Demonstration of a Testis-specific trans-Acting Factor Tet-1 in Vitro That Binds to the Promoter of the Mouse Protamine 1 Gene*

(Received for publication, July 8, 1991)

Taka-aki Tamura‡, Yasutaka Makino‡, Katsuhiro Mikoshiba‡, and Masami Muramatsu**

From the ‡Department of Biochemistry, Saitama Medical School, Moroyama-machi, Saitama 350-04, the §Division of Macromolecular Function, Institute for Protein Research, Osaka University, Yamada-oka, Suita 565, the ¶Department of Biochemistry, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108, and the **Department of Biochemistry, Faculty of Medicine, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

We have established testis-specific in vitro transcription of the mouse protamine 1 (MP1) gene using rat testis nuclear extracts. Addition of testis nuclear extracts to brain extracts enhanced transcription from the MP1 upstream sequence-carrying adenosivirus major late promoter. Moreover, the MP1 upstream region from positions -92 to -41 alone exhibited transcriptional activation in a tissue-specific manner. DNase I footprinting demonstrated the presence of a DNA-binding factor around position -60 (Tet-1) in testis nuclear extracts, but not in other tissues. Gel shift analysis also revealed the presence of testis-specific Tet-1. Since mutational analysis in transcriptional and binding assays demonstrates that the Tet-1 site is responsible for transcriptional activation, we suggest that Tet-1 is a novel tissue-specific trans-acting factor. The Tet-1-recognizing sequence was delineated to the 11-mer TGACTTCATAA at position -64. Although the first 8-mer in the Tet-1 11-mer shares homology with the cyclic AMP-responsive element, Tet-1 is demonstrated to be distinct from known cAMP-responsive element-binding factors.

The process of spermatogenesis represents a unique cellular differentiation pathway. During this process, many testis-specific genes and testis-specific variants of somatic genes are differentially expressed (1-4). During spermatogenesis in mammals, histones are ultimately replaced by protamines, which are small, highly basic proteins containing 40-70% arginine (5, 6). This change occurs during the late haploid phases of male germ cell differentiation and produces a DNA-protamine complex that is compact and genetically inactive (5-7). The nuclei of the mammalian sperm examined contained P1 protamine. Structural comparison of P1 protamines from several species has revealed strong sequence homologies (5-7). The nuclei of the mammalian sperm examined contained P1 protamine. Structural comparison of P1 protamines from several species has revealed strong sequence homologies and an identical length of 50 amino acids (8-15). Mouse protamine 1 (MP1) is similar to the P1 protamine of other mammalian species. In mice and hamsters, there is another minor protamine, protamine 2, the amino acid compositions of which are distinct from those of MP1 (12, 16, 17). Gene expression of mouse protamine is restricted in the testis (12, 18-21); the mRNAs are first detected in the round spermatids, the first haploid precursors of mature spermatozoa. These stable, very abundant mRNAs are then stored for up to 8 days before being translated in the elongating spermatids.

The study of gene expression in male germ cells has been hampered by the lack of a permanent spermatogenic germ cell line and primary cells in culture. Therefore, neither the cis-acting element nor the trans-acting factor of testis-specific genes has been analyzed in detail. Making a testis-derived in vitro transcription system has been considered an attractive alternative. In a dissociated population of cells from adult testes, ~80% of the nucleated cells are postmeiotic germ cells (22-25). Bunic et al. (24, 25) have established in vitro transcription using mouse testis nuclear extracts. They studied transcriptional regulation of the mouse protamine 2 gene and suggested a positive regulatory region between positions -170 and -82. In this study, we investigated the regulation of mouse protamine 1 gene transcription by a testis-specific cell-free system and showed a cis-acting element at position -64 and its cognate factor (Tet-1).

