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Expression of Differentiated Functions in Hepatoma Cell Hybrids: High Frequency of Induction of Mouse Albumin Production in Rat Hepatoma–Mouse Lymphoblast Hybrids*

(immunodiffusion/immuno-adsorption)

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ABSTRACT **We have studied the production of serum albumin by somatic hybrids between well-differentiated 2s and 1s rat hepatoma cells (Faza), which produce serum albumin, and sub-diploid mouse leukemic lymphoblasts (Lc), which do not produce albumin. We determined the rat or mouse origin of the albumin by double immunodiffusion, using immuno-adsorbed noncrossreacting antisera. Each of 12 karyologically identified 2s hybrid clones (Lc2F) produces both rat and mouse albumin. Moreover, unlike 1s hybrids reported previously, eight of nine 1s hybrids (LcF) also produce mouse albumin; six of them produce rat albumin as well. One clone from the 1s cross produces only rat albumin.**

In an effort to understand the control of cellular differentiation, somatic cells that express differentiated (luxury) functions have been hybridized with cells that do not express these functions (for review, see ref. 1); the major observations from such studies can be summarized as follows. *Extinction* of luxury functions, which was first observed for pigment formation in melanoma-fibroblast hybrids (2), is a common occurrence in hybrids that contain most of the chromosomes of both parents. *Re-expression* of extinguished luxury functions may occur upon the loss of chromosomes from the hybrid cells (3); the re-expression of any one of the liver-specific enzymes studied in intraspecific hepatoma hybrids may occur independently of that of the others (4–7). These findings imply that the presence of certain chromosomes is necessary to maintain extinction, and that a large number of regulatory elements must exist. Moreover, re-expression shows that the epigenotype of the differentiated parent can remain stable over many generations without being expressed.

Other observations demonstrate an effect of *gene dosage* on the phenotype of hybrid cells. For example, the inducibility of glycerol-3-phosphate dehydrogenase in 1s glial cells is completely extinguished when these cells are hybridized with fibroblasts, but only partially extinguished in hybrids between 2s glial cells and fibroblasts (8). Melanogenesis is systematically extinguished in hybrids between 1s melanoma cells and fibroblasts, but not when 2s melanoma cells are crossed with the same fibroblasts (9, 10).

In the case of albumin synthesis, a function that is not totally extinguished in hepatoma hybrids (11), gene dosage may be responsible for the *induction* of a parental genome to produce a protein foreign to its tissue of origin, as was first demonstrated in hybrids between rat hepatoma cells and 3T3 mouse fibroblasts (11). Each hybrid from the 1s hepatoma cross produced some rat albumin. However, among five hybrid clones in which the hepatoma parent was 2s, two produced no albumin, one both rat and mouse albumin, and two only mouse albumin.

In an attempt to clarify the gene dosage requirements for the induction of mouse albumin, we have undertaken a new cross, choosing as the nonexpressing parent a mouse leukemic lymphoblast line whose chromosome number is only about half that of 3T3, so that the balance of hepatoma and non-hepatoma chromosomes in 1s hybrids in this series is similar to that previously established in 2s hepatoma-3T3 hybrids. As a result, it has been possible to demonstrate the induction of mouse albumin in almost all of the 1s hepatoma hybrids, as well as in the 2s ones.

MATERIALS AND METHODS

Cultures were maintained in modified (12) Ham's F12 medium (13) supplemented with 5% or 10% fetal-calf serum as described (14). Two lines of rat hepatoma cells, derived from the Reuber H35 (15) line of Pitot *et al.* (16), have been used. Faza 967 (hereafter referred to as Faza) is a subclone of Fu5-5 (4) and is resistant to 6 μg/ml of 8-azaguanine. 2s Faza 967 cl. 9 (hereafter referred to as 2s Faza) is one of a series of 2s hepatoma clones isolated following treatment of Faza 967 by Sendai virus. The mouse parental cells, which grow in suspension, were derived from the lymphoblastic leukemia line L5178y (17). Dr. G. A. Fischer kindly provided us with the subline designated 2BF (resistant to 30 μg/ml of 5-bromodeoxyuridine), which we have subcloned to obtain a stable 1s line referred to below as Lc. All three parental lines fail to grow in HAT (18) medium.

