Proc. Natl. Acad. Sci. USA
Vol. 73, No. 6, pp. 2052–2055, June 1976
Cell Biology

Release by human chromosome 3 of the block at G_1 of the cell cycle, in hybrids between tsAF8 hamster and human cells

(Lesch–Nyhan fibroblast/Sendai virus fusion/Giemsa banding/temperature sensitive mutant)

PEN-MING L. MING, HELENA L. CHANG, AND RENATO BASERGA

Department of Pathology and Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Communicated by Earl P. Benditt, April 9, 1976

ABSTRACT **A temperature-sensitive mutant of Syrian hamster cells, AF8, was fused with simian-virus-40-transformed Lesch–Nyhan fibroblasts, LNSV, in the presence of β-propiolactone-inactivated Sendai virus. The AF8 cells grow well at 33.5° but are arrested in mid G_1 period when shifted to 39°. The LNSV cells are deficient in hypoxanthine guanine phosphoribosyltransferase. The hybrid clones were selected in hypoxanthine–aminopterin–thymidine medium at 39°. A total of 20 clones was isolated and karyotyped. All clones contained most of the hamster chromosomes and one to eight human chromosomes. The preferential retention of human chromosome 3 was observed in 100% of the metaphases of all clones. In nine of the clones, the only human chromosome present was chromosome 3. The results indicate that human chromosome 3 is responsible for conferring to the hybrid cells the ability to grow at nonpermissive temperature for AF8 cells, i.e., the ability to overcome the G_1 block. These findings, together with other reports in the literature, also raise the possibility that different steps in the cell cycle may be regulated by genes located on different chromosomes.**

Fusion of mouse peritoneal macrophages with Lesch–Nyhan fibroblasts transformed by simian virus 40 (LNSV) results in the formation of hybrid cells capable of growing under culture conditions that are nonpermissive for the parental cells. Croce and Koprowski (1) have isolated and karyotyped more than 20 hybrid clones between mouse peritoneal macrophages and LNSV. All clones had at least a complete set of murine chromosomes and a variable number of human chromosomes, but all clones had human chromosome C7 and 4 clones had C7 as the only human chromosome present. Regardless of the fact that this human chromosome was shown to contain the simian virus 40 (SV40) genome (2), these findings can be simply interpreted as indicating that human chromosome C7 from LNSV cells contains information that allows mouse peritoneal macrophages to grow under nonpermissive conditions.

We have recently studied a temperature-sensitive mutant cell line, AF8, which was originally isolated from hamster BHK cells by Meiss and Basilico (3). AF8 cells grow normally at 33.5°, but, when shifted to 39°, stop at a point in the cell cycle that has been located in mid-G_1 (4). Since mouse peritoneal macrophages are presumably arrested in G_0 (see *Discussion*), we have fused AF8 and LNSV cells, and isolated hybrid clones in order to determine whether the same C7 chromosome that releases mouse peritoneal macrophages from G_0 can also overcome the mid-G_1 block of AF8 cells growing at the nonpermissive temperature. The present communication reports the results of our studies.

MATERIALS AND METHODS

Cell Lines. The AF8 cell line is a temperature-sensitive mutant of BHK 21/13 cells isolated and described by Basilico and coworkers (3, 5). The AF8 cells used for this study were obtained directly from Dr. Basilico's laboratory at New York University Medical School, New York. These cells grow normally at 33°, but are arrested in G_1 when shifted to 39°. The temperature-sensitive block can be reversed by returning the cells to the permissive temperature. The cells were grown in Dulbecco's high glucose medium (Gibco) plus 10% calf serum (Flow Laboratories), 100 IU/ml of penicillin, and 50 μg/ml of streptomycin at 33° in a humidified atmosphere of 10% CO_2.

LNSV cells are human skin fibroblasts derived from a patient with Lesch–Nyhan syndrome, and transformed by SV40. These cells are deficient in hypoxanthine guanine phosphoribosyltransferase (HGPRT) and resistant to 15 μg/ml of 8-azaguanine (2, 6). The LNSV cells were obtained from Dr. Rovera's Laboratory at the Wistar Institute, Philadelphia, and were maintained in Eagle's medium containing 10% calf serum and 8-azaguanine at a concentration of 15 μg/ml.

Cell Hybridization. The hamster and human cells were fused in the presence of Sendai virus inactivated by β-propiolactone according to the technique described by Croce *et al.* (8).

