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HeLa cell poly(A)− mRNA codes for a subset of poly(A)+ mRNA-directed proteins with an actin as a major product

(mRNA·cDNA hybridization/cell-free translation/isoelectrofocusing/two-dimensional gel electrophoresis)

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ABSTRACT Poly(A)+ and poly(A)− mRNA from HeLa cells were separated and translated in heterologous messenger-dependent protein synthesizing systems. Two-dimensional electrophoretic analysis revealed three classes of polypeptides. At the level of detectability in the electropherograms, a small number (about 10) of proteins were detected only among the poly(A)− mRNA products, a larger number (about 40) were produced by both poly(A)− and poly(A)+ mRNA, and a large number of polypeptides were found exclusively in the poly(A)+ mRNA products. The major product of both poly(A)+ and poly(A)− mRNA was shown to be the β form of actin.

Previous cross hybridization measurements suggested little homology between poly(A)+ and poly(A)− mRNA populations. In view of the apparent identity of many poly(A)− products with those of poly(A)+, the homology between poly(A)+ and poly(A)− mRNA sequences was examined in greater detail. cDNA complementary to only the most abundant poly(A)+ message sequences was prepared. About 10% of this cDNA hybridized to abundant sequences in the poly(A)− fraction. This corresponded to only 2% of the total mass of poly(A)+ mRNA and accounted for the failure to detect cross hybridization in previous experiments. Thus, a small number of poly(A)+ sequences appear to be present in relatively high concentration in poly(A)− mRNA as evidenced by both the translation products and the cross hybridization results.

The majority of known eukaryotic mRNA species occur in a form that is polyadenylylated at the 3′ terminus (1–4). The functional significance of the poly(A) segment is not known. Recent studies have shown that, in addition to polyadenylylated mRNA, there are also messenger molecules in a number of eukaryotic cell types—including HeLa cells (5), L cells (6), plant cells (7, 8), and sea urchin embryos (9–12)—that fail to bind to substrates [poly(U) and oligo(dT)] that bind most mRNA molecules. This class is termed "poly(A)− mRNA" although only for histone mRNA is it known that the 3′ ends have no oligo(A) segment.

In HeLa cells, about 30% of the pulse-labeled polyribosome-associated mRNA is poly(A)−. The kinetics of appearance of poly(A)− mRNA in the cytoplasm parallel those of poly(A)+ mRNA (5) and indicate that poly(A)− mRNA could be a separate class not derived by cytoplasmic loss of poly(A). Other properties that distinguish poly(A)− from poly(A)+ mRNA are a somewhat larger modal sedimentation value (ref. 5; C. Milcarek, unpublished data) and a much lower sensitivity to inhibition by 3′-deoxyadenosine (cordycepin) (5). The cross hybridization of poly(A)+ cDNA to poly(A)− mRNA shows little apparent homology of the two populations (5), which also indicates that the poly(A)− molecules do not arise from simple random degradation.

In this report the coding sequences present in the poly(A)− mRNA from HeLa cells are examined by *in vitro* translation in two heterologous cell-free systems. The major poly(A)− product, which is also coded for by poly(A)+ message, is the β form of actin. A few proteins appear only as poly(A)− products whereas the majority of proteins coded for by the poly(A)− mRNA are also formed by poly(A)+ messages.

MATERIALS AND METHODS

HeLa Cells. HeLa (S3) cells were grown in suspension at 37° in Eagle's medium supplemented with 7% horse serum (Microbiological Associates, Inc.) and kanamycin (Grand Island Biological Co.). Cells were maintained at 4×10^5 cells per ml.

