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The scattered distribution of actin genes in the mouse and human genomes

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A hamster actin cDNA probe was used to localize actin genes on the major components of mouse and human DNAs, namely on the four families of fragments forming the bulk of these genomes. Over 20 *Eco*RI fragments hybridizing the probe could be detected; a different subset of these fragments was found in each component. Since the fragment families forming the major components of the mouse and human DNAs derive from very long chromosomal segments, the isochores, the presence of actin genes on all components provides evidence for their dispersion in both genomes. *In situ* hybridization of ^{125}I-labeled probe to metaphase chromosomes in the presence of dextran sulfate confirmed this dispersion by showing that the 29–30 actin gene sites so identified are distributed on almost all chromosomes. Moreover, some human actin genes could be mapped on specific chromosomal segments; in particular, one gene was localized on the long arm of the X chromosome. Finally, three different mouse actin genes were isolated from a recombinant DNA library and previously investigated interspersed repeated sequences were identified in the vicinity of these genes.

Key words: multigene family/main band components

Introduction

Actins are a family of well conserved proteins associated with subcellular, cellular, and organismal motility. Isoelectric focusing has allowed identification of several actins found either in muscle tissues (α-actins) or in microfilaments (β- and γ-actins); amino acid sequencing studies of actins from mammalian sources have further shown the presence of at least four distinct α-actins, bringing the minimal number of actin genes up to six (Vandekerckhove and Weber, 1979). Hybridizations using actin cDNA plasmids has accordingly shown that actin genes form multigene families, in mammals as well as in other metazoans (for a review, see Firtel, 1981). Some multigene families, such as the β-globin gene family (Lacy *et al.*, 1979), have been found to exist as clusters at single chromosomal loci, whereas actin genes have been shown by *in situ* hybridization to be scattered in *Drosophila* (Tobin *et al.*, 1980; Fyrberg *et al.*, 1980); in sea urchin, however, some clustering of actin genes occurs (Scheller *et al.*, 1981). Such data are not yet available for mammalian actin genes, although no tight gene clustering has been observed with individual human actin genes isolated from DNA libraries (Engel *et al.*, 1981; Humphries *et al.*, 1981).

The demonstration of linkage or dispersion of members of a multigene family may be investigated by somatic cell genetic approaches (Boone *et al.*, 1972) or by chromosome sorting (Davies *et al.*, 1981). An alternative method, which can provide information about close gene linkage, can be based upon the localization of gene family members on individual major DNA components. Main band DNA from higher vertebrates can be resolved into four major components, differing in their buoyant density (Filipski *et al.*, 1973; Thiery *et al.*, 1976; Macaya *et al.*, 1976; Cuny *et al.*, 1981). In the case of the mouse genome, the major components have buoyant densities of 1.699, 1.701, 1.704, and 1.708 g/cm^3 and represent 22%, 34%, 26%, and 7% of the DNA, respectively; in the case of the human genome, the densities are 1.698, 1.700, 1.704, and 1.708 g/cm^3 and the relative amounts 29%, 33%, 22%, and 9%, respectively (Cuny *et al.*, 1981). These components are families of DNA fragments derived from regions of relatively homogeneous base composition, the isochores, which are estimated to be at least 300 kb in size and which may correspond to chromosomal bands (Macaya *et al.*, 1976; Cuny *et al.*, 1981). The presence of individual members of the multigene family on several components will therefore strongly suggest that members are not closely linked. Yet another approach for assessing the linkage of multigene families is *in situ* hybridization to metaphase chromosomes, using methods allowing the localization of single-copy genes (Gerhard *et al.*, 1981).

In this study we report on the number and distribution of actin genes on both DNA components and chromosomes of mouse and man using a recombinant plasmid containing cDNA synthesized on Syrian hamster mRNA coding for β- or α-actin (Dodemont *et al.*, 1982). The results indicate that the actin genes, present at ~30 sites, are widely dispersed in both genomes. In addition, three different mouse actin genes have been isolated from a DNA library and repeated sequences flanking the genes have been characterized.

