RP58 Associates with Condensed Chromatin and Mediates a Sequence-specific Transcriptional Repression*

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Katsunori Aoki‡§, Gaoyuan Meng¶, Kenji Suzuki, Tohru Takashi‡‡, Yosuke Kameoka‡‡, Kazuhiro Nakahara‡, Reiko Ishida‡, and Masataka Kasai§§

From the ‡Departments of Immunology and ¶Pathology, ‡‡Division of Genetic Resources, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162, the §First Department of Internal Medicine and ¶¶Department of Laboratory Medicine, School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113, and the §§New Product Research Laboratories IV, Tokyo R&D Center, Daiichi Pharmaceutical Company, Limited, 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134, Japan

An approximately 120-amino acid domain present generally at the NH2 termini, termed the POZ domain, is highly conserved in various proteins with zinc finger DNA binding motifs. We have isolated a novel protein sharing homology with the POZ domain of a number of zinc finger proteins, including the human BCL-6 protein. By using a binding site selection technique (CAST), a high affinity binding site of the protein was determined to be (A/C)ACATCTG(G/T)(A/C), containing the E box core sequence motif. The protein was shown to repress transcription from a promoter linked to its target sequences and was hence named RP58 (Repressor Protein with a predicted molecular mass of 58 kDa). Immunogold electron microscopic study revealed that almost all RP58 is localized in condensed chromatin regions. These observations demonstrate for the first time that a protein mediating a sequence-specific transcriptional repression associates with highly condensed chromatin. We suggest that RP58 may be involved in a molecular link between sequence-specific transcriptional repression and the organization of chromosomes in the nucleus.

Increasing numbers of studies have demonstrated that various proteins containing zinc finger DNA binding motifs at the carboxyl terminus share an extensively conserved region at the amino terminus, termed the POZ domain (1) (also called ZIN (2, 3), TAB (4), or BTB (5, 6)). The POZ domain family includes the Drosophila developmental regulators Tramtrack (7, 8), Broad-Complex (9), and bric a brac (10) and the chromatin regulators GAGA factor (11) and E(var)3-93D (12). The mammalian POZ domain family includes the human BCL-6 protein, which is involved in chromosomal translocations in non-Hodgkin’s lymphoma (13–15), the human HIC-1 protein, a putative tumor suppressor gene activated by p53 (16), the human PLZF protein, which is involved in a variant chromosomal translocation in acute promyelocytic leukemia (17), the human KUP protein (18), the clone 18, a transcription factor that regulates the major histocompatibility complex II promoter (4), the human ZID protein (1), and the mouse ZF-5 (2), a transcriptional repressor.

In previous studies, we identified and cloned the gene coding for a DNA-binding protein, translin, exhibiting general binding activity to consensus sequences at breakpoint junctions of chromosomal translocations in many cases of lymphoid neoplasms (19–22). Further molecular analysis revealed that the native form of translin is a ring-shaped octamer connected by the leucine zipper motifs of each dimer and that this structure is responsible for the binding to target sequences situated only at single-strand DNA ends (23). Subsequently, to investigate the functional significance of translin, we examined whether it might interact with other proteins using yeast two-hybrid interaction analysis and identified an associated 33-kDa protein partner, TRAX, with extensive amino acid homology (28). During the course of screening for translin-associated molecules in the yeast two-hybrid system, which was designed to avoid self-association of translin to form the ring-shaped structure, we finally selected one clone D15 whose product gave the strongest activation only with the translin bait lacking the leucine zipper motif. The protein encoded by D15, named RP58, contains a POZ domain in its amino-terminal region and Kruppel-type zinc finger motifs separated by a short conserved motif, TGEKP(Y/P)/P/C (H/C link) in the carboxyl-terminal region.

The goal of this study was to elucidate the biological role of RP58 and, especially, its possible involvement in transcriptional regulation. In transient cotransfection experiments, RP58 was shown to repress transcription from a promoter linked to its target sequence containing the E box motif. Immunogold electron microscopic study revealed that RP58 is localized in condensed chromatin regions, suggesting a role for the sequence-specific transcriptional repression in the heterochromatin structure.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Cloning—DNA encoding the translin domain lacking a leucine zipper motif (amino acids 1–189) was cloned into the yeast GAL4 DNA binding domain vector, pGBT9 (CLONTECH). The resulting plasmid, GAL4bd-Tra (LZ delete), was used as bait to screen a human splenic cDNA library in two-hybrid interaction analysis which was performed following the Matchmaker Two-hybrid System Protocol (CLONTECH). Positive yeast clones were screened by activation of his and lacZ reporter genes. After transformation of yeast DNA into Escherichia coli, plasmids containing cDNA clones were identified by restriction mapping and further characterized by DNA sequencing.

