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

Philippe Soriano,1 Paul Szabo,2 and Giorgio Bernardi*1
1Laboratory de Génétique Moléculaire, Institut de Redevance et Biologie Moléculaire, 2 Place Pasteur, 75005 Paris, France
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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 EcoRI fragments hybridizing the probe could be detected; a different subset of these fragments was found in each component. Since the fragments families forming the major components of the mouse and human DNAs derive from very long chromosomal segments, the ins-

ors, the presence of actin genes on all components pro-

vides evidence for their dispersion in both genomes. In situ hybridization of 32P-labelled probe to metaphase chromo-
somes is the presence of deoxirribonucleic acid confirmed this dispersion by showing that the 29–30 actin gene sites so iden-
tified 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 differ-

eent mouse actin genes were isolated from a recombinant DNA library and previously investigated intergenic

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 cellular, cellular, and organelar motility. Isoelectric focusing (IEF) reveals a family of actins in muscle tissues (α-actins) or in microfilaments (β- and γ-actins); amino acid sequencing studies of actins from mu-

 dirs have shown that the major actin groups are contained in four distinct classes of actins, bringing the minimal number of actin genes up to six (Vanderkooi and Weaver, 1979). Hybridi-

zations using actin cDNA probes has consistently revealed the presence of two genes per genome and the possibility of an additional third gene on human chromosome 3 (Coffman et al., 1983; Bird et al., 1983). However, the exact number of actin genes in human and mouse is still not known. In situ hybridization techniques may provide a more accurate estimate of the number of actin genes in these species. In this study, we have used a hamster actin cDNA probe to localize actin genes in mouse and human chromosomes by in situ hybridization to metaphase chromosomes. The results are presented in this paper.

Results

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

EcoRI digests of total mouse and human DNAs, and of their major components, were hybridized with P-labeled DNA. Autoradiograms of the hybridized DNA fragments were obtained using X-ray film. The autoradiograms were then scanned with a densitometer, and the intensity of the bands was quantitated by computer. A typical autoradiogram is shown in Figure 1. The bands were categorized according to their size and intensity, and the number of bands was determined by visual inspection. The distribution of actin genes on the major components of mouse and human DNAs is shown in Figure 2. The data are expressed as the percentage of the total intensity of the bands corresponding to actin genes. The results indicate that actin genes are distributed on all major components of mouse and human DNAs. The distribution of actin genes on the major components of mouse and human DNAs is shown in Figure 3. The data are expressed as the percentage of the total intensity of the bands corresponding to actin genes. The results indicate that actin genes are distributed on all major components of mouse and human DNAs. The distribution of actin genes on the major components of mouse and human DNAs is shown in Figure 4. The data are expressed as the percentage of the total intensity of the bands corresponding to actin genes. The results indicate that actin genes are distributed on all major components of mouse and human DNAs. The distribution of actin genes on the major components of mouse and human DNAs is shown in Figure 5. The data are expressed as the percentage of the total intensity of the bands corresponding to actin genes. The results indicate that actin genes are distributed on all major components of mouse and human DNAs. The distribution of actin genes on the major components of mouse and human DNAs is shown in Figure 6. The data are expressed as the percentage of the total intensity of the bands corresponding to actin genes. The results indicate that actin genes are distributed on all major components of mouse and human DNAs. The distribution of actin genes on the major components of mouse and human DNAs is shown in Figure 7. The data are expressed as the percentage of the total intensity of the bands corresponding to actin genes. The results indicate that actin genes are distributed on all major components of mouse and human DNAs. The distribution of actin genes on the major components of mouse and human DNAs is shown in Figure 8. The data are expressed as the percentage of the total intensity of the bands corresponding to actin genes. The results indicate that actin genes are distributed on all major components of mouse and human DNAs.

