Drinkwater, N. R., Miller, J. A., Miller, E. C., & Yang, N. C. (1978) *Cancer Res. 38*, 3247–3255.
Gamper, H. B., Tung, S.-C. A., Straub, K., Bartholomew, J., & Calvin, M. (1977) *Science 197*, 671–674.
Gerchman, L. L., & Ludlum, D. B. (1973) *Biochim. Biophys. Acta 308*, 310–316.
Grunberger, D., & Weinstein, I. B. (1979) in *Chemical Carcinogens and DNA* (Grover, P., Ed.) pp 60–93, CRC Press, Boca Raton, FL.
Johnson, P. H., & Grossman, L. I. (1977) *Biochemistry 16*, 4217–4225.
Kakefuda, T., & Yamamoto, H. A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 415–419.
King, H. W., Osborne, M. R., & Brookes, P. (1979) *Chem.-Biol. Interact. 24*, 345–353.
Kohn, K. W. (1979) *Methods Cancer Res. 16*, 291–345.
Kohn, K. W., & Grimek-Ewig, R. (1973) *Cancer Res. 33*, 1849–1853.
Kriek, E. (1974) *Biochim. Biophys. Acta 355*, 177–203.
Kuhnlein, U., Tsang, S. S., & Edwards, J. (1979) *Mutat. Res. 64*, 167–182.
Lawley, P. D. (1976) *ACS Monogr. No. 173*, 83–244.
Lawley, P. D., & Brookes, P. (1963) *Biochem. J. 89*, 127–138.
Lawley, P. D., & Shah, S. A. (1972) *Biochem. J. 128*, 117–132.
Lawley, P. D., & Warren, W. (1976) *Chem.-Biol. Interact. 12*, 211–220.
Lin, J. K., Miller, J. A., & Miller, E. C. (1975) *Cancer Res. 35*, 844–850.
Lindahl, T. (1979) *Prog. Nucleic Acid Res. Mol. Biol. 22*, 135–192.
Lindahl, T., & Andersson, A. (1972) *Biochemistry 11*, 3618–3623.
Lotlikar, P. D., Scribner, J. D., Miller, J. A., & Miller, E. C. (1966) *Life Sci. 5*, 1263–1269.
Loveless, A. (1969) *Nature (London) 223*, 206–207.
McCann, J., Choi, E., Yamasaki, E., & Ames, B. N. (1975a) *Proc. Natl. Acad. Sci. U.S.A. 72*, 5135–5139.
McCann, J., Spingarn, N. E., Kobori, J., & Ames, B. N. (1975b) *Proc. Natl. Acad. Sci. U.S.A. 72*, 979–983.
Miller, E. C. (1978) *Cancer Res. 38*, 1479–1496.
Mizusawa, H., Tanaka, S., Kobayashi, M., & Koike, K. (1977) *Biochem. Biophys. Res. Commun. 74*, 570–576.
Osborne, M. R., Harvey, R. G., & Brookes, P. (1978) *Chem.-Biol. Interact. 20*, 123–130.
Phillips, D. H., Miller, E. C., & Miller, J. A. (1980) *Proc. Am. Assoc. Cancer Res. 21*, 65.
Poirier, L. A., Miller, J. A., Miller, E. C., & Sato, K. (1967) *Cancer Res. 27*, 1600–1613.
Shearman, C. W., & Loeb, L. A. (1979) *J. Mol. Biol. 128*, 197–218.
Shooter, K. V. (1976) *Chem.-Biol. Interact. 13*, 151–163.
Singer, B. (1975) *Prog. Nucleic Acid Res. Mol. Biol. 15*, 219–284.
Singer, B. (1977) *J. Toxicol. Environ. Health 2*, 1279–1295.
Singer, B. (1979) *J. Natl. Cancer Inst. 62*, 1329–1339.
Singer, B., Kroger, M., & Carrano, M. (1978) *Biochemistry 17*, 1246–1250.
Swenson, D. H., & Lawley, P. D. (1978) *Biochem. J. 171*, 575–587.
Tarpley, W. G., Miller, J. A., & Miller, E. C. (1980) *Cancer Res. 40*, 2493–2499.
Thielmann, H. W. (1977) *Z. Krebsforsch. Klin. Onkol. 90*, 37–69.
Wilkinson, R., Hawks, A., & Pegg, A. E. (1975) *Chem.-Biol. Interact. 10*, 157–167.
Wislocki, P. G., Miller, J. A., & Miller, E. C. (1975) *Cancer Res. 35*, 880–885.

Isolation and Characterization of Complementary Deoxyribonucleic Acid Complementary to the Highly Abundant Class of Poly(adenylic acid)-Containing Ribonucleic Acid from Oocytes of *Drosophila melanogaster*†

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ABSTRACT: The complementary deoxyribonucleic acid (cDNA) complementary to the highly abundant class of poly(adenylic acid)-containing ribonucleic acid [poly(A+) RNA] from *Drosophila malanogaster* oocytes has been isolated and characterized. Analysis of the kinetics of hybridization of this cDNA ($cDNA_{HA}$) to total poly(A+) RNA of oocytes indicates this class contains ~86 different sequences. Hybridization kinetics of $cDNA_{HA}$ annealed to poly(A+) RNA from 19-h-old embryos is essentially the same as that of oocyte poly(A+) RNA. This suggests the highly abundant class of poly(A+) RNA persists in approximately the same frequency through early development. Analysis of the hybridization of $cDNA_{HA}$ to genomic DNA suggests that the highly abundant poly(A+) RNA from oocytes is not enriched for transcripts from repetitive sequences of the genome.