The Lc line was chosen for this work because of its near-diploid karyotype. Lc cells show a sharp modal number (m) of 39 chromosomes, of which one is a large metacentric and the remainder are telocentric. The 1s hepatoma cells are hyperdiploid (m = 53), and, with the exception of one long metacentric, all chromosomes are like those of the normal rat complement and can be subgrouped as follows: large subtelocentric (2), small bi-armed (m = 22), and telocentric (m = 28). The cells of the 2s hepatoma clone show the same sub-

Abbreviations: RSA, rat-serum albumin; MSA, mouse-serum albumin; m, modal number.

* This is paper number eight in a series. Number seven is ref. 7.

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TABLE 1. *Chromosome numbers of parental and hybrid cells**

Cell line	Total number	Number of large sub-telocentrics	Number of large metacentrics	Number of small bi-armed†	Number of telocentrics	Corrected number of telocentrics‡
Parents						
Lc	38.8 (37-40)	0	1.1 (1-2)	0	37.7 (36-39)	
Faza	52.6 (49-57)	2	1.2 (1-2)	22.6 (20-26)	26.8 (22-31)	
2*s* Faza	103.2 (97-110)	3.6 (2-4)	2.3 (2-3)	44.8 (38-51)	52.6 (48-58)	
Hybrids						
Lc X 2*s* Faza						
Expected:	142.1 (134-150)	3.6 (2-4)	3.4 (2-5)	44.8 (38-51)	90.3 (84-97)	
Observed:						
Lc2F *3*	134.2 (126-142)	3.0 (2-4)	5.0 (4-9)	46.8 (40-55)	79.3 (67-91)	83.3 (76-97)
Lc2F *16*	133.2 (126-140)	2.9 (2-3)	4.5 (3-9)	45.6 (41-49)	80.6 (70-90)	83.7 (76-95)
Lc2F *1*	130.5 (124-135)	3.9 (2-4)	3.3 (3-4)	41.8 (36-45)	81.3 (71-90)	81.9 (73-90)
Lc2F *19*	129.8 (117-138)	3.6 (3-4)	4.0 (2-5)	45.6 (40-50)	76.5 (66-85)	78.7 (68-85)
Lc2F *12*	120.9 (111-129)	2.6 (3-4)	4.5 (2-10)	41.2 (36-45)	72.5 (56-78)	76.5 (72-83)
Lc2F *7*	110.8 (102-125)	3.6 (3-4)	5.5 (4-7)	40.7 (32-45)	60.4 (52-82)	65.7 (56-84)
Lc2F *22*	103.7 (93-117)	2.5 (2-4)	8.1 (6-11)	42.2 (26-52)	50.8 (46-61)	61.1 (54-73)
Lc2F *8*	102.2 (88-108)	2.8 (2-4)	8.1 (6-11)	42.5 (34-46)	48.5 (43-56)	58.7 (51-67)
Lc2F *25*	96.9 (93-101)	2.2 (2-3)	7.4 (4-10)	38.0 (32-44)	49.3 (44-55)	58.3 (54-63)
Lc2F *23*	96.3 (78-106)	2.1 (1-4)	5.0 (4-6)	23.0 (18-30)	66.1 (52-79)	70.1 (56-83)
Lc2F *21*	94.4 (85-104)	1.8 (1-4)	6.9 (5-9)	27.0 (17-35)	58.1 (50-65)	66.4 (58-74)
Lc2F *20*	78.2 (73-83)	1.0 (1-2)	3.3 (2-5)	19.9 (18-23)	53.9 (48-59)	55.0 (48-61)
Col. *14*	105.4 (101-111)	2.9 (2-3)	2.7 (2-4)	45.0 (42-48)	54.6 (50-60)	
Col. *5*	96.2 (94-98)	3.6 (3-4)	3.7 (3-6)	40.2 (37-44)	48.6 (45-52)	
Col. *18*	87.1 (83-94)	2.6 (2-3)	5.3 (2-10)	34.1 (28-39)	44.8 (40-52)	
Lc X Faza						
Expected:	91.4 (86-97)	2	2.3 (2-4)	22.6(20-26)	64.5 (58-70)	
Observed:						
LcF 1	80.8 (76-89)	2.1 (1-3)	5.7 (3-9)	19.0 (14-23)	53.9 (48-61)	61.4 (56-67)
LcF 5	80.7 (71-87)	2.0 (1-3)	4.9 (2-7)	19.8 (17-22)	53.9 (43-60)	59.6 (47-68)
LcF 6	79.5 (74-87)	1.9 (1-2)	2.7 (2-5)	21.0 (18-25)	53.9 (50-61)	55.3 (51-61)
LcF α	78.8 (76-83)	1.9 (1-2)	3.9 (2-6)	21.8 (20-24)	51.0 (47-57)	54.9 (51-59)
LcF 3	78.4 (74-83)	2.1 (2-4)	3.5 (3-5)	19.6 (16-24)	53.2 (45-59)	56.3 (47-63)
LcF 7	76.8 (69-84)	1.7 (1-3)	5.4 (4-8)	22.8 (19-25)	46.8 (45-52)	53.7 (47-60)
LcF β	71.3 (62-78)	1.5 (1-3)	2.5 (1-4)	13.0 (9-16)	54.0 (48-61)	55.3 (48-63)
LcF 4	55.0 (47-62)	1.0 (0-3)	3.5 (1-5)	10.9 (8-14)	39.4 (33-49)	42.5 (37-53)
LcF 2	95.6 (83-101)	3.2 (2-4)	5.3 (3-12)	33.2 (29-38)	53.8 (39-58)	60.3 (62-65)

