

## DNA CONTENT IN NORMAL, TRANSFORMED, AND REVERTANT MOUSE CELL LINES

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### ABSTRACT

Revertants of SV40-transformed 3T3 cells have been examined for the chromosome number and the amount of DNA per cell. All classes of revertants have more DNA and chromosomes per cell than 3T3 or SV40-transformed 3T3 cells.

The conversion of a normal cell to a malignant cell is often accompanied by alterations in the chromosome number.<sup>1</sup> Many tumors, either spontaneously arising<sup>2</sup> or induced by carcinogens,<sup>3-5</sup> display karyotypic abnormalities. The acquisition of the ability to grow in vitro is also associated with increases in the chromosome number, and many established cell lines will form tumors when injected into suitable hosts.<sup>6-8</sup> However, the chromosome change is not sufficient for tumorigenesis, because nontumorigenic, aneuploid established cell lines exist.<sup>9,10</sup> The mouse fibroblast line 3T3, an example of such an established cell line, has been used as the prototype "normal" cell line in studies of viral oncogenesis.

The growth of normal 3T3 cells is regulated by many environmental factors. They are unable to grow in low concentrations of serum (1%) and require high concentrations of serum for growth.<sup>11,12</sup> They grow to low saturation density in 10% calf serum and must be anchored to a solid substrate to grow.<sup>9,10,13</sup> Transformation by SV40 abolishes some or all of these forms of growth regulation.<sup>12-15</sup> Fully transformed SV3T3 (i.e., 3T3 transformed by SV40 virus) cells grow well in 1% calf serum, grow to high saturation density in 10% calf serum, and form colonies when single cells are suspended in a methyl-cellulose medium.

The SV40 transformation also leads to alterations in other cellular properties. Transformed cells have a SV40-specific intranuclear antigen.<sup>16</sup> Cyclic

AMP concentrations in growing cultures of transformed cells are lower than in similarly growing 3T3 cells.<sup>17,18</sup> Transformants are also more susceptible to agglutination by such plant lectins as concanavalin-A (con-A).<sup>19</sup>

The use of different selection procedures can result in the isolation of variant sublines from populations of SV3T3 cells that have reverted to a 3T3-like state.<sup>20-26</sup> Revertants are isolated by plating transformed cells in conditions that support the growth of transformants only and then adding a drug to kill dividing cells. The revertant cells are unable to divide and therefore survive the selection procedure. Various classes of revertants have been isolated (Tables 1 and 2). Density revertants are selected to have a low saturation density in excess serum concentrations. They are isolated by plating transformed cells at high density in 10% calf serum and adding FUDR, BUdR, or colchicine to kill the cells capable of dividing in dense culture<sup>20,22,25-26</sup> (Table 1). Serum revertants are selected to be unable to grow in 1% calf serum or in 10% agamma-depleted calf serum<sup>26</sup> (Table 1). Sublines resistant to con-A are isolated by exposing transformed cells to high concentrations of con-A.<sup>21,23-24</sup> Some of these survivors have altered growth properties. Revertants isolated in different ways have unique growth properties. In particular, selection for reversion in one growth property does not necessarily result in reversion in all growth properties (Table 3).

Chromosome studies have been performed on transformed and revertant cell lines to further investigate the relationship between alterations in growth control and karyotypic changes. A common finding among revertants is an increase in chromosome number compared to the transformed parents.<sup>22,27-29</sup> The revertants contain more chromosomes than the subtetraploid number found in

TABLE 1  
ISOLATION OF REVERTANTS OF SV40-TRANSFORMED 3T3 CELLS\*

Type of revertant	Growth conditions		Selective agent
	Cell density	Serum concentration	
Density	Dense	10%	FUDR
Density	Dense	10%	BUdR and ultraviolet light
Density	Dense	10%	Colchicine
Serum	Sparse	1%	BUdR and ultraviolet light
Serum	Sparse	10% agamma depleted	BUdR and ultraviolet light
Con-A resistant	Subconfluent	10%	Con-A

\*Transformed cells were plated in the conditions described and treated with various agents toxic to growing cells.

