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Tse-2: a *trans*-Dominant Extinguisher of Albumin Gene Expression in Hepatoma Hybrid Cells

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Serum albumin gene expression is generally extinguished in hepatoma × fibroblast hybrids. To define the genetic basis of this phenomenon, we screened a panel of hepatoma hybrids retaining different fibroblast chromosomes for albumin production by immunofluorescence. We report that albumin extinction in these clones was strictly correlated with the retention of mouse chromosome 1. Furthermore, albumin was systematically reexpressed in chromosome 1 segregants. These data define a tissue-specific extinguisher locus (*Tse-2*) that affects albumin gene expression in *trans*. Two other liver genes, those encoding liver alcohol dehydrogenase and liv-10, were coordinately extinguished with albumin in monochromosomal hybrids that specifically retained mouse chromosome 1.

Expression of the serum albumin gene is developmentally regulated and tissue specific, and this locus has served as a paradigm of mammalian gene control for many years. The albumin and α-fetoprotein genes are products of gene duplication and divergence; they have remained tightly linked through 300 million years of vertebrate evolution (21, 27). Developmentally, albumin and α-fetoprotein are first expressed in embryonic visceral endoderm; several days later, transcription of both genes can be detected in fetal liver and the gut (45). α-Fetoprotein gene activity in both tissues decreases shortly after birth, whereas albumin expression continues to increase in liver, reaching maximum levels 7 to 10 days after birth. Albumin mRNA is one of the most abundant steady-state transcripts of adult liver, whereas extrahepatic tissues such as brain or kidney accumulate fewer than 100 molecules of albumin mRNA per cell (22).

Sequence elements of the albumin gene required for regulated expression have been identified by assaying the activities of mutant promoters in in vitro transcription reactions (6, 22, 29, 32), in transfected tissue-culture cells (20, 23, 36), and in transgenic animals (40). Sequences that confer tissue specificity seem to reside within 170 and 150 base pairs of the mouse and rat albumin cap sites (22, 23), and elements able to promote (20, 23) or repress (36) albumin transcription have been defined. In contrast to these proximal elements, DNA sequences required for maximal transcription are located far upstream of gene (40). Cell-specific sites of DNase I hypersensitivity in the albumin transcription unit have been described (1), and a number of different *trans*-acting factors that bind to the albumin promoter have been identified by biochemical tests. Both ubiquitous and cell-specific factors have been detected (2, 6, 12, 13, 32), and the functions of these molecules in regulating albumin transcription in vivo and in vitro are now being defined.

Expression of the serum albumin gene in cultured hepatoma cells, dedifferentiated hepatoma variants, and hepatoma hybrids has been studied for many years in attempts to define genetic factors involved in albumin gene control. As with most liver genes (9), albumin expression is repressed (extinguished) in intertypic hybrids and heterokaryons formed by fusing hepatic cells with other cell types (16, 18, 35). On the other hand, silent albumin genes of nonhepatic cells can be activated in hybrids with many hepatoma cell chromosomes, the first documented case of heterologous gene activation in mammalian cells (33, 37). Thus, functional studies provide further evidence that both positive and negative factors control albumin gene activity, but the nature of those genetic factors has yet to be defined. This may be particularly informative in view of the emerging biochemical data regarding albumin gene control.

We report here that albumin extinction in hepatoma hybrids has a specific genetic basis and define a particular genetic locus, *Tse-2*, that represses albumin gene activity in *trans*. We assign murine *Tse-2* to chromosome 1 and demonstrate that two other liver genes are regulated by extinguisher loci on that particular mouse chromosome.

MATERIALS AND METHODS

Cell lines and culture conditions. FAO-1, Fado-2, FT-1, and FTO-2B are drug-resistant hepatoma lines derived from H4IIEC3 as described elsewhere (25, 26). Mouse embryo fibroblast donor cells were prepared from C57BL/6J embryos or from translocation stocks with specific Robertsonian translocations (5, 19). These parental lines were used to generate hybrid and microcell hybrid clones (Table 1) whose properties have been reported elsewhere (25, 31, 38).

