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INHIBITION OF SOMATIC EMBRYOGENESIS IN CULTURED CARROT CELLS BY MEVINOLIN

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Abstract

Mevinolin, an inhibitor of hydroxymethylglutaryl-coenzyme A reductase and subsequently reduces availability of mevalonic acid, strongly inhibited the somatic embryogenic process of the wild carrot *Daucus carota* L. from early stages. The inhibition was reversed by mevalonic acid if both mevinolin and mevalonic acid were added at the stage when the cells were transferred to the regeneration medium and removed 24 h after their application. Gibberellic acid (GA_3) or 2-isopentenyladenine (2-ip) did not have clear effects on the reversal of mevinolin inhibition.

Key words: Carrot; somatic embryogenesis; mevinolin; mevalonic acid.

Introduction

Cultured wild carrot cells can undergo somatic embryogenesis when 2,4-dichlorophenoxyacetic acid (2,4-D), the only growth regulator supplemented, is removed from the culture medium (Sung, 1979).

Many plant growth regulators (PGR) such as gibberellins, abscisic acid and the side chains of some cytokinins are biogenetically derived from mevalonic acid (Sembdner *et al.*, 1980). To understand how endogenous growth regulators are involved in regulating somatic embryogenesis, we applied mevinolin to the culture medium as an approach to affect the synthesis of endogenous isoprenoid growth regulators.

Mevinolin is a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase; EC 1.1.1.34) (Alberts *et al.*, 1980) and subsequently blocks the formation of mevalonic acid in isoprenoid pathway. In other organisms tested, mevinolin inhibits gibberellic acid (GA) formation in *Gibberella fujikuroi* and inhibits germination in *Oryza sativa* presumably by inhibiting its formation of

growth regulators (Shiao, 1983). A structurally related compound, namely compactin, is also known to inhibit the growth of tobacco callus (Hashizume *et al*, 1983).

Materials and Methods

Plant Material and Growth Conditions

Cultured wild carrot (*Daucus carota* L.) cell line W001C was a gift from Dr. Z. R. Sung at the University of California, Berkely, USA.

Cells grown on agar MS medium (Murashige and Skoog, 1962) supplemented with 0.1 mg/l 2,4-D were transferred to liquid medium of the same composition about three weeks prior to embryogenesis experiments, and kept shaking at a speed of 150 rpm.

Somatic Embryogenesis

The procedure to initiate somatic embryogenesis was established by Z. R. Sung (1979) that the cells were transferred to the same medium devoid of 2,4-D and diluted to a density about 2×10^4 cells/ml in sterilized plastic petri dish. The cells were kept under darkness without shaking.

Mevinolin Inhibition

Mevinolin was obtained from a liquid culture of *Aspergillus terreus* (ATCC20542) according to the procedure of Alberts *et al.* (1980) with modification (Shiao, 1983). Purified mevinolin was confirmed by spectroscopic methods and found identical with an authentic sample of mevinolin kindly donated to us by Merck Sharp and Dohme Research Laboratories. Mevinolin and other supplements such as mevalonic acid. 2-isopentenyladenine (2-ip) and GA_3 were added to the autoclaved medium by Nucleopore (0.2 μ) filtration.

To study the effects of mevinolin on embryogenesis, the inhibitor at various concentrations was added to the cell suspension right after the cells were transferred to the regeneration medium.

Mevalonic acid at concentration of 5 μM was supplemented in part of the mevinolin treatments to see its effects on the recovery of embryogenesis. Both mevinolin and mevalonic acid were either present throughout the embryogenic process or removed after 24 h of treatment. 2-Isopentenyladenine (0.1 mg/l) and/or GA_3 (3 μM) was also supplemented to a portion of cultures with mevinolin treatments to elucidate their effects on mevinolin antagonism.

To assay for the effects of these treatments on the embryogenesis of carrot cells, the cells were incubated in sterilized plastic petri dishes and later scored as callus (cell clumps), globular-stage, heart-stage, torpedo-stage embryos and plantlets.

Results and Discussion

Mevinolin was a highly potent inhibitor on the somatic embryogenesis of carrot cells. The inhibition started before the formation of the globular stage.

