A transposon-like DNA fragment interrupts a *Physarum polycephalum* histone H4 gene

Marcelle L. Wilhelm and François-Xavier Wilhelm*

Laboratoire de Biophysique, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue René Descartes, 67084 Strasbourg Cédex, France

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A recombinant DNA library was screened for histone H4 genes using a sea urchin probe. One recombinant was analysed by restriction enzyme mapping and Southern blotting. The complete DNA sequence of the H4 histone locus was determined. An 86 base pair interrupting sequence was found within the histone H4 coding sequence. The inserted DNA fragment has some characteristics of a transposable element.

Histone gene *Interrupting sequence* *Transposon* Physarum polycephalum

1. INTRODUCTION

The structural organization of histone genes from a variety of eukaryotes has been well investigated (review [1,2]). In most of the species the 5 histone genes are clustered and repeated. In eukaryotes such as sea urchin or *Drosophila melanogaster* [3,4] the histone clusters are tandemly duplicated whereas in *Xenopus laevis*, the newt *Notophtalmus*, chicken, mouse or man the histone clusters are separated by spacer regions [5–8]. The multiplicity of the histone genes varies considerably among the different species; it is as low as 2 copies in the yeast *Saccharomyces cerevisiae* and can reach several hundred copies (600 copies/hybrid genome) in sea urchin. The two copies of core histone genes of *S. cerevisiae* have now been sequenced [10–12] but the only other lower eukaryotes whose histone gene organization has been examined are the ciliate *Stylonychia mytilus* [13] and the ascomycete *Neurospora crassa* [15].

We report here the cloning and sequencing of a *Physarum polycephalum* histone H4 gene. DNA sequence analysis was used to identify the histone gene in recombinant clones. Surprisingly, we find that the coding sequence is interrupted by a short DNA sequence. In most of the histone gene studies up to now, electron microscopic observation, DNA sequencing of the protein coding region, and S_1 nuclease mapping have shown a lack of introns in the histone gene family. However, authors in [14] have discovered one chicken histone H3 gene which contains two intervening DNA sequences. Similarly, authors in [15] showed that the histone H3 and H4 genes of *Neurospora* were interrupted by intervening sequences. An unusual H1 pseudogene containing two-thirds of the coding sequence and at least 1800 base pairs (bp) of apparently non-coding sequence in place of the 5′-coding region has also been isolated from *X. laevis* [16]. Moreover, authors in [17] established that a cloned sea urchin H2B gene was interrupted by a transposable element. The interrupting sequence identified in the *P. polycephalum* H4 gene is not bounded by the consensus (5′GT, 3′AG) intron-exon splice junction, but has some characteristics of a transposable element with a terminal direct repeat at the junction of the inserted element and internal inverted repeats.

* To whom correspondence should be addressed

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2. MATERIALS AND METHODS

2.1. *Strains*

The source of the *P. polycephalum* DNA for cloning was strain M3C VIII [18]. The lambda cloning vector has been described in [19]. The strains used to prepare the phage packaging mixes were BHB 2688 and BHB 2690 [20]. The host for growing phage M13 was JM 103 [21], and the host for λ WES was strain 1046 [22].

2.2. Physarum polycephalum *library construction*

Physarum DNA prepared as in [23] was digested to completion with *Eco*RI. The λ gt WES arms were prepared by digesting the phage DNA with *Eco*RI and removing the λ B fragment by sucrose gradient sedimentation. The *Physarum* DNA was then ligated to the λ gt WES arms using T4 DNA ligase and a 3-fold excess of *Physarum* DNA fragments relative to phage DNA. Recombinant DNA molecules were packaged in vitro as in [20] and recovered as plaques on bacterial strain 1046.

