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SYNCHRONIZATION OF CELL PROLIFERATION IN SARCOMA 37 OF MICE BY HYDROXYUREA

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The synchronizing action of hydroxyurea on the passage of sarcoma 37 cells through the S phase and mitosis was investigated in mice, allowing for diurnal fluctuations in mitotic activity and the index of labeled nuclei. The degree of synchronization was estimated from changes in the number of proliferating or labeled cells and the rate of change of synchronization. The tumor consisted of at least two cell populations in which variations in the number of cells both in the S period and also, probably, in mitosis were out of phase. The degree of artificial synchronization of the cells in mitosis based on the rate of change of synchronization was much higher than the natural level in the tumor not divided into separate populations. However, the number of cells undergoing artificial synchronization was not significantly different not only from the number of cells in the tumor undivided into separate populations, but also from the number of cells naturally synchronized in one of the populations. A possible explanation of this fact is that hydroxyurea acted on only one group of cells, for fluctuations in the number of DNA-synthesizing cells in the separate populations also were out of phase.

KEY WORDS: *Sarcoma 37; mitosis; DNA synthesis; hydroxyurea; synchronization of cell proliferation.*

Cell systems synchronized with respect to proliferative processes can yield more accurate information about the mitotic cycle and its changes under different conditions. For this reason, besides the analysis of natural synchronization, in recent years artificial synchronization has been used, especially in systems whose natural synchronization is weak. Hydroxyurea, which temporarily blocks the passage of cells from the G_1 to the S phase and DNA synthesis, is an effective synchronizer of cell proliferation in tumors *in vivo* [6, 8].

In this investigation the synchronizing action of hydroxyurea on cell proliferation was studied in sarcoma 37, making allowance for diurnal variations in mitotic activity and in the number of DNA-synthesizing nuclei. The need for investigating diurnal rhythms during experimental procedures directed toward cell division is partly explained by the unequal action of certain cytostatics when administered at times of maximal or minimal proliferative activity [1, 2, 5].

EXPERIMENTAL METHOD

The tumor chosen for this investigation was sarcoma 37, inoculated into albino mice in which the synchronization of cell proliferation had been studied in the esophageal epithe-

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TABLE 1. Diurnal Changes in MI (in °/₀₀) and ILN (in °/₀₀) in Sarcoma 37 in Intact Mice and after Treatment with Hydroxyurea

Time of day	Intact animals		Animals after treatment with hydroxyurea	
	MI	ILN	MI	ILN
4 a.m.	7,4	62,4	5,8	111,0
6 a.m.	10,9	47,4	3,9	105,6
8 a.m.	10,4	111,0	8,1	115,0
10 a.m.	7,9	180,0	16,3	75,0
12 noon	8,5	74,7	21,2	67,2
2 p.m.	10,8	35,2	11,5	153,2
4 p.m.	12,0	167,5	19,1	68,1
6 p.m.	11,3	74,3	7,3	108,0
8 p.m.	11,5	91,0	11,5	46,5
10 p.m.	14,0	85,3	11,5	140,8
12 midnight	13,1	27,9	11,7	20,5
2 a.m.	10,2	6,5	17,0	38,0
Mean diurnal values	10,7	80,1	12,1	87,4

lium [4]. Ascites fluid containing sarcoma cells, diluted 1:1 with physiological saline, was injected subcutaneously into the axillary region in a dose of 0.1 ml per mouse. The tumor developed beneath the skin in a solid form.

