FEBS Letters 345 (1994) 225–228

FEBS 14058

Sequence analysis of the promoter region of the rat somatostatin receptor subtype 1 gene

Frank Hauser, Wolfgang Meyerhof**, Iris Wulfsen, Christiane Schönrock, Dietmar Richter*

Institut für Zellbiochemie und klinische Neurobiologie, UKE, Universität Hamburg, Martinistraße 52, D-20246 Hamburg, Germany

Received 11 April 1994

Abstract

Somatostatin receptor (SSTR) subtype genes are differentially expressed in brain and various peripheral tissues. RNA blotting and semiquantitative PCR analyses have revealed low levels of SSTR1 mRNA in the gastrointestinal tract and relatively high levels in GH_3 anterior pituitary cells. As a first step in the investigation of the regulation of SSTR1 gene expression, we isolated a genomic fragment that contains the promoter region and determined the transcriptional initiation site. The SSTR1 gene lacks introns and TATA and CAAT motifs, but possesses several consensus recognition sequences for the transcription factors GCF and AP-2. The presence, also, of two Pit-1 binding sites could explain the high SSTR1 mRNA levels in GH_3 cells.

Key words: Pit-1; Pituitary; GCF; AP-2

1. Introduction

The diverse biological activities of the peptide hormone somatostatin are mediated by at least five different receptors, termed SSTR1-5 [1–7], that belong to the superfamily of G-protein coupled receptors each of which contains seven transmembrane domains. When expressed individually in mammalian cells the five receptor subtypes exhibit distinct pharmacological properties, yet, when activated, all inhibit adenylate cyclase [8]. RNA blot analysis and nuclease protection and in situ hybridization experiments show a spatially and temporally different localization of the individual SSTR mRNAs in brain and peripheral tissues [9]. For instance, in rat brain areas such as the cortex and the magnocellular preoptic nucleus SSTR1 transcripts are first observed at prenatal day 14 and increase in number until prenatal day 20 [5]. However, SSTR1 mRNA levels then decrease in the magnocellular preoptic nucleus during further development being barely detectable by in situ hybridization in adult animals; in contrast, they remain unchanged in cortex. The heterogeneous mRNA distributions point to different physiological roles for the various receptor subtypes and reflect the complex regulation of expression of the five genes.

By RNA blot analysis and polymerase chain reaction (PCR) amplification we have further examined the distribution of SSTR mRNAs in peripheral organs. To gain a first insight into the mechanism of gene regulation, we have also isolated and characterized the rat SSTR1 gene.

2. Materials and methods

2.1. Isolation of genomic clones

A rat genomic library constructed in lambda DASH II (Stratagene) was screened by standard protocols [10] with a 1,276 bp fragment that contained the entire coding region of the rat SSTR1 cDNA [3]; this was obtained by PCR amplification using the primers 5′-TGAGCTGTGAGCTTGGAG-3′ and 5′-TCAAAGCGTGCTGATCCT-3′. Positive plaques were purified and one clone, λrg1.1, was chosen for further analysis.

2.2. Mapping, subcloning and sequencing

The 19 kb insert of λrg1.1 was analyzed by restriction mapping and Southern blot hybridizations. Appropriate restriction fragments were subcloned into pBluescript II SK+ (Stratagene) and sequenced either manually [11] or with the help of an Applied Biosystems model 373A DNA Sequencer.

2.3. Primer extension

Primer extension was performed essentially as described [10] using poly(A)$^+$ RNA from GH_3 cells and a ^{32}P end-labeled antisense 30-base oligonucleotide 5′-GACGCTCACCTTGCGCCCTTGCTGCCTGCG-3′ corresponding to nucleotides 94–123 of the cloned rat SSTR1 cDNA [3]. 20 μg RNA was mixed with 5×10^4 cpm of primer (specific activity 10^7 cpm/pmol) in 30 μl hybridization buffer which contained 80% (v/v) formamide, 40 mM PIPES (pH 6.4), 1 mM EDTA (pH 8.0) and 0.4 M NaCl. Following incubation at 80°C for 5 min the mixture was hybridized overnight at 32°C. After extension for 1 h at 37°C with 200 units M-MLV reverse transcriptase (Stratagene), products were analyzed in 6% denaturing polyacrylamide gels.

2.4. RNA blot analysis and semiquantitative PCR analysis

Preparation of total RNA, isolation of poly(A)$^+$ RNA, RNA blot and semiquantitative PCR analysis were carried out as described previously [12]. As hybridization probe for RNA blot analysis, the complete SSTR1 cDNA [3] was radiolabeled by the random priming method [10].