Our approach to the investigation of the early developmental processes in *Drosophila melanogaster* has centered on the programmed use of stored maternal messenger RNA. Early embryogenesis (pregastrular) is dependent upon the informational molecules placed in the oocyte during oogenesis (Lovett & Goldstein, 1977), since very little zygotic transcription is believed to occur during the first 2 h after egg deposition (Anderson & Lengyel, 1979; Lamb & Laird, 1976; Zalokar, 1976). Thus the oocyte provides an excellent opportunity to investigate the role of both maternal and zygotic

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messenger RNA in early development and will eventually lead to the elucidation of the mechanisms involved in their regulation.

To this end, this laboratory has been engaged in the characterization of the poly(A+) RNA[1] present in oocytes and the changes that occur during embryogenesis. We had previously shown by kinetic analysis that the poly(A+) RNA of mature stage 14B *Drosophila* oocytes contains ~14 500 different sequences that fall into three frequency classes: a highly abundant fraction of 102 sequences, an abundant fraction containing 2877 sequences, and a rare fraction containing 11 510 sequences (Arthur et al., 1979). Of the total poly(A+) RNA, only 40% is in polysomes; the remainder is found in nonpolysomal ribonucleoprotein particles (RNP's). During development, the percent of poly(A+) RNA found in polysomes increases to 70%. A comparison of sequences found in polysomes with those in nonpolysomal RNP particles indicates that the latter represents a surplus supply (Goldstein, 1978). During the development from oocyte to late prehatching embryo (19 h), 5.3% of the total number of sequences present in the oocyte disappear. In gastrulae 7.7% of the sequences present are not represented in the oocyte, and at late prehatching 11.0% of the poly(A+) RNA sequences are not present in the oocyte (Arthur et al., 1979). cDNA complementary to the 5.3% of the sequences that disappear (transient maternal sequences) has been isolated and found to represent 44 ± 14 different sequences (Goldstein & Arthur, 1979).

In order to further characterize the poly(A+) RNA complement of the *Drosophila* oocyte, we have isolated cDNA complementary to the highly abundant component. This class is represented by ~86 ± 21 different sequences. By 19 h of embryogenesis, essentially the same sequences remain at approximately the same frequency. Therefore, this class of gene products is an ubiquitous class of poly(A+) RNA sequences throughout embryonic development. In addition, we have evidence that this ubiquitous class of RNA is derived from nonrepetitive sequences in the genome.

Materials and Methods

Collection of Oocytes and Embryos. D. melanogaster used in this study was an Oregon R strain derived from a $y^+ \cdot Y \cdot B^s$ stock (Goldstein & Snyder, 1973). Stage 14B oocytes were collected from adults 3–4 days old that had been fed heavily on baker's yeast for 24 h. Flies were homogenized in insect Ringer's solution (Doane, 1967) and poured through sieves, and the oocytes were further purified by differential settling and adhesion to glass. The final purity of stage 14 oocytes isolated in this manner exceeds 95% (Lovett & Goldstein, 1977). Fertilized eggs were collected on agar plates smeared with baker's yeast. Four-hour collections aged for 17 h yielded 19 ± 2 h old embryos.

Isolation and Characterization of Poly(A+) RNA. Oocytes or embryos were rapidly homogenized in extraction buffer (35 mM K–Hepes, pH 7.4, 75 mM KCl, 5 mM $MgCl_2$, and 4 mM dithiothreitol) containing 2–4% diethyl pyrocarbonate, followed by centrifugation at 8000*g* for 10 min (Goldstein & Synder, 1972). The postmitochondrial supernatant fraction was extracted with phenol–chloroform, precipitated in ethanol, and resuspended, and the poly(A+) RNA was isolated by chromatography on oligo(dT)–cellulose (Collaborative Research) (Kaufman et al., 1976; Arthur et al., 1979).

The quantification of poly(A+) RNA was based on its hybridization with [^{3}H]poly(U) (22.3 mCi/mol, Schwarz/Mann), followed by digestion of single-stranded material with pancreatic RNase (Sigma Chemical Co.). Known poly(A) standards (Miles Laboratories), assayed in the same manner, served as the basis for the determination of cpm per nanogram of [^{3}H]poly(U) bound. To establish poly(A+) RNA size distribution, we centrifuged an aliquot of the poly(A+) RNA fraction at 24 °C on a linear 15–30% sucrose gradient in 0.1% $NaDodSO_4$ in a Spinco SW 27 rotor (Milcarek et al., 1974). The average nucleotide length of the RNA in each preparation was calculated from the distribution of [^{3}H]poly(U) binding across the gradient. Any preparation displaying either visible ribosomal RNA (rRNA) contamination as judged by absorbance at 260 nm or degradation as judged by distribution of poly(A+) RNA using a [^{3}H]poly(U) hybridization assay was not used. The size of the poly(A) tails was established by the digestion of total oocyte cytoplasmic A+ RNA with pancreatic RNase and T_1 RNase (Sigma), followed by electrophoresis in 10% polyacrylamide–$NaDodSO_4$ disc gels. Poly(A) tracts of 120, 54, and 28 (average) nucleotides (Miles Laboratories) served as size standards (Arthur et al., 1979).