Amplification of cDNA Ends— Amplification of cDNA ends was carried out using a 5’-RACE2 system (Life Technologies, Inc.). First-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) AJ001388.

§§ To whom correspondence should be addressed: Dept. of Immunology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162, Japan. Tel.: 81 3-5285-1112 (ext. 2130); Fax: 81 3-5285-1150; E-mail: masataka@nih.go.jp.

1 The abbreviations used are: RACE, rapid amplification of cDNA ends; nt, nucleotide(s); PCR, polymerase chain reaction; bp, base pair(s); GST, glutathione S-transferase; PBS, phosphate-buffered saline; ORF, open reading frame; kb, kilobase pair(s); EMSA, electrophoretic mobility shift assay.
strand cDNA was synthesized from poly(A) + RNA of NALL-1 cells (non-T and non-B-ALL) using Moloney murine leukemia virus reverse transcriptase and a RP58-specific primer, 5′-CATATGTCGTGCA- AACTGAC-3′ (primer R1, complementary to nt 524–544). An anchor sequence was then added to the 3′ end of the cDNA using terminal deoxynucleotidyltransferase. A nested PCR amplification was then performed using a primer complementary to the anchor and specific primers, 5′-CATGTTGCTGAATTTTATC-3′ (primer R2, complementary to nt 389–409) and 5′-GACAAGACCTAAATGGCAGAAG-3′ (primer R3, complementary to nt 333–355). The resulting PCR products (380 bp) were cloned into pBluescript II, and seven clones were selected for further sequencing.

**Bacterial Protein Expression**—A GST fusion protein containing the RP58 lacking a POZ domain (amino acids 122–552), GST-RP58 (∆POZ), was expressed using pGEX 4T-1 vectors (Amersham Pharmacia Biotech). After induction with 2 mM isopropyl-β-D-thiogalactopyranoside for 4 h, the bacteria were suspended in Lysis Buffer containing 50 mM sodium phosphate, pH 7.6, 300 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 2 mM dithiothreitol and lysed by sonication. The bacterial debris was removed by centrifugation at 6000 × g for 15 min at 4 °C, and the supernatant was incubated with glutathione (GSH)-Sepharose beads (Amersham Pharmacia Biotech) at 4 °C for 2 h, washed three times with the Lysis Buffer, and used for a CAST experiment. The full-length RP58 protein carrying a histidine hexamer tag (RP58-HIS) was also expressed using the pQE-9 plasmid (Qiagen) and purified by the Lysis Buffer, and the two bacterial proteins were used for a CAST experiment.

**Protein Purification**—The complex was then washed five times in the same buffer and subjected to DDSG (AGAGCGGTCCATTGCA(N)20CTGTAGGAATTCCGGATC) and CAST-15(2) (TCCGAAT) binding buffer (20 mM Tris-HCl, pH 7.6, 50 mM KCl, 10 mM MgCl₂, 100 mM sodium phosphate, pH 7.0, 300 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 2 mM dithiothreitol and lysed by sonication. The bacterial debris was removed by centrifugation at 6000 × g for 15 min at 4 °C, and the supernatant was incubated with glutathione (GSH)-Sepharose beads (Amersham Pharmacia Biotech) at 4 °C for 2 h, washed three times with the Lysis Buffer, and used for a CAST experiment.

