STABILIZATION OF HETEROGENEOUS NUCLEAR RNA BY INTERCALATING DRUGS

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SUMMARY: The effect of the intercalating drugs proflavine, ethidium and daunomycin on the rate of degradation of ^{14}C-labeled heterogeneous nuclear RNA (HnRNA) in KB cells was studied. All three drugs decreased the rate of degradation of ^{14}C-HnRNA to acid soluble products. The most striking effect was produced by proflavine which promptly and completely stabilized ^{14}C-HnRNA against degradation. Ethidium also produced complete stabilization after a delay of 30 to 60 min. Daunomycin decreased the rate of ^{14}C-HnRNA degradation but did not alter the fraction of ^{14}C-HnRNA which was ultimately degraded. The results are consistent with the view that base-paired sequences are present in HnRNA *in vivo* and play a role in the processing of HnRNA.

Several years ago a class of high molecular weight, heterogeneous nuclear RNA (HnRNA) distinct from 45 s ribosomal precursor RNA (pre-rRNA) was reported in mammalian cells (1,2). Although most HnRNA appears to be rapidly degraded without ever leaving the nucleus (3,4), recent studies suggest that portions of at least some HnRNA molecules serve as cytoplasmic messenger RNA (mRNA) (5-9). This implies that mechanisms exist for selecting those portions of an HnRNA molecule which are to function as mRNA while degrading the remainder of the HnRNA molecule. It follows, therefore, that HnRNA must contain specific cleavage sites which can be recognized by cellular nucleases.

Recently Snyder et al. (10) have shown that intercalating drugs can inhibit the processing of 45 s pre-rRNA into cytoplasmic rRNA. They suggested that the action of the intercalating drugs was due to binding by intercalation at hypothetical base-paired segments in the 45 s RNA molecule and proposed that such base-paired segments would then have to be at the sites of the specific cleavage of 45 s pre-rRNA. The recent report by Jelinek and Darnell (11) and by Ryckov

et al. (12) that HnRNA contains double-stranded regions which are absent from cytoplasmic mRNA suggests the possibility that base-paired regions might serve as specific recognition sites for cleavage of HnRNA. Consistent with this possibility we now report that intercalating drugs inhibit the degradation of HnRNA.

METHODS

All experiments were carried out with KB cells grown in suspension culture at 37° as described previously (13).

To determine the amount of ^{14}C-labeled acid-precipitable RNA remaining in pre-labeled KB cells after treatment with various drugs, a 5 ml sample of the culture was withdrawn at various times and rapidly chilled by dilution with 25 ml of cold (0-4°) 0.15 M NaCl - 0.01 M sodium phosphate, pH 7.2. The cell sample was then centrifuged 10 min at 160 x g and the cell pellet obtained was frozen at -20° until used. The frozen pellet when used was treated 30 min with 5 ml of cold 5% trichloroacetic acid, centrifuged and the sediment washed two additional times with 5 ml of cold 5% trichloroacetic acid. The sediment was then dissolved in 0.5 ml of 1 N NaOH and incubated 16 h at 37°. The solution was then neutralized with 0.5 ml of 1 N HCl and acidified by the addition of 1 ml of cold 10% trichloroacetic acid. The precipitate which formed after 30 min was removed by centrifugation and 1 ml of the supernatant solution containing the hydrolized RNA was counted in a liquid scintillation counter.

RESULTS

Effect of intercalating drugs on HnRNA degradation - The addition of high levels of actinomycin D to cells which have been pre-labeled with ^{14}C-uridine results in rapid degradation of a portion of the ^{14}C-labeled RNA to acid-soluble products. This degradation is accounted for, in part, by the nonconservative conversion of 45 s pre-rRNA to the 28 s and 18 s mature species of rRNA (14) and, in part, by the rapid degradation of a fraction of the ^{14}C-labeled HnRNA (4). Fig. 1A shows the degradation pattern of ^{14}C-labeled RNA observed after the addition of either actinomycin D alone or actinomycin D plus one of three different intercalating drugs to suspension cultures of KB cells. The degradation pattern seen in the

