Chromosomal localization of the mouse genes coding for α2, α3, α4 and β2 subunits of neuronal nicotinic acetylcholine receptor

Alain Bessis[1], Dominique Simon-Chazottes[2], Anne Devillers-Thiéry[1], Jean-Louis Guénet[2] and Jean-Pierre Changeux[1]

[1]*UA CNRS D1284, Neurobiologie Moléculaire, Département des Biotechnologies, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cédex 15*, and [2]*Unité de Génétique des Mammifères, Département d'Immunologie, Institut Pasteur, Paris, France*

Received 5 March 1990

The chromosomal localization of four neuronal nicotinic acetylcholine receptor subunit genes was performed by following the mendelian segregation of their corresponding alleles in backcrosses involving the mouse species *Mus spretus* and the laboratory strains C57BL/6 or BALB/c. A similar analysis previously performed with muscle nicotinic acetylcholine receptor subunits revealed that the genes coding for the α and β subunits are respectively located on chromosome 2 and 11, whereas the γ and δ subunit coding genes are linked and located on mouse chromosome 1. In this study, we show that the genes coding for the neuronal nicotinic acetylcholine receptor α2, α3 and β2 subunits are dispersed on three different mouse chromosomes, *viz.* 14, 9 and 3 respectively. Moreover, the α4 subunit gene is located on chromosome 2 but is not genetically linked to the α1 subunit gene.

Chromosomal localization; Nicotinic receptor; Mouse interspecies cross; Restriction lenght fragment polymorphism

1. INTRODUCTION

The nicotinic acetylcholine receptor (nAchR) from vertebrate neuromuscular junction and from *Torpedo* electric organ is composed of four homologous transmembrane subunits (α, β, γ and δ, the γ subunit being replaced in some species by an ε subunit in the adult). Each subunit is encoded by a single copy gene (reviewed in [1,2]) and assembled in an $\alpha_2\beta\gamma\delta$ oligomer (reviewed in [3]). The high level of sequence identities and gene structural homologies suggest the notion that the five muscle subunit coding genes form a multigene family. The acetylcholine binding sites are carried, at least in part, by the two α-subunits [4,5] and have been defined at the amino acid level [4,6]. In particular, a cysteine doublet is involved in the acetylcholine binding site [7] and is conserved in the muscle α subunit throughout evolution [8–12].

The occurrence of nAchR in the central nervous system has been demonstrated using several different techniques, such as electrophysiology [13] (references in [14]), autoradiography with radiolabeled ligands [15–17], immunocytochemistry with monoclonal antibodies [18] (references in [19]) and cerebral glucose utilization [20]. Recently, molecular cloning of genes and cDNAs has revealed at least seven homologous subunits of nAchRs from goldfish [21], chick [12] and rat [22–27]. The genes encoding these subunits are all members of the nAchR gene family and are expressed in the nervous system. DNA sequence analysis shows that four of these subunits display the cysteine doublet homologous to *Torpedo* α subunit cysteines 192–193 [8,9]. These four subunits were therefore termed α2 [12,24], α3 [12,22], α4 [12,23], α5 [28] in rat and chick using this structural basis. The three other neuronal subunits were called β2 in rat [25] or nα in chick [12], β3 in rat [26] or Gfnα2 in goldfish [21] and nα2 in rat [27] (nα2 has also been termed β4 in rat [29]).

In situ hybridization experiments using specific probes have demonstrated differential expression of these seven genes within the rat central nervous system [26,28,29,30,31]. Such different expression must therefore be under the control of fine regulatory mechanisms to assure cell-specific and coordinate expression of specific subunits to build functional receptors in cholinoceptive nuclei. In this study, we have analysed the putative genetic linkage between the members of this family by localizing the genes coding for the α2, α3, α4 and β2 nAchR subunits on mouse chromosomes.

Based on the established rules for nomenclature [32], and following the pattern adopted for the muscle subunit genes, we have used the following nomenclature: α2, α3 and α4 subunits genes are termed Acra-2, Acra-3, and Acra-4 respectively. The β2 subunit gene is termed Acrb-2.