**RESULTS**

**Molecular Cloning of RP58 in Two-hybrid Interaction Analysis**—To understand better the functional significance of translin and its relation to other proteins, we examined whether it might be a member of a multicomponent complex using a protein-protein interaction analysis. To avoid self-association of translin to form the ring-shaped structure, DNA encoding its domain lacking the leucine zipper motif (amino acids 1–189) was cloned into the yeast GAL4 DNA binding domain vector and used as bait to screen a human spleen cDNA library in two-hybrid interaction analyses. From screening 6.2 × 10⁸ individual colonies for activation of the lacZ reporter gene, 20 clones were obtained. Of these 20, nine were selected by activation of the lacZ reporter gene, but eight turned out to be the gene coding for TRAX showing extensive homology with translin (24). We finally selected one clone D15 (2024 nt cDNA) whose product gave the strongest activation with the translin bait. In contrast to the TRAX, which was selected by both the full-length and deleted translin, the D15 clone was obtained only when translin lacked its leucine zipper motif. In order to generate a full-length cDNA, we used 5′-RACE PCR to extend the D15 in the 5′ direction. Analysis of seven resultant clones resulted in the identification of an additional 207 nt sequences in all cases, preceding the 5′ end of the original clone, suggesting that this position might be a major transcription start site. Further screening of a human spleen cDNA library with the D15 probe resulted in the identification of several overlapping clones extending 2015 nt sequences in the 5′ direction. As a result, an additional 3′-PCR amplification with a primed 3′ end of the D15 clone was performed using a combination of cDNA (4246 nt) containing the AAATTA polyadenylation signal and poly(A) tract (Fig. 1A, GenBank™ accession number AJ001388). Nucleotide sequence analysis of the cDNA revealed an open reading frame (ORF) encoding a protein of 552 amino acids with a molecular mass of 58 kDa, RP58. Since no other favorable ORF was identified, we assumed that the ATG codon (nt 524–526) immediately 3′ of the in-frame termination codon (TGA) was the
FIG. 1. A, the nucleotide sequence of the RP58 cDNA and its deduced amino acid sequence. The isolated clone (D15; nt 208–2231), its 5'-RACE product (nt 1–355), and several overlapping clones were combined to construct the composite cDNA. The horizontal arrows indicate the sequences targeted by the specific primers (R1, R2, and R3) used for the 5'-RACE system. The 5' and 3' ends of the D15 clone and the 5'-RACE products are indicated by the vertical arrows. The 522-amino acid sequence deduced from the determined nucleotide sequence of the RP58 cDNA is shown. The in-frame translation stop codons (TGA; nt 488–490, TAA; 2090–2092) and polyadenylation signal are underlined. The four C2-H2 zinc finger motifs
translational initiation codon. The RP58 protein contains the putative nuclear localization signal in the amino-terminal region (KKKLKEK, amino acids 114–120). The carboxyl-terminal region of the RP58 protein contains 4 sets of Kruppel-type zinc finger motifs separated by a short conserved motif, TGEKP(Y/P)x (H/C link). Data base searches using BLAST and FASTA programs revealed that the amino-terminal region (amino acids 1–115), termed the POZ domain here, is highly conserved in a number of C2H2 zinc finger proteins (30–40% identity), including the human BCL-6 protein, the human HIC-1 protein, the human PLZF protein, the human KUP protein, clone 18, mouse ZF-5, and human ZID (Fig. 1B). On the other hand, the central region between the POZ domain and zinc finger motifs was not found to have any significant similarity with previously described proteins.

**Tissue-specific Expression of RP58**—To determine the size and expression pattern of RP58 mRNA, multi-tissue Northern blots were probed with a 1.5-kb RP58 ORF. A major transcript of approximately 4.3 kb was detected in lymphoid tissues, testis, heart, brain, skeletal muscle, and pancreas and at much lower levels in other tissues, along with a minor band at 9 kb (Fig. 1C). The size of the major transcript was consistent with that of RP58 cDNA, suggesting that the RP58 cDNA contains a full-length cDNA. Interestingly, a faint band at 7 kb appeared to be expressed only in brain. Our preliminary studies suggested that this 7-kb RNA may be derived from a particular portion of the brain. The expression of several different transcripts is suggestive of alternative splicing, as is frequently observed for mRNAs encoding zinc finger proteins (25, 26).

Fig. 1D illustrates expression of RP58 protein in various human tissues, studied by immunoblotting with an anti-RP58 antibody. Specific binding was found for a 60-kDa band which corresponds to the full length of RP58 protein. In addition, a 48-kDa band, thought to be the truncated form,2 was detected. The results clearly indicated that there is no direct correlation between the mRNA level and the amount of protein in the spleen, brain, and skeletal muscle (compare Fig. 1, C and D). Comparison of protein samples revealed that the 60-kDa protein level in brain is several hundredfold higher than in any other tissues (Fig. 1E), suggesting post-transcriptional control.

