MOLECULAR AND CELLULAR BIOLOGY, Sept. 1985, p. 2491–2494
0270-7306/85/092491-04$02.00/0
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Vol. 5, No. 9

# Chromosomal Assignment and *trans* Regulation of the Tyrosine Aminotransferase Structural Gene in Hepatoma Hybrid Cells

T. C. PETERSON, A. M. KILLARY, AND R. E. K. FOURNIER*

*Department of Microbiology and the Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles, California 90033*

Received 1 April 1985/Accepted 31 May 1985

**The structural gene encoding liver-specific tyrosine aminotransferase (TAT; EC 2.6.1.5) was assigned to mouse chromosome 8 by screening a series of hybrid cell lines for retention of murine *Tat-1* gene sequences by genomic Southern blotting. This assignment demonstrated that the *Tat-1* structural gene was not syntenic with *Tse-1*, a chromosome 11-linked locus that negatively regulates TAT expression in *trans* (A. M. Killary and R. E. K. Fournier, Cell 38:523–534, 1984). We also showed that the fibroblast *Tat-1* gene was systematically activated in hepatoma × fibroblast hybrids retaining fibroblast chromosome 8 in the absence of chromosome 11 but was extinguished in cells retaining both fibroblast chromosomes. Thus, the TAT structural genes of both parental cell types were coordinately regulated in the intertypic hybrids, and the TAT phenotype of the cells was determined by the presence or absence of fibroblast *Tse-1*.**

Tyrosine aminotransferase (TAT; EC 2.6.1.5) is an enzyme of hepatic gluconeogenesis whose expression is developmentally regulated and tissue specific (11, 14). Like many other liver-specific traits (7), TAT expression is primarily controlled at the level of gene transcription (16). The mechanisms responsible for tissue-specific differences in TAT gene activity remain largely unknown.

We recently identified a genetic locus that regulates hepatic TAT expression in *trans* (12). This locus, termed tissue-specific extinguisher-1 (*Tse-1*), was defined in the context of hepatoma × fibroblast hybrid cells. Thus, expression of TAT and TAT mRNA was specifically repressed ("extinguished" [6]) in hepatoma hybrids retaining mouse fibroblast chromosome 11 but was reexpressed in cells from which chromosome 11 had been removed. These data define and localize a genetic locus, *Tse-1*, that negatively regulates TAT expression in *trans*. As indicated above, murine *Tse-1* resides on chromosome 11; the analogous human locus has been assigned to human chromosome 17 (12).

*Tse-1* clearly acts in *trans* in hybrid cells, since the donor locus (mouse or human) affects expression of a host (rat) cell gene. Although *Tse-1* is detected in such hybrids, one presumes that it fulfills specific functions in the fibroblasts from which it was derived. One obvious possibility is that *Tse-1* plays a role in the suppression of TAT gene activity in fibroblasts and other nonhepatic cells. According to this view, *Tse-1* would represent one component of a regulatory system for restricting expression of tissue-specific gene sequences in heterologous cell types.

The studies described in this report were designed to provide information on the function of *Tse-1* in fibroblasts. First, we defined the linkage relationship between *Tse-1* and the TAT structural gene (*Tat-1*) by showing that the two loci reside on different mouse chromosomes. Thus, regulation of *Tat-1* by *Tse-1* in murine fibroblasts would necessarily occur in *trans*. Second, we assayed the expression of fibroblast TAT in hybrids in which murine *Tse-1* was either absent or retained and documented the apparent regulation of the fibroblast *Tat-1* gene by *Tse-1*.

The chromosomal location of the murine gene encoding TAT was determined by screening a series of hybrid cell lines containing different subsets of the mouse chromosome complement for retention of murine *Tat-1* gene sequences by genomic Southern blotting (17). DNA samples isolated from various cell lines were digested to completion with *Msp*I (New England BioLabs, Inc., Beverly, Mass.), electrophoresed on 0.7% agarose gels, and blotted onto Zetabind sheets (AMF). The blots were hybridized with nick-translated plasmid pcTAT-3, a cDNA clone containing a 600-base-pair insert complementary to the 3′ end of rat TAT mRNA (16). The labeled probe hybridized with a single 1.7-kilobase fragment of mouse genomic DNA (Fig. 1, lanes a and l), whereas the corresponding fragments in the rat (lanes b and m) and Chinese hamster (lane k) genomes were 1.1 and 3.9 kilobases in length, respectively. This interspecific polymorphism was used to screen for the presence of the murine *Tat-1* gene in rat-mouse and hamster-mouse hybrid cells.

