Localization of the Casein Gene Family to a Single Mouse Chromosome

P. GUPTA, J. M. ROSEN, P. D'EUSTACHIO, and F. H. RUDDLE
Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030; and Biology Department, Yale University, New Haven, Connecticut 06520

ABSTRACT A series of mouse-hamster somatic cell hybrids containing a variable number of mouse chromosomes and a constant set of hamster chromosomes have been used to determine the chromosomal location of a family of hormone-inducible genes, the murine caseins. Recombinant mouse cDNA clones encoding the α-, β-, and γ-caseins were constructed and used in DNA restriction mapping experiments. All three casein cDNAs hybridized to the same set of somatic cell hybrid DNAs isolated from cells containing mouse chromosome 5, while negative bybridization was observed to ten other hybrid DNAs isolated from cells lacking chromosome 5. A fourth cDNA clone, designated pCM δ40, which hybridized to an abundant 790 nucleotide poly(A)RNA isolated from 6-d lactating mouse mammary tissue, was also mapped to chromosome 5. The chromosomal assignment of the casein gene family was confirmed using a mouse albumin clone. The albumin gene had been previously localized to mouse chromosome 5 by both breeding studies and analogous molecular hybridization experiments. An additional control experiment demonstrated that another hormone-inducible gene, specifying a 620 nucleotide abundant mammary gland mRNA, hybridized to DNA isolated from a different somatic cell hybrid line. These studies represent the first localization of a peptide and steroid hormone-responsive gene family to a single mouse chromosome.

The caseins are a group of phosphoproteins whose synthesis and secretion are regulated by both peptide and steroid hormones (1). These milk proteins are the predominant proteins synthesized in the mammary gland during lactation and occur in milk as micellar complexes with calcium phosphate (2). The primary sequences of the four bovine caseins, designated α_{s1}, α_{s2}, β, and κ, have been determined (3, 4, 5). Sequence homology between the bovine α- and β-caseins, especially in the 15 amino acid signal peptide sequence, suggests that they may have evolved from a common ancestral gene (5–7). Consistent with this hypothesis, bovine α- and β-casein genetic polymorphisms are tightly linked (8, 9).

The hormonal induction of casein messenger RNA (mRNA) in murine mammary gland organ culture is a unique model system for studying peptide hormone regulation of gene expression and its modulation by steroid hormones (10, 11). Further, the large and rapid response to hormonal stimulation (>10-fold induction in 24 h) makes the system useful for investigating the role of transcriptional and post-transcriptional processes in regulating specific mRNA accumulation (12).

To determine whether the coordinate expression of casein genes in this system reflects a *trans* (13–15) or *cis* (16, 17) regulatory control mechanism, it is crucial to determine the chromosomal localization(s) of these genes. Individual complementary DNA (cDNA) clones for the three major rat caseins (designated α, β, and γ) from a rat mammary gland cDNA library have recently been isolated, characterized, and used to generate selective hybridization probes for studies of the structure, organization, and expression of the individual rat casein genes (18–20). Cross-hybridization between these rat probes and mouse genomic DNA sequences was weak, however, and we have used these probes to identify homologous mouse cDNAs. We then used the mouse probes to analyze genomic DNA from a panel of mouse × Chinese hamster somatic cell hybrids that allows an 18-fold division of the mouse genome (21–24). Three such casein genes can thus be identified and mapped to chromosome 5, as was the albumin gene (22).