Results

Distribution of actin genes on the major components of mouse and human DNA

*Eco*RI digests of total mouse and human DNAs, and of their major components, were hybridized with pAct-1. Both total DNAs display at least 15 hybridization bands on autoradiograms (Figure 1). Such bands display different intensities, due to the different levels of homology of the probe with different actin genes or to the co-migration of similarly sized fragments. Some of the latter could be unambiguously resolved because they were found on components having a very distinct density. Those which were present on components exhibiting close densities might be due to incomplete separation of the components. This explanation can, however, only hold for the case in which a weak hybridization band corresponds in size to a strong hybridization band on a component of close density, and not to the case in which equally strong hybridization bands identical in size are present on such components (Figure 1). Indeed, the latter case would

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imply levels of cross-contamination which are ruled out not only by the analytical ultracentrifugation work done during the preparation of the components (Cuny *et al.*, 1981), but also by the fact that a number of single-copy and multiple-copy genes (globin genes, histocompatibility genes, protein synthesis initiation factor genes) were found to be localized on restriction fragments specifically belonging to given components (data not shown). A more likely explanation for the strong similarly-sized hybridization bands found on components of close density is the presence of restriction sites at an identical location within some genes. Taking into account the above considerations, one can estimate that >20 distinct *Eco*RI fragments hybridizing the actin probe were present in either genome.

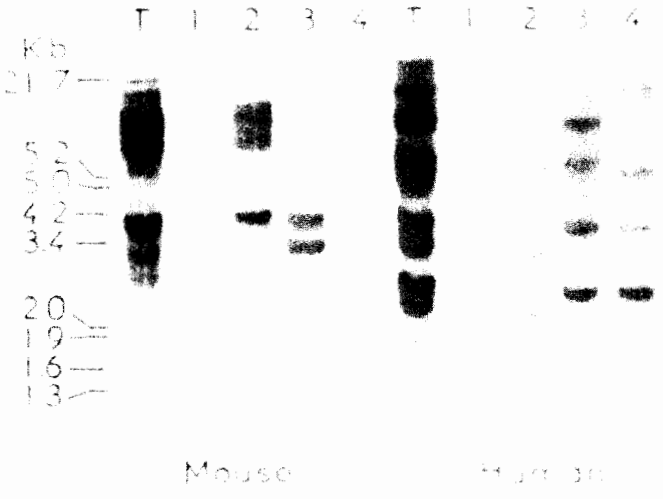

Fig. 1. Distribution of actin genes in mouse and human DNA components. Total mouse and human DNAs **(T)** and their major components (in order of increasing density, **1–4**), were digested with *Eco*RI, fractionated on a 0.6% agarose gel, transferred to nitrocellulose filters, and hybridized with nick-translated pAct-1. Components were loaded in quantities proportional to their amount in total DNA (Cuny *et al.*, 1981), with 1 μg of components 1.708 **(lanes 4)** loaded in each case. λ DNA digested by *Eco*RI and *Hind*III was used as mol. wt. marker.

In situ hybridization

^{125}I-labeled pAct-1 was hybridized to mouse and huma metaphase chromosome preparations under conditions whi promote the formation of probe networks (Gerhard *et a* 1981; Wetmur, 1975). As a control for background due to t pBR322 vector and to estimate the signal expected for a un que gene, a single copy human DNA clone, pAW (kindly pr vided by R. White; Wyman and White, 1980) was hybridize to metaphase chromosomes from: (a) A9/HRBC2 (Miller *al.*, 1971), a mouse:human hybrid cell line which contains t human X chromosome (Figure 2A); and (b) GM74 (Opi and Pallister, 1973), a human fibroblastic cell line. Under t hybridization conditions used, pAW yields a few silver grai and occasionally one or two clusters of silver grains p metaphase cell. In the case of the human cells it was possibl to show that this pAW sequence is located near the telome of the long arm of chromosome 14 (data not shown). Whe chromosomes from both cell lines were annealed with th pAct-1 probe (Figure 2B and 2C), a strikingly higher numbe of grains and grain clusters was observed. A comparison o the number of silver grains over interphase cells hybridize with either probe showed that the actin probe gave 8- t 9-fold more grains per cell (29.5 grains/day/cell *versus* 3. grains/day/cell) than pAW at the same concentration. This i a minimum difference since the grains over the actin gene were observed as large clusters even at short exposure tim (2–4 days) leading to a systematic underestimate of th number of grains because of coincidence of silver grai deposition (Gerhard *et al.*, 1981; Szabo *et al.*, 1977).