TABLE 2  
GROWTH PROPERTIES OF REVERTANT CELL LINES\*

Line	Anchorage Growth in Methocel†	Density Saturation density in 10% calf serum, 10 <sup>4</sup> (cells/cm <sup>2</sup> )	Serum	
			Doubling time, hr	
			1%	10%
3T3	0.001	5	85	21
SV101	20	>45 (peels)	30	16
Density revertants				
FISV101	0.01	9	36	25
BuSV2	0.02	13	50	22
ColSV4	0.07	14	>100	24
Serum revertants				
LsSV2	0.04	12	>120	22
A $\gamma$ SV5	11	10	>120	21
Con-A revertant				
CA <sup>+</sup> 32	0.005	10	36	22

\*Cells were inoculated at 0.1 to 0.2  $\times$  10<sup>4</sup> cells/cm<sup>2</sup> in 1 or 10% calf serum, and the number of cells per plate was determined daily by trypsinization and counting on a Coulter counter. The medium was changed every 3 days in all growth determinations.

†For Methocel growth, 10<sup>5</sup>, 10<sup>4</sup>, 10<sup>3</sup>, and 10<sup>2</sup> cells were plated in 4 ml of Methocel medium and incubated for 21 days with 4 ml of fresh Methocel medium added every week.<sup>2,5</sup> Only colonies larger than 0.2 mm in diameter were scored.

3T3 cells or SV40-transformed 3T3 cells. Using the Los Alamos flow microfluorometer, we have found that the revertants also contain more DNA than the transformed parent.<sup>2,5</sup>

## GROWTH PROPERTIES OF REVERTANTS

Lines of SV40-transformed 3T3 cells differ from 3T3 cells in many of their growth properties (Fig. 1 and Table 2). Revertants of SV3T3 cells have lost some or all of these transformed growth properties, and these sublines show varying combinations of normal and transformed phenotypes (Fig. 1 and Tables 2 and 3).

Density revertants isolated with FUDR (FISV101) or BUdR (BuSV2) grow to low saturation density in 10% calf serum and cannot form colonies in Methocel. However, they grow in 1% calf serum with a doubling time similar to SV3T3 cells (Fig. 1 and Table 2). Thus these variant sublines have reverted in only two of the three transformed growth properties. The con-A revertant CA<sup>+</sup>32 is also reverted in its density and anchorage properties but not in its

**TABLE 3**  
**GROWTH PROPERTIES OF REVERTANT CELL LINES**  
**ISOLATED FROM SV40-TRANSFORMED 3T3 CELLS**

Line	Saturation density*	Serum requirement†	Anchorage requirement‡
3T3	Normal	Normal	Normal
SV101	Transformed	Transformed	Transformed
Density revertants selected with			
FUDR	Normal	Transformed	Normal
BUdR	Normal	Transformed	Normal
Colchicine	Normal	Normal	Normal
Con-A revertant			
CA <sup>f</sup> 32	Normal	Transformed	Normal
Serum revertants selected in			
1% calf serum	Normal	Normal	Normal
A $\gamma$ -depleted calf serum	Normal	Normal	Transformed

\*Normal cells have saturation densities less than  $15 \times 10^4$  cells/cm<sup>2</sup> in 10% calf serum.

†Assayed by growth in 1% calf serum. Normal cells have doubling times greater than 80 hr; transformed cells double in 35 hr or less.

‡Assayed by ability to form a colony in Methocel. Normal cells do not form colonies in Methocel but transformed cells do.

serum requirement for growth (Table 2). Density revertants isolated with colchicine have reverted in all three growth properties since they also grow poorly in 1% calf serum (Fig. 1 and Table 2).