3. Results and discussion

Previous studies have shown that SSTR1 [3] and

* Corresponding author.

***Present address:* Universität Potsdam & Deutsches Institut für Ernährungsforschung, Arthur-Scheunert-Allee 114–116, D-14458 Bergholz-Rehbrücke, Germany.

Abbreviations: SSTR, somatostatin receptor; GCF, GC-factor; AP-2, activator protein 2.

SSDI 0014-5793(94)00444-Z

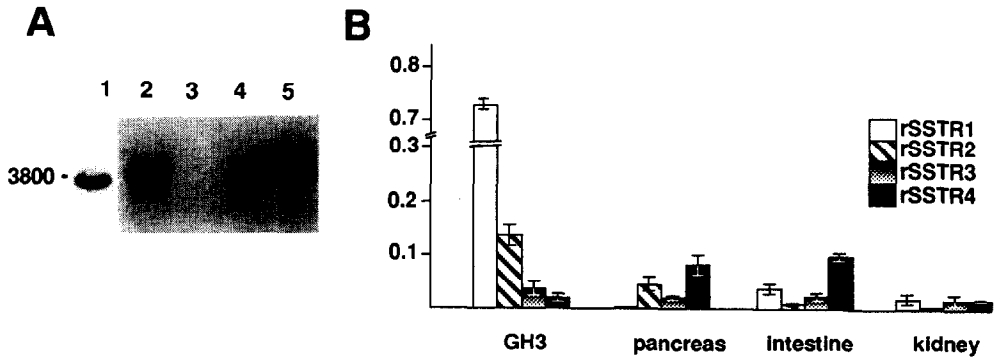


Fig. 1. (A) Northern blot analysis of rat SSTR1 mRNA in peripheral tissues. Poly(A)$^+$ RNA (10 μg) isolated from GH_3 cells (1), stomach (2), pancreas (3), duodenum (4) and jejunum (5) was electrophoresed, blotted and hybridized with a radiolabelled SSTR1 cDNA probe. (B) Relative abundance of SSTR1-4 mRNAs analyzed by semiquantitative PCR. Amplified cDNAs generated using specific primers were dot-blotted, hybridized with subtype-specific oligonucleotide probes and quantified using a PhosphoImager as described previously [12].

SSTR3 [5] mRNAs are present in various rat brain regions [5,12]. To investigate the possible physiological roles of the different SSTRs, the distributions of SSTR mRNAs in peripheral tissues were analyzed. RNA blot analysis revealed high levels of the 3.8 kb SSTR1 transcript in rat anterior pituitary GH_3 cells and lower levels in stomach, duodenum and jejunum (Fig. 1A). No SSTR1 transcripts were detected in the pancreas, and no SSTR3 mRNA has been observed in any of the above tissues when using this technique (data not shown). To analyze the SSTR mRNA distributions in greater detail and at higher sensitivity, semiquantitative PCRs have been employed. Fig. 1B reveals that the different tissues contain distinct sets of SSTR mRNAs. Thus, SSTR1 probably has an important role in anterior pituitary GH_3 cell function. SSTR2 also seems to be involved in pituitary function, while SSTR4 may be important for gastrointestinal and pancreatic physiology.

These results, which are in line with previous reports [9] strongly suggest that SSTR gene expression is subject to finely tuned regulatory mechanisms. In order to begin to examine the molecular details of this regulation, the SSTR1 gene was isolated. Sequence analysis of a 7.4 kb *Eco*RI–*Hin*dIII fragment revealed that it contains the entire previously reported cDNA sequence [3], which lacks introns, about 2 kb of 5′-flanking DNA and 1.7 kb of 3′-flanking DNA (Fig. 2).

The transcriptional start site of the SSTR1 gene was determined using poly(A)$^+$ RNA from GH_3 cells that contain a relatively large amount of the corresponding mRNA. Fig. 3 shows a major primer-extended product, corresponding to a guanosine residue, 178 bp upstream from the translation initiation site. In addition, three minor extension products are observed corresponding to positions −2, −1 and + 2 of the SSTR1 gene (Fig. 2).