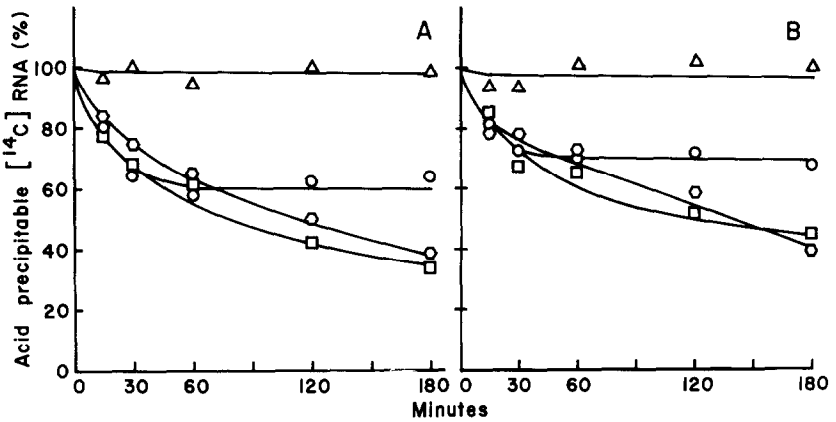

Fig. 1. Effect of intercalating drugs on ^{14}C-RNA degradation.

A. Four 50 ml cultures of KB cells (270 x 10^3 cells/ml) were each labeled 30 min with 0.15 ml of ^{14}C-uridine (2 μC/ml, 0.8 μmole/ml). At the time designated zero time on the figure, actinomycin alone or actinomycin plus an intercalating drug were added to each culture. Samples were withdrawn at various times and assayed for acid-precipitable ^{14}C-RNA as described in Methods. The zero time value (996 cpm) is normalized to 100%.

B. Four 50 ml cultures of KB cells (305 x 10^3 cells/ml) were each incubated 25 min with 0.04 μg/ml of actinomycin. The cultures were then labeled 30 min with ^{14}C-uridine and treated with actinomycin alone or actinomycin plus an intercalating drug as described in (A). The zero time value (540 cpm) is normalized to 100%.

□, actinomycin (4 μg/ml); ○, actinomycin (4 μg/ml) plus daunomycin (10 μg/ml); ○, actinomycin (4 μg/ml) plus ethidium bromide (500 μM); △, actinomycin (4 μg/ml) plus proflavine (80 μM).

presence of actinomycin D alone is similar to that previously reported by other workers. All three intercalating drugs tested affected this degradation pattern to some extent. The most striking effect was produced by proflavine which rapidly and completely stabilized the ^{14}C-labeled RNA against degradation. The addition of ethidium also resulted in complete stabilization but only after a delay of 30 to 60 minutes. Daunomycin, on the other hand, decreased the rate of ^{14}C-RNA degradation but did not alter the fraction of RNA which was ultimately degraded. These results are qualitatively similar to those obtained by Snyder, et al. (10) in their study of the effect of intercalating drugs on the processing of 45 s pre-rRNA. Since approximately one third of the ^{14}C-uridine incorporated into RNA during the 30-minute labeling period used in our experiments is present in

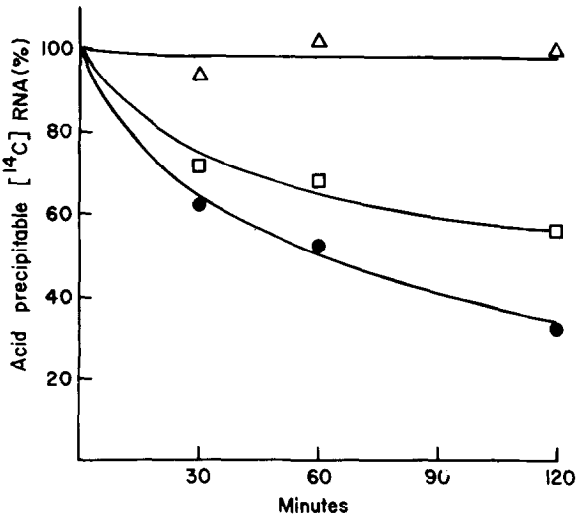

Fig. 2. Effect of cycloheximide on ^{14}C-RNA degradation. Three 40 ml cultures of KB cells (200 x 10^3 cells/ml) were each incubated 25 min with 0.04 μg/ml of actinomycin. The cultures were then labeled 30 min with 0.15 ml of ^{14}C-uridine (2 μC/ml, 0.8 μmoles/ml). At the time designated zero time on the figure, actinomycin alone or actinomycin plus proflavine or actinomycin plus cycloheximide were added to each culture. Samples were withdrawn at various times and assayed for acid-precipitable RNA as described in Methods. The zero time value (353 cpm) is normalized to 100%.