The actin genes are clearly dispersed in both the mouse an human genomes. In the case of the human metaphas chromosomes, which could be karyotyped with less difficult (Figure 3), an average of one silver grain cluster ($\geq$3 grains was observed per chromosome. The bulk of these cluste were at sites found to be repeatedly labeled. We analyze these preparations by determining the distribution of thes clusters rather than individual grains to minimize the distribu tion of random background silver grains which are mos often single grains. Also, since the number of silver grains at a site is proportional to probe network size and not to th degree of hybridization at that site, the frequency of labelin

Fig. 2. *In situ* hybridization. A9/HRBC2 metaphase chromosomes were hybridized with the single-copy human clone pAW **(A)** and with pAct-1 **(B)**, at 60 ng/ml probe concentration; exposure time 2 days. GM74 chromosomes were hybridized with (metaphase and interphase) pAct-1 **(C)**, at 30 ng/ml probe concentration; exposure time 6 days.

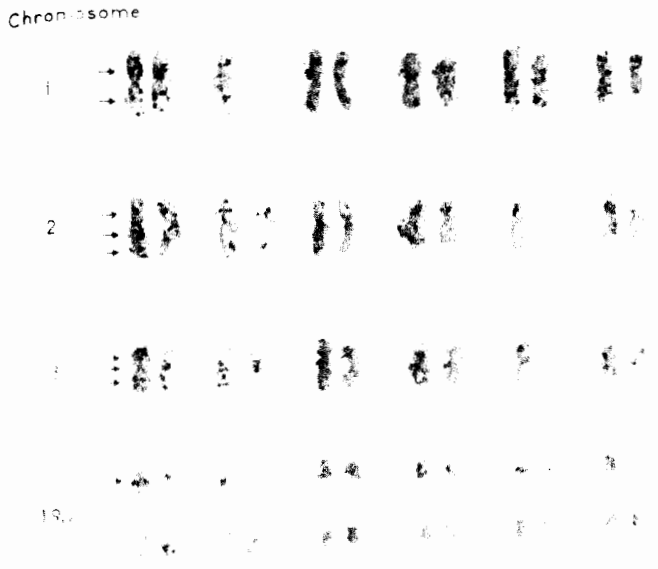

Fig. 3. Identification of actin gene sites on individual human chromosomes. A and F group GM74 metaphase chromosomes from 6 spreads (a-f) are displayed; some chromosomes are not displayed because they were covered by others in the original photograph. Arrows indicate the region of silver grain clusters.

is a more accurate measure of the significance of labeling at a site. Table I summarizes our observations on 11 metaphase cells hybridized with ^{125}I-labeled pAct-1. Listed are preliminary actin gene chromosome assignments, within the resolution afforded by ^{125}I autoradiography, and the frequency at which grain clusters were observed over these sites per chromatid. In these experiments, the genes could be mapped to chromosome subregions approximately the size of a human E group chromosome. The frequency of clusters at each labeled site varied between 0.13 and 0.6; outside of the regions indicated in the table (e.g., the Y, 6q, Xp) the frequency of grain clusters was ~0.03 for comparably sized areas. One chromosome of the F group (chromosomes 19 and 20), one of the G group (chromosomes 21 and 22), and the Y chromosome were the only clearly unlabeled human chromosomes. Of the 29 sites listed on Table I, 14 can be classified as strong sites (frequency ≤ 0.4), eight as intermediate sites (frequency $\geq 0.3 < 0.4$) and the remaining seven sites are classified as weak sites (frequency $\geq 0.1 < 0.3$). One strong site was localized on the long arm of the X chromosome. Since none of the strong actin sites was more significantly labeled than any other, we conclude that each of these sites represents either a single actin gene which shares a high degree of homology with the probe, or a cluster of intermediate and/or weak sites. The intermediate and weak sites are probably the result of pAct-1 hybridization of actin genes (or pseudogenes) having less homology with the probe.

In the case of the mouse actin genes, it was not possible to make many actin gene site assignments but it was apparent that the labeling pattern was specific and that the sites were dispersed over many chromosomes. However, the numerous marker chromosomes of the A9 mouse cell line (Miller *et al.*, 1971; Allderdice *et al.*, 1973) present in A9/HRBC2 included isochromosomes which were labeled at homologous positions on the chromosome arms allowing us to assign actin genes to some specific mouse chromosomes, including chromosomes 3, 8, and 18. In addition, the human X chromosome, which

