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Karyological Properties of Human–Mouse Somatic Hybrids

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ABSTRACT Karyological examinations performed 24 hr to 11 days after Sendai virus-induced fusion of mouse cells of the permanent line 3T3 and human diploid cells, suggest that hybrids with extensive loss of human chromosomes derive from fusion involving all the chromosomes of both parental cells. Examination of 39 independent clones isolated from two different crosses between L mouse cells and cells of two permanent human lines, D98/AH2 and VA2, show that the karyotypes of human–mouse hybrids are a function of the interaction between the parental cells: the mouse chromosomal complement may be doubled or not. For the first time, viable hybrids that have retained almost all of the human chromosomes have been isolated.

A survey of the published data on human–mouse somatic hybrids (1–8) shows that in all cases a massive loss of human chromosomes was observed, and that this holds whether the hybrids were formed spontaneously or after fusion induced by inactivated Sendai virus. Moreover, these data suggest that the hybrids can be grouped into two general classes:

(*i*) the first is characterized by the presence of a single complete set of mouse chromosomes and by early loss of the human chromosomes (2–7); only 0–15 of the human chromosomes are retained at the 20th hybrid cell generation and most of these are lost between the 20th and the 100th generation, either in standard or in 5-bromodeoxyuridine (BrdU) medium.

(*ii*) the second is characterized, with rare exceptions, by the presence of two sets of mouse chromosomes and by a slower rate of loss of human chromosomes (persistence of 10 or more human chromosomes at the 150th generation) (1, 3, 8).

The five types of hybrids that belong to the first class have in common the mouse parent (an L cell); their human parents are either diploid cells or an SV40-transformed aneuploid ("pre-crisis") cell (one case).

The second class comprises three types of hybrids, whose mouse parents are either diploid cells or cells from the permanent line 3T3; their human parents are cells from permanent lines.

These data are, however, quite imcomplete and represent the results reported by several authors, whose aim in most cases was the isolation of a few hybrid clones rather than the extensive karyological study of hybrids that are produced in a given cross. It therefore appeared of interest to try to establish the empirical rules governing both the variations of ploidy of the mouse genome and of the loss of human chromosomes, by the systematic study of a great number of hybrids that are formed in a given cross and by the observation of both the early mitoses of newly formed hybrids and the karyotypes of viable hybrid clones.

The present article describes observations on hybrids from three crosses: the first between mouse cells of the permanent line 3T3 and human diploid cells, and the two others between cells of the permanent mouse L line, LM (TK^-) Cl 1D, and human cells of the permanent lines D98/AH2 and VA2, respectively. The first cross was chosen for the favorable karyotype of the mouse cells; the two others, were chosen because of the presence in both parental lines of selective markers, which facilitate both earlier isolation and quantitation of hybrids†.

MATERIAL AND METHODS

Mouse cells. Clones 3T3-4E (3T3) (1) and LM(TK^-)Cl 1D (Cl 1D) (10), that are deficient in thymidine-kinase, are resistant to 30 μg of BrdU/ml.

Human Cells. FH10 is a strain of diploid cells isolated from an embryo. D98/AH2 and VA2 (3) are both permanent lines deficient in hypoxanthine–guanine–phosphoribosyl-transferase and resistant to 3 μg of 8-azaguanine/ml of medium.

The parental cells were maintained in Dulbecco's modified Eagle's medium, supplemented with 10% calf serum and, depending on the line concerned, with 30 μg of BrdU/ml or 3 μg of 8-azaguanine/ml. Hybrid cells were selected and maintained in HAT (the same medium as above supplemented with 100 μM hypoxanthine, 400 nM aminopterin, and 16 μM thymidine, ref. 11).

Hybridization Experiments. For crosses of FH10 x 3T3 and of D98 x Cl 1D, the technique of Sendai virus fusion described by Davidson (12) was used, with 5×10^5 cells of each parental population and 300 hemagglutinating units (HAU) of UV-inactivated Sendai virus. For some experiments, the parental cell ratio was modified (10 FH10:1 3T3 cell). 24 hr later, the cells were dispersed with trypsin; 5- and 10-fold dilutions were inoculated into 6-cm dishes with 5 ml of HAT.