□, actinomycin (4 μg/ml); ●, actinomycin (4 μg/ml) plus cycloheximide (20 μg/ml); △, actinomycin (4 μg/ml) plus proflavine (80 μM).

HnRNA (15), however, our results suggest that the stabilization of pre-labeled RNA is not limited to pre-rRNA.

To test this possibility more directly we studied the effect of intercalating drugs on the degradation pattern of RNA labeled in the presence of low levels (0.04 μg/ml) of actinomycin D. Since this concentration of actinomycin D selectively inhibits the synthesis of 45 s pre-rRNA (4,16), most of the ^{14}C-uridine incorporated into RNA under these conditions is present in HnRNA. The results of this experiment are shown in Fig. 1B where it can be seen that the degradation patterns obtained are virtually identical to those seen in Fig. 1A. Once again proflavine elicited a prompt and complete stabilization of the pre-labeled RNA while ethidium and daunomycin produced less extensive effects. We conclude from these results that intercalating drugs in general, and proflavine in particular, decrease the rate of degradation of HnRNA.

Effect of cycloheximide on HnRNA degradation - In addition to its other properties, proflavine is known to inhibit protein synthesis (17). Inhibition of protein synthesis by cycloheximide has been reported to inhibit the processing of 45 s pre-rRNA in HeLa cells (18,19). It is possible, therefore, that proflavine inhibits the degradation of HnRNA as a consequence of its ability to inhibit protein synthesis. We examined this possibility by testing the effect of cycloheximide on the rate of degradation of ^{14}C-labeled HnRNA. The results of this experiment are shown in Fig. 2. Cycloheximide at a concentration sufficient to inhibit protein synthesis by 94% increased rather than decreased the rate of ^{14}C-labeled HnRNA degradation. We conclude, therefore, that the stabilization of HnRNA elicited by proflavine and other intercalating drugs is not a secondary consequence of protein synthesis inhibition.

DISCUSSION

Our results demonstrate that the drugs proflavine, ethidium and daunomycin, which are all thought to intercalate between base pairs in helical regions of DNA (20-22), decrease the rate of degradation of HnRNA to acid-soluble products. Since cycloheximide does not cause a similar effect, this result cannot be attributed to the inhibition of protein synthesis by these drugs. Of the 3 drugs tested, proflavine is by far the most effective, eliciting a prompt and complete stabilization of HnRNA. Ethidium also elicits complete stabilization of HnRNA but only after a delay of 30 to 60 minutes. However, the fact that ethidium also inhibits RNA synthesis more slowly than proflavine (personal observation) suggests that this delay may simply reflect a low rate of either cell entry or intercalation by ethidium. Daunomycin, on the other hand, is clearly less effective in stabilizing HnRNA than either proflavine or ethidium. In this regard, it is interesting that proflavine and ethidium also inhibit the processing of 45 s pre-rRNA while daunomycin is totally ineffective. Snyder et al. (10) have suggested that the exceptional behavior of daunomycin may be related to the fact that it carries essential glycosidic side-chains which proflavine and ethidium do not.

Recently it has been shown that purified HnRNA contains intramolecular base-

paired sequences of sufficient length to exhibit resistance to pancreatic and T1 ribonuclease (11,12). The base-paired sequences are found in the larger species of HnRNA (>35 s) and are absent from smaller species of HnRNA as well as from cytoplasmic mRNA. Ryskov et al. (12) have suggested, on the basis of these observations, that these sequences are related to nucleus-restricted HnRNA sequences which are destroyed in the course of processing giant precursor molecules. Jelinek and Darnell (11), however, have cautioned that there is at the moment no proof that these intromolecular base-paired sequences exist as such inside the cell. They may exist only in purified RNA preparations. Our observation that intercalating drugs inhibit the degradation of HnRNA is consistent with the view that base-paired sequences are present in HnRNA *in vivo*. In addition, our results raise the possibility that such regions may serve as recognition sites for cleavage and subsequent degradation of HnRNA. Although we have no direct evidence that the stabilization effects we have observed are, in fact, the result of intercalation of the drugs into base-paired regions of HnRNA, the fact that both proflavine and ethidium are known to bind to double-stranded regions of RNA (23-28) makes this possibility attractive.

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