To directly resolve the question of whether the murine TAT gene was syntenic with *Tse-1* on chromosome 11, three monochromosomal microcell hybrids [F(11)J, F(11)N, and F(11)U] retaining that single mouse chromosome (Table 1) were screened for the presence of murine *Tat-1* gene sequences. As expected, the rat-specific 1.1-kilobase *Msp*I fragment was detected in DNA digests of each of the three rat-mouse hybrid clones. However, the diagnostic 1.7-kilobase murine fragment was not present in any of the hybrid DNAs (Fig. 1, lanes c, d, e, and v). These data established that the mouse structural gene encoding TAT was not located on chromosome 11 and therefore was not linked to *Tse-1*.

Hybrid clones retaining various other mouse chromosomes were then screened for the presence of murine *Tat-1*. ABm-31 is a hamster-mouse hybrid that retained eight different mouse autosomes (Table 1); its isolation and characterization have been described in detail elsewhere (9). Only hamster-specific TAT gene sequences were detected in ABm-31 (Fig. 1, lane j). In contrast, the rat-mouse hybrid FF4-3a, a subline of a clone (FF4-3) whose properties have been previously reported (12), retained both rat and mouse *Tat-1* gene sequences (Fig. 1, lane f). A4-3a, a derivative of FF4-3a that had segregated several mouse chromosomes (Table 1), no longer retained murine *Tat-1* (Fig. 1, lane g).

* Corresponding author.

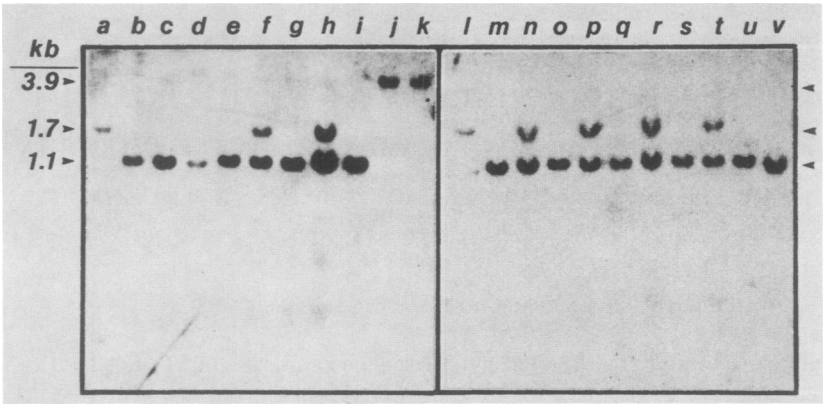


FIG. 1. Retention of murine *Tat-1* gene sequences by hybrid and microcell hybrid clones. DNA (5 μg) prepared from various cell lines (2) was digested to completion with *Msp*I (New England BioLabs), electrophoresed on 0.7% agarose gels, and blotted onto Zetabind (AMF) sheets in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Plasmid pcTAT-3 (16) was labeled by nick translation (1) and hybridized to the filters as described previously (18). The filters were washed in two changes of 2× SSC–0.1% sodium dodecyl sulfate at 25°C and in four changes of 0.2× SSC–0.1% sodium dodecyl sulfate at 55°C. Autoradiography was for 15 h with one intensifying screen. Lanes a and l show a mouse control (C57BL/6J, liver). Lanes b and m show a rat control (Fado-2 cells). Lane k shows a Chinese hamster control (CHO DR31 cells). Monochromosomal hybrids retaining mouse chromosome 11 [F(11)N, F(11)J, and F(11)U] are shown in lane c, lanes d and v, and lane e, respectively. Polychromosomal hybrids (Table 1) FF4-3a, A4-3a, and ABm-31 are shown in lanes f, g, and j, respectively. The microcell hybrids selectively ($APRT^+$) retaining mouse chromosome 8, F(8)E and F(8)D, are shown in lanes h and p and lane n, respectively; their respective $APRT^-$ backselectants, FB(8)E and FB(8)D are shown in lanes i and q and lane o, respectively. Lanes r and t show microcell hybrids F(8.14)Q and F(8.14)N, which retain Rb(8.14); their backselectants FB(8.14)Q and FB(8.14)N are shown in lanes s and u, respectively. Note the concordant segregation of murine *Tat-1* gene sequences with chromosome 8 in F(8) and F(8.14) hybrids versus their FB(8) and FB(8.14) backselectants.

