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The Inhibition of Ribosomal RNA Synthesis and Maturation in Novikoff Hepatoma Cells by 5-Fluorouridine[1]

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SUMMARY

The inhibition of ribosomal RNA (rRNA) maturation by 5-fluorouridine (FUrd) in Novikoff hepatoma cells appears to depend upon the incorporation of the analog into the 45 S rRNA precursor. Precursor synthesized in the presence of FUrd is not processed into mature rRNA, but precursor synthesized in the absence of the analog is processed normally after the addition of the drug. The effect of FUrd on rRNA maturation is concentration dependent. At a concentration of 1×10^{-4} M, the analog completely inhibits the formation of mature 18 S and 28 S rRNA; while at a concentration of 1×10^{-7} M, the analog has no significant effect on rRNA maturation. These results suggest that some minimum degree of analog substitution is necessary to inhibit the maturation process. In addition to its inhibition of maturation, FUrd also inhibits the transcription of 45 S rRNA precursor. However, this effect of the drug is less complete and more time dependent than the effect on maturation. The inhibition of rRNA maturation by FUrd persists after removal of the analog from the culture medium. Cells that had been exposed to FUrd for 2 hr were unable to process 45 S rRNA precursor 20 hr after removal of the drug from the medium.

INTRODUCTION

5-Fluorodeoxyuridine and FUra[3] have been used extensively in the treatment of certain types of cancer (9–11) since they were first synthesized in 1957 (5). It is generally thought that the cytotoxicity of these and several related compounds, such as 5-fluoroorotic acid and FUrd, is related to the fact that they are all metabolically converted to 5-fluoro-2′-deoxyuridine -5′-monophosphate, which is a potent inhibitor of thymidylate synthetase and, hence, of DNA synthesis (8). However, a number of recent reports have demonstrated that certain of these compounds are also potent inhibitors of rRNA metabolism. Since the synthesis of ribosomes during the G_1 phase of the cell cycle is a prerequisite for DNA synthesis during the S phase (1), the inhibition of rRNA maturation by these fluorinated pyrimidines may be of equal or even greater importance than the inhibition of DNA synthesis.

In mammalian cells, mature 28 S and 18 S rRNA are produced in the nucleolus from a common precursor by a complex maturation process (2). The initial rRNA transcript product is a 45 S molecule, which contains the sequences of both 28 S and 18 S rRNA, plus additional sequences the functions of which are currently undefined. The 45 S molecule is methylated, in both base and sugar moieties, either during or soon after its synthesis. After methylation, the 45 S molecule is converted to a 41 S molecule, which also contains the sequences for both 28 S and 18 S rRNA. The 41 S molecule is cleaved to a 32 S molecule and a 20 S molecule, which are the immediate precursors of mature 28 S and 18 S rRNA, respectively. Throughout the maturation process the 45 S rRNA precursor and the various intermediates exist as ribonucleoprotein particles. FUra inhibits rRNA synthesis in bacteria (7), yeast (4), and rat liver (26). 5-Fluoroorotic acid inhibits the production of mature 28 S and 18 S rRNA in the livers of rats (24) and mice (6). Recent studies demonstrated that both FUrd and FUra inhibit the maturation of rRNA in Novikoff hepatoma cells (25). In all of these studies the inhibition appeared to occur at a posttranscriptional site. This paper describes in detail the nature of the inhibition of rRNA maturation by FUrd in Novikoff hepatoma cells.

MATERIALS AND METHODS

Cells and Media. Novikoff hepatoma cells (strain N1-S1) were grown at 37° in suspension culture under an atmosphere of 5% CO_2:95% air in a gyratory incubator rotating at 150 rpm (12). The cells were grown in Swim's Medium 77, supplemented with calf serum (100 ml/liter) and Pluronic F-68 (1 g/liter) and modified to contain 4 mM L-glutamine. This medium is hereafter designated as Medium S69.

Extraction and Electrophoretic Analysis of RNA. Upon completion of the experimental incubation period, the cell suspensions were transferred to centrifuge tubes and chilled to 0°. The cells were harvested by centrifugation at $3000 \times g$ for 5 min at 4°. RNA extraction (25) and analysis by polyacrylamide:agarose gel electrophoresis (24) were performed as described previously. After electrophoresis, the gels were scanned at 260 nm with a recording spectrophotometer equipped with a linear transport module and then

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[3] The abbreviations used are: FUra, 5-fluorouracil; FUrd, 5-fluorouridine; Urd, uridine; PCA, perchloric acid.