Two classes of serum revertants exist with respect to these growth parameters. The revertant line selected not to grow in 1% calf serum (LsSV2) has reverted in the other two growth parameters as well. Serum revertant A $\gamma$ SV5, selected for its inability to grow in 10% agamma-depleted calf serum,<sup>30</sup> grows to low saturation density in excess serum but can form colonies in Methocel (Table 2). This line represents a unique class of cells which displays only a transformed anchorage property. Table 3 summarizes the growth properties of the revertants.

## CHROMOSOMES AND DNA CONTENT PER CELL

Diploid mouse-embryo fibroblasts (MEF) contain 40 chromosomes per metaphase. Line 3T3, a continuous cell line derived from mouse-embryo fibroblasts, is subtetraploid and contains a mean chromosome number of

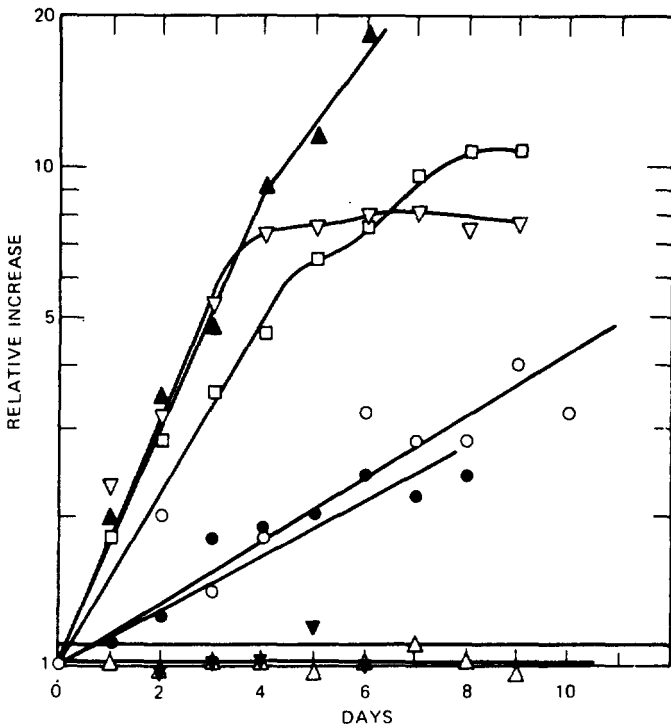


Fig. 1 Revertant growth in 1% calf serum. Growth determinations were done as described in Table 2. ○, 3T3; ▲, SV101; ▽, FISV101; □, BuSV3; ●, ColSV2; Δ, LsSV2; ▼, A<sub>7</sub>SV5.

approximately 70 chromosomes per metaphase (Fig. 2 and Table 4). The SV40-transformed 3T3 cells contain the same number of chromosomes per cell as the 3T3 cells. All revertants show an increase in chromosome number per cell (Fig. 2 and Table 4). Such increases can be marked, as in BuSV3 or FISV101 where the mean value has increased to 95 to 110 chromosomes per metaphase, or they can be slight, as in the colchicine or con-A revertants, where the mean value has increased to 75 to 85 chromosomes per metaphase.

The chromosome increase is accompanied by an increase in DNA content per cell (Table 4). The 3T3 and SV3T3 cells contain approximately three haploid amounts of DNA (Table 4). The DNA measurements were made on individual cells using the Los Alamos flow microfluorometer and cells stained specifically for DNA (acriflavine-Feulgen procedure). The histograms of DNA per cell are shown<sup>31</sup> in Fig. 3. The G<sub>1</sub> peaks are relatively narrow, even for FISV101, which has more than three times the amount of DNA of a diploid mouse-embryo fibroblast. With a few exceptions the increase in DNA content appears to be directly correlated with the increase in chromosomes (Fig. 4). The line with the