Thus, the murine *Tat-1* locus was necessarily located on one of the chromosomes present in FF4-3a but was absent in both A4-3a and ABm-31. Six mouse chromosomes (autosomes 8, 10, 13, 16, 18, and 19) satisfied this criterion. To determine which of these chromosomes carried murine *Tat-1*, karyotypically simple microcell hybrids were used (8).

Clones F(8.14)N and F(8.14)Q were generated by fusing mouse embryo fibroblast microcells with Fado-2 ($APRT^-$ $HPRT^-$ $OUA^R$) rat hepatoma recipients and selecting $APRT^+$ hybrids in medium containing adenine, aminopterin, and thymidine. The fibroblast donor cells used in this cross were prepared from Rb(8.14)16Rma, a mouse strain that carries a Robertsonian (Rb) translocation (centric fusion) between autosomes 8 and 14 (4). Since the murine *Aprt* gene resides on chromosome 8 (13), the $APRT^+$ microcell hybrids produced in this experiment selectively retained autosomes 8 and 14 as a specific Robertsonian translocation (8).

Clone F(8.14)Q retained six mouse chromosomes (Table 1), two of which (8 and 13) had been implicated as potential carriers of the murine *Tat-1* gene. In contrast, F(8.14)N retained the Rb(8.14) translocation as the only donor-derived mouse chromosome (Table 1). Both hybrids contained murine *Tat-1* gene sequences (Fig. 1, lanes r and t). These data established that chromosome 8 was the only mouse chromosome whose presence correlated with the retention of murine *Tat-1*. To confirm this assignment, microcell hybrids selectively retaining that single mouse chromosome were constructed and characterized.

Clone F(8)E was a microcell hybrid that retained mouse chromosomes 8 and 9, whereas F(8)D was a monochromosomal clone retaining chromosome 8 alone (Table 1). Both hybrids clearly contained the mouse *Tat-1* gene (Fig. 1, lanes h, n, and p). Furthermore, murine *Tat-1* gene sequences segregated concordantly with mouse chromosome 8 when the primary F(8) and F(8.14) microcell hybrids were backselected for the $APRT^-$ phenotype in medium containing 2,6-diaminopurine (Fig. 1, lanes i, o, q, s, and u). We conclude that *Tat-1* can be assigned to mouse chromosome 8.

The assignments of murine *Tat-1* to chromosome 8 and *Tse-1* to chromosome 11 were interesting in that both chromosomes encoded selectable markers (APRT and TK, respectively). Thus, hybrid cells retaining *Tat-1*, *Tse-1*, or both could be directly selected. We exploited this situation in an attempt to define conditions under which the fibroblast TAT gene might be activated in hepatoma hybrids and to determine whether expression of fibroblast *Tat-1* responded to control by *Tse-1*. To do this, hepatoma hybrids retaining fibroblast chromosome 8, 11, or both were screened for TAT expression with an assay capable of discriminating the rat (hepatoma) and mouse (fibroblast) forms of the enzyme. This was accomplished by electrophoresis of cell extracts on nondenaturing polyacrylamide gels followed by TAT-specific histochemical staining (10).

The rat (Fig. 2, lanes a and l) and mouse (Fig. 2, lanes b and m) TAT enzymes were resolved by electrophoresis on 7.5% polyacrylamide gels. When extracts prepared from hepatoma microcell hybrids specifically retaining fibroblast chromosome 8 were analyzed in this manner, three bands of TAT activity were observed (Fig. 2, lanes c and e). The anodal band comigrated with authentic rat TAT, while the cathodal band corresponded to the homodimeric mouse enzyme. The presence of an intermediate band, representing an enzymatically active interspecific heterodimer, conclusively established that both rat (hepatoma) and mouse (fibroblast) TAT were coexpressed in the hybrid cells. As expected, $APRT^-$ backselectants that had segregated mouse chromosome 8 no longer expressed murine TAT, although the rat enzyme continued to be expressed (lanes d and f). Thus, expression of the mouse fibroblast *Tat-1* gene was activated in hepatoma microcell hybrids retaining mouse chromosome 8 alone.

