Untucht-Grau, R., Schulz, G. E., & Schirmer, R. H. (1979) *FEBS Lett. 105*, 244–248.
van Muiswinkel-Voetberg, H., & Veeger, C. (1973) *Eur. J. Biochem. 33*, 271–278.
Visser, J., Voetberg, H., & Veeger, C. (1970) in *Pyridine Nucleotide-Dependent Dehydrogenases* (Sund, H., Ed.) pp 359–373, Springer-Verlag, West Berlin.
Visser, A. J. W. G., Grande, H. J., Muller, F., & Veeger, C. (1974) *Eur. J. Biochem. 45*, 99–107.
Weimar, W. R., & Neims, A. H. (1975) in *Riboflavin* (Rivlin, R. S., Ed.) pp 1–47, Plenum Press, New York.
Wilkinson, K. D., & Williams, C. H., Jr. (1979) *J. Biol. Chem. 254*, 852–862.
Williams, C. H., Jr. (1976) *Enzymes, 3rd Ed. 8c*, 90–174.
Williams, C. H., Jr., & Arscott, L. D. (1972) *Z. Naturforsch. B 27*, 1078–1080.
Williams, C. H., Jr., Zanetti, G., Arscott, L. D., & McAllister, J. K. (1967) *J. Biol. Chem. 242*, 5226–5231.

Small Circular Deoxyribonucleic Acid of *Drosophila Melanogaster*: Homologous Transcripts in the Nucleus and Cytoplasm†

Sharon W. Stanfield‡ and Judith A. Lengyel*

ABSTRACT: We have recently characterized small circular DNA of *Drosophila* cultured cells in terms of its average size, sequence complexity, and homology to intermediate repetitive DNA. We show here that transcripts homologous to small circular DNA are present in various RNA fractions. Nuclear poly(A+), nuclear poly(A−), and polysomal poly(A+) RNA drive 10, 7, and 20%, respectively, of in vitro labeled small circular DNA tracer into hybrid. Sequences complementary to small circular DNA are at least 10-fold more concentrated in nuclear poly(A+) RNA than in nuclear poly(A−) or polysomal poly(A+) RNA. We do not detect significant homology between poly(A−) cytoplasmic RNA and small circular DNA. Assuming that only the least complex component of small circular DNA is driven into hybrid and that transcription is asymmetric, we use the results obtained here and previously published data to calculate the sequence complexity and relative concentration of nuclear poly(A+), nuclear poly(A−), and polysomal poly(A+) RNA homologous to small circular DNA.

Heterogeneous small circular DNA is an intriguing class of molecules for which a number of genetic regulatory functions has been proposed. This type of DNA has been described in a wide variety of eukaryotic organisms, including *Neurospora*, *Euglena*, trypanosomes, yeast, tobacco, *Xenopus*, chickens, and boar, and in cell culture lines from monkey, mouse, and humans (Agsteribbe et al., 1972; Nass & Ben-Shaul, 1972; Ono et al., 1971; Billheimer & Avers, 1969; Wong & Wildman, 1972; Delap & Rush, 1970; Buongiorno-Nardelli et al., 1976; Hotta & Bassel, 1965; Smith & Vinograd, 1972; Delap et al., 1978).

We have previously described a number of properties of small circular DNA from cultured cells of *Drosophila melanogaster* (Stanfield & Helinski, 1976; Stanfield & Lengyel, 1979). This DNA, isolated as covalently closed circular molecules, is heterogeneous in contour length, with an average size of 3300 NTP.[1] It is localized predominantly, if not entirely, in the nucleus and has a buoyant density indistinguishable from that of the main band of nuclear DNA. The sequence complexity of the major component (82%) of the small circular DNA is 1.8×10^4 NTP. A majority (possibly all) of the small circular DNA is homologous to middle repetitive sequences of *Drosophila* chromosomal DNA. Thermal denaturation studies of hybridized small circular and chromosomal DNA demonstrate that there is ~2% mismatch between small circular DNA and its homologous chromosomal sequences.

In order to define the role(s) of small circular DNA within the cell, it is necessary to determine if small circular DNA, or genomic sequences homologous to it, is transcribed into RNA. Our studies described here demonstrate that transcripts homologous to small circular DNA are present in both nucleus and cytoplasm and that these transcripts are more concentrated in nuclear poly(A+) than in nuclear poly(A−) or polysomal poly(A+) RNA.

