, CCAAT and CCAAT-like sequences in the promoter regions of GRP94, GRP78, CR, and protein disulfide isomerase. ~ indicates the complementary strand. The top-most sequence of GRP94, GRP78, and CR contains a DNA footprint-protected sequence that is overlined. For ease of sequence comparisons, nucleotide doublings are in boldface, and higher base repetitions are in lower case. B, two different sequence alignments of the protected regions with greatest sequence homology. Upper case letters in consensus sequence represent a 3 out of 3 base match, and lower case letters represent a 2 out of 3 base match. The numbers reference nucleotide positions relative to the putative messenger RNA transcription initiation site. CCAAT and CCAAT-like sequences are underlined. Gaps are represented by - and nucleotide matches by :.

sequence repetitions in addition to the multiple CCAAT sequences and thus could conceivably have enhancer-like activity as well.

Previous DNA footprint analysis and chloramphenicol acetyltransferase transcriptional studies have localized a major regulatory element in the human GRP78 promoter (~160 to ~101) that is homologous to a major regulatory element in the human GRP94 promoter (~201 to ~164) (21,45). These elements are important in the constitutive expression of these two genes (21,45). Nucleic acid sequence comparison of these two regions with the CR gene promoter region revealed several homologous sequences centered around a CCAAT motif as shown in Fig. 5A. One of these homologous CR sequences was shown in Fig. 5B. This may suggest that CR and the GRPs may have a common regulatory sequence which would enable these genes to be coordinately expressed, as previous studies have suggested (39).

The specific retarded bands seen in the CR gel mobility shift assay (Fig. 3) are not likely to be the DNA-protein
complexes that occur at common transcriptional motifs such as CCAAT box, TATA box, or Sp1 sites, because these trans-factors are common to all mammalian cells. If those common DNA-protein complexes were detectable, we should have been able to observe the same retarda bands with all extracts. However, the complexes were not detectable with the murine nuclear extract as they were with both human cell nuclear extracts. It is interesting that the gel shift of the CR promoter fragment could be abrogated by a synthetic oligonucleotide corresponding to an area protected by footprint analysis (Fig. 2). This synthetic oligonucleotide is inclusive of a CCAAT sequence though it is less likely that a CCAAT trans-factor is responsible for the gel shift, as the BCL1 nuclear extract, which contains CCAAT box binding protein, causes no shift. The characteristics of these putative transcriptional regulatory factors remain to be further elucidated.

The DNA footprint analysis of CR revealed enhanced cleavage areas that might indicate a structural difference in the DNA segment resulting from the binding of trans-factors (46). These kinds of structural changes often aid in the opening of a DNA segment for the initiation of RNA polymerase II transcription.

The AP-2 (18) and H4TF-1 (17) recognition sequences found in the CR promoter region are typically present in genes that are active during cellular proliferation. Thus their presence in the CR gene is consistent with the finding that CR messenger RNA is expressed at higher levels in rapidly dividing lymphocytic cell lines and in lipopolysaccharide-stimulated peripheral blood leukocytes than in unstimulated peripheral blood leukocytes.2 The human GRP78 and GRP94 promoter regions also contain an AP-2 sequence (20, 21).

Interupted poly(G) sequences occur frequently in the CR promoter region, particularly the GGGNNGGG motif which may be a recognition sequence for transcriptional regulation, though none of these sequences were protected by our DNA footprint analysis. The GRP78 and GRP94 promoter regions are extremely GC-rich like the CR promoter region, but each has only the sequence GGGNNGGGNGGNGG that resembles two overlapping GGGNNGGG motifs. However, it is interesting to note that the rabbit calcequinin (CS) promoter region has six different GGGNNGGG motifs (47). Like CR, CS is an acidic calcium binding protein of the sarcoplasmic reticulum (47). Unlike CR, however, CS is not found in the ER (48). CS is thought to be a major storage depot of calcium in the ER analogous to the calcium binding function of CS in the ER (48). CR is thought to be a major storage depot of calcium (47). Unlike CR, however, CS is not found in the peripheral blood leukocytes.3 The human GRP78 and GRP94 promoter regions contain two overlapping GGGNNGGG motifs. However, it is less likely that a CCAAT trans-factor sequence though it is less likely that a CCAAT trans-factor fragment could be abrogated by a synthetic oligonucleotide extract. It is interesting that the gel shift of the CR promoter is responsible for the gel shift, as the BCL1 nuclear extract, which contains CCAAT box binding protein, causes no shift. The characteristics of these putative transcriptional regulatory factors remain to be further elucidated.

