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## GRAVITY, CHROMOSOMES, AND ORGANIZED DEVELOPMENT IN ASEPTICALLY CULTURED PLANT CELLS

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### Background and Hypothesis

Plant development entails an orderly progression of cellular events both in terms of time and geometry (dimensional space). Work done by us on Soviet Kosmos 782 and 1129 biosatellites using totipotent carrot cells which could undergo somatic embryo formation showed that while the broad events of asexual embryogenesis could and did occur, the transition from one stage to another was slowed down significantly. The cell system used for the Kosmos experiments involved the generation of so-called embryogenic cells, their induction on Earth to produce proembryos, and their subsequent exposure to space conditions so as to evaluate their capability of expressing their capacity to form later stage somatic embryos. The normalcy of the developmental pathway of cells to proembryos to later stages of embryogeny could thus be scored. Similarly, the broad temporal aspects could be traced. The experimental design was, however, not optimal insofar as the temporal aspects were concerned (there was no onboard fixation), neither was a 1-g centrifuge available on the 1129 flight. Moreover, no attempt was made to carry out karyological observations, chromosomal and detailed cell biological and biochemical analysis.

Since then, a much-improved in vitro system for carrot somatic embryogenesis has been developed by us. The advantages of the new system include: (1) simulation of zygotic

embryogenesis with high fidelity; we now have the ability to expose cells to the space environment that show no obvious polarity and to "turn on" the "embryogenic switch" in space by means of the very simple procedure of a change in medium; (2) not requiring external growth regulators to be manipulated at any stage of the entire process of obtaining or modulating embryogenic cells; (3) being 100% responsive; i.e., all preproglobular somatic embryos go on to yield proembryos and later stage embryos; (4) no selection or mechanical cell sorting is required to prepare test specimens; i.e., they are selected very early in the culture process; (5) being readily amendable to automation; (6) having potential for selection of adaptive cells (mutants?); (7) controllably providing an open-ended system; i.e., the system can be made to cycle so that new test specimens do not have to be prepared de novo for successive experiments. Not only will answers gotten from such a system be of interest to developmental plant biologists but they will have significance for those seeking to use biotechnological procedures and manipulations in space for a variety of reasons. Indeed, the ability to use and manipulate plant cells and other kinds of propagules in vitro reliably in space will be a necessary prerequisite to many projected or hypothesized commercialization schemes.

### Objectives

The more specific objectives of the PCR experiment are:

- To test the hypothesis that microgravity will in fact affect the pattern and developmental progression of embryogenically competent plant cells from one well-defined, critical stage to another.

- To determine the effects of microgravity in growth and differentiation of embryogenic carrot cells grown in cell culture.

- To determine whether microgravity or the space environment fosters an instability of the differentiated state.

- To determine whether mitosis and chromosome behavior are adversely affected by microgravity.

### Methods

The methods employed will consist of the following:

- Special embryogenically competent carrot cell cultures will be grown in cell culture chambers provided by NASDA.

- Four cell culture chambers will be used to grow cells in liquid medium.

- Two dishes (plant cell culture dishes) will be used to grow cells on a semi-solid agar support.

- Progression to later embryogenic stages will be induced in space via crew intervention and by media manipulation in the case of liquid grown cell cultures.

- Progression to later stages in case of semi-solid cultures will not need crew intervention.

- Embryo stages will be fixed at a specific interval (day 6) in flight only in the case of liquid-grown cultures.

- Some living cells and somatic embryos will be returned for continued post-flight development and "grow-out." These will derive from the semi-solid grown cultures.

### Post-Flight Analysis

Post-flight analysis will concentrate on the following general features:

- General cellular morphology
- Scoring of embryogenesis according to stage
- Light microscopy at the level of embryogenic cells
- Electron microscopy (SEM, scanning electron microscopy)
- Chromosome analysis and karyotype determination by examining directly fixed materials
- Evaluation of non-fixed materials by post-flight behavior in vitro for continued growth and adaptation post-flight.

The above should unequivocally establish whether there are developmental phase-related disturbances in a critical event stage of embryogenesis, i.e., from the preproglobular to the globular stage proembryo. Also, it should go far to establish whether the fidelity and frequency of embryonic progression both morphologically and cytologically (chromosomally) are adversely affected or enhanced.