PREMATURE CHROMOSOME CONDENSATION:
A MECHANISM FOR THE ELIMINATION OF
CHROMOSOMES IN VIRUS-FUSED CELLS

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SUMMARY

The fate of prematurely condensed chromosomes (PCC) that are induced following fusion
between a mitotic and an interphase cell was studied in the homokaryons of HeLa and
2 glycine-requiring mutants of Chinese hamster ovary cells. The data indicate: (1) the prema-
turely condensed chromosomes are often incorporated into daughter nuclei of the fused cells
and hence they are functionally retained by the progeny; (2) in general, the only viable products
of fusion between mitotic and interphase cells are those where PCC has been induced; (3) fusion
between mitotic and S-phase cells leads to the most rapid loss of chromosomes, while the
homophasic fusions tend to produce more stable hybrids with regard to their chromosome
number.

INTRODUCTION

Formation of visible, condensed chromosomes can be induced in an interphase
nucleus by placing it in a proper environment; that is, within a mitotic cell. The
visualization of interphase chromosomes achieved by the fusion of mitotic with inter-
phase cells is termed premature chromosome condensation or PCC (Johnson & Rao,
1970). The factors present in a mitotic HeLa cell are capable of inducing PCC in
differentiated or tissue culture cells from a wide variety of animal species including
mosquito cells (Johnson, Rao & Hughes, 1970). The condensed G₁ and G₂ chromo-
somes so produced are discrete units with one and two chromatids, respectively. The
condensed S chromosomes on the other hand, present a 'pulverized' appearance. In
the present study the fate of the prematurely condensed chromosomes from G₁,
S and G₂ nuclei is examined in heterophasic homokaryons which complete division
after fusion. The data presented indicate that the PCC are randomly segregated into
the daughter nuclei, and that in many instances are retained by the progeny of the
fused cells without loss of their genetic activity. The significance of these findings in
relation to the chromosome losses that are common among heterokaryons produced
by cell fusion is discussed.

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MATERIALS AND METHODS

HeLa cells and auxotrophic mutants of Chinese hamster ovary (CHO) cells were used in this study. The procedures for the maintenance of HeLa cells in suspension culture were described earlier (Rao & Engleberg, 1965). HeLa cells were synchronized by the use of excess thymidine (2.5 mM) double block technique (Rao & Engleberg, 1966). Synchronized populations of mitotic HeLa cells were obtained by the application of a nitrous oxide block (Rao, 1968). The reversal of the nitrous oxide block results in the rapid restoration of the mitotic spindle and the subsequent completion of the cell division.

Medium change
Harvest cells in

Colcemide added

M* G1 S G2

-2 0 2 3 9 13

Hours

Fig. 1. Synchronization procedure for CHO cells. A schematic presentation of the time schedules for obtaining synchronized populations of Gly-A and Gly-B mutants of Chinese hamster ovary cells. *Mitotic cells collected, colcemide block reversed and cells plated in fresh medium.

The 2 glycine-requiring mutants (Gly-A and Gly-B) of Chinese hamster ovary cells were derived from the parental culture CHO-K1 and were grown as described previously (Kao & Puck, 1967; Puck & Kao, 1967). They belong to different complementation groups so that their fusion hybrids will grow in the absence of glycine even though neither parental cell can multiply without glycine in the medium (Kao, Chasin & Puck, 1969a, b).

Cells of each mutant were synchronized by the reversal of a colcemide block. Mitotic cells of 96-97% purity were harvested by gentle shaking after the cultures were exposed to colcemide (0.05 µg/ml) for 2 h. The mitotic cells were centrifuged, resuspended in fresh medium and plated in several plastic dishes. Cells that failed to attach to the dish within 1 h after plating were removed by changing the medium in order to improve the degree of synchrony. About 2 h after the reversal of the colcemide block virtually all the cells had completed mitosis. These dishes were trypsinized at appropriate times to obtain \( G_1 \), \( S \) or \( G_2 \) populations as shown in Fig. 1. A 15-min exposure of the \( S \) cells to \(^3\)H-TdR resulted in the labelling of 94% of the population indicating a high degree of synchrony. However, the degree of synchrony among the \( G_2 \) population is rather poor in comparison to the other phased populations.