MATERIALS AND METHODS

Plasmid DNAs—The mouse MP1 plasmid pMP1-3.2 (21) was a kind of gift from Dr. R. D. Palmer and contains the mouse MP1 gene on a 3.2-kb PstI fragment extending from -1.7 to +1.5 kb relative to the transcription start site and cloned into pUC18. In pPR DNA, the MP1 sequence downstream from position +587 was removed (see Fig. 1). pML is an adenosivirus major late promoter (MLP) construct containing the TATA and initiator elements (26). Configuration of pML/PR series plasmids carrying the MLP and the MP1 upstream region is indicated in Fig. 3B. pML in pML/PR3M carries internal deletions in the Tet-1 site as (-64=T=C)AGAA. For pML/PR 2T construction, an oligonucleotide (5-GAATTCTGACTTCATACCTCATGCTGCTGAGATACGAGAATTC) containing 16 units of the Tet-1 site (underlined) connected tandemly through a 4-bp spacer, EcoRI linker, and XbaI-EcoRI linker was inserted into pML in a normal orientation. In pML/PR2TM, the Tet-1 11-mer is changed to TGGCGATACAA (underlined). Sequences of 11-mer T1 DNA and its derivatives are indicated in Table I. These sequences were flanked with PstI (at the 5′-terminus) and XbaI (at the 3′-terminus) sites and inserted into pUC119 (Fig. 7A).

Preparation of Nuclear Extracts and In Vitro Transcription—Nuclear extracts form rat and mouse tissues were prepared as described previously (27). For testes, 4-6-month-old animals were used. Freshly prepared testes were washed with 0.6 × phosphate-buffered saline and minced well, and cells were disrupted by a loosely fitted Teflon homogenizer. At the first centrifugation step for pelleting nuclei, the sucrose density was decreased from 2.0 to 1.94 M for better yield of nuclei. Other procedures were the same as the standard method (27). The concentration of nuclear extracts was adjusted to 10 mg of protein/ml. In vitro transcription was carried out at 30 °C for 45 min using 300 ng of closed circular DNA template (27, 28). Transcripts

*This work was supported by a grant-in-aid for scientific research on priority areas from the Ministry of Education, Science, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be addressed. Fax: 492-95-2784.

§The abbreviations used are: MP1, mouse protamine 1; kb, kilobase(s); bp, base pairs(s); MLP, major late promoter, HEPEIS, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; CRE, cAMP-responsive element.
were analyzed by a modified S1 nuclease mapping procedure using single-stranded DNA probe. Probe DNAs were designed to generate 590- and 316-base transcription signals for pPR and pML, respectively.

DNase I Footprinting—DNase I footprinting was carried out as previously described (29). 20 ng of 32P-labeled probe (2 × 10⁶ cpm, by kination), 0.4 μg of poly(dA-dT), 0.1 μg of poly(dI-dC), 12 mM HEPES/KOH (pH 7.6), 40 mM KCl, 0.6 mM dithiothreitol, 1 mM EDTA, 8% glycerol, and various amounts of extracts. DNase I digestion was carried out at 30°C in the presence of 3 mM CaCl₂, and DNA digests were analyzed on 8% sequencing gels.

Gel Shift Assay—For probe preparation unless described otherwise, T1 DNA carrying the Tet-1 11-mer or its derivative sequences (see Fig. 7A) was excised from the pUC119 vector with HindIII and BamHI and labeled with 32P by kination. The specific activity of the probe was adjusted to 4 kcpm/ng. Extract (0.2–2.5 μl) was incubated on ice for 10 min with 0.2 μg of poly(dA-dT) and 0.8 μg of poly(dI-dC) in 12 μl of a mixture containing 12 mM HEPES/KOH (pH 7.6), 80 mM KCl, 0.6 mM dithiothreitol, and 1 mM EDTA prior to binding. Binding reaction in the presence of probe DNA (2 kcpm) was performed on ice for 20 min, and the mixture was loaded onto a 4.5% polycrylamide gel in TAE buffer (6.7 mM Tris-HCl (pH 7.6), 3.3 mM sodium acetate, and 1 mM EDTA).

RESULTS

In Vitro Transcription from Mouse Protamine 1 Promoter—A template of the mouse MP1 promoter pPR (Fig. 1) for in vitro transcription was derived from pMP1-3.2 DNA (21). Nuclear extracts from rat testes were prepared by the standard method (27). In vitro transcription was carried out at 30°C for 45 min using closed circular DNA templates (27, 28). The adenovirus MLP in pML DNA was transcribed efficiently in the rat brain nuclear extracts and weakly but significantly in the testis nuclear extract (Fig. 2). Although we verified that the rat testis nuclear extract was essentially transcription-competent, the amount of transcripts from the MP1 promoter pPR was very low (Fig. 2, lane 2).