* Mean values (and ranges in parentheses) are given. These numbers are based upon the analysis of 17-24 metaphases (parental lines) or 15-20 metaphases (hybrid lines).

† Rat markers.

‡ This calculation is based upon the assumption that "new" large metacentric chromosomes are formed by centric fusion of telocentrics. The expected number of large metacentrics is subtracted from the observed, the remainder multiplied by two and added to the observed number of telocentrics.

groups: the numbers of each are approximately double those of the 1*s* cells, the ranges are somewhat greater, and the modes are less pronounced. Since in the hybrid cells, the small bi-armed chromosomes serve as rat markers, whereas telocentric chromosomes come from both parents, estimates of the contribution of rat and mouse chromosomes to the hybrid karyotype can be made (see footnotes, Tables 1 and 2).

Hybrids have been isolated from crosses of both 1*s* and 2*s* hepatoma cells with Lc cells. Virus fusion (19) was carried out in suspension, using either 600 or 1000 hemagglutinating units/ml of UV-inactivated or β-propriolactone-inactivated Sendai virus. After virus treatment, the cells were diluted and inoculated in HAT medium. From the cross of Lc with 2*s* Faza (parental cell ratio of 5:1), more than 50 hybrid colonies were obtained from a total of 12×10^6 parental cells. By contrast, only nine hybrid colonies were obtained from two different crosses of Lc with Faza: from the first, (16×10^6 parental cells, ratio 1:1), two independent hybrid colonies were obtained, and from the second (36×10^6 parental cells, 2.6:1), seven hybrid colonies.

Hybrid colonies, isolated in most cases from different culture dishes, were picked with micro-pipettes and grown out in HAT medium for standard karyological analysis (20) and for determination of albumin production. All analyses were performed between 25 and 35 cell generations after fusion.