Hybridizations between random and synchronized populations of Gly-A and Gly-B cells were carried out, using synchronization in the same or different phases of the life-cycle (Table 1). The procedures for cell fusion are essentially the same as followed by Kao et al. (1969b). About \( 5 \times 10^6 \) cells of each mutant were washed once in cold (4 °C) Hanks’s saline solution to remove the serum completely before adding 65 haemagglutinating units (HAU) of ultraviolet-inactivated Sendai virus. The fusion mixture was kept at 4 °C for 15 min and then transferred to a 37 °C water bath. After incubating for 45 min at 37 °C the fused cells were diluted and plated in F12 minus glycine medium (Kao et al. 1969a) in a number of plastic dishes. Each dish was inoculated with 4000 cells of the fusion mixture. This figure refers to the cell count taken before the cell fusion was initiated and hence does not represent the exact numbers of fused single cells plated. The plating efficiencies as measured by the number of colonies which developed in the selective medium were scored after 7 days.
Table 1. Protocol for the collection of G₁, S, G₂ and M cells from Gly-A and Gly-B mutants for fusion

<table>
<thead>
<tr>
<th>Dish no.</th>
<th>Phase in which cells are to be collected</th>
<th>Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2*</td>
<td>G₁</td>
<td></td>
</tr>
<tr>
<td>3, 4</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>5-7</td>
<td>G₂</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>G₁</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>G₂</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>G₁</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>G₁</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td></td>
</tr>
</tbody>
</table>

**Phase in which cells are to be collected:**
- **G₁:** Collect M cells, reverse colcemide block and plate
- **S:** Add colcemide
- **G₂:** Collect M cells, reverse colcemide block
- **M:** Add colcemide

**Cells:**
- **G₁:** Mutant-glycine A
- **G₂:** Mutant-glycine B
- **S:** Mutant-glycine A
- **M:** Mutant-glycine B

**Cells to be collected:**
- **M:** Trypsinize and collect M cells
- **G₁:** Trypsinize and reverse colcemide block
- **G₂:** Trypsinize and collect G₂ cells
- **S:** Trypsinize and collect S cells
- **M:** Trypsinize and collect M cells

**Add colcemide:**
- Add colcemide
- Trypsinize and reverse colcemide block

**Fuse:**
- (A) M₁/M₂
- (B) M₁/S₁
- (C) G₁/G₂
- (D) S₁/S₂
- (E) S₁/G₁
- (F) S₁/M₂
- (G) G₁/G₂
- (H) G₁/S₁
- (I) G₁/S₂
- (J) G₁/M₂

* 7 x 10⁵ cells were plated in 100-mm plastic plates at 44 h prior to the commencement of the experiment.

† Following the reversal of colcemide block the cells were resuspended in 2 ml of medium and plated in 30-mm plastic dishes.  a, Mutant Gly-A;  b, Mutant Gly-B.
RESULTS

**PCC and the cellular factors that influence its induction in HeLa cells**

When mitotic cells obtained by treating an exponentially growing culture of HeLa cells with colcemide \((6.4 \times 10^{-7} \text{ M})\) for 22 h were fused with a random population, PCC was induced in 50% of the mitotic-interphase fused cells. The frequency of induction could either be increased or decreased by adding suitable chemical compounds to the fusion mixtures (Rao & Johnson, 1971). Presently, we are concerned only with the possible causes for the failure of PCC induction in 50% of the mitotic-interphase fused cells. Earlier it was suggested that the rate of induction of PCC varied depending upon the location of the interphase cell in the cell cycle (Johnson & Rao, 1970). Thus the nuclei of \(G_1\) phase were more readily induced than those of \(S\) or \(G_2\) phase. Subsequently it occurred to us that the length of the period for which a cell is held in mitosis by colcemide might have some effect on its ability to induce PCC in an interphase nucleus following fusion. When colcemide is added to an exponentially growing culture and mitotic cells collected after 20 h it is clear that some cells are held in mitosis for a longer period than others. How does this factor affect the rate of PCC induction? HeLa cells were plated in plastic culture dishes and incubated overnight. The medium, along with the floating cells, was then removed and replaced by fresh medium containing colcemide \((6.4 \times 10^{-7} \text{ M})\); 4 h later mitotic cells were collected by the selective detachment technique. Half of these mitotic cells was immediately fused with interphase cells while the other half was used for similar fusion after another 20 h of colcemide treatment. The incidence of PCC was 88% when freshly collected mitotic cells were used in contrast to 10% when the cells were held in mitosis for 20 h or longer, indicating the loss of activity of the inducing factors as a function of time. Consequently in complementation studies with the glycine-requiring mutants of CHO cells only those mitotic cells which had arrived at mitosis within the preceding 2 h were used.