MP1 Upstream Region Enhances Transcription Tissue-specifically—To find a tissue-specific cis-acting element in the MP1 promoter, we transcribed three chimeric promoters having an MP1 upstream region and MLP (pML/PR series DNAs) (Fig. 3). MP1 upstream regions lacking sequences downstream from position −40 were joined to the MLP core promoter (26) (Fig. 3B). These DNA were transcribed in brain nuclear extract or in brain and testis mixed nuclear extract (Fig. 3A). The brain nuclear extract here works as a “base” extract that allows adequate MLP-directed transcription. Testis nuclear extracts were supposed to contain a potent inhibitor for basic transcription since pML signals in the mixed extract were lower than those in the brain extract. All chimeric promoters yielded weaker transcripts than pML in the brain nuclear extract, although we do not know exactly how these phenomena occur. In the mixed extract, however, chimeric promoters produced many more transcripts than pML, although enhancement by the longest MP1 fragment (pML/PR1) was not so striking. These results indicate that the MP1 upstream sequence alone can enhance transcription from a heterologous MLP promoter. Even a short MP1 stretch from positions −92 to −41 (pML/PR3) enhanced transcription.

The results shown above imply that the MP1 sequence in pML/PR3 enhances transcription in a testis-specific manner. To confirm this, we prepared nuclear extracts from various rat and mouse tissues including testis, brain, liver, spleen, and kidney. pML and pML/PR3 were transcribed in a reaction mixture containing rat brain nuclear extracts and one of the various rat or mouse nuclear extracts (Fig. 4). Only testis nuclear extracts elevated transcription regardless of the two species. Non-testis extracts were rather inhibitory, as seen in...
DNA-binding Factor Tet-1 in Testis Nuclear Extracts—Since the results shown above imply the presence of a testis-specific trans-acting factor for MP1 DNA between positions -92 and -41, we carried out DNase I footprinting analysis using MP1 upstream DNA and testis nuclear extracts (Fig. 5A). We found a pronounced protection at positions -57 to -63 by the testis nuclear extract as well as hyper-digested bands around position -96. However, these footprints characteristic of testis nuclear extract were not seen in non-testis extracts such as brain, liver, kidney, and spleen (Fig. 6A). Among the mouse tissues tested, only the testis nuclear extract gave the specific footprint (Fig. 6B). These results clearly demonstrate that rat and mouse testes contain tissue-specific DNA-binding factors that interact with the MP1 promoter. We designated this testis-specific factor Tet-1. The MP1 sequence from positions -64 to -54 (Table I) was inserted into pUC119 between PstI and XbaI sites using 3-bp spacers at both ends and subjected to DNase I footprinting (Fig. 5B). The testis nuclear factor again bound to this truncated 11-mer and its flanking sequences. Hence, we conclude that the 11-mer contains a required sequence for Tet-1 binding.

Tet-1 in Gel Shift Assay—We analyzed Tet-1 by gel shift assay. A Tet-1 11-mer-binding sequence was cloned into pUC119 through 3-mer flanking sequences to construct T1 DNA (Fig. 7A and Table I). A HindIII-BamHI fragment carrying T1 DNA was excised from the vector and used as a DNA-binding site for gel shift analysis. The results showed that Tet-1 binds specifically to the T1 DNA fragment, and this binding is not observed with non-testis nuclear extracts (Fig. 7B). The sequence of the T1 DNA fragment is given in Table I.

**Table I**

| DNA | 5'-Flanking sequence | Binding sequence | 3'-Flanking sequence |
|-----|----------------------|------------------|---------------------|
| T1  | TTC                  | (−64) TGACTTCTAA | GGC                 |
| T1-5A| TTC                  | GACTTCTAA        | GGC                 |
| T1-5B| TTC                  | ACTTCTAA         | GGC                 |
| T1-3A| TTC                  | ACTTCTAA         | GGC                 |
| T1-3B| TTC                  | TGACCTTAA        | GGC                 |
| T1A | TTC                  | TGACCTTAA        | GGC                 |
| T1B | TTC                  | TGACCTTAA        | GGC                 |
| T1C | TTC                  | TGACCTTAA        | GGC                 |
| SOM*| TCTCTGACGTGCA       |                  |                     |

*The rat somatostatin (SOM) CRE from positions -37 to -53 (30) was linked directly to PstI and XbaI adaptors. The core 8-mer of the CRE is represented by boldface letters.*
probe. Incubation of the testis nuclear extract with the T1 DNA yielded one major (arrowhead) and one minor (circle) shifted band (Fig. 7B, lane 4). A corresponding vector DNA fragment did not yield these complexes. We prepared two additional probes: a T1 DNA-carrying HindIII-EcoRI fragment containing longer vectors and a native MP1 sequence from positions -92 to -41. Gel shift assay revealed that these two probes also formed complexes similar to those formed by T1 DNA (Fig. 7C). Competition gel shift assay demonstrated that both major and minor complexes were Tet-1 site-specific (Fig. 7D). Moreover, we found that non-testis extracts did not yield the Tet-1-specific bands in the experiments of Fig. 8.