RNA Isolation. Total cytoplasmic RNA was isolated from HeLa cells as described (5). The only RNase inhibitor used in these preparations was diethylpyrocarbonate (Sigma). Two milligrams of total cytoplasmic RNA was fractionated into poly(A)+ and poly(A)− RNA by passage five times over a 1-ml oligo(dT) column (Collaborative Research, Inc.). This procedure yielded poly(A)− RNA that still contained 2–4% of the original poly(A) (5). Five passages over a second oligo(dT) column removed all poly(A) detectable by hybridizing to poly(U) (5). Also, analysis of the RNase-resistant fragments of this poly(A)− preparation showed no oligo(A) larger than A_{15}–A_{20}.

Protein Synthesis Assays. *Translation in wheat germ extracts.* Preincubated wheat germ S30 extracts were prepared according to Roberts and Paterson (13). Extracts (50 A_{280} units/ml) were centrifuged at 12,000 × *g* for 2 min, and the supernatant was used for assay. Reaction mixtures contained, in a final volume of 25 μl: 7 μl of the preincubated extracts, 20 mM K *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate (Hepes) (pH 7.5), 2 mM dithiothreitol, 1 mM ATP, 50 μM GTP, 8 mM creatine phosphate, creatine phosphokinase at 40 μg/ml, 25 μM each of 19 unlabeled amino acids (except methionine), 80 mM KOAc; 3 mM $Mg(OAc)_2$, 0.24 mM spermidine, 10–15 μCi of [^{35}S]methionine (Amersham, 400 Ci/mmol), and RNA as indicated. Reactions were incubated for 2 hr at 25°.

Translation in reticulocyte lysate. Micrococcal nuclease-treated reticulocyte lysate was a generous gift of B. Roberts. It was prepared by a modification of the procedure of Pelham and Jackson (14); 12 μl of reticulocyte lysate, containing hemin at 40 μg/ml and creatine phosphokinase at 160 μg/ml, was added to make the 25-μl reaction mixture. Other components were 20 mM K *N*-2-hydroxyethyl-*N*′-2-ethanesulfonate (pH 7.6), 2 mM dithiothreitol, 8 mM creatine phosphate, 25 μM each of

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19 unlabeled amino acids (omitting methionine), 80 mM KOAc, 0.65 mM $Mg(OAc)_2$, 0.5 mM spermidine, and 15 μCi of [^{35}S]-methionine. Incubation was for 60 min at 37°.

Gel Electrophoresis. Sodium dodecyl sulfate/10–15% polyacrylamide gradient slab gels (15) with a 3% stacking gel were used. Gels were stained with Coomassie brilliant blue R (Sigma), destained, dried, and fluorographed (16) and then were exposed to prefogged Kodak X-Omat films according to Laskey and Mills (17).

For two-dimension gel electrophoresis, isoelectrofocusing in the first dimension was performed according to the method of O'Farrell (18) with 100 × 1.5 mm gels containing 1.6% of pH 5–7 and 0.4% of pH 3.5–10 Ampholines (LKB). The gels were then extruded and equilibrated with sodium dodecyl sulfate sample buffer. The second dimension was electrophoresed as in the preceding paragraph.

Actin Purification. Actin was purified from HeLa cells by repolymerization according to Spudich and Watt (19). An acetone powder of HeLa cells was extracted in depolymerization buffer (0.5 mM ATP/0.5 mM 2-mercaptoethanol/0.2 mM $CaCl_2$/10 mM Tris·HCl, pH 7.4) for 30 min at 4°. The sample was then centrifuged at 50,000 × *g* for 1 hr in the cold. The supernatant was made 0.1 M in KCl and 1 mM in $MgCl_2$. After 2 hr at room temperature the sample was brought to 0.5 M KCl and left overnight at room temperature. The resulting F-actin was collected by centrifugation at 150,000 × *g* for 1 hr in the cold. The pellet was rinsed with depolymerization buffer and dissolved in the electrophoresis buffer.

