antigen they exhibit a pattern of RNA metabolism similar to that of other cell culture systems (*6, 7*) in which the largest single accumulation of pulse-labeled RNA is precursor to ribosomal RNA. The unusually rapid rate of synthesis of nonribosomal RNA seen after PHA stimulation is, therefore, not obligatory for growing lymphocytes, but must be a response to treatment with PHA.

We also have evidence that the continuous presence of PHA is required for this phenomenon. When PHA-stimulated lymphocytes are washed, the RNA metabolic pattern appears to revert to the predominant synthesis of 45 to 50*S* material, although cell growth does not diminish. This alteration sometimes occurs without washing, after 60 to 70 hours of incubation with PHA, especially if low starting concentrations are used. Addition of excess PHA restores the major production of 6 to 30*S* RNA, again demonstrating that PHA treatment is responsible for this pattern of RNA synthesis.

The precise nature and function of the abundant nonribosomal RNA produced by lymphocytes in the presence of PHA are unknown, but an obvious hypothesis is that it is messenger RNA being produced in large quantity, perhaps as a result of the abrogation of normal regulatory processes by PHA.

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13. Sedimentation constants were calculated by the method of R. Martin and B. Ames, *J. Biol. Chem.* **236**, 1372 (1961), a value of 18*S* for the lighter ribosomal RNA peak of rabbit or rat liver carrier RNA being assumed.
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15. In previous studies (*5*) the sedimentation pattern of labeled RNA remaining after the chase in PHA-stimulated cells showed good correspondence with the absorbancy profile. The difference in the present work is due to the more complete extraction of nonribosomal RNA achieved by our current methods.
16. Abbreviations: U, uridine monophosphate; G, guanosine monophosphate; A, adenosine monophosphate; C, cytidine monophosphate.
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3 March 1966

## Mitotic Waves in Laticifers of Euphorbia marginata

Abstract. *A successive pattern of nuclear divisions that result in mitotic waves has been observed within the coenocytic nonarticulated laticifers of embryos of* Euphorbia marginata *Pursh. These waves originate independently in the cotyledonary or hypocotyl portion of the laticifer and exhibit uni- or bidirectional movement at variable velocities. Individual nuclei or groups of neighoring nuclei in a laticifer were observed in a sequence of mitotic stages ranging from prophase to telophase; division activity varied with individual laticifers in an embryo. Two mitotic patterns were apparent in the embryo: a random pattern associated with various cells in the meristematic area, and a successive pattern restricted to the laticifer. A substance, synthesized by and restricted to the laticifer, may be associated with this mitotic pattern.*

Mitotic activity within organisms can be broadly categorized into random, synchronous, and successive patterns of division. Random distribution is the most common and characterizes mitotic activity in vegetative apices (*1, 2*). The simultaneous division pattern is most frequently observed in various megagametophytes (*3*), endosperms (*4*), proembryos (*5*), antheridia (*6*), sporangia (*7*) of plants, and eggs of animals (*8*). Frequency of the successive pattern of mitotic division is comparatively low and has been reported in endosperm (*9*), laticifers (*10*), and in some animal eggs where an initial synchronous pattern is followed by successive mitotic waves (*8*).

Successive division that results in a mitotic wave occurs in the nonarticulated laticifer, a specialized coenocytic cell in *Euphorbia marginata* Pursh. A limited number of laticifers, a cell type restricted to only a few families of flowering plants, originates during the early heart stage in embryo development. The laticiferous cell branches and it also becomes coenocytic by repeated divisions of its nucleus. In addition, some branches of the laticifer permeate downward into the hypocotyl and penetrate very close to the root meristem, while others extend into the developing epicotyl. The hypocotyl is an excellent location for study of the division pattern in the laticifer because a considerable number of nuclei occur in a row within a relatively short length of the cell.

Observations were made from paraffin-embedded histological sections of immature embryos of *E. marginata* in various stages of development.

Division sequence for a row of nuclei in the laticifer consistently follows the successive pattern of mitosis. Mitotic activity is not randomly distributed along a laticifer.

Two sites of origin of mitotic waves, one in the middle of the cotyledon and the other in the upper half of the hypocotyl, have been observed. The mitotic wave that originates in the cotyledon exhibits a unidirectional movement toward the cotyledonary node, while in the hypocotyl it may display either unidirectional or bidirectional flow.

Only one uni- or bidirectional wave was observed in any one laticifer. From the point of origin, a retrogressive sequence of divisional stages from telophase to prophase was observed in both directions along the laticifer, or in only one direction (Fig. 1). This successive pattern of mitotic activity suggests that division is controlled by a substance diffusing along the axis of the laticifer.

The mitotic waves in the cotyledonary and hypocotyl branches of the laticifer are out of phase. When nuclear divisions were observed in the cotyledonary portion of the laticifer, none were evident in the hypocotyl axis of the cell.