Correspondence address: A. Bessis, UA CNRS D1284, Neurobiologie Moléculaire, Département des Biotechnologies, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cédex 15, France

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

2. MATERIALS AND METHODS

2.1. *Mice*

Female of the laboratory inbred strain C57BL/6 or BALB/c were mated to male of a moderately inbred strain of *Mus spretus* (SPE/Pas). The F1 female offspring were then backcrossed with C57BL/6 or BALB/c male to produce backcross progeny of 75 individuals. These were subsequently analyzed for the segregation of 106 biochemical, coat color or genetic markers already localized on the mouse genetic map, as well as for the neuronal nAchRs subunit genes using RFLP analysis.

2.2. *Probe labelling*

Probes were made using plasmids HYP16(9) [24], PCA48E(3) [22], HYA23-1E(1) [23] and PCX49(1) [25] for the detection of the $\alpha 2$, $\alpha 3$, $\alpha 4$ and $\beta 2$ subunit genes respectively. All probes were made using the whole cDNA inserts as template. Specific DNA probes were synthesized at a specific activity of 3×10^9 cpm/μg using the Amersham Multiprime labelling kit.

2.3. *Southern blotting*

High molecular weight spleen DNA (5 μg) was digested with an appropriate enzyme and size fractionated on 0.7% agarose gel. DNA was transferred onto nylon membranes according to the technique of Southern [58]. Membranes were prehybridized and hybridized (5×10^6 cpm/ml) at 42°C in 50% formamide, 5 × SSC (1 × SSC = 0.15 M NaCl + 15 mM sodium citrate), 0.05% blotto, 0.5% SDS, 0.1 g·l^{-1} denatured salmon sperm DNA, and washed at 65°C in 2 × SSC. After hybridization and washing, filters were exposed 3–9 days to Kodak XAR-5 films.

3. RESULTS

3.1. *Acra-2, Acra-3, Acra-4 and Acrb-2 loci are not linked*

We have used the entire cDNA insert (cDNA probes were kindly provided by J. Boulter, J. Patrick and S. Heinemann, Salk Institute, San Diego, USA) corresponding to each gene as a specific probe to detect RFLPs between *Mus spretus* and the laboratory strains C57BL/6 or BALB/c. The $\alpha 2$ probe detects a 4.5 kb fragment in *Mus spretus* DNA and a 4 kb fragment in C57BL/6 or BALB/c DNA when both genomic DNA were digested with the restriction enzyme *Taq*I. This enzyme also generates an RFLP in the locus detected by the $\alpha 3$ probe. The $\alpha 4$ probe detects an RFLP when DNA preparations were digested with the restriction enzyme *Sac*I, revealing a 2.6 kb band with C57BL/6 or BALB/c genomic DNA and a 5.5 kb band in *Mus spretus* DNA (Fig. 1a). Finally, we found an RFLP for the $\beta 2$ subunit gene by digesting mice genomic DNAs with the restriction enzyme *Bgl*I (Fig. 1b).

The RFLPs detected between parental mouse species were used to follow the mendelian segregation of the four genes in the progeny (75 mice) of the backcrosses (C57BL/6 × *M. spretus*) × C57BL/6 or (BALB/c × *M. spretus*) × BALB/c. DNA preparations from the offspring were digested with the enzymes *Taq*I, *Sac*I, or *Bgl*I, transferred to nylon membranes (Hybond N, Amersham) and hybridized with the appropriate probe. Each animal was typed + or − according to whether the DNA fragment characteristic of *Mus spretus* species was present or not (i.e. whether the animal was heterozygote or homozygote for the studied gene). The +/− distribution panel was then matched to that of the already existing panel and linkage with other loci was detected (references in [33]). Table I shows that there is no significant cosegregation among the four neuronal nAchR genes, demonstrating the lack of genetic linkage between them.