MATERIALS AND METHODS

The somatic cell hybrids used in this study, their growth and karyotype analysis, and the isolation of high molecular weight DNA have been described in detail elsewhere (21–24). Somatic cell hybrids were formed between the Chinese hamster cell line E36 and either peritoneal macrophages from A/HeJ mice (MACH hybrid series), fibroblasts from BALB/c fetal mice (BEM hybrid series), cells from a tissue culture-adapted subline of Meth A murine fibrosarcoma (MAE hybrid series), or cells from the murine cell lines CT11c (hybrid ECm4e) or C1 1DH3 (hybrid R44-1). Karyotype analysis was performed using the sequential Giemsa-Viokase-Hoechst 33258 technique, and the hybrids were tested for the presence of 18 mouse isoenzyme markers whose chromosomal locations are known (24). DNA was digested with the restriction endonuclease *Eco*RI, and 30 µg was fractionated per track by electrophoresis on a 1% agarose gel in the presence of a *Hind* III digested λC*I*857 DNA standard (24). Fractionated DNA was transferred to nitrocellulose filters by the procedure of Southern (25) and hybridized with *Hha* I cleaved, cloned cDNA fragments labeled by nick translation with [^{32}P]deoxycytidine and [^{32}P]deoxyadenosine triphosphate (>400 Ci/mmol) to a specific activity of between 100 and 150 cpm/pg. Hybridizations were performed in a sealed bag using between 15 and 20 × 10^6 cpm/8–10 ml per filter for 12–16 h in the presence of 10% dextran sulfate as described by Wahl et al. (26). The filters were washed under stringent conditions as previously described (27, 28). A final wash in 10 mM Tris base was occasionally used to reduce nonspecific background radioactivity (29). Autoradiography was performed for 7–10 d at −80°C, using Kodak XR film and Dupont Quanta II screens. DNA blots were reused after washing at 68 °C for 30 min in 10 mM Tris base (29).

Recombinant cDNA clones were constructed using poly(A)RNA isolated from 6- to 8-d lactating mouse mammary tissue essentially as described by Richards et al., (18) with the omission of the cDNA fractionation by sucrose gradient centrifugation. The corresponding rat casein α-, β-, and γ- inserts isolated by *Pst* I or *Hpa* II digestion and sucrose gradient centrifugation were used in colony hybridization experiments to identify cDNA clones containing the homologous mouse α-, β-, and γ-casein inserts, designated pCMα11, pCMβ13, and pCMγ19, respectively. Two other abundant mRNA, mammary gland-specific cDNA clones, designated pCMδ40 and pXMI-14, were isolated and characterized by RNA blot hybridization (18). The detailed characterization of the five mouse mammary gland cDNA clones will be presented elsewhere (Gupta and Rosen, manuscript in preparation). A mouse albumin cDNA, pmalb2, containing a 700 base pair *Hind* III insert was kindly provided by Dr. Shirley M. Tilghman, Institute for Cancer Research, Fox Chase, PA (30).

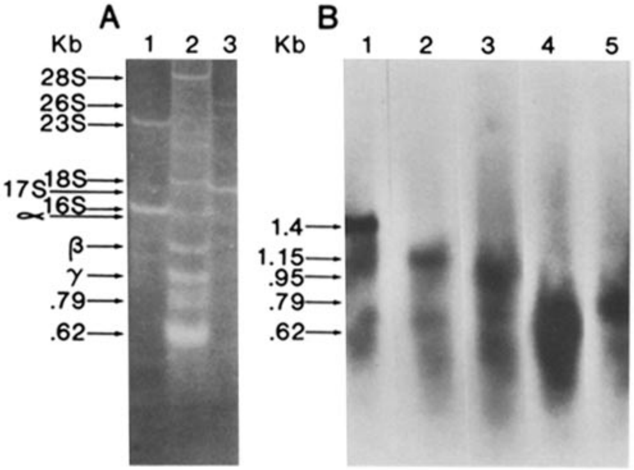

FIGURE 1 Analysis of mouse mammary gland cDNA clones by RNA blot hybridization. (*A*) lane *1*, *E. coli* rRNA standards; lane *2*, Poly(A)RNA (7 µg/lane) isolated from mice at 6–8 d of lactation was electrophoresed on 2% agarose gels containing 10 mM CH_3HgOH and visualized by staining with ethidium bromide; lane *3*, yeast rRNA standards. (*B*) Replicate tracks of RNA shown in *A* were transferred to diazobenzyloxymethylcellulose paper and hybridized with 5–10 × 10^6 cpm of ^{32}P-labeled cloned cDNA labeled by nick translation to a specific activity of between 50 and 100 cpm/pg. Lane *1*, pCMα11, lane *2*, pCMβ13, lane *3*, pCMγ19, lane *4*, pXMI-14, and lane *5*, pCMδ40. Autoradiography was performed for 16–24 h using Kodak XR film and Dupont Quanta II intensifying screen.

RESULTS

We initially attempted to use cloned rat casein cDNA probes to analyze DNA extracted from mouse × Chinese hamster somatic cell hybrids, using the technique of DNA blot hybridization. Only the rat β-casein cDNA clone displayed sufficient homology under the stringent conditions required in these total DNA mapping experiments to hybridize with fractionated mouse DNA, however. To construct homologous mouse casein cDNA clones, cloned rat casein cDNAs were used under less stringent conditions to select homologous mouse recombinants, using the technique of colony hybridization. The identity of each mouse cDNA clone was then confirmed by DNA and RNA blots, a positive translational analysis of cDNA-selected mRNA, and restriction endonuclease mapping.

