

PROPHASING OF INTERPHASE NUCLEI AND INDUCTION OF NUCLEAR ENVELOPES AROUND METAPHASE CHROMOSOMES IN HeLa AND CHINESE HAMSTER HOMO- AND HETEROKARYONS

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ABSTRACT

Fusing human HeLa metaphase cells with HeLa interphase cells resulted within 30 min in either of two phenomena in the resultant binucleate cell: either prophasing of the interphase nucleus or formation of a normal-appearing nuclear envelope around the metaphase chromosomes. The frequency of either occurrence was strongly dependent on environmental pH. At pH's of 6.6–8.0, prophasing predominated; at pH 8.5 nuclear envelope formation predominated. Additionally, the frequencies of the two events in multinucleate cells depended on the metaphase/interphase ratio. When the ratio was 0.33 nuclear envelope formation predominated; when it was 2.0 prophasing predominated. In their general features, the results with fused HeLa cells resembled those reported earlier with fused Chinese hamster Don cells. However, the results provided an indication that between pH 6.6 and 8.0 the HeLa metaphase cells possessed a much greater capacity than the Don metaphase cells to induce prophasing. Fusion of Don metaphase cells with HeLa interphase cells or of Don interphase cells with HeLa metaphase cells at pH 8.0 resulted in nuclear envelope formation or prophasing in each kind of heterokaryon. As in the homokaryons, the frequencies of the two events in the heterokaryons depended on the metaphase/interphase ratio. The statistics of prophasing and nuclear envelope formation in the homo- and heterokaryon populations were consistent with the notion that disruption or formation of the nuclear envelope depends on the balance attained between disruptive and formative processes.

INTRODUCTION

Sendai virus-mediated cell fusion of an interphase cell with a metaphase cell of the Chinese hamster Don line results mainly in either of two phenomena. On the one hand, the interphase nucleus undergoes a transformation, induced by factors in the metaphase cell, which resembles that seen in

prophase of a mononucleate cell and the process has, therefore, been termed by us "prophasing" (10, 11). Originally, this change in the interphase-metaphase (I-M)¹ binucleate cell was called "chromosome pulverization" (14) and later "premature chromosome condensation" in HeLa cells (6, 7). Alternatively, we have reported that the interphase nucleus of the Don I-M cell may remain unchanged but the metaphase chromosomes become enclosed by a normal-appearing nuclear envelope (NE). The resulting structure has been termed a telophase-like nucleus (TLN) (4). The major event in the TLN formation appears to be a production of a new NE (4). The frequency of occurrence of either phenomenon in fused Don cells can be controlled by adjusting the environmental pH during and shortly after cell fusion: at relatively low pH the frequency of prophasing predominates, whereas at relatively high pH TLN formation predominates (16). Prophasing and TLN formation are mutually exclusive events, since I-M cells showing both phenomena are not observed (4, 16).

Evidence has been presented (9) that protein synthesis must be unimpeded late in the G₂ period of the Don cell, which ultimately becomes the metaphase partner of the fused I-M cell, if prophasing is to occur. Some factor that is either a protein(s) or is dependent on protein synthesized in G₂ is needed for prophasing.

Whatever the chemical nature of this material needed for prophasing, it is not species specific. Heterokaryon I-M cells formed by fusion of Don cells with human hematopoietic cells or with monkey kidney cells lead to the same changes in both the Don chromosomes and the monkey or human chromosomes that are observed in the Don I-M homokaryon (5). In heterokaryons involving a HeLa metaphase component the interphase nucleus of several species also undergoes the same changes that take place in HeLa I-M homokaryons (7).

The current report is concerned with several questions: Is TLN formation a peculiarity of the Don line? If it is not, is TLN formation species specific? Can there be detected quantitative differences between cell lines in their abilities to induce prophasing or TLN formation?

¹ *Abbreviations used in this paper:* HAU, hemagglutinating units; I-M, interphase-metaphase; M/I ratio, ratio of mitotic to interphase nuclei; NE, nuclear envelope; PF, prophasing-inducing factor; TLN, telophase-like nucleus.

MATERIALS AND METHODS

Virus and Cells

UV-inactivated Sendai virus, suitably diluted in glucose-free Hanks' solution, was used to induce cellular fusion. The methods for proliferating the virus, inactivation of the virus, and the preparation of the stock virus have been described (8).