2.3. *Screening the library and hybridization*

The recombinant phage plaques were screened by in situ plaque hybridization as in [24]. The filter blots were hybridized with the radioactively labeled DNA probe, and the plaques that hybridized with the probe were detected by autoradiography. Putative histone gene clones were plaque purified and screened again by hybridization. The plaque purification was repeated twice. The histone probe was a kind gift from Dr M. Birnstiel and contains the region coding for sea urchin histone H4 between amino acid codon 3 and amino acid codon 94. It was prepared by digesting plasmid pHae 181 with *Eco*RI and subsequently by purifying the small *Eco*RI fragment.

Radioactively labeled DNA was prepared by nick translation. Relatively high stringency conditions were used for hybridization. Filters were incubated for 16–18 h at 65–68°C in 3 × SET, 10 × Denhardt solution, 0.5 M NaCl, 0.1% SDS, 50 μg/ml heat-denatured *E. coli* DNA and 10^6–10^7 cpm denatured labeled DNA/ml [20 × SET: 3 M NaCl, 0.6 M Tris–HCl (pH 7.6), 0.04 M EDTA; 50 × Denhardt solution: 1% Ficoll 400, 1% PVP 40, 1% BSA]. Following hybridization the filters were washed 4 times for 1–2 h each in solutions of decreasing ionic strength (0.5 M NaCl, 3 × SET, 0.1% SDS, 0.1% $NaPP_i$, and 5 × Denhardt solution; 3 × SET, 0.1% SDS, and 0.1% $NaPP_i$; 2 × SET, 0.1% SDS, and 0.1% $NaPP_i$; 1 × SET, 0.1% SDS, and 0.1% $NaPP_i$).

2.4. *Enzymes*

The restriction enzymes and T4 ligase were obtained from Boehringer. Nick translation was performed using a nick translation kit from Bethesda Research Laboratories. All the enzymes were used according to the manufacturer's instructions.

3. RESULTS AND DISCUSSION

3.1. *Isolation and characterization of a histone H4 genomic clone*

The evolutionary conservation of histone H4 suggests that the coding region of sea urchin and *Physarum* histone genes might cross-hybridize. *Physarum* DNA was digested with *Eco*RI and cloned in bacteriophage λ gt WES λ B. The *Physarum* genomic DNA library was screened for phages containing the H4 gene by the plaque hybridization procedure in [24] using radiolabeled sea urchin H4 DNA. After 3 rounds of screening of approx. 20000 primary recombinant plaques, one phage recombinant designated λφH 12 was detected. The recombinant phage λφH 12 carries an *Eco*RI restriction fragment of 6.6 kbp. This fragment corresponds to one of the bands observed in a genomic blot obtained after digestion of total *Physarum* DNA with *Eco*RI. The 6.6 kbp fragment was excised from the recombinant DNA by *Eco*RI digestion, and its restriction map determined by the use of single and multiple enzyme digestion. The structure and physical map of the *Physarum* fragment containing the histone H4 gene is presented in fig.1. To facilitate further

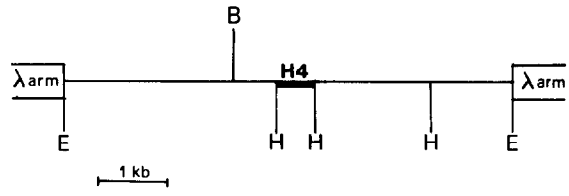

Fig.1. Restriction map of λφH 12 with restriction sites of *Hin*dIII (H), *Bam*HI (B) and *Eco*RI (E) indicated. There are no sites recognized by *Sal*I, *Xho*I, *Hpa*I, or *Pvu*I. The position of the H4 gene is shown.

work, the 2.5 kbp and 4.1 kbp *Bam*HI fragments were transferred to the bacterial plasmid pBR322 by ligation into the *Bam*HI-*Eco*RI site of the vector (subclones pφH 125 and pφH 121). The location of *Physarum* DNA sequences which hybridize to the sea urchin H4 histone gene probe was determined by blot hybridization [25]. *Hin*dIII and *Bam*HI restriction digests of the 6.6 kbp insert were separated by electrophoresis in agarose gels and transferred to nitrocellulose filters. The blots were then hybridized with ^{32}P-labeled nick-translated sea urchin H4 DNA.