The length of mitotic waves varied both in the same laticifer and in different ones. A wave consisted of as few as 4 (Fig. 1) or as many as 28 nuclei, representing all stages of mitosis. The shorter wave usually occurred in the upper half of the cotyledon or in the cotyledonary node. Temporally, metaphases and telophases were the

most persistent figures. Comparison of mitotic activity in several laticifers within an embryo revealed that each laticifer could be in a different phase of division. In one embryo, nuclei of three laticifers exhibited divisions, while the remaining approximately nine laticifers showed no dividing nuclei. Further, all of these three laticifers were out of phase in that the positions of similar mitotic stages were at different levels along the longitudinal axis. This aphasic pattern could be ascribed to several factors: The stimulus could be synthesized independently in each laticifer since some laticifers displayed no dividing nuclei; or, even if a stimulus was initiated simultaneously in several laticifers, the point of origin or velocity of the mitotic waves in laticifers of the same embryo could be variable.

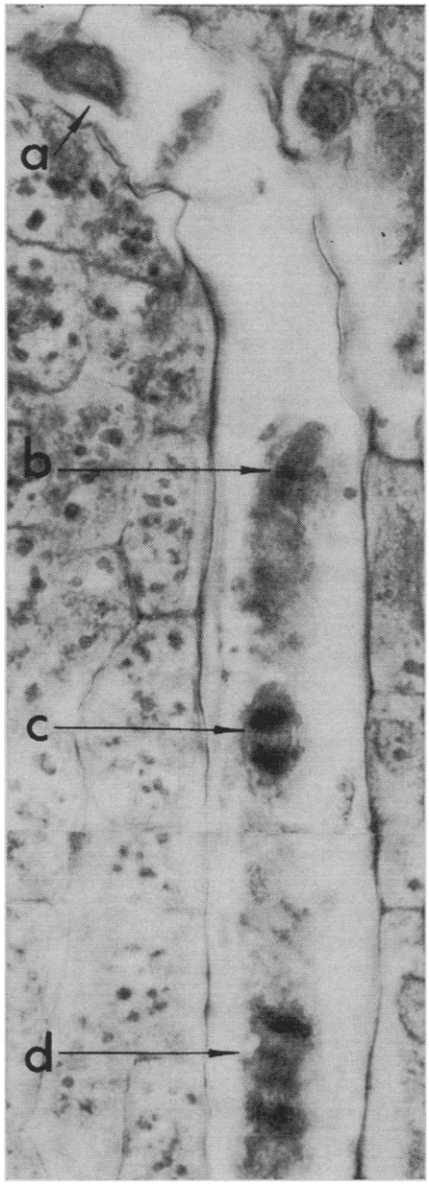


Fig. 1. A mitotic wave represented by four nuclei (*a–d*) in successive stages of division. The stimulus moved upwards in the laticifer from telophasic to prophasic nuclei (× 1200).

Since laticifers extend the entire length of the embryo axis, they are surrounded by other cells at various stages of differentiation. No immediate relation is apparent between mitotic activity in the laticifers and in the adjacent cells. Mitotic stages in other vertical rows of cells or across the entire hypocotyl axis do not exhibit any succession. Thus, two mitotic patterns occur in the embryonal axis of *Euphorbia*—one, a more random pattern involving the uninucleated cells; the other, a successive pattern within the coenocytic laticifer.

The successive pattern within the laticifer may be associated with a specific factor(s) which is triggering mitoses along the cell axis. Two points suggest that a mitotic stimulus is synthesized within the laticifer: (i) The successive pattern; a substance diffusing from the adjoining cells probably would not provide the regularity of division pattern, especially bidirectional waves, which are observed in the laticifer. (ii) Since the neighboring cells, through which a mitotic factor would have to diffuse to reach the laticifer, never undergo a successive pattern of division, it is improbable that this factor is synthesized elsewhere.

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## Radiosensitization of X Chromosome of Chinese Hamster Cells Related to Incorporation of 5-Bromodeoxyuridine

Abstract. *Selective incorporation of 5-bromodeoxyuridine into the late replicating arm of the X chromosome of Chinese hamster cells cultured in vitro caused a selective radiosensitization of the arm to ionizing radiation. Radiation damage was observed as chromosomal aberrations, and incorporation was studied by using tritiated 5-bromodeoxyuridine.*

We have undertaken to determine whether selective radiosensitization occurs in a region where there is selective incorporation of 5-bromodeoxyuridine (BUdR). Since in Chinese hamster cells the long arm of the X chromosome synthesizes its DNA during the latter portion of the DNA-synthesis phase (*1*), it was possible to incorporate BUdR (labeled with tritium) selectively into the long arm without appreciable incorporation into the short arm. Radiosensitization in the long arm containing BUdR was then compared with radiosensitization in the short arm, which contained relatively little BUdR.

Although it is generally believed that the sensitization of mammalian cells to ionizing radiation in terms of both chromosomal damage (*2–4*) and cell lethality (*5–9*) is related to the replacement of thymine in the DNA by the analog 5-bromouracil, there is some evidence to indicate that another effect, possibly metabolic in nature, may be involved in sensitization (*6, 10, 11*). Also, it has not been shown that radiosensitization occurs in the particular regions of the DNA in which incorporation of BUdR occurs.

Diploid male Chinese hamster cells of the Don strain (*12*) were grown as a monolayer on glass at 37°C in an atmosphere of 6 percent $CO_2$ in McCoy's 5a medium supplemented with fetal calf serum (20 percent). The cells were treated for 2 hours with medium containing 50 $\mu$g of either BUdR or thymidine per milliliter; tritium-labeled BUdR or thymidine (*13*) was added to the nonradioactive BUdR or thymidine, respectively, to give a specific activity