A representative RNA blot hybridization is shown in Fig. 1. A sample of total poly(A)-containing RNA isolated from 6- to 8-d lactating mouse mammary tissue was electrophoresed on a 2% agarose gel containing 10 mm CH_3HgOH. As illustrated in Fig. 1*A*, eleven prominent RNA bands were visualized by staining with ethidium bromide, including ones of 1,400, 1,150, and 950 nucleotides that specify the three major mouse caseins. These hybridized specifically with the mouse cDNA clones pCMα11, pCMβ13, and pCMγ19, respectively (Fig. 1*B*, lanes *1*, *2*, and *3*). The indistinct hybridization beneath each band probably reflects partial degradation of these major abundant mRNAs during isolation or electrophoresis, and not specific cross-hybridization with distinct minor mRNA species. To visualize the mRNAs by ethidium bromide staining, the gels were overloaded relative to the sensitivity of the RNA blot hybridization.

Two additional clones were isolated from the mouse cDNA library. Clones pCMδ40 hybridized to the 790 nucleotide RNA seen in the ethidium bromide–stained gel (Fig. 1*A*, lane *2*, and *B*, lane *5*), and clone pXMI-14 hybridized to the 620 nucleotide RNA (Fig. 1*A*, lane *2*, and *B*, lane *4*). Homologous RNAs have also been identified in poly(A)RNA isolated from the rat lactating mammary gland, although clone pCMδ40 hybridizes to an 840 nucleotide RNA in the rat. The protein encoded by clone pCMδ40 has not yet been identified. Clone pXMI-14 is homologous to a rat cDNA clone originally designated pLA32 (18), which selectively arrests the translation of a rat mRNA coding for a 20,000-dalton novel whey protein, and which is not α-lactalbumin (34; Hobbs, Hennighausen, Sippel, and Rosen, manuscript in preparation).

We then used these homologous cDNA clones to determine the chromosomal assignment of the mouse casein gene family. The sensitivity of the total DNA mapping experiments was improved by increasing from 15 to 30 µg the amount of *Eco*RI-digested DNA electrophoresed on 1% agarose gels and transferred to nitrocellulose filters. When the probes were used to analyze mouse, Chinese hamster, and hybrid cell genomic DNAs, each hybridized with a characteristic set of mouse bands (Figs. 2–5). All bands detected in the mouse were found in the hybrids with no alterations in their sizes or relative intensities (although absolute intensities were reduced, consistent with the subhaploid gene number in the hybrid cell populations). The high backgrounds observed in these DNA blots are also a consequence of the reduced signal-to-noise observed during the long exposure times required to detect unique DNA fragments in hybrid cell populations containing a subhaploid gene number.

The mouse-specific α, β, pCMδ40, and γ-casein band sets were detected in two hybrids (Figs. 2*A* and *B* and 3, lanes *5* and *11*; Figs. 4*A* and 5*A* and *B*, lanes *4* and *5*). Nine other hybrids totally lacked these bands. The absence in the hybrid lines of the 7.3- and 1.5-kb (kilobase pair) β-casein bands (Fig. 2*B*, lane *1* vs. lanes *5* and *11*) observed in the parental mouse line probably reflects restriction enzyme polymorphisms frequently observed in many inbred mouse strains. The weak hybridization signal observed in Fig. 2*A* and *B*, lane *11*, and

Fig. *3*, lane *11*, was the result of an incomplete digest of that DNA sample, as was evidenced both by the ethidium bromide-stained gel profile (data not shown) and by the weak 3.1-kb hamster DNA band (Fig. 2*B*, lane *11*). Reuse of one of the DNA blots shown in Fig. 2 with the mouse γ-casein cDNA clone (pCMγ19) also revealed hybridization to the same two positive lines that were observed with the α- and β-casein probes (data not shown). The DNA sequences detected by these probes therefore reside on only a single mouse chromosome. Karyotypic analyses showed that the only mouse chromosome present in both positive hybrids and absent from the nine negative ones was number 5 (Table I). To confirm the assignment of the genes to chromosome 5, a duplicate filter was hybridized with a probe, pmalb2, specific for mouse albumin (Fig. 4*B*). Albumin has previously been mapped to chromosome 5, both in Mendelian analyses and by the techniques discussed here (22). The probe reacted with the expected 6.4-kb DNA fragment in mouse DNA and in the same two hybrids. All others were negative, confirming the assignment. Reuse of the DNA blots shown in Fig. 4*A* and *B* with the α- and β-casein probes indicated that positive hybridization occurred only with the *Eco*RI-digested hybrid DNAs shown in Fig. 4*A* and *B*, lanes *4* and *5*, as expected (Fig. 5*A* and *B*, lanes *4* and *5*).