Table I. Preliminary localization of actin genes on human chromosomes

Chromosome	Labeled region	Frequency[a] of labelling	Type of site
1	1p13 – 1p34	0.55	Strong
	1q24 – 1qter	0.60	S
2	2p12 – 2p22	0.43	S
	2q11 – 2q22	0.30	Intermediate
	2q31 – 2qter	0.50	S
3	3p23 – 3pter	0.23	Weak
	3p13 – 3q13	0.31	I
	3q22 – 3qter	0.31	I
4	4p15 – 4q13	0.43	S
	4q28 – 4qter	0.32	I
5	5q14 – 5q31	0.55	S
6	6p11 – 6pter	0.30	I
7	7p12 – 7pter	0.22	W
	7q11 – 7q22	0.50	S
8	8q21 – 8qter	0.40	S
9	9p22 – 9q12	0.34	I
	9q22 – 9qter	0.45	S
10	10q22 – 10qter	0.32	I
11	11q12 – 11qter	0.39	I
12	12q11 – 12q22	0.46	S
13	13q14 – 13qter	0.29	W
14	14q11 – 14q23	0.25	W
15	15pter – 15q21	0.13	W
16	16q11 – 16qter	0.44	S
17	17pter – 17q22	0.29	W
18	18q11 – 18qter	0.56	S
19 or 20	–	0.50	S
21 or 22	–	0.45	S
X	Xq22 – Xqter	0.49	S

[a]Labeling was scored when a silver grain cluster (≥ 3 grains) was observed per chromatid.

could often be identified by the absence of C banding at the centromere (Miller *et al.*, 1971), was found to be labeled at a high frequency, as expected from the analysis of the GM74 cell line.

To obtain an estimate of the number of mouse actin genes, we counted the total number of silver grain clusters per cell. The average number of grain clusters per metaphase cell was 35.4 ± 2.5, based on 17 cells. Assuming that the average frequency of labeling is equivalent to that observed for the human preparations (0.39) and that the A9/HRBC2 cell line is essentially triploid (Miller *et al.*, 1971), we can estimate that the actin gene copy number is ~30 (35.4/0.39/3), similar to the number found in humans.

The *in situ* hybridization results show, moreover, the efficiency of the probe network formation. For these particular experiments, one can calculate that the maximum signal which could be obtained at individual actin sites without the formation of probe networks would be ~0.01 – 0.02 grains /day; this calculation assumes an average hybridization efficiency of 10% (Gerhard *et al.*, 1981; Szabo *et al.*, 1977). The actual signal observed at individual actin sites on murine chromosomes was ~2 grains/day of exposure on slides hybridized with pAct-1 at 60 ng/ml indicating that a 100- to 200-fold enhancement of the signal was obtained, comparable to the increase in signal observed for the annealing of double-stranded DNA probes to immobilized DNA on filters (Wahl *et al.*, 1979). At higher concentrations ($\geq 100 - 200$ ng/ml) non-specific background grain clusters become a pro-

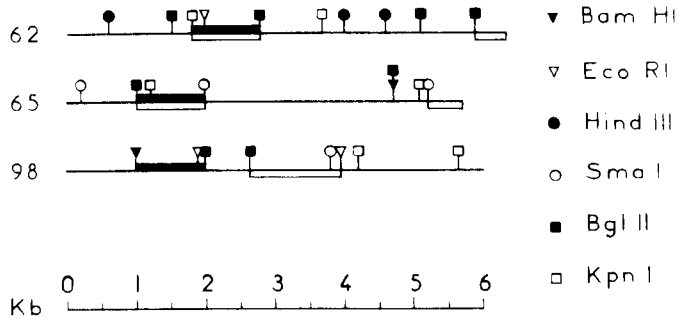

Fig. 4. Restriction maps of the isolated mouse and actin genes. Maps are oriented with the left arm of Charon 28 on the left. Black and white bars indicate segments hybridizing with pAct-1 and total mouse DNA and corresponding to actin gene and repeated sequences, respectively.

