RNA Synthesis Induction in Cell Culture by a Tumor Promoter

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SUMMARY

The potent tumor promoter, phorbol myristate acetate, enhances RNA synthesis in stationary cultures of 3T3 mouse fibroblasts leading to a release of density-dependent inhibition of cell division. RNA synthesis is unaffected by phorbol ester treatment in viral-transformed and chemical carcinogen-transformed cell lines. The nuclear RNA synthesis induced by phorbol myristate acetate in 3T3 cells is partially resistant to the inhibitory effects of actinomycin D.

INTRODUCTION

A notable property of the 3T3 line of mouse fibroblasts is its exceptional sensitivity to density-dependent inhibition of cell division (12, 14). Moreover, a decreased sensitivity to controls on cell division appears to be directly related to the tumorigenic potential of cultured cells (1, 4). We have observed that the outgrowth of SV40-3T32 passed at a low inoculation density (100 cells/50-mm dish) is partially inhibited by a 102 to 104 excess of untransformed 3T3 cells. This inhibition can be relieved and SV40-3T3 outgrowth enhanced in the mixed culture by the addition of nontoxic amounts (0.01 to 1.0 μg/ml) of PMA, a compound derived from croton oil and demonstrated to have extraordinary tumor-promoting activity for mouse skin (9, 15, 16). Thus, a cell culture system, which may reflect the phenomenon of tumor promotion in vivo, was available for a study of the response of normal and transformed cells to the phorbol esters. Futher, PMA causes rapid changes in cell configuration, probably caused by interaction at the cell membrane which may lead to altered control of macromolecular synthesis and cell division (8).

RESULTS AND DISCUSSION

The cell lines used in this study were 3T3 (12), SV40-3T3 (13), and a benzo(a)pyrene-transformed hamster embryo line (11). They were grown in 100-mm plastic dishes in Dulbecco's modification of Eagle's medium (3). The 3T3 cells were usually grown to saturation density and allowed to remain there for at least 3 days before PMA was added to the culture medium. Under these conditions, the culture becomes stationary in the G1 phase of the cell cycle where DNA synthesis and cell division have essentially stopped and RNA synthesis is considerably reduced (14). At various intervals after addition of the tumor promoter, the rate of RNA synthesis was measured with uridine-5-3H in the presence and absence of actinomycin D (Chart 1). Within 18 hr after addition of the tumor promoter to 3T3 cells without actinomycin D, the rate of RNA synthesis was doubled, and this 2-fold enhancement persisted for up to 11 days in the presence of PMA. This persisting effect is in sharp contrast...
to the rapid transient increases in RNA synthesis observed when stationary cultures are fed (14). A shorter time of PMA exposure of 3T3 cells from 1 to 5 hr did not induce an increase in RNA synthesis. Although the increase in RNA synthesis may be due to increased pool size of uridine caused by a possible permeability alteration, the results of these short PMA exposure experiments suggest that this is not the case. Moreover, the specific activity of the acid-soluble fraction (2) calculated on the basis of orcinol-positive compounds was similar in control to PMA-treated cells. The results of a typical experiment after PMA exposure for 48 hr were 723 cpm/µg nucleotide in the control and 738 cpm/µg nucleotide in the PMA-treated cells providing evidence that alteration in pool size is probably not the critical inducing factor in the increase in the rate of RNA synthesis.

The addition of actinomycin caused a 90% inhibition of RNA synthesis in otherwise untreated monolayers of 3T3. In contrast, treatment with PMA seemed to induce the synthesis of a species of RNA which was less sensitive to the inhibitory action of actinomycin by a factor of 3 after 18 hr of PMA treatment.

With populations of transformed cells (SV40-3T3 and benzo(a)pyrene-transformed hamster embryo) no significant enhancement of nuclear RNA synthesis was observed after exposure of these cell lines to PMA (Chart 1). With SV40-3T3, there appeared to be a slight increase in total and actinomycin-resistant RNA synthesis in a very dense culture exposed to PMA for 6 days. Earlier studies with untransformed 3T3 cultures showed that DNA synthesis was unaffected 24 hr after phorbol ester treatment of stationary cultures of 3T3 (10). However, the DNA synthesis as measured by thymidine-3H incorporation between 30 and 48 hr after PMA treatment was more than doubled from 4164 cpm/µg DNA to 9987 cpm/µg DNA, and the protein content of the cultures was increased by about 20% (Table 1). Longer exposure times resulted in a doubling of the protein content and cell density of the treated cultures. This indicated that a portion of the PMA-treated cell population was released from the G1 phase and progressed through cell replication.

We had suggested earlier, based on the amphipathic structure of the phorbol esters, that alteration in cell membrane properties may occur due to interaction of these agents with the membrane (9), and supporting evidence for this view has been obtained (8).

In the present communication, it was shown that the membrane-active phorbol esters can induce an increase in RNA synthesis. This leads to an increase in cellular mass and to cell division in a population that is prevented from progressing through the cell cycle as a result of controls exerted through cell surface interactions (1, 4—6). Further, the RNA synthesis induced by PMA appears to be of a special kind, because its formation is partially resistant to actinomycin D.

The most likely sequence of events compatible with the existing data is: interaction of the tumor promoter with a cell membrane site that may have a controlling influence on the cell cycle; synthesis of nuclear RNA providing messengers and ribosomal precursors, possibly for the initiation of the S phase; and increase in cell mass and passage through mitosis. 'In the usual initiation-promotion sequence on mouse skin, the initiated or preneoplastic cells retain their neoplastic potency unexpressed for periods of up to 1 year (15, 16). After treatment with phorbol esters, tumors arise rapidly and progress to invasive cancers. The data presented above demonstrate that these potent tumor-promoting agents for mouse skin can alter the cell replication controls in an in vitro system of mouse embryo cells and suggests that a similar situation may obtain in mouse skin.

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REFERENCES

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