Nucleolar transformation in plants grown on clinostats

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Summary. Cells of carrot calli (Daucus carota L.) grown on clinostats (simulated weightlessness) exhibit increases in nucleolar number and volume. In clinostat-grown whole barley plants (Hordeum vulgare L. cv. Steptoe), nucleoli in ~70% of root meristem and root cortical cells in the 1 mm root apex exhibit multiple nodulations after one day of growth. The nucleolar nodules (1.1 μm mean diameter) are densely and finely fibrous, distinctly different from the nucleolus in which the content is so compact that the granular component is masked. Control nucleoli (from vertically rotated and stationary seedlings) rarely exhibit nodule-like protrusions, are not compact, and contain a well defined granular component. Proteins that are heat soluble, characteristic of many stress responses, rapidly increase in barley grown on clinostats. Barley growth on clinostats is slowly and steadily inhibited. There is no difference between vertically rotated and stationary controls for any of the parameters measured, indicating that clinostat motion per se does not affect significantly barley development. The evidence taken together suggests that barley plants germinated and grown on clinostats are stressed, the effects of which are expressed sequentially by alteration of nucleolar morphology, increased production of heat-soluble proteins, and decreased plant growth. Similar stress-related changes may be expected to occur in plants subjected to weightlessness during space flight. At the cellular level, clinostat treatment has been shown to facilitate a uniform distribution of amyloplasts (Shen-Miller and Hinchman 1974), and to result in an increased number and volume of nucleoli (Hinchman and Shen-Miller 1968).

The nucleolus, an organelle composed of fibrillar and granular component, fibrillar centers, and vacuoles, is the site of rDNA transcription and ribosome assembly and processing (Alberts et al. 1989, Goessen 1984, Lewin 1987, Risueño and Medina 1986). It is a dynamic subunit of the nucleus and a monitor of environmental perturbation. Heat stress in dicotyledonous plants results in rapid changes of nucleolar fine structure (Dylenski et al. 1991, Mansfield et al. 1988, Neumann and Nover 1991). In monocots, growth temperature and mechanical removal of rootcaps can affect nucleolar size and morphology (Grundwag and Barlow 1973, Jordan et al. 1985). In addition, growth temperature can alter nucleolar fine structure and simultaneously change RNA polymerase I activity in rDNA transcription (Morcillo et al. 1978). This

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nucleolar-associated enzyme is much more sensitive to Act D than is nuclear-associated RNA polymerases II or III activity in mRNA synthesis in mammalian cells (Purvis-Dutilleul et al. 1992). Treatment with Act D (in animal cells) and other drugs (in plant cells; Olmedilla et al. 1987), even at very low concentrations, rapidly alters the ultrastructure of nucleoli. In flax, the amount of rDNA can evidently be altered “permanently” by environmental changes (e.g., of nutrient levels; Cullis 1982).

Unusual nucleolar changes in morphology are noted in the present study in barley roots in particular and in carrot tissue-cultures grown on clinostats. What effect might such changes have on plant growth? Because the nucleolus is the site of production of ribosomes involved in protein synthesis, we have analyzed changes in protein content and documented changes of growth in barley seedlings associated with the change in nucleolar morphology. We observe that there is a rapid increase in heat-soluble proteins, accompanied by a gradual but statistically significant decrease in barley seedling growth. Apparently, the morphological alteration of nucleoli in barley grown on clinostats is a stress-related phenomenon. Environmental stress is known to inhibit plant growth and to rapidly initiate the synthesis of stress proteins (Jacobsen and Shaw 1989, Nover et al. 1991, Patrusky 1990, Vierling 1991), some of which move into the nucleolus and many of which are heat soluble. In addition to showing that barley plant growth is stressed in the simulated weightless environment of clinostats, we show also that wheat roots grown in space contain morphologically altered nucleoli unlike those observed in the ground controls.

