proves to have no great biological significance, then other substrates might be sought to clarify the function of this glycosylase.

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References

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Brent, T. P. (1975) *Biochim. Biophys. Acta 407*, 191–199.
Brent, T. P. (1976) *Biochim. Biophys. Acta 454*, 172–183.
Brent, T. P. (1977) *Nucleic Acids Res. 4*, 2445–2454.
Cerutti, P. A. (1975) in *Molecular Mechanisms for Repair of DNA* (Hanawalt, P. C., & Setlow, R. B., Eds.) p 3–12, Plenum Press, New York.
Espejo, T. R., & Canelo, E. S. (1968) *Virology 34*, 738–747.
Espejo, R. T., Canelo, E. S., & Sinsheimer, R. L. (1969) *Proc. Natl. Acad. Sci. U.S.A. 63*, 1164–1168.
Friedberg, E. C., Ganesan, A. K., & Minton, K. (1975) *J. Virol. 16*, 315–321.
Goth-Goldstein, R. (1977) *Nature (London) 267*, 81–82.
Kirtikar, D. M., & Goldthwait, D. A. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 2022–2026.
Laval, J. (1977) *Nature (London) 269*, 829–832.
Lawley, P. D., & Orr, D. J. (1970) *Chem.-Biol. Interact. 2*, 154–157.
Lawley, P. D., & Warren, W. (1975) *Chem.-Biol. Interact. 11*, 55–57.
Lawley, P. D., & Warren, W. (1976) *Chem.-Biol. Interact. 12*, 211–220.
Lindahl, T. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3649–3653.
Margison, G. P., & O'Connor, P. J. (1973) *Biochim. Biophys. Acta 331*, 349–356.
Riazuddin, S., & Lindahl, T. (1978) *Biochemistry 17*, 2110–2118.
Schaller, H., Nusslein, C., Bonhoeffer, F. J., Kurtz, F. J., & Neitzman, I. (1972) *Eur. J. Biochem. 26*, 474–481.
Sekiguchi, M., Hayakawa, H., Makino, F., Tanaka, K., & Okada, Y. (1976) *Biochem. Biophys. Res. Commun. 73*, 293–299.
Teebor, G., Goldstein, M., Frenkel, K., Duker, N., & Brent, T. P. (1978) in *DNA Repair Mechanisms* (Hanawalt, P. C., & Friedberg, E. C., Eds.) Academic Press, New York (in press).
Walker, I. G., & Ewert, D. F. (1973) *Mutat. Res. 19*, 331–341.

Analysis of the Message-Sequence Content of the Pulse-Labeled Poly(A)+ Heterogeneous Nuclear RNA from HeLa Cells by cDNA-Excess Hybridizations†

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ABSTRACT: The message-sequence content of pulse-labeled poly(A)+ HeLa heterogeneous nuclear RNA (hnRNA) has been examined by hybridizations to an excess of message cDNA. Control experiments show that the message cDNA accurately reflects the sequence distribution of the complex mixture of poly(A)+ messages present in the HeLa cytoplasm. Pulse-labeled poly(A)+ molecules in both the lamina-associated and shnRNA fractions contain message sequences, and approximately 65% of the poly(A)-adjacent hnRNA sequences are homologous to the 3′ ends of mRNA. The majority of the pulse-labeled hnRNA molecules contain abundant message sequences. By use of these techniques it is also shown that some pulse-labeled polyadenylated message sequences are still synthesized in the presence of the adenosine analogue 5,6-dichloro-β-D-ribofuranosylbenzimidazole under conditions where little or no new cytoplasmic mRNA is produced.

The synthesis of heterogeneous nuclear RNA (hnRNA)[1] and its relationship to cytoplasmic messenger RNA (mRNA) have been studied for many years (for reviews see Lewin, 1975a,b; Perry, 1976). While the metabolism of hnRNA is quite complex and only a small portion is exported to the cytoplasm (Perry et al., 1974; Herman & Penman, 1977), several lines of evidence suggest that mRNA is derived from the nuclear transcripts. Various experiments have shown that hnRNA is posttranscriptionally modified at both the 5′ and 3′ termini and these modified structures can be found in cytoplasmic mRNA (reviewed by Perry, 1976). Hybridization experiments have shown that steady-state poly(A)+ hnRNA contains the sequences for poly(A)+ mRNA (Herman et al., 1976; Levy et al., 1976; Jacquet et al., 1978). In addition, nuclear precursors, larger than the mature products, have been identified for several specific cellular messages (Ross et al., 1976; Curtis & Weissmann, 1976; Kwan et al., 1977; Bastos & Aviv, 1977; Gilmore-Herbert & Wall, 1978).