On the ninth day after inoculation of the tumor the animals were divided into two groups. The mice of the experimental group received an intraperitoneal injection of hydroxyurea in a dose of 100 mg/kg body weight at 2 and again at 4 a.m., whereas mice of the control group received no such injection. On the basis of certain observations [7, 8] it could be assumed that hydroxyurea, which has low toxicity, if injected in this way would block the cells at the end of the G_1 phase and in the S phase until 7-9 a.m. According to our own observations [3], a sharp increase in the index of labeled nuclei (ILN) in sarcoma 37 begins at this same time. It could therefore be expected that synchronization in this case would be more effective than natural synchronization or artificial caused by injection of hydroxyurea at other times. Five mice of each group were killed at intervals of 2 h during the 24-h period from 4 a.m. (first sacrifice) to 2 a.m. (12th sacrifice). Mice of the experimental group killed at 4 a.m. received one injection of hydroxyurea, unlike the mice of the other groups, each of which received two injections. All the animals were given an injection of thymidine-^{3}H (specific activity 16 Ci/g) in a dose of 30 μCi per mouse 1 h before sacrifice.

Histological sections of the tumor were coated with type M photographic emulsion. Nuclei above which there were at least five grains of silver were regarded as labeled. ILN and the mitotic index (MI) were expressed in promille, and determined by counting 6000-9000 cells in each case (sections were examined over the whole area from the capsule to the central zone, which was usually necrotic).

The degree of synchronization was estimated by means of two mutually complementary criteria [4]: 1) the ratio between the increase in the number of cells in mitosis or in the S phase during the period of synchronization and the total number of cells in the same phases of the cycle during the 24 h under natural conditions, in percent; 2) the rate of increase of the number of cells passing through the S phase or mitosis synchronously during the couse of synchronization. This rate for both MI and ILN was expressed in promille per hour.

EXPERIMENTAL RESULTS

The values of ILN for the tumor of the control animals (Table 1) were maximal at 10 a.m. and 4 p.m. and minimal at 2 p.m. and 2 a.m. ($P_{10a.m.-2p.m.} = 0.022$, $P_{10a.m.-noon} = 0.008$, $P_{2-4p.m.} = 0.044$, $P_{4p.m.-2a.m.} = 0.016$). MI reached a maximum at 10 p.m. and a minimum at 2-4 a.m. ($P_{4a.m.-10p.m.} = 0.007$, $P_{10p.m.-2a.m.} = 0.037$). The absence of a maximum of MI in the period between the two maxima of ILN suggests that the maxima of ILN characterized DNA synthesis in two different cell populations and not in two consecutive mitotic cycles.

No synchronizing action of hydroxyurea on ILN could be detected in the experimental animals: The level of labeled nuclei during the period of action of the synchronizing agent (until 6-7 a.m.) was high, and the subsequent fall and rise were not significant. However, the larger values of ILN during the period of action of hydroxyurea than in the control suggest that the cells were nevertheless all synchronized in the S phase.

Changes in the value of MI can be interpreted as the result of artificial synchronization, for at the end of the period of action of hydroxyurea the values of MI rose sharply from 6 a.m. to noon ($P_{6a.m.-noon} = 0.004$). Except for a decrease between 4 and 6 p.m. ($P_{4-6p.m.} = 0.015$), the subsequent fluctuations of MI were not significant. Since hydroxyurea, according to the literature, acts on cells in the S phase and inhibits DNA synthesis, it can be assumed that in this experiment the cells were synchronized in that period of the mitotic cycle. The high values of ILN during the action of the synchronizing agent would thus indicate that the compound probably caused a temporary lengthening of the phase of DNA synthesis, which would lead to the accumulation of cells in that phase, followed by synchronization of their passage into mitosis.

There are definite grounds for the assertion that the level of ILN during the period of action of hydroxyurea corresponded to the sharp increase in MI after the end of its action if the ratio between the mean ILN and the mean MI in these time intervals was equal to the ratio between the mean diurnal values of these parameters. The ratio between the mean ILN from 4 to 8 a.m. and the mean MI from 8 a.m. to noon was in fact equal to the ratio between the mean diurnal values of these parameters (7.2:1.0), and it can accordingly be concluded that ILN from 4 to 8 a.m. and MI from 8 a.m. to noon characterized the same group of cells.