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cut with a Mickel gel slicer (Brinkmann Instruments, Inc., Westbury, N. Y.) into 2-mm slices. The gel slices were incubated overnight at 37° in 0.5 ml of 0.5 N NaOH and then assayed for radioactivity after the addition of 0.5 ml of 0.5 N HCl and either 5 ml of PCS solubilizer or 10 ml of Scintiverse.

Incorporation of [^{3}H]Guanosine into the Acid-soluble Fraction. Cells were exposed to [8-^{3}H]guanosine under various conditions. Upon completion of the incubation period, the cells were harvested by centrifugation at 270 × *g* for 3 min. The cell pellets were washed by mixing with a vortex mixer in fresh Medium S69, and the cells were collected again by centrifugation at 27,000 × *g* for 5 min. The washed cell pellets (containing approximately 25 × 10^6 cells) were suspended in 2 ml of cold 0.2 N PCA and allowed to sit at 0° for 10 min. The resulting precipitate was collected by centrifugation (27,000 × *g*, 5 min) and washed twice with 1 ml of cold 0.2 N PCA. The combined supernatant fluids are the acid-soluble fraction.

Chromatographic Analysis of Urd Nucleotides in the Acid-soluble Fraction. Cells were exposed to [5-^{3}H]uridine under various conditions. The acid-soluble fraction was prepared as described above, except that trichloroacetic acid (6 g/100 ml) was used in place of 0.2 N PCA. The combined supernatant fluids for each sample were extracted with ether until the pH was raised to approximately 5. Samples were then lyophilized, and the residue was dissolved in water (5 μl/ml of acid-soluble fraction). Five μl of each sample were spotted on Whatman No. 3MM paper (23 x 57 cm). The uridine nucleotides were separated by descending chromatography, using a solvent containing 1 M ammonium acetate:95% ethanol (30:70, v/v) for 14 hr. After air drying, the chromatograms were cut into 1- x 3-cm segments, which were placed in scintillation vials and rocked with 1 ml of 0.01 N HCl for 1 hr at 37°. Five ml of a dioxane-based counting cocktail were added for the determination of radioactivity in the eluate.

Radioactivity Measurements. All radioactivity measurements were performed by a Packard Model 3375 liquid scintillation spectrometer. Automatic external standardization was used to correct for quenching. The dioxane-based counting solution contained 8 g of Omnifluor (New England Nuclear, Boston, Mass.) and 60 g of naphthalene (J. T. Baker *Chemical Co.*, Phillipsburg, N. J.) per liter of dioxane (ICN Isotope and Nuclear Division, Cleveland, Ohio).

Materials. Swim's Medium 77 and calf serum were purchased from Grand Island Biological Co., Grand Island, N. Y., and International Scientific Industries, Inc., Cary, Ill., respectively. Pluronic F-68 was a gift from the Wyandotte Chemical Co., Wyandotte, Mich. PCS solubilizer, [8-^{3}H]guanosine (15.8 Ci/mmole), and [5-^{3}H]Urd (5 Ci/mmole) were obtained from Amersham/Searle Corp., Arlington Heights, Ill. [2-^{14}C]Urd (45 mCi/mmole) was purchased from Calatomic, Los Angeles, Calif. Scintiverse was obtained from Fisher Scientific, Pittsburgh, Pa. Actinomycin D was supplied by Calbiochem, La Jolla, Calif. Urd was purchased from Sigma Chemical Co., St. Louis, Mo. FUrd was a generous gift from Hoffmann-LaRoche, Inc., Nutley, N.J.

RESULTS

Concentration Dependence of the Inhibition of rRNA Maturation by FUrd. Identical cultures of Novikoff hepatoma cells were exposed to [^{3}H]Urd for 90 min in the presence of various concentrations of FUrd. Chart 1 shows the results of the electrophoretic analysis of the RNA extracted from these cultures. At a concentration of 1 × 10^{-7} M (Chart 1*A*), FUrd had little or no effect on the incorporation of the labeled nucleoside into mature 18 S or 28 S rRNA, since essentially the same radioactivity profile was obtained in control cultures treated with 1 × 10^{-7} M Urd (results not shown). Although some inhibition of mature rRNA formation was observed at 1 × 10^{-6} M FUrd (Chart 1*B*), significant amounts of 32 S, 28 S, and 18 S rRNA were still produced. There was essentially no formation of mature 18 S or 28 S rRNA at 1 × 10^{-5} M FUrd (Chart 1*C*), although significant production of the 45 S and 32 S rRNA precursors was observed. At 1 × 10^{-4} M FUrd