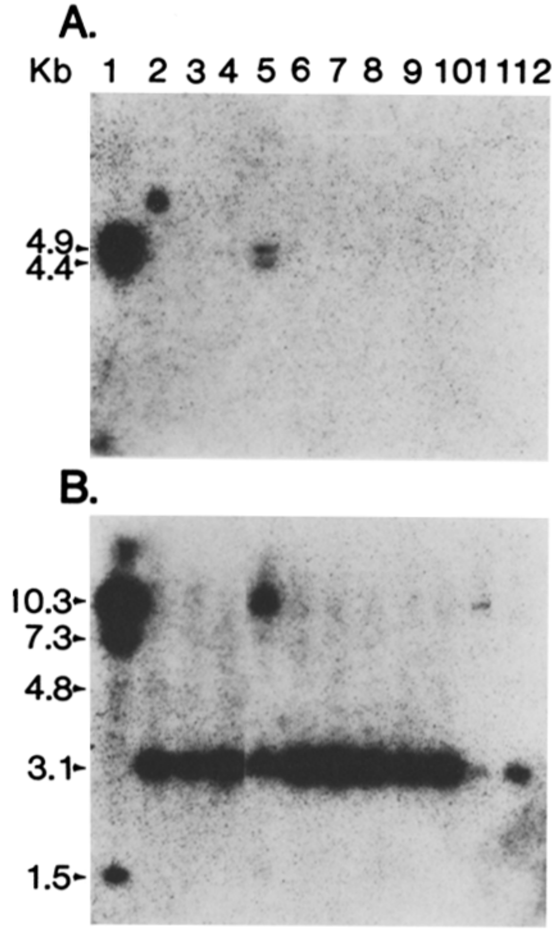

FIGURE 2 Chromosomal localization of the mouse α- and β-casein genes in mouse-hamster somatic cell hybrid DNA. Approximately 30 μg of *Eco*RI-digested DNA were electrophoresed on a 1% agarose gel and transferred to nitrocellulose filters as described by Swan et al. (21). Hybridization was performed with either the mouse α-casein cDNA (*A*) or β-casein cDNA (*B*) as described in Materials and Methods. DNA was isolated from the following cell lines: lane *1*, parental mouse line A-9; lane *2*, parental hamster line E36; lanes *3–13*, hybrid cells BEM 1-6, MACH 4A64A-1, MACH 7A13-3B3, MACH 4A63, MACH 4B31A23, MAE 28A, MAE 32, R44-1, BEM 1-4, and ECm4e, respectively. The weak hybridization signal observed in lane *11* in both *A* and *B* was the result of an incomplete digest of that DNA sample, as was evidenced both by the ethidium bromide-stained gel profile (not shown) and by the weak 3.1-kb hamster DNA band (*B*, lane *11*). A water condensation spot observed in lane *2* of *A* is the cause of the apparent hybridization signal. This spot is not the result of specific hybridization and is frequently encountered even in the best Southern's assays.

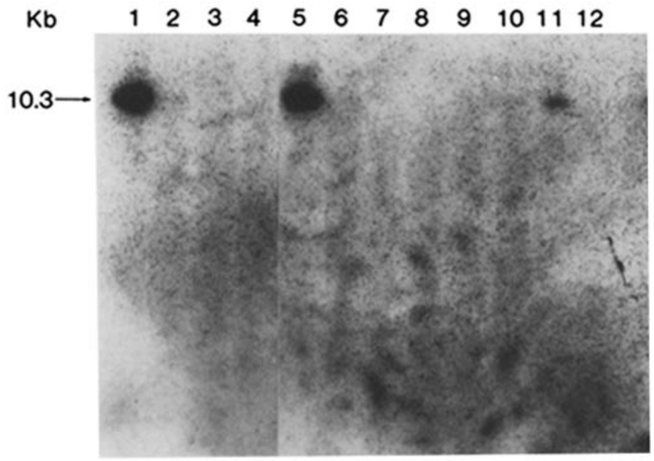

FIGURE 3 Chromosomal localization of the mouse mammary gland pCMδ40 gene in hybrid cell DNA. A DNA blot containing the identical samples described in the legend to Fig. 2 was hybridized to clone pCMδ40.