All cell lines were cultured in 1:1 Ham F12-Dulbecco modified Eagle medium containing 10% fetal bovine serum. Selective media contained either hypoxanthine-aminopterin-thymidine (HAT) or adenine-aminopterin-thymidine (AAT) for $HPRT^+$ TK^+ or $APRT^+$ TK^+ selection, respectively. Antibiotics were not used, and all cell lines were free of mycoplasma, as judged by staining with Hoechst 33258 (8).

Microcell fusions. F(1.11) series microcell hybrids were prepared in two steps, using donor cells from Wmp/Pas mice, a translocation stock whose karyotype consists entirely of Robertsonian translocations, including Rb(1.11). Peritoneal macrophages from Wmp adults were fused with TK^- L cells (Ltk^-) by using 50% polyethylene glycol 1540, and TK^+ hybrids retaining the Wmp-derived Rb(1.11) were selected in HAT. Polyclonal mixtures of HAT-resistant hybrids were then used as donors for microcell fusions with

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TABLE 1. Serum albumin expression in a hepatoma × fibroblast hybrid panel

Clone	Passage	Fraction of cells retaining given mouse chromosome																			Fraction of ALB$^+$ cells
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	
Hybrid																						
FF5-1	11	1.00	1.00	1.00	0.92	0.96	1.00	0.96	1.00	0.96	1.00	0.96	0.92	0.96	1.00	0.92	0.92	1.00	0.96	0.96	0.83	0.01
FF3-3	10	1.00	1.00	0.94	0.81	0.97	1.00	0.97	0.94	0.94	0.94	0.97	0.97	1.00	0.94	0.97	1.00	1.00	0.94	0.97	0.91[a]	0.02
FF1-9	6	0.66	0.60						0.87	1.00	0.80	0.90		0.87			0.93					0.24
FF1-9	14	0.22				0.22	0.60	0.27	0.57	0.77			0.22	0.22		0.77	0.55			0.44	0.33	0.83
FF4-3	5								0.47	0.47	0.33		0.47	0.40	0.53	0.67	0.27		0.60	0.40	0.67	0.92
FF4-3	23									0.40	0.40			0.47	0.80	0.73			0.40		0.80	0.99
Microcell hybrid																						
F(2.8)D	9		0.93[b]						0.93[b]													0.98
F(3.8)8-6	6			1.00[b]					1.00[b]													0.98
F(4.11)A	10				0.76[b]							0.83[b]										0.94
F(7.8)A	3							0.88[b]	0.88[b]													0.97
F(8)D	8								1.00[a]													0.94
F(8.9)A	2								0.96[b]	0.96[b]												1.00
F(10.11)E	14										0.64[b]	0.75[b]										1.00
F(11)J	7											0.91[a]										1.00
F(11)Y	7									0.76		0.83[a]										1.00
F(11)O	7											0.80[a]		0.74		0.50						0.93
F(11)G	3						0.66					0.86[a]		0.71	0.79	0.83						1.00
F(11.13)B	4						0.74				0.48	1.00[b]		1.00[b]		0.63	0.69					0.95
F(8.14)1	9								0.96[b]				0.55	0.59	0.96[b]							0.99
F(X)A	4																				0.81[a]	0.99

[a] Chromosome selectively retained.
[b] Chromosome selectively retained as Robertsonian translocation.

FTO-2B rat hepatoma recipients as described previously (25). TK^+ microcell hybrids were selected in medium containing HAT plus 3 mM ouabain.

F(3.8,X) microcell hybrids, rat hepatoma cells containing a murine Rb(3.8) translocation plus the mouse X chromosome, were generated by fusing microcells from diploid C57BL/6J fibroblasts with a microcell hybrid [F(3.8)8-6] that selectively retained Rb(3.8). $HPRT^+$ clones were selected in HAT.

Cytogenetic Analyses. Fixed metaphase spreads were analyzed by using a sequential staining protocol in which Giemsa-trypsin banding was followed by Hoechst 33258 centric heterochromatin staining (25).