The autoradiographs showed that the 4.1 kbp *Bam*HI fragment and the 0.6 kbp *Hin*dIII fragment hybridize with the probe. In a control experiment we made a genomic blot after digestion of total *Physarum* DNA with *Hin*dIII, and found that one of the bands which hybridizes with the histone probe has a size of 0.6 kbp. The DNA sequence of the 0.6 kbp *Hin*dIII fragment was then determined by the chain termination method [26] after subcloning in the M 13 mp 10 phage vector. The nucleotide sequence of the *Physarum* H4 gene locus is shown in fig.2. The sequence was determined twice and nearly 50% of the sequence was confirmed by sequencing both strands. The most striking feature of the result presented in fig.2 is that the H4 coding region is interrupted at amino acid 46 by an 86 bp sequence.

3.2. *Histone H4 coding region*

The amino acid sequence of *Physarum* H4 deduced from the DNA nucleotide sequence differs slightly from other published H4 histones. Compared to the prototype sequence of calf thymus histone H4 there are 4 amino acid substitutions between the two sequences at position 48 (Asn⟷Gly), 49 (Thr⟷Leu), 60 (Thr⟷Val) and 77 (Arg⟷Lys). Authors in [27] have determined a partial amino acid sequence of *Physarum* H4 histone and confirm that there is an Arg⟷Lys exchange at position 77. The same

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AAGGACGAAAAAAAAGGACCAGAGAATGTATGGCCGAAAAAGTTAGAAGAAAGCAGGGTTTATGCGATTTTGACTTAGGCCCATGTCACGAGCCCAGCGGCA

AGAGCGTTTAAGATGGCCCGACGTGCGATGTCCGTTGCTCAACATCACATGCACTACACAGACA  ATG TCT GGA CGT GGT AAA GGA GGC AAG
                                                                   atg ser gly arg gly lys gly gly lys
                                                                        1   2   3   4   5   6   7   8

GGA CTC GGC AAG GGA GGC GCC AAG AGG CAC AGG AAG GTG CTC CGT GAT AAC ATC CAG GGT ATT ACC AAG CCT GCT ATC
gly leu gly lys gly gly ala lys arg his arg lys val leu arg asp asn ile gln gly ile thr lys pro ala ile
 9  10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25  26  27  28  29  30  31  32  33  34

CGC AGA TTG GCT CGC CGT GGT GGT GTG AAG CGT ATC TTTCCTTACCTTGCAAATTCATTGCTTTGTCTATTTCGTTTATGTGCGTCCAAAGCAAT
arg arg leu ala arg arg gly gly val lys arg ile
35  36  37  38  39  40  41  42  43  44  45  46
                                gly leu                                                   val
 TTAGTTCCTTTGATGAAACGAAGTATC AGC AAC ACC ATC TAC GAG GAG ACC CGT GGA GTC CTG AAG ACC TTC TTG GAG AAC GTC
                             ser asn thr ile tyr glu glu thr arg gly val leu lys thr phe leu glu asn val
                              47  48  49  50  51  52  53  54  55  56  57  58  59  60  61  62  63  64  65
                                                  lys
ATC CGT GAC GCT GTG ACC TAC ACT GAG CAT GCC CGC CGC AAG ACA GTG ACT GCC ATG GAC GTT GTC TAT GCC CTC AAA
ile arg asp ala val thr tyr thr glu his ala arg arg lys thr val thr ala met asp val val tyr ala leu lys
66  67  68  69  70  71  72  73  74  75  76  77  78  79  80  81  82  83  84  85  86  87  88  89  90  91

