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.

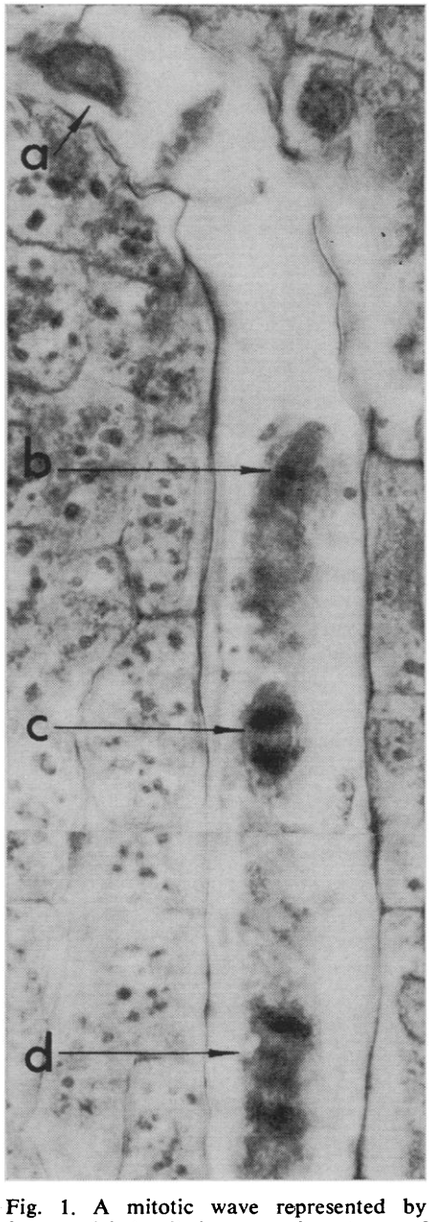

Fig. 1. A mitotic wave represented by four nuclei (*a–d*) in successive stages of division. The stimulus moved upwards in the laticifer from telophasic to prophasic nuclei (× 1200).

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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14 February 1966

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 μg 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

of 0.2 μc/μg. After 2 hours of treatment the cells were washed with fresh medium containing unlabeled thymidine (10 μg/ml); then, conditioned medium (*3*) containing thymidine (10 μg/ml) was returned to the flasks. Immediately, some of the flasks were irradiated with 200 r of x-rays (*14*) at a dose rate of 186 r/min. Two hours before fixation, Colcemid (0.06 μg/ml) was added to the medium to arrest the mitotic cells in metaphase. Control cells were fixed 2 hours after treatment by the hypotonic method (*15*). Irradiated cells were fixed 4 hours after treatment; the irradiated samples were fixed 2 hours later than the controls because of a 2-hour mitotic delay induced by irradiation (*4*). After the cells had been fixed and stained with acetic orcein, slides were prepared for autoradiography by the squash technique (*16*). A thin layer of Formvar was applied over the cells (*17*), and AR-10 autoradiographic stripping film was applied and exposed for 50 days. In two replicate flasks the amount of H^3BUdR incorporated into the cells was determined by liquid-scintillation counting (*18*).

Table 1. Aberrations observed in the X chromosome of cells treated with either H^3BUdR or H^3-thymidine.

Arm	Aberrations (No.)				Chromosomes (No.)	Aberrations per chromosome (No.)
	Isolocus	Chromatid		Total		
		Breaks and terminal deletions	Exchanges			
			H^3-thymidine only			
Labeled	0	2	0	2	65	0.03
Unlabeled	0	0	0	0	65	0
			H^3-thymidine and 200 r			
Labeled	0	4	0	4	67	.06
Unlabeled	1	2	0	3	67	.04
			H^3-BUdR only			
Labeled	0	5	0	5	67	.07
Unlabeled	0	0	0	0	67	0
			H^3-BUdR + 200 r			
Labeled	3	22	2	27	84	.32
Unlabeled	1	3	0	4	84	.05