A Chinese hamster embryonic lung cell line (Don) and HeLa cells were used in the present study. The former originated from a cell stock of the American Type Cell Culture Collection, Rockville, Md., and the latter was kindly supplied by Dr. William Munyon of our institute. The Don line used has a modal chromosome number of 22, which is euploid and characteristic of the Chinese hamster. The HeLa cells exhibited a modal chromosome number of 60. These cell lines were grown as monolayer cultures in RPMI 1640 medium (13) supplemented with 10% fetal calf serum, and containing 100 µg/ml each of penicillin and streptomycin. Cells in log phase were obtained about 15–16 h after subculture of the Don line and about 20–21 h in the case of HeLa cells. The cultures were treated with Colcemid, 0.08 µg/ml, for 5 h. After trypsinization for 3 min at 37°C (16) the cells were washed once with prewarmed pH-adjusted medium containing Colcemid and resuspended in fresh medium at 37°C at the same pH (16).

Metaphase cells were prepared essentially by the method of Stubblefield and Klevecz (20) as applied in our laboratory (11). After exposure of the log phase monolayers to Colcemid as above, the flasks were rocked gently for 40–50 times in the case of Don and several times in the case of HeLa. The detached metaphase cell suspension was chilled to 1°C and the cells were recovered by centrifugation at 800 g for 3 min and suspended in prewarmed, preadjusted fresh medium containing Colcemid. In most cases the mitotic index of the Don cells was over 90% and over 80% in the case of HeLa. Thus, in the fusion of interphase Don cells with metaphase HeLa cells the probability is better than 4:1 that the interphase cell will not be of HeLa origin. The situation is similar for the fusion of Don metaphases with HeLa interphase cells. Interphase cells came from confluent monolayer cultures.

Cell Fusion and Slide Preparation

Procedures for cell fusion and slide preparation were essentially the same as those described in previous papers (4, 16). Approximately 10⁷ log phase cells or a mixture of metaphase and interphase cells (5 × 10⁶ cells of each) were suspended at 37°C in a total of 1.0 ml of pH-adjusted medium containing 2,000 hemagglutinating units (HAU) of inactivated Sendai virus and 0.08 µg of Colcemid. The cell-virus suspension was allowed to stand for 10 min at about 1°C. The suspension was transferred to an incubator maintained at 37°C and shaken gently for 10 min. It was then diluted sixfold with prewarmed medium of the same pH containing 0.08 µg/ml of

Colcemid and incubated at 37°C for an additional 20 min. The cells were recovered by centrifugation and suspended in 0.5 ml of 15 mM sodium citrate for 5 min at room temperature and then fixed by the addition of 0.5 ml of Carnoy's fixative (acetic acid/methanol, 1:3). After centrifugation the supernate was discarded and the cells were resuspended in fresh fixative, and spread on glass slides without flaming. The air-dried cells were stained with Giemsa's.

Electron Microscopy

For electron microscopy, fused HeLa cells incubated with virus as described above were centrifuged at 800 g for 3 min, and the resulting pellets were fixed in chrom-osmium (3, 16) at pH 7.3 for 1–2 h. The pellets were rapidly dehydrated with graded ethyl alcohol and then embedded in Epon-Araldite mixtures (12). Thin sections were cut on an LKB ultramicrotome and stained in 2% uranyl acetate for 20 min followed by lead citrate (17) for 10 min. The specimens were examined with a JEM 7 electron microscope and photographs taken at accelerating voltage of 80 kV with instrumental magnification of 3,000–15,000 times in diameter.

RESULTS

TLN Formation in Binucleate HeLa I-M Cells as a function of pH: Comparison to Don Cells

Prophasing of the interphase nucleus arising from fusion of HeLa interphase cells with HeLa metaphase cells was readily observed after fusion had occurred at pH 8.0. Fig. 1 is a typical example. A new finding, as regards fused I-M HeLa cells, was the formation of TLN in such cells. Its appearance in the light microscope is given by Fig. 2 and in the electron microscope by Fig. 3. The uniform metaphase chromatin was surrounded by an envelope that closely resembled the NE of the interphase nucleus in the same I-M cell, just as in the case of Don I-M cells (4, 16).