Preparation of Drosophila DNA. Frozen embryos 2–20 h old were thawed and homogenized in DNA extraction buffer (50 mM K–Hepes, pH 7.4, 100 mM KCl, and 15 mM $MgCl_2$) with a motor-driven Teflon pestle in a Potter homogenizer. The homogenate was poured through sieves and rinsed with 0.1 M KCl, and the nuclei were isolated by differential centrifugation. The nuclear preparation was homogenized in 1 × NaCl/Cit containing 1% $NaDodSO_4$ and 2–4% diethyl pyrocarbonate and gently extracted 3 times with phenol–chloroform. The DNA was wound out of ethanol, resuspended in 1 × NaCl/Cit, and treated successively with pancreatic RNase (Sigma) and proteinase K (Merck). After a second extraction in phenol–chloroform, the DNA was wound out of ethanol and resuspended in column buffer (0.5 M NaCl, 50 mM Tris-HCl, pH 8, and 1.0 mM EDTA) to an approximate concentration of 1 mg/mL. The DNA was sonicated at 0 °C at the maximum setting for 9 min with a Bronwill sonicator. Sonicated DNA (~5 mg) was then applied to a 2.5 × 45 cm column of Sepharose CL-2B-300 (Pharmacia). Fractions were collected and analyzed by electrophoresis in 1.4% agarose gels using a *Hin*dIII digest of phage λ (Miles Laboratories) as size markers. The gels were visualized by using ethidium bromide, and appropriate fractions containing DNA of 500–1500 base pairs in length were pooled, precipitated, and resuspended in hybridization buffer (0.24 M sodium phosphate buffer and 2.0 mM EDTA).

Synthesis of cDNA. [^{3}H]cDNA was prepared by using oocyte poly(A+) RNA as a template for avian myoblastosis virus (AMV) reverse transcriptase (the generous gift of Dr. J. H. Beard). Globin cDNA was synthesized from rabbit globin mRNA (Miles Laboratories). The reaction mixture consisted of 500 μCi of [^{3}H]dCTP (20.9 Ci/mmol, ICN Pharmaceuticals), 10 mM dithiothreitol, 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, 5 μg/mL oligo$(dT)_{12-18}$ (Collaborative Research), 10 μg/mL actinomycin D, and 1 mM each of dATP, dGTP, and dTTP (Sigma) in a final reaction volume of 2.0 mL. Oocyte poly(A+) RNA (20 μg) and AMV reverse transcriptase (150 units) were added to the reaction vial, and the mixture was incubated at 37 °C for 1 h (Goldstein & Arthur, 1979). An aliquot of the cDNA was centrifuged at 37 000 rpm for 24 h at 5 °C in a Spinco SW 41 rotor on

[1] Abbreviations used: poly(A+) RNA, poly(adenylic acid)-containing RNA; cDNA, complementary deoxyribonucleic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; NaCl/Cit, standard saline citrate; kb, kilobase; Hb, hemoglobin.

alkaline sucrose gradients (8–18% w/v in 0.1 N NaOH). Size determination was made by comparison to a parallel gradient containing a *Hin*dIII digest of phage λ. Separate analyses in 1.4% agarose gels were also performed and confirmed the size determined by centrifugation analysis. All cDNA reactions were carried out in siliconized glassware.

Fractionation of cDNA. cDNA complementary to the highly abundant class of poly(A+) RNA was isolated from preparations containing cDNA made from total oocyte poly(A+) RNA. Alternately, preparations previously depleted for sequences present in the oocyte but absent in the late embryo (transient maternal sequences) were used (Goldstein & Arthur, 1979). Isolation of abundant fractions was accomplished by hybridization of the cDNA with a 500–1000-fold excess of total oocyte poly(A+) RNA to an appropriate R_0t in 0.24 M sodium phosphate buffer, pH 6.8, 0.2% $NaDodSO_4$, and 2.0 mM EDTA (hybridization buffer) at 70 °C. Distilled water at 0 °C was added to terminate hybridization and to reduce the buffer concentration to 0.03 M sodium phosphate buffer. Hydroxylapatite (0.5 g) in 0.03 M sodium phosphate buffer was immediately added and the suspension was gently shaken at room temperature for 30 min (Hames & Perry, 1977). An aliquot of the hybridization mixture was analyzed for double-stranded material with S-1 nuclease (Sigma) (Milcarek et al., 1974). The flask was then placed in a 60 °C water bath to allow temperature equilibration, and the suspension was pipetted into a 60 °C water-jacketed column. The column was washed with four 5-mL fractions of 0.12 M sodium phosphate buffer until the amount of radioactivity in each successive wash leveled off. The double-stranded material was eluted with 5 mL of 0.5 M sodium phosphate buffer, brought to 100 °C in 0.3 M NaOH for 10 min, neutralized in HCl, and dialyzed overnight against 100 mM NaCl and 10 mM Tris-HCl, pH 7.4, at room temperature. The dialysate was precipitated in ethanol and recovered by ultracentrifugation in polyallomer tubes. The cDNA was resuspended in a small volume of hybridization buffer.

Hybridization Reactions. Poly(A+) RNA–[^{3}H]cDNA hybridizations were carried out in hybridization buffer in siliconized 2-mL reactivials (Pierce Chemicals) overlaid with paraffin oil. The reaction solution contained a 500–1000-fold excess of poly(A+) RNA. All reactions were initiated by heating the solutions to 100 °C for 5 min and then allowed to proceed at 70 °C. Aliquots containing ~2000 cpm of [^{3}H]cDNA were taken at appropriate time intervals during the course of the reaction and assayed for double-stranded material by S-1 nuclease digestion (Milcarek et al., 1974).