3.2. *Chromosomal localization of the four neuronal nAchR genes*

In order to map precisely the chromosomal loci of these four genes, we have analyzed the cosegregation of each gene with 106 autosomal marker genes already

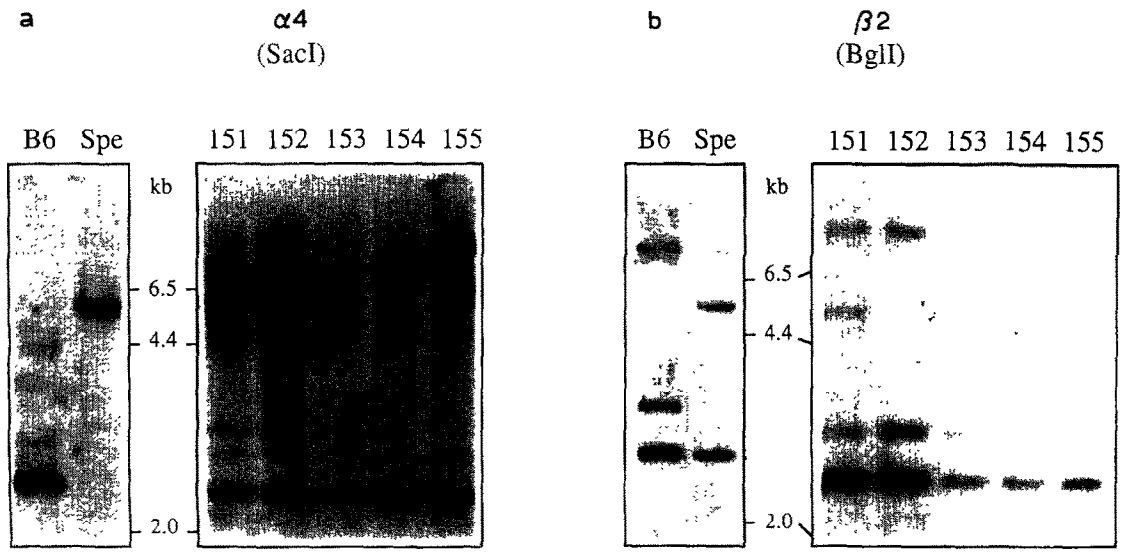

Fig. 1. Southern blots of (a) *Sac*I digests ($\alpha 4$ probe) or (b) *Bgl*I digests ($\beta 2$ probes) of DNA from C57BL/6 or BALB/c (B6), *Mus spretus* (Spe), and of 5 of the 75 offsprings of the backcrosses (C57BL/6 × *M. spretus*) × C57BL/6 or (BALB/c × *M. spretus*) × BALB/c. None of the probes gave cross-hybridization with heterologous subunit restriction fragments.

Table I

Analysis of the cosegregation of the four neuronal nAchR subunit genes in the backcross (C57BL/6 × *M. spretus*) × C57BL/6 or (BALB/c × *M. spretus*) × BALB/c

	Acra-2	*Acra-3*	*Acra-4*	*Acrb-2*
Acra-2		59	62	56
Acra-3	23/39		51	44
Acra-4	28/35	20/39		51
Acrb-2	20/34	14/32	26/51	

Results are shown, in the lower left part of the matrix, as cosegregant per total number of animals analyzed and, in upper right part, as percentages of cosegregants

assigned to a mouse chromosome. Such a procedure detects linkage in 80% of the cases. Furthermore, recombination events between the nAchR subunits genes and the chromosomic markers allowed us to map the genes relatively to the other known loci, and also to determine genetic distances.

As shown in Table IIa, we observed a high degree of cosegregation between *Acra-2* locus and a number of loci localized on chromosome 14. Specifically, we observed 100% cosegregation with the *Ctla-1* locus (cytotoxic T-lymphocyte-associated protein-1), shown to be located on chromosome 14 by in situ hybridization [34]. We observed 98% cosegregation with *Gdh-x* and *Np-1* loci (respectively glutamate-deshydrogenase human X and nucleoside phosphorylase). The latter two loci have been located on chromosome 14 following their mendelian segregation [35,36]. The most likely order is: *Np-1–Acra-2/Ctla1–Gdh-x* in accordance to [37]. The genetic distances between *Acra-2/Ctla-1* and *Np-1*, and *Acra-2/Ctla-1* and *Gdh-x* loci are the same as already reported, i.e., 2.2 ± 2.2 cM.

