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Colchicine acts as a progression factor to initiate DNA synthesis in quiescent Balb/c 3T3 cells

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In quiescent Balb/c 3T3 cells, competence factors such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and platelet-derived growth factor (PDGF) synergize with progression factors such as insulin to initiate DNA synthesis. In this study, we found that colchicine, a microtubule-disrupting agent, acted synergistically with TPA, but not with insulin, to induce the maximal stimulation of DNA synthesis. Colchicine also synergized with PDGF in the presence of epidermal growth factor to elicit nearly the optimal induction of DNA synthesis. Moreover, it acted synergistically with fibroblast growth factor, another competence factor. These results suggest that colchicine acts as a progression factor like insulin in quiescent Balb/c 3T3 cells.

Microtubule depolymerization; DNA synthesis; Progression factor; (Balb/c 3T3 cell)

1. INTRODUCTION

Previous reports have demonstrated that there are at least two distinct regulatory phases that control the cell cycle traversal of density-arrested Balb/c 3T3 cells [1–3]. A group of growth factors classified as competence factors could induce a competent state, then the competent cells could enter the S phase by the addition of another group of growth factors, progression factors, such as insulin or insulin-like growth factor-1. For example, PDGF acts as a competence factor and synergizes with insulin in the presence of EGF to initiate DNA synthesis [4]. Another competence factor TPA can act synergistically with insulin to induce DNA synthesis in the absence of EGF [5,6]. Classification of growth factors could help our understanding about the pathway of mitogenic signal transduction. It has been shown that the competence factors cause the activation of protein kinase C and enhance the expression of proto-oncogenes c-myc and c-fos similarly [7,8]. The existence of the progression factor-induced mRNAs has also been reported [9]. Although much information has accumulated, the mechanisms of signal transduction are not fully understood.

Microtubule integrity has been suggested to be involved in proliferative regulation based on the observations that microtubule depolymerizing agents such as colchicine acted synergistically with some growth factors to stimulate DNA synthesis in quiescent cells [10–17]. However, since detailed investigation with Balb/c 3T3 cell lines has not been carried out, whether colchicine acts like a competence factor or a progression factor has not been determined. Here, we present evidence that colchicine acts as a progression factor in quiescent Balb/c 3T3 cells. Several lines of evidence suggest that colchicine exerts its effect by depolymerizing cytoplasmic microtubules.

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2. MATERIALS AND METHODS

2.1. *Materials*

Colchicine, colcemid, insulin and TPA were purchased from Sigma. EGF, bovine pituitary FGF and PDGF were purchased from Collaborative Research (Waltham, MA). Taxol was the gift from Dr Mattew Suffness of the Natural Product Branch,

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Division of Cancer Treatment, National Cancer Institute (USA). Fetal calf serum was purchased from Gibco.

2.2. *Cell culture*

Balb/c 3T3 cells were from Drs I. Yahara and S. Koyasu (The Tokyo Metropolitan Institute of Medical Science). Cells were plated at 1.5–2.0 × 10^4 per 35 mm dishes in 2.5 ml of Dulbecco's modified Eagle's (DME) medium supplemented with 10% fetal calf serum. They were kept at 37°C in an atmosphere containing 5% CO_2. 3 days after seeding when the cells were nearly confluent, they were given 1 ml of fresh DME medium containing 0.25% fetal calf serum and then allowed to become quiescent in 2 days.

2.3. *Incorporation of [^{3}H]thymidine into DNA*

The quiescent Balb/c 3T3 cells in 35 mm dishes were incubated with anti-microtubule drugs (or related agents) and/or peptide growth factors for 40 h at 37°C in the presence of [^{3}H]thymidine (1 μCi/ml). The cells were then rinsed in cold phosphate-buffered saline twice and incubated in 10% trichloroacetic acid for 30 min at 0°C. The trichloroacetic acid-precipitable material was solubilized in 1 ml of 0.4 M NaOH at 4°C. After neutralization, the radioactivity was counted in a liquid scintillation counter.

2.4. *Preparation of lumicolchicine*

Colchicine dissolved in 95% ethanol was converted to lumicolchicine by ultraviolet irradiation as described [18].