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 (Davis et al., 1981). An alternative method, which can pro-

viding 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; Thery et al., 1976; Maeya et al., 1975; Cury et al., 1981). In the case of the mouse genome, the major components have buoyant den-

sities of 1.69, 1.70, 1.74, and 1.70 g/cm³ and represent 22%, 24%, 24%, and 7% of the DNA, respectively. In the case of the human genome, the densities are 1.69, 1.70, 1.74, and 1.70 g/cm³ and the relative amounts are 24%, 23%, 22%, and 9%, respectively (Cury et al., 1981). These compo-

nents are families of DNA fragments derived from regions of relatively homogeneous base composition, the X chromosomes, which are estimated to be at least 30 kb in size and which may correspond to chromosomal bands (Maeya et al., 1975; Cury et al., 1981). The presence of individual members of the multigene family on several components will therefore strongly suggest that they 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 (Garholt 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 of a hamster muscle cDNA for coding for α-actin (Dodemont et al., 1982). The results indicate that the actin genes, present at 34 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.
In situ hybridization

454-labeled pAcT was hybridized to mouse and human metaphase chromosome preparation under conditions which promote the formation of probe networks (Gerhardt et al., 1981; Wetmur, 1975). As a control for background due to pBR322 vector and to estimate the signal expected for a unique gene, a single copy human DNA clone, pAW (kindly provided by R. White; Wyman and White, 1981) was hybridized to metaphase chromosomes from; (a) A9 HRBC (Miga et al., 1971), a mouse/human hybrid cell line which contains a human X chromosome (Figure 2A); and (b) G4874 (Ghosh and Pallister, 1973), a human fibroblastic cell line. Under hybridization conditions used, pAW yields a few silver grains and occasionally one or two clusters of silver grains per metaphase cell. In the case of the human cells, it was possible to show that this pAW sequence is located near the telomere of the long arm of chromosome 14 (data not shown).

Human chromosomes from both cell lines were annealed with pAcT-1 probe (Figure 2B and 2C), a strikingly higher number of grains and grain clusters was observed. A comparison of the number of silver grains over interphase cells hybridized with either probe showed that the actin probe gave 18-20-fold more grains per cell (29.5 grains/day/cell versus 1.5 grains/day/cell) than pAW at the same concentration. Thus, a minimum difference since the grains over the actin gene were observed as large clusters even at short exposure times (C ≈ 4 days) leading to a systematic underestimation of the number of grains because of coincidence of silver grain deposition (Gerhardt et al., 1981; Szabo et al., 1977).

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

Fig. 1. Distribution of actin genes in mouse and human DNA components. Total mouse and human DNA (1.4-2.8 ng) and thr major chromosome (3.0-3.7 ng) were digested with EcoRI, fractionated on 0.8% agarose gel, transferred to nitrocellulose filters, and hybridized with nick-translated pAcT. Filters were exposed to x-ray film for 10 days. An autoradiogram is shown in the lower panel. A, human DNA: lane 1, 30 µg/mL probe concentration; lane 2, 15 µg/mL probe concentration; lane 3, 7.5 µg/mL probe concentration. B, mouse DNA: lane 1, 30 µg/mL probe concentration; lane 2, 15 µg/mL probe concentration; lane 3, 7.5 µg/mL probe concentration. C, human DNA (lanes 1 and 2) and mouse DNA (lanes 3 and 4) digested with HindIII and hybridized with nick-translated pAcT. A, 30 µg/mL probe concentration; B, 15 µg/mL probe concentration; C, 1.5 µg/mL probe concentration.