From the cross Lc $\times$ 2*s* Faza (hybrid series Lc2F), 15 clones from 11 different dishes have been analyzed. Of these 15, only 12 were identified as hybrids on the basis of their karyotypes. The other three (colonies *5*, *14*, and *18*) are either 2*s* Faza revertants, able to grow in HAT medium, or hybrids which have undergone extensive loss of telocentric chromosomes.

From the cross Lc $\times$ Faza (hybrid series LcF), nine

TABLE 2. *Hybrid cell lines: karyotypes and albumin production*

Cell line	Estimated percent of expected chromosome number*			Type of albumin produced	
	Total	Rat	Mouse	Rat	Mouse
Lc2F *3*	94	104	97	+	+
Lc2F *16*	94	102	101	+	+
Lc2F *1*	92	93	106	+	+
Lc2F *19*	91	102	88	+	+
Lc2F *12*	85	92	94	+	+
Lc2F *7*	78	91	66	+	+
Lc2F *22*	73	94	50	+	+
Lc2F *8*	72	95	43	+	+
Lc2F *25*	68	85	54	+	+
Lc2F *23*	68	51	125	+	+
Lc2F *21*	66	60	96	+	+
Lc2F *20*	55	44	94	+	+
LcF 1	88	84	111	−	+
LcF 5	88	88	104	−	+
LcF 6	87	93	91	+	+
LcF α	86	96	88	+	+
LcF 3	86	87	97	+	+
LcF 7	84	101	83	+	+
LcF β	78	57	112	+	+
LcF 4	60	48	84	+	+
LcF 2	105	150	70	+	−

* Obtained by dividing the actual mean ($n \geq 15$) by the expected one, and multiplying by 100. Calculations of "Rat" and "Mouse" chromosomes are made assuming equal probability of loss of rat small bi-armed and rat telocentric chromosomes. *"Rat" chromosomes:* The estimate consists of the number of small, bi-armed, marker chromosomes. *"Mouse" chromosomes:* Since the rat parental cells contributed approximately equal numbers of bi-armed and telocentric chromosomes, the number of mouse telocentrics is estimated for each metaphase by subtracting the number of small, bi-armed (rat) chromosomes from the corrected total number of telocentrics.

hybrid clones have been isolated and analyzed. Of these nine, four arose in different dishes or bottles; five of the hybrid clones (LcF4, LcF5, LcF6, LcFα, and LcFβ) were isolated from a single bottle. However, as shown below, among the hybrids in both series for which the independence of origin cannot be guaranteed, only LcF6 and LcFα are similar in both karyotype and albumin production.

For the assay of albumin in the culture medium, an aliquot of cells was inoculated in 60 ml of medium (in four 10-cm Falcon petri dishes) and grown for 72 hr. The medium was collected, centrifuged, dialyzed against bi-distilled water, and lyophilized. The residue was redissolved in normal saline to a concentration 100 times that of the volume dialyzed, and subsequently diluted as necessary.

After each incubation, the cells were detached, pooled, and counted. The number of hybrid cells generally increased 2- to 4-fold during the 72-hr incubation. The inocula varied between 0.5 and 1.5 $\times$ 10^5 cells per ml. The numbers of cells ($\times$ 10^{-5}) per ml after 72 hr are noted in parentheses for each of the clones studied. Lc2F: *3* (1.5), *16* (1.5), *1* (1.5), *19* (2.5), *12* (1.8), *7* (3.3), *22* (4.2), *8* (1.6), *25* (1.7), *23* (4.2), *21* (4.0), *20* (4.7). LcF: 1 (4.5), 5 (2.7), 6 (3.1), α (2.7), 3 (4.6), 7 (3.8), β (1.4), 4 (1.7), 2 (3.0). The three clones which cannot be identified as hybrids are colonies *14* (1.4), *5* (3.6), and *18* (3.2). Counts for parental cells, each inoculated at 1.3 $\times$ 10^5/ml, were as follows after 72 hr: 2*s* Faza (4.8), Faza (11.9), Lc (17.1).