In HeLa cells two distinct subfractions of hnRNA have been observed. When isolated HeLa nuclei are exposed to high ionic strength ammonium sulfate, 10% of the mass of the pulse-labeled hnRNA is eluted (shnRNA) while the other 90% remains firmly attached to the nuclear lamina (Price et al., 1974). These two fractions differ both in their sedimentation profile and in their metabolic stability (Price et al., 1974;

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[1] Abbreviations used: hnRNA, heterogeneous nuclear RNA; shnRNA, small heterogeneous nuclear RNA subfraction; C_0t, the product of the concentration times the time of incubation; cDNA, complementary DNA; EDTA, ethylenediaminetetraacetate; $NaDodSO_4$, sodium dodecyl sulfate; DRB, 5,6-dichloro-β-D-ribofuranosylbenzimidazole.

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Herman & Penman, 1977). The nature of the relationship between the two fractions and with messenger RNA is not fully understood at the present time.

The use of certain drugs which inhibit the synthesis or posttranscriptional modification of hnRNA has provided additional information about the conversion of nuclear RNA into cytoplasmic species. The adenosine analogue 5,6-dichloro-β-D-ribofuranosylbenzimidazole (DRB) has been shown to inhibit the synthesis of hnRNA in eukaryotic cells from widely divergent origins (Egyházi et al., 1970; Egyházi, 1974, 1975; Granick, 1975; Sehgal et al., 1976). While it was originally believed that DRB acts by inhibiting the initiation of hnRNA synthesis, recent experiments suggest an alternative mode of action (Fraser et al., 1978). Nevertheless, DRB treatment reduces hnRNA synthesis by 65–70% and mRNA synthesis by more than 95% in both HeLa and L cells. Since posttranscriptional polyadenylation does not appear to be directly affected, it has been proposed that DRB selectively inhibits the synthesis of the true precursors to mRNA (Sehgal et al., 1976).

The technique of complementary DNA (cDNA) excess hybridizations has been used here to determine the sequence content of pulse-labeled hnRNA and to examine the effects of DRB on the synthesis of nuclear molecules containing message sequences. While it is shown that message sequences are still synthesized in the presence of DRB, their synthesis appears to be relatively more sensitive than is the synthesis of the total hnRNA population. Furthermore, it is suggested that the poly(A)+ hnRNA synthesized in the presence of DRB is deficient in scarce message sequences.

Materials and Methods

(*a*) *Cell Culture.* HeLa S3 cells were grown in suspension culture at 37 °C in Joklik's modified minimum essential medium supplemented with 7% horse serum (Microbiological Associates). Density was maintained at 4×10^5 cells/mL.

(*b*) *Labeling and Fractionation.* Cells were concentrated 10-fold into 50% fresh and 50% conditioned media and treated at 37 °C with actinomycin D (Calbiochem) (0.04 μg/mL) for 30 min to suppress nucleolar RNA synthesis. When used, DRB was added to a final concentration of 75 μM 30 min prior to the addition of the radioactive precursors. The cells were pulse-labeled for 15 min to label hnRNA or 90 min to label mRNA with [^{3}H]uridine, [^{3}H]cytidine, and [^{3}H]guanosine (New England Nuclear), each at 100 μCi/mL. Incorporation was stopped by pouring the cells over crushed frozen Earle's saline. The cells were fractionated as described previously (Herman et al., 1976; Herman & Penman, 1977). The shnRNA fraction was released from the detergent-washed nuclei by the procedure of Price et al. (1974).

(*c*) *RNA Extraction and Purification.* RNA was extracted from nuclear and cytoplasmic fractions by the phenol–chloroform method (Singer & Penman, 1973; Herman & Penman, 1977). Purified hnRNA was DNase 1 (Worthington) treated as described by Herman et al. (1976). Alkaline cleavage and affinity chromatography on either oligo(dT)-cellulose (Collaborative Research) or poly(U)-Sepharose (Pharmacia) were performed according to published procedures (Singer & Penman, 1973; Molloy et al., 1974; Herman et al., 1976).