Experimental Procedures

Cell Fractionation and RNA Purification. Drosophila Schneider line 2 cells (Schneider, 1972) were collected and lysed, and the cytoplasmic fraction was prepared as described by Levis & Penman (1977), with the substitution of Mg^{2+} for Ca^{2+} in the wash and lysis buffers. Nuclei were freed of contaminating cytoplasm by sedimentation through mixed-detergent sucrose (0.64 M sucrose, 1% Tween-40, 0.5% sodium deoxycholate, and 0.25% diethyl pyrocarbonate, in 0.85 × lysis buffer) and then digested with DNase and ethanol precipitated (Levis & Penman, 1977).

Polysomal RNA was prepared by EDTA release from polysomes, a technique designed to minimize contamination of mRNA by hnRNA (Galau et al., 1974). Cytoplasm was prepared as described above, with the exceptions that emetine (25 μg/mL) was added to the cells a few seconds before

† From the Molecular Biology Institute (S.W.S. and J.A.L.) and the Department of Biology (J.A.L.), University of California, Los Angeles, Los Angeles, California 90024. *Received January 14, 1980.* This work was supported by National Cancer Institute Training Grant CA 09056 and National Institutes of Health Postdoctoral Fellowship GM 06176 to S.W.S, by National Institutes of Health Grant HD 09948 and National Science Foundation Grant PCM 04197 to J.A.L., and by U.S. Public Health Service Grant RR 07009.

‡ Present address: Biology Department, University of California, San Diego, La Jolla, CA 92093.

[1] Abbreviations used: NTP, nucleotide pairs; NT, nucleotides; NaDodSO$_4$, sodium dodecyl sulfate.

0006-2960/80/0419-3873$01.00/0

harvesting (to prevent polysome runoff), and cells, poured onto frozen wash buffer, were cooled rapidly in a −20 °C bath prior to centrifugation. The cytoplasmic fraction was brought to 0.025 M EGTA and 0.035 M magnesium acetate and centrifuged on a 5–40% sucrose gradient in polysome buffer (0.1 M NaCl, 0.01 M Tris, pH 7.4, 0.025 M EGTA, 0.035 M magnesium acetate, 0.35 mg/mL heparin, and 25 μg/mL emetine). Gradients were collected through an ISCO UA-5 absorbance monitor, and greater than 100S material was pooled and ethanol precipitated. The pellet was resuspended in polysome buffer plus 0.1 M EDTA and centrifuged through a 5–40% sucrose gradient in polysome buffer plus 0.1 M EDTA. Material of less than 80 S was pooled and ethanol precipitated. This constituted the EDTA-released polysomal RNA fraction.

Ethanol-precipitated nuclear, cytoplasmic, and EDTA-released polysomal fractions were digested with proteinase K in $NaDodSO_4$ buffer (0.01 M Tris, pH 7.4, 0.1 M NaCl, 0.001 M EDTA, and 0.5% $NaDodSO_4$) for 1 h at room temperature and then extracted to completion with phenol–chloroform (Lengyel & Penman, 1977). RNA was separated into poly(A)-containing and poly(A)-lacking fractions by chromatography on oligo(dT)–cellulose (T3; Collaborative Research) as described previously (Anderson & Lengyel, 1979). Prior to binding, samples were denatured in 15% LSB (0.01 M Tris, pH 7.4, 0.001 M EDTA, and 0.1% $NaDodSO_4$)–85% Me_2SO for 5 min at 65 °C and then diluted 20-fold with binding buffer. Samples were carried through two cycles of chromatography before use in hybridization experiments. RNA which consistently bound to oligo(dT)–cellulose is termed poly(A+) and that which consistently did not bind is referred to as poly(A−).

DNA was removed from nuclear poly(A−) RNA by digestion with RNase-free DNase (Federoff et al., 1977). After phenol extraction the sample was passed over Sephadex G-100, and material in the excluded volume was pooled and redigested. This removed more than 99.98% of contaminating DNA. The nuclear poly(A+) fraction was not treated with DNase as it contained less than 0.05% of contaminating DNA. Nuclear RNA was contaminated with 12% of the total cytoplasmic RNA, as determined by comparing rRNA A_{254} peaks in sucrose gradients of nuclear and cytoplasmic RNA.