Since the tumor in these experiments consisted of at least two cell populations in which fluctuations both in ILN and, evidently, in MI were out of phase, it was very difficult to detect correlation between the control and experimental series. Artificial synchronization, which could be detected only for MI, was accordingly compared with natural synchronization, as revealed by MI in the tumor regarded as a homogeneous population, and by ILN in the separate tumor populations. For instance, the increase in the number of cells in mitosis from 6 a.m. to noon during artificial synchronization was 20.2% of the total number of cells in this phase under natural conditions, whereas the rate of increase in the number of cells passing through mitosis synchronously was 2.88 °/₀₀/h. The increase in the number of cells in mitosis in the control during the period from 4 a.m. to 10 p.m. was 21.5%, and the rate of increase in the number of cells passing through mitosis synchronously (if the increase was represented by an averaged straight line) was 0.34 °/₀₀/h.

During artificial synchronization the rate of change in the number of cells passing through mitosis synchronously was thus considerably higher than during natural synchronization if the tumor was regarded as a homogeneous population. The number of cells producing the maximum of MI during artificial synchronization (20.2%) did not differ significantly either from the number of cells naturally synchronized in the tumor regarded as a homogeneous population (21.5%) or from the number of cells naturally synchronized with one of the populations (the decrease in the number of cells in the S phase in the control animals from 10 a.m. to 2 p.m. was 15.1%, indirectly indicating a similar increase in the number of cells in this phase, which could not be directly estimated because of the low degree of significance of the increase in ILN between 6 and 10 a.m.).

The absence of appreciable differences in the number of cells undergoing artificial and natural synchronization can be explained on the grounds that only one group of cells was artificially synchronized as a result of the phase shift between the fluctuations in the number of cells in the S period in the two different tumor populations.

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DIFFERENCES IN THE FRACTIONAL COMPOSITION OF CYTOPLASMIC RNP PARTICLES IN ZAJDELA ASCITES HEPATOMA CELLS AND THE LIVER CELLS OF ANIMALS WITH TUMORS

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The relative content of poly(A)-RNA in the cytoplasm is higher in cells of Zajdela's ascites hepatoma and of the liver of rats with tumors than in normal rat liver cells. Another distinguishing feature of the tumor cells (and also, to a lesser degree, of the liver cells of animals with tumors) is a change in the ordinary ratio between polyribosomes and monoribosomes (and, correspondingly, between mRNP particles and informosomes) for normal liver cells in favor of the latter, indicating the occurrence of definite changes in their protein-synthesizing apparatus. According to some of the indices investigated, cells of tumor-bearing animals occupy an intermediate position between normal and tumor cells.

KEY WORDS: *Cytoplasmic mRNA; cytoplasmic RNP particles; hepatoma; liver of tumor-bearing animals.*

Selective transport of template RNA (mRNA) is known to take place in the cells of eukaryotes, as a result of which only a very small proportion of the molecules synthesized in the cell nucleus enters the cytoplasm. There is reason to suppose that the spectrum of mRNA transported into the cytoplasm in tumor cells is broader than in normal cells [5, 8-10] and that this decrease in selectivity of transport may have some relation to the different manifestations of tumor growth. These considerations have motivated investigations of the further fate of cytoplasmic messenger RNAs. Another aspect of the problem of malignant growth is the explanation of the molecular mechanisms of the harmful effect of tumors on the host organism, which necessitates the investigation of various tissues of tumor-bearing animals [5].

The object of this investigation was a comparative analysis of the fractional composition of cytoplasmic ribonucleoprotein (RNP) particles of cells from normal rat liver, from Zajdela's ascites hepatoma, and from the liver of animals with primary tumors (Zajdela's ascites hepatoma).

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 150-200 g, deprived of food for 24 h before the experiments, were used. Rat liver mRNA was labeled with ^{14}C-orotic acid and of Zajdela's ascites hepatoma with ^{14}C-uridine (100 μCi per rat in each case) under conditions of selective suppression of ribosomal RNA synthesis (1 h before radioactive labeling the animals were given an injection of 80 μg actinomycin D). Cytoplasmic poly(A)-containing RNAs were selectively adsorbed on a poly(U)-sepharose column [11]. Cytoplasmic RNP particles were iso-

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