Activation of the fibroblast *Tat-1* gene was also observed in hybrid clones retaining substantial numbers of other

mouse chromosomes [FF4-3a, F(8.14)Q; Table 1]. For example, clone FF4-3a retained 10 mouse chromosomes in addition to chromosome 8 and clearly expressed both rat and mouse TAT (Fig. 2, lane k). In marked contrast, TAT expression was never observed in hepatoma hybrids retaining fibroblast chromosome 11 (i.e., retaining fibroblast *Tse-1*), regardless of their karyotype with respect to retention of other fibroblast chromosomes. As described in detail elsewhere (12), TAT expression was extinguished in monochromosomal hybrids retaining fibroblast chromosome 11 (Fig. 2, lane g) but was reexpressed in backselectants from which chromosome 11 had been removed (Fig. 2, lane h). In hybrids retaining fibroblast chromosomes 8 (*Tat-1*$^+$) plus 11 (*Tse-1*$^+$), neither rat nor mouse TAT was expressed (FF1-9, FF2-2; Table 1 and Fig. 2, lanes i and j). However, segregant subclones that retained fibroblast chromosome 8 (*Tat-1*$^+$) but had segregated chromosome 11 (*Tse-1*$^-$) reexpressed both rat and mouse TAT (A1-9v, A1-9u, and A1-9l; Fig. 2, lanes n, o, and p, respectively). Thus, expression of the fibroblast *Tat-1* gene in hepatoma hybrids exactly paralleled expression of the resident hepatoma gene, and both loci were regulated in *trans* by fibroblast *Tse-1*.

The extinction of tissue-specific traits in intertypic hybrids is a general phenomenon first described nearly 20 years ago (6). Less frequently, the activation of tissue-specific products encoded by a heterologous parental genome has been observed. These cases have been largely restricted to hybrids in which gene dosage was strongly biased; for example, the activation of liver-specific enzymes (3) or serum proteins (5) in hybrids retaining a double complement of hepatoma

TABLE 1. Karyotypic analysis of hybrid and microcell hybrid clones[a]

| Clone | No. of passages | Mouse chromosomes retained (fraction of cells) | | | | | | | | | | | | | | | | | | | No. of cells karyotyped | Mouse *Tat-1* retention[b] | Mouse TAT expression[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | X | | | |
| F(11)N | 5 | | | | | | | | | | | 0.93 | | | | | | | | | | 30 | − | − |
| F(11)J | 7 | | | | | | | | | | | 0.91 | | | | | | | | | | 23 | − | − |
| F(11)U | 6 | | | | | | | | | | | 0.88 | | | | | | | | | | 33 | − | − |
| ABm-31 | 2 | | 0.95 | | | | 0.40 | 0.51 | | | | | 0.65 | | 0.43 | 0.27 | | 0.73 | | | | 37 | − | − |
| FF4-3a | 5 | | | | | | | | 0.47 | 0.59 | 0.33 | | 0.47 | 0.40 | 0.53 | 0.67 | 0.27 | | 0.60 | 0.40 | 0.67 | 15 | + | + |
| A4-3a | 61 | | | | | | | | 1.00 | 0.47 | | | | 0.81 | 0.62 | | | | | | 0.90 | 21 | − | − |
| F(8.14)Q | 9 | 0.23 | | | | | | 0.95 | | | | | | 0.95 | | | | | | | 0.32 | 22 | + | + |
| F(8.14)N | 7 | | | | | | | 0.81 | | | | | 0.55 | 0.59 | 0.81 | | | | | | | 31 | + | + |
| F(8)E | 8 | | | | | | | 0.90 | 0.90 | 0.31 | | | | | | | | | | | | 29 | + | + |
| F(8)D | 8 | | | | | | | 1.00 | | | | | | | | | | | | | | 19 | + | + |
| FF2-2 | 10 | 0.95 | | | | | | 0.80 | 0.95 | 0.95 | 0.95 | 0.85 | 0.55 | 0.90 | | | | | | | | 20 | + | − |
| FF1-9 | 4 | 0.52 | 0.61 | | | 0.52 | 0.35 | 0.52 | 0.87 | 0.91 | 0.83 | 0.91 | | 0.87 | | | 0.91 | | | | | 23 | + | − |
| A1-9v | 11 | | | | | | | 0.58 | 0.58 | 0.95 | | | | | | | | | | | | 19 | + | + |
| A1-9u | 14 | | | | | | 0.62 | | 0.90 | 1.00 | | | | | | | 0.76 | | | | 0.90 | 21 | + | + |
| A1-9l | 19 | | | | | | 0.47 | | 0.59 | 0.88 | 0.65 | | | | | 0.47 | 0.82 | | | | 0.82 | 17 | + | + |

[a] Karyotyping was accomplished by a sequential staining technique in which G-banding was followed by Hoechst 33258 C-banding as described previously (12).
[b] Determined from genomic Southern blots; see legend to Fig. 1.
[c] Determined by electrophoresis of cell extracts and specific histochemical staining; see legend to Fig. 2.