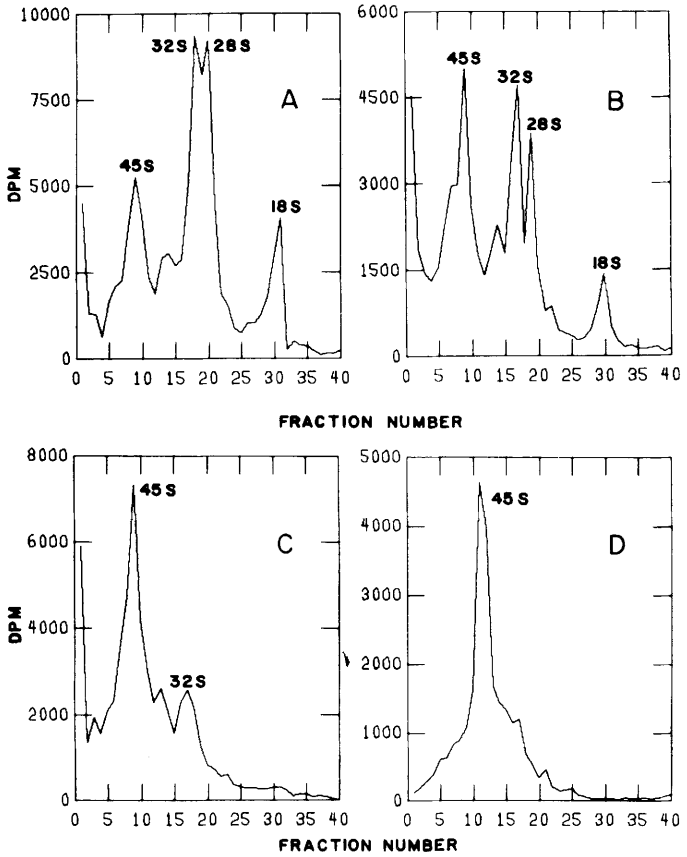

Chart 1. Effect of different concentrations of FUrd on the incorporation of [^{3}H]Urd into rRNA. Novikoff hepatoma cells at a density of 1.42 × 10^6/ml were harvested and resuspended in one-fifth the original volume of fresh Medium S69. Identical 5-ml cultures were treated with [^{3}H]Urd (1 μCi/ml) and different concentrations of FUrd (*A*, 10^{-7} M; *B*, 10^{-6} M; *C*, 10^{-5} M; *D*, 10^{-4} M). After incubation for 90 min, total cellular RNA was extracted and 1 A_{260} unit of each sample was applied to a composite gel containing 2.4% acrylamide and 0.6% agarose. Gels were subjected to electrophoresis for 3 hr at 6 ma/gel, scanned at 260 nm, and cut into 2-mm slices; each slice was assayed for radioactivity. The location of the various rRNA peaks was verified by comparing the radioactivity profile to the A_{260} scan.

(Chart 1*D*), the initial 45 S rRNA precursor was the only species into which a significant amount of labeled nucleoside was incorporated. For each of the FUrd-treated cultures discussed here, control cultures were run that contained the corresponding concentrations of Urd. In each case the radioactivity profile was qualitatively identical to that seen in Chart 1*A*.

Effect of FUrd on the Rate of 45 S rRNA Synthesis. Identical cultures were exposed to [^{3}H]guanosine for 15 min in the presence of either 1×10^{-4} M Urd or 1×10^{-4} M FUrd. In one-half of the cultures, the nucleosides were added simultaneously with the labeled precursor; in the other half, the nucleosides were added 1 hr before the addition of labeled precursor. The electrophoretic analysis of RNA extracted from these cultures is shown in Chart 2. When FUrd was added simultaneously with the [^{3}H]guanosine, the incorporation of radioactivity into 45 S rRNA precursor was not inhibited. However, when FUrd was added 1 hr before the addition of [^{3}H]guanosine, the incorporation of radioactivity into 45 S rRNA precursor was significantly inhibited. The data in Table 1, which shows the incorporation of radioactivity into the acid-soluble pool, indicate that decreased uptake of [^{3}H]guanosine was not responsible for the decreased incorporation of radioactivity into 45 S rRNA precursor.