3. RESULTS AND DISCUSSION

Measurements of [^{3}H]thymidine incorporation in quiescent Balb/c 3T3 cells stimulated by TPA and/or insulin have shown that TPA alone initiated DNA synthesis only slightly (fig.1A, ●), but in the presence of 1 μM insulin, TPA markedly induced DNA synthesis (fig.1A, ○), the level of which was comparable to that induced by 10% fetal calf serum. This result was consistent with the previous reports [5,6]. The addition of colchicine (1 μM) instead of insulin enhanced the stimulative effect of TPA on DNA synthesis (fig.1A, ×); the thymidine incorporation reached the maximal level that was induced by TPA plus insulin. In contrast, colchicine did not act synergistically with insulin (fig.1B). Colchicine alone, like insulin alone, stimulated DNA synthesis only slightly (fig.1).

FGF, like TPA, synergized with insulin to induce DNA synthesis in quiescent Balb/c 3T3 cells (fig.2, ○), as was previously reported [3]. Colchicine enhanced DNA synthesis stimulated by FGF as effectively as insulin did (fig.2, ×). These data suggest that colchicine acts as a progression factor, because colchicine synergized with a competence factor, TPA or FGF, and does not synergize with a progression factor, insulin.

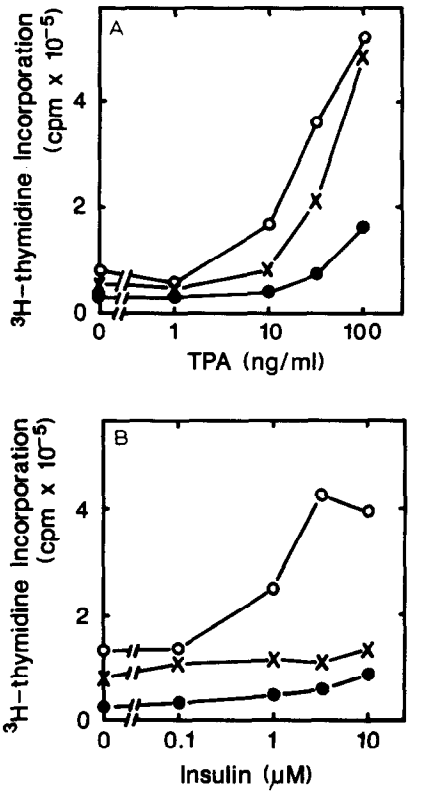

Fig.1. Effects of TPA, insulin and colchicine on the initiation of DNA synthesis in quiescent Balb/c 3T3 cells. Quiescent cells were treated with agents and [^{3}H]thymidine for 40 h. Thymidine incorporation into trichloroacetic acid-insoluble material was measured as described in section 2.3. (A) Dose response curve for TPA in the presence of insulin or colchicine. Cells were exposed to indicated concentrations of TPA with no addition (●), 1 μM insulin (○) or 1 μM colchicine (×). (B) Dose response curve for insulin in the presence of TPA or colchicine. Cells were exposed to indicated concentrations of insulin with no addition (●), 30 ng/ml TPA (○) or 1 μM colchicine (×). Thymidine incorporation in cells stimulated by 10% fetal calf serum was 4.2–4.8 × 10^5 cpm and this level was referred to as the optimal or maximal stimulation level in this paper.

Colcemid, another microtubule-depolymerizing agent, also caused the enhancement of thymidine incorporation stimulated by TPA (table 1). Lumicolchicine, a photoisomer of colchicine which does not depolymerize microtubules, did not enhance DNA synthesis stimulated by TPA (table 1). Taxol, a microtubule-stabilizing agent, inhibited the stimulation of DNA synthesis induced by col-

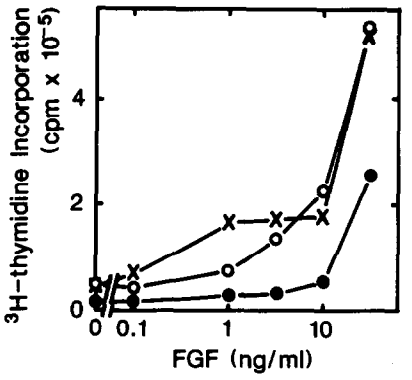

Fig.2. Dose response curve for FGF in stimulating DNA synthesis in the presence of insulin or colchicine. Quiescent Balb/c 3T3 cells were incubated for 40 h with various concentrations of FGF with no addition (●), 1 μM insulin (○) or 1 μM colchicine (×). [^{3}H]Thymidine incorporation was measured.

chicine (table 1). These data suggest that colchicine exerts its stimulative effect on the initiation of DNA synthesis through microtubule depolymerization.