The fate of PCC during cell division immediately following fusion

HeLa cells, reversibly blocked in mitosis by nitrous oxide treatment (Rao, 1968), were separately fused with \(G_1\), \(S\) and \(G_2\) HeLa cells each of which was prelabelled with \(^{3}H\)thymidine. After the completion of cell fusion the cells were resuspended in culture medium and plated in plastic dishes. One of the dishes was trypsinized every hour until 7 h after fusion, and cells were prepared for microscopic examination using the cytocentrifuge as described earlier (Rao & Johnson, 1970). In most cases the first mitosis following fusion was completed by 7 h. At this time, colcemide was added to the remaining dishes to see whether the PCC, following incorporation into the daughter nuclei of the dividing cell, would enter into mitosis during the second cycle after fusion.

**Distribution of PCC into daughter cells.** Immediately following fusion between mitotic and interphase cells the PCC of \(G_1\), \(S\) or \(G_2\) types formed discrete entities which were not interspersed with the metaphase plate of the mitotic component. They often lay alongside the metaphase plate (Fig. 3). We have no evidence to suggest that
the PCC of any type are integrated into the mitotic spindle. As metaphase progressed
the discrete bodies of PCC became scattered and this was most commonly seen in
the PCC of the S type (Fig. 4A). At this stage some of the PCC become intermingled
with the mitotic chromosomes. During anaphase the PCC were distributed at random.
The PCC were incorporated into one or both of the groups of anaphase chromosomes
or neither of them (Fig. 4B, C). The random distribution and incorporation of PCC
into daughter cells became evident by the end of telophase. Often only one of the
daughter cells received the PCC (Fig. 5A, B). In these interphase nuclei silver grains
were localized in certain areas indicating the incorporation of labelled PCC (Fig. 5C).

Mitosis in PCC during the second cycle. Among the cells of M1G1 or M1G2 fusions
to which colcemide was added during the second mitotic cycle, chromosome spreads
were observed where some chromosomes were labelled while others were not (Fig. 6A).
This suggests that the chromatin derived from the PCC and incorporated into daughter
nuclei would produce normal chromosomes during the subsequent mitosis.

Formation of micronuclei in mitotic-interphase fused cells. When there was no induc-
tion of PCC in mitotic-interphase fused cells, the mitotic chromosomes, in a majority
of the cases, did not complete division but formed micronuclei (Fig. 6B). This
phenomenon occurred even when the mitotic:interphase ratio was 1:1. These micro-
nuclei derived from the mitotic chromosomes did not synthesize either DNA or RNA
during the next cycle and were probably genetically inactive (Fig. 6B). However, in
some fused cells, particularly in the M1G2 fusion where there was no induction of PCC,
the mitotic chromosomes underwent division (Fig. 6C).

Short-term survival studies of heterophasic HeLa cells. In order to demonstrate that
heterophasic homokaryons could survive and multiply, the following experiment was
done. Labelled and unlabelled synchronized populations were fused and the propor-
tions of labelled to unlabelled mono- and multinucleate cells were scored immediately
after fusion. The fusion mixture was plated in plastic dishes containing coverslips.
After 2 days the coverslips were fixed and processed for radioautography. Colonies
containing 2 or more cells were scored for the presence or absence of label. The pro-
portion of labelled to unlabelled colonies after 2 days was very similar to the initial
ratio (Table 2). This suggests that most of the heterophasic cells which constituted
between 13 and 20% of the fusion mixtures completed one or two divisions.

Hybrids of Gly-A and Gly-B mutants of Chinese hamster ovary cells. The long-range
effects of PCC induction with regard to the survival of hybrids and the elimination of
chromosomes in their progenies were studied by making various types of homophasic
and heterophasic fusions between the synchronized populations of Gly-A and Gly-B
mutants of the CHO cells (Table 1, p. 497). Use of the these mutants has an advantage
in that cell growth is impossible unless genetic components from each parent are active
in the hybrid cell.