Concentration of RNA Drivers. RNA concentrations of poly(A−) fractions were determined by absorbance at 260 nm. For poly(A+) fractions, poly(A) content was determined by the [^{3}H]poly(U) assay as described by Milcarek et al. (1974). This was converted into an amount of RNA by assuming that the poly(A) tract is 8% of the mass of the nuclear and 11% of the mass of polysomal poly(A+) RNA. These values are based on the number-average sizes of these molecules (see Results) and the assumption that the steady-state sizes of poly(A) tracts in *Drosophila* hnRNA and mRNA are the same as those in mammalian cells, namely, 140 and 80 nucleotides, respectively (Bantle & Hahn, 1976). [Poly(A) tracts of pulse-labeled *Drosophila* hnRNA and mRNA are the same size respectively as those of pulse-labeled HeLa cell hnRNA and mRNA (Levis, 1977; J. A. Lengyel, unpublished experiments).]

Purification and in Vitro Labeling of Small Circular DNA. Small circular DNA was prepared as described previously (Stanfield & Lengyel, 1979) and labeled in vitro with ^{32}P to a specific activity of 1.8×10^8 cpm/μg (Rigby et al., 1977). DNase was present in the reaction mixture at a final concentration of 0.1 μg/mL. This high concentration was required to nick the very small supercoiled DNAs (average size 3000 NTP) and thus to obtain the necessary high specific activities. The majority of contaminating mitochondrial DNA was removed by hybridization of the labeled preparation to a large excess of unlabeled mitochondrial DNA as previously described (Stanfield & Lengyel, 1979).

Table I: Characteristics of in Vitro Labeled Small Circular DNA Preparations

prepn	label	size (NT)	total reactability[a] (%)	reactable mitochondrial DNA[b] (%)	reactable small circular DNA[c] (%)
1	^{32}P	90	40	14	26
2	^{32}P	130	75	46	29
3	^{32}P	70	50	4	46

[a] Total reactability was determined by reaction with a >500 000-fold excess of total cellular DNA driver. [b] Reactability of mitochondrial DNA was determined by hybridization to pure mitochondrial DNA driver. [c] The difference between the total reactability and the reactability of the mitochondrial DNA determined the percent reactable small circular DNA.

RNA Excess Hybridization. In vitro ^{32}P-labeled small circular DNA was mixed with a $[(1-500) \times 10^4]$-fold excess of RNA, denatured at 105 °C, and incubated at 70 °C in 0.24 M sodium phosphate, 0.001 M EDTA, and 0.1% $NaDodSO_4$. Reactions were terminated and assayed on hydroxylapatite at 65 °C as described by Stanfield & Lengyel (1979).

To correct for self-annealing of the tracer DNA sequences, we performed control hybridization reactions in which trace amounts of small circular DNA or mitochondrial DNA were permitted to react in the presence of excess *Escherichia coli* ribosomal RNA. The corresponding points in this control curve were subtracted from each point in the RNA-driven reactions. All R_0t values were corrected to equivalent R_0t (ER_0t) conditions (60 °C; 0.12 M phosphate buffer) by multiplying by 2.93 (Britten et al., 1974). This correction has been found to apply to RNA–DNA hybridizations as well as to DNA–DNA hybridizations (R. B. Goldberg, unpublished results).

Results

Hybridization of Small Circular DNA to Cellular RNA Fractions. The homology between small circular DNA and various cellular RNA fractions was investigated by using excess unlabeled RNA to drive ^{32}P-labeled circular DNA tracer (containing 26–46% reactable small circular DNA) into hybrid. This tracer will be referred to as "^{32}P tracer" in what follows. The characteristics of the various ^{32}P tracers used are described in Table I. Polysomal RNA was prepared by EDTA release of polysomes to minimize contamination by nuclear RNA. A representative distribution of polysomes and their release by EDTA are shown in Figure 1. All RNA fractions used as driver were carried through two cycles of denaturation and binding to oligo(dT)–cellulose to separate poly(A+) from poly(A−) RNA. The number-average size of the nuclear and polysomal poly(A+) RNA drivers, determined by sedimentation in $NaDodSO_4$–sucrose gradients, was 1700 and 730 NT, respectively (Figure 2). Nuclear poly(A−) (nonribosomal) RNA was assumed to be the same size as nuclear poly(A+) RNA (Levis & Penman, 1977). Other details of the preparation and characterization of DNA tracers and RNA drivers are presented under Experimental Procedures.