Rat serum albumin (RSA) and mouse serum albumin (MSA) (Pentex, fraction V) were further purified by electrophoresis as described (11). The purified MSA, which was used for immunization of rabbits, contained two minor impurities (demonstrated with goat anti-mouse-serum antiserum, Hyland), which did not raise antibodies. Therefore, new MSA was prepared by sequential electrophoresis, first in agarose and then in acrylamide-agarose gels (21). The final MSA (used for all subsequent tests) and RSA showed no evidence of impurities against rabbit anti-mouse-serum or anti-rat-serum antisera (Pentex), respectively. The prepared antisera (see below) showed only a single band on immunoelectrophoresis against the whole homologous sera.

Rabbits were immunized by three monthly subcutaneous injections of 2 ml of emulsion containing equal volumes of either RSA (1.5 mg/ml) or MSA (1.0 mg/ml) and complete Freund's adjuvant, followed by a final intravenous injection of 0.1 ml of RSA or MSA alone, 6 days before the final bleeding. Because rabbit anti-RSA antiserum crossreacts with MSA, and vice versa, noncrossreacting antisera were prepared by adsorption of anti-RSA antiserum with mouse-serum proteins that had been polymerized with glutaraldehyde (22), and vice versa. The resulting immuno-adsorbed anti-RSA antiserum (undiluted or diluted 1:2) showed no reaction against any concentration (1.5–480 μg/ml) of MSA that was tested; the converse was true for the immuno-adsorbed anti-MSA antiserum.

Double immunodiffusion was performed in 1-mm thick slabs of 0.8% agarose in normal saline. After 12–24 hr of incubation at room temperature, gels were processed and stained as described (11). The gels shown in Fig. 1 demonstrate the standard test system: noncrossreacting antiserum in the center well, the homologous albumin in opposite wells, and controls or unknowns in the remaining wells. Initially, the antigen and antibody wells used were 3 mm in diameter, and were separated by 3 mm; each well held up to 15 μl of sample. Owing to the weakness of the immuno-adsorbed antisera, the sensitivity of the system was increased by using a center well of 4 mm (about 25 μl), and by decreasing the distance between antibody and antigen wells to 1.5 mm.

In this system the minimal detectable concentration of both MSA and RSA is 6 μg/ml. Therefore, when it is stated that there is no detectable MSA or RSA in a 100-fold concentrated medium, this means that there was less than 0.06 μg/ml in the unconcentrated growth medium. For a given culture, the concentration of albumin is a function of the number of cells per ml (see above); the volume of medium (60 ml) and time of incubation (72 hr) were constant.

RESULTS

All of the 12 identifiable 2*s* hepatoma $\times$ mouse leukemic lymphoblast hybrids (Lc2F) produce both MSA and RSA. Of this series, five clones (Lc2F *1*, *3*, *16*, *19*, and *25*) secrete albumin in amounts roughly comparable to that produced by the 2*s* Faza parental cells, as judged from the dilutions required to approach antigen-antibody equivalence in the immunodiffusion system (see, for example, Fig. 1*D*, Lc2F *3* [*3'*] and 2*s* Faza [2*s'*]; note that both are concentrated only 12.5$\times$). Moreover, these five hybrid clones each produce