Indirect immunofluorescence assay for albumin production. The method of Mevel-Ninio and Weiss (35) was used. Cells (1×10^5 to 5×10^5) were plated onto 12-mm-diameter glass cover slips and incubated for 24 h. The cover slips were rinsed in phosphate-buffered saline (PBS) and fixed in ice-cold 3% formaldehyde for 1 min. Two equal volumes of methanol were added, the fixative was removed, and the cover slips were dehydrated in absolute methanol for 20 min. The cover slips were rinsed in PBS and incubated with rabbit anti-rat albumin (1:200; Organon Teknika) for 30 min. After a rinse in PBS, the cover slips were incubated with fluorescein-conjugated secondary antibody (sheep anti-rabbit immunoglobulin G; Organon Teknika) as described above. The cover slips were mounted with 50% glycerol in PBS and viewed under epifluorescence illumination with a Zeiss photomicroscope.

DNA blot hybridization. Genomic DNA from parental and hybrid cells was digested to completion with *Eco*RI, fractionated on 0.7% agarose gels, and transferred to Zetabind membranes in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) by the method of Southern (43). Plasmids a_1p_1 (44), Hf677 (11), GLC1 (41), pPS1.25 (4), r49 (10), and M*a*Sp1 (24), containing sequences from the *Hba*, *Col1A1*, *Myl-1*, *Emv-17*, *Ren-2*, and *Sph* loci, respectively, were labeled with ^{32}P to 10^9 cpm/μg by random hexamer-primed synthesis and hybridized to membrane-bound DNA as described elsewhere (46). The filters were washed sequentially in 2× SSC–0.1% sodium dodecyl sulfate (15 min, room temperature) and 0.1× SSC–0.1% sodium dodecyl sulfate (15 min, room temperature) and twice in 0.1× SSC–0.1% sodium dodecyl sulfate (30 min, 60°C). Autoradiography was for 1 to 4 days at −70°C, using Kodak XAR film (Eastman Kodak Co.) with a single intensifying screen.

RNA blot hybridization. Cytoplasmic RNA was fractionated on formaldehyde-agarose gels, transferred to Zetabind, and hybridized with radiolabeled probes as described previously (9). Cloned DNA sequences from the α-tubulin (Kα-1 [14]), serum albumin (pRSA57 [42]), alcohol dehydrogenase (ADH) (pZK6-6 [17]), phosphoenolpyruvate carboxykinase (PEPCK) (pPCK-10 [47]), transferrin (pliv6), liv-7 (pliv-7), liv-9 (pliv-9), and liv-10 (pliv-10) (15) loci were used as probes.

RESULTS

Albumin expression in a hepatoma hybrid panel. We used an indirect immunofluorescence assay to screen hybrid cell lines for expression of the serum albumin gene. This assay allowed us to score albumin production on a single-cell basis; thus, extinguished cells could be detected even in phenotypically heterogeneous hybrid populations. Typical phase-contrast and fluorescence images of stained cells are shown in Fig. 1. Albumin-specific immunofluorescence in albumin-expressing (ALB^+) cells was perinuclear, representing albumin polypeptides in the Golgi apparatus prior to secretion (35). For parental hepatoma cells (Fig. 1A and B), >95% of cells stained positive in this assay. In contrast, fibroblasts (Fig. 1C and D) and extinguished hepatoma hybrids (Fig. 1E and F) failed to exhibit albumin-specific immunofluorescence.

A panel of rat hepatoma × mouse fibroblast hybrids and microcell hybrids was screened for albumin production in an attempt to correlate albumin extinction with retention of a particular fibroblast chromosome. The karyotypes and albumin phenotypes of these clones are summarized in Table 1.