Because microtubule-depolymerizing agents inhibit the formation of mitotic spindle, in order to investigate whether treatment with these agents in the presence of TPA induces the initiation of cell division as well as DNA synthesis, the microtubule-depolymerizing agents should be removed before M phase. To this end, colcemid was used since it can be removed much more easily

Table 1

Effects of anti-microtubule agents on the initiation of DNA synthesis in quiescent Balb/c 3T3 cells in the presence or absence of TPA (50 ng/ml)

Treatment	[^{3}H]Thymidine incorporation (cpm × 10^{-4})	
	− TPA	+ TPA
No addition	2.5	13.4
1 μM colchicine	8.6	42.5
1 μM lumicolchicine	2.5	14.2
1 μM colcemid	9.3	45.9
1 μM colchicine + 12 μM taxol	2.9	23.7

Taxol, when present, was added to the cells 1 h before colchicine treatment. Thymidine incorporation was measured after 40 h incubation

than colchicine by a thorough wash. After pulse treatment (6 h) with various concentrations of colcemid in the presence of 100 ng/ml TPA, the cells were further incubated in the presence of TPA for 40 h and then the cell number was counted. The result depicted in fig.3 shows that about 30% increase in the cell number was induced with 1 or 3 μM colcemid treatment, while without colcemid treatment the increase induced by TPA alone was very small.

It has been shown that in quiescent Balb/c 3T3 cells, insulin synergizes with PDGF in the presence of EGF to stimulate the thymidine incorporation into DNA, and that the DNA synthesis induced by PDGF plus EGF plus insulin is nearly equal to that induced by 10% fetal calf serum [4]. Colchicine could replace insulin in this system (fig.4). PDGF plus colchicine, EGF plus colchicine, or PDGF plus EGF had only additive effect on the initiation of DNA synthesis, whereas PDGF plus EGF plus colchicine elicited the maximal, synergistic induction of DNA synthesis, the level of which was equal to that induced by 10% fetal calf serum (fig.4).

In this study, we have shown that colchicine synergized with competence factors such as TPA, FGF and PDGF plus EGF, but not with a progression factor insulin, to initiate DNA synthesis in

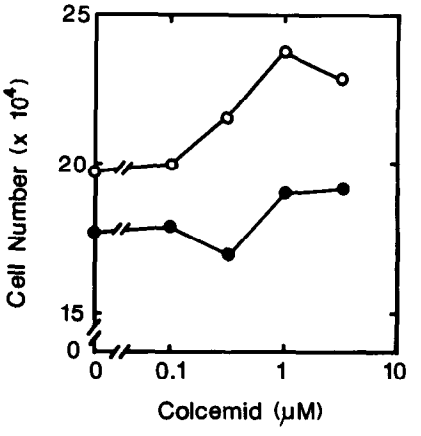

Fig.3. Initiation of cell division by pulse treatment with colcemid in the presence of TPA. Quiescent cells were treated with indicated concentrations of colcemid for 6 h in the absence (●) or presence (○) of 100 ng/ml TPA. Colcemid was then removed by washing three times. After 72 h incubation in DME medium in the absence (●) or presence (○) of TPA, cell number was counted.

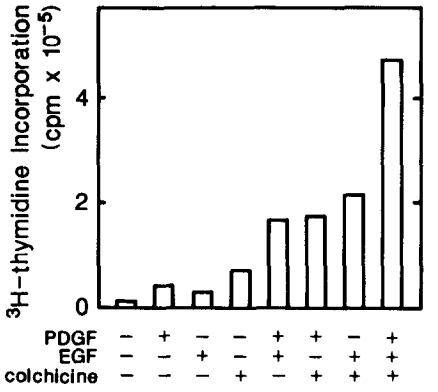

Fig.4. Effects of PDGF, EGF and colchicine on the initiation of DNA synthesis. Quiescent Balb/c 3T3 cells were incubated for 40 h with or without 10 ng/ml PDGF, 0.3 nM EGF and 1 μM colchicine as indicated. Thymidine incorporation was measured.

quiescent Balb/c 3T3 cells. Because this effect of colchicine was not mimicked by lumicolchicine and was inhibited by taxol pretreatment, it seems that colchicine exerts its effect by depolymerizing cytoplasmic microtubules. Thus, colchicine, i.e., microtubule depolymerization, acts as a progression factor in Balb/c 3T3 cells. Since Bockus and Stiles reported that insulin caused transient microtubule depolymerization [19], it seems possible that insulin initiates DNA synthesis by depolymerizing cytoplasmic microtubules. It is of interest to examine whether insulin and colchicine commonly elicit certain other cellular responses essential for the induction of DNA synthesis. Such examination could help our understanding about signal transduction mechanisms of progression factors.

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