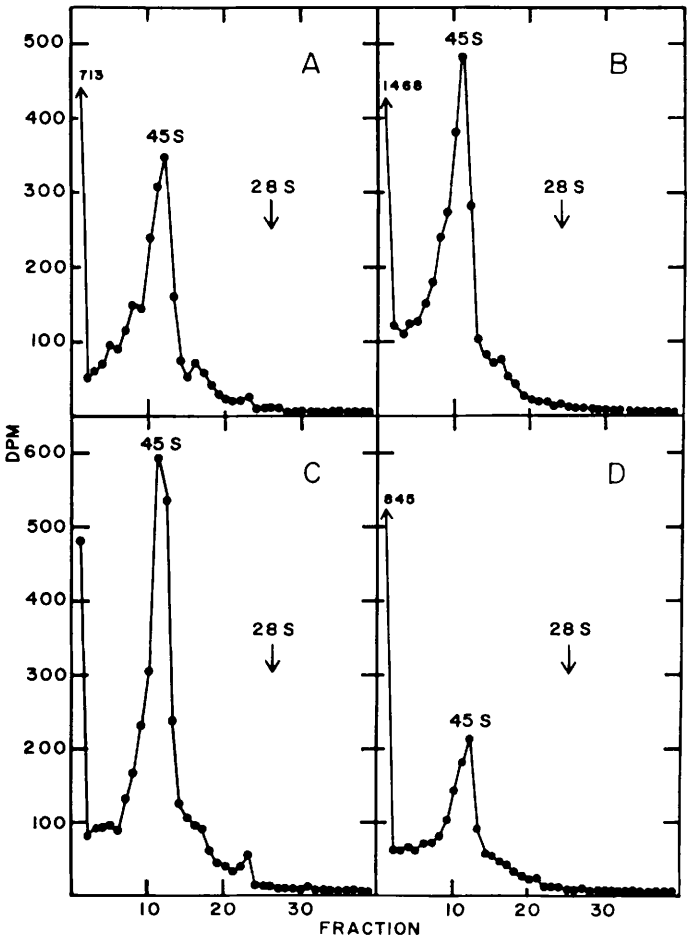

Chart 2. Inhibition of rRNA synthesis by FUrd. Cells were exposed to [^{3}H]guanosine for 15 min. Urd (*A* and *C*) or FUrd (*B* and *D*) was added either simultaneously with (*A* and *B*) or 1 hr before (*C* and *D*) the addition of the radioactive compound. RNA was extracted and analyzed by electrophoresis as described in the legend of Chart 1.

Table 1

Effect of FUrd on the incorporation of [8-^{3}H]guanosine into the acid-soluble fraction

Eight 25-ml aliquots from a stock culture containing 0.93×10^6 cells/ml were centrifuged, and the cell pellet derived from each aliquot was resuspended in 5 ml of fresh medium. Each experimental 5-ml culture was exposed to [^{3}H]guanosine (1 μCi/ml) for 15 min. In addition to the labeled precursor, either 1×10^{-4} M Urd or 1×10^{-4} M FUrd was added to each culture according to the protocol indicated below. At the end of the 15-min pulse, the amount of radioactivity in the acid-soluble fraction was determined.

Treatment	Preincubation (min)	cpm/10^6 cells
Urd	0	17,099 ± 114[a] (100)[b]
Urd	60	17,761 ± 112 (104)
FUrd	0	18,619 ± 219 (109)
FUrd	60	24,809 ± 927 (145)

[a] Mean ± the average deviation for values obtained from duplicate cultures.

[b] Numbers in parentheses, each value expressed as the percentage of the corresponding value obtained in the cultures that received Urd and [^{3}H]guanosine simultaneously.

Effect of FUrd on the Urd Nucleotide Pool. Identical cultures were incubated with either 1×10^{-4} M Urd or 1×10^{-4} M FUrd. Both cultures contained [^{3}H]Urd, 1.25 μCi/ml. After various periods of time, the acid-soluble pool was analyzed by paper chromatography. The results obtained are shown in Chart 3 and Table 2. The relative distribution of radioactivity among the various uridine nucleotides was not affected by the presence of FUrd. Approximately 75% of the radioactivity was in the form of UTP in each culture at all time points examined.

Effect of FUrd on the Maturation of 45 S rRNA Precursor Labeled in the Absence of Analog. Identical cultures were exposed to [^{3}H]Urd for 15 min. This pulse length allows label to be incorporated only into the 45 S rRNA precursor (Chart 2). At this point, one culture was made 1×10^{-4} M with respect to Urd and another was made 1×10^{-4} M with respect to FUrd. Both cultures received actinomycin D at the time of addition of the nucleoside to inhibit further RNA synthesis. Twenty min following the addition of actinomycin and unlabeled nucleoside, RNA was extracted and analyzed by gel electrophoresis. In both control (Chart 4*A*) and FUrd-treated (Chart 4*B*) cultures, essentially all of the 45 S molecules that had been labeled during the initial 15-min pulse were processed to 32 S, 28 S, or 18 S rRNA. Since nucleosides are transported into the cell and phosphorylated in the presence of actinomycin D (14), these data indicate that the presence of FUrd or its low-molecular-weight metabolites did not prevent the maturation of 45 S molecules synthesized prior to its addition.