The hybridization of ^{32}P tracer with different classes of RNA is shown in Figure 3. In order to determine the extent

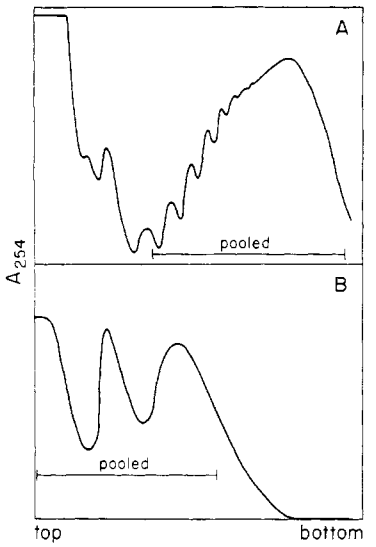

FIGURE 1: Preparation and EDTA release of polysomal RNA. Details of the methods used are presented under Experimental Procedures. (A) Preparative sedimentation of polysomes from cultured *Drosophila* cells. Polysomes sedimenting at >100 S were collected and treated with EDTA. (B) Preparative sedimentation of EDTA-treated polysomes. Material sedimenting at <80 S was pooled and treated as described under Experimental Procedures.

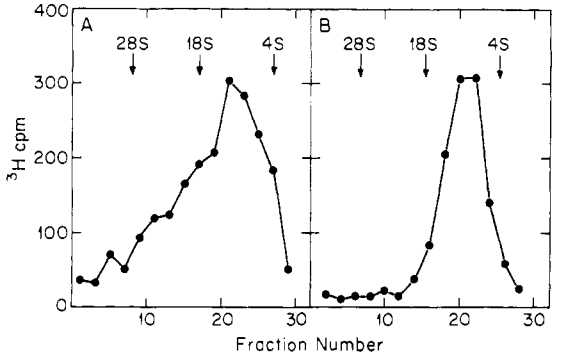

FIGURE 2: Size distribution of polyadenylated nuclear and polysomal RNA. Nuclear poly(A+) RNA was prepared from unlabeled cells as described under Experimental Procedures. Polysomal poly(A+) RNA was prepared by oligo(dT)-cellulose chromatography (see Experimental Procedures) of EDTA-released polysomal RNA (Figure 1). An aliquot of each RNA preparation was sedimented in a 15–30% sucrose gradient in 0.01 M Tris, pH 7.4, 0.1 M NaCl, 0.001 M EDTA, and 0.1% $NaDodSO_4$ for 17 h at 26 500 rpm in an SW40 rotor. The gradients were fractionated and aliquots were assayed for poly(A) content by hybridization with [^{3}H] poly(U). The arrows indicate the positions of ^{14}C-labeled HeLa rRNA sedimented in the same gradient. (A) Nuclear poly(A+) RNA. (B) Cytoplasmic poly(A+) RNA. The number-average size (in NT) of the RNA in each gradient was determined as $\bar{N} = \sum(C_i N_i)/\sum C_i$ where C_i is the number of cpm of [^{3}H]poly(U) hybridized to each fraction in the gradient and N_i is the length in NT of the RNA of each fraction. N_i was determined from the s value, which was estimated by interpolation between the ^{14}C markers, which were assumed to be 5100 and 2000 NT (Loening, 1968). For cytoplasmic poly(A$^+$) RNA, $\bar{N}$ = 730 NT; for nuclear poly(A+) RNA, $\bar{N}$ = 1700 NT. Because the rRNA marker, due to its secondary structure, sediments in these gradients more rapidly (relative to its molecular weight) than mRNA, these sizes may be underestimates, by up to 50%.

of homology with small circular DNA, we have normalized the data in Figure 3A–C to the total reactable small circular DNA in the ^{32}P tracer (since different tracers contained different amounts of reactable small circular DNA; see Table I). These data were then fit by computer to one component, since increasing the number of components did not significantly decrease the root mean square error of the fit.