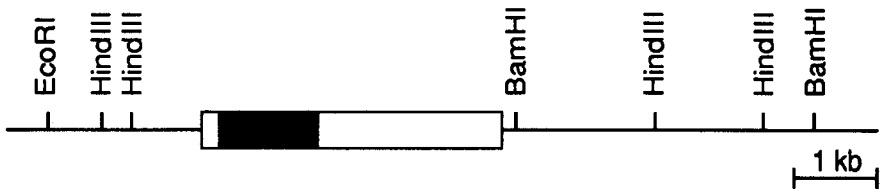
The nucleotide sequence upstream of the SSTR1 mRNA start site contains neither a canonical TATA box nor a CAAT box but possess sequences having a high GC content. These features are reminiscent of promoters of ubiquitously-expressed housekeeping genes [13,14] such as the human dehydrofolate reductase, hypoxanthine phosphoribosyl transferase and phosphoglycerate kinase genes [15–17]. However, promoters lacking a TATA box but containing GC rich sequences can also be found in genes involved in growth control such as the gene for the human epidermal growth factor receptor [18] and the cellular proto-oncogene Ha-ras [19]. In addition, it is worth noting that the promoter for another G-protein coupled receptor, namely the rat serotonin 5-HT_2 receptor, also lacks a TATA motif [20].

Table 1
Putative regulatory elements in the 5′-flanking region of the rat SSTR1 gene

Pit-1		5′						3′		
consensus		A/T	T	A	T	C/T	C	A	T	
	-1952	T		T	A	T	T	C	A	T -1959
	-110	A		T	A	T	C	C	A	T -117
GCF		5′							3′	
consensus		G/C	C	G	G/C	G/C	G/C	C		
	-1084	G	C	G	G	C	G	C		-1078
	-1054	G	C	G	C	G	G	C		-1060
	-1010	C	C	G	C	G	C	C		-1004
	-954	G	C	G	G	G	G	C		-960
	-656	G	C	G	C	C	G	C		-650
	-233	C	C	G	C	C	C	C		-239
	-200	G	C	G	C	C	C	C		-194
	-192	C	C	G	C	C	G	C		-186
AP-2		5′							3′	
consensus		C	C	C	A/C	N	G/C	G/C	G/C	
	-1374	C	C	C	A	T	C	C	C	-1381
	-1343	C	C	C	C	A	G	G	C	-1350
	-1127	C	C	C	C	A	C	C	C	-1120
	-906	C	C	C	C	C	C	C	C	-913
	-197	C	C	C	C	C	C	C	G	-190
	-99	C	C	C	A	G	C	G	C	-106

Consensus sequences recognized by the transcription factors are from [26]. The numbering of the putative regulatory elements corresponds to that of Fig. 1.