Materials and methods

Carrot tissue-culture initiation and growth on clinostats

Carrot calli were initiated from phloem tissues of carrot roots (Daucus carota L.) and carried through several subcultures on an agar medium (Linsmaier and Skoog 1965) containing 10% coconut milk and 1 ppm 2,4-D. Small callus explants (~30 mg) were placed in 125 ml Erlenmeyer flasks containing 50 ml media and plugged. The flasks were divided into three treatments: C, clinostat rotation (for experimental gravity compensation); R, vertical rotation (control for vertical rotation, for experimental gravity compensation); and S, stationary (control for horizontal rotation). The 1 π clinostats used were designed and built at the Argonne National Laboratory (Gordon et al. 1964); in essence, they are derivatives of the clinostat classically used in plant physiological experiments, modified to enable numerous vessels to rotate horizontally at 2 rpm, each on a single axis. The cultures were grown under ~11 lux of fluorescent illumination, at 22.5 °C.

Barley growth on clinostats

Barley grains (Hordeum vulgare L. cv. Steptoe) were dehusked, soaked in tap water (initially 50 °C) for 2 h, decanted, and planted immediately or stored moist at 5 °C overnight. The grains were planted in Whatman # 2 filter paper cylinders, moistened in test tubes, centered in 125 ml Erlenmeyer flasks, covered with Saran wrap (cellophane). The grains were germinated and grown, under the three clinostat treatment conditions, for periods from 1–7 d in darkness at 15 °C.

Light and electron microscopy

Carrot tissue culture

After 40 d of growth, carrot calli were harvested, fixed in FAA (formalin-acetic acid-alcohol; Jensen 1962), dehydrated in a graded series of alcohol solutions, embedded in paraffin, and sectioned. Tissue sections were stained with safranin-fast green (Jensen 1962) and examined by LM.

Barley roots

1 mm long spicas of primary root of barley were harvested after various times of growth, fixed for 3 h in 2% glutaraldehyde and 2% paraformaldehyde buffered in 0.05 M sodium cacodylate (for LM), or in 0.05 M PIPES (piperazine-N,N'-bis[2-ethanesulfonic acid]) plus 5% sucrose (for EM), and the buffer or fixative pH was adjusted to 6.9 (Dannahofffer and Shen-Miller 1993). Root tips were postfixed in buffered 2% OsO₄ for 2 h, at room temperature or on ice, then dehydrated in acetone solutions. The tissues were infiltrated and embedded in Spurr’s resin (1969).

For LM examination of nucleolar volume and number, three root tips per treatment were sectioned longitudinally through the central region of the apex. Sections of 4 μm thickness were prepared and stained with 0.1% Toluidine Blue O. For EM examination, 100 nm thick sections were prepared and collected on formvar-coated grids, with saturated aqueous uranyl acetate (~8%) for 30 min at 60 °C, followed by Reynolds lead citrate (Hayat 1986) for 2 min. For study of nucleolar morphology in barley, six grids of serial sections collected after trimming one-third of the total root width were examined, and were photographed using either a JEOL 100 (80 kV) or a Zeiss 10 (60 kV) electron microscope.

Wheat roots from space flight

1 mm tips of fresh wheat roots (Triticum aestivum L. cv. Broom), obtained from the space flight JML-1 mission (experiment of D. G. Heathcote, Gravitational Plant Physiology Laboratory, Philadelphia) and from ground controls (using the same planting methods (growth chamber, soil, moisture content), and growth temperature (22.5 °C), growth duration (5 d), and growth conditions (darkness) as those for the 5 d old space flight roots), were processed for EM examination by the same protocol as that used for barley roots. During the space flight, the plants were subjected to 2 d of growth in a 1 g centrifuge and 3 d of growth in weightlessness.

Nucleolar number and volume

Carrot calli

Slides containing the carrot tissues were blind coded for LM examination. Three distinct cell-types, EP ("epimeristematic"), EN ("endomeristematic"), and PA (parenchymatous) were found in calli of all three treatments. The EP cells, densely cytoplasmic and dark-