(*d*) *Synthesis of cDNA.* cDNA was synthesized in microgram quantities in a 1.4-mL reaction mixture containing (final concentrations) dCTP, dATP, dGTP, and dTTP (each at 350 μM); ATP (500 μM); Tris-HCl, pH 8.3 (70 mM); dithiothreitol (14 μM), magnesium acetate (8 mM); oligo-$(dT)_{10}$ (35 μg/mL); bovine serum albumin (35 μg/mL); poly(A)+ HeLa mRNA (360 μg/mL); actinomycin D (100 μg/mL); [^{14}C]dCTP (5 μCi; Amersham/Searle); and reverse transcriptase (Life Sciences Inc.; 350 units/mL). The mixture was incubated at 43 °C for 3 h in a siliconized glass tube. $NaDodSO_4$ was added to a final concentration of 0.5% to terminate the reaction. The cDNA was purified as described (Milcarek et al., 1974). The cDNA was estimated to be 600–700 nucleotides long by sedimentation in alkaline sucrose (Williams & Penman, 1975) by use of the 500 nucleotide Bg12-HpA1 SV40 DNA restriction fragment (provided by Dr. B. Roberts) as marker.

(*e*) *cDNA-Excess Hybridizations.* Cleaved poly(A)+ RNA fragments sedimenting at about 5–9 S in a 15–30% sucrose gradient were mixed with an excess of cDNA in 0.24 M sodium phosphate (equimolar) containing 0.2% $NaDodSO_4$ and 2 mM EDTA. Aliquots (0.2–1 μL) were sealed in siliconized glass capillaries. The capillaries were boiled for 5 min and then incubated at 70 °C. At various times the contents of a capillary were expelled into 1.1 mL of 0.3 M NaCl, 0.01 M Tris-HCl, pH 7.4. Two 0.5-mL portions were removed and pancreatic RNase A (Worthington) was added to one to a final concentration of 20 μg/mL. Both portions were incubated at 37 °C for 30 min. Acid-insoluble material was collected on Millipore filters. The data were corrected for background (usually less than 5%) by subtraction of the fraction of each RNA preparation which was resistant to digestion in the absence of cDNA. The concentration of the cDNA was determined from the specific activity of the dCTP in the reaction mixture. For this estimation it was assumed that dCMP constituted 25% of the nucleotides incorporated into cDNA. We calculated mRNA concentrations by assuming that poly(A)+ mRNA comprises 2.5–3% of the total cytoplasmic RNA. hnRNA concentrations were calculated on the assumption that 10^8 HeLa nuclei contain 25 μg of hnRNA (Pederson, 1974).

Results

Much useful information has been obtained from experiments in which a cDNA probe of high specific activity was hybridized to a large excess of unlabeled RNA or DNA. Unfortunately, this type of experiment, in general, only permits the examination of *steady-state* populations. The availability of cDNA in amounts sufficient to drive hybridizations would make it possible to characterize pulse-labeled as well as steady-state RNA. To date, large amounts of cDNA have been obtained for only the most abundant messages such as globin. Recently, 75 μg of cDNA was synthesized from 500 μg of HeLa poly(A)+ cytoplasmic RNA (see Materials and Methods), and this cDNA has been used to drive hybridizations with both pulse-labeled mRNA and hnRNA.

The ability of the message cDNA to drive hybridizations is shown in Figure 1 by the annealment of 90-min pulse-labeled poly(A)+ HeLa mRNA to a 40-fold excess of cDNA. Since the cDNA was estimated to be complementary to only the 600–700 nucleotide sequence at the 3′ end of the message, the pulse-labeled mRNA was alkaline-cleaved (Molloy et al., 1974; Herman et al., 1976) into fragments of this size and those containing poly(A) selected by affinity chromatography (Singer & Penman, 1973). Hybridizations between the pulse-labeled poly(A)+ RNA fragments and an excess of message cDNA were performed in 0.24 M phosphate buffer at 70 °C (Williams & Penman, 1975). At saturation 70% of the pulse-labeled poly(A)+ mRNA fragments entered RNase A resistant hybrids (Figure 1). This result indicates that the cDNA is capable of driving a significant portion of the input complementary sequences into hybrid molecules. The kinetics of hybridization are virtually identical with those previously