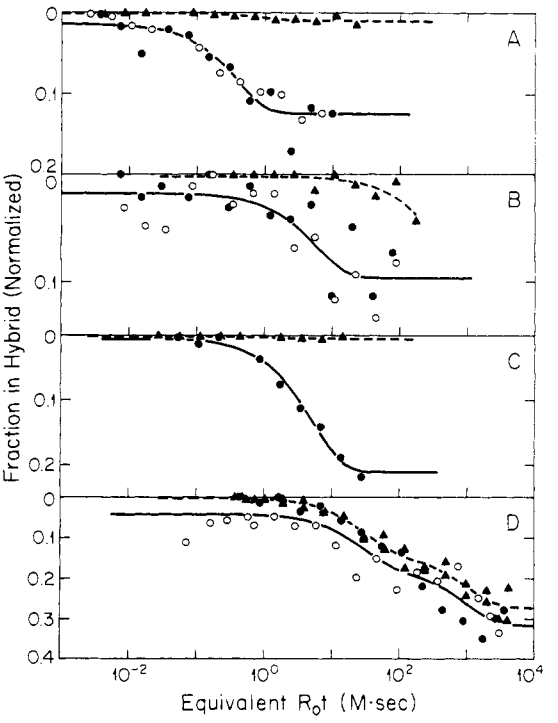

FIGURE 3: Hybridization of circular DNA tracer with different classes of cellular RNA. (A) Nuclear poly(A+) driver (two different RNA driver preparations were used). ^{32}P circular DNA tracer preparation 2 (●); ^{32}P circular DNA tracer preparation 1 (○); ^{3}H mitochondrial DNA tracer (▲). (B) Nuclear poly(A−) driver. ^{32}P circular DNA tracer preparation 2 reacted with two different preparations of nuclear poly(A−) RNA (● and ○); ^{3}H mitochondrial DNA tracer (▲). (C) Polysomal poly(A+) driver. ^{32}P circular DNA tracer preparation 3 (●); ^{3}H mitochondrial DNA tracer (▲). (D) Cytoplasmic poly(A−) driver (two different RNA driver preparations were used). ^{32}P circular DNA tracer preparation 2 (●); ^{32}P circular DNA tracer preparation 1 (○); ^{3}H mitochondrial DNA tracer (▲). ^{32}P circular DNA tracer, containing different amounts of reactable small circular DNA (see Table I), was hybridized at 0.3–0.8 ng/mL, 500–1000 cpm/reaction point, with a [(6–500) × 10^4]-fold excess of different cellular RNA fractions. As a control, pure ^{3}H-labeled mitochondrial DNA (6–8 ng/mL; 500–1000 cpm/reaction point) was also hybridized with the various RNA drivers to determine what fraction of the ^{32}P tracer hybridization was due to its content of contaminating mitochondrial DNA. Samples were counted for 10 min and backgrounds of <10 and <30 cpm were subtracted from the ^{3}H and ^{32}P tracers, respectively. The hybridization of the mitochondrial tracer was normalized to its own reactability (90%) and then multiplied by the fraction of reactable mitochondrial DNA contained in the ^{32}P tracer (see Table I). The hybridizations of ^{32}P tracer in panels A, B, and C were normalized to the reactable small circular DNA in the ^{32}P tracer (see Table I). The data of (D) were normalized to the reactability of the mitochondrial DNA in the ^{32}P tracer, since the large amount of mitochondrial DNA driven into hybrid by cytoplasmic poly(A−) indicated that most of the reacting material in the ^{32}P tracer preparation was mitochondrial DNA (see the text). The solid and dotted lines indicate the best least-squares solution to the data assuming pseudo-first-order kinetics (Pearson et al., 1977).

Because the ^{32}P tracers used in the hybridization reactions were contaminated to varying extents with mitochondrial DNA, pure ^{3}H-labeled mitochondrial DNA was reacted with different RNA drivers as a control. To allow comparison with the normalized ^{32}P tracer reaction, we normalized the mitochondrial hybridization as described in the legend to Figure 3.

It is apparent that nuclear poly(A+), nuclear poly(A−), and polysomal poly(A+) RNAs all contain sequences homologous to small circular DNA (parts A, B, and C of Figure 3). All of these RNA fractions react to a significant extent with the ^{32}P tracer but very little with the ^{3}H mitochondrial tracer (Figure 3). The great majority of the hybridization of nuclear

Table II: Observed Rate of Reaction and Extent of Homology of Small Circular DNA with Various RNA Fractions in Excess

RNA driver	k_{obsd}[a] (M^{-1} s^{-1})	% in hybrid[b]	extent of homology (%)[c] (M^{-1} s^{-1})
nuclear poly(A+)	2.5	10	20
nuclear poly(A-)	0.17	7	14
polysomal poly(A+)	0.19	20	40

[a] Obtained by computer fit of the data in Figure 3. [b] Percent in hybrid = (F_{tc} - F_{mit}) × 100, where F_{tc} = the normalized fraction of total circular DNA which hybridizes in the transition defined by the rate constant k_{obsd} and F_{mit} = the normalized fraction of mitochondrial DNA which hybridizes (from Figure 3). Because there is slightly more hybridization than that defined by the observed rate constant (see Figure 3), the maximum percent in hybrid is actually somewhat greater than that given in the table. [c] Extent homology (%) = (percent in hybrid) × 2. The factor of 2 assumes that transcription is asymmetric (see the text).

poly(A+), nuclear poly(A-) and polysomal poly(A+) RNA with ^{32}P tracer is therefore due to the homology between sequences in the RNA driver and small circular DNA in the tracer.