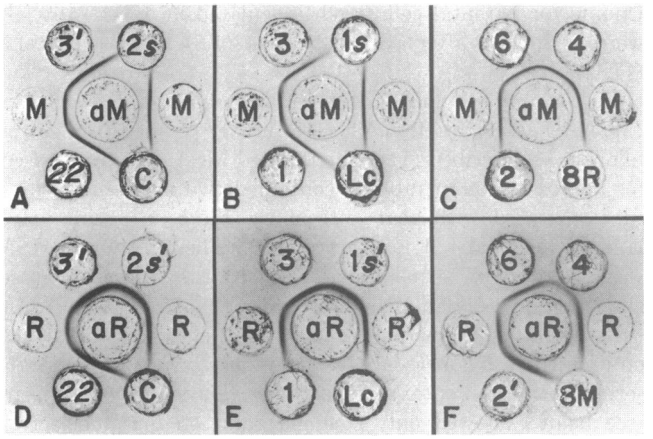

FIG. 1. Double immunodiffusion tests are shown for mouse (*A–C*) and rat (*D–F*) albumin production by parental and hybrid cells, using noncrossreacting rabbit anti-MSA (aM, diluted 1:2 in center wells of *A–C*) and anti-RSA (aR, undiluted in center wells of *D–F*). *Controls.* Homologous-albumin controls: M (MSA, 30 μg/ml), R (RSA, 30 μg/ml). Heterologous-albumin controls: 8R (RSA, 240 μg/ml), 8M (MSA, 240 μg/ml). Control medium: *C* (culture medium concentrated 100×), which demonstrates the absence of interference by fetal calf serum in medium never exposed to cells. *Concentrated media from cell cultures.* Parental cells: 1*s* (Faza), 2*s* (2*s* Faza), and Lc. 2*s* hybrids: *3* (Lc2F *3*) and *22* (Lc2F *22*), shown in *A* and *D*. 1*s* hybrids: 1 (LcF 1) and 3 (LcF 3) in *B* and *E;* 6 (LcF 6), 4 (LcF 4), and 2 (LcF 2) in *C* and *F* All media were concentrated 100× (except 1*s*, 110×), or 12.5× for the samples designated by a prime (′) (except 1*s*′, 14×).

amounts of MSA and RSA of the same order of magnitude (compare *3′* in Fig. 1*A* [MSA] and Fig. 1*D* [RSA]). It has not yet been determined whether some of them produce even more total albumin than 2*s* Faza.

The other seven Lc2F hybrid clones produce less albumin (see, for example, *22* in Fig. 1*A* and *D*, each concentrated 100×). For these hybrids also, the amounts of the two albumins are in the same general range, with the notable exception of Lc2F *23* (not shown in Fig. 1), which secreted moderate amounts of RSA, but only barely detectable amounts of MSA. Since the smallest detectable concentration of albumin (in media concentrated 100×) was 6 μg/ml and the highest final number of cells per ml after 72 hr was 4.7×10^5, we can say that every Lc2F hybrid produces at least 0.13 μg/10^6 cells of both MSA and RSA, and some of them much more.

Of the nine hybrids from the 1*s* cross (LcF), eight also produce MSA (Fig. 1*B* and *C*). Although each of these clones produces one or both albumins, for two of them there was no detectable RSA (LcF1, <0.13 μg/10^6 cells per 72 hr, and LcF5, <0.22 μg/10^6 cells per 72 hr; see Fig. 1*E* for the former), and for one (LcF2; see Fig. 1*C*), no detectable MSA (<0.20 μg/10^6 cells per 72 hr). Except for this last hybrid (see below), none of the LcF hybrids secretes amounts of albumin comparable to that of their Faza parent.

The karyotypes of the Lc2F hybrids are extremely variable, and most of them fail to show clear modes. The first four Lc2F hybrid clones indicated in Table 1 are characterized by little chromosomal loss, by morphological characteristics very similar to those of the hepatoma parental cells, and by a high albumin production. (Only one of the segregated clones, Lc2F *25*, shows both a strong hepatoma-like morphology and a high albumin production.) The remaining clones show more significant chromosome loss (15–45% of the expected number, Table 2), which, judging by the loss of rat markers, seems to be due in three cases to the loss of rat chromosomes, and in four to the loss of mouse chromosomes (deficiency of telocentrics). Most of these clones are characterized by the formation of "new" long metacentric chromosomes, indicating chromosomal rearrangements (see footnote, Table 1). The cells of the last clone of this series, Lc2F *20*, have undergone very extensive loss of chromomes, probably mostly of rat origin, and show a karyotype very similar to that of several observed in the LcF series. It is of interest that within the wide range of karyotypes observed in this series of hybrids, all clones produce detectable amounts of both rat and mouse albumin.