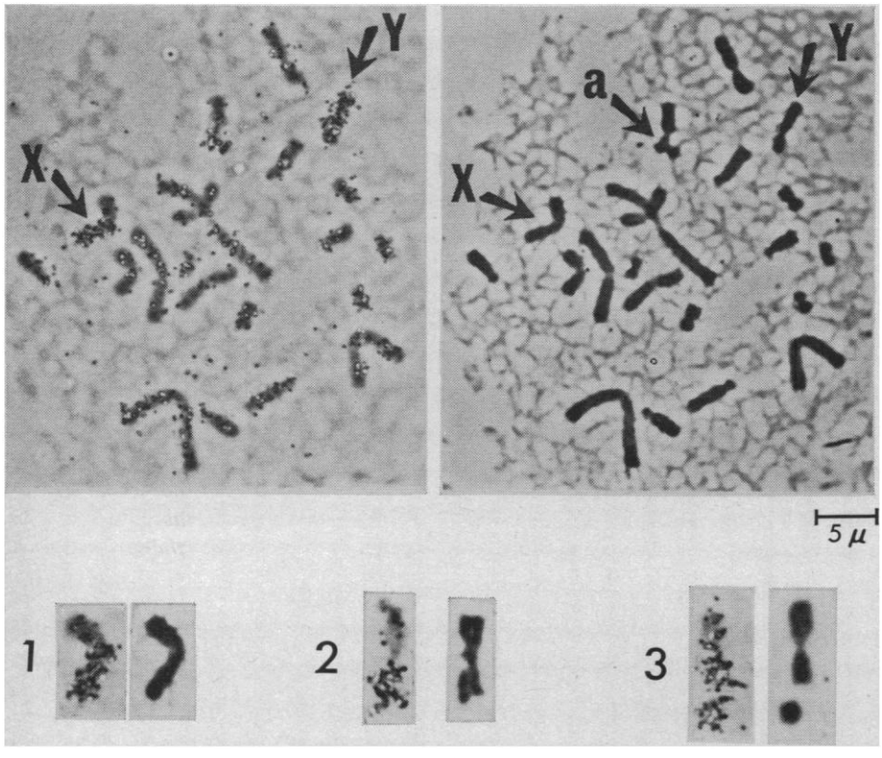

Fig. 1. Photomicrographs illustrate the distribution of H^3BUdR in the chromosomes of Chinese hamster cells labeled and irradiated during the latter part of the DNA-synthesis phase. On the left, the distribution of autoradiographic grains is shown, and on the right the chromosomes are shown after the autoradiographic film was removed. Normal X and Y chromosomes are illustrated in a cell, and it can be seen that both the long arm of the X chromosome and both arms of the Y chromosome were much more heavily labeled than the other chromosomes. In many cells, showing the replicating pattern of the late synthesis phase, the small metacentric chromosomes were also heavily labeled. A chromatid interchange (*a*) was also observed. Enlargements of individual chromosomes are shown also. Chromosome 1 is the X chromosome from the cell above; chromosomes 2 and 3 are X chromosomes from two other cells and illustrate chromosomal aberrations in the heavily labeled arms (a chromatid break or large gap in No. 2 and an isolocus break in No. 3). (In chromosome 3 it is possible that the fragment was not completely separated and that the aberration is a chromatid break.)

Metaphase cells (65 to 84 from each sample) which clearly showed that one arm of the X chromosome was much more heavily labeled than the other arm were selected for analysis; an example is shown in Fig. 1. Some of these cells were photographed; for others the distribution of the label in the chromosomes was recorded by drawings. Then the layer of film was removed and the same metaphase cells were analyzed for chromosomal aberrations. The damage was scored separately in the labeled and unlabeled arms of the X chromosome and was observed as chromatid breaks or large gaps, terminal deletions, isolocus deletions, and chromatid intrachanges and interchanges (*19*).

Since the time interval (G_2) from the end of the DNA-synthesis phase to mitosis is 2 to 3 hours (*1*), the labeled metaphase cells sampled were primarily in the latter part of DNA synthesis at the time of the treatment. Many of these metaphases showed the replicating pattern of the late synthesis phase (*1*), in which the long arm of the X chromosome is heavily labeled while the short arm is very lightly labeled (see Fig. 1). The Y chromosome was also more heavily labeled than the other chromosomes. In 43 cells labeled with H^3BUdR and showing the replicating pattern of the late synthesis phase, there was an average of 140 grains per cell, with 21 per Y chromosome, 15 per long arm of the X chromosome, and only 3 per short arm of the X chromosome. If the grains were uniformly distributed throughout the chromosomes, only 2.7 percent of the grains should lie over the long arm of the X chromosome (*20*). However, the long arm of the X chromosome, which on the average is only 1.3 times longer

than the short arm (*20*), contained 11 percent of the grains in the cell, and the short arm contained only 2 percent (*21*).