A



B

```
-1985  GAATTCCACTCTAACATTTTTTTCTCATGAATAATTCAGCATCTGTTACACACACTCTTAGGGAGTCTGTGGTCATTGATTTTAATCTGACTGCCTCTGCCATCATGGACACCGCACGTG
-1865  ATTCTTTCTCTCCTAGCAGCACTGAGTTTGGGAGAGCGAGGAATCCATGTGTAGGCAATTTCTGAGAACAAGCAACAAGCCATTGTTGGATTGATGCTTTACTTTTAATGCGGTGTCCAA
-1745  CTCTGTGCACTCTAGACTGTCTGGTAGGGACAGCTTCGGTAAATTTGGGTTTAAATTTACCTCCTCTTGGTGGAGATGTTAGAGGGAGACTAGGAAGACAGCAATTGTGAGCAGCCCAGC
-1625  TCCAGGTGAGCACTGGACTGCAGCGGCAAGTCTTCCTGAGACTTGGCATGCTTCCTGCAAAGACTTGCAGGGATTTCCGTGGAAGGAAATTCCTTTCATGAAAAGGAATAGGTGGTTGAA
-1505  TCATTTAGCAGAGAGTAGCTGTAAGGCCAAAGAGACCCTAGGTTCAGAGTCCTGGGAACTCCCAAGGTCCCTTAAAAATCAACATCCATTCTTTCTCAATCAAGAGCCTGTAGTTCCCAG
-1385  ACTTGGGATGGGAACTGCAGGTCGATGAGCTTTGAGCCTGGGGCTTTAATAACTATATCTTAATGGTTTTAGCATTTGGAACACAGAGGGGGTTATCCCTCAAGGCTCTACTATCCCTTA
-1265  GTATTCCATAGGGGAAAGCTTGAAAGTCTTGCCACTGTTCCCAGGCTTGATGACAGTCCATCAGTTGCTCAGCCAGCCACGCGTGGATTAAGACTGTCGCGCCTGCCTATTCTGGCCTTG
-1145  GCGCTCTCCACCTCCATCCCCCACCCCGCCAACTCTCCCGGGTACAAAGTACAGCCGGGACGCGGCGCGGGAGCAGCTGTTCCAAGCCGCGCAGCCTAATCTTTCCCCAGTGAACACCTA
-1025  CATGGATTTCCAATACCGCGCCTGGCAGGTTCCGGAGGGAGTGCCAGCCTCCTCCTGAGCTCTCTGCCCCGGCATTCTGACTTCTGCAGTGGATATCCCTAGGGGAGGTGGGGGGGGGGG
 -905  GGCAGGGCTCAGGGAATTACAAGCTTTGTTAGACAGTCGCAATGGTTCCACTGAGTCCCCCTGAACACTCCCTGCTGCAGAGTGGAGGGCGATGGATGTTGTAACTCAGCATCCCTTAAC
 -785  ACACCACTGTAGTCCTCCGTGCTCATAATGCTATTGGCTTAGGTCTGGTTTCCACCGCTCAGTGTTTGCGTGGGCACTTGTGCAGCAGCCAGCCCCAAAGGGAGTTGGACCTCGCAAAAG
 -665  AACGTCAGAGCGCCGCCAGACCTCACAAGCACTTTGCAAGGGCCATCTGGAGACATCGATTTAGGGACCCGACGACCTGAAATCTCCAGGTACAGTTTTGAAGAACTGGCAAGCAGGAAA
 -545  GGAGGTAGGTGTTTAGGGTCCGGTCCTGAAGAGTGTCTTAGGGACAGGTGAAGTCGCCCCCATCACCAGAAGAGAGCAGCAGCAGAAAGAAATGAAGAGGAACTCCAGACATGTATGTCT
 -425  GGGGTTTGCTTCGGTCTTTACTTAGAGTAGGAGTGGTGTGGAACGGGACAGAATCTACATTCGTGACTGGCACCTACCCTTTAGAAAGGAAGAGAAACTCCTCAGGTCTGTCCTCGGAAT
 -305  GACTGAAGATGCTATGAGAGAAATCTGAAGCAATCTTTGAGTTGAGCTTAGAGGGGACTAGAATTGGGGGCGGGAGCGATGACGGGAGAATAAACCACCTATGTGGCGCCCCCCCGCCGC
 -185  TTTTTCGTAGCTGCTGACGCGATGGTGCTGCTTATTAATCATTCATCAGTCCAGAGCCTTTCCACTTAATGGATATGTCGCGCTGGGCTCTAGCCTCTTGACCTCTCCTCTCCTCTTCTT
  -65  TCCACGCTCCAGTGTATCCAGGACCCCCAGAGCTGCAAGAGACAGCGCTCAAGCAAGCAGCCGCCGGGCGTTGCGCCAGACGGCCTCAGTTGGAGCCGC..3615 bp cDNA..TGTTG
 3655  CAAGTTGTCTGGCTTCCATTTGAATCTTCGCTGTTTCAAGGGGTTATTTGAATGCATGGGTATGATTGACATGCTTAGTTTTTAGGAAAATTTCAAGTTAACTTTGAAATTTCACAAGAT
 3775  TGCCTCATCCTTTGAATTGTTTTCTTTTAAATGTGGATCCTGGTGTGCATGTCCTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTACTAGACAAGGAGGG
 3895  AAGAAGGAGGGTAAAGAGGAGGGTAAGGAGGAGGAGGAATAAAAGAAGGAAGCAATAATGTCCCTTGAATGAGTTTGAACAGGAAGCAATGCCCTAGGTTACACTCTGTTCTCACTACAC
 4015  TAACCAGACAATGTCTCTGTTTTTCACAGATTAAAATTACTGGCTGACCTCATTTATGACCTCAGATTTTGCCCTCGAGTTTAGTGACCGCTGCTTTGCATTAGAGACATAGCAATGGGA
 4135  GGGAGAAACATCTCTTAAAGTGTTGGAAGGGAAGTGGAGAAAAGAAATAGAAAAACCAGGTTTTGAGGAGGACACATTTTATGCCTTCCTCAGTTTTGACCATTTCCATGATGGAGGCCA
 4255  TAACAAGGCTTGGTGGAGATCATTTTTTTAAGCAGGTTCTGTGATTTATAGCAGCTTTATTTTCACAGCAAAATGAATGAAAGGAGCATTCCTCAAGCATTTGAGAGCAGTAAGTCTACT
 4375  GTGATAGTGGTTGAAAGTGTCAAAAGCCCTGCAACCCCAGCTTCATGTAGTTATAGACATCAGCTCACAATGACTAAGGCAAGGGATGCTTTTACATTTTCACCATTTCTCAAAGTCGTA
 4495  GCAAGGTAGCAAATGCAAAGTTAAGTTTACAACTTAGATTTGTAACATCCTTTTCTTAGTGCTGCAATTGGGCCCAGTTCCTCTACACATAATTTGTTAATTTAGTCTTAACTACTCTAA
 4615  ACATTCCGAAAACCCTTCAAACTAGTTTTCAAACTGGGAGCAAAGAGACAGTTGAGCTAATAATTTAAATAGCTATTTTTAAAGTTGCTTTTATTTAGGTTTGTACAGTATCACAGCTAG
 4735  CACCTCTCTCTTATAACTAAAATGGCACTAAATGGTGCTTATATGGTGTAGGATGCACTACATACCCTAGTGGGTAAATATGACTGGGTTGAGCCCTTGGGGAAAGTGGCCCTCCAGGTC
 4855  CTTCCCCTTTTGTGAAATGTTTTCCCAGCTGCAGTTGGGCAACATTTGCAATTCTAGAACTGGGAGCCTCCATCCATCTGACAGATCCAGGTCGGGTTGTAGCCCTGGAGTCCGGTGGCT
 4975  CTCACACTGATCACCAGTCCAGTGGATAACACAGACTGCACAGAGTCTGACGCCTGCCATGGCAGCTCCTGGCTCAGTGGCTAGAGGGGACCTAGGATAGCTGGTACTAGATGTGCTCCA
 5095  GCATCACCGTGGTCTGCTCTTGTCTCCCTCTGCCAAAGGCTGTCTCAAATCTTCTGTATTTATCTTTGTTTCTGTCAACCTCACTTACTGAACATTTCCTATTCTCTATAACTGCTTTGT
 5215  ACTAGAGGAAAACCATGAGTTAAATCCTGTTCCTTAGCCCTGGGTCCCATGCAGAAGGAAAGTGTTAAGCTAATTCCTACTCCTTCCCCAACCAGGCTTACACCTGATTCCCAAGAAATT
 5335  TTCATTTCCTAACTATAAACCCTTCTCCTATCCTCAGGTGATAAGCTT
```