We do not detect significant homology between poly(A-) cytoplasmic RNA and small circular DNA. Hybridization of cytoplasmic poly(A-) RNA to the ^{32}P tracer appears to be almost entirely to the mitochondrial DNA contaminant of the tracer. The reaction of cytoplasmic poly(A-) RNA with the ^{32}P tracer, when normalized to the reactable mitochondrial rather than to the reactable small circular DNA in the tracer, is almost identical with its reaction with pure mitochondrial tracer (Figure 3D). This indicates that the bulk of the hybridization between cytoplasmic poly(A-) RNA and ^{32}P tracer is due to mitochondrial DNA contaminating the tracer. The small difference in the total extent of hybridization, however, permits the possibility that up to 5% of the small circular DNA could be homologous to cytoplasmic poly(A-) RNA (assuming asymmetric transcription).

The observed and corrected rate constants for the reaction of small circular DNA with different driver RNAs are summarized in Table II. A comparison of the rate constants for the different hybridization reactions shows that nuclear poly(A+) RNA drives small circular DNA into hybrid at a significantly faster rate than does nuclear poly(A-) or polysomal poly(A+) RNA. Contamination of nuclear RNA by cytoplasmic RNA thus cannot be responsible for the hybridization observed between small circular DNA and poly(A+) nuclear RNA. We must, however, consider whether contamination of the cytoplasm by nuclear RNA might be responsible for the hybridization driven by polysomal poly(A+) preparations. This is very unlikely since, of the label incorporated into RNA in a 3-min pulse (which is primarily in nuclear RNA), less than 0.1% is found in EDTA-released, polysomal poly(A+) RNA under our methods of preparation (Lengyel & Penman, 1977; Graham and Lengyel, unpublished experiments). We conclude from the relationship between the rate constants in Table II that transcripts homologous to small circular DNA are significantly (at least 10-fold) more concentrated in nuclear poly(A+) than in nuclear poly(A-) or polysomal poly(A+) RNA.

The percent of small circular DNA which hybridized with each RNA driver is given in Table II. This number was obtained by subtracting the control mitochondrial hybridization from the ^{32}P tracer transition defined by the computer-fitted rate constant. The extent of homology of small circular DNA with different driver RNAs is also summarized in Table II. We assume that all the RNA which hybridizes to small circular DNA has been transcribed asymmetrically. This is a reasonable assumption because polysomal poly(A+) RNA, which is presumably mRNA and thus transcribed asymmetrically, hybridizes to the greatest extent to small circular DNA. We therefore multiply the percent of small circular DNA in hybrid with different RNA fractions by 2 to calculate that nuclear poly(A-), nuclear poly(A+) and polysomal poly(A+) RNA are homologous to 14, 20, and 40%, respectively, of small circular DNA. We note that these numbers may be underestimates for the homology of the poly(A+) RNA fractions if any degradation of the poly(A+) driver occurred prior to its isolation on oligo(dT)-cellulose.

Discussion

Homology of Small Circular DNA with Various RNA Fractions. By hybridization of small circular DNA tracer with various RNA fractions in excess, we have shown that sequences homologous to small circular DNA are present in both nuclear RNA and polysomal RNA (Figure 3). Thus, the small circles themselves and/or genomic sequences homologous to them are transcribed into RNA in Schneider line 2 cultured cells. The observed rate constants for the hybridization of small circular DNA with different RNA fractions indicate that sequences homologous to small circular DNA are at least 10-fold more concentrated in nuclear poly(A+) than in nuclear poly(A-) and polysomal poly(A+) RNA.