Colonies *14*, *5*, and *18* cannot be identified as hybrids on the basis of their karyotypes, since the ratios of small bi-armed to telocentric chromosomes fall within the range observed for the 2*s* Faza cells. Since none of these colonies produces MSA, and all produce RSA in amounts comparable to that of their hepatoma parent, independent tests will be required to determine their exact nature.

The LcF hybrid clones show less karyotypic heterogeneity than those of the Lc2F series. The ranges are narrower, although the modes (which are close to the means) tend to be weak. All clones (except LcF2, see below) show fewer chromosomes than expected; only two clones (LcFβ and LcF4) show extensive loss of (probably rat) chromosomes. The two clones that produce only MSA have the highest chromosome numbers; those which produce albumins of both species contain between 83 and 112% of the expected mouse chromosomes, and 48–101% of the expected rat markers (Table 2). The production of both types of albumin by LcF4, which appears to have lost half of the rat chromosomes, is of special interest since it shows that the maintenance of the complete 1*s* hepatoma complement (and a high ratio of rat:mouse chromosomes) is not required for the continued production of both types of albumin.

LcF2 is unique among the LcF clones in three respects: it secretes no detectable MSA (Fig. 1*C* [2]), it produces RSA in amounts roughly equivalent to that of its hepatoma parent (Figs. 1*F* [2′] and 1*E* [1*s*′]), and it contains an estimated 50% more rat chromosomes than expected (Table 2). One can only speculate about the mechanism responsible for its origin.

DISCUSSION

These experiments demonstrate (*i*) that the expression of a "silent" gene can be induced in somatic hybrids resulting from fusion of 1*s* as well as 2*s* differentiated cells, (*ii*) that this induction can occur with great regularity, and (*iii*) that the amounts of both the "natural" and the induced protein produced by the hybrids can approach that of the natural protein produced by the expressing parent.

The demonstration of the induction of mouse-albumin production in LcF hybrids supports earlier observations on gene-dosage effects (11); by using as the mouse parent a cell line with only about half the number of chromosomes of the fibroblast line used previously, a similar balance of rat hepatoma and mouse chromosomes has been achieved in 1*s* hybrids of this cross as in the 2*s* hybrids of the previous cross. This balance may explain the induction of mouse-albumin production in our 1*s* hybrids, which in previous work was seen only in 2*s* hybrids. Other interpretations cannot be ruled out, such as

special properties of the lymphoblast parent (e.g., its secretory activity). Possible effects due to the malignancy of the parental cells must also be kept in mind in interpreting all of these results.

Induction in the Lc2F and LcF hybrids is almost general: 20 of the 21 unequivocally hybrid clones produce detectable amounts of mouse albumin. This finding strengthens the interpretation (11) that rat regulatory signals for albumin production are recognized by the mouse genome.

The one hybrid that does not make detectable mouse albumin, LcF2, is peculiar in that it has almost 50% more than the expected number of rat marker chromosomes, and is the only LcF hybrid to make large quantities of (rat) albumin (Fig. 1*F*). The stage at which these extra chromosomes appeared is unknown, and their presence raises the possibility that early post-fusion events are crucial for the induction of mouse-albumin production, and that irregularities in the early divisions of the LcF2 cells may have been responsible for their unique phenotype.

The only clear correlation detected between karyotype and albumin production is that the four Lc2F hybrid clones with the most complete complements of both rat and mouse chromosomes, Lc2F *3*, *16*, *1*, and *19* (Table 1), include all but one of the clones which produce large amounts of both types of albumin. In the 1*s* hybrid series, LcF4 is noteworthy in that it has lost more than half of the expected rat marker chromosomes but still produces both mouse and rat albumin. Hybrid clones of this type offer promise for a more precise analysis of the karyotypic requirements for the maintenance of albumin production.