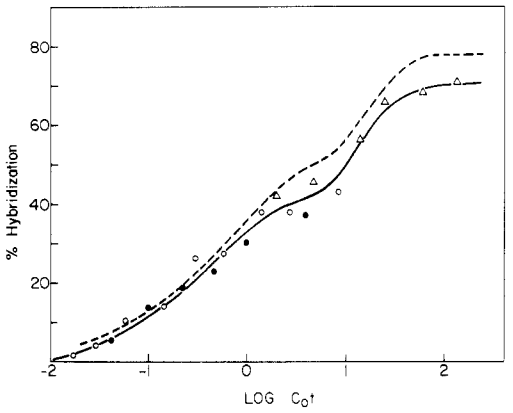

FIGURE 1: Hybridization of pulse-labeled poly(A)+ mRNA. HeLa cells were concentrated 10-fold and incubated for 30 min with actinomycin D (0.04 μg/mL). The cells were then pulse-labeled for 90 min with [^{3}H]uridine, [^{3}H]cytidine, and [^{3}H]guanosine (each at 100 μCi/mL). mRNA was isolated and alkaline-cleaved. Poly(A)+ fragments were isolated by affinity chromatography on oligo-(dT)-cellulose. Hybridizations were performed and assayed as outlined under Materials and Methods. cDNA concentration: (○, ●) 0.030 mg/mL, fivefold weight excess over mRNA; (△) 0.388 mg/mL, 40-fold excess. Concentrations and excesses were calculated as described under Materials and Methods. The dashed line shows the hybridization of high specific activity message cDNA to a vast excess of steady-state poly(A)+ HeLa mRNA. (Data reproduced from Herman et al., 1976.)

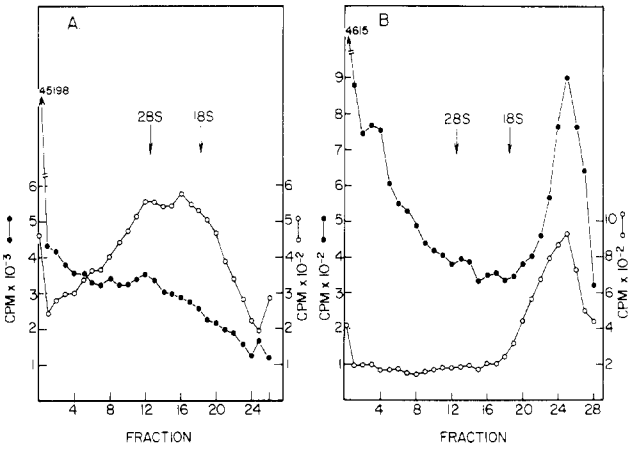

FIGURE 2: Sedimentation distribution of hnRNA. Cultures of 4 × 10^7 cells were concentrated 10-fold and treated with actinomycin (0.04 μg/mL) for 30 min at 37 °C (A) and for an additional 30 min with 75 μM DRB (B). They were then pulse-labeled for 15 min as described in the legend to Figure 1. Nuclei were isolated and exposed to 0.4 M ammonium sulfate. hnRNA was sedimented at 22000 rpm in the SW41 rotor at 25 °C for 15 h in 15–30% sucrose in 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.4, 1 mM EDTA, 0.5% $NaDodSO_4$. (A) One percent of each fraction sedimented. (B) One percent of the unreleased (nuclear lamina bound) and 20% of the shnRNA fraction sedimented. Acid-precipitable radioactivity is plotted. (●) Unreleased hnRNA; (○) shnRNA.