Effect of colcemide on plating efficiency. When random populations of Gly-A and
Gly-B were fused the rate of recovery of colonies of hybrids was 1:56%. However,
the exposure of these populations to colcemide at a concentration of 0.05 μg/ml for
2 h reduced the plating efficiency to a third of the control (Table 3). This suggests
that not only the cells temporarily blocked in mitosis but also nearly 60% of the
interphase cells suffered damage even though colcemide was removed from the medium after a 2 h treatment. This is indicative of the fact that even a brief exposure of interphase cells to colcemide has some residual effect on these cells causing a decrease in plating efficiency.

Rate of survival of hybrids among homophasic and heterophasic fusions of Gly-A and Gly-B. The average rate of survival of hybrids among the various homophasic fusions (i.e. fusion between cells in the same phase of the cell cycle) was 0.46% and comparable to the 0.5% survival of the control (Table 3). The control in this case was the fusion (no. 2 of Table 3) involving random populations of Gly-A and Gly-B which were exposed to colcemide for a period of 2 h. The extremely low rate of survival was due to the low fusion index of about 15%. Of this 15% only half were products of fusion between Gly-A and Gly-B which can grow in the selective medium. About 50% of these hybrids were multinucleate cells with more than 2 nuclei and would presumably fail to develop into colonies due to anomalies in mitosis. These factors brought the plating efficiency of the fused cells down to 3%. A brief exposure to colcemide during the synchronization procedures further reduced the plating efficiency to 0.5-1% and this was close to the value we actually obtained.

The average plating efficiency of the heterophasic fusions, i.e. $S^+/G_2^b$, $S^+/G_2^b$ and $G_1^+/G_2^b$, was 0.28% (Table 3). The plating efficiencies were variable in fusions involving mitotic/interphase cells. The rate of survival in the $M^+/S^b$ fusion was only 0.062% and was significantly lower than that of any other fusion, including $M^+/G_1^b$ and $M^+/G_2^b$ fusions (Table 3). The possibility of PCC induction among the heterophasic fusions such as $S/G_1$, $S/G_2$ and $G_1/G_2$ is relatively greater than among the

<table>
<thead>
<tr>
<th>Type of fusion</th>
<th>% labelled and unlabelled cells immediately after fusion</th>
<th>% labelled and unlabelled colonies after 2 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unlabelled</td>
<td>Labelled</td>
</tr>
<tr>
<td>$M/G_1^*$</td>
<td>35.7</td>
<td>64.3</td>
</tr>
<tr>
<td>$M/S^*$</td>
<td>39.6</td>
<td>60.4</td>
</tr>
<tr>
<td>$M/G_1^*$</td>
<td>41.6</td>
<td>58.4</td>
</tr>
<tr>
<td>$S/G_1^*$</td>
<td>30.8</td>
<td>69.2</td>
</tr>
<tr>
<td>$S/G_2^*$</td>
<td>38.5</td>
<td>61.5</td>
</tr>
<tr>
<td>$G_1/G_2^*$</td>
<td>34.6</td>
<td>65.4</td>
</tr>
</tbody>
</table>

* Prelabelled with $[^3H]$thymidine.
† The figures in parentheses indicate the percentage of heterophasic cells in the fusion mixture.
homophasic fusions. The highest incidence of PCC, ranging from 80 to 90%, was observed among the binucleate cells of the M/G₁, M/S and M/G₂ fusions. It thus appears that the high incidence of PCC among mitotic/interphase fused cells is a contributing factor to the low rate of hybrid survival.

Table 3. Rate of survival of hybrids in homo- and heterophasic fusions between synchronized populations of Gly-A and Gly-B mutants

<table>
<thead>
<tr>
<th>Type of fusion</th>
<th>No. of colonies per 1000 cells of each parental type</th>
<th>Relative plating efficiency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>R₁/R₂</td>
<td>15.6</td>
</tr>
<tr>
<td>2</td>
<td>R₁/R₂</td>
<td>15.6</td>
</tr>
<tr>
<td>3</td>
<td>R₁ + R₂ (w/o virus)</td>
<td>0.0</td>
</tr>
<tr>
<td>Homophasic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M₁/M₂</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>G₁/G₂</td>
<td>5.5</td>
</tr>
<tr>
<td>6</td>
<td>G₁/G₂</td>
<td>4.6</td>
</tr>
<tr>
<td>7</td>
<td>G₁/G₂</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Average: 18.5/4 = 4.6</td>
<td>100.0</td>
</tr>
<tr>
<td>Heterophasic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Mitosis/interphase</td>
<td>M₁/G₁</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>M₁/S</td>
<td>0.62</td>
</tr>
<tr>
<td>10</td>
<td>M₁/G₂</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>Average: 8.4/3 = 2.8</td>
<td>61.0</td>
</tr>
<tr>
<td>(ii) Interphase/interphase</td>
<td>S₁/G₁</td>
<td>3.2</td>
</tr>
<tr>
<td>12</td>
<td>S₁/G₂</td>
<td>2.7</td>
</tr>
<tr>
<td>13</td>
<td>G₁/G₂</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Average: 8.4/3 = 2.8</td>
<td>61.0</td>
</tr>
</tbody>
</table>