DNA–[^{3}H]cDNA hybridizations were carried out at 60 °C in 0.5-mL siliconized reactivials (Pierce Chemicals) overlaid with paraffin oil in hybridization buffer or, alternately, for reaction rate acceleration, in high-salt sodium phosphate buffer containing 0.743 M NaCl. Aliquots containing 2000 cpm of [^{3}H]cDNA were stored at −90 °C in capped 2-mL plastic tubes. The samples were diluted into 0.12 M sodium phosphate buffer and loaded onto hydroxylapatite columns preequilibrated in 0.12 M sodium phosphate buffer at 60 °C. Double-stranded material was eluted with 0.5 M sodium phosphate buffer, collected on Millipore filters, and counted in a liquid scintillation counter.

Results

Characterization of RNA and DNA. Accurate analysis of RNA- or DNA-driven hybridization kinetics requires that the size of these nucleic acids be determined. The poly(A+) RNA fractions used in this study were characterized for size by $NaDodSO_4$–sucrose gradient sedimentation analysis. The length of the RNA in all preparations was determined to be 1500–2000 nucleotides as judged by the distribution of A_{260nm} material and by hybridization of [^{3}H]poly(U) to poly(A) tails. The length of the poly(A) sequences was determined by analysis on polyacrylamide gels by using standard poly(A) nucleotide sequences of 28, 54, and 120 (average) residues for comparison (Hirsch & Penman, 1974). In these determinations, the average number of nucleotides in the poly(A) tail was found to be 52, or ~2.5% of total poly(A+) RNA length. This value was then used to calculate the amount of poly(A+) RNA in subsequent preparations from [^{3}H]poly[U] hybridization data. The average length of cDNA in each preparation used for hybridization studies was determined separately by alkaline sucrose gradient ultracentrifugation analysis. A *Hin*dIII digest of phage λ was run on parallel gradients, and, based on the position of the 2.2- and 2.5-kilobase fragments, the length of the cDNA used in these experiments averaged 450–620 nucleotides for different preparations.

Table I: Sequence Complexity of cDNA Complementary to Highly Abundant Poly(A$^+$) RNA of Mature Oocyte

expt	$R_0t_{1/2}$(app)	$R_0t_{1/2}$(real)	no. of different sequences[a]
1	0.054	0.041	89
2	0.130	0.085	57
3	0.062	0.043	94
4	0.090	0.068	105
av			86 ± 21

[a] Computation of the number of sequences is calculated as follows: $R_0t_{1/2}$(Hb std)/$R_0t_{1/2}$(*Drosophila*) = 1/x, where the $R_0t_{1/2}$ of the Hb standard is corrected for the length of cDNA and mRNA used in each individual preparation (Bishop et al., 1974; Hereford & Roshbash, 1977), such that $(4.0 \times 10^{-4})^a(2000/1320)^b(470/620)^c = 4.61 \times 10^{-4}$, where a is the $R_0t_{1/2}$ of Hb standard (α and β), b is the RNA length correction, and c is the cDNA length correction.

Fractionation and Characterization of Highly Abundant cDNA. The cDNA corresponding to the highly abundant class of poly(A+) RNA ($cDNA_{HA}$) was isolated from cDNA made to the total oocyte poly(A+) RNA complement or from total cDNA from which a middle abundant class had been removed. The kinetics of hybridization of the total cDNA fractions to its homologous poly(A+) RNA is shown for comparison in Figure 1a (Arthur et al., 1979). A computer analysis (UCLA Biomedical Program Series BMD P3R) of the data had indicated that the highly abundant class of poly(A+) RNA comprised ~22% with a transition point at a R_0t of 2.0. To isolate the $cDNA_{HA}$ fraction, we terminated hybridization between the cDNA and total poly(A+) RNA at a total RNA R_0t of 2.0. The double-stranded sequences were isolated by hydroxylapatite chromatography. The proportion of double-stranded material was determined by assaying the S-1 sensitivity of an aliquot taken from the reaction solution prior to chromatography.

The results of the hybridization of total oocyte poly(A+) RNA to the $cDNA_{HA}$ fraction are shown in Figure 1b. Both the total poly(A+) RNA R_0t and the R_0t of the highly abundant RNA sequences (22% of the total R_0t) are indicated on the abscissa. The reaction of α and β globin mRNAs with their homologous cDNAs is included as a sequence complexity standard. Examination of the curve constructed from the kinetic analysis reveals a single RNA component; no superabundant class of RNA is evident. The hybridization reaction reaches a plateau within 2 log units of R_0t, indicative of a single frequency class.