The data in Table 1 indicate that the long, labeled arm of the X chromosome was selectively sensitized to irradiation by the incorporation of BUdR. In the long arm, the frequency of aberrations was five times higher in the BUdR-treated cells than in the thymidine-treated cells. In contrast, no radiosensitization was apparent in the short, unlabeled arm of the X chromosome (*22*).

The radiosensitization associated with BUdR incorporation is *not* caused by 5-bromouracil increasing the absorption of energy from ionizing radiation (calculated to be an increase of less than 1 percent) (*8*). Also, the amount of tritium incorporated in the cells (22 percent more for H^3-thymidine than for H^3BUdR) did not introduce any apparent complications. In control cells labeled with H^3-thymidine (50 μg/ml) at a specific activity of either 0.2 μc/μg or 0.05 μc/μg there were 0.21 and 0.27 aberration per cell, respectively. For the higher specific activity, an average of 17 H^3 disintegrations would have occurred in the cells prior to fixation. This is equivalent to about 5 rads of x-rays and has been shown to produce a negligible amount of chromosomal damage in the Chinese hamster cell (*18*).

These results clearly illustrate that the late replicating arm of the X chromosome both incorporated about five times as much BUdR and sustained about five times as much chromosomal damage as the early replicating arm. In the irradiated cells treated with thymidine, however, the two arms sustained about the same amount of chromosomal damage. Therefore, where selective incorporation of the BUdR occurred, there was also selective radiosensitization. This correlation is consistent with previous studies (*3, 5, 6, 23*) showing that an increase in the incorporation of thymine analogs causes an increase in the radiosensitization of the cell. Furthermore, this correlation supports the hypothesis that BUdR sensitization of chromosomes to ionizing radiation is specific for the regions of incorporation and is caused by the replacement of thymine with 5-bromouracil. As discussed before (*3, 6, 10, 23, 24*), it is possible that sensitization is caused by 5-bromouracil in the DNA either interfering with repair of lesions induced near the site of incorporation or reducing the amount of energy required to damage the DNA.

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21. From the tritium radioactivity of the cells, measured by liquid-scintillation counting (*3, 18*), it was calculated that the amount of thymine replaced by 5-bromouracil was 2.8 percent in the metaphase cells and 11 percent in the long arm of the X chromosome.
22. For thymidine treatment only, thymidine treatment plus 200 r, BUdR treatment only, and BUdR treatment plus 200 r there were, respectively, 0.21, 1.9, 0.44, and 3.4 aberrations per cell and 0.05, 0.13, 0.05, and 0.22 aberration per Y chromosome.
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3 March 1966

Biosynthesis and Composition of Histones in Novikoff Hepatoma Nuclei and Nucleoli

Abstract. *Histones were prepared from isolated nuclei and nucleoli of the Novikoff ascitic hepatoma at several time points after the injection of L-lysine uniformly labeled with C^{14} into tumor-bearing rats. Amino acid analysis and starch-gel electrophoresis failed to reveal any differences between the nuclear and nucleolar histones, although both fractions were more acidic in composition than calf thymus histones. However, the nucleolar histones were a metabolically distinct fraction, and their rate of synthesis was approximately twice that of the total nuclear histones.*

The importance of the nucleolus for synthesis and methylation of ribonucleic acid (RNA) has been established by many authors (*1*). The possibility that nucleoli may have a significant role in protein synthesis has also been investigated. Birnstiel and Hyde (*2*) and Birnstiel and Flamm (*3*) demonstrated rapid synthesis of proteins in pea seedling nucleoli; among other nucleolar proteins, histones became labeled to a significant extent. Rees, Rowland, and Varcoe (*4*) have noted incorporation of labeled amino acids into proteins of a nuclear fraction containing nucleoli. They concluded that the lipid-rich material, probably the heterochromatin associated with the nucleoli, was the active site for the incorporation of amino acids by rat liver nuclei. However, Busch and his co-workers were unable to provide evidence for synthesis of histones in nucleoli of mammalian tissues either in vivo or in vitro, although acidic nuclear proteins were actively labeled (*5*). Since histones seem to play an important role in regulating the biosynthesis of ribosomal RNA in nucleoli (*6*), we have attempted to determine whether the biosynthesis of histones by nucleoli is a special feature of plants or whether similar biosynthesis also occurs in mammalian cells.

Male albino rats (200 to 250 g) bear-