**The Consensus Binding Sequence for RP58**—We have employed a CAST protocol (27) to determine the consensus binding sequence for RP58. Based on previous reports (1, 2) showing inhibition of its DNA binding ability by the amino-terminal domains of ZID and ZF5, a truncated form of RP58 (a GST fusion protein lacking the POZ domain), GST-RP58 (APoz), was immobilized on GSH-Sepharose beads and incubated with a pool of synthetic oligonucleotides. The bound complexes were subjected to PCR. The consensus sequence for the RP58-binding sites is shown in the open box. The H/C link, TGEKP(Y/P)x, is underlined. B, alignment of the amino-terminal domains of RP58 and various zinc finger proteins. The amino-terminal regions of RP58 (amino acids 1–115) and the zinc finger proteins, Bcl-6 (1–123), HIC-1 (1–124), PLZF (1–120), KUP (6–111), clone 18 (5–111), ZF-5 (17–124), and ZID (12–119), were aligned using FASTA programs. Conserved amino acids are indicated by gray shading, K. Aoki, G. Meng, R. Ishida, and M. Kasai, manuscript in preparation.

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![FIG. 2](image-url)

**Fig. 2A.** The RP58-binding sites selected by CAST. After cloning by CAST, the 56 fragments obtained were sequenced and classified into 22 independent clones. The approximately 20-bp sequences of the central portions between CAST-16 (+) and CAST-15 (−) primers are shown. The perfect match for the binding core sequence (A/ACACATCTG) and its antisense are indicated with a black background and gray shading, respectively. One base mismatched sequences are underlined. The lowercase letters indicate the bases derived from primer sequences used for PCR. B, the consensus sequence for the RP58-binding sites. The percentage compositions of bases around the binding core sequences were calculated for the 44 sequences in the 22 independent clones. The nucleotides derived from the primer sequences were excluded from the calculation.

Fig. 2A lists the sequences of the central portions of each clone obtained after cloning using the CAST protocol. Some of the clones, especially CAST4, overlapped 26 out of the total of 56 clones. All of the sequences contained a (A/ACACATCTG core within a conserved 10-bp (A/ACACATCTG/G/T)(A/C) sequence.
Interestingly, the most conserved sequence of the core was found to be the consensus E box sequence, CANNTG (28). To determine whether the consensus binding sites determined by CAST really bind to the RP58, an EMSA was performed with the recombinant RP58 using the various sequences listed in Fig. 2A. A competition assay was performed using the CAST4 fragment as a probe. Increasing amounts of cold CAST4 or N20 fragments (10, 50, and 250 ng) and anti-RP58 antibody (rabbit IgG), anti-RP58, or normal rabbit IgG, NR-IgG (0.1 and 0.5 μg), were added as competitors.

(Fig. 2B). Interestingly, the most conserved sequence of the core was found to be the consensus E box sequence, CANNTG (28). To determine whether the consensus binding sites determined by CAST really bind to the RP58, an EMSA was performed with the recombinant RP58 using the various sequences listed in Fig. 2A. In this experiment, to avoid possible steric hindrance by GST, full-length RP58 containing a six-histidine tag was used instead of the GST fusion protein. Of six fragments studied, CAST4 or CAST2-2, which have a perfect match for the core sequence, bound to the RP58 with the highest affinity. The binding affinity decreased with the increase of mis-match sequences and the pool of unselected sequences, N20, did not form the complex, suggesting RP58 binding specificity (Fig. 3A). In competition assays using cold CAST4 or N20 fragments, the binding specificity of RP58 was again confirmed (Fig. 3B). Furthermore, DNA-protein complex formation was efficiently inhibited by the addition of anti-RP58 antibody, but not by normal rabbit IgG, suggesting the involvement of RP58 protein in the complex.