TABLE 4  
MEAN CHROMOSOME NUMBER AND DNA CONTENT  
IN REVERTANT CELL LINES\*

Line	Number of metaphases counted	Mean number of chromosomes	Standard deviation	DNA per cell†
MEF		40		2
3T3	15	68.4	14.5	3
SV101	25	69.6	6.9	3
SV3T3(B)	18	75.9	12.0	3.5
A $\gamma$ 61	31	64.0	20.9	3.0
A $\gamma$ 256	25	58.4	11.8	3.3
Density revertants				
BuSV2	30	98.6	22.9	6.0
BuSV3	28	96.5	20.3	6.0
FISV101	29	111.0	23.2	7
ColSV1	30	83.0	12.5	5
ColSV3	32	78.4	9.4	3.5
Con-A revertants				
CA <sup>r</sup> 30	25	78.8	12.9	5.8
CA <sup>r</sup> 32	28	78.8	16.8	5.8
CA <sup>r</sup> 41	23	84.0	16.7	4.4
Serum revertants				
LsSV1	30	95.7	22.3	4.2
LsSV2	30	94.0	18.3	3.5
A $\gamma$ SV4	30	100.3	12.9	4.7
A $\gamma$ SV5	30	92.6	18.1	4.4

\*Chromosome analysis was done as described in Ref. 27.

†The DNA content per cell was measured on the Los Alamos flow microfluorometer as described in Ref. 25.

most chromosomes per cell, FISV101, also contains the most DNA per cell. Con-A revertants and ColSV1, however, contain five haploid amounts of DNA without manifesting a large increase in chromosome number (Table 4).

This increase in DNA content per cell is specific for the revertant phenotype and is not the result of cells simply surviving the selection procedure. The DNA per cell does not increase in lines surviving BUdR treatment, which still maintain all transformed properties (A $\gamma$ 12d2, A $\gamma$ 61, and A $\gamma$ 256) (Fig. 2 and Table 4). Furthermore, rerevertants of FISV, selected for their ability to form colonies on 3T3 monolayers, have also reverted in their chromosome number to a value similar to the transformed parent line (Fig. 5).

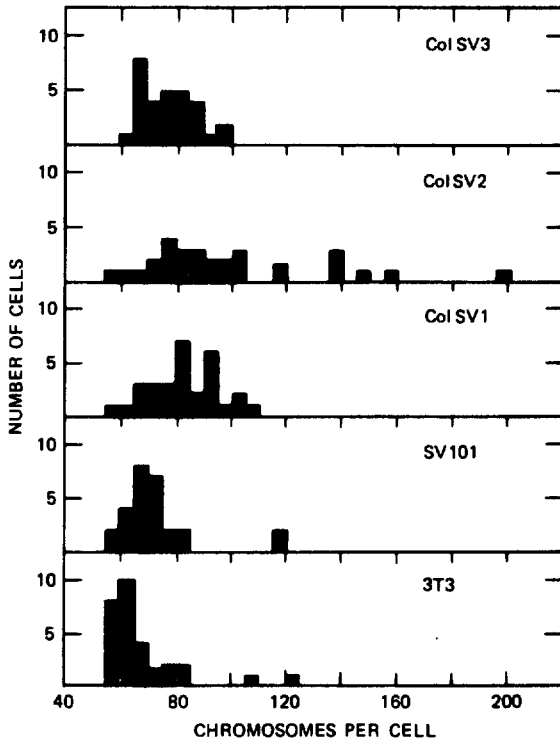


Fig. 2 Chromosomes per cell in various mouse cell lines. Chromosomes were analyzed as described in Ref. 27.

## DISCUSSION

Each revertant has been selected for alteration in a specific growth property, and the selected alteration may or may not be accompanied by reversion in other growth parameters. However, in our hands the reversion process always leads to an increase in DNA, independent of the particular phenotype of the revertant.