Selection of Hybrids. Based on the fact that AF8 cells do not grow at 39° and that LNSV cells are deficient in hypoxanthine guanine phosphoribosyltransferase, the hybrid cells were selected in hypoxanthine–aminopterin–thymidine (HAT) medium (9) at 39°. Hybrid cells growing in the selective medium were propagated and cloned in HAT medium at 39°. Each clone originated from a different hybrid cell colony.

Karyological Analysis. Chromosomes of hamster and human parental cells and of hybrid clones were analyzed and identified by Giemsa banding staining using a method described by Seabright (10) with modifications. The chromosome preparations were made by air-dry technique (11) and treated with a mixture of trypsin (0.05%) and disodium EDTA (0.02%) for 5 min, rinsed with medium containing 5% fetal calf serum, and then rinsed with Hanks' solution. The slides were stained with 2% Giemsa (pH 6.8) for 10 min, rinsed with Gurr's buffer, pH 6.8, and air dried at room temperature. A minimum of 20 metaphases of each hybrid clone and of the parental cells was photographed and analyzed.

Expression of SV40 T Antigen. Hybrid and parental cells were stained for SV40 antigen by indirect immunofluorescence as previously described (1).

RESULTS

Karyotype of AF8 cells

The chromosome composition of AF8 cells was variable, ranging from 36 to 45 chromosomes per cell with a modal number of 40. A representative karyotype is shown in Fig. 1. Pseudodiploidy, monosomy, and aberrant chromosomes such as extra-large submetacentric or acrocentric chromosomes and

Abbreviations: SV40, simian virus 40; HAT, hypoxanthine–aminopterin–thymidine.

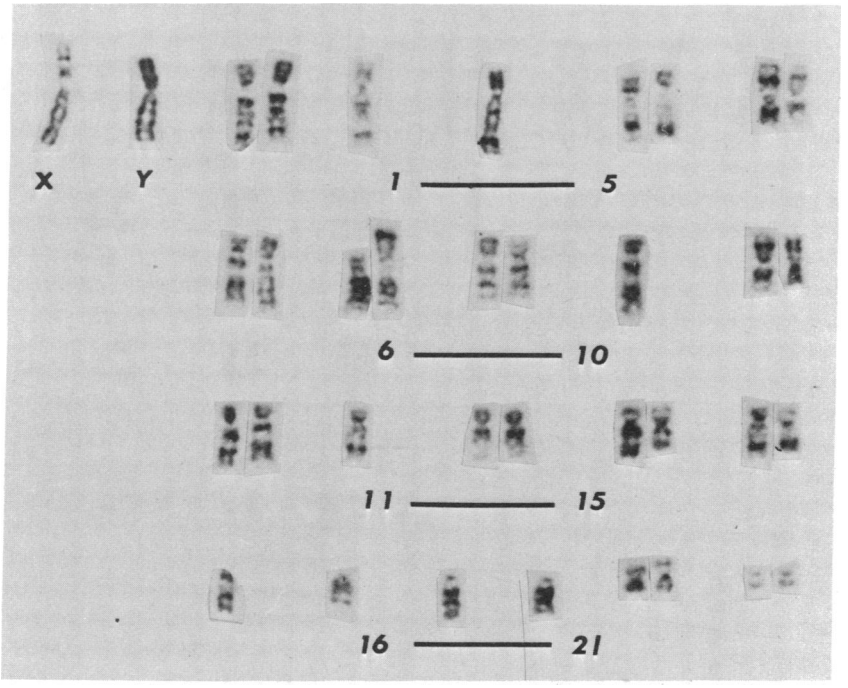

FIG. 1. Karyotype of a tsAF8 cell. The chromosome composition of tsAF8 cells deviates considerably from normal Syrian hamster cells ($2n = 44$). This karyotype shows 36 chromosomes with partial deletion of a short arm of chromosome 7. Aneuploidy and various aberrant chromosomes are often present in tsAF8 cells.

minute acentric fragments were occasionally present. Less than 5% of the cells were quasi-triploid or tetraploid. These results are consistent with the findings reported by Meiss and Basilico (3).

Karyotype of LNSV cells

This is illustrated in Fig. 2. Approximately 70% of the LNSV cells were in the diploid–triploid range, with an average number of 60 chromosomes; the remaining were quasi-tetraploid with an average number of 86 chromosomes. These findings are similar to those previously described by Croce and Koprowski (7).