For crosses of VA2 x Cl 1D, a suspension mixture containing 1×10^6 of each parental type was exposed to 300

Abbreviations: BrdU, 5-bromodeoxyuridine; HAT, Dulbecco's modification of Eagle's medium with 10% calf serum and hypoxanthine, aminopterin, and thymidine; HAU, hemagglutinating unit.

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† A preliminary account of some of these results has been published in *C. R. Acad. Sci.* (9)

TABLE 1. *Early metaphases of FH10 × 3T3 hybrid cells*

Time after fusion	Metaphase cells examined	Human karyotype						Aberrant karyotypes
		Complete			Incomplete			
		T*	1s†	2s†	T	1s	2s	
24 hr	88	51	*43*	*8*	9	*6*	*3*	28
48 hr	12	10	*10*	*0*	2	*2*	*0*	0
3–6 days	23	11	*8*	*3*	10	*9*	*1*	2
9–11 days	28	4	*2*	*2*	24	*16*	*8*	0
Total	151							

* T: Total number of metaphases.
† 1s, 2s: mouse chromosomes

HAU of Sendai virus in 0.3 ml of serum-free medium. The cells were plated in six 10-cm dishes with 7 ml of HAT. All media were renewed twice a week.

Hybrid colonies were picked and transferred to one 3-cm dish with 1.5 ml of HAT. As soon as the cell layer was confluent, the cells were dispersed with trypsin and plated into two 3-cm dishes. Karyological examinations were made on one of the dishes by the technique of Rothfels and Siminovitch (13).

Karyological Characteristics of the Parental Lines. 3T3 has 76 [64–80] chromosomes, all telocentrics.

Cl 1D has 52 [50–55] chromosomes of which 44 [39–47] are telocentrics and 8 [7–11] long metacentrics and submetacentrics. Among the latter, one is characterized by a secondary constriction ("D" chromosome). The frequency of 2s cells is less than 1%.

Diploid human FH10 cells have 46 chromosomes.

D98 has 62 [58–64] chromosomes. Application of the Denver classification leads to the following distribution: 5 [3–8] chromosomes of group A, 1 [0–2] of group B, 25 [22–30] of group C, 7 [4–8] of group D, 11 [8–15] of group E, 7 [4–9] of group F, and 5 [2–8] of group G.

VA2 has 74 [67–80] chromosomes, of which 6 [4–6] are of group A, 4 [2–5] of group B, 36 [34–40] of group C, 6 [5–7] of group D, 10 [9–14] of group E, 6 [6–9] of group F, and 5 [2–6] of group G.

Karyological Analysis of the Hybrids‡. In the hybrids, about 10 human chromosomes of group D and G can be confused with mouse telocentrics, since when their satellites are not clearly visible, these chromosomes appear as telocentrics. Therefore, all telocentric chromosomes were classified as mouse chromosomes, except when the presence of satellites allowed the identification of D or G chromosomes.

Small and medium submetacentric chromosomes were classified as human.

It was not possible to distinguish the human chromosomes of group A and the largest of group C from the submetacentric chromosomes of Cl 1D. In a given metaphase, the length of the mouse "D" chromosome was taken as a standard: the ratio of lengths of the smallest submetacentric of Cl 1D and of the "D" chromosome is 0.7–0.8. In the hybrid cells therefore, only those submetacentric chromosomes with a length <0.7 times that of the "D" chromosome were classified as human chromosomes; the A and the largest C chromosomes, if present in a hybrid metaphase, were confused with Cl 1D chromosomes.

Human chromosomes of group B were identified in the hybrids by their length and very marked inequality of arm length.

‡ This work was performed before the quinacrine mustard fluorescence technique made it possible to identify almost all of the human chromosomes in this type of hybrid (14).

RESULTS

FH10 × 3T3. This cross was chosen because almost all the human chromosomes can be identified in the hybrid cells. Table 1 summarizes the results of a karyological study made during the first days after cell fusion. Analysis of 88 hybrid cells in metaphase, 24 hr after virus-induced fusion, shows that the majority of cells contain the complete genome of both parents. The nonrandom topographic distribution of the chromosomes of the two species in many of the cells in metaphase makes it highly probable that they represent indeed the first mitosis of the hybrid cell. These observations indicate that hybrids with extensive loss of human chromosomes derive from fusions involving all the chromosomes of both parental cells.