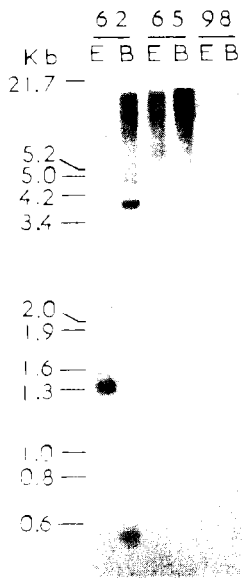

Fig. 5. Identification of repeated sequences in the isolated mouse actin clones. 1 μg of total mouse DNA was digested by *Eco*RI (E) and BamHI(B), fractionated on a 0.8% agarose gel, transferred to nitrocellulose filters, and hybridized with nick-translated λMac62, λMac65, and λMac98. λ DNA digested by *Eco*RI and *Hind*III was used as mol. wt. marker.

blem for the analysis (Gerhard *et al.*, 1981); in this experiment slides were hybridized with pAct-1 at several concentrations and only those with relatively low background (30–60 ng/ml) were analyzed.

Isolation of individual mouse actin genes

Three different mouse actin genes were isolated from a recombinant DNA library of A9/HRBC2. For reasons that remain unclear, most of the recombinant phages recovered after amplification of the library contained the dispensable phage *Bam*HI fragment and consequently inserts were shorter than expected, averaging 6 kb. Fifteen phages were initially selected from the library, and after streaking, six which hybridized more strongly (yet differently) to pAct-1 were selected. Of these six phages, four contained the same gene, as shown by detailed restriction mapping. All of the purified phages contained, in addition to actin sequences, repeated mouse DNA sequences, as determined by hybridization with ^{32}P-labeled mouse DNA. None of the actin gene-containing phages annealed with total human DNA, confirming the murine origin of these genes.

Figure 4 shows the restriction maps of the three isolated mouse actin genes, λMac62, λMac65, and λMac98. Sequences homologous to the actin probe and repeated sequences were localized on the maps by hybridization with pAct-1 and total mouse DNA, respectively. The repeated sequences found in the proximity of the genes were in addition characterized by hybridizing nick-translated phage DNAs to total mouse DNA cleaved by *Eco*RI or *Bam*HI, and transferred to nitrocellulose filters (Figure 5). λMac62 contained a member of the *Bam*HI family of interspersed repeats (Meunier-Rotival *et al.*, 1982) and both λMac65 and λMac98 gave hybridization patterns similar to those obtained with B1 repeats (Krayev *et al.*, 1980).

Discussion

The present work shows that the actin genes are scattered in the mouse and human genome. Evidence for this is based on the presence of actin genes on all four major components of both genomes, as well as on most chromosomes. Moreover, three mouse actin genes have been isolated from a DNA library, and repeated sequences flanking the genes have been mapped. In all cases, the repeated sequences found close to the genes belong to previously investigated families of interspersed repeated sequences.

Since the major components of mammalian DNAs derive from chromosomal stretches characterized by a fairly homogeneous base composition and a length of at least 300 kb, the isochores (Macaya *et al.*, 1976; Cuny *et al.*, 1981), the actin gene distribution on the four components is inconsistent with a tight clustering of all the genes; rather, this distribution indicates dispersion of the genes. Quite a different distribution on the components is indeed observed for the members of clustered multigene families (such as some immunoglobulin genes or genes coding for histocompatibility antigens), since they are only found on one or two components (data not shown). The scattering of the actin genes was further demonstrated by *in situ* hybridization on metaphase chromosomes since most of these were found to contain an actin gene site.

The total number of actin genes can be estimated from the *in situ* hybridization results, which revealed 29 and 30 hybridization sites on human and mouse chromosomes, respectively; cases of chromosomal linkage were observed, but no clear example of close linkage (e.g., sites that were particularly heavily labeled) was encountered. Actin gene sites were however labeled with different frequencies, presumably because the cDNA probe distinguishes between actin gene sequences according to their sequence divergence. The number of gene sites found by *in situ* hybridization is similar to the total number of *Eco*RI hybridizing fragments found on the components; this may be the result of a compensation between factors which tend to underestimate the number of genes (presence of several genes on one large DNA fragment, the fact that components may not resolve all actin gene fragments, especially if these are similarly sized and are on the same component) and others which tend to overestimate the number (several fragments may originate from a single gene). For these reasons, the number of *Eco*RI bands observed can only give an estimate of the number of actin genes, and not the definitive number, as suggested (Humphries *et al.*, 1981). The *in situ* hybridization results strongly suggest that: (a) the actin genes are dispersed, perhaps completely, if the strong gene sites do not correspond to clusters of weaker ones, in both the mouse and human genomes, as is found in

Drosophila (Tobin *et al.*, 1980; Fyrberg *et al.*, 1980), but unlike the situation encountered in sea urchin (Scheller *et al.*, 1981); and (b) the total number of actin genes in the mouse and human genomes is of the order of 29 – 30, with the same limitation as in (a).