CGC CAG GGA CGC ACT CTG TAC GGA TTC GGC GGC TAA TTACCTTTGCCCCCCCGA
arg gln gly arg thr leu tyr gly phe gly gly term
92  93  94  95  96  97  98  99 100 101 102
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Fig.2. Nucleotide sequence of the *Physarum* histone H4 locus. The amino acid sequence specified by the coding region is shown under the nucleotide sequence. Amino acid residues of calf thymus H4 which differ from those of *Physarum* are given above the DNA sequence. In the 5′-flanking region the putative TATA and CAP boxes as well as the GATCC-like sequence are underlined. In the interrupting sequence the inverted repeated sequences are indicated by arrows.

conservative substitution at position 77 also occurs in pea and wheat [28,29]. In agreement with our results, authors in [27] have found that the sequence from residue 46 to 59 contains two variations from calf thymus H4. Gly and Leu in position 48 and 49 are replaced by Asx and Thr, but the position of Asx and Thr was not assigned. The DNA sequence shows that Asn is found in position 48 and Thr in position 49. The effect of the 3 non-conservative substitutions at positions 48, 49 and 60 on the protein structure is not known, but it should not greatly affect the properties of the protein. Somewhat similar non-conservative substitutions occur in the H4 histone of yeast [12] where Ala in positions 69 and 83 and Val in position 60 are replaced by Ser, and Thr in position 54 is replaced by Val. The patterns of amino acid exchange between the H4 histones of different eukaryotes show that the replacements occur largely in the carboxy-terminal half of the protein. In the case of *Physarum* H4, the 4 replacements occur indeed after residue 48, and there are no amino acid differences in the N-terminal region.

3.3. *Codon usage*

In prokaryote and eukaryote gene coding sequences synonymous codons are not used randomly, a result also found for the H4 histone gene of *Physarum* (table 1). The biased codon usage is particularly striking for the triplets AUC which codes for Ile and AAG which codes for Lys. Authors in [30] have studied the pattern of codon recognition by tRNA isoacceptors of *Physarum* and have found that several different amino aminoacyl tRNAs responded poorly to codons with G in the 3′-position (e.g., ala GCG, arg CGG, gly GGG, ser UCG) and that the most abundant glutamine isoacceptor recognized CAG. This result is in agreement with the pattern of codon usage found in the H4 histone gene where the codons UCG, CGG, GCG and GGG are never used. The codon usage for several other H4 genes is also given in table 1 to permit interspecies comparison. The pattern of codon usage of *Physarum* is more similar to those observed in *Xenopus*, chicken and man than to those in wheat or yeast. As in mammalian genes the use of A in the third position is reduced to half that of U; 64% of the codons end with C or G compared to a GC content of 58% for the H4 gene. Thus there is a slight overuse of G and C at the third position, but it is not as pronounced as in *Xenopus*, chicken or man [31,32]. In eukaryotes, the dinucleotide GpC is used preferentially to CpG [33], and this known tendency is also observed in

Table 1

Codon usage in the *Physarum* histone H4 gene in comparison with those of other species sequenced

