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Influence of both coculture and BrdU on NOR activity of mouse, rat and human cells

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Summary. The coculture of mouse PG19 cells with human MGC cells can significantly suppress nucleolar organizer region (NORs) activity of both PG19 and MGC cells. 5′-bormodeoxyuridine (BrdU) can also significantly suppress the NOR activity of rat RC cells, human MGC and Hela cells, and mouse PG19 cells: i.e. the average number of Ag-NORs and the number of chromosomes bearing Ag-NORs per cell decrease significantly. The degree of the suppression increases with increase in both BrdU concentration in the culture medium and BrdU treatment time. The suppressed NOR activity of the PG19 cells can gradually be restored when the BrdU-treated cells are transferred into BrdU-free medium for 50 h. In PG19 cells deoxycytidine (dC) can reverse the suppression of NOR activity caused by BrdU. Coculture plus BrdU treatment suppress the NOR activity of PG19 cells more severely than BrdU treatment alone. In coculture medium containing 30 μg BrdU/ml, dC can also reverse the suppression of the NOR activity of PG19 cells but not that of the MGC cells. The degree of the reversion in the coculture plus BrdU treatment is significantly lower than that found with BrdU-treatment alone.

Key words: Cell genetics – NOR activity – Coculture inhibition – BrdU inhibition – dC reversion

Introduction

Gap junctions are dynamic components of cellular membranes found in all multicellular organisms. Most cells in both organ and tissue culture show cell-cell coupling via gap junctions demonstrated by injected fluorescent dyes or radioactively labelled metabolites between contiguous cells (Hooper et al. 1981).

Genetic defects in the metabolic cooperation via gap junctions have been described in mouse cells, human cells (Vitkauskas et al. 1983) and Chinese hamster cells (Willecke et al. 1983). Cell-cell contact promotes DNA synthesis in retial glia cells (Burke 1983). While coculture decreases the frequency of sister chromatid exchange (Giuseppe et al. 1982) there is evidence that ouabain resistance can be communicated from ouabain-resistant to ouabain-sensitive cells (Corsaro et al. 1982).

NORs are the sites where the transcription of rRNA genes takes place within a cell; silver-stained NORs (Ag-NORs) are believed to be the sites of the genes coding for 18s and 28s rRNA on the chromosomes (Goodpasture et al. 1975; Stocker 1978). It was demonstrated that only the NORs with functionally active rRNA genes can be stained (Miller 1976; Miller et al. 1976). While Ag-NORs number and location on the chromosomes are more or less constant within a given karyotype (Hsu et al. 1975). NOR activity can change in spermatogenesis (Hofgärtner et al. 1979), somatic cell hybrids (Miller 1976; Arrighi et al. 1980; Yan Yongshan et al. 1983) and interphase nucleus treated with RNA inhibitor (Hofgärtner et al. 1979). In a previous paper we reported that BrdU inhibits the NOR activity of Chinese hamster cells and that dC reverses this suppression (Yan Yongshan et al. 1985). Can BrdU also inhibit NOR activity in other species? Is the suppressed NOR activity irreversible? Can dC reverse the suppression of the NOR activity caused by BrdU in other species? Can coculture influence the NOR activity by the cell communication? How about the NOR activity in the coculture plus BrdU treatment?

In the present paper we report on BrdU inhibition on the NOR activity of rat, mouse and human cells, dC reversion on the suppression of NOR activity caused by BrdU, and the influence of coculture of human cells with mouse cells on their NOR activity.

Materials and methods

1 Cell lines

PG19 is a mouse cell line (HGPRT$^-$) which has a mean chromosome number of 39, two of which are metacentric (Rb 12.12)

and submetacentric (Rb 5.15) chromosomes; the others are acrocentric (Jonasson et al. 1977; Yan Yongshan 1985).

MGC is a human cell line derived from a stomachus cancer with a mean chromosome number of 65 most are metacentric and submetacentric chromosomes. The chromosome characteristics of the PG19 cells are thus very different from those of the MGC cells (Figs. 1 and 2).

Hela is a well-known human cell line.

All three cell lines grow very well in Minimal Essentia Medium (MEM) supplemented with 15% new born calf serum at 37 °C.

RC is a rat tetraploid cell line established in our lab from a sarcoma. The cell grow very well in RPMI1640 medium containing 10% calf serum at 37 °C.