The fifth probe, pXMI-14, reacts with a 7.7-kb band in mouse genomic DNA. This fragment was not detected in any of the eleven hybrids, suggesting its possible assignment to chromosome 11, the only autosome absent from all members of our panel (its assignment to Y can be excluded by the presence of the gene and the gene product in female cells). We could detect the pXMI-14 fragment in a twelfth hybrid, MACH 3B9C4-1 (Fig. 4*C*, lane *9*). This hybrid also lacked an intact chromosome 11 but had undergone extensive chromosomal rearrangement. The possibility that it had retained portions of the chromosome in the form of translocation, therefore, cannot be excluded.

DISCUSSION

Rodent mammary tissue has been widely used in studies of the hormonal regulation of milk protein synthesis, but the proteins themselves and the genes that encode them remain poorly characterized. While the majority of the cell and molecular biological studies of casein gene regulation have been performed in the murine model systems, the casein proteins have been well-characterized only in bovine and other ruminant species. For comparative purposes, the α, γ, and β-murine caseins appear to be homologous to α_{s1}, α_{s2}, and β-bovine caseins, respectively (7). No rodent protein or cDNA clone has yet been identified that is analogous to the major bovine milk protein, κ-casein. Detailed sequence analysis of both the murine and bovine casein genes will be needed to define their relationship precisely. Here, we describe the generation of cDNA clones corresponding to murine α-, β-, and γ-casein mRNAs, and to two other mammary gland mRNAs of ~620 and 790 nucleotides, respectively. Each of the homologous mouse α-, β-, and γ-casein cDNA clones used in this study was selected with previously characterized rat cDNA clones (18, 19). Under the stringent conditions used in these studies the murine casein clones each hybridize to unique mRNA species and display no cross-hybridization (19). Using these clones as probes to analyze genomic DNA from a panel of mouse × Chinese hamster somatic cell hybrids containing various combinations of mouse chromosomes on a constant hamster background, we have mapped the α-, β- and γ-caseins and a fourth abundant mammary gland cDNA clone, pCMδ40, to chromosome 5. The relatively simple pattern observed in the total DNA blots shown in Figs. 2–5 also suggests that each of the murine caseins is encoded by one gene, or at most a few genes, and, regardless of the gene copy number, the entire gene family maps to chromosome 5. The hormone-regulated pXMI-14 gene maps elsewhere in the genome, possibly to chromosome 11.

We hypothesize that the cDNA clone pCMδ40 may also be a member of the casein gene family and we have tentatively designated this cDNA clone as β-like. Clone pCMδ40 both contains an internal *Pst* I site and hybridizes to murine genomic DNA fragments similar in size to those that contain the authentic murine β-casein gene. This putative β-like probe does not cross-hybridize with authentic α-, β- or γ-casein mRNAs or cDNA probes. The definitive confirmation of its identity will require detailed restriction mapping and sequence analysis of both genomic and cDNA clones. The second novel gene, defined by the pXMI-14 clone, has no detectable homology to any other murine milk protein gene. The facts that the pCMδ40 and pXMI-14 probes correspond to hormone-inducible, abundant RNA species of murine mammary tissue, and that at least pXMI-14 appears to have a polypeptide product, argue against their being pseudogenes.