		1	2	3	4	5	6	7^1	7^2	8			1	2	3	4	5	6	7^1	7^2	8			1	2	3	4	5	6	7^1	7^2	8			1	2	3	4	5	6	7^1	7^2	8
Phe	UUU										Ser	UCU	1	1	2	1	1		3	3	1	Tyr	UAU	1			1	1	1		1	2	1	Cys	UGU									
	UUC	2	2	2	2	2	2	2	2	2		UCC	1					1	3	3			UAC	3	4	3	3	3	4	3	2	3		UGC					1	1			
Leu	UUA							2	2			UCA					1	1					UAA											UGA									
	UUG	1			2	1		5	5	2		UCG		1				1					UAG										Trp	UGG									
Leu	CUU	2	1	1	1						Pro	CCU			1	1	1				1	His	CAU	1			1	1		2	2	1	Arg	CGU	1		1	4	4		2	2	6
	CUC	3	5	4	2	2	6			3		CCC											CAC	2	2	2	1	1	2			1		CGC	10	11	6	2	3	10			6
	CUA			2	1	2	1	2	2			CCA	1						1	1		Gln	CAA				1	1		2	2			CGA	1			4	3				
	CUG	2	2	1	2	2	1			2		CCG	1					1					CAG	1	2	2	1	1	2			2		CGG	2	1	3			5			
Ile	AUU	1	1	1				3	3	1	Thr	ACU	1		2	1	1		4	5	3	Asn	AAU	1	1	1	1	1					Ser	AGU									
	AUC	5	5	5	6	6	7	4	4	5		ACC	4	4	5	2	2	7	2	1	5		AAC	1	1	1	1	1	3	1	1	3		AGC									1
	AUA											ACA	1			3	3				1	Lys	AAA	4	1	3	2	2		4	4	2	Arg	AGA		1	1	1	1		12	11	1
Met	AUG	1	1	1	1	1	1			1		ACG	1	3									AAG	7	10	8	9	9	9	7	7	8		AGG		1	3	3	3		0	1	2
Val	GUU			2	1	1		4	4	1	Ala	GCU	1		2	1	2		4	3	3	Asp	GAU	1		1	1	2	2	2	2	1	Gly	GGU	3	2		6	7		16	16	4
	GUC	2	6	4	5	5	6	4	4	4		GCC	4	5	4	3	2	4	2	3	4		GAC	2	3	2	2	1	1	1	1	2		GGC	12	10	7	5	4	14			5
	GUA	3			1	1						GCA			1	3	3					Glu	GAA			1	1	1		4	4			GGA		1	7	6	6	1			7
	GUG	4	3	3	2	2	2			3		GCG	1	2				3					GAG	4	4	3	3	3	4			4		GGG	2	4	3			2			

(1) Man [32]; (2) chicken [34]; (3) *Xenopus* [31]; (4) sea urchin, *P. miliaris* [35]; (5) sea urchin, *S. purpuratus* [36]; (6) wheat [29]; (7^1 and 7^2) Copy I and II from yeast [12]; (8) *Physarum*

Physarum. We find, for example, that CpG is never used in position 2–3 in the codons of *Physarum* H4 whereas GpC is used 12 times. The same result was also found for *Xenopus* histone genes, but the bias against such use of CpG in the codons is not understood.

3.4. *5′-Flanking region of the H4 gene*

The sequences with a putative regulatory function for histone genes have been described in detail [1]. In the 5′-flanking sequence of the H4 gene we find a TATA box-like sequence, 5′TTTAAGA3′, 51 base pairs upstream from the ATG initiation codon (with the A of ATG as +1). A similar putative TATA box has been reported for the wheat H4 gene [29]. Thirteen nucleotides upstream from the TATA sequence we find a sequence 5′GAGCC3′ similar to the consensus sequence, 5′GATCC3′, characteristic of histone gene loci but not necessarily of other eukaryotic genes. A potential cap box cannot be clearly assigned, but two regions are in reasonable agreement with the consensus cap site, 5′CATTC3′. Thus, 17 and 12 nucleotides upstream from the initiation codon we find the sequences 5′CATCA3′ and 5′CATGC3′, respectively. Further experiments will be necessary to identify accurately the transcriptional initiation site of this gene.

3.5. *Interrupting sequence*

The coding sequence of the *Physarum* H4 gene cloned in λφH 12 is interrupted at amino acid 46 by an 86 bp sequence. This sequence is not bounded by the consensus (5′GT, 3′AG) intron-exon splice junction. As a transposable element was found recently within a histone H2B pseudogene in the sea urchin *Strongylocentrotus purpuratus* [17], we have examined whether the DNA segment interrupting the *Physarum* H4 gene could also have some characteristics of a transposable element. When a transposable element is inserted into the genome, direct repeats of the recipient DNA sequence flank the transposon. The nucleotide sequence at the junction of the inserted 86 bp element shows that a 5 bp segment, 5′GTATC3′, of the H4 sequence is duplicated as a direct repeat resulting in duplication of codons corresponding to amino acids 45 and 46. The inserted segment has 3 inverted repeat sequences: 5′TTTC3′, 5′CAAA3′ and 5′ATTGCTTTG3′. Fig.3 shows