The frequency of prophasing and TLN formation in HeLa I-M cells as a function of the pH of the medium was studied. The results are shown in Fig. 4. Although TLN formation increased from pH 6.6 to 8.0, prophasing at all pH's studied was appreciably in excess of TLN formation; even at pH 8.0 prophasing in a given population of fused I-M cells occurred about twice as often as TLN formation. These results are in contrast with the findings in Don cells (16) where at pH 6.6 prophasing predominated and at pH 8.0 TLN formation was about three times as frequent as prophasing.

Fig. 4 shows the difference between HeLa and Don I-M cells at pH 8.0.

Thus, compared with the Don metaphase cell at pH 8.0, the HeLa metaphase cell would appear to have a much greater capacity to induce prophasing. Alternatively, the Don interphase cell may have a much greater capacity than the HeLa interphase cell at this pH to induce TLN formation.

In HeLa I-M cells TLN formation could be the predominant result, provided the environmental pH was raised above 8.0. Results at pH 8.5 are contrasted with those at pH 8.0 in Table I. Log phase monolayer cultures were used at each pH in any single experiment. At the lower pH prophasing was about three times as prevalent as TLN formation; at pH 8.5 TLN formation was at least three times as frequent as prophasing.

Over 90% of the binucleate I-M cells exhibited either TLN formation or prophasing. Fused cells with both phenomena were not detectable, as was also the case with Don cells (4, 16).

TLN Formation and Prophasing in Multinucleate HeLa and in Multinucleate Don I-M Cells: Effect of the Metaphase/Interphase Ratio at pH 8.0

Johnson and Rao (6) reported that the frequency of "premature chromosome condensation" in fused HeLa multinucleate I-M cells depended on the metaphase/interphase (M/I) ratio. With Don cells fused at pH 7.2, the higher the M/I ratio the greater was the percentage of fused cells that exhibited prophasing (4). Conversely (4), the lower the M/I ratio in fused Don cells the greater was the percentage of fused cells that exhibited TLN formation. That is to say, prophasing and TLN formation exhibit dose-response properties that are dependent on the metaphase and interphase components, respectively, in the fused cells.

If the Don and HeLa metaphase cells differ in their ability to cause prophasing, as suggested by the results given in the present report so far, there should be differences between the dose-response properties of Don multinucleate I-M cells and those of HeLa multinucleate I-M cells. The results obtained at pH 8.0 are given in Fig. 5 and, indeed, demonstrate a distinct difference between Don and HeLa cells. For example, at an M/I ratio of 2 in the Don I-M population, prophasing and TLN formation occurred at about the same frequency,