Karyotype of hybrid cells

A total of 20 clones was isolated by selection in HAT medium at 39°. All clones retained most of the hamster chromosomes. The hamster chromosomes varied both in number and in configuration from clone to clone, and there was no pattern consistent in all the hybrid clones examined. The number of human chromosomes in hybrid cells ranged from one to eight in different clones, as shown in Table 1. Note that all hybrid clones contained human chromosome 3 and, in fact, nine clones contained human chromosome 3 as the only extra chromosome. The karyotype of one such clone is shown in Fig. 3. Note that the human chromosome 3 is readily distinguishable, by its

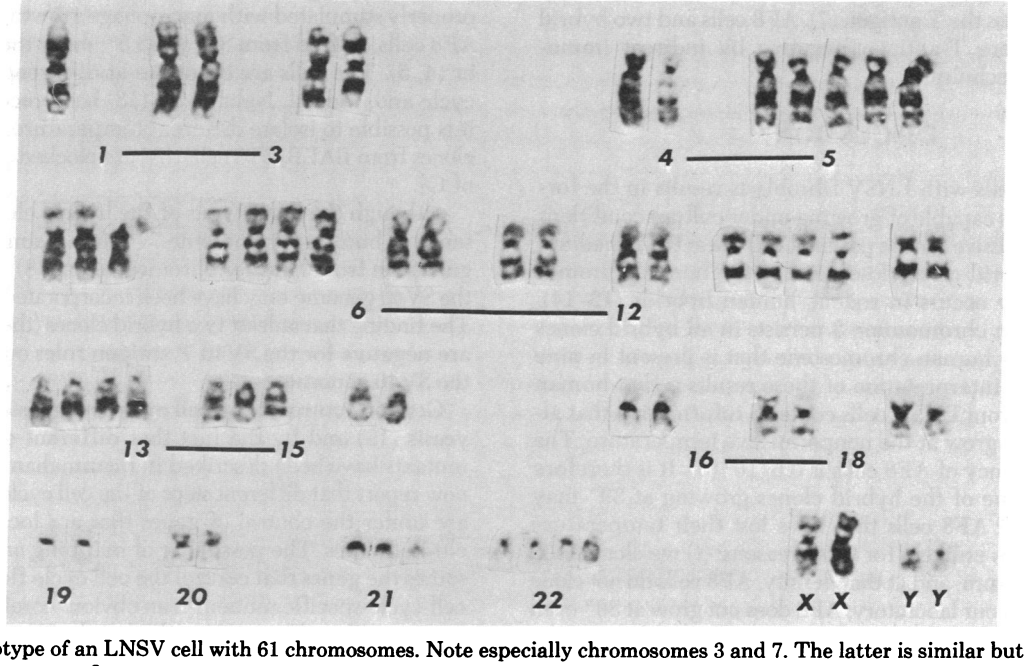

FIG. 2. Karyotype of an LNSV cell with 61 chromosomes. Note especially chromosomes 3 and 7. The latter is similar but distinguishable from hamster chromosome 8.