These results are significantly different from those shown in Chart 4*D*, which were obtained from a culture in which 1×10^{-4} M FUrd was present together with the [^{3}H]Urd during the initial 15-min pulse. As before, actinomycin D was added 15 min after the addition of label and RNA was extracted 20 min after the addition of actinomycin D. The

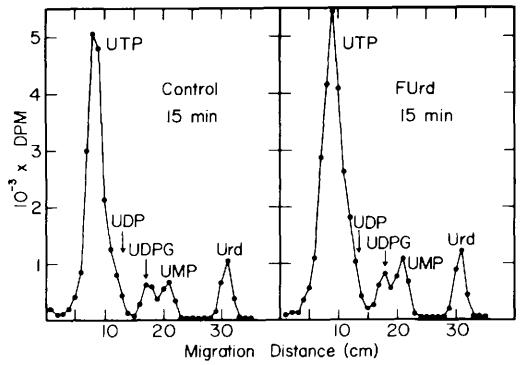

Chart 3. Effect of FUrd on the relative distribution of Urd nucleotides in the acid-soluble pool. Cells that had grown to a density of 1.38×10^6/ml were harvested and resuspended in one-fifth the original volume of fresh Medium S69. Identical cultures were then exposed to [^{3}H]Urd (1.25 μCi/ml) in the presence of 1×10^{-4} M Urd (control) or 1×10^{-4} M FUrd. After incubating for 15 min, the acid-soluble fraction was prepared and analyzed by paper chromatography.

Table 2

The effect of FUrd on the distribution of Urd nucleotides in the acid-soluble pool

Two 40-ml aliquots from a stock culture containing 1.38×10^6 cells/ml were centrifuged, and the cell pellet derived from each aliquot was resuspended in 8 ml of fresh medium. One culture was treated with 1×10^{-4} M Urd and the other with 1×10^{-4} M FUrd. Both cultures contained [^{3}H]Urd (1.25 μCi/ml). One-ml aliquots were removed after 15, 30, and 60 min of incubation. The trichloroacetic acid-soluble fraction was extracted with ether, lyophilized, and chromatographed. The amount of radioactivity associated with each nucleotide fraction is reported as the percentage of the total counts recovered from the chromatogram.

Treatment	Distribution of radioactivity			
	UTP + UDP	UDP glucose	UMP	Urd
		15 min		
Urd	77	8	6	9
FUrd	76	7	8	9
		30 min		
Urd	73	12	9	6
FUrd	71	14	8	7
		60 min		
Urd	78	4	13	5
FUrd	72	5	20	3

presence of FUrd during the initial labeling period resulted in the synthesis of 45 S rRNA precursor which was not converted to 32 S, 28 S, or 18 S rRNA during the subsequent actinomycin D treatment. In the control culture, which contained 1×10^{-4} M Urd during the initial pulse, all of the labeled 45 S rRNA precursor was converted to these more mature rRNA species during the 20-min actinomycin D treatment (Chart 4*C*).

Persistent Effects of FUrd on rRNA Maturation. Identical cultures were exposed to [^{3}H]guanosine in the presence of either 1×10^{-4} M Urd or 1×10^{-4} M FUrd for 2 hr. From each culture, an aliquot of cells was taken from which RNA was extracted and analyzed by electrophoresis (Chart 5, *A* and *D*). The cells in the remaining portion of each culture were harvested by centrifugation, washed twice, resuspended in fresh medium devoid of [^{3}H]guanosine or unlabeled nucleoside, and allowed to incubate for an additional 20 hr. Each culture was then incubated with [^{14}C]Urd in the absence or presence of actinomycin D, after which incubation RNA was extracted and analyzed by electrophoresis. After the initial 2-hr incubation period, [^{3}H]guanosine was

