Synthesis of DNA Complementary to Cellular Slime Mold Messenger RNA by Reverse Transcriptase†

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ABSTRACT: Messenger RNA isolated from *Dictyostelium discoideum* could act as template for synthesis of complementary DNA in the presence of a primer. Oligo(dT) served as the most efficient primer but both oligo(dC) and oligo(dG) could also prime DNA synthesis. The DNA product initiated with oligo(dT) sedimented through an aqueous sucrose gradient slightly more slowly than the template. Both template and product sedimented through dimethyl sulfoxide sucrose gradients at identical rates but these gradients appear to accelerate DNA sedimentation rate relative to that of RNA. Over 90% of the product DNA could be hybridized back to the template RNA. A kinetic analysis of the hybridization of the complementary DNA to cell DNA showed that the product DNA was similar in its nucleotide sequence representation to the template RNA. Excess complementary DNA could protect over 95% of the template RNA against ribonuclease digestion. When DNA complementary to mRNA obtained after 18 hr of development was annealed with mRNA from vegetative stage cells, only 55% of the DNA was rendered resistant to digestion by single-strand specific nuclease. This result implies that some of the mRNA at the 18-hr stage of development represents nucleotide sequences transcribed since differentiation was initiated.

The cellular slime mold *Dictyostelium discoideum* is an ideal organism to study the control of development at the level of transcription. It undergoes a defined morphological cycle of development in the presence or absence of food source (Bonner, 1967). The specific activity of certain enzymes increases and then decreases at specific stages during development (see Sussman and Sussman, 1969; Newell, 1972). Furthermore, RNA excess hybridization to single-copy DNA indicates that there are qualitative and quantitative changes during development in the relative concentrations of the RNA transcripts from the single-copy portion of the genome (Firtel, 1972). Thus it appears that the regulation of development occurs at least in part at the level of gene activity.

The heterogeneous nuclear RNA (HnRNA)[1] of *Dictyostelium* is the precursor of mRNA and is about 20% larger than mRNA (Firtel and Lodish, 1973). Hybridization kinetics data indicate that approximately 90% of the nucleotide sequences of polysomal mRNA are transcribed from the single-copy portion of the genome. In contrast 75% of HnRNA is transcribed from the single-copy and 25% from repetitive sequences (Firtel *et al.*, 1972; Firtel and Lodish, 1973). Recent experiments indicate that the 20% of each HnRNA molecule excluded during the processing and transport of mRNA to cytoplasm represents nucleotide sequences which are transcribed from repetitive DNA sequences and are present at the 5′ end of the HnRNA (Firtel and Lodish, 1973). Like other eukaryotic mRNAs, the *Dictyostelium* mRNA also contains a poly(A) sequence at its 3′ end (Firtel *et al.*, 1972; Firtel and Lodish, 1973; Jacobson *et al.*, 1974).

If one could obtain DNA transcripts complementary to individual or groups of stage-specific mRNAs, they could serve as valuable tools for studying the control of the biosynthesis, structure, and metabolism of mRNA and HnRNA. Synthesis of DNA complementary to many individual eukaryotic mRNAs has been successfully achieved (Verma *et al.*, 1972; Ross *et al.*, 1972; Kacian *et al.*, 1972; Zassenhaus and Kates, 1972; Diggelman *et al.*, 1973; Berns *et al.*, 1973). In this paper we will show that total mRNA from *Dictyostelium* can be utilized as template to synthesize complementary DNA (cDNA) by purified DNA polymerase from avian myeloblastosis virus (AMV). The requirements of synthesis of cDNA, its size, the effect of various primers, and the hybridization properties will be discussed.

Experimental Section

Materials

^{3}H- and ^{32}P-containing deoxyribonucleoside triphosphates were purchased from New England Nuclear Corp. (Boston, Mass.) and ICN (Irvine, Calif). The primers were obtained from Collaborative Research (Waltham, Mass.).

Methods

Purification of AMV DNA Polymerase. AMV was provided by Dr. J. Beard, Duke University, N. C., through the Virus Cancer Program of the National Cancer Institute. The DNA polymerase was purified as described before (Verma and Baltimore, 1973).