Karyotypically complete hepatoma × fibroblast hybrids (FF5-1 and FF3-3) were extinguished for serum albumin expression: these populations contained <2% albumin-positive cells (Table 1). In marked contrast, all other hybrid and microcell hybrid clones assayed consisted predominantly of ALB^+ cells. Of these clones, 14 were microcell hybrids that, as a group, selectively retained 11 different mouse chromosomes (2, 3, 4, 7, 8, 9, 10, 11, 13, 14, and X). In addition, six other mouse chromosomes (6, 12, 15, 16, 18, and 19) were nonselectively retained at frequencies of >40% in individual hybrid clones. As >90% of cells in each hybrid population expressed the serum albumin gene, these data eliminate 17 of the 20 mouse chromosomes as potential sites of an albumin extinguisher locus.

Albumin extinction in F(1.11) microcell hybrids. Mouse chromosomes 1, 5, and 17 were not well represented in the hybrid panel described above and remained potential sites of an albumin extinguisher locus. To determine whether loci on chromosome 1, 5, or 17 were involved in albumin extinction, we sought to generate hybrids that specifically retained each of these murine chromosomes.

Wmp/Pas mice contain many different Robertsonian translocations, including Rb(1.11), a chromosome that can be fixed in TK^- cultured cells by selecting for chromosome 11-encoded murine thymidine kinase. We transferred the Rb(1.11) translocation from Wmp/Pas donor cells to rat hepatoma recipients by the two-step procedure outlined in Materials and Methods. Twenty-six independent F(1.11) series hybrids were obtained and assayed for albumin expression by immunofluorescence. Twenty-two of these clones expressed albumin in >80% of the cells, but four of them were extinguished for albumin expression.

The F(1.11) hybrids were screened for retention of the Rb(1.11) translocation by staining fixed metaphase spreads with Hoechst 33258. Under these conditions, the translocation was apparent as a large metacentric chromosome with highly fluorescent constitutive heterochromatin on each side of the centric constriction. Surprisingly, most of the hybrids had deleted most of chromosome 1: they retained only the chromosome 11 arm that conferred HAT resistance. In

FIG. 1. Indirect immunofluorescence analysis of albumin expression in parental and hybrid cells. Fixed cells were stained as described in Materials and Methods and photographed under phase-contrast (A, C, E, G, and I) or epifluorescence (B, D, F, H, and J) illumination. Parental hepatoma cells (FTO-2B [B]) displayed albumin-specific immunofluorescence that was not apparent in mouse embryo fibroblasts (D) or hepatoma × fibroblast hybrids (FF5-1) [F]). Albumin immunofluorescence was extinguished in microcell hybrid F(1.11)J (H) but reexpressed in its backselectant FB(1.11)J (J). Note the perinuclear staining in positive cells.