Synthesis of cDNA. A mixture of 75 μCi of [^{3}H]dCTP (New England Nuclear, 27 Ci/mmol) and 5 μg of actinomycin D (Calbiochem) was dried in a siliconized tube; 40 μl of the reaction mixture containing 50 mM Tris·HCl (pH 8.3), 20 mM dithiothreitol, 12 mM $Mg(OAc)_2$, 4 mM Na pyrophosphate, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dTTP, and oligo$(dT)_{12-18}$ (15 μg/ml) was added followed by 5 μg of isolated poly(A)+ mRNA and 13 units of avian myeloblastosis virus reverse transcriptase (RNA-directed DNA nucleotidyltransferase) (kindly supplied by J. Beard). Incubation was at 43° for 1 hr, and the cDNA was purified as described (5). The length of this cDNA was estimated by sedimentation in alkaline sucrose to be 400–500 nucleotides (20). The hybridization of RNA with cDNA was as described (21).

RESULTS

Translation Efficiency of Poly(A)− mRNA in Heterologous Systems. Poly(A)− mRNA stimulates a heterologous protein synthesis system with nearly the same efficiency as poly(A)+ mRNA. Total HeLa cytoplasmic RNA was fractionated into poly(A)+ and poly(A)− RNA as described in *Materials and Methods*.

The rRNA is present only in the poly(A)− fraction and partially inhibits its translation in the *in vitro* system. Therefore, to compare the stimulation by poly(A)− and poly(A)+ mRNA, an equivalent amount of purified rRNA, free of endogenous mRNA, was added to the isolated poly(A)+ mRNA fraction. Message-free rRNA was prepared by selectively degrading the mRNA in polyribosomes with the endogenous ribonucleases in HeLa cytoplasmic extract. This results in the fragmentation of mRNA but rRNA is left intact and can be easily purified by sedimentation.

To 0.05 μg of purified poly(A)+ mRNA was added 1.45 μg of rRNA to obtain the same concentration of rRNA as is present in the poly(A)− fraction. This resulted in a 35% inhibition (Table 1) of translation of the poly(A)+ RNA in wheat germ extract. The inhibition was fairly constant over the range 1.25–3.0 μg of added rRNA.

Table 1. Stimulation of [^{35}S]methionine incorporation in wheat germ extracts

RNA fraction	RNA, μg	Stimulation, cpm × 10^{-4}	Relative activity	
Total cytoplasm	1.50*	36.2	100.0	
rRNA	1.50†	1.0	2.7	
Poly(A)+ RNA	0.05	44.7	123.6	
Poly(A)+ RNA and rRNA	0.05 1.45†	30.2	83.5	103
Poly(A)− RNA	1.50* ‡	7.1	19.8	

Incorporation of [^{35}S]methionine was measured as detailed in *Materials and Methods*. Amounts of RNA and stimulation of incorporation are for a 25-μl reaction mixture. The endogenous activity of the wheat germ extract, 2.5 × 10^4 cpm/25 μl, was subtracted. The concentration of poly(A)+ mRNA was determined by hybridizing [^{3}H]poly(U) assays (5) and found to be 0.05 μg of poly(A)+ mRNA per 1.5 μg of total cytoplasmic RNA. The concentration of other RNA fractions was determined by their absorbance at 260 nm; as noted, the poly(A)− mRNA concentration could not be determined directly. The poly(A)− RNA preparation did not contain tRNA; the contribution of tRNA to the total cytoplasmic RNA concentration was subtracted.

* Mostly rRNA.

† Only rRNA.

‡ Poly(A)− mRNA was prepared from the same number of cells that yielded the 0.05 μg of poly(A)+ mRNA used above.

Table 1 compares the stimulation of protein synthesis in a wheat germ extract by total HeLa cytoplasmic RNA, by poly(A)+ RNA with added message-free rRNA, and by poly(A)− with rRNA. Poly(A)+ mRNA accounted for 80% of the stimulation of total RNA and poly(A)− mRNA, for 20%. Poly(A)− mRNA has been estimated in previous pulse-labeling experiments to comprise 30% of total mRNA on a mass basis and possibly somewhat less on a molar basis (5). Thus, poly(A)− mRNA is translated with an efficiency similar to that of poly(A)+ mRNA.