Isolation and Purification of Dictyostelium mRNA. Poly(A)-containing cytoplasmic heterogeneous RNA (mRNA) was isolated from vegetative and developing cells of *Dictyostelium discoideum* as described previously (Firtel and Lodish, 1973). It was purified away from rRNA and tRNA by chromatography on poly(U)-Sepharose columns. The RNA eluted from the columns, however, served as a very poor template for AMV DNA polymerase in the presence of any primer, possibly due to the presence of small quantities of poly(U) eluted from

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[1] Abbreviations used are: cDNA, complementary DNA, HnRNA, heterogeneous nuclear RNA; mRNA, messenger RNA; AMV, avian myeloblastosis virus; TES, *N*-tris(hydroxmethyl)methyl-2-aminoethanesulfonic acid.

TABLE I: Requirements for DNA Synthesis using *Dictyostelium* mRNA.[a]

Reaction Components	pmol dAMP Incorporated in 180 min
Complete	70
Without $(dT)_{12-18}$	6
With ribonuclease	<1
With actinomycin D	41
Without dCTP	<1
Without TTP	<1
Without dGTP	9

[a] The complete reaction mixture consisted of the following in 0.1 ml: 50 mM Tris-HCl (pH 8.3), 10 mM dithiothreitol, 6 mM magnesium acetate, 60 mM NaCl, 0.6 mM dCTP, 0.6 mM dTTP, 0.6 mM dGTP and 0.1 mM [^{3}H]dATP (160 cpm/pmol), 1 μg of $(dT)_{12-18}$, 450 pmol of *Dictyostelium* mRNA, and 0.2–0.5 μg of AMV DNA polymerase. Actinomycin D was added at a concentration of 100 μg/ml. Ribonuclease-treated samples were prepared as described before (Verma *et al.*, 1972). Reactions were carried out in sealed tubes under an N_2 atmosphere, and incubated at 37° for 180 min. Acid-precipitable radioactivity was determined as previously described (Baltimore *et al.*, 1970).

the column. To eliminate any poly(U) eluted from the poly(U)-Sepharose columns, the RNA was further sedimented on 99% Me_2SO gradients (Strauss *et al.*, 1968; Firtel and Lodish, 1973). Material sedimenting faster than 5S rRNA and tRNA markers was precipitated with ethanol. The precipitate was dissolved in distilled water and used in subsequent experiments. It was stored at −70°.

Enzyme assays and sucrose gradient centrifugation were performed as described before (Verma *et al.*, 1972, 1973). The details are described in the legends to tables and figures.

Hybridizations were performed in capillary tubes as previously described (Firtel, 1972; Firtel and Lodish, 1973). Specific conditions are given in the legends to tables and figures. The fraction of DNA present in hybrid or duplex was determined by using single-strand specific nuclease from *Aspergillus oryzae* (S_1 nuclease). The enzyme was purified using modifications of the procedures of Vogt (1973), in association with Dr. H. Fan.

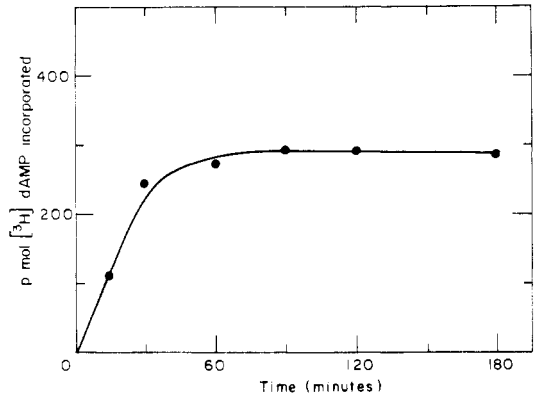

FIGURE 1: Kinetics of complementary DNA synthesis using *Dictyostelium* mRNA as template. The complete reaction mixture described in Table I was used with 2500 pmol of mRNA and 100 μg/ml of actinomycin D.

TABLE II: Effect of Various Primers on the Synthesis of DNA Complementary to *Dictyostelium* mRNA.[a]

Primer	pmol dAMP Incorporated in 180 min
$(dT)_{12-18}$	55
$(dC)_{12-18}$	23
$(dG)_{12-18}$	13
$(dA)_{12-18}$	3
Without primer	4

[a] The complete reaction mixture as described in Table I with 100 μg/ml of actinomycin D and 1 μg of primer was used.