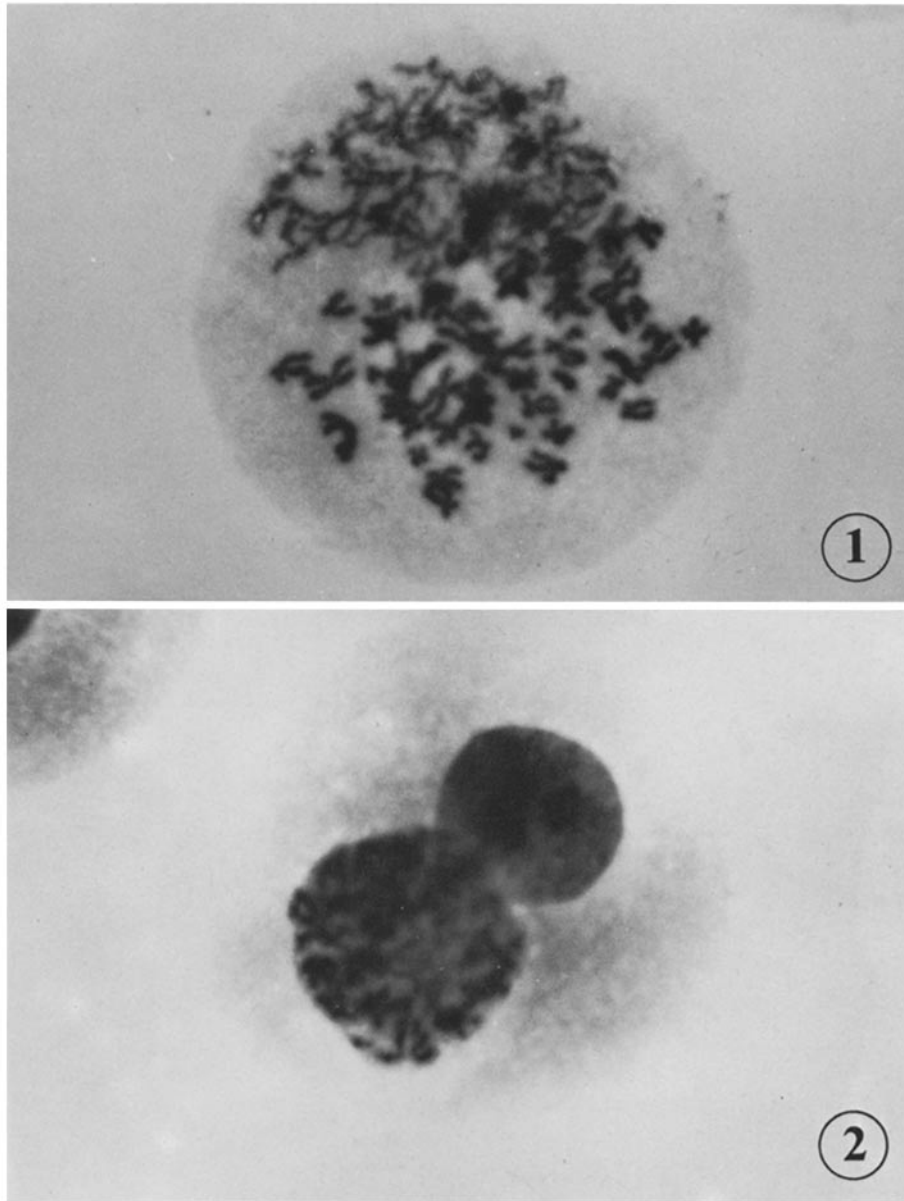


FIGURE 1 Prophasing of the interphase nucleus in a fused HeLa I-M binucleate cell. Cells were fused at pH 8.0 after exposure of a log phase culture to Colcemid for 5 h and fixed 30 min after incubation with virus. \times 1,300.

FIGURE 2 TLN formation in a HeLa I-M binucleate cell fused at pH 8.0. This cell originated from the same sample as the cell shown in Fig. 1. \times 1,300.

whereas in the HeLa population with the same ratio almost all the I-M cells exhibited prophasing. At an M/I ratio of 0.5, prophasing was almost undetectable in the Don population whereas 15%

of the HeLa population exhibited prophasing. These data also indicate that at pH 8.0 the HeLa chromosomes do not suffer from an incapacity to become incorporated into a TLN, since at an M/I