Effect of Tet-1 Site on Transcriptional Activation—In vitro transcriptional and DNA binding assays suggest that the Tet-1 sequence is a testis-specific cis-acting element of the MP1 promoter. To obtain more evidence about the function of the Tet-1 site, we carried out various transcriptional experiments. A mutant template (pML/PR3M) was constructed in which 8 bp within the Tet-1 sequence were deleted from pML/PR3. Plasmids pML/PR3 and pML/PR3M were transcribed in the brain or brain/testis mixed extract (Fig. 9A). In the brain extract, Tet-1 site-mediated transcriptional stimulation was not observed. We do not know exactly why pML/PR3M and pML showed similar transcriptional efficiency in the brain nuclear extract. In the mixed extract, the MP1 sequence from positions -92 to -41 (pML/PR3) activated transcription from MLP, but the mutated sequence (pML/PR3M) exhibited only a small effect. This suggests that the Tet-1 site itself is responsible for transcriptional activation. When a competitor DNA fragment carrying the Tet-1 site was added to the transcription mixture, it significantly decreased transcription of pML/PR3 (Fig. 9B). Addition of the nonspecific DNA (pUC) instead increased transcription slightly. We further transcribed pML/2T (2 × wild type) and pML/2T (2 × mutant) DNAs in the brain/testis mixed extract (Fig. 9C). The mutated sequences did not bind to the Tet-1 factor (data not shown). pML/2T was transcribed much more efficiently than pML, but pML/2TM was not. In the brain extract, transcriptional activities of pML and pML/2T were almost the same (data not shown). These results indicate that the Tet-1 site itself is responsible for transcriptional activation of the MP1 promoter.

Tet-1 Site Overlaps Cyclic AMP-responsive Element—To delineate the sequences required for Tet-1 binding, we constructed several T1 DNA-derived 3' and 5' deletion mutants (Table I) and used them in gel shift assay (Fig. 10). T1 DNA yielded one major shifted band as demonstrated above. All deletion mutants exhibited weaker signals than those of T1 DNA. 3'-Deletions seemed to affect the binding affinity more severely than 5'-deletions. Interestingly, however, we found that T1-3A formed a smeared “super” retarded band as indicated (Fig. 10A, lane 6, asterisk). From these analyses, we delineated the 11-mer as a required sequence for strong binding.

Since the first 8-mer of the Tet-1 sequence (TGACTTTCA) was similar to the cyclic AMP-responsive element (CRE) (TGACGTCGA), we examined the relationship between the Tet-1 site and the CRE. We constructed three T1 DNA-derived base substitution mutants in which the fifth T residue was altered to A (T1A), G (T1G), or C (T1C) as well as the rat somatostatin CRE sequence (GGCTGACGTCAAG) (30) (Table I). All T1 DNA-derived mutants resulted in significant amounts of the Tet-1-related complex in gel shift assay, although the bands were weaker than those of T1 DNA (Fig. 10B), suggesting that the fifth T residue is not critical for Tet-1 binding. We found, however, that T1A formed considerable amounts of complexes that were more retarded than those of Tet-1. Moreover, T1G DNA carrying the consensus CRE yielded more of an upper band than T1A DNA. The somatostatin CRE probe did not yield a detectable Tet-1-specific complex, but did yield large amounts of the upper band. The positions of somatostatin CRE-derived upper
Testis-specific Protamine Gene Transcription

Fig. 9. Effect of Tet-1 site on in vitro transcription. Transcription was performed in both rat brain (6 µl) and testis (2 µl) nuclear extracts. A, three templates were transcribed in the presence of the rat brain (lanes 1-3) or brain plus testis (lanes 4-6) nuclear extract as described under “Materials and Methods,” and transcripts (arrowhead) from the MLP were analyzed. B, 220 ng of pML or pML/PR3 DNA was transcribed in the mixed extract in the presence of 5 ng of competitor DNA fragments. Competitor (pUC119 or T1 DNA) from HindIII to BamHI was added during the preincubation period. C, pML, pML/2T (dimerized Tet-1-carrying pML), and pML/2TM (Tet-1 site mutant of pML/2T) were transcribed in the mixed extract.