Nuclease digestions were carried out in 0.5 ml containing 0.5 M NaOAc, 1 mM $ZnSO_4$, NaCl to bring the final (Na^+) to 0.2 M, 10 μg/ml of denatured DNA, and enough S_1 nuclease to digest 10 μg of denatured DNA in 5–10 min. Nuclease digestions were performed for 35 min at 45°. Nuclease resistant material was precipitated with 10% Cl_3CCOOH. Precipitates were collected on HAWP Millipore filters and counted.

Results

(*a*) *Requirements and Kinetics of the Synthesis of Complementary DNA.* Table I shows that the synthesis of DNA was primer-dependent and required all four deoxyribonucleoside triphosphates. In the presence of actinomycin D (100 μg/ml), which prevents the synthesis of double-stranded DNA (McDonnell *et al.*, 1970; Manly *et al.*, 1971), only 55–58% of the total DNA synthesis could be detected. The reaction was inhibited by the addition of ribonuclease.

Figure 1 depicts the rate of incorporation of [^{3}H]dAMP. The reaction reached completion after 60 min of incubation at 37°. At completion, approximately 30–100% (depending on the individual preparation) of the input nucleotides of the template were transcribed into cDNA. The amount of DNA synthesized was linearly related to the amount of the template within a range of 200–6000 pmol of nucleotides. In all of the hybridization experiments reported here only those cDNA preparations synthesized in the presence of actinomycin D (100 μg/ml) were used where over 80% of the input nucleotides were transcribed.

(*b*) *Effect of Various Primers on the Synthesis of Complementary DNA.* Oligomers of dT, dC, and dG were capable of initiating the synthesis of cDNA while oligo(dA) did not serve as a primer (Table II). Both the rate of synthesis and the yield of the product was higher with oligo(dT) than with oligo(dC) or oligo(dG) (Figure 2, inset). It is assumed that oligo(dT) served as primer by hydrogen bonding to the poly(A) sequences on the mRNA (Verma *et al.*, 1972). In order to explain the role of oligo(dC) and oligo(dG) as primers, one has to postulate the presence of G-rich and C-rich regions of the mRNA (Figure 2, inset). The slow rate of synthesis of DNA stimulated by the oligo(dC) and oligo(dG) primers suggests that the complementary oligo(G) and oligo(C)-rich regions in the mRNA are perhaps small in length and may therefore require a longer period of time to form a stable initiation complex.

(*c*) *Size of the Complementary DNA Synthesized by Various Primers.* Figure 2 depicts the relative sizes of DNA synthesized utilizing oligo(dT), oligo(dC), and oligo(dG) primers. The oligo(dT)-stimulated DNA was largest in size. Since oligo(dT) presumably acted as primer by binding to the poly(A) sequence at the 3′-OH end of the mRNA, the large size of

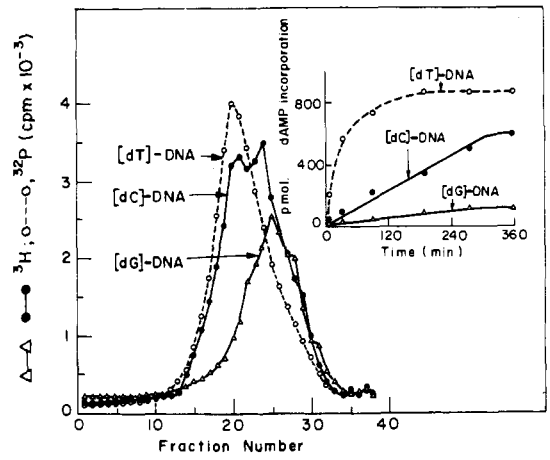

FIGURE 2: Comparison of rate of synthesis and size of complementary DNA synthesized with various primers. Inset: The complete reaction mixture described in Table I was used. In addition each reaction mixture contained approximately 6000 pmol of mRNA, 100 μg/ml of actinomycin D, and 0.2 μg of the respective primer. In cases where oligo(dT) was used as primer, [^{3}H]dATP was substituted with [α-^{32}P]dATP. The specific activity of both isotopes was 1600 cpm/pmol. Alkaline sucrose gradient sedimentation analysis: For size analysis reaction mixtures were allowed to incubate at 37° for 6 hr. The DNAs synthesized with various primers were then purified as described before (Verma *et al.*, 1972). Samples containing either oligo(dT)-stimulated [^{32}P]DNA and oligo(dC)-stimulated [^{3}H]DNA or oligo(dT)-stimulated [^{32}P]DNA and oligo(dG)-stimulated [^{3}H]DNA were centrifuged on 5–20% alkaline sucrose gradients made in 0.3 M NaOH, 0.7 M NaCl, and 0.001 M EDTA (pH 12.4) for 16 hr at 4° in rotor SW50.1 of Spinco ultracentrifuge. The gradients were collected and radioactivity counted as described before (Verma *et al.*, 1973). The figure represents a composite picture of two separate gradients. In both gradients, oligo(dT)-stimulated [^{32}P]DNA sedimented at the same position.