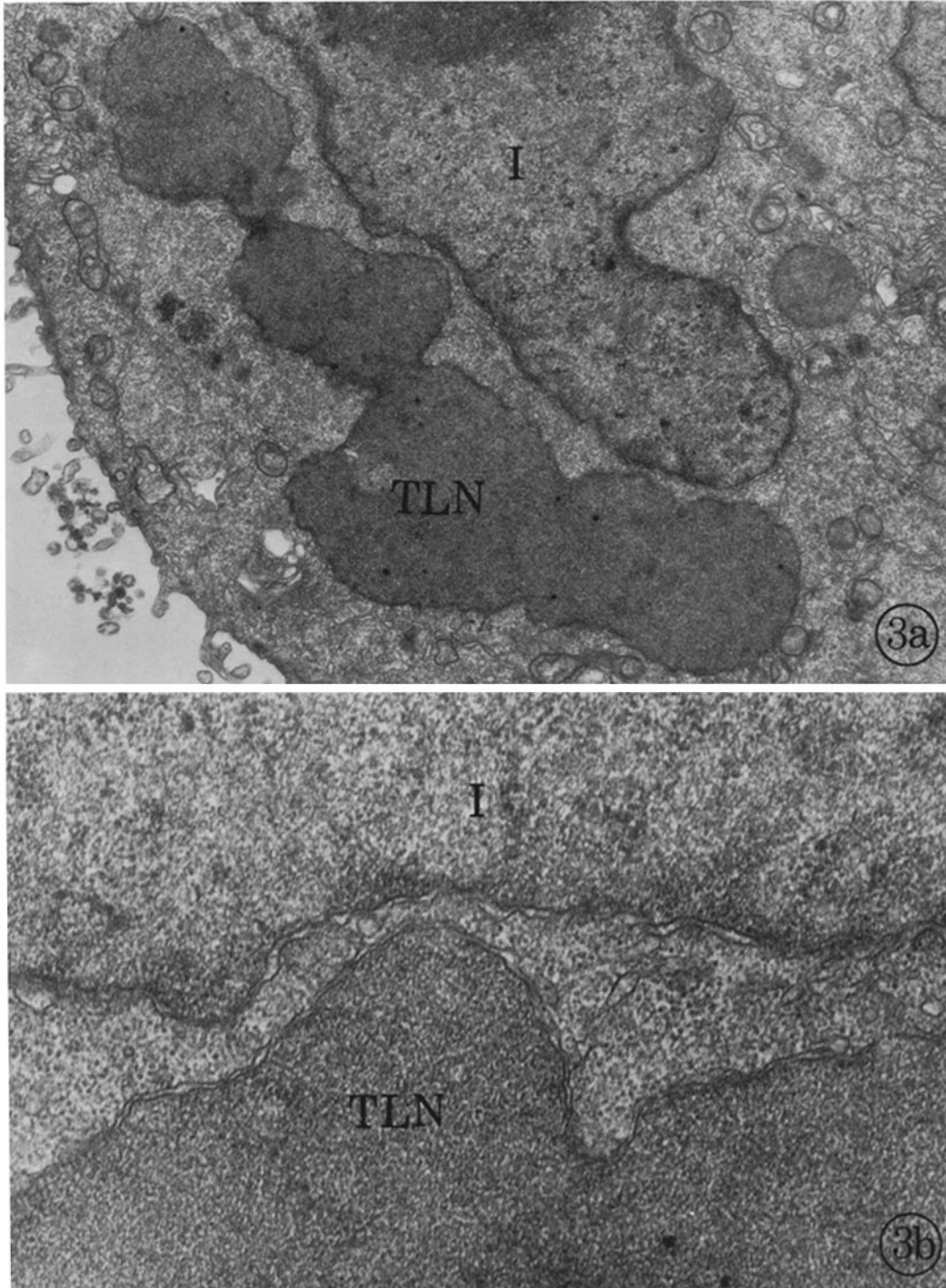


FIGURE 3 Electron micrographs of TLN formed in a HeLa I-M binucleate cell fused at pH 8.0. (a) Overall appearance of TLN with a portion of an interphase nucleus (*I*). $\times 8,000$. (b) Enlargement of a region of Fig. 3 *a*. Membranes of both TLN and interphase nucleus (*I*) are morphologically indistinguishable. $\times 30,000$.

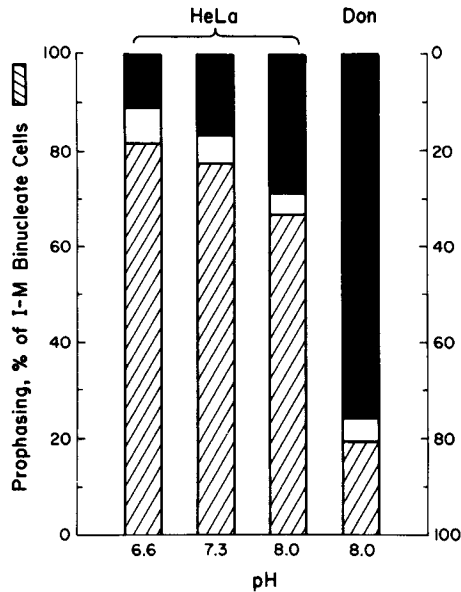


FIGURE 4 Frequency of cells with prophaning and of cells with TLN as a function of the pH of the medium during fusion. HeLa I-M binucleate cells are compared with Don I-M binucleate cells. The blank areas in the middle part of each bar show the small percentage of I-M binucleate cells without TLN or prophaning. The experiments were performed at least three times and 300–500 I-M binucleate cells were examined at random at each pH. The average results are recorded. The range of standard deviations was ± 1.5 to $\pm 4.5\%$.

