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Study of biochemical mechanism of embryogenesis in watermelon by using molecular marker

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Somatic embryogenesis in cultured cells is a useful system for research on expression of totipotency. Fujimura and Komamine (1979) established a system of somatic embryogenesis in which selected cell clusters differentiated into embryos at high frequency in an auxin-free medium. However, it may be that embryogenetic differentiation was already determined in the initial cell clusters in their system. It is important in understanding the initiation mechanism of somatic embryogenesis to reveal the development from single cells to such embryogenic cell clusters. In the present finding, it is reported on the establishment of a system in which embryo-genesis occurs from single cell at high frequency, and also on some aspects of molecular mechanisms of embryogenesis in the earliest stage.

A cell culture derived from the hypocotyls of a watermelon seedling was subcultured in a medium containing 2, 4-D (5 x 10^7 m). Embryo formation was induced by transfer of the cell clusters to medium containing zeatin (1 x 10^{-7} m) but no auxin. Cell clusters which could differentiate into embryos in the embryo-inducing medium were regarded as having expressed potency for embryogenesis. For autoradiography, cells were labeled with ^{3}H-thymidine or ^{3}H-uridine. They were then embedded, sectioned and fixed on the glass slide, and covered with photographic emulsion. The distribution of poly (A) +RNA was followed by *in situ* hybridization using a ^{3}H-poly (U) probe (Raghavan, 1981).

Cells of 10 to 16 µm were collected by sieving with nylon screens and fractionated by density-gradient centrifugation in Percoll solutions; spherical single cells were picked up manually. When this population was transferred to the embryo-inducing medium, no embryos were formed, indicating that totipotency was not expressed in these single cells. However, when they were cultured in a medium containing 2,4-D (5 x 10^8 m), zeatin (1 x 10^6 m), and mannitol (0.2 m) for seven days, followed by transfer to the embryo-inducing medium, 85-90 per cent of these single cells differentiated into embryos.

During the process from single cells to embryogenic cell clusters, polarized localization of DNA synthesis and RNA synthesis was observed. The results of *in situ* hybridization revealed that poly (A) + RNA synthesis occurred locally in cell clusters after the second division of single cells. The frequency of polarized localization of poly (A) +RNA synthesis in cell clusters correlated with that of embryo formation from cell clusters during culture of single cells. This polarity was cancelled when embryogenic clusters were cultured under a condition where embryo formation was inhibited. These observations indicated that polarity may play a critical role in embryogenesis in its early stage.

The system established here may also be useful for cell technology and gene manipulation for *in vitro* improvement of higher plants, because a genetically manipulated single cell can differentiate into a whole plant at high frequency.

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