Sequence-specific Transcriptional Repression Mediated by RP58—Some of the POZ/zinc finger proteins including BCL-6, PLZF, and ZF5 are known to function as sequence-specific transcriptional repressors (2, 32–34). Based on the consensus binding sequence for RP58, we asked whether RP58 can also repress the transcription of reporter genes from promoters linked to the DNA target site, BS10, containing 10 copies of the binding core sequence for RP58 (Fig. 4A). The RP58 expression vector, pcDNA3.1-RP58, and the BS10-pGL2C reporter vector containing the luciferase gene driven by the SV40 early promoter (SV) linked to the DNA target site, BS10, were cotransfected into COS-7 cells (Fig. 4B). In this experiment, no significant difference was detectable between the basal levels of luciferase expressions of BS10-pGL2C and pGL2C (data not shown).
shown). The results indicate that the pcDNA3.1-RP58 led to a strong and dose-dependent repression of the reporter gene expression, whereas the plasmid expressing the TRAX protein, pcDNA3.1-TRAX, did not affect the transcriptional activity (Fig. 4C). The trans-repression activity of RP58 proved to be specific and dependent upon the presence of the RP58 domain in the effector vector and the BS10 sequence in the reporter vector.

Association of RP58 with Condensed Chromatin—The properties intrinsic to RP58, sequence-specific DNA-binding protein, transcriptional repression, and the presence of nuclear localization signal in the amino-terminal region, raise the possibility that it may localize to a particular region of the nucleus. Therefore, we examined localization of RP58 in the nucleus of IMR32 cells (human neuroblastoma) by immunofluorescence experiments. Indirect immunofluorescence of RP58 proteins revealed a punctate nuclear staining pattern, especially in regions adjacent to the nuclear membrane (Fig. 5). To investigate further its distribution at the ultrastructural level, we performed immunogold cytochemistry. When sections of IMR32 cells were labeled with anti-RP58 antibody, followed by the second antibody conjugated with 15-nm gold particles, over 90% of the gold particles were found to be distributed in the electron-dense chromatin (Fig. 6).

These results suggest that the transcriptional repression activity of RP58 might be important in controlling heterochromatin-mediated gene inactivation processes.

**DISCUSSION**

We have isolated a novel zinc finger protein, RP58, sharing homology with a number of transcription regulators at the amino terminus and determined the RP58 consensus binding site using the CAST technique. Interestingly, the preferred binding sequence consensus for RP58, \((A/C)ACATCTG(G/T)(A/C)\), contains the E box sequence of CANNTG, with most clones containing a CATCTG core. E boxes were first identified as in vivo protein-binding sites in the immunoglobulin heavy chain (IgH) enhancer (29–31). Subsequently, E box binding activity was found for members of the helix-loop-helix class of proteins (28) including a number of developmentally relevant examples like MyoD and c-Myc, and the proteins encoded by the *Drosophila* genes *Daughterless*, *Twist*, and *Achaete-scute*. The presence of an E box motif in the RP58-binding site raises the possibility that RP58 competes with other transcriptional regulators such as the helix-loop-helix class of proteins carrying the E box binding activity.

We have demonstrated that in transient transfection assays the RP58 protein can mediate a sequence-specific transcriptional repression. Transcriptional repression activities have been proposed for other POZ/zinc finger proteins including BCL-6 (32, 33), ZF5 (2) and PLZF (34). Although a number of the POZ/zinc finger proteins studied so far have displayed a consistent transcriptional repression activity, some POZ/zinc finger proteins display transcriptional activation (4) or the ability to modify nucleosomal structure (35), suggesting multifunctional aspects of this protein family. Other lines of evidence have indicated that the POZ domain acts in specific protein-protein interactions serving to organize higher order macromolecular complexes and thereby affect transcriptional activity (1, 6). These results suggest that transcriptional repression is not an intrinsic property of the POZ domain. Therefore, the repressor activity of RP58 protein might be regulated by controlling the affinity to its target sequence through interaction with the POZ domains of other partner proteins.

A search of the eukaryotic promoter data base using the BLAST program revealed that the binding core sequence of various genes

| Gene name                  | Promoter region sequence | Position |
|----------------------------|--------------------------|----------|
| Bovine Preproctachykinin   | AACCCAAATTGGTTCCAGAGATGTTATGCGC | –172 to –165 |
| Rat Preproctachykinin      | AACCCAAATTGGTTCCAGAGATGTTATGAC | –168 to –161 |
| Human anion exchanger 1    | GACTCAACGCTTCCAGAGATGGGTAAG | –182 to –175 |
| Mouse anion exchanger 1    | GATGGCACAGCTGTTCCAAGATGGGGAAG | –53 to –46 |
| Rat anion exchanger 1      | GATGGCACAGCTGTTCCAAGATGGGGAAG | –168 to –165 |
| Human Na/H exchanger       | TTATGGAGACTGCAGAGATGGGGAAG | –252 to –245 |
| Human protein C            | AAATGAGACCAATCTGGTCAGGTTTT | –176 to –169 |