TABLE I
The Effect of pH on TLN Formation and Prophaning in HeLa I-M Binucleate Cells

pH	Experiment number	Frequency of I-M cells with		
		TLN	Prophaning	No change
		%	%	%
8.0	1	20.0	75.0	5.0
	2	26.7	64.3	9.0
	3	26.0	69.7	4.3
8.5	1	67.0	26.0	7.0
	2	77.0	16.3	6.7
	3	77.7	18.0	4.3

A fresh culture of log phase cells was used from experiment to experiment and treated with Colcemid for 5 h. In each of the experiments the same culture was fused at pH 8.0 or 8.5 as described in Materials and Methods. After fixing and staining, 100 I-M cells were examined at random in the first experiment and 300 were examined at random in each of the remaining experiments.

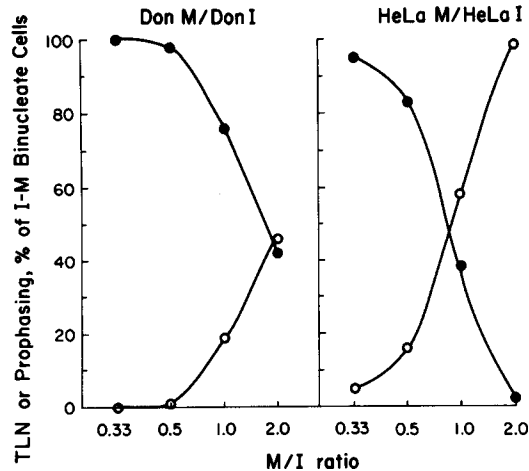


FIGURE 5 Characteristic patterns at pH 8.0 of prophaning and TLN formation in Don and HeLa I-M homo-karyons as a function of the M/I ratio in multinucleate cells. Log phase cultures arrested for 5 h with Colcemid were used. ●—●, TLN; ○—○, prophaning.

ratio of 0.5, 83% of the HeLa I-M cells exhibited TLN formation.

TLN Formation and Prophaning in Heterokaryons at pH 8.0

Prophaning of an interphase nucleus of one species can be brought about by fusion with a metaphase cell of another species (5, 7). Experiments were performed to determine whether or not TLN formation can also take place in heterokaryons.

HeLa metaphase or interphase cells were fused with Don interphase or metaphase cells, respectively, at pH 8.0. The fusion frequency at pH 8.0 using 5×10^6 cells each per ml containing 2,000 HAU of virus was approximately 32% under the conditions described in Materials and Methods. Fig. 6 a shows a multinucleate cell in which three or possibly four Don interphase nuclei have undergone prophaning. In Fig. 6 b there appears a binucleate cell containing a HeLa TLN and, in all likelihood, a Don interphase nucleus. When the interphase cell component was HeLa, Don chromosomes also became enclosed in a TLN indistinguishable from that described earlier in Don homokaryons (16).

The results with homokaryons suggested that at pH 8.0 HeLa metaphase cells have a much greater capacity than Don metaphase cells to cause prophaning. If this were the case, then in a heterokar-

yon population in which the metaphase component of the fused cells came from HeLa and the interphase component from Don, the frequency of prophasing at relatively low M/I ratios should be greater than at the same ratios in Don I-M homokaryons. In Fig. 7 the results of fusing HeLa

metaphase cells with Don interphase cells are shown. Prophasing predominated strongly at M/I ratios of 1.0 and 0.5, and even at 0.33 it was equal to TLN formation. Comparison with the results in Fig. 5 bears out these predictions.