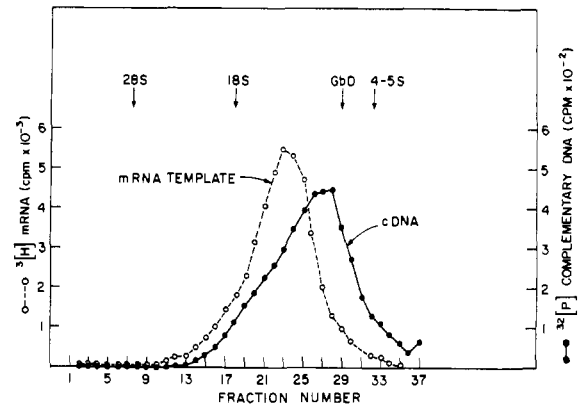

FIGURE 3: Neutral sucrose gradient sedimentation analysis of complementary DNA. [^{3}H]Uracil-labeled mRNA from vegetative cells (Firtel and Lodish, 1973) (approximately 1.5 × 10^5 cpm/μg) and ^{32}P-labeled oligo(dT)-stimulated DNA complementary to vegetative mRNA obtained as described in Figure 2 were centrifuged in 15–30% sucrose gradients prepared in 0.01 M NaCl, 0.01 M Tris-HCl (pH 7.6), and 0.05% sodium dodecyl sulfate. The sample contained in 0.4 ml of 1 mM EDTA (pH 7.1) was layered and the gradients were centrifuged in rotor SW 27.1 of Spinco ultracentrifuge at 25,000 rpm for 22 hr at 22°. *Dictyostelium* rRNA and DNA complementary to 10S rabbit reticulocyte mRNA (GbD) were centrifuged in a parallel gradient. The gradients were collected and analyzed as described before (Firtel *et al.*, 1972). Similar results were obtained if cDNA to vegetative cells mRNA and vegetative cells mRNA template were sedimented separately. (O - - - O) [^{3}H]mRNA template; (●——●) [^{32}P]cDNA.

much of the oligo(dC)-stimulated DNA suggests that the postulated stretch of G-rich region on the mRNA is close to the poly(A) sequence on the mRNA. The stretchs of C-rich regions, on the other hand, appear to be located further from the poly(A) sequences because of the small size of the oligo(dG)-stimulated DNA. Alternately, it is possible that the configuration of the templates somehow is responsible for the variation in size of the DNAs synthesized by various primers.

Figure 3 portrays the sedimentation through a neutral sucrose gradient of the oligo(dT)-stimulated DNA along with the mRNA template. Although some of the cDNA sequences were of the same size as the template, the average size of the DNA molecules appeared to be about 500–600 nucleotides long when compared with complementary globin DNA which is about 450–550 nucleotides long[2] (Verma *et al.*, 1972, 1973). The same comparison indicated that the average size of the mRNA template was about 1000 nucleotides.

When the DNA product was analyzed on Me_2SO sucrose gradients it cosedimented with the template and thus appeared to be much larger in size. However, DNAs appear to sediment faster than RNAs through Me_2SO sucrose gradients (R. A. Firtel, unpublished observations). For instance, when fdDNA, which comigrates with 28S rRNA in neutral sucrose gradients containing either 0.1 M Na^+ or 0.5 mM Na^+, was analyzed in Me_2SO sucrose gradients, it sedimented with 35S poliovirus RNA. Therefore, we assume that aqueous sucrose gradients are more appropriate for comparing the template with the product and that the average product is somewhat smaller than the template.

(*d*) *Hybridization Properties of the Complementary DNA.* To determine whether the DNA product was a faithful transcript of the RNA template, hybridization experiments were performed. Table III shows that over 90% of the oligo(dT)-stimulated DNA product was rendered resistant to S_1 nuclease from *A. oryzae* when annealed to excess RNA template. This nuclease digests only single-stranded polynucleotides. The oligo(dG)-stimulated DNA product was rendered only 75% resistant to digestion by S_1 nuclease under similar conditions. This incomplete resistance of (dG)-DNA product to S_1 nuclease remains unexplained.