Similar analysis on 43 progeny (see Table IIb) allowed us to map the Acra-3 gene to mouse chromosome 9, close to the *Es-14* locus (esterase 14) at 9.5 ± 3.3 cM [38]. The *Acra-3* locus also cosegregates with two other markers on chromosome 9: *Mpi-1* (mannose phosphate isomerase) [35] and *Mod-1* (malic enzyme supernatant) [39,40], the genetic distance between *Acra-3* and *Mpi-1* was determined to be 4.8 ± 3.3 cM. According to this analysis, the most likely genetic order on chromosome 9 is: *Es-14–Acra-3–Mpi-1–Mod-1*.

The Acra-4 gene locus shows 71% cosegregation in the 58 animals analyzed with the agouti locus previously located on mouse chromosome 2 [32]. The genetic distance between these two loci is 29.3 ± 6.0 cM. Combination of the data obtained by [41], and [42] reveals that the gene coding for muscle α1 subunit (*Acra-1* locus) is located on this chromosome. (Initially *Acra-1* was located on chromosome 17 on the basis of its linkage with the α-cardiac actin gene, which had been incorrectly assigned to chromosome 17 [43]. Recent work [42] demonstrated linkage of the α-cardiac actin gene with the β_2-microglobulin gene, unambiguously

Table II

Analysis of the cosegregation of the four neuronal nAchR subunit genes and other chromosomal markers in the backcross

(a)	*Acra-2*	*Np-1*	*Ctla-1*	*Gdh-x*
Acra-2		98	100	98
Np-1	45/46		98	93
Ctla-1	46/46	59/60		93
Gdh-x	45/46	56/60	70/75	

(b)	*Acra-3*	*Mpi-1*	*Mod-1*	*Es-14*
Acra-3		93	95	91
Mpi-1	40/43		83	81
Mod-1	41/43	62/75		86
Es-14	39/43	60/74	64/74	

(c)	*Acra-4*	*Hox-5*	*a*
Acra-4		50	71
Hox-5	27/54		61
a	41/58	31/51	

(d)	*Acrb-2*	*Pk-1*	*Hao-2*	*Amy*
Acrb-2		88	93	86
Pk-1	35/40		90	79
Hao-2	52/56	44/49		90
Amy	48/56	39/49	67/74	

(a) Cosegregation of the gene encoding the α2 subunit (*Acra-2*) with genes coding for nucleoside phosphorylase 1 (*Np-1*), cytotoxic T-lymphocyte associated protein (*Ctla-1*) and the glutamate-deshydrogenase human X pseudogene (*Gdh-X*). (b) Cosegregation of the gene encoding the α3 subunit (*Acra-3*) with genes coding for mannose phosphate isomerase (*Mpi-1*), malic enzyme (*Mod-1*) and esterase 14 (*Es-14*). (c) Cosegregation of the gene encoding the α4 subunit (*Acra-4*) with the non agouti gene (*a*) and the Homeo box-5 (*Hox-5*) gene. (d) Cosegregation of the gene encoding the β2 subunit (*Acrb-2*) with genes coding for the pyruvate kinase 1 (*Pk-1*), the hydroxyacid oxidase-2 (*Hao-2*) and the salivary amylase (*Amy*)

located on chromosome 2.) However, our analysis shows that the *Acra-4* locus does not significantly cosegregate with loci previously mapped close to the *Acra* loci such as the *Hox-5* locus (Homeobox-5) [44] (see Table IIc). Hence, the α4 subunit gene is located on chromosome 2, linked to the non agouti gene but not to the α1 muscle subunit coding gene.

Finally, the *Acrb-2* locus was found to cosegregate with the *Hao-2* locus (hydroxyacid oxidase-2) [45], the *Pk-1* locus (pyruvate kinase-1) [46] and the *Amy* locus (salivary amylase) [47] (see Table IId). These three loci have previously been mapped to chromosome 3. Our segregation data show that the *Acrb-2* locus is most probably located between *Pk-1* (at 10.0 ± 4.7 cM) and *Hao-2* (at 3.7 ± 2.6 cM).