The results with homokaryons at pH 8.0 also

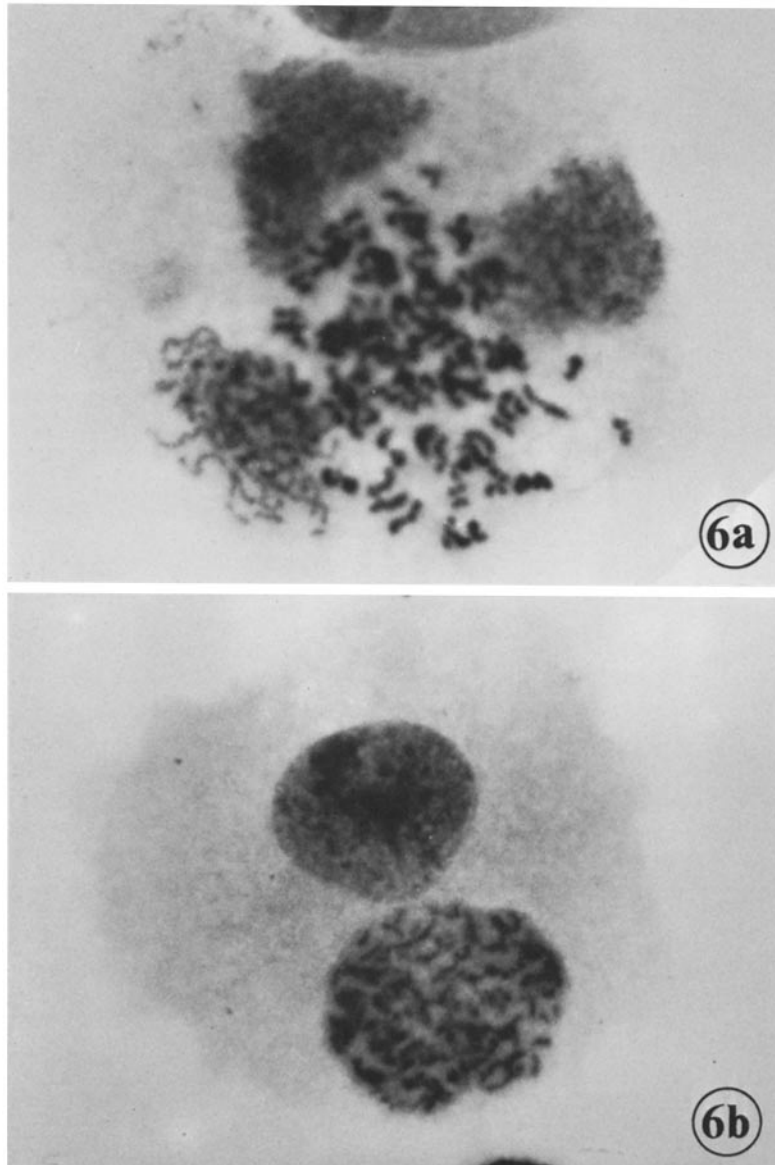


FIGURE 6 (a) Prophasing induced in a Don/HeLa heterokaryon consisting of three Don interphase nuclei and one HeLa metaphase. In spite of the low M/I ration (0.33) all of the interphase nuclei have undergone prophasing. $\times 1,300$. (b) Binucleate heterokaryon containing a HeLa TLN and a probable intact Don interphase nucleus. At pH 8.0 the frequency of TLN in such fused cells having a M/I ratio of 1.0 is less than 4% (see Fig. 7). $\times 1,300$.

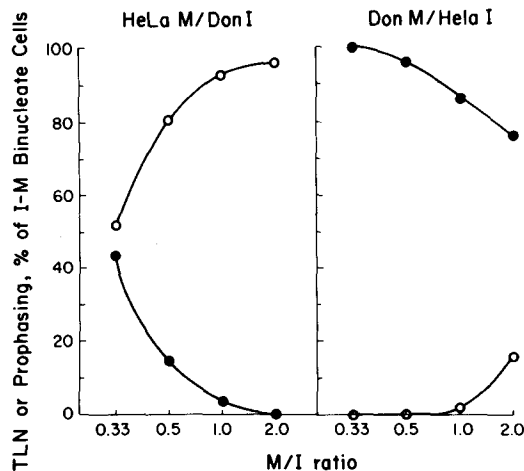


FIGURE 7 Contrasting patterns at pH 8.0 of prophasing and TLN formation in Don/HeLa I-M heterokaryons as a function of the M/I ratio in multinucleate cells. Interphase cells were obtained from confluent monolayers. Metaphase cells were obtained from log phase cultures arrested for 5 h with Colcemid. ●—●, TLN; ○—○, prophasing.

indicated an alternative possibility, i.e., that the Don interphase cell had a much greater capacity than the HeLa interphase cell to induce TLN formation. If this were the case, then in a heterokaryon population, where the interphase component came from HeLa, TLN formation should be less frequent than in a Don I-M homokaryon population. The results of fusing Don metaphase cells with HeLa interphase cells are given in Fig. 7 and should be compared to the results in Fig. 5 with Don I-M homokaryons. Contrary to the expectation, the HeLa interphase component was as potent or more so than the Don interphase component in inducing TLN formation enclosing Don chromosomes at all M/I ratios.