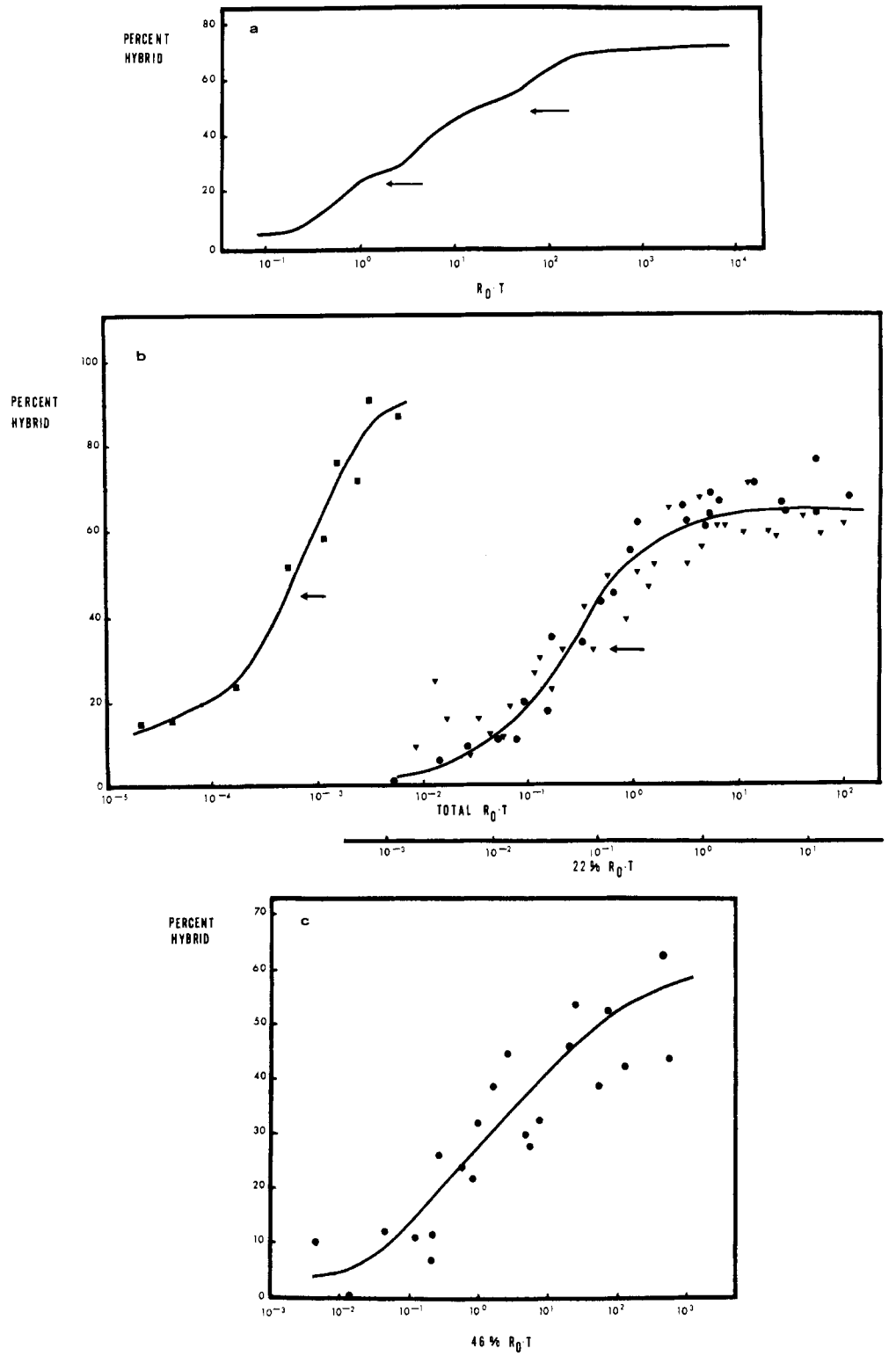

FIGURE 1: Hybridization of cDNA to poly(A+) RNA. All reactions were performed as indicated under Materials and Methods. The percent double-strandedness was determined by sensitivity to S-1 nuclease digestion. (Abscissa) R_0t; (Ordinate) percent double-strandedness. (a) Hybridization of oocyte cDNA to oocyte poly(A+) RNA. Arrows indicate the computer-derived transitions between each class (Arthur et al., 1979). (b) Hybridization of cDNA to poly(A+) RNA. Hybridization of $cDNA_{HA}$ to oocyte poly(A+) RNA (●) and to poly(A+) RNA from 19-h-old embryos (▼). Hybridization of globin mRNA (α and β) to globin cDNA (■). The R_0t values on the abscissa represent two R_0t scales: the R_0t for total poly(A+) RNA and the R_0t for the 22% of the poly(A+) RNA that is the highly abundant class. (c) Hybridization of the cDNA that remains following the isolation of $cDNA_{HA}$ to oocyte poly(A+) RNA. The R_0t values shown are for the 46% of the poly(A^+) RNA that remains.

The cDNA that remains after the highly abundant cDNA has been isolated should contain sequences of the middle abundant and rare frequency classes. This fraction was subjected to kinetic analysis by hybridization to total oocyte poly(A+) RNA. The results of this analysis are presented in Figure 1c. The kinetics of hybridization contrast sharply with that displayed by the $cDNA_{HA}$ fraction. The remaining cDNA appears to represent more than one frequency class since terminal hybridization requires more than 4 log units of R_0t. It appears that some rapidly hybridizable material remains.