2 Cell treatment and chromosome preparation

In order to make the cells contact well in coculture, the PG19 cells and MGC cells were mixed in equal numbers (about 10^6 cells for each cell line in a culture flask of 25 cm^2).

Twenty-four hours after inoculation, some of the PG19, MGC, Hela, RC and the coculture group were transferred into MEM (RPMI1640 for the RC cells) containing BrdU (Sigma, 3.30 μg/ml), others grew in BrdU-free medium as control groups. The cells were grown in the medium containing BrdU under dark conditions for 24 h and 48 h, respectively.

Some PG19 cells were transferred into BrdU-free MEM for 50 h after an initial 48 h incubation in the MEM containing BrdU.

To test whether dC can reverse the BrdU-suppressed NOR activity of the human and mouse cells, some of the PG19 cells and the coculture group were transferred into the MEM containing both BrdU (30 μg/ml) and dC (30 μg/ml) for 48 h in a dark room.

Two hours before cell harvest, colcemid (Sigma) was added into the medium to a final concentration of 0.02 μg/ml. Chromosome preparations were carried out according to Yan Yongshan et al. 1984.

3 Silver-staining

Three to six day-old slides were treated with silver-staining as described by Yan Yongshan et al. 1984. Metaphases which stained well were used for determining Ag-NOR number. In the PG19 cell, there are three chromosomes each of which bears four Ag-NORs-called A_4 chromosomes (Yan Yongshan et al. 1983). All data were treated statistically according to the t-test.

Results

1 Influence of BrdU on the NOR activity of PG19, MGC, Hela and RC cells

As shown in Table 1, when the MGC cells were grown in the medium containing 3 μg BrdU/ml (Table 1), the NOR number and the number of the chromosomes bearing Ag-NORs per cell was similar to that found in the control group ($P > 0.05$). However, if the BrdU concentration in the culture medium was increased to 30 μg/ml, the NOR numbers per cell for PG19, MGC, Hela and RC cells decreased, respectively, to 67%, 82%, 70% and 57% of their control groups. The numbers of chromosomes bearing Ag-NORs also decreased, respectively, by 33%, 18%, and 25% for PG19, MGC and Hela cells. The number of A_4 chromosomes per cell decreased from 2.80 to 0.17 for the PG19 cells. It is clear that BrdU can inhibit the NOR activity of the PG19, Hela, MGC and RC cells.

When the PG19 cells initially incubated in MEM containing BrdU for 48 h were transferred into the BrdU-free MEM for 50 h, the Ag-NOR number and the

Table 1. Inhibiting effect of BrdU on the NOR activity of PG19, MGC, RC and Hela cells (means ± SE)

Cell type	BrdU		dC		No. of cells examined	Ag-NORs/cell	No. of chromosomes bearing Ag-NORs/cell	No. of A_4 chromosomes/cell
	Concentration (μg/ml)	Time (h)	Concentration (μg/ml)	Time (h)				
PG19	0	0	0	0	68	20.78±0.78	6.69±0.17	2.80
	30	48	0	0	114	7.05±0.46	4.51±0.26	0.17
	30[a]	48	0	0	53	14.51±0.39	7.77±0.35	0.93
	30	48	30	48	60	14.10±0.49	6.85±0.20	0.98
MGC	0	0	0	0	100	8.97±0.21	4.69±0.15	0
	3	48	0	0	54	8.72±0.28	4.56±0.15	0
	30	48	0	0	228	7.39±0.29	3.84±0.11	0
Hela	0	0	0	0	54	12.55±0.35	6.98±0.20	0
	30	48	0	0	53	8.77±0.33	5.26±0.20	0
RC	0	0	0	0	64	13.50±0.54		
	30	24	0	0	50	11.90±0.48		
	30	48	0	0	40	7.68±0.52		

[a] PG19 cells were cocultured in MEM containing BrdU (30 μg/ml) for 48 h and then allowed to grow in BrdU-free MEM for another 50 h