Fig. 10. Mutational analysis for Tet-1 site by gel shift assay. The positions of Tet-1-specific and super retarded upper bands are indicated by arrowheads and asterisks, respectively. Nucleotide sequences of Tet-1-derived DNAs are listed in Table I. A, T1 DNA-derived deletion mutants were analyzed using 1.5 µl of rat testis nuclear extract (lanes 2-7). Lane 1, no extract. B, effects of base substitution at the 50th nucleotide of the Tet-1 site. SOM, rat somatostatin CRE. Lane 1, no extract; lanes 2, 4, 6, 8, and 10, 0.3 µl of rat testis nuclear extract; lanes 3, 5, 7, 9, and 11, 0.7 µl of rat testis nuclear extract.

bands were not distinguishable from those generated by T1A and T1G. The super retarded band detected with the T1-3A probe (Fig. 10A, lane 6) seemed to be similar to one of these upper bands. We suggest that factors in the upper bands are related to CRE-binding factors and that the Tet-1 site contains a CRE-like sequence.

DISCUSSION

In this work, we studied transcriptional regulation of the MP1 gene to understand molecular mechanisms of spermatogenesis. MP1 mRNA is expressed exclusively in postmeiotic testis cells (6, 21, 26, 31, 32), and transcriptional regulation of the trout protamine gene has been studied using nonspecific assay systems such as HeLa cell-free transcription (33) and transfection with Cos-1 cells (34). In this study, however, we established testis-specific in vitro transcription of the MP1 gene and successfully identified a testis-specific cis-acting element.

From the experiments in Fig. 3, we suggested that the MP1 sequence from positions −92 to −41 confers testis specificity to MLP-driven transcription. However, some points in Fig. 3 remain to be resolved. Since in vitro transcription is sensitive to the presence of cis-acting elements in template DNA, if template DNA has a promoter-like sequence (pseudo promoter), transcription may decrease by titrating out of general transcription factors. We suppose that MP1 sequences from positions −293 to −93 and −92 to −41 contain strong and weak pseudo promoter sequences, respectively. If this is the case, lower transcription of pML/PR1−3 in the brain extract compared to pML could be explained. The pseudo promoter in pML/PR3 DNA may overlap the Tet-1 site since the Tet-1 sequence and its surrounding are AT-rich (a potential TATA box) (see Fig. 5B). This hypothesis may explain why transcription of pML/PR3M was higher than that of pML/PR3 in the brain extract (Fig. 9A, lanes 2 and 3) and why the mutation did not severely affect pML/PR3M-driven transcription in the mixed extract (lane 6). We can speculate the existence of a testis-specific inhibitory sequence between −1.7 and −293 kb from weak pML/PR1 transcription in the mixed extract compared to pML/PR2−3 (Fig. 3A). Purification of transcription factors may be required to clarify these issues.

In this study, we identified a testis-specific DNA-binding factor (Tet-1) from DNase I footprinting and gel shift analyses. Since transcriptional activation is dependent on the presence of the Tet-1 sequence (Fig. 8), we conclude that Tet-1 is a testis-specific trans-acting factor. Our experimental evidence that Tet-1 was detected in both rat and mouse testes suggests that other mammals also have Tet-1 or a related factor. Many studies have identified tissue-specific factors in a variety of cells and tissues (36-46). However, there has been no report on a testis-specific transcription factor. Since the Tet-1 11-mer sequence was found to be unique among known factor-binding sequences including testis-specific genes (47), we suggest that Tet-1 is a novel tissue-specific trans-acting factor.