The mouse genome is 1s in about 80% and 2s in about 20% of these cells in metaphase. However, already at this time, 10% of the cells in metaphase that contain either one or two mouse genomes are deficient in human chromosomes and the frequency of their occurrence increases markedly during the following days. On the other hand, about 30% of the cells in metaphase have aberrant numbers of chromosomes of either one or both parents (81–114 mouse chromosomes, 52–69 human chromosomes). The proportion of these aberrant chromosomes clearly diminishes in the following days (see Table 1).

The slow rate of development of hybrids, as compared to that of the human parental cells, did not permit their further study. A more extensive analysis was therefore performed on hybrids from the D98 x Cl 1D and VA2 x Cl 1D crosses.

D98 × Cl 1D. In the cross D98 x Cl 1D, the first hybrid colonies were detected 9 days after fusion, while other colonies were detected later and developed more slowly. A total of 32 presumed clones, from 23 different dishes were isolated, 15–32 days after fusion. The frequency of viable hybrid formation was $1–4 \times 10^{-4}$.

Karyological analysis of these 32 hybrid colonies showed that all had lost large numbers of human chromosomes, retaining 1–41; only three of them contained a single set of

mouse chromosomes; the 29 others had a higher number of mouse chromosomes, generally slightly more or less than 2*s*. Moreover, a preliminary analysis showed that while some of these 32 hybrid colonies showed only a narrow range in numbers of human and mouse chromosomes, others showed an unexpectedly great variability. A careful study was made of 12 of the clones, care was taken to ensure that this sample included all of the types mentioned above, i.e., those showing great or small variability, many or few human chromosomes, and a 1*s* or 2*s* Cl 1D complement.

Fig. 1 shows the distribution of chromosomes in the parental cells and in the 12 hybrid clones. The number of human chromosomes is different from clone to clone and from one metaphase to another within a clonal population. This variation is small in some cases (e.g., clones "121", AH111, and AH17) and more pronounced in others (clones AH133 and AH162).

The number of telocentric chromosomes in clone "121" is exactly within the expected range. In all the other clones that are considered as 2*s* mouse, the majority of metaphases contained fewer telocentric chromosomes than expected (78–94; particularly evident in clone AH101), while a few metaphases had higher numbers of telocentric chromosomes than expected (AH162, AH211). However, hybrid clone "128" represents an exception, since every metaphase presented more telocentric chromosomes than expected for a 2*s* mouse.

The same remarks can be made concerning the long metacentric chromosomes, but the confusion with some human chromosomes makes the distribution less obvious; moreover, the presence of two "D" marker chromosomes of the mouse was not always observed in the cells in metaphase of hybrid clones classified as 2*s* mouse.

In the hybrid clones containing a 1*s* mouse complement, the loss of human chromosomes is almost total after 20–25 generations (only 1–4 remained in three independent clones); when the mouse complement exceeds 1*s*, many metaphases have a higher number of human chromosomes (1–41 in 29 independent clones).

VA2 × Cl 1D. Crosses of Cl 1D with a second permanent human cell line, VA2, gave results somewhat different from those just described. The presumed hybrid colonies appeared later, being detectable only 15 days after fusion, and showing great variations in growth rate and viability: some grew rapidly, others degenerated and disappeared, and some remained stationary for several weeks and subsequently resumed rapid growth. Among 108 hybrid colonies marked on day 18, only eight were successfully propagated. The frequency of viable hybrid formation was low, about 1×10^{-5}.

The karyotypes of seven hybrid clones, picked 18–46 days after fusion, were analyzed. Four had a single set of Cl 1D chromosomes and a very small number of identifiable human chromosomes [0–12]; in these clones, the numbers of telo-