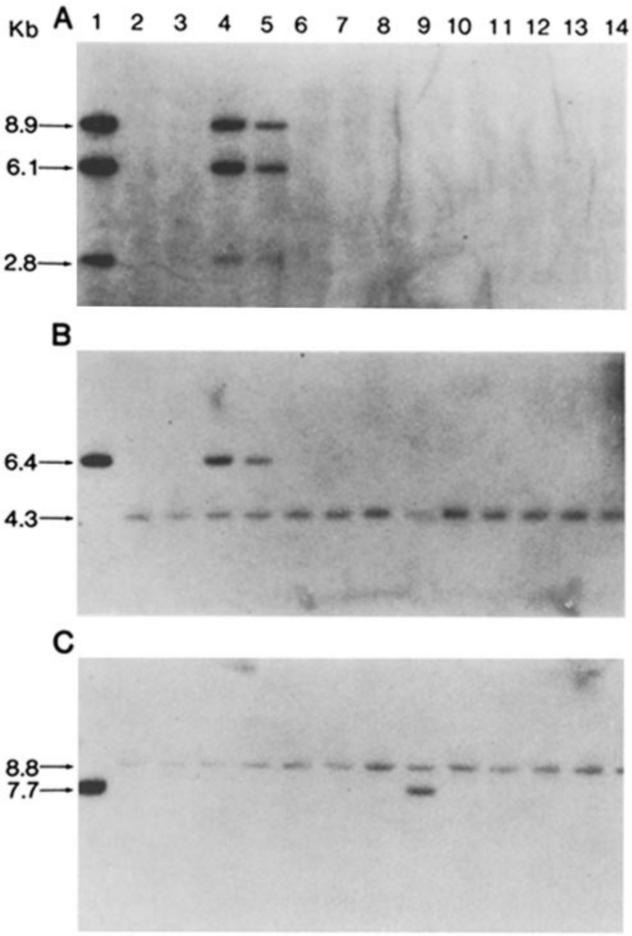

FIGURE 4 Chromosomal localization of the mouse γ-casein, albumin, and pXMI-14 genes. The hybridization probes were: (*A*) pCMγ19, (*B*) pmalb2, and (*C*) pXMI-14. The conditions of electrophoresis and hybridization were as described inthe legend to Fig. 2. The *Eco*RI-digested DNAs are shown in the following order on each filter: lane *1*, mouse cell line A9; lane *2*, hamster cell line E36; lanes *3–14*, hybrid lines BEM 1-6, BEM 1-4, MACH 7A13-3B3, MACH 4A63, MACH 4A64-A1, MACH 4B31A23, MACH 3B9C4-1, MACH 2A2, MAE 28A, MAE 32, ECm4e, and R44-1, respectively.

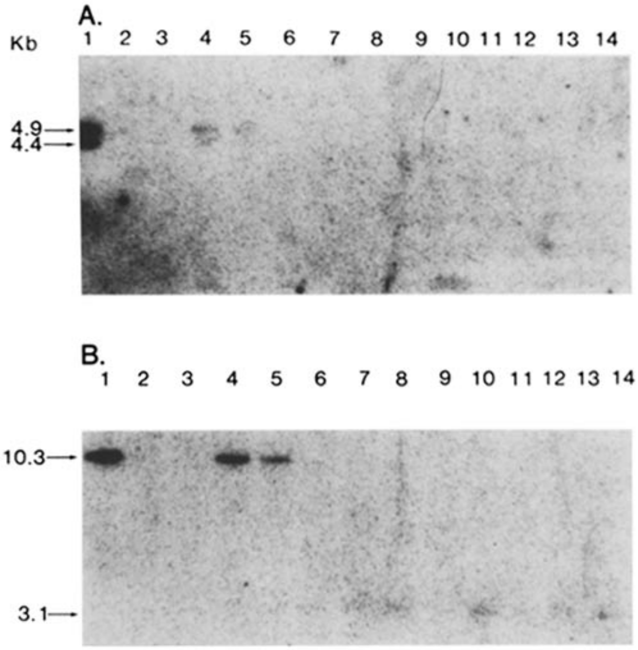

FIGURE 5 Reuse of DNA blots to map the α- and β-casein genes. The same DNA blots described in Fig. 4 *A* and *B* were rehybridized to pCMα11 (*A*) and pCMβ13 (*B*) as described in Materials and Methods. A reduction in signal intensity is observed routinely when DNA blots are reused as evidenced by the weaker signal observed in lanes *4* and *5* and the reduction in the hamster specific 3.1-kb band in *B*. Note that lane *4* contains hybrid line BEM 1-4, which gave the weaker signal in Fig. 2 *A* and *B*, lane *11*.