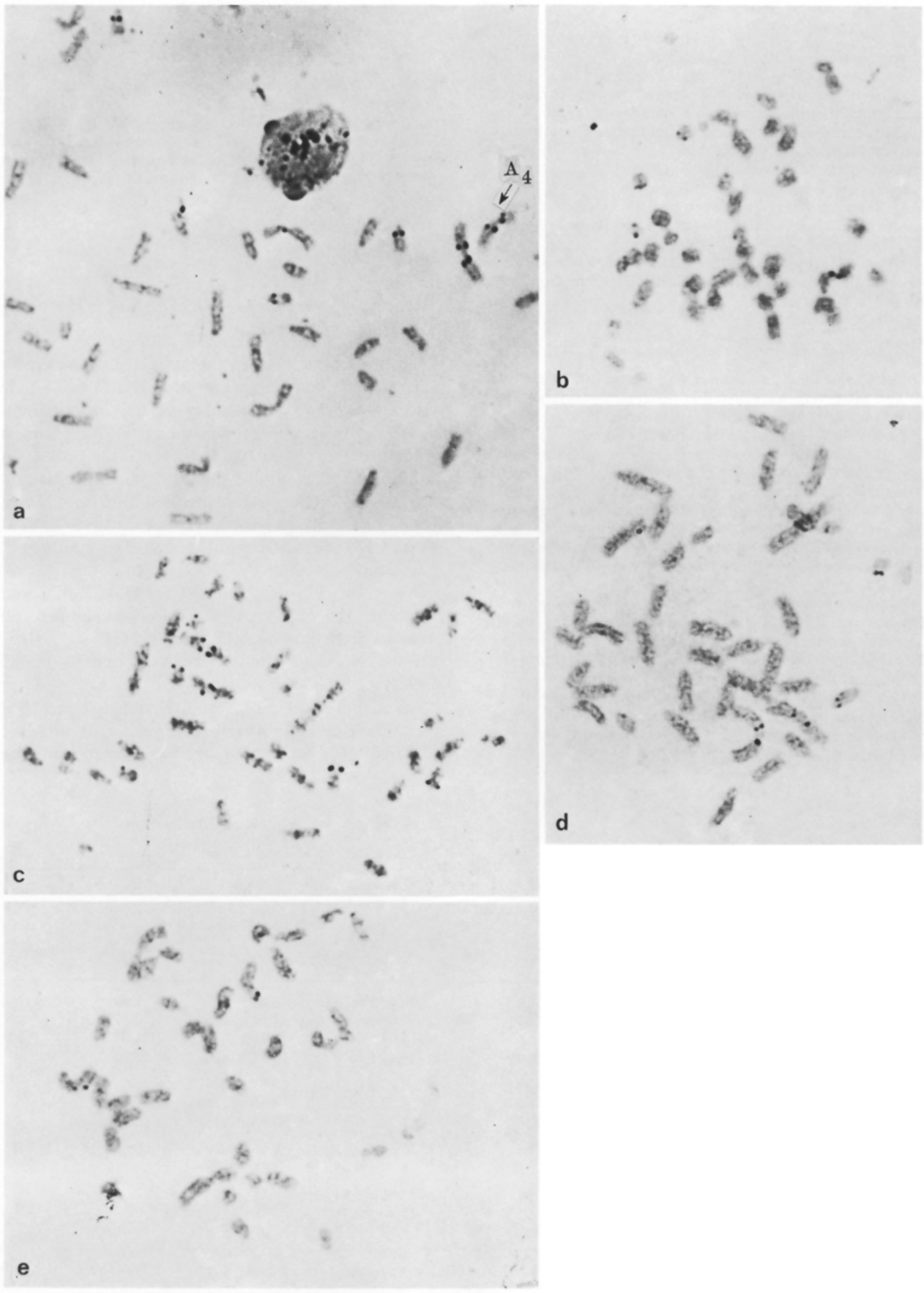

Fig. 1 a–e. Ag-NOR distribution of PG19 cells. **a** Untreated cell; **b** BrdU-treated cell; **c** BrdU plus dC-treated cell; **d** cocultured cell; **e** coculture plus BrdU-treated cell