DISCUSSION

The structural (1, 6, 11, 18) and biochemical features (2, 9, 11, 19) of prophasing strongly support the idea that this phenomenon in fused I-M cells represents a normal mitotic event triggered by the circumstance of the interphase nucleus finding itself in a milieu to which the metaphase cell contributes essential material leading to prophasing of the interphase nucleus. In this environment there appears to be a protein(s), prophasing-inducing factor (PF) (9), that had been

made during the G_2 period of the metaphase cell, or some product dependent on protein synthesis in that period, before fusion with the Don interphase cell, and which is responsible for prophasing. Additional support for the notion that prophasing reflects a normal event is found in the similarity of its pH dependence to that of the normal G_2 to metaphase progression (16) of the Don cell.

The basis for considering TLN formation, that is to say, NE formation around metaphase chromosomes in Don I-M cells, as representing a normal mitotic event is threefold: structural similarity to normal NE (4, 16, 18), the similarity of its pH dependence to that of the normal metaphase to G_1 progression, which includes normal telophase (16), and the fact that the chromatin of the Don TLN can progress to interphase chromatin with attendant new RNA synthesis and with the reformation of nucleoli (15). Direct evidence that a factor(s) in the interphase cell is responsible for the formation of NE of the TLN in the fused I-M cell is lacking. Nevertheless, the relation of its frequency of formation at pH 7.2 to the M/I ratio (4), found again in the present work at pH 8.0, strongly indicates that such a factor is resident within the interphase cell. Additionally, the finding that TLN can form in a human cell line, HeLa, as well as in a hamster cell line, with dependencies on environmental pH and M/I ratio, encourages the belief that mammalian interphase cells, in general, contain agents that are responsible for the formation of the normal NE.

That there were found quantitative differences between Don I-M and HeLa I-M cells with regard to frequency of prophasing and of TLN formation at pH 8.0 is not surprising. It is likely that certain enzymes, as yet unidentified, are responsible, at least in part, for the effects seen and it would not be unexpected that their pH optima would vary from species to species.

If it is postulated that prophasing in a variety of mammalian cells is the outcome of the action of similar chemically constituted agents (enzymes?) on similarly chemically constituted structures, then the occurrence of prophasing in heterokaryons is not surprising. The same consideration would apply to TLN formation. Construction of a similar NE around similar chromatin would require agents (enzymes?) that might not distinguish between the substrates leading to phospholipid components of the NE in one cell and those of another, nor might they distinguish between the

metaphase chromatin of one cell and that of another. Thus, TLN formation would not be species specific and this was found to be the case in the present studies.

We have proposed (4, 16) that NE formation in a Don cell is the outcome of a balance between the action of two sets of agents: one that is disruptive of the NE and another that is formative for the NE. This hypothesis was initially based on the results of varying the M/I ratio (4), but was strengthened by the finding that prophasing predominates at pH 6.6, whereas TLN formation predominates at pH 8.0 (16). The identical proposal can be advanced with regard to the HeLa cell, because prophasing is favored by a high M/I ratio, whereas TLN formation is favored by a low M/I ratio, i.e., high I/M ratio. Additionally, at pH 8.0, prophasing is favored but at pH 8.5 TLN becomes the dominant event.

The hypothesis receives support when the results obtained with the heterokaryon I-M cells are compared with those obtained with the homokaryon I-M cells. At pH 8.0, the HeLa metaphase cell appears to have a more potent PF than does the Don cell. If formation of the NE of the TLN depended on a balance between the postulated antagonistic agents, then in a HeLa M/Don I binucleate cell at pH 8.0, prophasing should predominate over TLN formation and this was found to be the case. Most striking was the result of fusing Don metaphase cells with HeLa interphase cells at pH 8.0. In this case, TLN formation was far in excess of prophasing. This result indicates that the HeLa interphase cell does have a potent TLN forming capacity, but that it cannot be expressed in the HeLa homokaryon I-M cell because of the antagonism exhibited by the HeLa PF. Only at pH 8.5 in HeLa homokaryon I-M binucleate cells was TLN formation, with its attendant NE, present in excess over prophasing, either because the pH optimum for prophasing was exceeded or the pH optimum for NE formation was more closely approached.

The indirect nature of the evidence for the existence of factors that cause formation of the NE has prompted us to seek evidence of a more conclusive nature, as was done in the case of PF (9). The outcome of such experimentation will be the subject of a future report.

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