These variant sublimes are selected for reversion to a more "normal" state of growth control. It is surprising that, in every case, this reversion to a "normal" type of growth control is accompanied by what may be thought of as an "abnormal" increase in the amount of DNA per cell, which in some cases can be very marked (FISV101). It should be emphasized that a variant line with less DNA per cell than the transformed parent was never found by us, although such variants of polyoma-transformed BHK cells have been reported by others.<sup>28</sup>

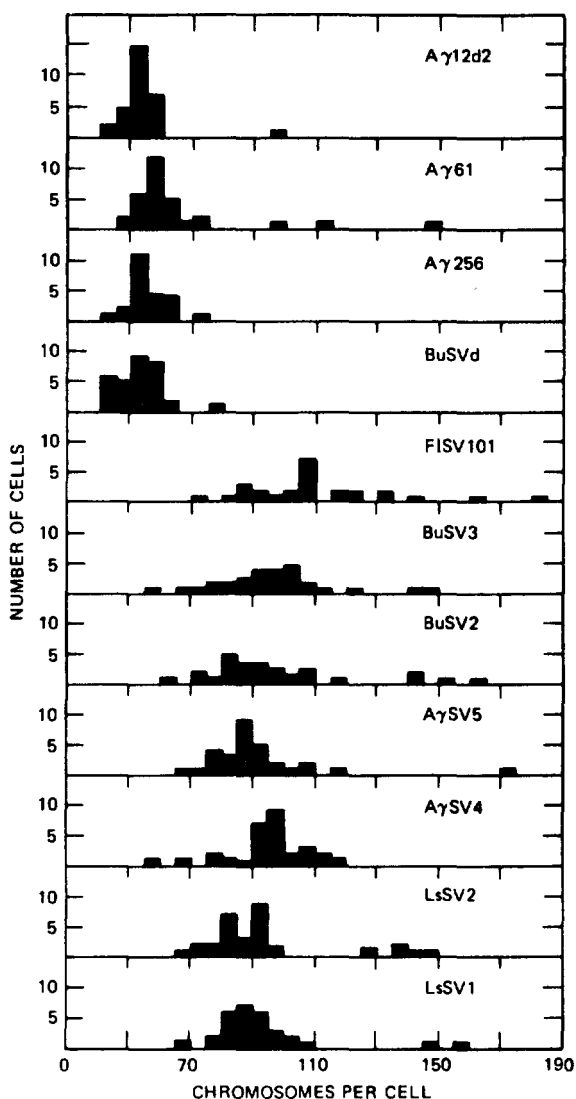


Fig. 2 (Continued).

We as yet do not understand the relationship of the increase in DNA to the reversion process. This phenomenon has been reported in many laboratories.<sup>21-22,25-29</sup> The revertants that we have isolated to date still contain the SV40 genome since they are SV40 T antigen positive and contain SV40 DNA and RNA.<sup>20,25,29,32</sup> It has been difficult to rescue SV40 from the revertants by fusion with permissive monkey cells, but the virus that has been rescued is wild type with respect to its ability to transform 3T3 cells.<sup>24-25</sup> Thus the