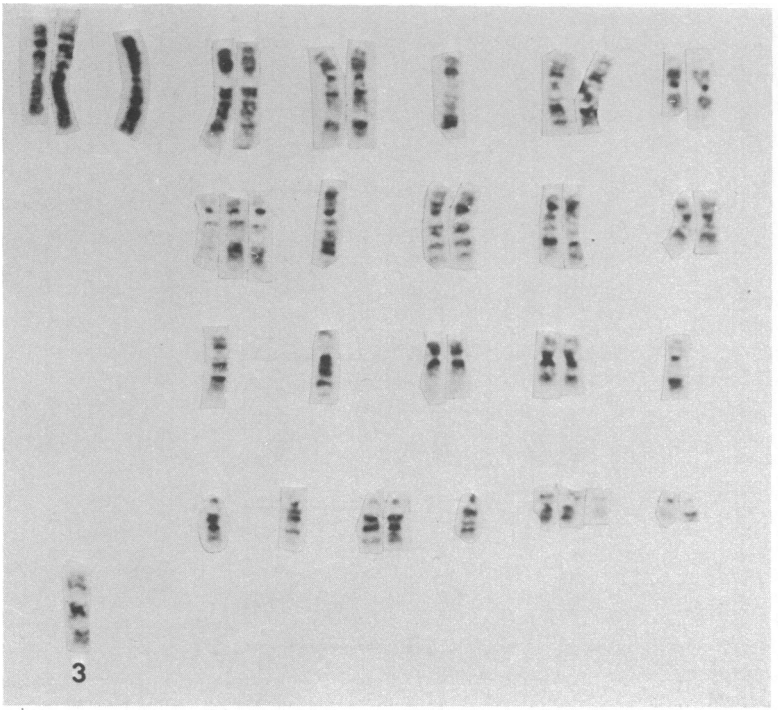

FIG. 3. Karyotype of hybrid clone 106. Chromosome 3 is the only human chromosome present in this hybrid. All the other chromosomes are of hamster origin. There is no pattern of hamster chromosomes consistent in all the hybrid clones examined.

specific banding pattern, from hamster chromosomes (compare Figs. 1 and 2).

It is known that human–rodent hybrid cells lose, unidirectionally, human chromosomes which segregate in various clones (12, 13). The loss of human chromosomes in human–hamster hybrids is even faster than that in human–mouse hybrids (14). In our experiment, substantial loss of human chromosomes was observed as early as the fourth passage after isolation of the clones, and the chromosome composition of the hybrids remained stable during the next seventeen passages.

Expression of the SV40 T antigen

LNSV cells express the T antigen (7). AF8 cells and two hybrid clones tested were T-antigen negative by indirect immunofluorescence techniques.

DISCUSSION

Fusion of AF8 cells with LNSV fibroblasts results in the formation of hybrids capable of growing under culture conditions that are nonpermissive for the parental cell lines (HAT medium at 39°). The hybrid clones lose most of the human chromosomes, as usually occurs in rodent–human hybrids (12–14). However, human chromosome 3 persists in all hybrid clones and it is the only human chromosome that is present in nine clones. A simple interpretation of these results is that human chromosome 3 from LNSV cells contains information that allows AF8 cells to grow at the nonpermissive temperature. The revertant frequency of AF8 cells is $0.6/10^6$ (3). It is therefore possible that some of the hybrid clones growing at 39° may simply represent AF8 cells that have lost their temperature sensitivity. This is unlikely for three reasons: (*i*) we cloned 100 cells per dish, 60 mm, and at that density, AF8 cells do not clone at 39°. In fact, in our laboratory, AF8 does not grow at 39° even when seeded at a density up to 4×10^4 cells per dish; (*ii*) no clone was isolated that did not contain one or more human chromosomes; and (*iii*) the presence of human chromosome 3 in all clones makes it highly unlikely that its presence is fortuitous.

We were surprised to find that it is a human chromosome other than chromosome 7 that is necessary to overcome the mid-G_1 block of AF8 cells, although chromosome 7 allows mouse peritoneal macrophages to grow at nonpermissive conditions. A possible explanation may be that AF8 cells are blocked (at 39°) in mid-G_1, while mouse peritoneal macrophages are in G_0. Whatever one may think of the distinction between G_0 and G_1 cells (15, 16), the fact is that mouse peritoneal macrophages take 20 hr to go into DNA synthesis when properly stimulated with macrophage growth factor (17) while AF8 cells, shifted from 39° to 33.5°, enter the S phase in 6–12 hr (4, 5). The cells are therefore at different steps of the cell cycle and, indeed, Naha *et al.* (18) have recently shown that it is possible to isolate different temperature-sensitive variant clones from BALB/3T3 cells that are blocked at different points of G_1.

Although the karyotypes of the hybrid clones show the absence of human chromosome 7 (which is similar to but distinguishable from hamster chromosome no. 8), it is possible that the SV40 genome may have been incorporated into hybrid cells. The finding that at least two hybrid clones (the only ones tested) are negative for the SV40 T antigen rules out the presence of the SV40 genome.

Genetic control of the cell cycle is suggested by the work in yeasts (19) and by the fact that different cell-cycle-specific mutants have been described in mammalian cells (20–24). We now report that different steps of the cell cycle (G_0 and mid-G_1) are under the control of genes that are located on different chromosomes. The possibility of mapping on human chromosomes the genes that control the cell cycle flow by using other cell-cycle-specific mutants is an obvious corollary. Less obvious is an answer to the question whether the SV40 genome (or other transforming genes) is necessary for the $G_0 \rightarrow G_1$ transition.