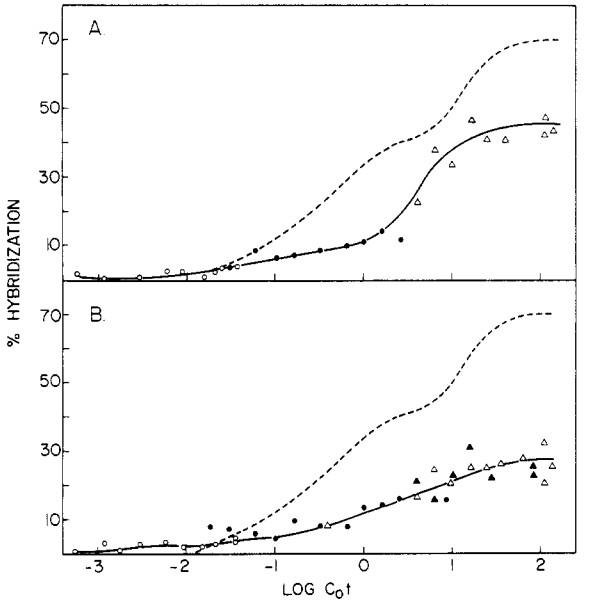

FIGURE 3: Hybridization of pulse-labeled hnRNA. The hnRNA was labeled and isolated as described in the legend to Figure 2. After alkaline cleavage the poly(A)-containing fragments were isolated by affinity chromatography on poly(U)-Sepharose. Hybridizations were performed as described under Materials and Methods. (A) Nuclear lamina bound poly(A)+ hnRNA. cDNA concentration: (○) 0.003 mg/mL, sixfold excess; (●) 0.030 mg/mL, 40-fold excess; (△) 0.388 mg/mL, 265-fold excess. Different symbols also indicate different hnRNA preparations. (B) Poly(A)+ shnRNA. cDNA concentration: (○) 0.003 mg/mL, sixfold excess; (●) 0.030 mg/mL, 50-fold excess; (▲) 0.291 mg/mL, 230-fold excess; (△) 0.388 mg/mL, 350-fold excess. Different symbols also indicate different shnRNA preparations. The dashed lines show the hybridization of pulse-labeled poly(A)+ mRNA to an excess of message cDNA. (Data reproduced from Figure 1.)

obtained for the hybridization of high specific activity message cDNA to a large excess of steady-state poly(A)+ HeLa mRNA (Williams & Penman, 1975; Herman et al., 1976). This clearly demonstrates that the cDNA accurately reflects the sequence distribution of the complex mRNA population from which it was transcribed even though the sequences are not all present at the same relative abundance (Bishop et al., 1974). It can also be concluded that the pulse-labeled (short-lived) poly(A)+ HeLa mRNA consists of abundant and scarce message sequences in the same proportions as are present at steady state. (For additional discussion of this point, see Lenk et al., 1978.) The data presented above show that cDNA-driven hybridizations can be used to determine the message-sequence distribution in a pulse-labeled mRNA population and should also provide a way to examine pulse-labeled hnRNA.

HeLa cells were pulse-labeled for 15 min in the presence of a low concentration of actinomycin D which selectively inhibits nucleolar RNA synthesis (Penman et al., 1968). The nuclei were isolated as described previously (Herman et al., 1976) and then exposed to 0.4 M ammonium sulfate to elute the shnRNA fraction (Price et al., 1974). The sedimentation profile of the RNA purified by phenol extraction from the two fractions is shown in Figure 2A. Under nondenaturing conditions the vast majority of the hnRNA is quite large and sediments to the bottom of the gradient. In contrast, the shnRNA fraction sediments heterogeneously as a broad peak of only 18–28 S.

About 20–30% of the pulse-labeled hnRNA in HeLa cells contains a poly(A) segment at the 3′ end of the molecule which is about 200 AMP residues in length (Derman & Darnell, 1974). A brief exposure to alkaline pH was used to cleave the hnRNA into 600–700 nucleotide fragments, and those containing poly(A) were isolated by affinity chromatography on poly(U)-Sepharose. Fragments containing the internal oligo(A) stretches were eluted from the column with 5% formamide while those containing the 3′ poly(A) were eluted with 90% formamide (Molloy et al., 1974; Herman et al., 1976).

The hybridization of the pulse-labeled poly(A)+ hnRNA fragments isolated from the unreleased fraction is shown in Figure 3. Even when annealed in the presence of a 265-fold excess of message cDNA, only 45% of the poly(A)-adjacent sequences were driven into hybrid molecules. In contrast, 70% of the pulse-labeled poly(A)+ mRNA formed hydrids at

saturation when annealed with a 40-fold excess of cDNA. Thus, only about 65% (45/70) of the pulse-labeled poly(A)-adjacent sequences in this hnRNA fraction are homologous to the 3′ ends of poly(A)+ mRNA. A similar result was obtained for steady-state HeLa hnRNA by use of RNA-excess hybridization techniques (Herman et al., 1976). Of the pulse-labeled hnRNA which does contain message sequences, 60–65% anneals by log C_0t = 0.7 and therefore contains abundant message sequences while the remaining 35–40% contains scarce message sequences.