Previous studies of hybridization kinetics indicated that approximately 90% of mRNA sequences are transcribed from the single-copy DNA while the remainder are transcribed from re-

TABLE III: Protection of cDNA with the mRNA Template.[a]

Primer used for cDNA Synthesis	mRNA Present during Annealing	% S_1 Nuclease Resistant after Annealing
Oligo(dT)	−	5
	+	95
Oligo(dG)	−	6
	+	75

[a] Approximately 0.01 μg of oligo(dT)-stimulated [^{3}H]DNA (2832 cpm) or 0.01 μg of oligo(dG)-stimulated [^{3}H]DNA (1907 cpm) was hybridized together with 30 μg of mRNA from 18-hr cells in 1.2 M NaCl, 0.2 M EDTA, and 2 mM TES (pH 6.8) for 20 hr at 67°. The details are described in Materials and Methods.

[2] When analyzed by sedimentation on alkaline sucrose gradients along with restriction enzyme, treated DNA fragment H of polyoma virus DNA (kindly provided by M. Vogt) of average molecular weight of 1 × 10^5 (about 150 base pairs long), globin DNA (GbD) was found to have an average molecular weight of 1.75 × 10^5 (about 500 nucleotides long) (I. M. Verma, unpublished results).

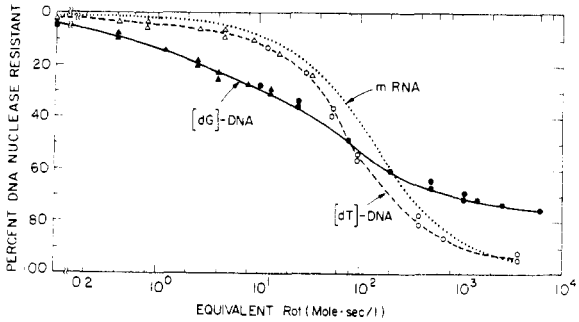

FIGURE 4: Hybridization kinetics of complementary DNA to *Dictyostelium* nuclear DNA. Oligo(dT)-primed and oligo(dG)-primed DNA complementary to mRNA from 18-hr development cells (labeled with [α ^{32}P]ATP and [^{3}H]ATP were hybridized with vast excess of *Dictyostelium* nuclear DNA (25 μg/point, ratio 25,000:1) until various C_0t values were obtained. These reactions were quenched by plunging the capillary tubes into an ice-water-NaCl solution. The fraction of the cDNA present in the DNA–RNA duplex was assayed with S_1-nuclease as described in Materials and Methods. (. . . .) expected curve with mRNA; (△, ○) oligo(dT)-primed DNA; (▲, ●) oligo(dG)-primed DNA; (△ and ▲) reaction containing 250 μg/ml of DNA in 0.36 M NaCl, 0.1 mM EDTA, and 1 mM TES at 61°; (○ and ●) reaction containing 1500 μg/ml of nDNA in 1.2 M NaCl, 0.2 mM EDTA, and 2 mM TES at 67°.

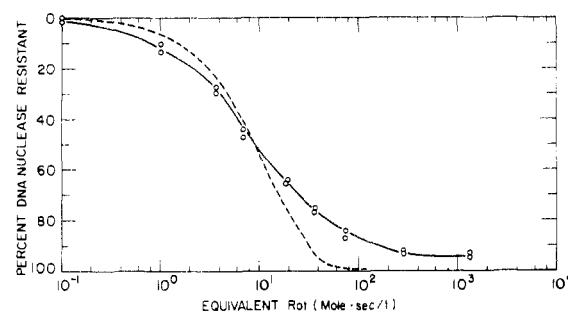

FIGURE 5: Kinetics of protection of complementary DNA by excess RNA template. ^{32}P-labeled DNA complementary to mRNA from vegetative cells (approximately 0.01 μg) was hybridized with vast excess of vegetative cell mRNA (30 μg, ratio of 3,000:1) in either 0.1 ml of 0.36 M NaCl, 0.1 mM EDTA, and 1 mM TES at 61° or 0.02 ml of 1.2 M NaCl, 0.2 mM EDTA, and 2 mM TES at 67°, until the appropriate R_0t values were achieved (equivalent R_0t = the product of initial RNA concentration and time, corrected for salt concentration) (Britten and Smith, 1970). The reaction product was analyzed for cDNA in DNA–RNA hybrids as described in the legend to Figure 4 and Materials and Methods. (- - - - -) a theoretical curve with a rate constant of $k = 0.078$ is drawn according to Figure 4 in Fan and Baltimore (1973); the principles of the derivation are given in the text.