R, Random population; *, parent Gly-A; b, parent Gly-B; +, exposed to colcemide (0.05 μg/ml) for 2 h before fusion.

Chromosome loss in the progenies of homophasic v. heterophasic fusions. On day 7 after fusion, cells from a number of hybrid colonies of each fusion were separately pooled into dishes, incubated for another 5 days and chromosome numbers determined for the bulk populations on day 12. A total of 100–150 chromosome spreads were counted for each type of fusion. The frequencies of cells with various chromosome numbers expressed as a percentage of the total chromosome spreads counted are shown as a histogram (Fig. 2).

The patterns of distribution of the various chromosome frequencies are similar to both homophasic and heterophasic fusions with the exception of one; that is, the M/S fusion. The modal value for the M/S fusion is 37 while it is 39 or 40 chromosomes for the rest of them. It is necessary to emphasize here that the lowest plating efficiency was observed in the M/S fusion indicating a possible relationship between chromosome loss and hybrid survival. The modal chromosome numbers obtained from Fig. 2
are in good agreement with the mean chromosome numbers for each sample of the population examined (Table 4).

_Chromosome anomalies in the progenies of the different hybrids._ The procedure of synchrony using a colcemide block has contributed to certain chromosomal anomalies among the progenies of all the types of fusions between Gly-A and Gly-B. The most common of them all are the chromosome breaks at the kinetochore region which appeared in approximately 2% of the cases. Dicentric chromosomes were obtained in 5 out of 1000 chromosome spreads examined. In the progeny of the $M/S$ fusion numerous chromosome fragments were observed in one of the cells. A ring chromosome was found in only one of the spreads from $M/G_1$ fusion. No such anomalies could be seen in 116 chromosome spreads of the control, $R/R$ fusion, where the cells were not exposed to colcemide.

![Chromosome frequency distributions for various types of fusions involving mutants of CHO cells.](image)
Fate of prematurely condensed chromosomes

DISCUSSION

The data presented in this paper indicate that in mitotic/interphase fusions where induction of PCC occurs the $G_1$, $S$ or $G_2$ chromosomes are wholly or partly incorporated into the progeny of the hybrid cells. The PCC are either directly incorporated into the daughter nuclei and/or remain as fragments of chromatin in the cytoplasm.

Table 4. Variability of chromosome numbers among the progenies of the various types of fusions between Gly-A and Gly-B mutants

<table>
<thead>
<tr>
<th>Type of fusion</th>
<th>No. of chromosomes per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest recorded</td>
</tr>
<tr>
<td>$R/R$</td>
<td>31</td>
</tr>
<tr>
<td>$M/M$</td>
<td>27</td>
</tr>
<tr>
<td>$M/G_1$</td>
<td>19</td>
</tr>
<tr>
<td>$M/S$</td>
<td>26</td>
</tr>
<tr>
<td>$G_1/G_1$</td>
<td>20</td>
</tr>
<tr>
<td>$G_1/S$</td>
<td>33</td>
</tr>
<tr>
<td>$S/S$</td>
<td>22</td>
</tr>
</tbody>
</table>

NOTE. Because of the difficulties in obtaining even a reasonable degree of synchrony among the $G_1$ population, the $M/G_2$ fusion for chromosome studies was not attempted.