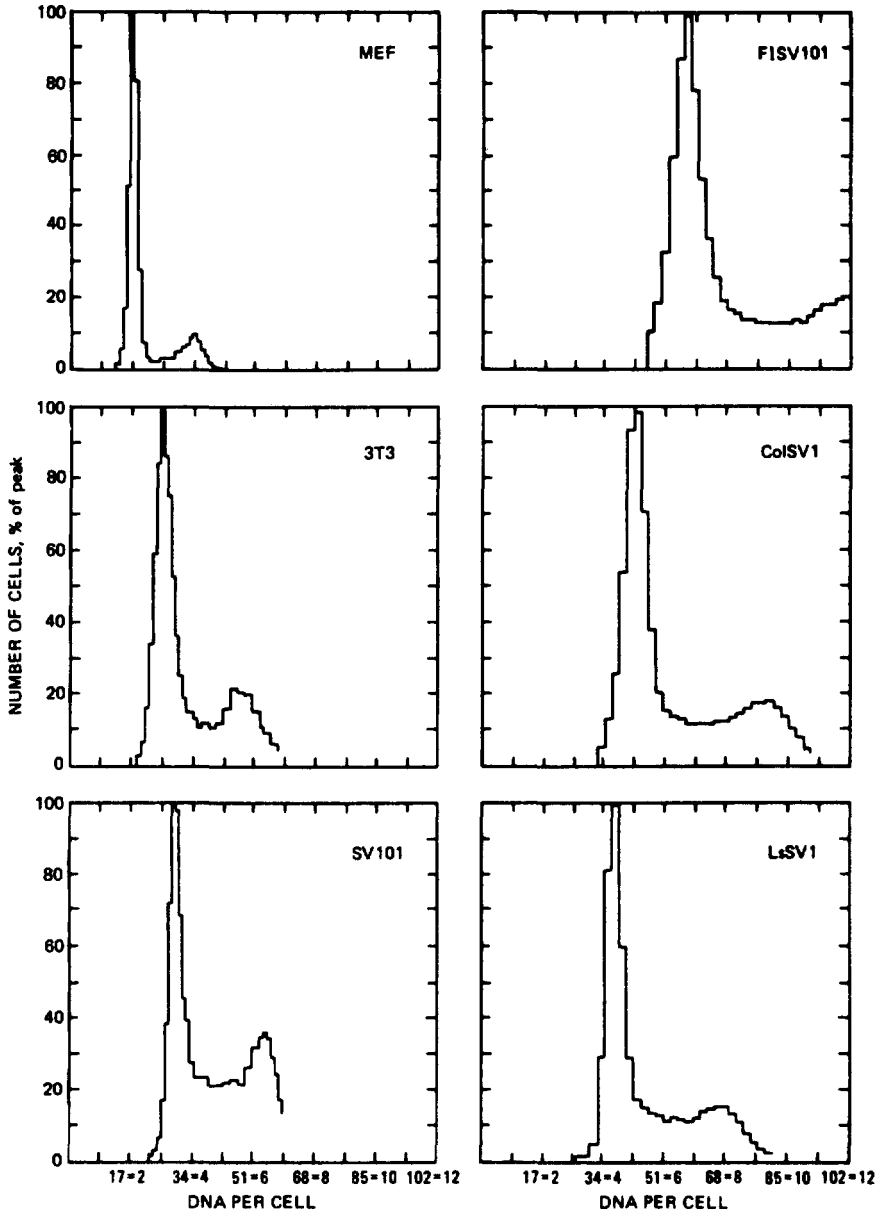


Fig. 3 Histograms of DNA content per cell in normal, transformed, and revertant mouse cell lines. The DNA content is measured in haploid equivalents of cell DNA, relative to mouse-embryo fibroblasts. (From A. Vogel, J. Oey, and R. Pollack, Two Classes of Revertants Isolated from SV40-Transformed Mouse Cells, in *Control of Proliferation of Animal Cells*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1974.)