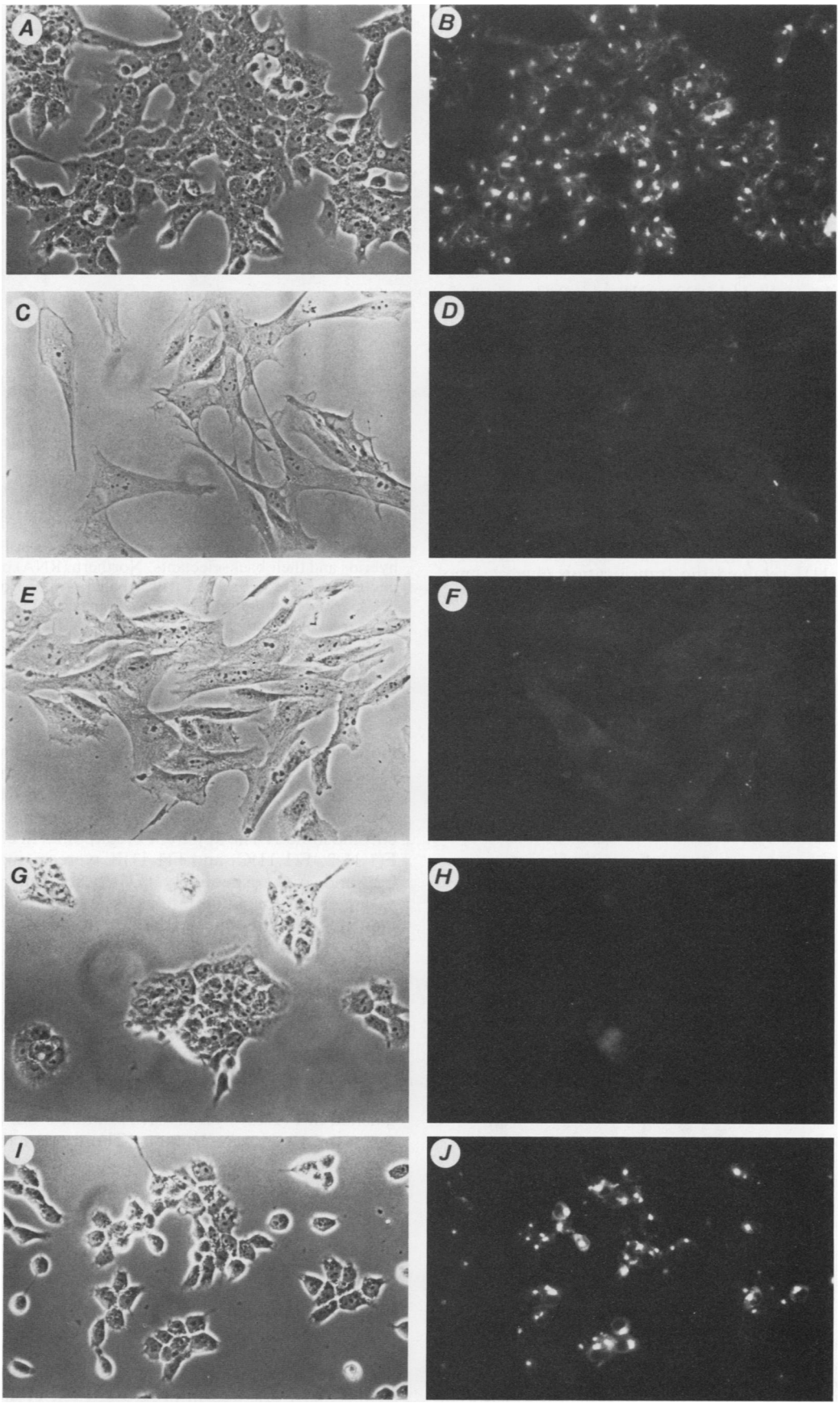
A
B
C
D
E
F
G
H
I
J

TABLE 2. Properties of F(1.11) series microcell hybrids and their backselectants

Clone	Passage	Mouse chromosome selectively retained	Fraction of cells	Fraction of ALB$^+$ cells
F(1.11)A	1	Rb(1.11)	0.92	<0.01
FB(1.11)A	5	None		0.85
F(1.11)J	2	Rb(1.11)	0.94	0.04
FB(1.11)J	5	None		0.81
F(1.11)8	1	Rb(1.11)	0.78	0.14
FB(1.11)8	5	None		0.79
F(1.11)6	1	Rb [del 1(E3-ter):11]	0.92	0.08
FB(1.11)6	6	None		0.90
F(1.11)2	1	Rb [del 1(D-ter):11]	0.87	0.90
FB(1.11)2	4	None		0.91
F(1.11)C	2	Rb [del 1(C2-ter):11]	0.83	1.0
F(1.11)K	3	Rb [del 1(A1-ter):11]	0.92	1.0

marked contrast, the four extinguished clones retained both arms of the translocation.