The conservation of a number of upstream sequence elements within mammalian protamine genes suggests that upstream sequences are involved in the regulation of protamine gene expression (17, 35). In a transgenic mouse study (21), the MP1 sequence from positions −880 to +625 is demonstrated to be necessary for efficient and tissue-specific MP1 gene transcription. It has been further demonstrated that, at most, 0.94 kb of the MP1 upstream sequence is sufficient to direct testis-specific gene expression (21, 32). Moreover, Behringer et al. (31) reported that sequences downstream from position +95 are dispensable in testis-specific MP1 gene expression. These observations have suggested that the full information needed for testis-specific MP1 gene transcription...
resides within sequences of several hundred base pairs around the transcription start site. Since the location of the Tet-1 sequence is consistent with the previous transgenic mouse studies (see above), the Tet-1 site is supposed to also function in vivo. At present, we do not know whether Tet-1 is a common cis-acting element that confers testis specificity since the 11-mer sequence has not been observed in the testis-specific genes reported so far. Further analysis may be required to generalize the role of Tet-1 in testis-specific gene expression.

We delineated the 11-mer sequence at position -64 of the MP1 promoter required for Tet-1 binding. In the beginning, we supposed that Tet-1 was a member of the CRE-binding factor because the first 8 bp in the Tet-1 sequence were similar to the consensus CRE, and T1G (having the consensus CRE) like T1, formed significant amounts of the Tet-1-specific complex. However, we now suggest that Tet-1 is a class of trans-acting factors distinct from a family of CRE-binding factor according to the following reasons. First, the fifth T residue in the Tet-1 site was not critical for binding, and T1G did not generate many more specific complexes than T1 DNA. We noted that the dense upper bands detected using T1A, T1G, and T1-3A probes were related to CRE-binding factors since the somatostatin CRE yielded large amounts of these upper bands. Second, the eleventh A residue (downstream of the CRE-related 8-mer) was found to be much more important than the fifth T residue (within the 8-mer) for strong Tet-1 binding. It remains unclear why T1-3A (but not T1-3B) yields the upper band. The affinity of Tet-1 for the 11-mer could be possibly higher than that for factors in the upper band, but drops sharply if the eleventh A residue is deleted. Moreover, the upper band-related factors may bind to the 10-mer, but not to the 9-mer, even though the fifth T residue does not fit the CRE-related 8-mer.) was found to be much more important.

Acknowledgments—We wish to thank Dr. R. D. Palmer for the gift of a mouse MP1 plasmid and Drs. U. Schibler, H. Fujimoto, and Y. Nakahashi for valuable discussions and critical reading of this manuscript.