Because the techniques used in the present work allow only a rough estimation of the amounts of albumin produced, we are currently turning to the precision and sensitivity of radioimmunoassay, in order to determine (*i*) whether any of the hybrid clones produce as much, or more, of either or both albumins than the total produced by the hepatoma parent, and (*ii*) whether hybrid clones LcF1 and 5, both of which appear to secrete only MSA, produce very small amounts of RSA which could be detected by a more sensitive method. As pointed out earlier (11), activation of the mouse genes for albumin, in the absence of expression of the hepatoma gene, implies "the existence of a heritable regulatory element for the activation and maintenance of albumin synthesis that is independent of the structural gene."

Another question worth pursuing in these hybrids can be stated as follows: we know that the maintenance of extinction requires the presence of genetic material of the nonexpressing parent (1); does the continued expression of the induced protein require the presence of chromosomes of the expressing parent? In other words, we wonder whether the induction of MSA production is the consequence of a stable change in the epigenotype of the lymphoblast parent, or whether it is due to the contribution by the hepatoma of an "activator" element, the maintenance of which is required for the continued production of the induced protein, as is the case for the activation by cell hybridization of Chinese hamster esterases (23). This question is amenable to study, and an answer will be of importance in the understanding of regulatory mechanisms in cell differentiation.

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1. Ephrussi, B. (1972) *Hybridization of Somatic Cells* (Princeton University Press, Princeton, N.J.).
2. Davidson, R., Ephrussi, B. & Yamamoto, K. (1966) *Proc. Nat. Acad. Sci. USA* **56**, 1437–1440.
3. Klebe, R. J., Chen, T. & Ruddle, F. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 1220–1227.
4. Weiss, M. & Chaplain, M. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3026–3030.
5. Bertolotti, R. & Weiss, M. C. (1972) "Cell differentiation," in *Proc. of the 1st Int. Conf. on Cell Differentiation*, eds. Harris, R. & Viza, D., pp. 202–205.
6. Bertolotti, R. & Weiss, M. C. (1972) *Biochimie* **54**, 195–201.
7. Sparkes, R. S. & Weiss, M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 377–381.
8. Davidson, R. & Benda, P. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1870–1877.
9. Fougère, C., Ruiz, F. & Ephrussi, B. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 330–334.
10. Davidson, R. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 951–955.
11. Peterson, J. A. & Weiss, M. C. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 571–575.
12. Coon, H. G. & Weiss, M. C. (1969) *Proc. Nat. Acad. Sci. USA* **62**, 852–859.
13. Ham, R. G. (1965) *Proc. Nat. Acad. Sci. USA* **53**, 288–293.
14. Schneider, J. A. & Weiss, M. C. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 127–131.
15. Reuber, M. D. (1961) *J. Nat. Cancer Inst.* **26**, 891–900.
16. Pitot, H. C., Peraino, C., Morse, P. A. & Potter, V. R. (1964) *Nat. Cancer Inst. Monogr.* **13**, 229–242.
17. Fischer, G. A. (1958) *Ann. N.Y. Acad. Sci.* **76**, 673–680.
18. Littlefield, J. (1964) *Science* **145**, 709–710.
19. Harris, H. & Watkins, J. F. (1965) *Nature* **205**, 640–646.
20. Rothfels, K. H. & Siminovitch, L. (1958) *Stain Technol.* **33**, 73–77.
21. Uriel, J. (1966) *Bull. Soc. Chim. Biol.* **48**, 969–982.
22. Avrameas, S. & Ternynck, T. (1969) *Immunochemistry* **6**, 53–66.
23. Kao, F. T. & Puck, T. T. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3273–3277.