The properties of several F(1.11) hybrids are summarized in Table 2. Three of these clones retained Rb(1.11) in an intact and apparently unrearranged form in 80 to 90% of the cells (Fig. 2). F(1.11)A was a polychromosomal hybrid that retained several different mouse chromosomes in addition to Rb(1.11), F(1.11)J retained the translocation plus chromosome 9 in 30% of the cells, and F(1.11)8 was monochromosomal and retained Rb(1.11) alone. Each hybrid contained DNA sequences from the murine *Hba* and *Col1A1* loci, located on chromosome 11, and from the chromosome 1 loci *Myl-1*, *Emv-17*, *Ren-2*, and *Sph* (data not shown). These hybrid populations were extinguished for serum albumin expression, as judged by immunofluorescence (Fig. 1G and H), but albumin was reexpressed in backselectant cells that had segregated Rb(1.11) (Fig. 1I and J). The backselectants no longer contained marker genes from either mouse chromosome 1 or 11. Thus, the albumin extinction phenotype of these clones segregated with the Rb(1.11) translocation.

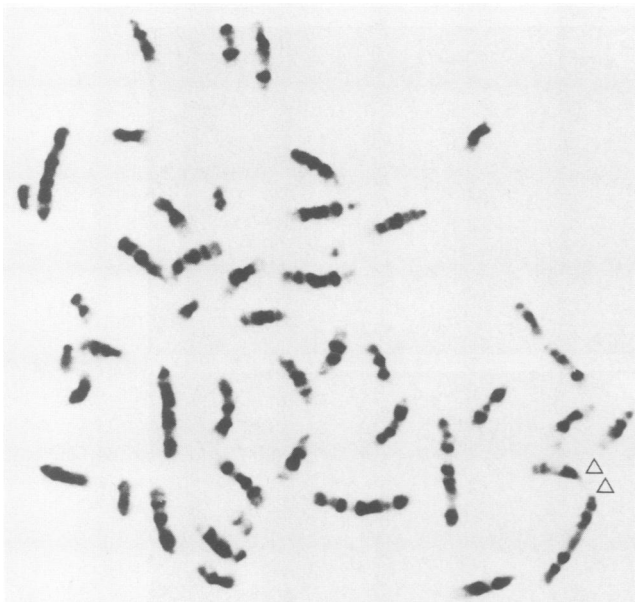

FIG. 2. Giemsa-trypsin-banded metaphase of microcell hybrid F(1.11)J. The only mouse chromosome retained is the Rb(1.11) translocation. Arrows indicate areas of murine constitutive heterochromatin on Rb(1.11) that were visualized by staining with Hoechst 33258.

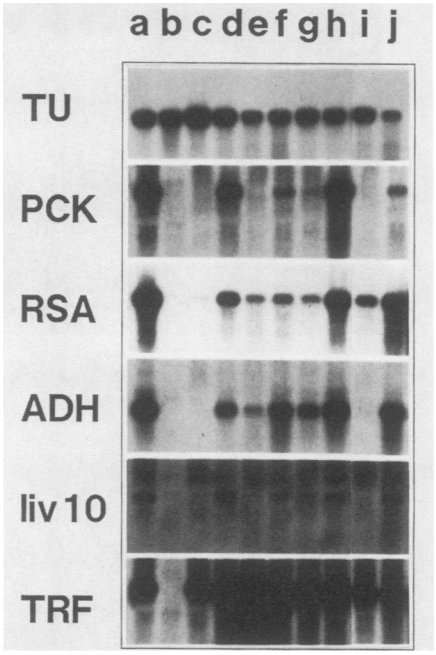

FIG. 3. Expression of liver-specific mRNAs in F(1.11) microcell hybrids and their backselectants. Northern (RNA) blots of cytoplasmic RNAs were hybridized with labeled probes from the α-tubulin (TU), phosphoenolpyruvate carboxykinase (PCK), serum albumin (RSA), alcohol dehydrogenase (ADH), liv-10 (liv10), and transferrin (TRF) genes as described in Materials and Methods. Lanes: a, FTO-2B rat hepatoma cells; b, mouse embryo fibroblasts; c, F(1.11)A; d, FB(1.11)A; e, F(1.11)J; f, FB(1.11)J; g, F(1.11)6; h, FB(1.11)6; i, F(1.11)8; j, FB(1.11)8.