4. DISCUSSION

Chromosomal localization analysis of the four

neuronal nAchR genes was performed by following the mendelian segregation of the corresponding alleles in backcrosses involving the mouse species *Mus spretus* and the laboratory strains C57BL/6 or BALB/c. The allelic forms of the genes were distinguished by RFLP analysis [48]. This very powerful genetic method has already been used for chromosomal assignment of many mouse genes [33]. Using this method, Heidmann et al. [41] have previously localized the genes coding for the α, β, γ and δ muscle nAchR subunits. Recently, it has been shown that the genes coding for $\alpha 3$, $\alpha 5$ and $\beta 4$ are tightly linked on the same 60 kb pair DNA fragment [28]. We have now localized the genes coding for the $\alpha 2$, $\alpha 3$, $\alpha 4$ and $\beta 2$ subunits of neuronal nAchR on mouse chromosomes. Thus, the chromosomal localization of ten members of the nAchR subunit gene family up to date identified in the mouse is the following:

Chromosome	1	2	3	9	11	14
Subunit	γ/δ	$\alpha 1$ $\alpha 4$	$\beta 2$	$\alpha 3/\alpha 5/\beta 4$	$\beta 1$	$\alpha 2$

Though the $\alpha 1$ muscle subunit gene and the $\alpha 4$ subunit gene have both been located on mouse chromosome 2, the present data unambiguously show that these genes are not linked.

The partial dispersion of the members of the nAchR subunit gene family is of interest. At variance with some other gene families, the nAchRs subunit genes are not contiguous on the same DNA fragment (see [49] for the histone gene family, [50] for the globin gene family, [51] for the NGF genes, [52] for the myosin heavy chain gene family and [53] for keratine intermediate filament genes). In situ hybridization experiments in rat central nervous system with $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\beta 2$ and $\beta 4$ probes [30,31] further show that some of these subunit genes are coexpressed in some nuclei of the central nervous system, but not in others. The partial dispersion of the corresponding genes on four different mouse chromosomes supports the notion that the regulation of the neuronal nAchR subunit genes expression is not simply accounted for by a single common DNA regulatory region (at variance with β and ϵ globin genes expression, see [54]). Nevertheless, common aspects of the regulation of these genes such as the 'neural specificity' may involve specific *trans*-acting factors interacting with similar DNA motifs. An example of such mechanism is provided in liver, muscle and pituitary cells where several tissue-specific promoters show a conserved DNA motif responsible for the binding of the tissue-specific factors (references in [55–57]). Identification of the DNA regulatory elements associated in *cis* with these neuronal receptor genes will provide essential information about their differential expression upon neuronal differentiation.

Acknowledgements: We thank Jacques Piette for helpful discussion, Joe Hill for critical reading of the manuscript. We thank Philippe Daubas for his patient and consistent aid throughout the duration of this work. This work was supported by a research grant from the Association Française contre les Myopathies, Collège de France, the CNRS, the Ministère de la Recherche, the EEC, the INSERM (Contract 872004), and the DRET (Contract 87/211).