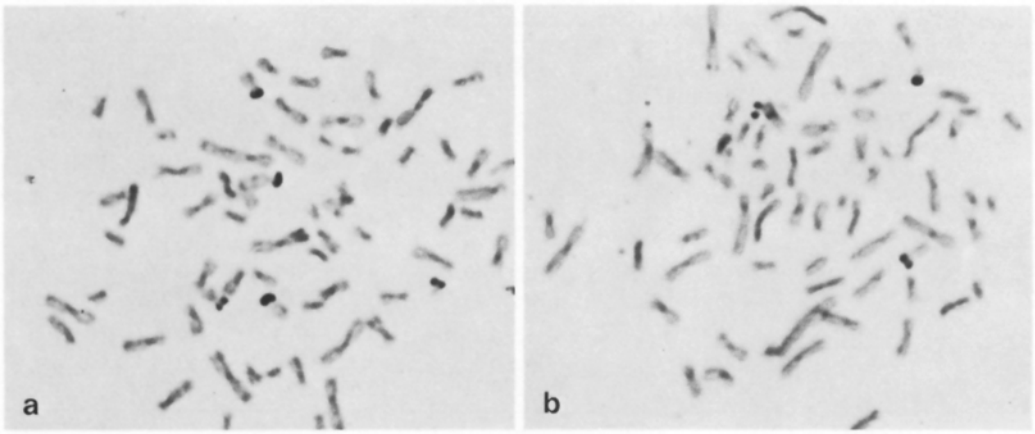

Fig. 2 a, b. Ag-NOR distribution of MGC cells. **a** Untreated cell; **b** cocultured plus BrdU-treated cell

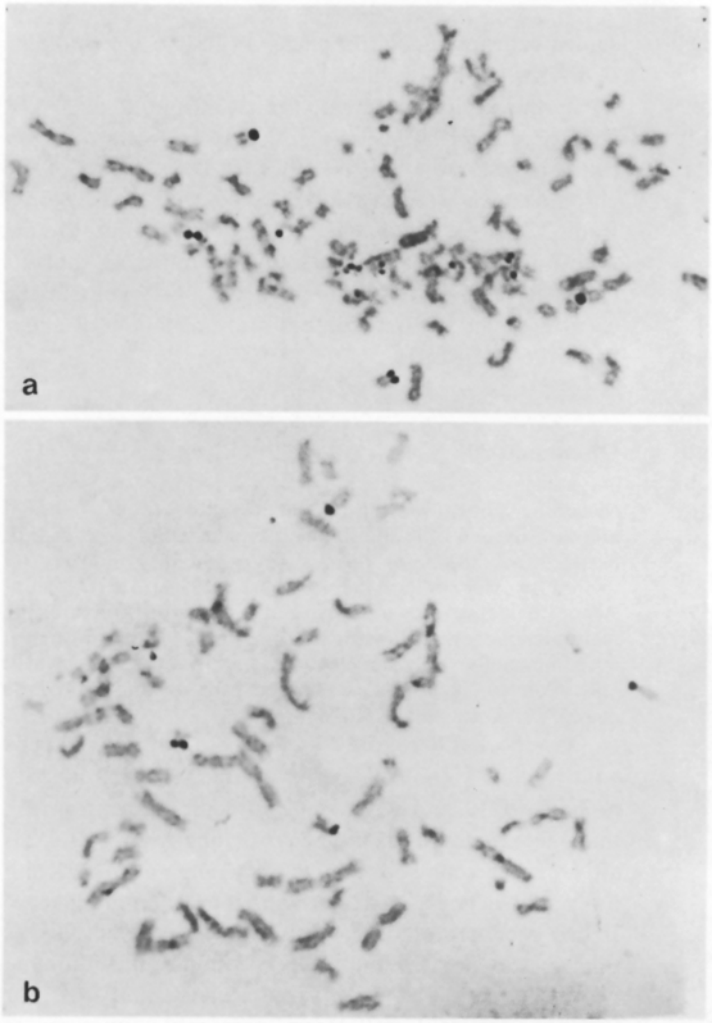

Fig. 3 a, b. Ag-NOR patterns of Hela cells. **a** Untreated cell; **b** BrdU-treated cell

Table 2. Influence of coculture on NOR activity of PG19 and MGC cells (means ± SE)

Cell type	BrdU		dC		No. of cells examined	Ag-NORs/cell	No. of chromosomes bearing Ag-NORs/cell	No. of A_4 chromosomes/cell
	Concentration (μg/ml)	Time (h)	Concentration (μg/ml	Time (h)				
PG19	0	0	0	0	112	9.53±0.55	4.52±0.24	0.71
	30	48	0	0	186	4.04±0.42	2.23±0.24	0.17
	30	48	30	48	68	9.10±0.46	4.04±0.19	0.77
	30	24	0	0	53	6.70±0.46	3.21±0.39	0.25
MGC	0	0	0	0	53	8.15±0.21	4.06±0.10	0
	30	48	0	0	226	7.34±0.22	3.85±0.11	0
	30	48	30	48	58	7.07±0.21	3.63±0.11	0

[a] PG19 cells were cocultured with the MGC cells

number of the chromosomes bearing Ag-NORs per cell recovered from 7.05 to 14.51, and from 4.51 to 7.77, respectively (Table 1). The number of A_4 chromosomes per cell also increased from 0.17 to 0.93. Our results indicate that the suppressed NOR activity of the PG19 cells is reversible.