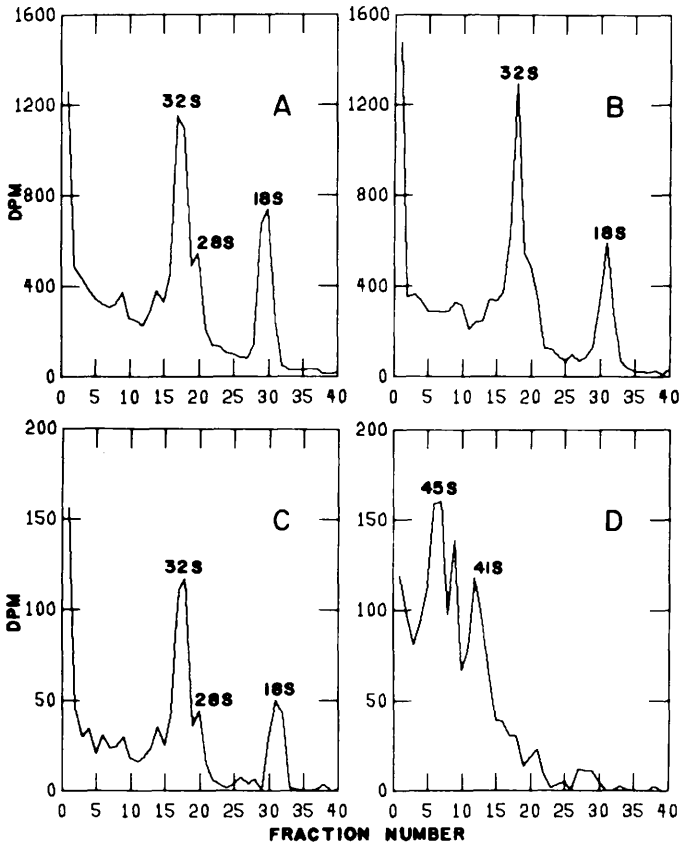

Chart 4. Effect of FUrd on the maturation of 45 S rRNA precursor during a 20-min treatment with actinomycin D. Novikoff cells were grown to a density of 1.14×10^6/ml, harvested, and resuspended in one-fifth the original volume of fresh Medium S69. Five ml of the resulting cell suspension were placed in each of 4 experimental flasks. At 0 time, 1 culture was treated with 1×10^{-4} M Urd (*C*) and another was treated with 1×10^{-4} M FUrd (*D*). All 4 cultures received [^{3}H]Urd (0.5 μCi/ml) at 0 time. Fifteen min following the addition of [^{3}H]Urd, actinomycin D (5 μg/ml) was added to each culture. At this time, in addition to the actinomycin D, 1 culture also received 1×10^{-4} M Urd (*A*) and another received 1×10^{-4} M FUrd (*B*). Twenty min after the addition of actinomycin D, RNA was extracted and analyzed by electrophoresis as described in the legend of Chart 1. A diagram of the experimental protocol is shown below.

Culture flask	[^{3}H]Urd	Actinomycin D	Extract RNA
A	↓	↓+ Urd	↓
B	↓	↓+ FUrd	↓
C	↓+ Urd	↓	↓
D	↓+ FUrd	↓	↓
	← 15 min →	← 20 min →	