**Table I**

Consensus binding sequence for RP58 in the putative promoter regions of various genes

The binding core sequence for RP58 is indicated by dots, and one-base mis-matched sequences are underlined. The translation start site is numbered +1.
RP58 is present in the putative promoter regions of various genes including the rat and bovine preprotachykinin genes (GenBank™ accession numbers E30009 and E11122), the human, mouse, and rat anion exchanger (AE1 or Band3) genes (GenBank™ accession numbers X77739, E25042, and L02942), the human Na/H exchanger (NHE-1) gene (GenBank™ accession number E40005), and the human protein C gene (GenBank™ accession number E320001) (Table I). The binding core sequences for RP58 were found 46–252 bp upstream from the transcription start sites, suggesting that these genes are possible targets for the RP58 protein. Since levels of RP58 protein vary in human tissues, it is likely that transcription of target genes is regulated by the differential expression. Considering the extraordinary amount of RP58 protein expressed in brain, further studies focused on brain might provide a clue as to its functional significance.

A number of heterochromatin-associated proteins contain a conserved domain, designated the “chromodomains” that is necessary for chromosome binding and sufficient to define binding specificity (36). The chromodomains-containing proteins are not thought to bind to DNA directly but probably associate with heterochromatin through protein-protein interactions. Since RP58 does not contain the chromodomains, it is of particular interest to determine whether it associates with condensed chromatin regions through interaction with other domains of partner proteins or binds to DNA even when the DNA is highly compacted in the mitotic chromosome. Our observations also support the previous studies indicating that the Drosophila GAGA transcription factor, which belongs to the zinc finger protein family with POZ domain, can associate with specific DNA regions of heterochromatin throughout the cell cycle (11). In contrast to the GAGA factor functioning as a transcriptional activator, RP58 is a sequence-specific DNA-binding protein with a transcriptional repression activity, the first to be reported to associate with condensed chromatin.

RP58 was originally found by screening for translin-associated molecules in yeast two-hybrid interaction analyses, the protein demonstrating interaction with translin lacking its leucine zipper motif, but not with full-length translin. Although it seems likely that the interaction occurs in the processing of multimeric translin structure, final conclusions can only be drawn when the interaction of these molecules is proven in vivo.

Transcriptional repression is an important component of gene regulation, but its mechanism is not yet as fully understood as that for transcriptional activation. The experiments reported here provide the molecular basis for addressing many intriguing questions such as the relation between gene-specific repression and the organization of chromosomes in the nucleus (37, 38), and its implications for human diseases associated with the silencing mechanisms involved in genetic imprinting.

Further investigation of RP58 functions is certainly warranted to acquire a better understanding of these problems.