Table 1. Chromosome composition of clones hybrid between tsAF8 hamster cells and SV40-transformed human cells*

Hybrid clones	Average no.of chromosomes of hybrids (range)	Human chromosomes present in hybrids†
7A	40 (37–43)	3, 11, 12
11A	41 (37–44)	3
101‡	44 (39–49)	3, 10, 17, 22
102	41 (39–43)	3
103	37 (34–40)	3, 20
104	41 (36–46)	3
106	40 (35–45)	3
107‡	43 (41–45)	3, 16
111	41 (38–44)	3
120	41 (39–43)	3, 20, 21
125	42 (39–45)	3, 16, 20
126	42 (40–44)	3
127‡	41 (39–43)	3, 16
128	41 (39–44)	3
130	40 (38–42)	3, 16
132	42 (42–44)	3
133	75 (72–78)	1, 3, 11, 12, 17, 18, 20, 21
134	42 (40–44)	3, 20
135	42 (40–44)	3
136	42 (39–45)	3, 12, 16, 19, 21

* The hybrid clones were selected in HAT medium at 39°.
† Twenty to 25 metaphases per clone were examined and karyotyped.
‡ Clones 101, 107, and 127 contained two human chromosomes 3.

Recently there have been some indications that only normal cells can go into G_0, while transformed cells can become stationary but cannot enter a true G_0 state (25). Our data, together with those of Croce and Koprowski (1, 2), seem to offer the methodology to answer these questions.

The authors wish to thank Dr. Carlo Croce, Wistar Institute, for performing the T-antigen tests; and Agnes Ernst, Betty Byrd, and Nancy Cyanovich for technical assistance. This work was supported by USPHS Grants CA-12923 and CA-08373.

1. Croce, C. M. & Koprowski, H. (1974) *J. Exp. Med.* **140,** 1221–1229.
2. Croce. C. M., Girardi, A. J. & Koprowski, H. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3617–3620.
3. Meiss, H. K. & Basilico, C. (1972) *Nature New Biol.* **239,** 66–68.
4. Kane, A., Basilico, C. & Baserga, R. (1976) *Exp. Cell Res.*, in press.
5. Burstin, S., Meiss, H. K. & Basilico, C. (1974) *J. Cell. Physiol.* **84,** 397–407.
6. Nyhan, W. L., Bakay, B., Connor, J. D., Marks, J. F. & Kelle, D. K. (1970) *Proc. Natl. Acad. Sci. USA* **65,** 214–218.
7. Croce, C. M. & Koprowski, H. (1974) *Science* **184,** 1288–1289.
8. Croce, C. M., Koprowski, H. & Eagle, H. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1953–1956.
9. Littlefield, J. W. (1964) *Science* **145,** 709–710.
10. Seabright, M. (1971) *Lancet* ii, 971–972.
11. Rothfels, K. H. & Siminovitch, L. (1958) *Stain Technol.* **33,** 73–77.
12. Weiss, M. & Green, H. (1967) *Proc. Natl. Acad. Sci. USA* **58,** 1104–1111.
13. Croce, C. M., Kieba, I. & Koprowski, H. (1973) *Exp. Cell Res.* **79,** 461–463.
14. Kao, F. T. & Puck, T. T. (1970) *Nature* **228,** 329–332.
15. Rossini, M., Lin, J. C. & Baserga, R. (1976) *J. Cell. Physiol.*, in press.
16. Rubin, H. & Steiner, R. (1975) *J. Cell. Physiol.* **85,** 261–270.
17. Virolainen, M. & Defendi, V. (1967) *Wistar Inst. Symp. Monogr.* **7,** 67–85.
18. Naha, P. M., Meyer, A. L. & Hewitt, K. (1975) *Nature* **258,** 49–53.
19. Hartwell, L. H., Culotti, J., Pringle, J. R. & Reid, B. J. (1974) *Science* **183,** 46–51.
20. Scheffler, I. E. & Buttin, G. (1973) *J. Cell. Physiol.* **81,** 199–216.
21. Smith, B. J. & Wigglesworth, N. M. (1973) *J. Cell. Physiol.* **80,** 253–259.
22. Roscoe, D. H., Robinson, H. & Carbonell, A. W. (1973) *J. Cell. Physiol.* **82,** 333–338.
23. Wang, R. J. (1974) *Nature* **248,** 76–78.
24. Liskay, R. M. (1974) *J. Cell. Physiol.* **84,** 49–55.
25. Baserga, R., Costlow, M. & Rovera, G. (1973) *Fed. Proc.* **32,** 2115–2118.