The hybridization between a 350-fold excess of message cDNA and the pulse-labeled poly(A)-adjacent sequences isolated from the shnRNA fraction is shown in Figure 3B. Approximately 65% of the hybridizable shnRNA also annealed by log C_0t = 0.7 to the abundant message sequences and the remaining 35% annealed to the scarce sequences. The data show that the shnRNA contains abundant and scarce message sequences in about the same proportions as are present in the unreleased, nuclear lamina associated hnRNA fraction. However, it has not been determined whether the two hnRNA fractions contain the sequences for the same or different messages. Only about 40% (27/70) of the poly(A)-adjacent shnRNA sequences is present in poly(A)+ cytoplasmic RNA while at least 65% of those in the unreleased fraction is homologous to the 3′ ends of poly(A)+ mRNA.

Effects of DRB on the Synthesis of Message Sequences. The incorporation of [^{3}H]nucleosides into total HeLa hnRNA was reduced approximately 10-fold after a 30-min pretreatment with 75 μM DRB. After correcting for the decreased uptake of nucleosides by the cells (Sehgal et al., 1976), we estimated that hnRNA synthesis was reduced by 70–75%. The sedimentation profile of the pulse-labeled hnRNA purified from the nuclear lamina fraction after DRB treatment is shown in Figure 2B. Aside from the peak of acid-insoluble material near the top of the gradient, the sedimentation distribution did not appear to be affected by the DRB treatment. The additional peak near the top contains the novel small nuclear RNA species recently identified by Benecke & Penman (1977) (data not shown). Also shown in Figure 2B is the sedimentation profile of the RNA eluted from the nuclei of DRB-treated cells by ammonium sulfate. In replicate experiments only 5–6% of the incorporated radioactivity was released by this treatment. The sedimentation profile characteristic of the shnRNA was not observed, and more than half of the eluted material sedimented as a broad peak near the top of the gradient.

To determine directly whether nuclear RNA molecules containing message sequences were synthesized after DRB treatment, the pulse-labeled poly(A)+ hnRNA isolated from the nuclear lamina fraction was hybridized to an excess of message cDNA (Figure 4). At low values of C_0t this poly(A)+ hnRNA hybridized with similar kinetics and to the same extent as did control hnRNA. However, of the poly(A)+ hnRNA synthesized after DRB treatment, only 30% (21/70) annealed to the message cDNA. Thus, while the synthesis of total hnRNA is reduced about threefold after DRB treatment, the synthesis of polyadenylated message sequences may be reduced as much as sixfold. It is interesting to note that the scarce message sequences constituted only 15–20% of the hybridizable poly(A)+ hnRNA pulse-labeled after DRB treatment but were almost 40% of the hybridizable RNA labeled in the absence of this drug.

Discussion

The results presented here show that cDNA excess hybridizations can be used to examine the sequence content of

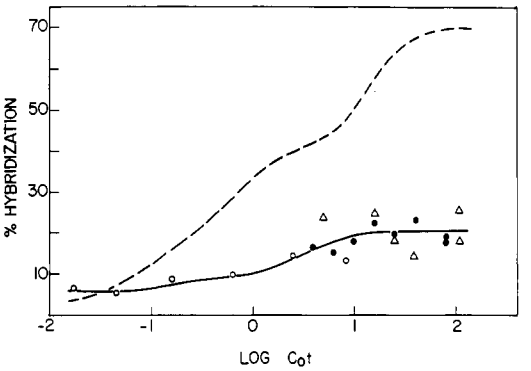

FIGURE 4: Hybridization of hnRNA pulse-labeled after DRB treatment. Cells were concentrated and treated with actinomycin as outlined. After treatment for an additional 30 min with 75 μM DRB, they were pulse-labeled for 15 min and hnRNA was isolated from the nuclear lamina fraction as outlined in the legends to Figures 2 and 3. Hybridizations were performed as described under Materials and Methods. cDNA concentrations: (○) 0.030 mg/mL, fivefold excess; (●) 0.290 mg/mL, 25-fold excess; (△) 0.390 mg/mL, 40-fold excess. Different symbols also indicate different hnRNA preparations. The dashed line shows the hybridization of pulse-labeled poly(A)+ mRNA to an excess of message cDNA. (Data from Figure 1.)