At a prolonged exposure to mevinolin, between 4×10^{-8} M to 10×10^{-8} M, its inhibition had a profound concentration dependency (Fig. 1). In the presence of mevinolin at 4×10^{-8} M, more than 35% of the carrot cell clumps reached beyond globular stage; at 8×10^{-8} M, only 15% succeeded; while above 1×10^{-7} M, rarely any proceeded beyond the globular stage.

A continuous treatment of mevinolin above 1 μm completely blocked the embryogenic process (Fig. 2A). If mevinolin at 1 μM was removed from the culture medium after 24 h since its application, a few embryos could form in each petri dish (Fig. 2B).

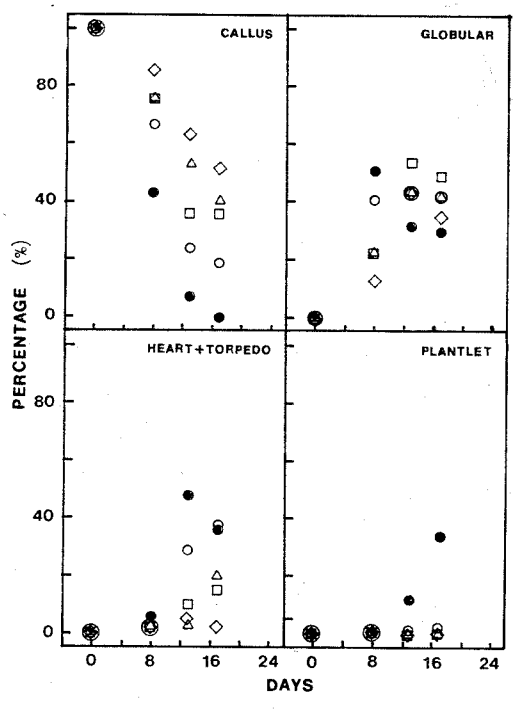
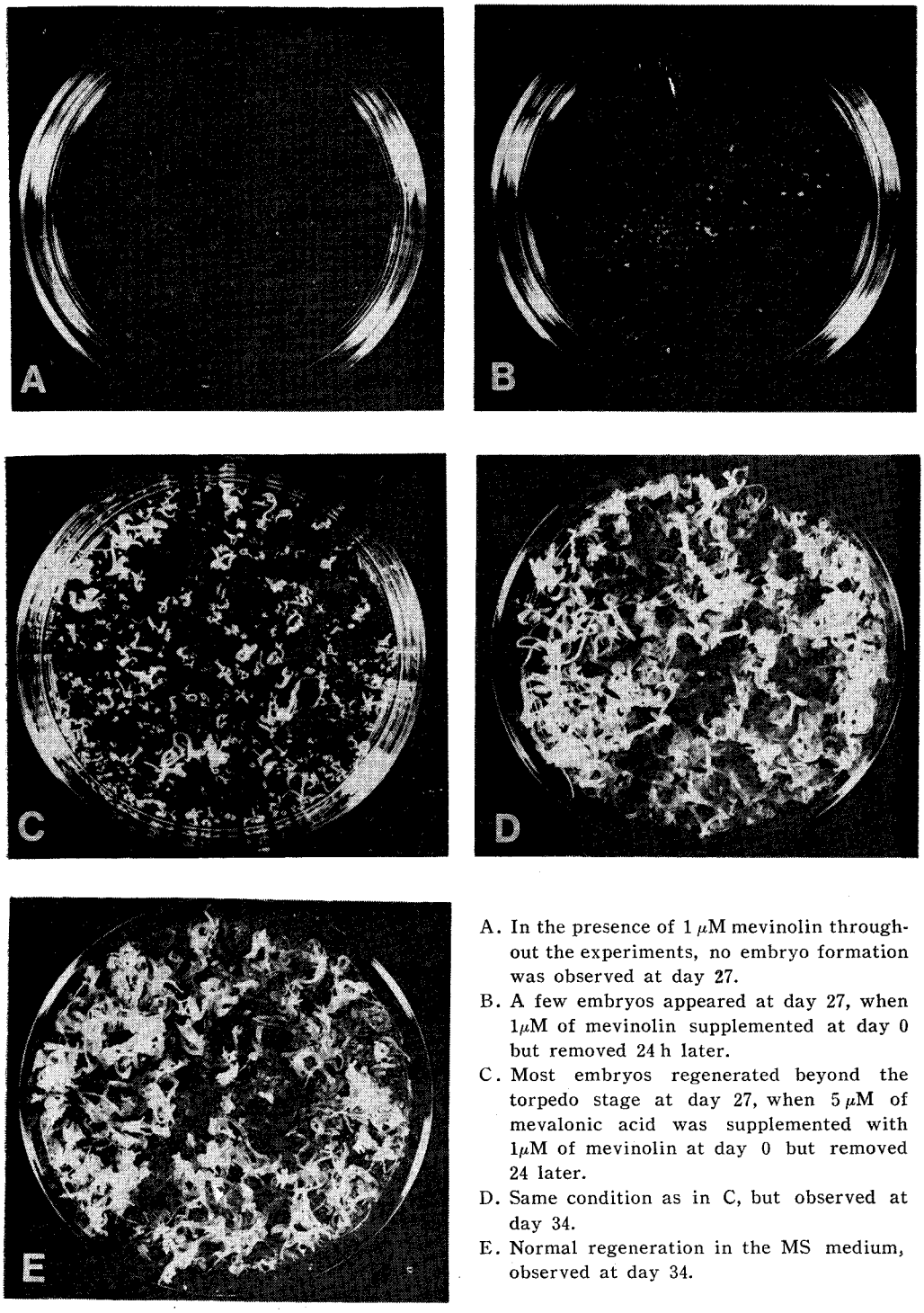