Depending on the RNA fraction used as driver, from 14 to 40% of the small circular DNA was homologous (assuming asymmetric transcription). Theoretically, all the sequences present in polysomal poly(A+) RNA should be present in both poly(A+) and poly(A-) nuclear fractions; both nuclear fractions should therefore drive at least as much small circular DNA into hybrid as did polysomal poly(A+) RNA. The fact that the nuclear RNA fractions drove only half as much, or less, of the small circular DNA into hybrid as did the polysomal poly(A+) RNA indicates that sufficiently high R_0t values were not achieved with the nuclear RNA drivers. Attempts to achieve higher R_0t values resulted in degradation of the RNA driver during prolonged hybridization.

It is of interest to determine the complexity of the small circular DNA which is homologous to cellular RNA. Small circular DNA has been previously shown to be composed of a major component (82% of the total mass) with a sequence complexity of 1.8×10^4 NTP and a minor component (18% of the total mass) with a higher but undefined sequence complexity (Stanfield & Lengyel, 1979). The fact that 40% of small circular DNA is homologous to polysomal poly(A+) RNA (assuming asymmetric transcription) suggests that a significant portion of the low sequence complexity component of small circular DNA is homologous to RNA. Assuming that the majority of the small circular DNA driven into hybrid is the low sequence complexity component, we calculate that 4400, 3100, and 8800 NTP of small circular DNA are homologous to nuclear poly(A+), nuclear poly(A-), and polysomal poly(A+) RNA, respectively.

The proportion of the different RNA fractions which are homologous to small circular DNA can be estimated by comparing the sequence complexity and rate constant for the reaction of the tracer with the sequence complexity and rate constant for the reaction of a pure DNA sequence. The rate constant for the reannealing of single-copy *Drosophila* DNA (Stanfield & Lengyel, 1979) corrected for RNA-DNA hybridization (Galau et al., 1974, 1977) was used as a standard.

The k_{obsd} values of Table II were corrected for tracer–driver disparity (Chamberlin et al., 1978), to the fragment length of the *Drosophila* DNA standard (Wetmur & Davidson, 1968), and for the contamination of nuclear RNA by cytoplasmic RNA (Experimental Procedures) assuming that 2.6% of the total RNA of the cell is poly(A+) (Falkenthal, 1980), that 6% of the poly(A) of the cell is in the nucleus (Levis & Penman, 1977), and that 10% of the nuclear RNA is poly(A+) (Levy et al., 1976). On the basis of these assumptions, we calculate that 12% of nuclear poly(A+), 1.4% of nuclear poly(A−), and 0.8% of polysomal poly(A+) RNA are homologous to small circular DNA. Taking into consideration the sources of error in the assumptions used to make the calculations, minimum estimates for the percent of nuclear poly(A+), nuclear poly(A−), and polysomal poly(A+) RNA homologous to small circular DNA are 0.67, 0.06, and 0.07%, respectively.

Possible Function of Small Circular DNA. We speculated earlier that small circular DNA might contain ribosomal, tRNA, or 5S genes (Stanfield & Helinski, 1976). Since we did not detect more than 5% homology between poly(A−) cytoplasmic RNA and small circular DNA, however, it is unlikely that rRNA, tRNA, or 5S genes constitute a significant fraction of the mass of small circular DNA.

Small circular DNA does appear to contain structural genes for mRNA. This is deduced from the fact that polysomal poly(A+) RNA is homologous to 40% of small circular DNA. The presence of poly(A+) mRNA transcribed from 2-μm circles of yeast has been described, and the protein products of some of the mRNAs have been identified (Broach et al., 1979). The transcription of mRNA from small circular DNA may therefore be a general phenomenon.

Whether nuclear RNA homologous to small circular DNA has any function other than as a precursor to polysomal mRNA is unclear at present. It may be significant that sequences homologous to small circular DNA are more abundant in nuclear poly(A+) than in nuclear poly(A−) or polysomal poly(A+) RNA. It is possible that these nuclear transcripts might be involved in the control of gene expression, as has been suggested for the transcripts of middle repetitive DNA (Davidson et al., 1977; Boncinelli, 1978; Scheller et al, 1978).

The homology of small circular DNA to intermediate repeat DNA (Stanfield & Lengyel, 1979) suggests that it may have the capacity to integrate into and excise from chromosomal DNA and thereby affect gene expression, as is the case for some translocatable elements of prokaryotes [reviewed by Kleckner (1977)]. Evidence in support of this possibility is that several repeated DNA sequence elements in *Drosophila* have been shown to be capable of insertion at many chromosomal sites (Potter et al., 1979; Strobel et al., 1979). Nuclear RNA homologous to small circular DNA might then arise from transcription of structural genes starting from a promoter within integrated circular DNA.