2 Ag-NORs distribution in coculture

Since the chromosome characteristics and NOR distribution on their chromosomes are quite different between PG19 and MGC cells (Figs. 1, 2, 3), it is very easy to identify their metaphase cells under the microscope. It can be seen from Table 2 that the Ag-NOR number and the number of the chromosomes bearing Ag-NORs per cell for the PG19 cells in the coculture decreased from 20.78 (in the control group) to 9.53 and from 6.69 to 4.52, respectively. The number of A_4 chromosomes per cell also decreased from 2.80 to 0.71. For MGC cells, coculture also decreased their Ag-NOR number and the number of chromosomes bearing Ag-NORs per cell. It is obvious that coculture inhibits the NOR activity of both PG19 and MGC cells.

When BrdU was added into the coculture group, Ag-NOR number and the number of the chromosomes bearing Ag-NORs per cell decreased more severely than in the BrdU treatment alone ($P < 0.001$) for PG19 cells: e.g., the Ag-NOR number per cell in the BrdU plus coculture (Table 2) is 4.04, but that in the BrdU treated group (Table 1) is 7.05. However, there is no difference between the BrdU treated group and the coculture plus BrdU treatment group for MGC cells ($P > 0.05$).

3 dC reversion on suppressed NOR activity

It is shown in Table 1 that dC addition restored NOR number (7.05 to 14.10) and the number of chromosomes bearing Ag-NORs per cell (4.51 to 6.85) for the BrdU-treated PG19 cells.

In the coculture group, the dC restored the NOR activity of the PG19 cells, i.e., the Ag-NOR number per cell was restored from 4.04 to 9.10, the number of the chromosomes bearing Ag-NORs per cell was restored from 2.23 to 4.04 (Table 2), which is similar to that found in the BrdU-free coculture. It indicates that dC can reverse the NOR activity of the PG19 cells in the coculture plus BrdU treatment, but not the NOR activity of the MGC cells.

Discussion

Metabolic cooperation can occur between a variety of normal and transformed cells and in heterotypic combinations of cells derived from different tissue and mammalian species (reviewed by Hooper and Subak-Sharpe 1981). There is substantial evidence that a specialized membrane structure, the gap junction, is the ultrastructural pathway for the transfer of ions, metabolites (e.g., nucleotides, amino acids and cAMP) and other small molecules between cells in contact (Gilula et al. 1972; Azarnia et al. 1974).

As reported here, the coculture of mouse PG19 cells with human MGC cells could inhibit the NOR activity of both PG19 and MGC cells and therefore implying that the expression of the genes coding for 18s and 28s rRNA can be controlled by the exchange of some small molecules between cells derived from different species. It has been reported that cell type is more important than species for the metabolic cooperation within the warm-blooded vertebrates (Marchase et al. 1976). For instance, mouse L cells form permeable junctions at low frequencies with most cell types and at high frequencies in certain combinations (Hooper et al. 1981). Recently, Burke (1983) reported that coculture promotes DNA synthesis in retinal glia cells but not in fibroblasts. The coculture between PG19 and MGC

cells that we reported here might thus be used for studying the control of the expression of genes coding for 18s and 28s rRNA.

Our results demonstrated that BrdU not only suppress NOR activity of Chinese hamster cells, but also suppresses that of rat, mouse and human cells. The suppressed NOR activity could be restored when the BrdU-treated cells were transferred into BrdU-free medium for a period thus BrdU suppression is reversable. The BrdU suppression on the NOR activity may be due to BrdU toxicity.

The results indicate once more that dC can reverse the NOR suppression caused by BrdU. Under coculture conditions, dC reverses the NOR number per cell for PG19 cells from 4.04 to 9.10, which is similar to that found in BrdU-free coculture (9.53) (Table 2), and not to 20.78 (Table 1), thus implying that the mechanism for the BrdU inhibition on the NOR activity might be different from that of coculture inhibition. There is no dC reversion for the NOR suppression in the MGC cells that can be explained on the basis of differences in nucleotide pool size between the PG19 and MGC cells.

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