Fig. 1. Effects of mevinolin concentration on frequencies of embryo formation in cultured wild carrot cells. The frequency was presented as percentage of the total cell clumps or embryos at the particular stage(s). Experiments began on day 0 when cells were washed and incubated in petri dishes containing MS medium or MS+mevinolin.
● MS; ○ MS+4×10^{-8} M mevinolin; △ MS+6×10^{-8} M mevinolin; □ MS+8×10^{-8} M mevinoliu; ◇ MS+10×10^{-8} M mevinolin.



A. In the presence of 1 μM mevinolin throughout the experiments, no embryo formation was observed at day 27.
B. A few embryos appeared at day 27, when 1μM of mevinolin supplemented at day 0 but removed 24 h later.
C. Most embryos regenerated beyond the torpedo stage at day 27, when 5 μM of mevalonic acid was supplemented with 1μM of mevinolin at day 0 but removed 24 later.
D. Same condition as in C, but observed at day 34.
E. Normal regeneration in the MS medium, observed at day 34.

Fig. 2. Mevinolin inhibition reversed by mevalonic acid.

Mevalonic acid at 5 μM could reverse most of the inhibition caused by 1 μM of mevinolin, if both were applied at the initial medium transfer to start embryogenic process and removed by washing after 24 h (Figs. 2C, D, E). The mode of mevinolin inhibition appeared to be affecting mevalonic acid biosynthesis in carrot cells.

The reversal of mevinolin inhibition by mevalonic acid was limited if both remained in the culture medium throughout the embryogenic process (Fig. 3). In the prolonged treatment of 6×10^{-8} M mevinolin, a supplement of mevalonic acid at 5 μM caused only 13% more cells to reach heart or torpedo stage at the 17th day after their presence.

2-Isopentenyladenine at concentration of 0.1 mg/l and/or GA_3 at 3 μM supplemented with mevinolin through-out the embryogenic process did not seem to have significantly positive effects on the reversal of mevinolin inhibition Fig. 4).