petitive sequences (Firtel *et al.*, 1973). To determine if the cDNA products represent the same distribution of nucleotide sequences as the mRNA, hybridization kinetics were performed using excess cell DNA and tracer amounts of cDNA product. Figure 4 shows the hybridization kinetics of the oligo(dT)- and oligo(dG)-primed products. The oligo(dT)-primed product showed hybridization kinetics similar to those of mRNA but with a slightly increased portion of the nucleotide sequences hybridizing to repetitive sequences. The second-order rate constant of hybridization for the cDNA products to nDNA is approximately two times that for mRNA to DNA. This discrepancy has also been reported for other systems (Bishop, 1972). The oligo(dG)-primed product, however, shows quite different kinetics indicating approximately 35% of the sequences are complementary to repetitive DNA, 40% to the single-copy DNA, and 25% are not represented in cell DNA. The reason for the odd behavior of oligo(dG)-primed DNA is not clear.

(*e*) *Excess DNA–RNA Hybridization.* In order to determine what fraction of the RNA template sequences are transcribed into a cDNA product, hybridization experiments were performed using excess DNA product. In these experiments a large excess of cDNA was hybridized to mRNA and the fraction of mRNA hybridized to the DNA was determined by treatment with ribonuclease. The results in Table IV indicate that approximately 93% of the mRNA sequences were protected with an excess of cDNA product. This result indicates that the majority of the sequences of the template are copied. It is not clear from these data whether some of the RNA sequences are represented in the DNA in a concentration higher than they are present in the template RNA.

(*f*) *Genetic Complexity of RNA Template.* In the experiment depicted in Figure 5, labeled cDNA was hybridized to an excess of the vegetative mRNA template from which it was copied. Under these conditions the rate of hybridization of any cDNA sequence is proportional to the concentration of the complementary mRNA sequence; the fraction of DNA hybridized is plotted *vs.* the product of RNA concentration and time. In order to interpret this experimental curve, a theoretical curve was derived assuming that all mRNAs occur in equimolar amounts, that 10% of the nuclear single-copy DNA sequences are represented in the mRNA (*i.e.*, that the kinetic complexity of the mRNA is 10% that of single-copy *Dictyostelium* DNA), and that the cDNA is a representative copy of the mRNA. The value of 10% was chosen so that the theoretical curve would cross the experimental curve at 50% hybridization. This 10% value is close to the experimental value of 15% transcription of single-copy DNA found previously (Firtel, 1972).

Comparing the experimental and theoretical curves, it appears that about 20% of the labeled cDNA hybridizes at a faster rate than predicted. This means that the mRNA sequences complementary to these cDNA sequences are present in the mRNA in greater than average proportion. Likewise, about 30% of the cDNA hybridizes to RNA at about a fivefold slower rate than predicted; this means that about 30% of the mRNA sequences are present in a concentration only about 1/5th that of the average. The difference between the theoretical and experimental curves is meaningful because a comparison of such curves for RNA tumor virus 70S RNA showed an almost exact overlap of the theoretical and experimental curves (Fan and Baltimore, 1973).

RNA-driven hybridization experiments have previously indicated the presence of RNA sequences occurring in low concentrations (Firtel, 1972). It should be noted, however, that the previous experiments (Firtel, 1972) were done with total cell

TABLE IV: Protection of the mRNA with cDNA.[a]

cDNA Present during Annealing	% RNase Resistant after Annealing
−	8.5[b]
+	96

[a] 1.2 μg of oligo(dT)–DNA complementary to mRNA from vegetative cells was hybridized to 1–3 ng of vegetative [^{32}P]-mRNA (4837 cpm) in 3 μl of 0.6 M phosphate buffer. After hybridization for 20 hr at 67° (approximate equivalent C_0t of 550), the hybrids were treated with 20 μg of RNase A/ml and 20 units of RNase T_1/μl. [b] RNase resistance is due to poly(A).