Table 5. The genetic consequences of fusion between mitotic and interphase cells

<table>
<thead>
<tr>
<th>Type of fusion</th>
<th>PCC induction</th>
<th>Probability of gene complementation</th>
<th>No PCC induction</th>
<th>Probability of gene complementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M/G_1$</td>
<td>PCC are wholly or partly incorporated into daughter nuclei of hybrid cells (Figs. 4, 5)</td>
<td>+</td>
<td>The uninduced $G_1$ nucleus becomes part of one of the daughter cells following completion of mitosis by the mitotic component (Fig. 6c) or</td>
<td>+</td>
</tr>
<tr>
<td>$M/S$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M/G_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCC not incorporated into daughter nuclei - The chromosomes of the mitotic component form pycnotic micronuclei which are genetically inactive (Fig. 6b) -

Such fragments are likely to be eliminated during subsequent mitoses. When there is no induction of PCC in mitotic/interphase fused cells the mitotic component commonly becomes pycnotic and will presumably be eliminated. Only rarely does the mitotic component undergo normal division in these cells. Immediately following fusion the mitotic/interphase cell will probably follow one of the variety of courses listed in Table 5. The question remains whether the prematurely condensed chromo-
some of the fused cell are retained in the progeny. From the experiments dealing with short-term survival of mitotic/interphase HeLa cells it is evident that both the mitotic and the PCC components are retained in the progeny for at least 2 days (Table 2, p. 500).

To examine whether the PCC become genetically integrated into the progeny of the fused cells experiments were performed using glycine-requiring mutants of CHO cells. The rate of recovery of hybrids between Gly-A and Gly-B mutants is dependent upon the type of fusion. Homophasic fusions yielded almost twice the number of hybrids of heterophasic (S/G1, S/G2 and G1/G2) fusions. The lowest rate of survival of hybrids (0.062%) occurred in the M/S fusion closely followed by M/G2 and M/G1 (Table 3). The differences in the rates of hybrid survival between homophasic and heterophasic fusions can be explained partly on the basis of the frequency of PCC induction. The incidence of PCC which is nearly 90% in the mitotic/interphase fusions probably accounts largely for the low rate of survival of these hybrids. The pycnosis and micronucleation of the mitotic component in cells where there is no induction of PCC also contributes to the low rate of survival of these cells since the micronuclei, which synthesize neither RNA nor DNA, are probably genetically inactive. PCC is completely absent among homophasic fusions where the rate of survival is markedly greater than in the mitotic/interphase fusions. The rare occurrence of PCC in the heterophasic interphase/interphase fusions (Johnson & Rao, 1970) suggests that there are other factors involved in reducing the rate of survival to about 50% of the homophasic fusions.

It is pertinent to ask whether all the hybrid cells with PCC fail to develop into colonies. From the experiments with HeLa cells there is conclusive evidence that in many cases the prematurely condensed chromosomes become part of the daughter nuclei of the fused cell and are thereby retained (Fig. 5). When no PCC occurs the mitotic component becomes genetically inactive by the process of micronucleation and under these conditions no genetic complementation seems possible. This observation lends further support to the conclusion that most of the surviving colonies in the mitotic/interphase fusions were derived from homokaryons in which the interphase nuclei had undergone PCC. Since the mitotic populations of CHO cells used in these experiments contained 4% of interphase cells it is possible that a small proportion (approximately 4% of 0.28% = 0.01%; refer to lines 11, 12 and 13 of Table 3) of the surviving colonies was derived by fusion between interphase cells of the two mutants.

The pulverization of chromosomes observed in the M/S fusion was not present in the M/G1 or M/G2 fusions. The high degree of scattering of the prematurely condensed chromosomes of the S phase and the subsequent failure of substantial incorporation into the hybrid nucleus may contribute to the loss of chromosomes and to a lower rate of survival of the M/S hybrids. On the other hand the PCC of the G1 or G2 phases usually remain as intact groups and are incorporated into one or more of the daughter nuclei. The modal chromosome numbers of 40 for the M/G fusion and 37 for the M/S fusion support this point of view (Table 4). This leads us to the conclusion that hybrid cells with PCC of G1 or G2 phases have a better chance
Fate of prematurely condensed chromosomes

of survival than those with PCC of S phase. A modal chromosome number of 40 might be expected for all the fusions involving interphase cells. However, in the light of Table 5 one would expect a significant loss of chromosomes within a short time in the progenies of mitotic/interphase fusions. The fact that we did not find a great deal of chromosome loss in the progenies of the mitotic/interphase fusions suggests that for complementation to occur between these 2 mutants retention of a majority of the prematurely condensed chromosomes is necessary.