Fig. 2. (A) Restriction map of the cloned genomic DNA fragment containing the rat SSTR1 gene. The open box represents the relative position of the previously reported cDNA sequence [3] and the black box indicates the coding region. The fragment from the *Eco*RI to the first *Hin*dIII restriction site downstream of the coding region was sequenced. (B) Nucleotide sequence of the rat SSTR1 gene and flanking regions. The sequence is numbered from the major transcriptional initiation site (+1) which is indicated by an arrow. The putative regulatory elements in the 5′-flanking region are underlined (GCF), doubly underlined (AP-2) and boxed (Pit-1), respectively.

Analysis of the SSTR1 gene sequence reveals the presence of many potential regulatory DNA elements in the 5′-flanking region (Table 1). There are eight putative binding sites for the GC-factor (GCF), three of them in close proximity to the transcriptional initiation site. GCF is a transcriptional repressor that binds to GC-rich sequences or GC boxes that are often found in promoters lacking a TATA motif [21]. In addition, six putative recognition sequences for another transcription factor interacting with GC boxes, AP-2 [22], are observed. Two of these are located within the first 200 bp upstream of the mRNA initiation site. Interestingly, two elements that have the consensus sequence of the binding site for the transcription factor Pit-1 (also called growth hormone factor 1) [23,24], are found at positions −107 and −1,951. Pit-1 is a POU-domain protein, which is

Fig. 3. Primer-extension analysis of the 5'-terminus of the rat SSTR1 mRNA. Poly(A)$^+$ RNA from the GH_3 cell-line (1) and an equal amount of yeast tRNA (2) were hybridized with a ^{32}P-labelled oligonucleotide complementary to part of the rat SSTR1 cDNA sequence and then subjected to reverse transcription. The same oligonucleotide was used in a sequencing reaction with the genomic clone. Synthesized DNA products of the C reaction (C; exposure time 16 h) were run as a marker in parallel with the primer-extended products (exposure time 48 h).