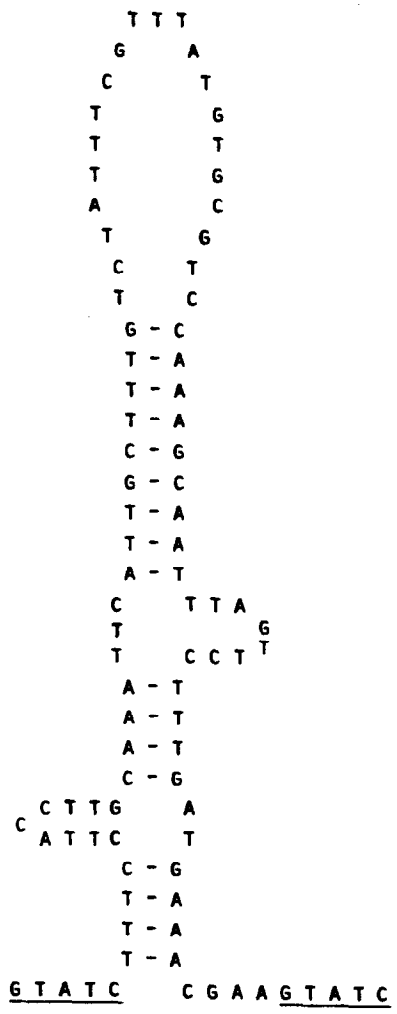

Fig.3. Theoretical secondary structure formed by one strand of the interrupting DNA segment after it has been allowed to fold back.

the configuration of a theoretical snap-back structure illustrating the presence of the inverted sequences. Thus, the DNA sequence interrupting the H4 gene has several characteristics of a transposable element, but other data will be needed to determine whether it is a transposon and whether it plays a role in the expression of the gene. Indeed, the discovery of interrupted histone genes poses the question of whether such genes are expressed or whether they are transcriptionally inactive pseudogenes. In the case of the H4 gene of *Physarum* described here, the DNA sequence predicts a polypeptide sequence very similar to that

of other H4 histones and DNA sequences playing a regulatory role are found in the 5'-flanking region of the gene. Therefore, it is not unreasonable to believe that this gene could be expressed. Experiments are now in progress to characterize other *Physarum* histone genes to determine whether they also contain such a transposon-like DNA fragment.