Although the loss of chromosomes among the progeny of the M/S fusion is not very extensive, it is significant. Kao et al. (1969b) produced hybrids by fusing random populations of 2 CHO mutants. Among the progenies of these hybrids it required 116 generations for the modal chromosome number to drop from 40 to 37. In the M/S hybrids of the present study a similar state is reached within the duration of about 20 generations (Fig. 2).

The relatively small loss of chromosomes observed in this study and that of Kao et al. (1969b), in which the modal value decreased from 40 to 37 chromosomes and remained stable even after 144 generations, may be related to the common origin of the cells involved. The degree of chromosomal loss in the hybrid progenies appears to depend largely upon the species differences between the 2 parental types. These auxotrophic mutants of CHO cells are identical except for the specific genes regulating their nutritional requirements and hence their hybrids are relatively more stable. The extensive loss of chromosomes in the hybrids from different species is illustrated by the mouse–human and human–Chinese hamster heterokaryons (Migeon & Miller, 1968; Matsuya & Green, 1969; Kusano, Long & Green, 1971; Kao & Puck, 1970). The loss of chromosomes among hamster–hamster or human–human cell hybrids is not as great as in the interspecific hybrids (Kao et al. 1969b; Siniscalco et al. 1969). In the hybrids of A9 cells and chick erythrocytes the genetic material of the latter cell type was virtually eliminated except for a small fragment carrying the gene that is necessary for the survival of the hybrids under the selective environment (Schwartz, Cook & Harris, 1971). In this case the authors suggested that the loss of chick genetic material was probably due to premature condensation of the chick nucleus. When cells of the same strain or species are fused the nuclei in the homokaryons become rapidly synchronized either at the time of the initiation of DNA synthesis or mitosis during the first mitotic cycle (Rao & Johnson, 1970). The achievement of synchrony and subsequent fusion of nuclei largely eliminate the chance of induction of PCC leading to the formation of stable hybrids with two complete genomes.

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*Fig. 3. PCC in M/G₁, M/S and M/G₄ fusions of HeLa cells at 30 min after fusion. The mitotic cells were obtained by the application of N₂O block. The interphase cells were prelabelled with [³H]thymidine prior to cell fusion.*

a. M/G₁, PCC of the G₁ type which is lightly stained lying alongside the metaphase plate (darkly stained) of the mitotic cell.

b. M/S. A radioautograph showing labelled S type PCC (indicated by arrows) lying around the metaphase plate.

c. M/G₄. A radioautograph showing labelled PCC of the G₄ type (indicated by the arrow) lying adjacent to the metaphase chromosomes of the mitotic cell.*

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3A

3B

3C
Fig. 4. Scattering of PCC in M/S fused HeLa cells at 4 h after fusion. S-phase cells prelabelled with \(^3\)H-TdR were fused with mitotic cells reversibly blocked with N\(_2\)O.

A, the mitotic chromosomes are completing anaphase while the PCC are scattered around them. PCC will probably be distributed randomly between the daughter nuclei.

B, radioautograph of an M/S fused cell during anaphase where the labelled PCC become localized to one side of the dividing cell.

C, in this M/S fused cell the PCC form a ring around one of the anaphase chromosome groups and in all probability will become part of that daughter cell.
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Fig. 5. Distribution of PCC during anaphase and telophase.

A, M/S fused cell. The S type of PCC is going to be incorporated exclusively into one of the daughter cells.

B, M/G1 fused cell in telophase. The labelled PCC are incorporated into the larger of the 2 telophase chromosome groups.

C, M/G1 fused cell at the end of the first mitosis after fusion. The G1-PCC are incorporated into 2 of the 3 daughter nuclei as indicated by the presence of label.
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Fig. 6. 

a, $M/G_2$ hybrid cell during the second mitosis after fusion showing labelled and unlabelled chromosomes on the same metaphase plate. The labelled chromosomes were derived by the incorporation of labelled PCC of the $G_2$ type.

b, $M/S$ fused cell in which there was no induction of PCC of the $S$ nuclei. The mitotic chromosomes were transformed into micronuclei under the influence of the interphase nuclei. The presence of $^3$H-TdR in the growth medium following fusion resulted in an increase in the number of grains on the $S$ nuclei.

c, $M/G_1$ cell in which the $G_1$ nucleus failed to undergo PCC induction. The mitotic component (shown by the arrow) has completed anaphase. The $G_1$ nucleus may be incorporated into one of the daughter cells.
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