In preliminary experiments in which human chromosomes were pre-treated for R-banding and hybridized without further denaturation, only a subset of the actin gene sites were as heavily labeled as with uniformly denatured slides (e.g., the actin sites on 1p, 6p, 11q, and chromosomes 16, 19 or 20, 21 or 22), the labeling frequency at most sites was reduced and no significant labeling was observed at some sites (e.g., human X-chromosome). The presence of actin genes on both G and R bands suggests that these genes are embedded in chromosomal stretches with different T_ms. Since there is evidence that G and R bands do in fact differ in their base composition (Comings, 1978), this result is in agreement with the presence of actin genes in all four major components of both genomes and with the suggestion that isochores correspond to G and R bands (Cuny *et al.*, 1981).

Concerning the chromosomal distribution of the actin genes, it may be noted that a strong site has been mapped to the terminal half of the q arm of the human X chromosome. This raises the question of the relationship of this gene with Beckers muscular dystrophy, which also maps in this region.

Some of the mouse actin genes were isolated from a DNA library for further characterization. The repeated sequences found in the proximity of the actin genes belong either to the *Bam*HI family of interspersed repeats in the case of λMac62 (Meunier-Rotival *et al.*, 1982) or gave a hybridization pattern similar to the one obtained with the B1 probe Mm31 (Krayev *et al.*, 1980, in preparation), in the case of λMac65 and λMac98. In view of the proximity to the actin coding sequence, some of these repeats might well be included in the actin transcription units.

Materials and methods

DNA components

The major components of mouse DNA were prepared by Cs_2SO_4/BAMD density gradient centrifugation (Cortadas *et al.*, 1979) and were generously provided by J. Cortadas, G. Cuny, and A. Huyard of our laboratory; the average size of these preparations was 100 kb. The major components of human DNA, obtained from a single placenta, were preparations already described, averaging 25 kb (Cuny *et al.*, 1981).

DNA blot hybridization

Restriction endonucleases were purchased from New England Biolabs (Beverly, MA) or prepared by L. Mallet and A. Meier of our Institute. DNA restriction fragments were fractionated on horizontal agarose (Type II, Sigma, St. Louis, MO) gels, and transferred to nitrocellulose filters (BA85, Schleicher and Schüll, Dassel, FRG) after treating the gel with 0.2 N HCl. Filters were prehybridized at 65°C in 1 M NaCl, 50 mM sodium phosphate, pH 6.8, 5 M EDTA, 250 μg/ml sheared denatured salmon sperm DNA, and 0.2% each of ficoll, polyvinylpyrrolidone, and bovine serum albumin. Hybridization was performed in the same buffer containing $10^5 - 5 \times 10^5$ c.p.m./ml of nick-translated probes ($10^8 - 5 \times 10^8$ c.p.m./μg). Filters were washed at 65°C in 1 M NaCl, 50 mM sodium phosphate, pH 6.8, 5 mM EDTA, and then in 0.2 M NaCl, 10 mM sodium phosphate, pH 6.8, 1 mM EDTA.

In situ hybridization

Metaphase spreads were prepared from A9/HRBC2 (Miller *et al.*, 1971) and from GM74 (Opitz and Pallister, 1973) cell lines. The preparation of slides and details of the *in situ* hybridization method have been described (Gerhard *et al.*, 1981). The plasmid probes used in these experiments were labeled by nick-translation with [^{125}I]dCTP (Radiochemical Center, Amersham, UK) to a specific activity of 3×10^8 d.p.m./μg.

Recombinant DNAs

Plasmid DNAs were purified by centrifugation in CsCl/ethidium bromide density gradients. The A9/HRBC2 recombinant library was constructed by ligation of partial *Sau*3A fragments into the *Bam*HI arms of the vector Charon 28 (Williams and Blattner, 1980). Recombinant phages containing actin sequences were identified by plaque hybridization (Benton and Davis, 1977) with the actin cDNA plasmid pAct-1 (Dodemont *et al.*, 1982), ^{32}P-labeled by nick-translation, and purified by three successive streakings.