REFERENCES

1. Gold, B., Fujimoto, H., Kramer, J. M., Erickson, R. P., and Hecht, N. B. (1985) Deu. Biol. 98, 392-399
2. Allen, R. L., O'Brien, D. A., Jones, C. C., Rockett, D. L., and Eddy, E. M. (1988) Mol. Cell. Biol. 8, 3260-3266
3. Goto, M., Koji, T., Mizuno, K., Tamura, M., Koikeda, S., Nakane, K., Mori, N., Masumune, Y., and Nakahashi, Y. (1990) Exp. Cell Res. 186, 273-278
4. Keshet, E., Itin, A., Fischman, K., and Nir, U. (1990) Mol. Cell. Biol. 10, 5021-5025
5. Belý, A. R. (1979) Oxf. Reprod. Biol. 1, 159-261
6. Hecht, N. B. (1986) in Experimental Approaches to Mammalian Embryonic Development (Rossant, J., and Pedersen, R., eds) pp. 151-193, Cambridge University Press, London
7. Bedford, J. M., and Calvin, H. I. (1974) J. Exp. Zool. 188, 137-156
8. Kolk, A., and Samuel, T. (1975) Biochim. Biophys. Acta 395, 307-319
9. Tobita, T., Tsutsumi, H., Kato, A., Suzuki, H., Nomoto, M., Nakano, M., and Ando, T. (1983) Biochim. Biophys. Acta 744, 141-146
10. Balhorn, R., Weston, S., Thomas, C., and Wyrobeck, A. J. (1984) Exp. Cell Res. 199, 298-308
11. Sautièrè, P., Belaiche, D., Martnagis, A., and Lior, M. (1984) Eur. J. Biochem. 144, 121-125
12. Kleene, K. C., Distel, R. J., and Hecht, N. B. (1985) Biochemistry 24, 719-722
13. McKay, D. J., Rennau, B. S., and Dixon, G. H. (1985) Biochi. Rep. 5, 383-391
14. Kretwetz, S. A., Connor, W., and Dixon, G. H. (1987) DNA (N. Y.) 6, 47-57
15. Lee, C. H., Mansouri, A., Hecht, W., Hecht, N. B., and Engel, W. (1987) Bioch. Chem. Hoppe-Seyler 368, 131-135
16. Yelick, P. C., Balhorn, R., Johnson, P. A., Corzett, M., Mazzirmas, J. A., Kleene, K. C., and Hecht, N. B. (1987) Mol. Cell. Biol. 7, 2175-2179
17. Johnson, P. A., Peschon, J. J., Yelick, P. C., Palmieri, R. D., and Hecht, N. B. (1988) Biochim. Biophys. Acta 950, 45-53
18. Kleene, K. C., Distel, R. J., and Hecht, N. B. (1985) Dev. Biol. 98, 455-464
19. Kleene, K. C., Distel, R. J., and Hecht, N. B. (1984) Dev. Biol. 105, 71-79
20. Hecht, N. B., Bower, P. A., Water, S. H., Yelick, P. C., and Distel, R. J. (1986) Exp. Cell Res. 164, 183-190
21. Peschon, J. J., Behringer, R., Brinster, R., and Palmiter, R. D. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5316-5319
22. Belli, A. R., Millette, C. F., Bhattacharyya, Y. M., and O'Brien, D. A. (1977) J. Histochem. Cytochem. 25, 480-494
23. Meistrich, M. L. (1977) Methods Cell Biol. 15, 15-54
24. Bunick, D., Balhorn, R., Stanker, L. H., and Hecht, N. B. (1990) Mol. Cell. Biol. 10, 4332-4339
25. Bunick, D., Johnson, P. A., Johnson, T. R., and Hecht, N. B. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 891-895
26. Tamura, T., Miura, M., Ikenaka, K., and Mikoshiba, K. (1988) Nucleic Acids Res. 16, 11441-11450
27. Tamura, T., Ohya, Y., Miura, M., Aoyama, A., Inoue, T., and Mikoshiba, K. (1989) Technique 1, 35-39
28. Tamura, T., Aoyama, A., Inoue, T., Miura, M., Okano, H., and Mikoshiba, K. (1989) Mol. Cell. Biol. 9, 3122-3126
29. Aoyama, A., Tamura, T., and Mikoshiba, K. (1990) Biochem. Biophys. Res. Commun. 187, 645-653
30. Montminy, M. R., and Bilezikjian, L. M. (1987) Nature 328, 175-178
31. Behringer, R. R., Peschon, J. J., Messing, A., Gartsisde, C. L., Hauschka, S. D., Palmitore, R. L., and Brinster, R. L. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2648-2652
32. Braun, R. E., Peschon, J. J., Behringer, R. R., Brinster, R. L., and Palmitore, R. D. (1989) Genes & Dev. 3, 789-802
33. Jankowski, J. M., and Dixon, G. H. (1984) Can. J. Biochem. Cell Biol. 62, 291-300
34. Jankowski, J. M., and Dixon, G. H. (1987) Biochim. Biophys. Acta 950, 519-529
35. Scheidereit, C., Comliah, J. A., Gerster, T., Kawakami, K., Balmaceda, C. G., Currie, R. A., and Roeder, R. G. (1988) Nature 336, 551-557
36. Tappasott, S. J., Davis, R. L., Thayer, M. J., Cheng, P.-F., Weintraub, H., and Lassar, A. B. (1988) Science 242, 405-411
37. Evans, T., and Felsenfeld, G. (1989) Cell 58, 877-886
38. Prat, M., Swart, G., Monaci, P., Nicosia, A., Stampili, S., Frank, R., and Cortese, R. (1989) Cell 59, 145-157
39. Lenardo, M. J., and Baltimore, D. (1989) Cell 58, 227-229
40. Mangalam, H. J., Albert, V. R., Ingram, H. A., Kaplito, M., Wilson, L., Nelson, C., Elebho, H., and Rosenfeld, M. G. (1989) Genes & Dev. 3, 946-958
41. Wright, V. E., Sassoon, D. A., and Lin, V. K. (1989) Cell 56, 607-617
42. Mueller, C. R., Maire, P., and Schibler, U. (1990) Cell 61, 279-293
43. Michiels, F., Gasch, A., Kalschmidt, B., and Renkawitz-Pohl, R. (1989) EMBO J. 8, 1559-1565