RNA while the present one utilizes purified mRNA and it is not necessarily true that the results will be comparable.

In the present report the mRNA has been defined as the polysomal poly(A) containing RNA species. Any mRNA devoid of a poly(A) stretch at its 3′-OH end will not be utilized as a template to synthesize cDNA.

(*g*) *Cross Hybridization of Development Stage Complementary DNA to mRNA from Vegetative Stage.* The ability to synthesize cDNA from mRNA with a high efficiency enables us to examine changes in the DNA sequences transcribed at various stages in differentiation. Complementary DNA synthesized from poly(A)-containing cytoplasmic heterogeneous RNA (mRNA) from cells at 18 hr of development (early culmination stage) was hybridized to 18-hr mRNA (template) and vegetative mRNA to determine the differences between the sequences present at both stages. The results in Table V show that the mRNA template isolated from 18-hr cells protects 93–94% of the cDNA while mRNA from vegetative cells protects only 53%. These results indicate that vegetative RNA contains some nucleotide sequences of the total mRNA sequences present at 18 hr of development. More importantly, it appears that some of the 18-hr mRNA represents mRNA sequences transcribed since differentiation was initiated. A more detailed study of hybridization kinetics should be able to determine the precise changes in the patterns of mRNA sequences.

TABLE V: Hybridization of Developmental Stage cDNA to mRNA from Vegetative and Developmental Cells.[a]

Type of mRNA Present during Annealing	% Nuclease Resistant after Annealing
None	7
18-hr mRNA	94
Vegetative mRNA	54

[a] Approximately 0.01 μg of oligo(dT)-stimulated ^{32}P-labeled DNA complementary to mRNA from cells at 18 hr of development was hybridized with 30 μg of mRNA from either 18-hr cells or vegetative cells as described in the legend to Table III.

Discussion

The results described in this paper show that *Dictyostelium* mRNA can be faithfully transcribed into complementary DNA with AMV DNA polymerase. The size of the (dT) and (dC)-primed DNA products suggests that at least some of the DNA molecules are approximately the same size as the mRNA template and represent complete transcripts. Hybridization to nuclear DNA indicates that the DNA made using a (dT)-primer has essentially the same sequence representation as does the mRNA template. The cDNA also contains over 90% of the nucleotide sequences present in the mRNA as shown by the ability of DNA to protect labeled mRNA from RNase digestion after hybridization. It is not clear if this result is due to the presence of some molecules of the same length of the mRNA template or due to internal starts other than at the 3′ poly(A) sequence. The high efficiency of DNA synthesis may be due to low G + C content of the mRNAs (R. A. Firtel and A. Jacobson, unpublished observations) and hence less secondary structure. The inability of mRNA to render oligo(dG)–DNA completely resistant to S_1 nuclease is unexplained.

We have shown that hybridization of 18-hr mRNA to DNA complementary to 18-hr mRNA protects over 93% of labeled cDNA while mRNA sequences from vegetative cells protect only 53–54% of cDNA to 18-hr mRNA. Thus a portion of mRNA nucleotide sequences is the same in both stages. RNA driven hybridizations of purified single-copy DNA to whole cell RNA had indicated a similar difference between the RNA present in vegetative cells and 18-hr cells (Firtel, 1972). The main difficulty in comparing these numbers is that the RNA driven hybridizations examines the vast majority of RNA sequences regardless of their relative concentrations. The hybridizations with labeled cDNA are biased for those mRNA sequences present at high concentrations or those preferentially transcribed into cDNA.

The ability to synthesize DNA complementary to *Dictyostelium* mRNA will serve as a very potent tool for studying the control of mRNA biosynthesis and metabolism. The fact that one can achieve a very high efficiency of synthesis will enable the cDNA to be used as a probe for newly synthesized RNA sequences. We have already shown by hybridization of cDNA to HnRNA (Lodish *et al.*, 1973) that the *Dictyostelium* HnRNA contains sequences not transported to the cytoplasm and that these sequences are covalently associated with the transported sequences. The results agree well with other evidence on the fraction of the sequences of the HnRNA which are not transported (Firtel and Lodish, 1973).

The ability to faithfully synthesize transcripts of total mRNA provide a very useful method to examine the regulation of development particularly when stage specific mRNAs are isolated.