complex mixtures of newly synthesized RNA molecules. While the cDNA was estimated to be only 600–700 nucleotides long, it, nevertheless, accurately reflected the abundance distribution of the template mRNA and was capable of driving a significant proportion of complementary sequences into hybrid molecules. By use of this approach it is shown that 65% of the pulse-labeled poly(A)+ nuclear lamina associated hnRNA and 40% of the poly(A)-containing shnRNA molecules contain message sequences adjacent to the poly(A). These results are entirely consistent with the earlier observation that only about 70% of the steady-state hnRNA in the lamina fraction contains message sequences adjacent to the poly(A) (Herman et al., 1976). Thus, it seems unlikely that the pulse-labeled hnRNA that failed to hybridize comprises a class of message sequences which are inadequately represented in the message cDNA. However, this possibility cannot be completely ruled out. On the basis of kinetic data, Price et al. (1974) suggested that the shnRNA possessed the characteristics of a precursor to mRNA. The significance of the finding that the poly(A)+ shnRNA contains a lower proportion of message sequences is not currently known but the results presented support the suggestion made by Price et al. (1974) that these molecules comprise a separate and distinct class of hnRNA.

Molecules containing abundant message sequences constitute nearly 65% of the pulse-labeled polyadenylated hnRNA even though these abundant sequences represent only about 5% of the total HeLa message complexity of 10000 (Williams & Penman, 1975). This overrepresentation of the abundant sequences is probably not due to the transcription of reiterated genes because the vast majority of the abundant cytoplasmic messages are transcribed from sequences present only once per haploid genome (Hastie & Bishop, 1976). It has recently been shown that the polyadenylated HeLa hnRNA is composed of several distinct kinetic components with half-lives ranging from 35 to perhaps 200 min (Herman & Penman, 1977). (The observed half-lives are the results of simple degradation plus processing and export.) Since the molecules with the shortest half-lives are labeled the most rapidly, it seems likely that those containing the abundant message sequences are short-lived in the nucleus. At steady state the population would be composed mainly of the more stable (long-lived) molecules. The analysis of the message sequence

content of the steady-state HeLa hnRNA shows that the majority of the polyadenylated molecules are, in fact, scarce message sequences (Herman et al., 1976). Thus, the relatively large proportion of abundant sequences which is labeled in a short pulse may indicate that the genes coding for the abundant messages are transcribed more frequently than those for the scarce sequences. Because there is no obvious correlation between lifetime and relative abundance in the HeLa cytoplasm (Figure 1; Lenk et al., 1978), their high abundance in the cytoplasm and their apparently short lifetime in the nucleus may indicate that the abundant sequences are also processed and exported from the nucleus more efficiently. Similar conclusions regarding the processing of abundant and scarce sequences were recently suggested by Tobin (1978).

DRB treatment reduces the synthesis of total hnRNA about threefold. While message sequences are still synthesized in the nucleus in the presence of this analogue, only about 30% of the poly(A)-adjacent sequences is homologous to the 3′ ends of mRNA. The pulse-labeled poly(A)+ hnRNA isolated from the nuclear lamina fraction contains the abundant message sequences in about the same porportions as are found in hnRNA from cells treated solely with a low concentration of actinomycin D. Even though this hnRNA appears to be deficient in only scarce message sequences, little or no new mRNA appears in the cytoplasm (Sehgal et al., 1976). The failure to export even the abundant messages may result from the DRB-induced disruption of the processing of hnRNA (Herman and Penman, unpublished observations). These effects cannot be due to the length of treatment with actinomycin D (Figure 2). As shown by Penman et al. (1968), there is at most only a 20% difference between the rate of hnRNA synthesis after treatment for 30 and 60 min with a low concentration of actinomycin. This small difference is insufficient to account for either the 70% decrease in the synthesis of total hnRNA or the sixfold decrease in the synthesis of polyadenylated message sequences. Sehgal et al. (1976) have also shown that DRB has the same effect on hnRNA synthesis in cells treated with a low concentration of actinomycin for as little as 25 min or as long as 75 min. Of course, the detection of newly synthesized poly(A)+ message sequences in the nucleus after DRB treatment does not prove that these molecules are the precursors to messenger RNA. A role for hnRNA in the maintenance of nuclear architecture has recently been suggested (Herman et al., 1978), and these newly synthesized molecules may be used preferentially for that alternative function.