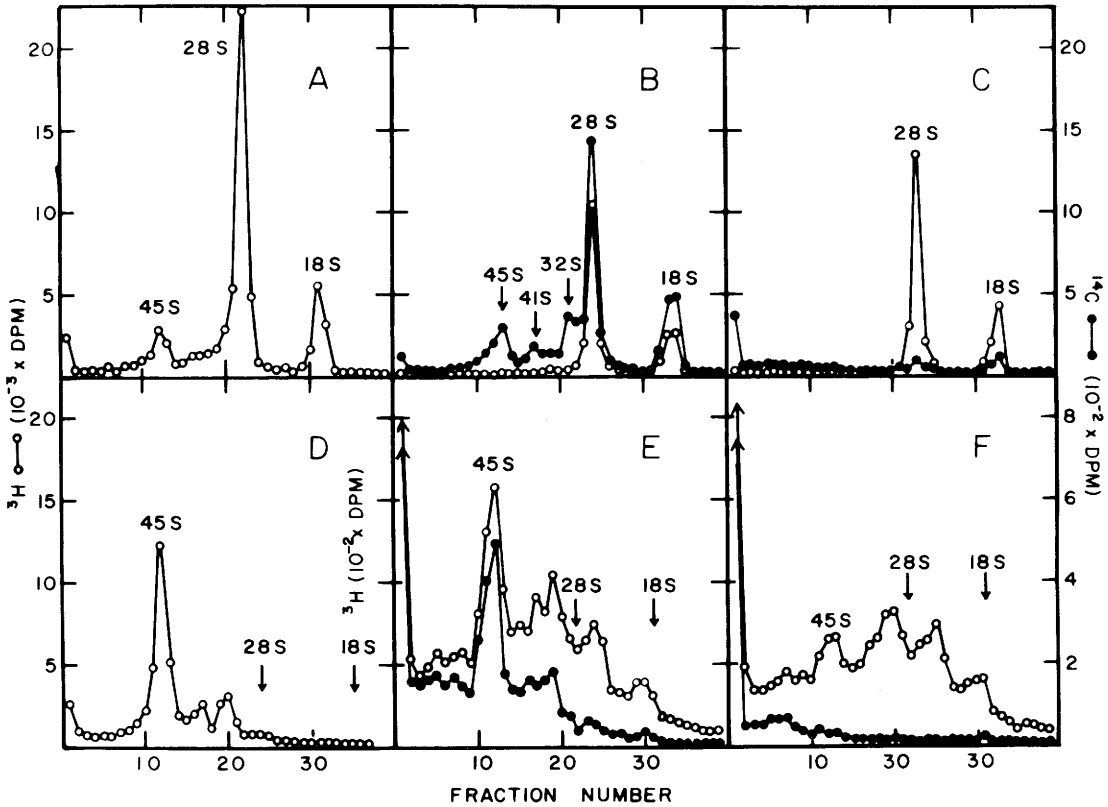

Chart 5. Persistent effects of FUrd on rRNA maturation. Two experimental cultures were prepared by harvesting cells from a stock culture containing 0.67×10^6 cells/ml and resuspending the cell pellets in one-fifth the original volume of fresh medium. One culture received 1×10^{-4} M Urd (*A* to *C*) and the other culture received 1×10^{-4} M FUrd (*D* to *F*). Both cultures received [^{3}H]guanosine (1 μCi/ml). After incubation for 2 hr, aliquots were taken from each culture for the extraction of RNA (*A* and *D*). Cells were then harvested by centrifugation from the remaining portions of each culture, washed twice in cold medium, and resuspended at a density of 0.67×10^6 cells/ml in medium devoid of [^{3}H]guanosine or unlabeled nucleosides. After incubating overnight, the cells in each culture were harvested by centrifugation and resuspended in one-fifth the original volume of fresh medium. Both cultures were divided into 2 equal portions. Exactly 20 hr following the removal of [^{3}H]guanosine from the medium, each culture received [^{14}C]Urd (0.1 μCi/ml) either in the presence (*C* and *F*) or absence (*B* and *E*) of actinomycin D (0.04 μg/ml). Two hr after the addition of [^{14}C]Urd (22 hr after the removal of [^{3}H]guanosine) RNA was extracted and analyzed by electrophoresis as described in the legend of Chart 1.

incorporated predominantly into mature 28 S and 18 S rRNA in the Urd-treated cultures (Chart 5*A*). Little or no [^{3}H]guanosine was incorporated into these mature rRNA species in the FUrd-treated cultures (Chart 5*D*). The tritium present in the RNA extracted from the Urd-treated cultures 22 hr after removal of [^{3}H]guanosine from the medium was found to reside almost exclusively in 28 S and 18 S rRNA (Chart 5*B*). No tritium was associated with the 45 S region. In contrast, significant amounts of tritium were still associated with the 45 S rRNA precursor in the FUrd-treated cells (Chart 5*E*). Furthermore, there was no distinct association of tritium with mature 18 S or 28 S rRNA in the analog-treated cultures. The significant decrease in the amount of tritium associated with the 45 S region observed after exposure of the FUrd-treated cells to actinomycin D (Chart 5*F*) indicates that the FUrd-substituted precursor undergoes metabolic turnover even in the absence of a normal maturation pathway. The actinomycin D treatment did not affect the distribution of tritium in RNA extracted from the Urd-treated cells (Chart 5*C*). This finding may be attributed to the relatively slow turnover of the mature 18 S and 28 S rRNA species.

Exposure of the cells to [^{14}C]Urd during the last 2 hr of incubation resulted in a ^{14}C profile that was strikingly similar to the tritium profile seen after the initial 2-hr exposure to [^{3}H]guanosine. This result demonstrates directly the continued ability of the Urd-treated cells to both transcribe and process rRNA (Chart 5*B*) and the persistent inhibition of rRNA processing in the FUrd-treated cells (Chart 5*E*). However, the FUrd-treated cells retained the ability to synthesize significant amounts of 45 S rRNA precursor. The lack of [^{14}C]Urd incorporation into 45 S rRNA precursor during the actinomycin D treatment in either the Urd-treated cultures (Chart 5*C*) or the FUrd-treated cultures (Chart 5*F*) verifies that the concentration of actinomycin D used was sufficient to inhibit new rRNA transcription.