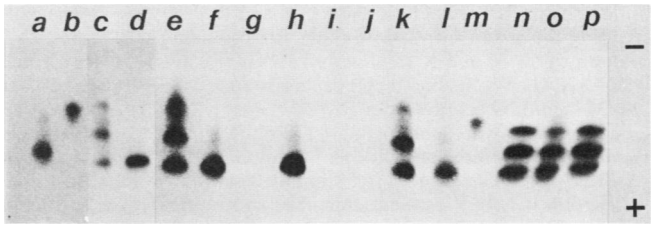


FIG. 2. Expression of rat and mouse TAT in hepatoma × fibroblast hybrids and microcell hybrids. Late-log-phase cultures were exposed to $10^{-6}$ M dexamethasone for 24 h, and cell extracts were prepared as described previously (12) except that the sonication buffer contained 1 mM α-ketoglutarate and 0.1 mM pyridoxal phosphate. The extracts were incubated at 65°C for 5 min and centrifuged at 39,000 × *g* for 30 min. The cleared supernatants were applied to 7.5% polyacrylamide gels and electrophoresed at 15 mA for 5 h. After electrophoresis, bands of TAT activity were visualized by specific histochemical staining (10). Lanes a and l show Fado-2 rat hepatoma parental cells; lanes b and m show C57BL/6J mouse liver cells. Microcell hybrids retaining fibroblast chromosome 8 expressed both rat and mouse TAT [F(8)D, lane c; F(8)E, lane e]. In addition, an enzymatically active rat-mouse heterodimer was detected in all lines expressing both rat and mouse TAT. Mouse TAT expression segregated with murine chromosome 8; APRT$^-$ backselectants of F(8)D and F(8)E expressed only rat TAT [FB(8)D, lane d; FB(8)E, lane f]. F(11)J, a monochromosomal clone retaining fibroblast chromosome 11, was extinguished for TAT expression (lane g), whereas removal of chromosome 11 by backselection resulted in reexpression of rat TAT [FB(11)J, lane h (see reference 12)]. In polychromosomal hybrids retaining fibroblast *Tat-1* (chromosome 8) but not *Tse-1* (chromosome 11), both rat and mouse TAT were expressed (FF4-3a, lane k). In contrast, hybrids with both fibroblast chromosomes 8 and 11 expressed neither rat nor mouse TAT (FF1-9, lane i; FF2-2, lane j). Segregation of mouse chromosome 11 from such clones resulted in reexpression of both rat and mouse TAT (A1-9u, lane n; A1-9v, lane o; A1-9l, lane p).

chromosomes. The results described in this report provide evidence that extinction and activation are related phenomena. In particular, our studies suggest that when gene dosage (3, 5) or chromosome segregation (12, 15) results in a lack of extinction, heterologous gene activation is systematically observed. The obvious implication is that in this system, activation is not a separate phenomenon; it is the simple absence of extinction.

The *Tat-1* gene appears to be completely inactive in fibroblasts (12). Despite this, mouse fibroblast *Tat-1* can be systematically activated and expressed at a level comparable to that achieved in adult liver cells, provided that the fibroblast chromosome carrying *Tat-1* is transferred into hepatic recipient cells and that the fibroblast *Tse-1* locus is not retained. The latter requirement indicates that *Tat-1* expression only occurs in the absence of negative regulation by *Tse-1*. The former requirement raises the possibility that positive factors encoded by the hepatic genome are also required for high levels of TAT expression. While *trans* regulation of both sorts seems likely to exist, negative regulation is clearly the dominant effect in genetic crosses between different cell types.

Finally, our results demonstrate that fibroblast *Tat-1* can be regulated in *trans* by fibroblast *Tse-1*. This observation suggests that *Tse-1* is involved in restricting *Tat-1* gene activity in nonhepatic cells in general. To critically evaluate the function of *Tse-1* in hepatic versus nonhepatic cells, the structure and expression of the locus will need to be investigated directly. This will be a formidable task but one that may provide new information concerning *trans* regulation of tissue-specific genes in mammalian cells.

We thank R. J. Leach, R. Maxson, and R. J. Deans for critically reading the manuscript, G. Schutz for providing the TAT cDNA clone pcTAT-3, and R. Stellwagen for advice on TAT gel electrophoresis. The valuable technical assistance of F. R. Parker and M. M. Smith is acknowledged.

This study was supported by Public Health Service grant GM26449 from the National Institute of General Medical Sciences. A.M.K. received postdoctoral support from the National Institutes of Health. R.E.K.F. is the recipient of an American Cancer Society Faculty Research Award.

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