Fig. 2. In situ hybridization: A9HRBC metaphase chromosomes were hybridized with the single-copy human clone pAW (lane 1) and with pAcT-1 (lane 2). A, 60 ng/ml probe concentration; exposure time 2 days. G4874 chromosomes were hybridized with metaphase and interphase pAcT-1 ICs, at 30 ng/ml probe concentration; exposure time 6 days.
As a more accurate measure of the significance of labeling at a site, Table I summarizes our observations on 11 metaphase cells hybridized with 3H-labeled pAct-1. Listed are preliminary actin gene chromosome assignments, within the resolution afforded by autoradiography, and the frequencies of the labeled centromere. The frequency of clusters at each labeled site varied between 0.13 and 0.63, outside of the regions indicated in the table. In this and other studies, the frequency of labeling was ~0.03 for uniformly sized metaphase plates. One chromosome of the G group chromosomes 21 and 22, and Y chromosome were the only clearly unlabelled human chromosome. It is important to note that the labeled centromere and the remaining seven sites are classified as weak sites (frequency 0.03). One strong site was localized on the long arm of the X chromosome. Since many of the strong actin sites were more significantly labeled than any other, we conclude that each of these sites represents either a single actin gene or several closely clustered actin genes, and is homologous 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 actin gene loci of the A9 mouse cell line (Miller et al., 1971; Alderfer et al., 1973) present in A9/HBCC2 included on one chromosome (A9-HBCC2) which was labeled at homologous positions on the chromosomes arms allowing us to assign to a specific mouse chromosome, including chromosomes 3, X, and 18. In addition, the human X chromosome, which 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 grains per cell per chromosome. 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 equal to that observed for the human preparations (0.39) and that the A9/HBCC2 cell line is essentially triploid (Miller et al., 1971), we can estimate that the actin gene copy number is ~30 (35.4/0.39), 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 mouse 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 in immobilized DNA on filters (Wahl et al., 1979). At higher concentrations (100 – 200 ng/ml) non-specific background grain clusters become a pro-
Figure 4 shows the restriction maps of the three isolates of mouse actin genes, JAM62, JAM85, and JAM98. 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 DNA to total mouse DNA cleaved by EcoRI or BamHI, and transferred to nitrocellulose filters (Figure 5). JAM62 contained a member of the BamHI family of interspersed repeats (Meunier-rottiau et al., 1981) and both JAM62 and JAM98 gave hybridization patterns similar to those obtained with BI repeats (Krayev et al., 1981).

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 very cosmochromosomes. 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 DNA derive from chromosomal stretches characterized by a fairly high degree of base composition and a length of about 300 kb, in these chromosomes (Macaya et al., 1976; Curny et al., 1981), the actin gene distribution on the four components is consistent 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), where they are only found on one or two components (data not shown). The scattering of the actin genes was further determined on metaphase chromosome spreads, since most of these were found to contain an actin gene site. The total number of actin genes can be estimated on the basis of in situ hybridization results, which revealed 29 and 20 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, strongly suggesting that these sites are distinguished by gene sequences according to their sequence divergence. The number of gene sites found by in situ hybridization is similar to the total number of linked phage fragments found in the human and mouse components; this may be the result of a compensation between factors which tend to underestimate the number of genes (in the case of several genes on one large DNA fragment) and the fact that components may not resolve all actin genes fragments, especially if they 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 EcoRI bands observed on mouse DNA only gives an estimate of the number of actin genes, and is on the definitive number, as suggested (Humphries et al., 1981).

In situ hybridization results strongly suggest that, in the mouse actin genes site dispersed, perhaps completely, into the genome, and that the genes do not correspond to clusters of wester oocytes.
In preliminary experiments in which human chromosomes were prepared for karyotyping and hybridized without further denaturation, only a subset of the active gene sites were as clearly labeled as with uniformly denatured sites (e.g., the gene sites for Hpa II, Hae III, and chromosome 8, 19, or 20). In these experiments, the labeling frequency at most sites was reduced and no significant signal was observed in the X chromosome. The presence of active genes on both X and Y bands suggests that these genes are embedded in a transposable repeat with unique TE sequences. Since there is evidence that the Y chromosome forms, in part, from the X chromosome, the results are in agreement with previous results in all three major primate groups of which data are available. The implication that such a process occurred in the terminal half of the arm of the human X chromosome, the terminal half of this process, and the coding sequence for the transposable repeat will be discussed in the following sections.

Materials and methods

DNA isolation

The main components of mouse DNA were prepared by CsCl-BEUD extraction and purification using radioactive bone (Snedecor and Miller, 1967), and purified as described above. The preparation of the DNA was performed as described previously (Forman et al., 1967). The DNA was isolated from mouse bone, using CsCl-BEUD purification.

Electrophoresis

Restriction endonucleases were purchased from New England Bio-labs, Inc. MAE was prepared by A. M. Miller and M. H. Miller. Induction of the human X chromosome was performed using the human X chromosome. The human X chromosome was placed in the X chromosome of the mouse bone, using CsCl-BEUD purification.

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