Proteins Produced by Poly(A)− and Poly(A)+ mRNA. The major proteins formed by translation of the two RNA populations in wheat germ S30 and in reticulocyte lysates treated with micrococcal nuclease (14) are compared on sodium dodecyl sulfate/polyacrylamide gels (Fig. 1). The patterns of products coded by poly(A)− and poly(A)+ RNA were similar in both cell-free systems and most of the major peaks appeared coincident at this resolution. In particular, a major peak (marked "G") comigrated with purified actin. It will be shown below that it is actin that is formed by both poly(A)− and poly(A)+ mRNA.

Analysis of the *In Vitro* Products of Poly(A)− and Poly(A)+ mRNA on Two-Dimensional Polyacrylamide Gels. The similarity between poly(A)− and poly(A)+ RNA products on one-dimensional electrophoretic gels (Fig. 1) suggested that similar proteins are coded for by the two forms of mRNA. A more detailed comparison of the two sets of *in vitro* products was afforded by the higher resolution of the two-dimensional gel electrophoresis system of O'Farrell (18). Fig. 2 shows the analysis of products synthesized in the wheat germ extract by equal amounts of poly(A)− and poly(A)+ mRNA. At least 50 different proteins were visible on the poly(A)− RNA plate, and a much more complex pattern appeared on the poly(A)+ RNA plate. The actual complexity of the poly(A)+ mRNA products was even greater than shown because longer exposure revealed many additional products. The coincidence of spots was de-

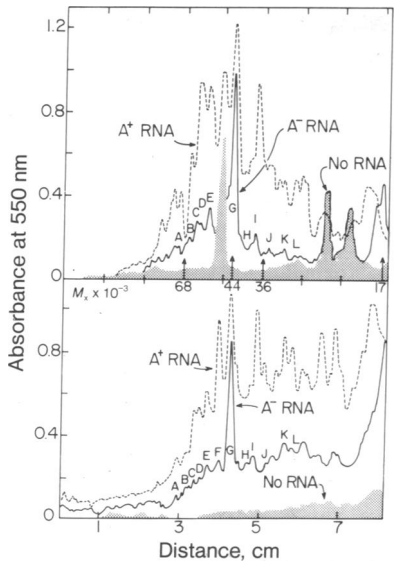

FIG. 1. Electrophoretic analysis of *in vitro* translation products directed by poly(A)− or poly(A)+ RNA in wheat germ S30 (*Lower*) or reticulocyte lysate (*Upper*). Three samples were tested in each extract: (*i*) no RNA added; (*ii*) 2.1 μg of poly(A)− RNA added; (*iii*) 0.25 μg of isolated poly(A)+ RNA added. The products were subjected to electrophoresis in a sodium dodecyl sulfate/polyacrylamide slab gel. The samples taken from the wheat germ extract contained: (*i*) 3600 cpm; (*ii*) 22,000 cpm; (*iii*) 61,000 cpm, and from the reticulocyte lysate: (*i*) 9000 cpm; (*ii*) 22,000 cpm, and (*iii*) 31,000 cpm. The dry gels were exposed to x-ray films, for 5 days for *i* and *ii* and for 1 day for *iii*. The autoradiograms were scanned at 550 nm.