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Characterization of a Pair of Isopycnic Twin Crustacean Satellite Deoxyribonucleic Acids, One of Which Lacks One Base in Each Strand†

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ABSTRACT: Two double-stranded DNAs (satellites I and II) of identical density, both containing unusually large porportions of deoxyguanylate and deoxycytidylate, have been isolated from four tissues of the hermit crab, *Pagurus pollicaris.* They have been separated from each other by the use of Ag^+ + Hg^{2+} – Cs_2SO_4 gradients and characterized as to t_m; the densities of the native double stranded and denatured single stranded DNAs have been determined. Base compositional analyses indicate that satellite DNA I has one strand containing 50% deoxyguanylate residues but is essentially free of deoxycytidylate (<3%); compositional analyses of the other strand give 47% deoxycytidylate and less than 2% deoxyguanylate. The distribution of the radioiodinated deoxyguanylate-rich strand of satellite I in alkaline CsCl indicates that deoxycytidylate is present in only trace amounts, if at all, in that strand. Assuming complementarity, the opposite strand should therefore be lacking in deoxyguanylate residues. In the other satellite (II), the divergence of the composition of the two strands is less severe (dG:dC = 36%:26% and 28%:32%, respectively).

We have previously described crustacean "satellite" DNAs rich in either dA + dT or dG + dC residues, the single strands of which are either nonbiased (Skinner, 1967; Beattie and Skinner, 1972) or biased (Beattie and Skinner, 1972) in base composition. We report here the physical and chemical characteristics of two (dG + dC)-rich satellites that make up less than 1% of the total DNA of various tissues (gonads, epidermis, hemocytes, and nerve) of the hermit crab, *Pagurus pollicaris.* Although the two satellite DNAs have identical densities in neutral CsCl gradients (such that we refer to them as "isopycnic twins"), they can be completely separated from each other in Cs_2SO_4 gradients containing both mercury and silver salts (Skinner and Beattie, 1973). The complementary strands of satellite I have very different base compositions, one being essentially free of guanine residues, the other of cytosine residues; the base-compositional bias of the individual strands of satellite II is less extreme.

Included here are data on the characterization of the iospycnic twins, including thermal dissociation and reassociation characteristics and the densities of the separated strands and their base compositions. In another paper (Skinner *et al.,* 1974), we present the nucleotide sequence of satellite I which is a repeating tetramer (5′-T-A-G-G-3′), and its complement, identical with part of the hexameric sequence that accounts for 50% of the sequences of the α satellite of the guinea pig, *Cavia porcellus* (Southern, 1970), and that may also be present in the HS-α satellite of the kangaroo rat, *Dipodomys ordii* (W. Salser and K. Fry, in press).

Materials and Methods

Animals. Hermit crabs were obtained from the staff of the Marine Biological Laboratory, Woods Hole, Mass. Guinea pig tissues were the kind gift of Dr. M. G. Hanna.

Isolation of DNAs. DNA was isolated from nervous tissue (eyestalk ganglia), testes, and hemocytes of hermit crabs according to methods previously described (Skinner, 1967; Skinner *et al.,* 1970) and from ovaries and epidermis by a modification of the Marmur (1961) method. Ovaries and epidermis were homogenized in 0.1 M NaCl–0.05 M EDTA (pH 8) and centrifuged at 9750*g* for 15 min to collect nuclei, which were resuspended in 0.1 M NaCl–0.05 M EDTA and made 8% in sodium dodecyl sulfate and 1 M in $NaClO_4$. Repeated deproteinizations with isoamyl alcohol–chloroform (Sevag *et al.,* 1938) followed. Preparations were then treated with RNase and Pronase as described previously (Skinner, 1967). Guinea pig DNA was isolated according to the procedure of Britten *et al.* (1970). Liver and kidney (20 g) from an adult male were mixed, as were testes and vas deferens (5 g). Tissues were finely minced and rinsed three times in 0.1 M NaCl–0.05 M EDTA to remove blood. They were then homogenized in an Omnimixer (Sorvall) in 5 volumes of a solution containing 8 M urea, 0.2 M sodium phosphate buffer (pH 6.8), 0.25% sodium dodecyl sulfate, and 3 mM EDTA. To the homogenate was added 5 volumes of hydroxylapatite (Bio-Rad, Bio-Gel HT). The mixture was shaken for 5 min to permit the DNA to bind to the hydrox-

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