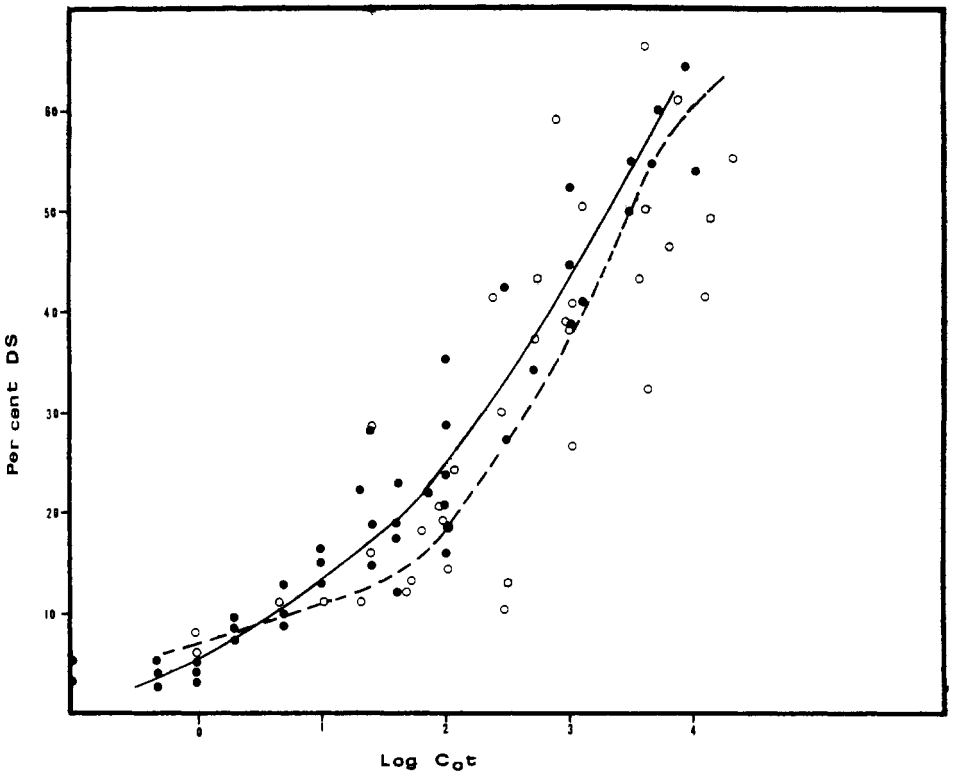

FIGURE 2: Hybridization of cDNA to sheared genomic DNA. Hybridizations were performed as described under Materials and Methods. Percent double-strandedness was determined by using hydroxylapatite chromatography. Hybridization of sheared DNA to total oocyte cDNA (●) and to $cDNA_{HA}$ (○).

However, this is not unexpected since the termination of the isolation hybridization occurred at a point slightly below 22%.

The sequence complexity of the highly abundant cDNA fraction was calculated from R_0t values based on the 22% of the poly(A+) RNA which is highly abundant by using the globin standard. Table I is a summary of the data derived from four kinetic analyses using four different DNA_{HA} preparations. Calculations based on these data indicate that the highly abundant fraction of oocyte poly(A+) RNA is represented by 86 ± 21 different sequences, a value consistent with previous sequence complexity determinations using total rather than fractionated cDNA.

For further characterization of the distribution of sequences within the highly abundant class, the cDNA that became double-stranded was isolated from a reaction that was terminated at a R_0t of 1×10^{-1}, which corresponds to 10% hybridization. Kinetic analysis of the hybridization of this fraction to total oocyte poly(A+) RNA yielded results very similar to the results from an analysis using the 20% cDNA fraction. This would suggest that no continuum of frequency classes exists within the highly abundant class, and the different sequences appear to be nearly equally represented.

$cDNA_{HA}$ was hybridized to the poly(A+) RNA extracted from 19-h-old embryos to determine if the highly abundant oocyte sequences change in frequency during development. The results of this experiment are presented in Figure 1b. When the kinetics of this heterologous hybridization are compared to the kinetics of hybridization between $cDNA_{HA}$ and oocyte poly(A+) RNA, small differences are observed. However, these are no greater in magnitude than differences observed between two different homologous hybridization reactions using two different $cDNA_{HA}$ preparations. Therefore, it appears that the highly abundant class of poly(A+) RNA in oocytes changes very little in composition, if at all, between the oocyte and 19 h of development.

C_0t Analysis. The proportion of single- and multiple-copy DNA sequences which serve as templates for cytoplasmic RNA can be determined by hybridization kinetics. cDNA transcribed from total oocyte poly(A+) RNA was hybridized to 1-kb (av) embryonic genomic DNA. Figure 2 presents the results of this analysis. The first 20–30% of the cDNA in this reaction hybridizes at a rapid rate, indicative of repetitive sequences, while the remainder reacts with the kinetics characteristics of unique sequence DNA. When $cDNA_{HA}$ is used as a driver in a similar reaction, it appears to react with similar kinetics. Thus, the $cDNA_{HA}$ does not appear to be enriched for sequences representative of repetitive genomic DNA.

Discussion

In this paper we report the isolation and characterization of cDNA complementary to the major abundance class of poly(A+) RNA from mature stage 14B oocytes of *D. melanogaster*. Analysis of the kinetics of hybridization of this cDNA to excess oocyte poly(A+) RNA has indicated that the poly(A+) RNA in this class is composed of ~86 different sequences. Heterologous hybridization with the poly(A+) RNA from 19-h-old prehatching embryos shows that these sequences are still present and, moreover, are in approximately the same frequency as in the oocyte. This suggests that they comprise an ubiquitous class and may perform housekeeping functions, at least during prelarval development.

An examination of the curve constructed from the hybridization of the isolated cDNA to total poly(A+) RNA indicates that only one frequency class is represented. A single kinetic component is discerned, occupying only 2 log units of R_0t. The hybridization kinetics of this cDNA fraction contrast with the kinetic analysis of the cDNA that remained following the isolation of the highly abundant sequences. The unreacted cDNA contains the middle abundant and rare class sequences,

and the kinetics of hybridization reflect this complex composition. Terminal hybridization of this complex class requires more than 4 R_0t log units, an expected result based on the assumption of more than one frequency class.

The number of sequences comprising the highly abundant class as determined from $R_0t_{1/2}$ data should be considered an approximation. A variety of assumptions have been made in the determination of the number of sequences that compose this class. The terminal point of hybridization in the original isolation of the highly abundant cDNA was determined from the kinetic analysis of the hybridization of total oocyte cDNA with total oocyte poly(A+) RNA. In this computer-assisted analysis (Arthur et al., 1979), it was determined that ~22% of the oocyte cDNA represented the highly abundant RNA sequences. Therefore, the number of sequences included in the highly abundant class is a function of the R_0t value that corresponds to this 22% hybridization value. The assumption has also been made that the AMV reverse transcriptase copies polyadenylated RNA regardless of its relative abundance, thus producing a population of cDNA molecules that truly reflects the composition of the RNA molecules. Although this supposition is probably not totally valid, the premise will allow the derivation of approximate sequence complexity number.