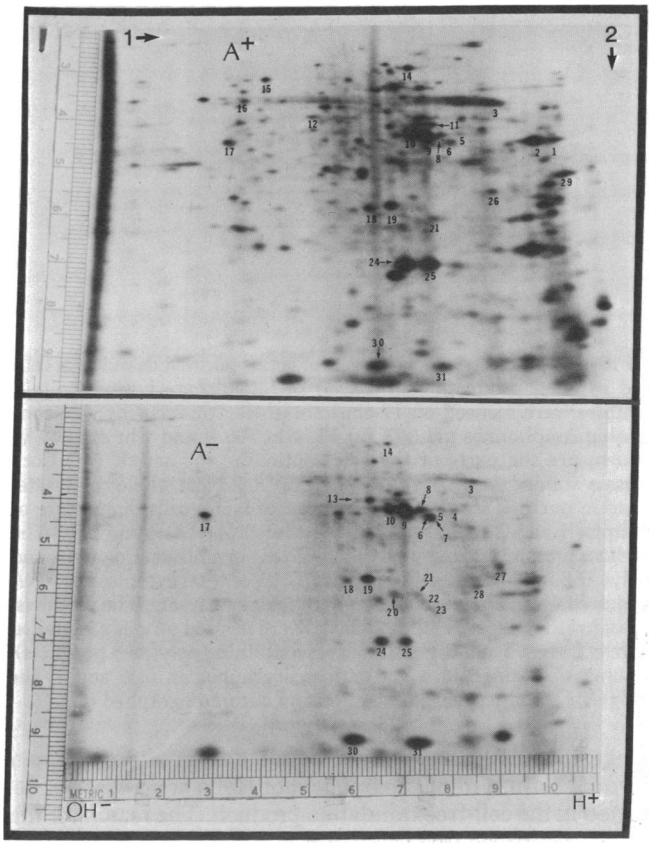

FIG. 2. Fluorographs of two-dimensional gel electrophoresis of the poly(A)− and poly(A)+ RNA *in vitro* translation products. Wheat germ S30 was used for translation of poly(A)− RNA (*Lower*) or an isolated poly(A)+ RNA (*Upper*). A sample containing 15,000 cpm of poly(A)− RNA products (including 3000 cpm of background incorporation) or 30,000 cpm of poly(A)+ RNA products mixed with 3000 cpm of background mixture was taken and 2 μg of unlabeled actin purified from HeLa cells or 1 μg of unlabeled actin from chicken thigh muscle was added to each sample. The first dimension was isoelectric focussing (1) and the second dimension was on sodium dodecyl sulfate/10–15% gradient polyacrylamide gels (2). The gels were stained, fluorographed, dried, and exposed to x-ray films for 21 days. Numbers refer to spots located above the numbers. Products 30 and 31 are synthesized in the "background" mixture. Unlabeled purified actin from HeLa coincided, on these gels, with product 9 and α form of actin was located between products 8 and 9.

termined by analyzing a mixture of poly(A)− and poly(A)+ RNA products (not shown). Most of the detectable poly(A)− products also appeared among the poly(A)+ RNA products (for example, see refs. 3, 8, 9, 10, 14, 17, 19, 24, and 25). At this sensitivity, a few proteins (4, 7, 13, 20, 22, 23, 27, and 28) were found only among the poly(A)− RNA products. In contrast, many proteins, including several major ones (see refs. 1, 2, 11, 12, 15, 16, 26, and 29) appeared exclusively in the poly(A)+ RNA products. Adding rRNA back to the poly(A)+ mRNA reaction mixture had no effect on the pattern (not shown). Also, poly(A)− RNA obtained from polysomes yielded the same translation products as poly(A)− RNA from total cytoplasm.

Identification of Actin as the Major Product of Poly(A)− and Poly(A)+ mRNA. One protein seemed to be the major translation product of both poly(A)− and poly(A)+ mRNA. It appeared as a major band at approximately 44,000 daltons in one-dimensional electrophoresis and a major spot on the acidic side of the two-dimensional electropherogram (9 in Fig. 2). This material was identified as the β form of actin by the following criteria. Actin was purified from HeLa cells by repolymerization (19). This procedure yielded virtually a single band migrating at 44,000 daltons in a sodium dodecyl sulfate gel. The major translation product of both mRNA preparations copolymerized and comigrated with the unlabeled presumptive HeLa cell actin and with chick skeletal muscle actin. In two-dimensional analysis, the main radioactive product from *in vitro* translation of poly(A)− and poly(A)+ RNA (spot 9) was coincident with unlabeled HeLa cell actin and slightly displaced toward basic pH compared to chick skeletal muscle actin (see legend to Fig. 2).