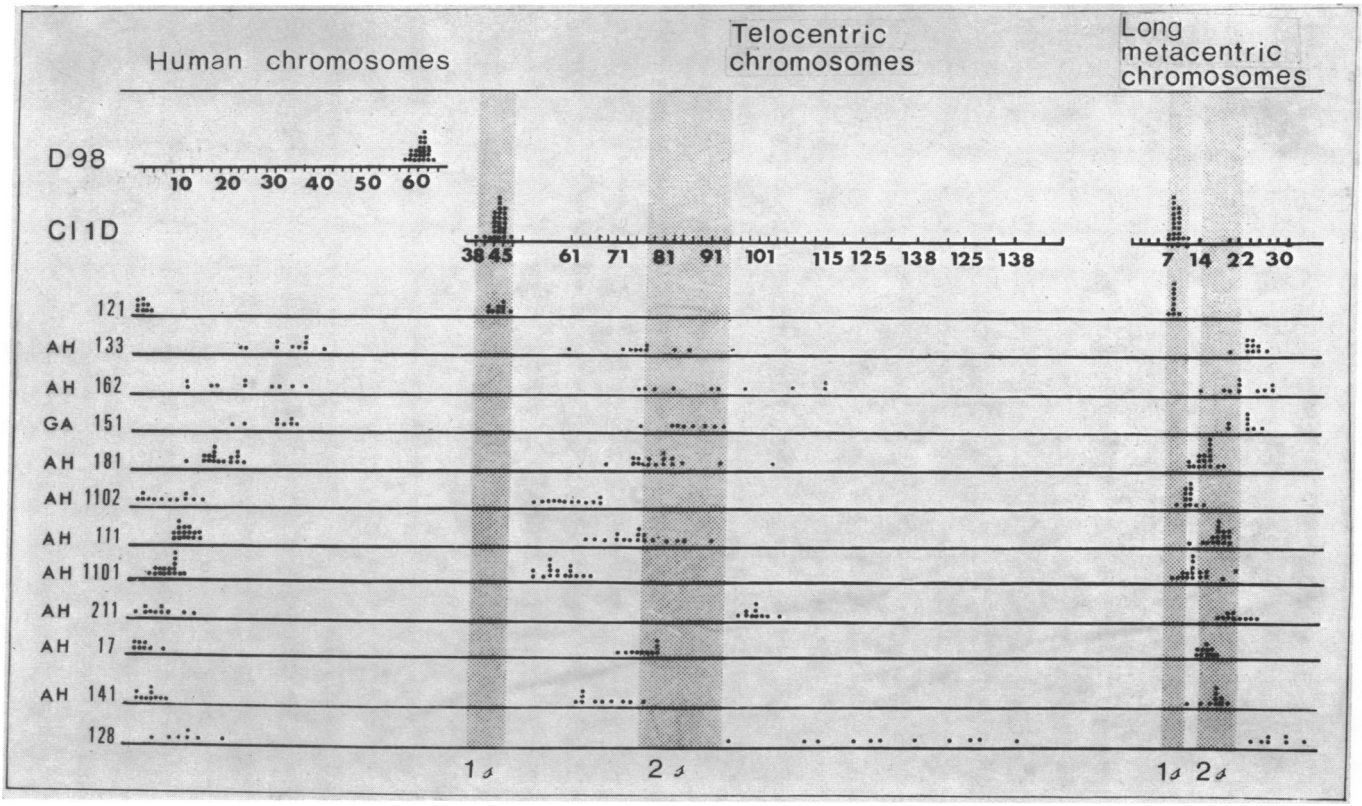

FIG. 1. Distribution of chromosomes in metaphases of parental and hybrid cells of 12 clones from D98 x Cl 1D cross*,†.

* 20–25 generations after fusion.

† Each point represents the number of human chromosomes (*left*), of mouse telocentric chromosomes (*middle*), and of long metacentric chromosomes (*right*) in one hybrid cell in metaphase, as compared to the distribution of these chromosomes in D98 and Cl 1D parental cells. The shaded areas correspond to the expected ranges of telocentric and long metacentric chromosomes in hybrid cells containing, respectively, a 1*s* or 2*s* mouse complement.

centric chromosomes [40–47] and long metacentric chromosomes [7–11] corresponded exactly to the distribution of these chromosomes in the parental mouse cells. One hybrid clone had a 2*s* mouse complement and retained a higher and more variable number of human chromosomes [10–34].

Two hybrid clones are very unusual in that they retained almost all human chromosomes. One of these (S61) had also almost all mouse chromosomes. The expected total number of chromosomes was 126 [110–135]. This hybrid had no clear mode (range 102–120) with 35–51 telocentric chromosomes, 9–14 long metacentric chromosomes, and 50–66 human chromosomes (Fig. 2*a*). The second unusual clone (F51) had lost a very large number of mouse chromosomes. Cells in metaphase of this clone showed a great deal of heterogeneity. Most of them contained a number of human chromosomes [54–80] slightly less or equal to the parental number, as well as 6–30 mouse chromosomes. However, a few metaphases had a much higher number of human chromosomes [105–146] and/or mouse chromosomes [55–66]. The presence of the mouse "D" marker and of long telocentric chromosomes provided unequivocal proof of the hybrid nature of cells of this clone (Fig. 2*b*).