DISCUSSION

FUrd is but 1 of a number of analogs which can inhibit the formation of mature rRNA (3, 13, 15, 16, 18, 20, 21). It has been postulated (22, 25) that the ability of analogs to inhibit rRNA maturation may be related to their ability to be incorporated into the 45 S rRNA precursor and the extent to which that incorporation alters the physiocochemi-

cal characteristics of that molecule. Recently, Weiss and Pitot (22) demonstrated that the incorporation of 5-azacytidine into the 45 S and 32 S rRNA precursors alters their secondary structure in such a way that the analog-substituted molecules possess a decreased electrophoretic mobility. If incorporation is the basis of their inhibitory effect, there should be an obligatory requirement for RNA synthesis in the presence of the analogs. The data shown in Chart 4 suggest that the inhibition of rRNA maturation by FUrd does depend upon the incorporation of the analog into 45 S rRNA precursor. Precursor molecules synthesized before the addition of FUrd were processed normally. In contrast, certain intercalating agents inhibit the maturation of normal 45 S rRNA precursor (17). However, these compounds can bind directly to double-helical regions of RNA, thereby disrupting its secondary structure.

Wellauer and Dawid (23) have shown with the electron microscope that HeLa cell 45 S rRNA precursor contains a series of hairpin loops. Formaldehyde denaturation studies indicate that base pairing is responsible for loop formation. It is possible that the arrangement of loops is one of the signals that the maturation enzymes recognize. Anything that interferes with base pairing, such as analog substitution, could alter the loop morphology, thereby altering the maturation process. Our results are consistent with the hypothesis that FUrd incorporation disrupts the physicochemical characteristics of the precursor molecule, rendering it unfit for proper processing. The concentration dependence of the inhibition by FUrd indicates that a certain degree of substitution may be necessary to elicit the effect.

In addition to its ability to inhibit maturation, FUrd also inhibits the transcription of rRNA. However, this effect of the drug is much less complete and does not manifest itself as rapidly as the effect on maturation. Therefore, it may be an indirect consequence of impaired maturation, whereby inhibited processing of 45 S precursor decreases the synthesis of new 45 S molecules. Studies on the acid-soluble fraction indicate that impaired nucleoside transport or uridine nucleotide metabolism is not responsible for the inhibition of 45 S rRNA synthesis by FUrd.

There is no significant accumulation of 45 S rRNA precursor in the presence of FUrd, despite the continuous synthesis and impaired processing. This implies that nonspecific nucleases are constantly degrading the fraudulent RNA or that normal cleavage enzymes are breaking down the analog-substituted RNA in a nonspecific fashion. The nonproductive degradation of FUrd-substituted 45 S rRNA precursor in the presence of actinomycin D supports this hypothesis.

The inhibition of rRNA maturation by FUrd persisted even after prolonged incubations following the removal of the drug from the medium. This finding is probably related to the fact that the disappearance of FUrd nucleotides from the acid-soluble fraction is extremely slow (unpublished data). This persistent effect on the formation of ribosomes must be taken into account when considering the overall cytotoxic activity of FUrd. However, the inhibition of rRNA maturation is probably not the primary site of action of all of the fluorinated pyrimidines. We have found, for example, that even after preincubation with the drug for 2 hr 1×10^{-4} M fluorodeoxyuridine had no effect on rRNA maturation. Essentially all of the cell-killing activity of this compound is probably related to the inhibition of DNA synthesis. One must also be cautious about extrapolating from our results with FUrd in one strain of Novikoff cells to other cell lines. As suggested previously by Umeda and Heidelberger (19), the exact mechanism of toxicity may well vary according to the cell line and culturing conditions. For example, 1×10^{-7} M FUrd, which had no effect on rRNA maturation in our study, was sufficient to prevent the growth of a fluorodeoxyuridine-sensitive Novikoff cell line (19); but 1×10^{-4} M FUrd, which completely inhibited rRNA maturation in our system, was required to suppress completely the growth of a flurodeoxyuridine-resistant Novikoff cell line. In the sensitive cells, the inhibition of DNA synthesis may have been the critical site of inhibition, while in the relatively resistant cells, the inhibition of rRNA maturation may have been the critical site.

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The Inhibition of Ribosomal RNA Synthesis and Maturation in Novikoff Hepatoma Cells by 5-Fluorouridine

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