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REFERENCES

[1] Hentschel, C.C. and Birnstiel, M.L. (1981) Cell 25, 301–313.
[2] Kedes, L.H. (1979) Annu. Rev. Biochem. 48, 837–870.
[3] Gross, K.W., Schaffner, W., Telford, J. and Birnstiel, M.L. (1976) Cell 8, 471–478.
[4] Lifton, R.P., Goldberg, M.L., Karp, R.W. and Hogness, D.S. (1977) Cold Spring Harbor Symp. Quant. Biol. 42, 1047–1056.
[5] Van Dongen, W., De Laaf, L., Zaal, R., Moorman, A. and Destrée, O. (1981) Nucleic Acids Res. 9, 2297–2311.
[6] Stephenson, E.C., Erba, H.P. and Gall, J.G. (1981) Cell 24, 639–647.
[7] Crawford, R.J., Krieg, P., Harvey, R.P., Hewish, D.A. and Wells, J.R.E. (1979) Nature 279, 132–136.
[8] Sittman, D.B., Chiu, I.M., Pan, C.J., Cohn, R.H., Kedes, L.H. and Marzluff, W.F. (1981) Proc. Natl. Acad. Sci. USA 78, 4078–4082.
[9] Heintz, N., Zernik, M. and Roeder, R.G. (1981) Cell 24, 661–668.
[10] Wallis, J.W., Hereford, L. and Grunstein, M. (1980) Cell 22, 799–805.
[11] Choe, J., Kolodrubetz, D. and Grunstein, M. (1982) Proc. Natl. Acad. Sci. USA 79, 1484–1487.
[12] Smith, M.M. and Andresson, O.S. (1983) J. Mol. Biol. 169, 663–690.
[13] Elsevier, S.M., Lipps, H.S. and Steinbrück, G. (1979) Chromosoma 69, 291–306.
[14] Engel, J.D., Sugarman, B.J. and Dodgson, J.B. (1982) Nature 297, 434–436.
[15] Woudt, L.P., Pastink, A., Kempers-Veenstra, A.E., Jansen, A.E.M., Mager, W.H. and Planta, R.J. (1983) Nucleic Acids Res. 11, 5347–5360.
[16] Turner, P.C., Aldridge, T.C., Woodland, H.R. and Old, R.W. (1983) Nucleic Acids Res. 11, 4093–4107.
[17] Liebermann, D., Hoffman-Liebermann, B., Weinthal, J., Childs, G., Maxson, R., Mauron, A., Cohen, S.N. and Kedes, L. (1983) Nature 306, 342–347.
[18] Daniel, J.W. and Rusch, H.P. (1961) J. Gen. Microbiol. 25, 47–59.
[19] Leder, P., Tiemeier, D. and Enquist, L. (1977) Science 196, 175–176.
[20] Hohn, B. (1979) Methods Enzymol. 68, 299–309.
[21] Messing, J.R., Crea, R. and Seeburg, P.H. (1981) Nucleic Acids Res. 9, 309–321.
[22] Cami, B. and Kourilsky, P. (1978) Nucleic Acids Res. 5, 2381–2390.
[23] Wilhelm, M.L., Wilhelm, F.X., Toublan, B. and Jalouzot, R. (1982) FEBS Lett. 150, 439–444.
[24] Benton, D.W. and Davis, R.W. (1977) Science 196, 180–182.
[25] Southern, E.M. (1975) J. Mol. Biol. 98, 503–515.
[26] Sanger, F., Coulson, A.R., Barrell, B.G., Smith, A.J.H. and Roe, B.A. (1980) J. Mol. Biol. 143, 161–178.
[27] Waterborg, J.H., Fried, S.R. and Matthews, H.R. (1983) Eur. J. Biochem. 136, 245–252.
[28] De Lange, R.J., Fambrough, D.M., Smith, E.L. and Bonner, J. (1969) J. Biol. Chem. 244, 5669–5679.
[29] Tabata, T., Sasaki, K. and Iwabuchi, M. (1983) Nucleic Acids Res. 11, 5865–5875.
[30] Hatfield, D., Rice, M., Hession, C.A. and Melera, P.W. (1982) J. Bacteriol. 151, 1013–1021.
[31] Moorman, A.F.M., De Boer, P.A.J., De Laaf, R.T.M., Van Dongen, W.M.A.M. and Destrée, O.H.J. (1981) FEBS Lett. 136, 45–52.
[32] Zhong, R., Roeder, R.G. and Heintz, N. (1983) Nucleic Acids Res. 11, 7410–7425.
[33] Grantham, R. (1980) FEBS Lett. 121, 193–199.
[34] Sugarman, B.J., Dodgson, J.B. and Engel, J.D. (1983) J. Biol. Chem. 258, 9005–9016.
[35] Busslinger, M., Portman, R., Irminger, J.C. and Birnstiel, M.L. (1980) Nucleic Acids Res. 8, 957–977.
[36] Grunstein, M., Diamond, K.E., Knoppel, E. and Grunstein, J.E. (1981) Biochemistry 20, 1216–1223.