REFERENCES

[1] Changeux, J.-P., Devillers-Thiéry, A. and Chemouilli, P. (1984) Science 225, 1335–1345.
[2] Stroud, R.M. and Finer-Moore, J. (1985) Annu. Rev. Cell Biol. 1, 317–351.
[3] Popot, J.-L. and Changeux, J.-P. (1984) Physiol. Rev. 64, 1162–1239.
[4] Kao, P., Dwork, A., Kaldany, R., Silver, M., Wideman, J., Stein, S. and Karlin, A. (1984) J. Biol. Chem. 259, 11662–11665.
[5] Blount, P. and Merlie, J.-P. (1989) Neuron 3, 349–357.
[6] Dennis, M., Giraudat, J., Kotzyba-Hibert, F., Goeldner, M., Hirth, C., Chang, J.-Y., Lazyre, C., Chrétien, M. and Changeux, J.-P. (1988) Biochemistry 27, 2346–2357.
[7] Kao, P.N. and Karlin, A. (1986) J. Biol. Chem. 261, 8085–8088.
[8] Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., Furutani, Y., Hirose, T., Asai, M., Inayama, S., Miyata, T. and Numa, S. (1983) Nature 299, 793–797.
[9] Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Tanabe, T., Shimizu, S., Kikyotani, S., Kayano, T., Hirose, T., Inayama, S. and Numa, S. (1983) Nature 305, 818–823.
[10] Devillers-Thiéry, A., Giraudat, J., Bentaboulet, M. and Changeux, J.-P. (1983) Proc. Natl. Acad. Sci. USA 80, 2067–2071.
[11] Boulter, J., Luyten, W., Evans, K., Mason, P., Ballivet, M., Goldman, D., Stengelin, S., Martin, G., Heinemann, S. and Patrick, J. (1985) J. Neurosci. 5, 2545–2552.
[12] Nef, P., Oneyser, C., Alliod, C., Couturier, S. and Ballivet, M. (1988) EMBO J. 7, 595–601.
[13] Curtis, D.R. and Ryall, R.W. (1966) Exp. Brain Res. 2, 81–96.
[14] Mulle, C. and Changeux, J.-P. (1990) J. Neurosci. 10, 169–175.
[15] Williams, H. and Robinson, J.-L. (1984) J. Neurosci. 4, 2906–2911.
[16] London, E.D., Walter, S.B. and Wamsley, J.K. (1985) Neurosci. Lett. 53, 179–184.
[17] Clarke, P.B.S., Schwartz, R.D., Paul, S.M., Pert, A. and Pert, A. (1985) J. Neurosci. 5, 1307–1315.
[18] Swanson, L., Lindstrom, J., Tzartos, S., Schmued, L.C., O'Leary, D.D.M. and Cowan, W.M. (1983) Proc. Natl. Acad. Sci. USA 80 4532–4536.
[19] Lindstrom, J., Schoepfer, R. and Whiting, P. (1987) Mol. Neurobiol. 1, 281–337.
[20] London, E.D., Connoly, R.L., Szikszay, M., Wamsley, J.K. and Dam, M. (1988) J. Neurosci. 8, 3920–3928.
[21] Cauley, K., Agranoff, B.W. and Goldman, D. (1989) J. Cell Biol. 108, 637–645.
[22] Boulter, J., Evans, K., Goldman, D., Martin, G., Treco, D., Heinemann, S. and Patrick, J. (1986) Nature 319, 368–374.
[23] Goldman, D., Deneris, E., Luyten, W., Kochhar, A., Patrick, J. and Heinemann, S. (1987) Cell 48, 965–973.
[24] Wada, K., Ballivet, M., Boulter, J., Connolly, J., Wada, E., Deneris, E.S., Swanson, L.W., Heinemann, S. and Patrick, J. (1988) Science 240, 330–334.
[25] Deneris, E.S., Connolly, J., Boulter, J., Wada, E., Wada, K., Swanson, L.W., Patrick, J. and Heinemann, S. (1988) Neuron 1, 45–54.
[26] Deneris, E.S., Boulter, J., Swanson, L.W., Patrick, J. and Heinemann, S. (1989) J. Biol. Chem. 264, 6268–6272.
[27] Isenberg, K.I. and Meyer, G.E. (1989) J. Neurochem. 52, 988–991.