The assignment of four of these genes, α-, β-, and γ-caseins, and pCMδ40, to chromosome 5 is a straightforward continuation of previous mapping experiments (e.g., 21–25, 31, 32). Two observations buttress the assignment. The first is the exact correspondence in the panel between reactivity with these probes and reactivity with a cDNA probe corresponding to the albumin gene. The second is the preliminary result of a screen of an independently derived panel of mouse × hamster somatic cell hybrids provided by Drs. J. Hilgers, J. Hilkens, and A. Sonnenberg (33). In this panel a concordance of positive hybridization for the three casein and albumin genes in seven hybrid lines was observed, while thirteen other hybrids were negative for all four markers (Gupta, Rosen, Hilkens, Sonnenberg, and Hilgers, unpublished observations), supporting the assignment of the casein genes to mouse chromosome 5.

The assignment of genes encoding four functionally and structurally related proteins to a single chromosome raises the possibility that they are members of a multigene family. In cattle, genetic analysis of polymorphic caseins variants (8, 9) has suggested close linkage of these genes. Such experiments have not been carried out in rodents. In the rat, we have so far

TABLE I

Karyotype Analysis of Mouse-Hamster Somatic Cell Hybrids *

Mouse chromosome number	BEM 1-6	BEM 1-4	MACH 7A13-3B3	MACH 4A63	MACH 4A64-A1	MACH 4B31A23	MACH 2A2	MAE 28A	MAE 32	ECm4e	R44-1
1	+	+	−	−	+	−	+	−	−	−	−
2	+	+	+	+	+	+	+	−	−	−	−
3	+	+	−	−	−	−	+	−	−	−	−
4	+	−	−	−	−	−	−	−	−	−	−
5	−	+	+	−	−	−	−	−	−	−	−
6	+	+	−	−	−	−	+	−	−	−	−
7	−	−	+	+	+	+	+	−	−	−	−
8	+	+	−	−	−	+	+	−	−	−	−
9	+	−	+	−	−	−	+	−	−	−	−
10	+	+	−	−	−	−	+	−	−	−	−
11	−	−	−	−	−	−	−	−	−	−	−
12	+	+	+	+	+	−	+	+	−	−	−
13	+	+	+	+	−	−	+	−	−	−	−
14	+	+	+	−	−	−	+	−	−	+	−
15	+	+	+	+	+	−	+	−	−	+	−
16	+	+	−	+	−	+	+	−	+	−	−
17	+	+	+	+	+	+	+	−	−	−	+
18	+	+	+	+	−	−	+	−	−	−	−
19	+	+	+	+	+	+	+	−	−	−	−
X	+	+	−	−	−	−	+	+	+	−	−
Reaction with pCM α, β, γ, pmalb2, and pCMδ40 probes	−	+	+	−	−	−	−	−	−	−	−

* The frequency of each chromosome in the 11 hybrid lines used in the study is shown in reference 24. The data here are summarized as positive (+), chromosome present, or negative (−), chromosome absent. For a chromosome to be designated +, it had to have a frequency of ≥0.20.

isolated and characterized a total of 62 kb of genomic DNA fragments containing α-, β-, and γ-casein genes. No fragment yet characterized has contained more than part of one of these genes (Yu and Rosen, unpublished observations). This is not unexpected, given the large size of the genes, probably 17–25 kb each (20), and further gene walking experiments will be required to determine the location and orientation of these genes with respect to one another in the rat genome.

An intriguing possibility raised by these results is that the casein genes are in fact a portion of an extended family of developmentally regulated genes encoding secreted proteins. As noted above, α-fetoprotein and albumin have also been mapped to chromosome 5 in the mouse (22), as has the J protein, which mediates the polymerization of secreted IgA and IgM immunoglobulins (Yagi, D'Eustachio, Ruddle, and Koshland, unpublished observations). DNA and protein sequence data are incomplete, but there is as yet no evidence for homology among these proteins. At the same time, the large divergence known to have occurred between caseins (see above), or between α-fetoprotein and albumin (30), may be sufficient to explain the lack of homology. Homology might rather be preserved most faithfully at the level of the organization of intervening and coding sequences, and within only some of the coding sequences. Clearly, the crucial tests of this speculation will come as the organization of these genes in the genome is worked out and their sequences are determined.

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Note added in proof: Complete sequence analysis of both rat and mouse pXmRNAs has suggested that they encode a novel murine whey protein described recently by Piletz, J. E., M. Heinlen, and R. E. Ganschow, 1981. Biochemical characterization of a novel whey protein from murine milk. *J. Biol. Chem.* 256:11509–11516.

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