On the basis of the altered sedimentation profile, it seems unlikely that the shnRNA molecules are synthesized after DRB treatment. The majority of the material eluted by the ammonium sulfate is composed of the small nuclear species which sediment at about 4–10 S (not shown) (Benecke & Penman, 1977). Thus, in addition to differences in sedimentation profile (Price et al., 1974) and kinetic behavior (Herman & Penman, 1977), the shnRNA fraction also appears to possess a higher sensitivity to DRB than does bulk hnRNA. This higher sensitivity to DRB cannot be attributed to the length of treatment with actinomycin D since Price et al. (1974) showed that this fraction is relatively less sensitive to inhibition by the latter drug than is bulk hnRNA. A more detailed examination of the relationship between the two hnRNA fractions and their differential sensitivity to DRB may enhance our understanding of the synthesis and processing of nuclear RNA. Since the manner in which DRB differentiates between resistant and sensitive transcripts is not known at the present time, additional experimentation will be required to distinguish between the various mechanisms which can be proposed for this process.

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References

Bastos, R. N., & Aviv, H. (1977) *Cell 11*, 641–650.
Benecke, B.-J., & Penman, S. (1977) *Cell 12*, 939–946.
Bishop, J. O., Morton, J. G., Rosbash, M., & Richardson, M. (1974) *Nature (London) 250*, 199–204.
Curtis, P. J., & Weissmann, C. (1976) *J. Mol. Biol. 78*, 321–334.
Derman, E., & Darnell, J. E. (1974) *Cell 3*, 255–264.
Egyházi, E. (1974) *J. Mol. Biol. 84*, 173–183.
Egyházi, E. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 947–950.
Egyházi, E., Daneholt, B., Edström, J.-E., Lambert, B., & Ringborg, U. (1970) *J. Cell Biol. 47*, 516–520.
Fraser, N. W., Sehgal, P. B., & Darnell, J. E. (1978) *Nature (London) 272*, 590–593.
Gilmore-Herbert, M., & Wall, R. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 342–345.
Granick, D. (1975) *J. Cell Biol. 65*, 398–417.
Hastie, N. D., & Bishop, J. O. (1976) *Cell 9*, 761–774.
Herman, R. C., & Penman, S. (1977) *Biochemistry 16*, 3460–3465.
Herman, R. C., Williams, J. G., & Penman, S. (1976) *Cell 7*, 429–437.
Herman, R., Weymouth, L., & Penman, S. (1978) *J. Cell Biol. 78*, 663–674.
Jacquet, M., Affara, N. A., Robert, B., Jakob, H., Jacob, F., & Gros, F. (1978) *Biochemistry 17*, 69–79.
Kwan, S., Wood, T. G., & Lingrel, J. P. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 178–182.
Lenk, R., Herman, R., & Penman, S. (1978) *Nucleic Acids Res. 5*, 3057–3070.
Levy, W. B., Johnson, C. B., & McCarthy, B. J. (1976) *Nucleic Acids Res. 3*, 1777–1789.
Lewin, B. (1975a) *Cell 4*, 11–20.
Lewin, B. (1975b) *Cell 4*, 77–93.
Milcarek, C. A., Price, R. P., & Penman, S. (1974) *Cell 3*, 1–10.
Molloy, G. R., Jelinek, W., Salditt, M., & Darnell, J. E. (1974) *Cell 1*, 43–49.
Pederson, T. (1974) *J. Mol. Biol. 83*, 163–183.
Penman, S., Vesco, C., & Penman, M. (1968) *J. Mol. Biol. 34*, 49–69.
Perry, R. P. (1976) *Annu. Rev. Biochem. 45*, 605–629.
Perry, R. P., Bard, E., Hames, B. D., & Kelley, D. E. (1974) *Fed. Eur. Biochem. Soc. Meet. [Proc.] 33*, 17–34.
Price, R. P., Ransom, L., & Penman, S. (1974) *Cell 2*, 253–258.
Ross, J. (1976) *J. Mol. Biol. 106*, 403–420.
Sehgal, P. B., Darnell, J. E., & Tamm, I. (1976) *Cell 9*, 473–480.
Singer, R. H., & Penman, S. (1973) *J. Mol. Biol. 78*, 321–334.
Tobin, A. (1978) *Dev. Biol.* (in press).
Williams, J. G., & Penman, S. (1975) *Cell 6*, 197–206.