[28] Boulter, J., O'Shea-Greenfield, A., Duvoisin, R.M., Connolly, J.G., Wada, E., Jensen, A., Gardner, P.D., Ballivet, M., Deneris, E.S., McKinnon, D., Heinemann, S. and Patrick, J. (1990) J. Biol. Chem., in press.
[29] Duvoisin, R.M., Deneris, E.S., Patrick, J. and Heinemann, S. (1989) Neuron 3, 487–496.
[30] Wada, E., Wada, K., Boulter, J., Deneris, E., Heinemann, S., Patrick, J. and Swanson, L.W. (1989) J. Comp. Neurol. 284, 314–335.
[31] Wada, E., McKinnon, D., Heinemann, S., Patrick, J. and Swanson, L.W. (1990) Brain Research, in press.
[32] Lyon, M.F. and Searle, A.G. (1989) in: Genetic Variants and Strains of the Laboratory Mouse, 2nd edn, edited by Lyon and Searle for the International Committee on Standardized Genetic Nomenclature for Mice, Oxford University Press.
[33] Guénet, J.-L., Simon-Chazottes, D. and Avner, P.R. (1988) Current Topics Microbiol. Immunol. 137, 13–17.
[34] Brunet, J.F., Dosseto, M., Denizot, F., Mattei, M.G., Clark, W.R., Haqqi, T.M., Ferrier, P., Nabholz, M., Schmitt-Verhulst, A.-M., Luciani, M.-F. and Golstein, P. (1986) Nature 322, 268–271.
[35] Nichols, E.A., Chapman, V.M. and Ruddle, F.H. (1973) Genetics 8, 47–53.
[36] Womack, J.E., Davidson, M.T., Eicher, E.M. and Kendal, D.A. (1977) Biochem. Genet. 15, 347–355.
[37] Harper, K., Mattéi, M.-G., Simon, D., Suzan, M., Guénet, J.-L., Haddad, P., Sasportes, M. and Golstein, P. (1988) Immunogenetics 28, 439–444.
[38] Britton-Davidian, J. and Bonhomme, F. (1979) CR Acad. Sci. Paris 288D, 1419–1422.
[39] Shows, T.B., Chapman, V.M. and Ruddle, F.H. (1970) Biochem. Genet. 4, 707–718.
[40] Antonucci, T.K., Von Deimling, O.H., Rosenblum, B.B., Skow, L.C. and Meisler, M.H. (1984) Genetics 107, 463–475.
[41] Heidmann, O., Buonanno, A., Geoffroy, B., Robert, B., Guénet, J.-L., Merlie, J.P. and Changeux, J.-P. (1986) Science 234, 866–868.
[42] Crosby, J.L., Phillips, S.J. and Nadeau, J.H. (1989) Genomics 5, 19–23.
[43] Czosnek, H., Nudel, U., Mayer, Y., Barker, P.E., Pavtcheva, D.D., Ruddle, F.H. and Yaffe, D. (1983) EMBO J. 2, 1977–1979.
[44] Feathererst, D.S., Baron, A., Gaunt, S.J., Mattei, M.-G. and Duboule, D. (1988) Proc. Natl. Acad. Sci. USA 85, 4760–4764.
[45] Holmes, R.S. (1978) Heredity 41, 403–406.
[46] Moore, K.J. and Bulfield, G. (1981) Biochem. Genet. 19, 771–781.
[47] Hjorth, H. (1979) Biochem. Genet. 17, 665–682.
[48] Robert, B., Barton, P., Minty, A., Daubas, P., Weydert, A., Bonhomme, F., Catalan, J., Chazottes, D., Guénet, J.-L. and Buckingham, M. (1985) Nature 314, 181–183.
[49] Hentschel, C.C. and Birnstiel, M.L. (1981) Cell 25, 301–313.
[50] Karlsson, S. and Nienhuis, A.W. (1985) Annu. Rev. Biochem. 54, 1071–1108.
[51] Evans, B.A. and Richards, R.I. (1985) EMBO J. 4, 133–138.
[52] Weydert, A., Daubas, P., Lazaridis, I., Barton, P., Gardner, I., Leader, D.P., Bonhomme, F., Catalan, J., Simon, D., Guénet, J.-L., Gros, F. and Buckingham, M.E. (1985) Proc. Natl. Acad. Sci. USA 82, 7183–7187.
[53] Powell, B.C., Graham, R.C., Fietz, M.J. and Rogers, G.E. (1986) Proc. Natl. Acad. Sci. USA 83, 5048–5052.
[54] Choi, O.-R. and Engel, D.J. (1988) Cell 55, 17–26.
[55] Courtois, G., Baumhueter, S. and Crabtree, G.R. (1988) Proc. Natl. Acad. Sci. USA 85, 7937–7941.
[56] Lassar, B.A., Buskin, J.N., Lockshon, D., Davis, R.L., Apone, S., Hauschka, S.D. and Weintraub, H. (1989) Cell 58, 823–831.
[57] Nelson, C., Albert, V.R., Elsholtz, H.P., Lu, L.I.W. and Rosenfeld, M.G. (1988) Science 239, 1400–1402.
[58] Southern, E. (1975) J. Mol. Biol. 98, 503–517.
[59] Nef, P., Mauron, A., Stadler, R., Alliod, C. and Ballivet, M. (1986) Proc. Natl. Acad. Sci. USA 81, 7975–7979.