restricted to the adult anterior pituitary and has been implicated in the regulation of the tissue specific expression of both the prolactin and growth hormone genes [25]. The presence of Pit-1 recognition sequences in the rat SSTR1 gene might explain the relatively high levels of SSTR1 mRNA in the pituitary [9] and the pituitary-derived, prolactin- and growth hormone-secreting GH_3 cell-line (Fig. 1). This hypothesis is attractive because it suggests that peptide hormone genes and the gene for a receptor involved in the control of peptide hormone secretion are subject to common transcriptional regulatory mechanisms. However, evidence in support of a functional role for the identified sites must await further experiments.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft (SFB 232/B4 to W.M. and D.R.). Some of the data presented here are part of a thesis by I.W.

References

[1] Epelbaum, J., Dournaud, P., Fodor, M. and Viollet, C. (1994) Crit. Rev. Neurobiol. 8, 25–44.
[2] Bell, G.I. and Reisine, T. (1992) Trends Neurosci. 16, 34–38.
[3] Meyerhof, W., Paust, H.-J., Schönrock, C. and Richter, D. (1991) DNA Cell Biol. 10, 689–694.
[4] Yamada, Y., Post, S.R., Wang, K., Tager, H.S., Bell, G.I. and Seino, S. (1992) Proc. Natl. Acad. Sci. USA 89, 251–255.
[5] Meyerhof, W., Wulfsen, I., Schönrock, C., Fehr, S. and Richter, D. (1992) Proc. Natl. Acad. Sci. USA 89, 10267–10271.
[6] O'Carrol, A.-M., Lolait, S.J., König, M. and Mahan, L.C. (1992) Mol. Pharmacol. 42, 939–946.
[7] Bruno, J.F., Xu, Y., Song, J. and Berelowitz, M. (1992) Proc. Natl. Acad. Sci. USA 89, 11151–11155.
[8] Raynor, K., Wang, H.-L., Dichter, M. and Reisine, T. (1991) Mol. Pharmacol. 40, 248–253.
[9] Bruno, J.F., Xu, Y., Song, J. and Berelowitz, M. (1993) Endocrinology 133, 2561–2567.
[10] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, NY).
[11] Sanger, F.S., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[12] Wulfsen, I., Meyerhof, W., Fehr, S. and Richter, D. (1993) J. Neurochem. 61, 1549–1552.
[13] Dynan, W.S. (1986) Trends Genet. 2, 196–197.
[14] Bird, A. (1986) Nature 321, 209–213.
[15] Patel, I., Framson, P.E., Caskey, C.T. and Chinault, A.C. (1986) Mol. Cell Biol. 6, 393–403.
[16] Chen, M., Shimada, T., Moulton, A.D., Cline, A., Humphries, R.K., Maizel, J. and Nienhuis, A.W. (1984) J. Biol. Chem. 259, 3933–3943.
[17] Singer-Sam, H., Keith, D.H., Tani, K., Simmer, R.L., Shively, L., Lindsay, S., Yoshida, A. and Riggs, A.D. (1984) Gene 32, 409–417.
[18] Ishii, S., Xu, Y.-H., Stratton, R.H., Roe, B.A. and Merlino, G.T. (1985) Proc. Natl. Acad. Sci. USA 82, 4920–4924.
[19] Ishii, S., Merlino, G.T. and Pastan, I. (1985) Science 230, 1378–1380.
[20] Ding, D., Toth, M., Zhou, Y., Parks, C., Hoffman, B. and Shenk, T. (1993) Mol. Brain Res. 20, 181–191.
[21] Kageyama, R. and Pastan, I. (1989) Cell 59, 815–825.
[22] Williams, T. and Tjian, R. (1991) Genes Dev. 5, 670–682.
[23] Bodner, M., Castrillo, J.-L., Theill, L.E., Deerinck, T., Ellisman, M. and Karin, M. (1988) Cell 55, 505–518.
[24] Schaufele, F., West, B.L. and Reudelhuber, T. (1990) Nucleic Acids Res. 18, 5235–5243.
[25] Mangalam, H.J., Albert, V.R., Ingraham, H.A., Kapiloff, M., Wilson, L., Nelson, C., Elsholtz, H. and Rosenfeld, M.G. (1989) Genes Dev. 3, 946–958.
[26] Faisst, S. and Meyer, S. (1992) Nucleic Acids Res. 20, 3–26.