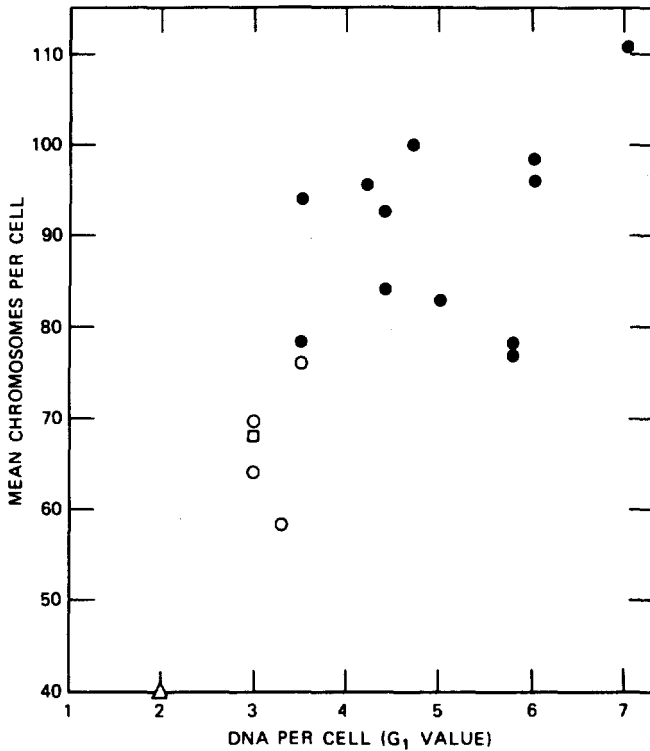


Fig. 4 Chromosomes per cell vs. DNA content per cell. The DNA content is expressed in haploid equivalents of DNA.  $\triangle$ , MEF;  $\square$ , 3T3;  $\circ$ , transformant;  $\bullet$ , revertant.

revertants may represent classes of cells which have managed to overcome the effects of the SV40 still present in these cells. If this is the case, then the increase in DNA may be involved in this cellular alteration.

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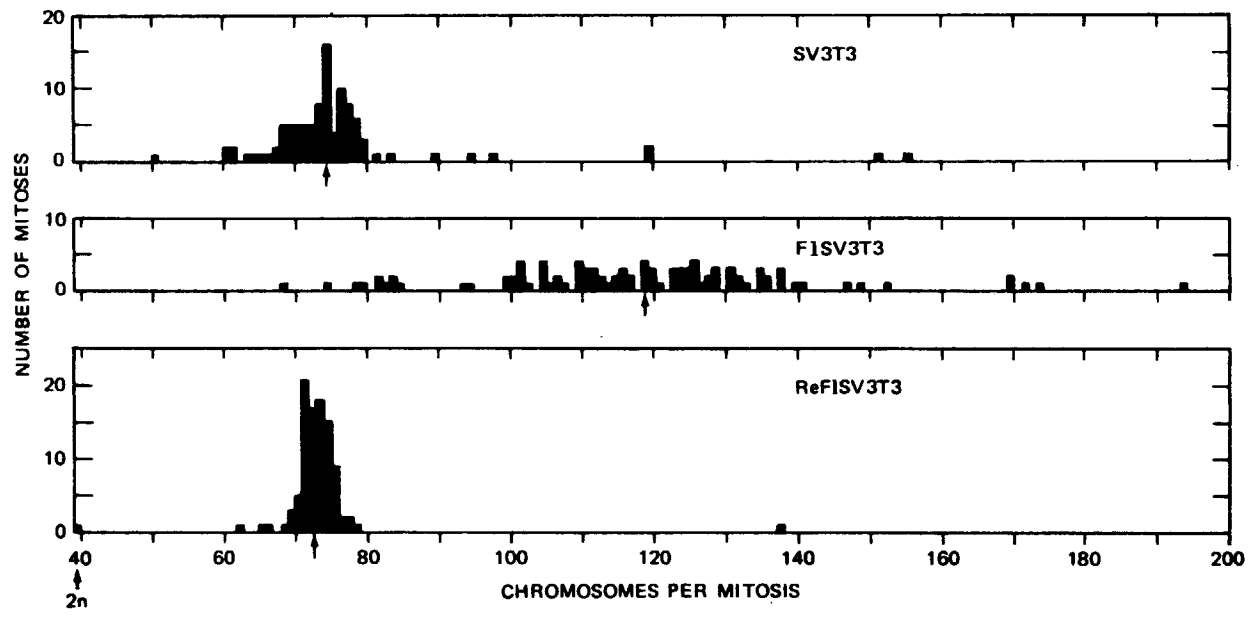


Fig. 5 Chromosomes per cell in SV3T3, F1SV3T3, and a rerevertant of F1SV3T3.

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