Phage DNAs were prepared by CsCl density gradient centrifugations, followed by treatment with proteinase K (Merck, Darmstadt, FRG) and extraction with phenol-chloroform-isoamyl alcohol (50:48:2). They were mapped using single and double restriction enzyme digests; the location of the actin gene sequences and of repeated DNA sequences were determined by transferring the DNA fragments to nitrocellulose filters, and hybridizing the filters with ^{32}P-labeled pAct-1 and total mouse DNA, respectively.

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References

Alderdice,P.W., Miller,O.J., Miller,D.A., Warburton,D., Pearson,P.L., Klein,G., and Harris,H. (1973) *J. Cell Sci.*, **12**, 263-274.
Benton,W.D., and Davis,R.W. (1977) *Science (Wash.)*, **196**, 180-182.
Boone,C., Chen,T.R., and Ruddle,F.H. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 510-514.
Comings,D.E. (1978) *Annu. Rev. Genet.*, **12**, 25-46.
Cortadas,J., Olofsson,B., Meunier-Rotival,M., Macaya,G., and Bernardi,G. (1979) *Eur. J. Biochem.*, **99**, 179-186.
Cuny,G., Soriano,P., Macaya,G., and Bernardi,G. (1981) *Eur. J. Biochem.*, **115**, 227-233.
Davies,K.E., Young,B.D., Elles,R.G., Hill,M.E., and Williamson,R. (1981) *Nature*, **293**, 374-376.
Dodemont,H.J., Soriano,P., Quax,W.J., Ramaekers,F., Lenstra,J.A., Groenen,M.A.M., Bernardi,G., and Bloemendal,H. (1982) *EMBO J.*, **1**, 167-171.
Engel,J.N., Gunning,P.W., and Kedes,L. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 4674-4678.
Filipski,J., Thiery,J.P., and Bernardi,G. (1973) *J. Mol. Biol.*, **80**, 177-197.
Firtel,R.A. (1981) *Cell*, **24**, 6-7.
Fyrberg,E.A., Kindle,K.L., Davidson,N., and Sodja,A. (1980) *Cell*, **19**, 365-378.
Gerhard,D.A., Kawasaki,E.S., Carter Bancroft,F. and Szabo,P. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 3755-3759.
Humphries,S.E., Whittall,R., Minty,A., Buckingham,M., and Williamson,R. (1981) *Nucleic Acids Res.*, **9**, 4895-4908.
Krayev,A.S., Kramerov,D.A., Skryabin,K.G., Ryskov,A.P., Bayev,A.A., and Georgiev,G.P. (1980) *Nucleic Acids Res.*, **8**, 1201-1215.
Lacy,E., Hardison,R.C., Quon,D., and Maniatis,T. (1979) *Cell*, **18**, 1273-1283.
Macaya,G., Thiéry,J.P., and Bernardi,G. (1976) *J. Mol. Biol.*, **108**, 237-254.
Meunier-Rotival,M., Soriano,P., Cuny,G., Strauss,F., and Bernardi,G. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 355-359.
Miller,O.J., Cook,P.R., Meera Khan,P., Shin,S., and Siniscalco,M. (1971) *Proc. Natl. Acad. Sci. USA*, **68**, 116-120.
Opitz,J., and Pallister,P.D. (1973) *Cytogenet. Cell Genet.*, **12**, 291-292.
Scheller,R.H., McAllister,L.B., Crain,W.R., Jr., Durica,D.S., Posakony, J.W., Thomas,T.L., Britten,R.J., and Davidson,E.H. (1981) *Mol. Cell. Biol.*, **1**, 609-628.
Szabo,P., Elder,R.T., Steffensen,D.M., and Uhlenbeck,O.C. (1977) *J. Mol. Biol.*, **115**, 539-563.
Thiéry,J.P., Macaya,G., and Bernardi,G. (1976) *J. Mol. Biol.*, **108**, 219-235.
Tobin,S.L., Zulauf,E., Sanchez,F., Craig,E.A., and McCarthy,B.J. (1980) *Cell*, **19**, 121-131.
Vandekerckhove,J., and Weber,K. (1979) *J. Mol. Biol.*, **126**, 783-802.
Wahl,G.M., Stern,M., and Stark,G.R. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3683-3687.
Wetmur,J.G. (1975) *Biopolymers*, **14**, 2517-2524.
Williams,B.G. and Blattner,F.R. (1980) in Setlow,J.K., and Hollaender,A., (eds.), *Genetic Engineering*, vol. 2, Plenum Press, NY, pp. 201-283.
Wyman,A.R., and White,R. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 6754-6758.