Several of the F(1.11) hybrids contained Rb(1.11) derivatives that appeared to have suffered terminal deletions of chromosome 1. The apparent breakpoints in clones F(1.11)6, F(1.11)2, F(1.11)C, and F(1.11)K were in subbands E3, D, C2, and A1, respectively. Of these, only F(1.11)6 was extinguished for albumin expression (Table 2). These data indicate that albumin extinction in hepatoma hybrids is mediated by a discrete genetic locus that maps to a particular region of mouse chromosome 1. We refer to this locus as tissue-specific extinguisher 2 (*Tse-2*).

To determine whether other genes were extinguished by loci on mouse chromosome 1, we screened the F(1.11) hybrids for expression of liver-specific transcripts by RNA blot hybridization (Fig. 3). In agreement with the immunofluorescence data, albumin mRNA expression was extinguished in F(1.11) hybrids A, J, 6, and 8 but reexpressed in the respective backselectants. Three other liver genes, encoding PEPCK, ADH, and liv-10, displayed the same pattern of extinction and reexpression in these clones. The PEPCK extinction phenotype of these hybrids was expected; it is mediated by an extinguisher locus (*Tse-1*) on mouse chromosome 11 (30). As neither albumin, ADH, nor liv-10 expression is affected by *Tse-1* (30), their extinction in the F(1.11) hybrids must be due to loci on mouse chromosome 1. Transferrin, S-ADH, liv-7, liv-9, and a number of other liver genes continued to be expressed in the F(1.11) microcell hybrid clones (Fig. 3 and data not shown).

Effects of chromosomes 3 and X on albumin expression. While this work was in progress, Petit et al. (39) reported that albumin extinction in rat hepatoma × mouse L-cell

TABLE 3. Properties of F(3.8,X) series dichromosomal microcell hybrids

Clone	No. of mouse chromosomes	Relative plating efficiency (fraction of cells)			Fraction of ALB$^+$ cells
		HAT	AAT	GAMAT	
F(3.8,X)A	2	0.94	1.4	0.95	0.98
F(3.8,X)B	3	1.1	1.3	1.2	0.93
F(3.8,X)C	2	0.92	<0.01	<0.01	1.0
F(3.8,X)F	2	0.91	0.67	0.65	1.0
F(3.8)8-6	1		0.98	<0.01	1.0
Mouse embryo fibroblasts	40	0.88	0.97	0.92	<0.01

microcell hybrids was correlated with the presence of an L-cell marker chromosome (M1) that resembled an X.3 centric fusion. However, the data summarized above clearly show that mouse chromosome 1 mediates albumin extinction in hepatoma hybrids, and neither chromosome X nor 3 alone induced albumin extinction in our clones [F(3.8)8-6 and F(X)A, Table 1]. To explain this apparent discrepancy, it is necessary to assume that either (i) the M1 marker chromosome contains sequences from mouse chromosome 1, including *Tse-2*, or (ii) chromosomes 3 and X in combination induce an albumin extinction phenotype distinct from but similar to that encoded by murine *Tse-2*. To test the latter possibility, we constructed microcell hybrids that retained both chromosomes 3 and X.

Microcell hybrid clone F(3.8)8-6 was prepared by transferring a Rb(3.8) translocation from Rb2Rma donors (19) into Fado-2 hepatoma recipients and selecting the chromosome 8-encoded APRT$^+$ phenotype. This clone retained Rb(3.8) at high frequency, and no other mouse chromosomes were retained (Table 1). F(3.8)8-6 recipients were fused with microcells from diploid C57BL/6J donors, and HPRT$^+$ clones were selected in HAT. The properties of four such hybrids are summarized in Table 3.

All four F(3.8,X) hybrids plated with >90% efficiency in HAT, as expected for HPRT$^+$ clones retaining the murine X chromosome. In addition, clones F(3.8,X)A, -B, and -F plated with high efficiency under conditions of APRT$^+$ (AAT) or APRT$^+$ HPRT$^+$ (GAMAT) selection. Thus, these hybrids expressed the APRT$^+$ HPRT$^+$ phenotype expected of cells retaining both Rb(3.8) and the mouse X chromosome. Retention of those specific mouse chromosomes was confirmed by cytogenetic analyses (data not shown). In contrast, F(3.8,X)C did not survive selection for the APRT$^+$ phenotype; it had segregated Rb(3.8) during the period of HAT selection.