In the previous study from this laboratory using total oocyte cDNA and poly(A+) RNA, hybridization kinetic analysis had estimated the number of sequences in the highly abundant class at ~100 (Arthur et al., 1979). Kinetic analysis of the isolated fraction as reported here yields a value close to this value (86 ± 21 sequences). It is felt that the latter, rather than the former, value more accurately represents the number of sequences present in the highly abundant class, since a $R_0t_{1/2}$ value is more readily ascertained when a cDNA population of lower sequence complexity is utilized for hybridization analysis.

Comparisons of the sequence complexity number of the abundant class obtained here with values derived from other studies are difficult. Experiments that utilize a similar isolation technique deal with differentiated cells or tissues (Ryffel & McCarthy, 1975a,b; Hastie & Bishop, 1976; Paterson & Bishop, 1977). In most systems where three kinetic classes have been resolved, the number of sequences in the most abundant class has been reported to be in the range of 1–10, although some have placed this value nearer the one reported here (Hastie & Bishop, 1976; Paterson & Bishop, 1977; Jacquet et al., 1978). Levy & McCarthy (1975), using the highly abundant cDNA isolated from a *Drosophila* tissue culture line for kinetic studies, reported this class to be composed of only four different sequences. The meaning of the difference between the *Drosophila* tissue culture line and the *Drosophila* oocyte is not clear at this time. One possible explanation would be the diversity between sequences necessary to direct the early developmental program of an embryo and those essential for maintanence of life processes in culture.

Hybridization kinetics of the highly abundant cDNA to genomic DNA indicates that a majority of these sequences are derived from nonrepetitive portions of the genome. The results of the C_0t analysis of the abundant cDNA fraction were not expected. Few, if any, of the sequences composing the highly abundant poly(A+) RNA fraction appear to be derived from repetitive sequences of the genome. Recent investigations utilizing cloned *Drosophila* DNA complementary to abundant poly(A+) RNA of culture cells and larval tissues have disclosed that they represent various families of repetitive sequences, i.e., "copia", 412, and 297 (Finnegan et al., 1977; Ilyin et al., 1977; Tschurikov et al., 1978; Strobel et al., 1979; Potter et al., 1979). We had expected that some of the highly abundant sequences isolated here would contain comparable sequences. The reason for the discrepency can only be speculated upon but may arise from basic differences between an oocyte and other specialized tissue types.

We report here the isolation of another class of cDNA, that complementary to the ~86 highly abundant poly(A+) RNAs from oocytes of *D. melanogaster*. Earlier, this laboratory had reported the isolation of another class of cDNA, that composed of 44 middle abundant sequences that disappear during development, the transient maternal sequences (Goldstein & Arthur, 1979). We are presently engaged in the characterization of recombinant DNA plasmids containing *Drosophila* DNA. Some of these plasmids contain DNA complementary to the $cDNA_{HA}$ sequences. These plasmids will be used for in situ hybridization studies to identify the specific loci from which the RNA was transcribed.

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References

Anderson, K. V., & Lengyel, J. A. (1979) *Dev. Biol. 70*, 217–236.

Arthur, C. G., Weide, C. M., Vincent, W. S., III, & Goldstein, E. S. (1979) *Exp. Cell Res. 121*, 87–94.

Bishop, J. O., Morton, J. G., Rosbash, M., & Richardson, M. (1974) *Nature (London) 250*, 199–204.

Doane, W. W. (1967) *Methods in Developmental Biology* (Wilt, F. H., & Wessels, N. K., Eds.) pp 219–244, Cromwell, New York.

Finnegan, D. J., Rubin, G. M., Young, M. W., & Hogness, D. J. (1977) *Cold Spring Harbor Symp. Quant. Biol. 42*, 1053–1063.

Goldstein, E. S. (1978) *Dev. Biol. 63*, 59–66.

Goldstein, E. S., & Snyder, L. A. (1972) *Biochim. Biophys. Acta 281*, 130–139.

Goldstein, E. S., & Snyder, L. A. (1973) *Exp. Cell Res. 81*, 47–56.

Goldstein, E. S., & Arthur, C. G. (1979) *Biochim. Biophys. Acta 565*, 265–274.

Hames, B. D., & Perry, R. P. (1977) *J. Mol. Biol. 109*, 437–453.

Hastie, N. D., & Bishop, J. O. (1976) *Cell 9*, 761–774.

Hereford, L. M., & Rosbash, M. (1977) *Cell 10*, 453–462.

Hirsch, M., & Penman, S. (1974) *J. Mol. Biol. 83*, 131–142.

Ilyin, Y. V., Tchurikov, N. A., Ananiev, E. V., Ryskov, A. P., Yenikolopov, G. N., Limborska, S. A., Maleeva, N. E., Gvoydev, V. A., & Georgiev, G. P. (1977) *Cold Spring Harbor Symp. Quant. Biol. 42*, 959–969.

Jacquet, M., Affara, N. A., Benoît, R., Hederidge, J., Jacob, F., & Gros, F. (1978) *Biochemistry 17*, 69–79.

Kaufmann, Y., Goldstein, E. S., & Penman, S. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1834–1838.

Lamb, M. M., & Laird, C. D. (1976) *Dev. Biol. 52*, 31–42.