DISCUSSION

The purpose of these experiments has been to attempt to establish the empirical rules that govern the karyological characteristics of human–mouse hybrids. It has been shown that, contrary to earlier observations, a mouse L cell derivative is subject to the phenomenon of "doubling up" of its chromosomal complement when crossed with human cells of permanent lines, an observation previously limited to diploid mouse cells and to cells of mouse line 3T3. Clearly the karyotype of human–mouse hybrids is a function not uniquely of the mouse parent, but of the interaction between the two parental cells. It is also clear that from a single cross, hybrids of the two general classes (defined in the *Introduction*) can be obtained, and moreover, that a third class also occurs, i.e., hybrids that retain essentially the complete human genome and may show preferential loss of mouse chromosomes.

The hybrids that contained a 1*s* mouse complement had exactly the expected number of mouse chromosomes: this fact allows us to assume that, in these hybrids, the loss of human chromosomes is not the result of unequal mitoses. By contrast, hybrid cells with more than one parental mouse genome had an irregular and variable number of mouse chromosomes, and in addition, the number of human chromosomes present in these cells was greater and more variable. These observations suggest that (*a*) human chromosomes are better "tolerated" when the genic balance is modified by an excess of mouse chromosomes; (*b*) karyotypes are not stabilizated as yet after 20–25 generations. The karyotype of the clone with inverted loss also showed great variability.

Hybrid clones with two sets of mouse chromosomes are very frequent in D98 x Cl 1D as in D98 x 3T3 (1) and VA2 x 3T3 (8) crosses. These cells may result from (*a*)