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REFERENCES
1. McCauliffe, D. P., Lux, F. A., Lieu, T.-S., Sanz, I., Hanke, J., Newkirk, M. M., Bachinski, L. L., Itoh, Y., Siciliano, M. J., Reichlin, M., Sontheimer, R. D., and Capra, J. D. (1990) J. Clin. Invest. 85, 1378-1393
2. McCauliffe, D. P., Zappi, E., Lieu, T.-S., Michalak, M., Sontheimer, R. D., and Capra, J. D. (1990) J. Clin. Invest. 86, 332-335
3. Smith, M. J., and Koch, G. L. E. (1989) EMBO J. 8, 3581-3586
4. Fliege, L., Burns, K., MacLennan, D. H., Reithmeier, R. A., and Michelak, M. (1988) J. Biol. Chem. 264, 21522-21528
5. Ausubel, F. M. (1990) Current Protocols in Molecular Biology, John Wiley and Sons, New York
6. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
7. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene (Amst.) 33, 103-119
8. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475-1489
9. Hanke, J. H., Landolfi, N. F., Tucker, P. W., and Capra, J. D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3560-3564
10. Kuwabara, M. D., and Sigman, D. (1987) Biochemistry 26, 7234-7238
11. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 499-560
12. Trout, B. T. (1989) Proc. Natl. Acad. Sci. U. S. A. 85, 2944-2948
13. Paget, R. A., Grabowski, P. J., Komarska, M. M., Seller, S., and Sharp, P. A. (1986) Annu. Rev. Biochem. 55, 1119-1150
14. Water, J. D., Hopkins, N. H., Roberts, J. W., Steitz, J. A., and Weiner, A. M. (1987) Molecular Biology of the Gene, pp. 274-277 and 706, Benjamin/Cummings Publishing Co., Menlo Park, CA
15. Dynan, W. S., and Tjian, R. (1985) Nature 316, 774-778
16. Imagawa, M., Chiu, R., and Gilbert, W. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9354-9358
17. Platt, T. (1986) Proc. Natl. Acad. Sci. U. S. A. 84, 10672-10676
18. Reichlin, M., Sontheimer, R. D., and Capra, J. D. (1990) J. Clin. Invest. 85, 1378-1393
19. Neumann, R. (1986) Annu. Rev. Biochem. 55, 339-372
20. Ting, J., and Lee, A. S. (1988) DNA 7, 275-286
21. Chang, S. C., Erwin, A. E., and Lee, A. S. (1989) Mol. Cell. Biol. 9, 2153-2162
22. Takan, K., Parkkonen, T., Chow, L. T., Kiviirikko, K. I., and Pihlajama, T. (1988) J. Biol. Chem. 263, 16218-16224
23. Sorge, P. K., and Pelham, H. R. B. (1987) J. Mol. Biol. 194, 341-344
24. Nicholson, R. C., Williams, D. B., and Moran, L. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 1159-1163
25. Rothman, J. E. (1989) Cell 59, 291-301
26. Hendershot, L. M., Ting, J., and Lee, A. S. (1988) Mol. Cell. Biol. 8, 4250-4266
27. Bole, D. G., Hendershot, L. M., and Kerney, J. F. (1986) J. Cell. Biol. 102, 1556-1566
28. Nakao, T., Deans, R. J., and Lee, A. S. (1989) Mol. Cell. Biol. 9, 2233-2238
29. Drummond, I. A., Lee, A. S., Resendez, E., Jr., and Steinhardt,
R. A. (1987) J. Biol. Chem. 262, 12801-12805
30. Shiu, R. P. C., Pouyssegur, J., and Pastan, I. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3840-3844
31. Lee, A. S. (1987) Trends Biochem. Sci. 12, 20-23
32. Chang, S. C., Wooden, S. K., Nakaki, T., Kim, Y. K., Lin, A. Y., Kung, L., Attenello, J. W., and Lee, A. S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 680-684
33. Freedman, R. B. (1989) Cell 57, 1069-1072
34. Freedman, R. B., and Hillson, D. A. (1980) in The Enzymology of Post-translational Modification of Proteins (Freedman, R. B., and Hawkins, H. C., eds) pp. 167-212, Academic Press, London
35. Hillson, D. A., Lambert, N., and Freedman, R. B. (1984) Methods Enzymol. 107, 281-294
36. Freedman, R. (1987) Nature 329, 196-197
37. Normington, K., Kohn, K., Kozutsumi, Y., Gething, M.-J., and Sambrook, J. (1989) Cell 57, 1223-1236
38. Munro, S., and Pelham, H. R. B. (1986) Cell 46, 291-300
39. Booth, C., and Koch, G. L. E. (1989) Cell 59, 729-737
40. Smith, M. J., and Koch, G. L. E. (1989) EMBO J. 8, 3581-3586
41. Pelham, H. R. B. (1989) EMBO J. 8, 3171-3176
42. Bienz, M., and Pelham, H. R. B. (1986) Cell 45, 753-760
43. Riddihough, G., and Pelham, H. R. B. (1986) EMBO J. 5, 1653-1658
44. Lin, A. Y., Chang, S. C., and Lee, A. S. (1986) Mol. Cell. Biol. 6, 1235-1243
45. Resendez, E., Jr., Wooden, S. K., and Lee, A. S. (1988) Mol. Cell. Biol. 8, 4579-4584
46. Tullius, T. D., Dombroski, B. A., Churchill, E. A., and Kam, L. (1987) Methods Enzymol. 155, 537-558
47. Zarain-Herzberg, A., Fliegel, L., and Maclennan, D. H. (1988) J. Biol. Chem. 263, 4807-4812
48. Koch, G. L. E. (1990) BioEssays 12, 527-531
49. Macer, D. R. J., and Koch, G. L. E. (1988) J. Cell Sci. 91, 61-70
50. Kemper, B., Jackson P. D. and Felsenfeld, G. (1987) Mol. Cell. Biol. 7, 2059-2069
51. Emerson, B. M., Lewis, C. D., and Felsenfeld, G. (1985) Cell 41, 21-30
52. Young, R. A., and Elliott, T. J. (1989) Cell 59, 5-8
53. Winfield, J. B. (1989) Arthritis Rheum. 32, 1497-1504
54. Lodish, H. F., and Kong, N. (1990) J. Biol. Chem. 265, 10893-10899
55. Furukawa, F., Kashihara-Sawami, M., Lyons, M. B., and Norris, D. A. (1990) J. Invest. Dermatol. 94, 77-85