HeLa cell actin and the major *in vitro* translation product were compared by isoelectric focusing with the α, β, and γ forms of actin obtained from fusing rat myoblasts (kindly supplied by R. Singer). A clear separation of the three forms of actin was achieved by using 17-cm-long gels. Fig. 3*M* shows part of these longer gels, stained with Coomassie blue, containing repolymerized HeLa cell actin, rat myoblast actin, and a mixture of the two preparations. The purified actin from HeLa cells contained two components, a major one focused with β actin and a minor one focused with γ actin. No α form was detected. Fig. 3 also shows an autoradiogram of the isoelectrofocused *in vitro* translation products of poly(A)− and poly(A)+ mRNA in a reticulocyte lysate. One band, which focused with the β form of actin, was produced in comparable amounts by both types of mRNA. Second-dimension analysis of the products synthesized in both reticulocyte and wheat extracts showed similar patterns in the "actin region" (not shown). The major *in vitro* product overlapped the marked position of Coomassie blue-stained rat myoblast β actin. However, the separation between the isozymic β and γ forms of actin in the second dimension was inferior to isoelectrofocusing alone. We conclude that, by the criteria of copolymerization with unlabeled actin, electrophoretic mobility, and isoelectric point, the major translation product of both mes-

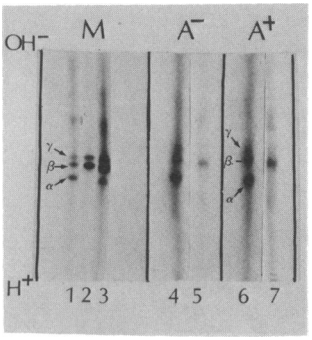

FIG. 3. Isoelectric focusing gels of actins purified from HeLa cells and *in vitro* products synthesized in reticulocyte lysate. Protein samples were focused on 17-cm-long isoelectric focusing gels containing Ampholines pH 5–7, for 17 hr at 500 V and 1 hr at 1000 V. Shown are the parts of the gels containing the actins. (*M*) Coomassie-stained gels. Lanes: 1, fusing rat myoblast whole cell lysate; 2, actin purified from acetone powder of HeLa cells; 3, mixture of samples 1 and 2. (A^- and A^+) ^{35}S-Labeled poly(A)− and poly(A)+ *in vitro* products mixed with unlabeled rat myoblasts. Poly(A)− and poly(A)+ RNA were prepared from 0.5 μg of total cytoplasmic RNA and used for *in vitro* translation in reticulocyte lysate. The products, mixed with unlabeled rat myoblasts, were focused on gels as detailed above. Lanes: 4 and 5, the same gel (containing poly(A)− products), Coomassie-stained in 4 and autoradiographed in 5; 6 and 7, the poly(A)+ products gel stained in 6 and autoradiographed in 7.

senger populations appears to be the β form of actin. The γ form of actin, a minor component of HeLa actin, was not detected in the cell-free translation products. The reason for this is not understood (see *Discussion*).

Homology between Poly(A)− mRNA and Abundant Poly(A)+ mRNA Sequences. A number of proteins, identical by isoelectric point and electrophoretic mobility, were coded for by both poly(A)− and poly(A)+ mRNA. Therefore, some degree of homology between the two RNA populations might be expected. In previous experiments, very little cross homology was found when cDNA synthesized from poly(A)+ mRNA was hybridized to poly(A)− mRNA (5). Two possible explanations could account for this seemingly paradoxical result. Identical proteins could be coded for by dissimilar mRNA sequences. Alternatively, a small number of sequences in poly(A)+ mRNA could be present at high abundance in the poly(A)− RNA fraction. Thus, a small amount of poly(A)+ mRNA, and hence of its cDNA, would be complementary to a large amount of poly(A)− message. This would result in very little cross hybridization because the few sequences common to poly(A)+ and poly(A)− would generate only a small portion of the poly(A)+ cDNA. This latter possibility appears to be the case, at least partially.