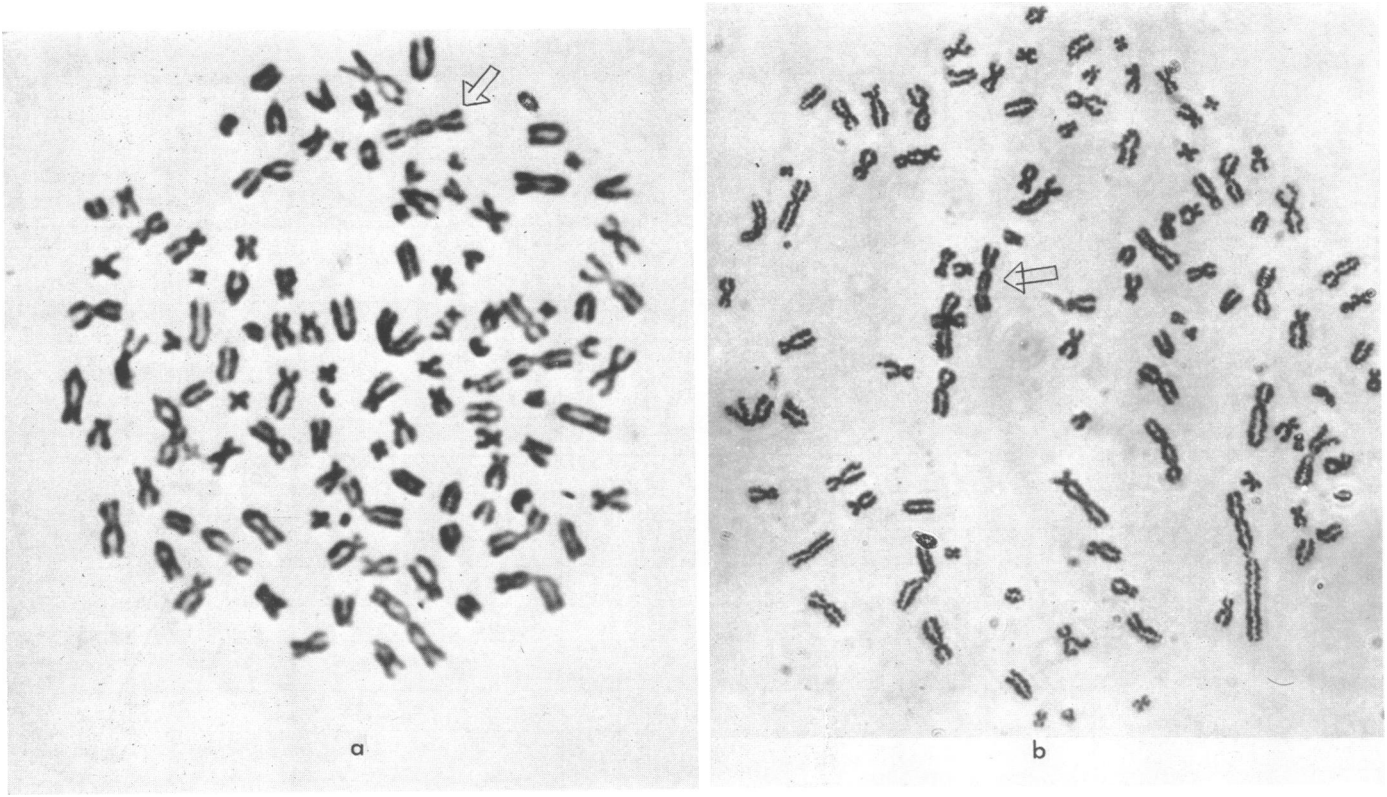

FIG. 2. Metaphases of cells of two clones from *VA2* × *Cl 1D* cross*,†.

* 20–25 generations after fusion.

† Human chromosomes were not segregated. (*a*) in S61, mouse chromosomes are present; in this metaphase 37 telocentric chromosomes, 12 long metacentric chromosomes, and 61 human chromosomes are present. (*b*) in F51, mouse chromosomes were segregated; in this metaphase 16 telocentric chromosomes, 9 long metacentric chromosomes, and 72 human chromosomes are present. *Arrows* indicate the mouse "D" marker. Magnification ×1000.

multiple fusions; (*b*) preferential fusion and/or hybrid formation of preexisting 2*s* mouse parental cells; (*c*) failure of cytokinesis of a hybrid cell that has already undergone extensive loss of human chromosomes; (*d*) difference in phase of parental cell cycles, before or after fusion.

(**a**) Some observations appear to contradict the first hypothesis: hybrid cells with a 2*s* mouse complement have been observed in spontaneous as well as in virus-induced crosses (1, 3); hybrid cells with only a single mouse genome have been derived from certain crosses performed with Sendai virus (4, 5), which induces the formation of considerable numbers of polykaryocytes; the mouse genome was indeed doubled in hybrids derived from crosses in which the ratio of parental cells was favorable to human cells (10FH10:1 3T3). While direct demonstration of multiple fusion is reported by Ricciuti and Ruddle (15), who described one hybrid clone between two different mouse cells and human WI38, these authors did not give quantitative data that would permit an evaluation of the frequency of this phenomenon.

(**b**) The second hypothesis appears unlikely, since the frequency of 2*s* Cl 1D cells is less than 1% in the parental population, and yet the frequency of hybrid clones, most of which contained a 2*s* Cl 1D complement, was in the expected range. If only 1% of the parental cells gave rise to hybrids, an extremely low frequency of hybrid colonies would most likely be observed.

(**c**) The third hypothesis is compatible with the observation that there are more human chromosomes in cells with two mouse-genomes than in those with only one. However, it does not account for the presence, 24 hr after cell fusion, of cells in metaphase with 2*s* mouse and one complete human diploid complement (FH10 x 3T3).

(**d**) All of the crosses described above were performed with nonsynchronized parental populations. As fusion of parental cells possibly occurs at random, while each may be engaged in a different phase of its cycle (16), it is possible to hypothesize that a G2 mouse cell fused with a G1 or S human cell may resume chromosome replication in response to signals for synthesis of human DNA, and if so, the mitosis would separate four sets of mouse chromosomes and two sets of human chromosomes. Such a resumption of DNA synthesis was indeed observed in nuclei of *Amoeba proteus* in G2 transplanted into *Amoeba* in S (17). However, in binucleate human cells, DNA synthesis is regular though asynchronous and metaphases are simultaneous (18). Moreover, in dikaryons obtained by Sendai-virus-induced fusion of two nonsynchronized (16) or synchronized (19) HeLa-cell populations, coordination of DNA synthesis is observed, while resumption of DNA synthesis by G2-nuclei fused with G1- or S-phase nuclei is not observed.

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