Immunofluorescent staining demonstrated that each F(3.8,X) hybrid population expressed serum albumin in >90% of the cells (Table 3). This finding indicates that albumin extinction in hepatoma hybrids does not involve loci on mouse chromosome 3 or X, either singly or in combination. We conclude that the M1 marker chromosome of Petit et al. (39) contains sequences from other mouse chromosome(s) and suggest that the relevant sequences are derived from mouse chromosome 1.

DISCUSSION

The experiments described in this report demonstrate that albumin extinction in rat hepatoma × mouse fibroblast hybrids is a specific genetic effect mediated by an extinguisher locus on mouse chromosome 1. As this is the second tissue-specific extinguisher to be mapped in the murine genome, we designate this locus *Tse-2*. Two other liver genes were coordinately extinguished and reexpressed with albumin in F(1.11) microcell hybrids and their backselectants, but it is not yet clear whether *Tse-2* encodes all three extinction phenotypes. Hybrids containing fragments of chromosome 1 may be useful for determining whether these phenotypes can be dissociated. As noted below, such hybrids should not be difficult to obtain.

The karyotypic instability of the F(1.11) microcell hybrid family was remarkable. Only 3 of 26 primary hybrid clones retained the Rb(1.11) chromosome under selection in an apparently intact form, whereas 23 clones had deleted sequences from the chromosome 1 arm of the translocation. Such instability has not been seen in microcell hybrids retaining other Robertsonian translocations. Furthermore, the hybrids that initially retained Rb(1.11) intact were rapidly overgrown by cells that had deleted most of chromosome 1. The derivative chromosomes so produced, consisting predominantly of chromosome 11 arms alone, were stably maintained in subsequent generations. These observations suggest that mouse chromosome 1 contains sequences that are unstable in rat-mouse hybrids. The basis of this phenomenon, noted previously for mouse chromosome 11 in hamster-mouse hybrids (28), is not known.

DNA sequences required for cell-specific expression of the serum albumin gene are concentrated within 200 base pairs of the major site of transcription initiation (6, 20, 22, 23, 29, 32, 36), and a number of cellular factors that interact with sequences in this region have been identified (2, 6, 7, 12, 13, 29, 32). A liver-specific factor that seems to play a major role in activating albumin transcription both in vivo and in vitro binds to a relatively proximal sequence element (6, 7, 13, 29); this factor has been designated hepatocyle nuclear factor 1 (HNF1). Interestingly, HNF1 binds to promoter sequences of several liver genes (13). Furthermore, whereas HNF1 has been detected in a number of ALB$^+$ hepatoma cell lines, a different factor (variant HNF1 [v-HNF1]) that binds to the same sequence is present in ALB$^-$ hepatoma variants and extinguished hepatoma hybrids (3, 6). Finally, extract mixing experiments have shown that the factor-binding profile of nonexpressing cells is dominant to that of expressing cells (7), a situation reminiscent of the phenotypes of somatic cell hybrids.

The mechanism(s) by which *Tse-2* extinguishes albumin gene activity in hybrid cells is not known, but the results summarized above are consistent with several possibilities. Perhaps the most economical model is one in which the *Tse-2* gene product is variant HNF1. This model predicts that v-HNF1 is encoded by a gene on mouse chromosome 1. Alternately, *Tse-2* may encode a factor that activates the expression or function of v-HNF1, with negative effects on HNF1 synthesis or activity. This mechanism would be similar to that proposed for growth hormone gene extinction, whereby growth hormone factor 1 expression is (34). In any case, it will be interesting to determine whether other liver genes regulated by HNF1/v-HNF1 are extinguished by *Tse-2* and whether those specific transcription factors are altered in extinguished microcell hybrids.

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