The translation pattern, which shows common poly(A)− and poly(A)+ mRNA products, reflects mainly the proteins coded for by the most abundant mRNAs. Therefore, the sequence homology between poly(A)− poly(A)+ RNA might be expected to occur in the abundant class of messages (22).

cDNA complementary to abundant poly(A)+ sequences was obtained by hybridizing the total (A)+ cDNA with an excess of poly(A)+ mRNA to a R_0t[§] of 0.13. At this point, the most abundant 20% of the (A)+ cDNA was hybridized to mRNA. The hybridized cDNA was selected on hydroxylapatite, repurified, and hybridized both to its template and to poly(A)− RNA. The hybridization is shown in Fig. 4. As is apparent from the *inset*, approximately 10% of the abundant (A)+ cDNA

§ R_0t is concentration of total RNA (mol of nucleotide per liter) × time (sec).

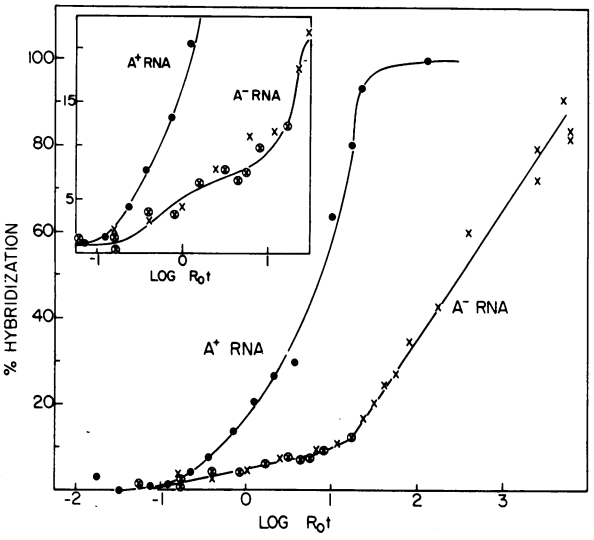

FIG. 4. Hybridization of abundant (A)+ cDNA to nonadenylylated and polyadenylylated RNA. The abundant sequences in poly(A)+ RNA were selected from the total (A)+ cDNA by hybridizing with an excess of isolated poly(A)+ RNA to R_0t of 0.13. Of the total (A)+ cDNA, 20% was in S_1 nuclease-resistant hybrids at this R_0t. The hybrids were isolated on a hydroxylapatite column at 68° and the cDNA was purified from the hybrids (20). This abundant (A)+ cDNA was used for hybridization with an excess of poly(A)− or poly(A)+ RNA as described (5). For comparing the poly(A)+ driven hybridization with poly(A)−, the poly(A)+ curve is shifted toward higher R_0t by a factor of 32, which is the estimated rRNA concentration in the poly(A)− RNA preparation. Two different poly(A)− RNA preparations were used. A background of 6% was substracted. (*Inset*) An enlargement of the initial region of the hybridization curve.

hybridized rapidly to the poly(A)− RNA fraction. Thus, 10% of the most abundant poly(A)+ message, or about 2% of the total adenylylated message, showed homology with the poly(A)− RNA fraction. The remaining abundant cDNA sequences eventually hybridized with poly(A)− RNA but at much higher R_0t. values. These shared sequences were diluted in the poly(A)− RNA by 100-fold as compared to their concentration in poly(A)+ mRNA and may represent cross contamination of the mRNA preparations. The abundance of the rapidly hybridizing sequences in the poly(A)− fraction cannot be determined by the available data, but they are present in relatively high concentrations and code for common poly(A)− and poly(A)+ mRNA products.