Acknowledgments

We are grateful to Dr. Judith Strommer for assistance with in vitro labeling of DNA, to Dr. A. J. Tobin for helpful comments on the manuscript, and to M. Uchiyamada for help in its preparation.

References

Agsteribbe, E., Kroon, A. M., & Van Bruggen, E. F. J. (1972) *Biochim. Biophys. Acta 269*, 299–303.

Anderson, K. V., & Lengyel, J. A. (1979) *Dev. Biol. 70*, 217–231.

Bantle, J. A., & Hahn, W. E. (1976) *Cell 8*, 139–150.

Billheimer, F. E., & Avers, C. J. (1969) *Proc. Natl. Acad. Sci. U.S.A. 64*, 739–746.

Boncinelli, E. (1978) *J. Theor. Biol. 72*, 75–79.

Britten, R. J., Graham, D. E., & Neufeld, B. R. (1974) *Methods Enzymol. 29E*, 363–406.

Broach, J. R., Atkins, J. F., McGill, C., & Chow, L. (1979) *Cell 16*, 827–839.

Buongiorno-Nardelli, M., Amaldi, F., & Lava-Sanchez, P. A. (1976) *Exp. Cell Res. 98*, 95–103.

Chamberlin, M. E., Galau, G. A., Britten, R. J., & Davidson, E. H. (1978) *Nucleic Acids Res. 5*, 2073–2094.

Davidson, E. H., Klein, W. H., & Britten, R. J. (1977) *Dev. Biol. 55*, 69–84.

Delap, R. J., & Rush, M. G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5855–5859.

Delap, R. J., Rush, M. G., Zouzias, D., & Khan, S. (1978) *Plasmid 1*, 508–521.

Falkenthal, S. V. (1980) Ph.D. Thesis, University of California at Los Angeles.

Federoff, N., Wellauer, P. K., & Wall, R. (1977) *Cell 10*, 597–610.

Galau, G. A., Britten, R. J., & Davidson, E. H. (1974) *Cell 2*, 9–20.

Galau, G. A., Britten, R. J., & Davidson, E. H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1020–1023.

Hotta, Y., & Bassel, A. (1965) *Proc. Natl. Acad. Sci. U.S.A. 53*, 356–362.

Kleckner, N. (1977) *Cell 11*, 11–23.

Lengyel, J. A., & Penman, S. (1977) *Dev. Biol. 57*, 243–253.

Levis, R. (1977) Ph.D. Thesis, Massachusetts Institute of Technology.

Levis, R., & Penman, S. (1977) *Cell 11*, 105–113.

Levy, W. B., Johnson, C. B., & McCarthy, B. J. (1976) *Nucleic Acids Res. 3*, 1777–1789.

Loening, U. E. (1968) *J. Mol. Biol. 38*, 355–365.

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

Nass, M. M. K., & Ben-Shaul, Y. (1972) *Biochim. Biophys. Acta 272*, 130–136.

Ono, T., Ozeki, Y., Okubo, S., & Inoki, S. (1971) *Biken J. 14*, 203–215.

Pearson, W. R., Davidson, E. H., & Britten, R. J. (1977) *Nucleic Acids Res. 4*, 1727–1737.

Potter, S. S., Brorein, W. J., Jr., Dunsmuir, P., & Rubin, G. M. (1979) *Cell 17*, 415–427.

Rigby, P. W. J., Dieckmann, M., Rhodes, C, & Berg, P. (1977) *J. Mol. Biol. 113*, 237–251.

Scheller, R. A., Constantini, F. D., Kozlowski, M. R., Britten, R. J., & Davidson, E. H. (1978) *Cell 15*, 189–203.

Schneider, I. (1972) *J. Embryol. Exp. Morphol. 27*, 353–365.

Smith, C. A., & Vinograd, J. (1972) *J. Mol. Biol. 69*, 163–178.

Stanfield, S., & Helinski, D. R. (1976) *Cell 9*, 333–345.

Stanfield, S. W., & Lengyel, J. A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 6142–6146.

Strobel, E., Dunsmuir, P., & Rubin, G. M. (1979) *Cell 17*, 429–439.

Wetmur, J. G., & Davidson, N. (1968) *J. Mol. Biol. 31*, 349–370.

Wong, F. Y., & Wildman, S. G. (1972) *Biochim. Biophys. Acta 259*, 5–12.