Levy, B. W., & McCarthy, B. J. (1975) *Biochemistry 14*, 2440–2446.

Lovett, J. A., & Goldstein, E. S. (1977) *Dev. Biol. 61*, 70–78.

Milcarek, C., Price, R., & Penman, S. (1974) *Cell 3*, 1–10.

Paterson, B. M., & Bishop, J. O. (1977) *Cell 12*, 751–765.
Potter, S. S., Brorein, W. J., Jr., Dunsmuir, P., & Rubin, G. M. (1979) *Cell (Cambridge, Mass.) 17*, 415–427.
Ryffel, G. U., & McCarthy, B. J. (1975a) *Biochemistry 14*, 1379–1385.
Ryffel, G. U., & McCarthy, B. J. (1975b) *Biochemistry 14*, 1385–1389.
Strobel, E., Dunsmuir, P., & Rubin, G. (1979) *Cell (Cambridge, Mass.) 17*, 429–439.
Tschurikov, N. A., Yu, V., Ilyin, Y. V., Ananeiv, E. V., & Georgiev, G. P. (1978) *Nucleic Acids Res. 5*, 2169–2187.
Zalokar, M. (1976) *Dev. Biol. 49*, 425–437.

Identification and Biochemical Analysis of Mouse Mutants Deficient in Cytoplasmic Malic Enzyme†

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ABSTRACT: During the biochemical screening of mutant enzymes in mice, individuals with an apparent nonfunctional allele at the locus (*Mod-1*) responsible for cytoplasmic malic enzyme were identified by starch gel electrophoresis and by enzyme activity measurements. A series of matings and genetic analyses were made, and mice homozygous for the nonfunctional or null allele (*Mod-1*^{n}) were produced. The mutation appeared to occur spontaneously in the C57BL/6J strain. By double-immunodiffusion and enzyme immunoinactivation assays, the null mutants were shown to express no proteins that cross-react with the antiserum to cytoplasmic malic enzyme (CRM-negative). In liver homogenates of homozygous null mutants, lack of protein components that form complexes with IgG from the cytoplasmic malic enzyme specific antiserum was further demonstrated by passage of the original serum through a mutant liver homogenate–Sepharose column, where the postadsorbed serum retained its titer and specificity. The residual malic enzyme activity (<10% of the normal) observed in various tissue homogenates of the homozygous null mutants was attributed to that of mitochondrial isozyme of malic enzyme. Assays of enzymes from tissues of different genotypes revealed no significant differences in activities of six other enzymes in the related metabolic pathways. However, in liver from mutant mice, a lower NADPH/NADP$^+$ ratio was consistently observed in comparison to that from control mice. Both the mutant and the control mice of the same age were found to have comparable body weight and lipid content.

Malic enzyme [L-malate:NADP$^+$ [1] oxidoreductase (decarboxylating), EC 1.1.1.40] catalyzes the NADP$^+$–NADPH-dependent interconversion between L-malate and pyruvate (Ochoa et al., 1950). In mammalian species, two main isozymes have been identified and characterized by their localization in cytoplasm and in mitochondria (Henderson, 1966, 1968), respectively. The genetics and biochemistry of two isozymes of malic enzyme in mice have been well characterized (Shows et al., 1970; Lee et al., 1978; Bernstine, 1979). The structural genes of cytoplasmic (*Mod-1*) and mitochondrial (*Mod-2*) malic enzyme have been mapped to chromosomes 9 and 7 (Shows et al., 1970), respectively. Cytoplasmic malic enzyme has been purified in our laboratory by general ligand affinity chromatography (Lee et al., 1978). It was shown to be a tetramer with a native molecular weight of 27 000. Kinetic studies revealed that this enzyme has a much lower K_m for L-malate (K_m = 50 μM) than for pyruvate (K_m = 5 mM) (Lee et al., 1978). Judging from intracellular metabolite and coenzyme concentrations, this enzyme would appear to catalyze preferentially the oxidation of L-malate to pyruvate with a concomitant generation of NADPH (Guynn et al., 1972). The tissue levels of malic enzyme are increased by dietary carbohydrate and repressed by dietary lipid, paralleling the rate of lipogenesis (Geer et al., 1978, 1979; Silpananta & Goodridge, 1971; Li et al., 1975). Therefore, malic enzyme was postulated to be important for lipogenesis, but not for glucogenesis (Young et al., 1964; Wise et al., 1964; Geer et al., 1978).

Mice apparently having an inactive allele at the locus responsible for cytoplasmic malic enzyme were discovered in the course of screening for germinal mutations (F. M. Johnson, G. T. Roberts, R. K. Sharma, F. Chasalow, R. Zweidinger, A. Morgan, R. W. Hendren, and S. Lewis, unpublished experiments). The purpose of the present investigation was to provide detailed genetic analysis and to characterize the biochemical as well as immunological consequences of the deficiency of cytoplasmic malic enzyme in order to gain understanding of the impact of the mutation on the physiology and viability of this organism and to seek information regarding the function of the normal enzyme.

Materials and Methods

Chemicals. The following chemicals were purchased from Sigma Chemical Co.: NADP$^+$ (acid form), NADPH (sodium salt), 6-phosphogluconic acid, ascorbic acid, L-malate, pyruvate, NADH (sodium salt), oxaloacetate, oxidized glutathione, isocitrate, phosphoenolpyruvate, glucose 6-phosphate, α-ke-

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[1] Abbreviations used: NADH, reduced nicotinamide adenine dinucleotide; NADP, NAD phosphate; NADPH, reduced NADP; ADP, adenosine 5′-diphosphate; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid.

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