DISCUSSION

The poly(A)− messenger population of HeLa cells examined in this study shows some unexpected properties. This RNA codes for a number of relatively abundant proteins, with actin as the major product. Most, although not all, of these proteins, are also abundant products of poly(A)+ mRNA. Thus, it appears that mRNA coding for a portion of the abundant proteins can appear in two forms: adenylylated and nonadenylylated. The reason for this polymorphism in messenger structure is not known. The cross hybridization data indicate that 10% of the abundant poly(A)+ messages are present at relatively high concentration in the poly(A)− population. About 40 proteins, visible in the two-dimensional gel analysis of the poly(A)− RNA products, are also found among the poly(A)+ RNA products. Because poly(A)− is homologous with $^1/_{10}$ of the abundant poly(A)+ sequences, it might be expected that abundant poly(A)+ mRNA would code for at least 400 proteins. The es-

timate of 400 products for the abundant messages of poly(A)+ RNA does not seem unreasonable, either from cDNA hybridization (22) or from an estimation from two-dimensional gel electrophoresis. The data do not permit an unambiguous determination as to whether all of the poly(A)− sequences are found in the poly(A)+ RNA. Certainly, the few proteins coded for only by poly(A)− RNA represent mRNA sequences either unique to poly(A)− mRNA or present in the poly(A)+ mRNA in relatively low amounts.

Translatable poly(A)− mRNA has been studied in several cell types. Nonadenylylated RNA from sea urchin eggs and embryos stimulates protein synthesis (23) and gives rise to a wide range of nonhistone proteins (12). Histone mRNA has been found in both adenylylated and nonadenylylated forms in sea urchin embryos (12) and in amphibian ovaries and oocytes (12, 24). Gedamu and Dixon (25) reported that poly(A)− and poly(A)+ RNA from a trout testis direct cell-free synthesis of three protamines, and a plant enzyme was also found to be synthesized *in vitro* from nonadenylylated mRNA (8). Recently, Sonenshein *et al.* (26) reported that a major polypeptide of 45,000 daltons is coded for by poly(A)− RNA from mouse sarcoma and myeloma cells.

The experiments reported here indicate by several criteria that the major product of poly(A)− RNA from HeLa cells is actin. However, this protein is not exclusively coded for by nonadenylylated RNA as was suggested by Sonenshein *et al.* (26). In HeLa cells, approximately the same amount of actin-like material was produced *in vitro* by the poly(A)+ RNA (Figs. 2 and 3). It has been recently shown by Whalen *et al.* (27) and Rubinstein and Spudich (28) that actin can exist in three isozymic forms called α, β, and γ, having identical molecular weights but slightly different isoelectric points. The actin with the most acidic isoelectric point is α, the major component of skeletal muscle; γ has the most basic isoelectric point and is the major actin in smooth muscle.

The actin-like poly(A)+ and poly(A)− RNA products synthesized in the reticulocyte lysate and in the wheat germ extract appear to be identical. Both types of mRNA directed the synthesis of a polypeptide that cofocuses and comigrates with the β form of actin from fusing rat myoblasts. There was no evidence of α actin either in the translation products or in the repolymerized unlabeled actin from HeLa cells. The γ form, which is present in purified actin preparation from HeLa cells, was not detected among the products of the cell-free systems. The reason for this is not clear. Either the γ actin mRNA is not efficiently translated in the cell-free systems or the γ form, found in HeLa cells, is produced by a post-translational modification that does not occur in the heterologous *in vitro* systems.

It is worth noting that the poly(A)− mRNA in HeLa cells and other systems is defined operationally by inability to bind to appropriate affinity materials and by the absence of oligo(A) stretches of more than 15 to 20 bases. The possible existence of a smaller oligo(A) sequence at the 3′ terminus has not been determined. Also, the possible origin of poly(A)− in rapidly deadenylylated poly(A)+ molecules cannot be ruled out although this would require selective mechanisms that are unproven at present. The significance of two forms of some abundant messages is unknown as is, indeed, the significance of poly(A).

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