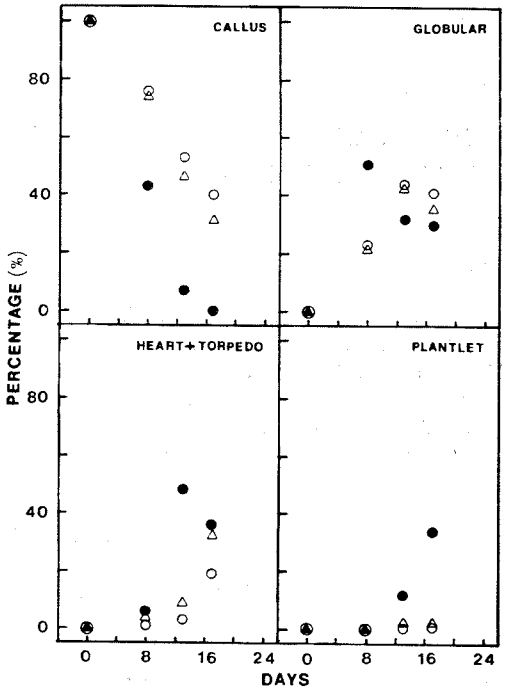


Fig. 3. Effects of mevalonic acid on the mevinolin inhibition of carrot embryogenesis. Mevinolin at a concentration of 6×10^{-8} M and/or 5 μM mevalonic acid was supplemented in the MS medium through out the experiments.
● MS; ○ MS+6×10^{-8} M mevinolin; △ MS+6×10^{-8} M mevinolin + 5 μM mevalonic acid.

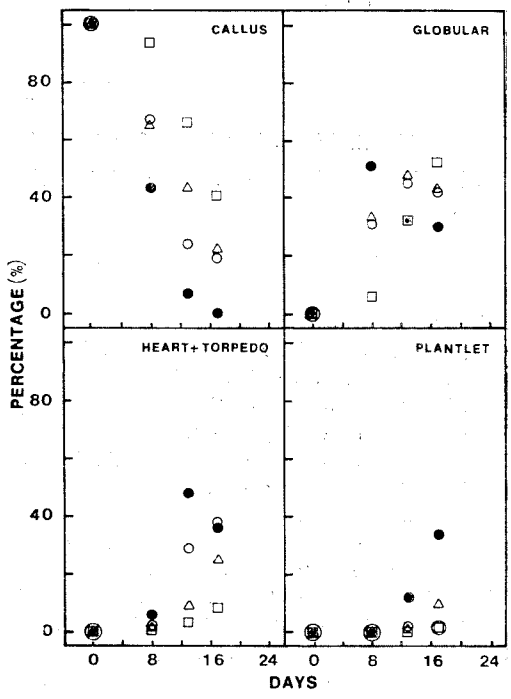


Fig. 4. Effects of 2-ip and GA_3 on the mevinolin inhibition of carrot embryogenesis. The supplement(s) was in the culture medium through out the experiments.
● MS; ○ MS+4×10^{-8} M mevinolin; △ MS+4×10^{-8} M mevinolin+0.1mg/l 2-ip; □ MS+4×10^{-8} M mevinolin + 0.1 mg/l 2-ip+3 μM GA_3.

For the difference in efficacy on the short term or long term reversal of mevinolin inhibition by mevalonic acid, our tentative explanation was either mevinolin had other inhibitory effects on the cells than simply blocking mevalonic acid and these effects manifested after prolonged culturing period, or a constant exposure to high concentration of mevalonic acid was not favorable for embryogenesis which required delicate regulation of endogenous growth regulators.

As for the lack of efficiency in reversing mevinolin inhibition by 2-ip or especially gibberellic acid (Fig. 4), it was likely that cells can still synthesize some cytokinins not subjected to mevinolin inhibition and gibberellic acid was not the critical positive growth regulator essential for carrot cells to undergo somatic embryogenesis. Noma and coworkers (Noma *et al.*, 1982) have pointed out that high levels of biological active endogenous polar GA has negative effects on embryogenesis of carrot cells. Furthermore, other growth regulating substances might be derived from mevalonic acid which could not be substituted by 2-ip or gibberellic acid.

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Mevinolin 抑制胡蘿蔔體胚分化

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Mevinolin 因抑制 HMG-CoA reductase 而降低 3,5-二羥基-3-甲基戊酸之供應，對組織培養野生胡蘿蔔 *Daucus carota* L. 的體胚分化有極强的抑制作用。在胡蘿蔔細胞被轉移入再生培養液時，如果將 mevinolin 及 3,5-二羥基-3-甲基戊酸一起添加並在24小時後洗淨，可將 mevinolin 的抑制作用完全抵消。外加徒長素 (GA_3) 或 2-異戊二烯基腺鹼 (2-ip) 對 mevinolin 的抑制作用無明顯的緩和功能。