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REFERENCES
1. Bardwell, V. J. & Treisman, R. (1994) Genes Dev. 8, 1664–1677
2. Numoto, M., Niwa, O., Kaplan, J., Wong, K.-K., Merrell, K., Kamiya, K., Yanagihara, K. & Calame, K. (1993) Nucleic Acids Res. 21, 3767–3775
3. Kaplan, J. & Calame, K. (1997) Nucleic Acids Res. 25, 1108–1116
4. Sugawara, M., Scholl, T., Ponath, P. D. & Strominger, J. L. (1994) Mol. Cell. Biol. 14, 8434–8450
5. Zollman, S., Godt, D., Prive, G. G., Coudere, J. L. & Laski, F. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10717–10721
6. Albagli, O., Dhurandhar, P., Deweindt, C., Lecocq G. & Leprince, D. (1995) Cell Growth Differ. 6, 1393–1400
7. Harrison, S. D. & Travers, A. A. (1990) EMBO J. 9, 207–216
8. Xiong, W. C. & Montell, C. (1993) Genes Dev. 7, 1085–1096
9. DiStefano, P. S., Withers, D. A., Bayer, C. A., Fristrom, J. W. & Guild, G. M. (1991) Genetics 129, 385–397
10. Chen, W., Zollman, S., Coudere, J. L. & Laski, F. A. (1995) Mol. Cell. Biol. 15, 3424–3429
11. Raff, J. W., Kellum, R. & Alberts, B. (1994) EMBO J. 13, 5977–5983
12. Dorn, R., Krauss, R., Gerber, T. & Baumweber, H. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11376–11380
13. Iwaki, T., Kawamura, N., Hirota, S. & Aoki, N. (1994) Blood 83, 26–32
14. Ye, B. H., Lister, F., Coo, P. L., Knowles, D. M., Offret, K., Chaganti, R. S. K. & Dalla-Favera, R. (1993) Science 262, 747–750
15. Kerekzaert, J. P., Deweindt, C., Tilly, H., Quief, S., Lecocq G. & Bastard, C. (1993) Nat. Genet. 5, 66–70
16. Wades, M. S., Bie, M. A., Deir, W. E., Nelkin, B. D., Isa, J.-P., Cavenne, W. K., Kuerbitza, S. J. & Baylin, S. B. (1995) Nat. Med. 1, 570–577
17. Chen, Z., Brand, N. J., Chen, A., Chen, S.-J., Tong, J.-H., Wang, Z. Y., Waxman, S. & Zelent, A. (1993) EMBO J. 12, 1161–1167
18. Chardigny, P., Courtous, G., Mattei, M. G. & Gisselbrecht, S. (1991) Nucleic Acids Res. 19, 1431–1436
19. Aoki, K., Suzuki, K., Sugano, T., Tsuchiya, T., Nakahara, K., Kage, O., Omeri, A. & Kasai, M. (1995) Nat. Genet. 10, 167–174
20. Kakeya, M., Mazzarz, R. T., Aoki, K., Sai, Y., Euston, E. & Strominger, J. L. (1992) Mol. Cell. Biol. 12, 4731–4737
21. Kasai, M., Aoki, K., Matsu, Y., Minowada, J., Mazzarz, R. T. & Strominger, J. L. (1994) J. Immunol. 6, 1017–1025
22. Aoki, K., Nakahara, K., Hirota, S., Sato, M., Takahashi, T., Minozawa, J., Strominger, J. L., Mazzarz, R. T. & Kasai, M. (1994) Oncogene 9, 1109–1115
23. Kasai, M., Matsu, T., Katayanagi, K., Omori A., Mazzarz, R. T., Strominger, J. L., Aoki, K. & Suzuki, K. (1997) J. Biol. Chem. 272, 11402–11407
24. Aoki, K., Ishida, R. & Kasai, M. (1997) FEBS Lett. 401, 109–112
25. Schneider-Gudicke, A., Beer-Romero, P., Brown, L. G., Mardon, G., Lush, S. W. & Page, D. C. (1989) Nature 342, 708–711
26. Bordereaux, D., Fitch, S., Tabernier, P. & Gisselbrecht, S. (1990) Oncogene 5, 925–927
27. Wright, W. E., Binder, M. & Funk, W. (1991) Mol. Cell. Biol. 11, 4104–4110
28. Kadesch, T. (1992) Immunol. Today 13, 31–36
29. Ephrussi, A., Church, G. M., Tonogawa, S. & Gilbert, W. (1985) Science 227, 154–140
30. Sen, R. & Baltimore, D. (1986) Cell 46, 705–716
31. Lenardo, M., Pierce, J. W. & Baltimore, D. (1987) Science 236, 1573–1577
32. Chang, C. C., Ye, B. H., Chaganti, R. S. K. & Dalla-Favera, R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6947–6952
33. Seyfort, V. S., Allman, D., He, Y. & Staudt, L. M. (1996) Oncogene 12, 2331–2342
34. Hong, S. H., David, G., Song, C. H., Dejean, A. & Privalsky, M. L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9028–9033
35. Tsukiyama, T., Becker, P. B. & Wu, C. (1994) Nature 367, 525–532
36. Komori, E. V., Zhou, S. & Luchesi, J. C. (1995) Nucleic Acids Res. 23, 4229–4233
37. Hecht, A., Strahl-Bolsinger, S. & Grunstein, M. (1996) Nature 383, 92–96
38. Grunstein, M. (1997) Curr. Opin. Cell Biol. 9, 383–387