Characterization of Polypyrimidines in *Drosophila* and L-Cell DNA†

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ABSTRACT: Unusually long pyrimidine tracts (polypyrimidines), ranging from 100 to over 1000 nucleotides in length, have been found in *Drosophila melanogaster* DNA. They are compared to shorter pyrimidine tracts (25–150 nucleotides) which have previously been found in L-cell DNA. Both species were able to anneal to homologous DNA; *Drosophila* polypyrimidines formed stable hybrids while L-cell polypyrimidines formed hybrids of lower thermal stability. In both cases, the kinetics of the reaction was rapid, suggesting that these tracts are part of highly repeated DNA.

DNA can be degraded selectively by appropriate acid treatment to liberate purine bases and a series of oligonucleotides containing only pyrimidine residues (Shapiro, 1967; Burton, 1967). Conventional analysis of these fragments has involved ion-exchange chromatography, and has been limited to oligonucleotides less than 15–20 residues in length (e.g., Spencer et al., 1969). The frequency distribution of pyrimidine isostichs in this size range has been examined for several DNA samples and small differences from random have been reported (Bellett et al., 1972; Sneider, 1971; Tate and Peterson, 1974). We have recently analyzed HeLa cell DNA and L-cell DNA to see if longer pyrimidine tracts are present (Birnboim et al., 1973; Straus and Birnboim, 1974). Since fragments of this size might be lost on an ion-exchange column, polyacrylamide gel electrophoresis was used for size separation. Unexpectedly, long pyrimidine tracts were found. Both the amount and size of these polynucleotides differed markedly from that predicted assuming a random distribution of bases. Because of their distinctly nonrandom nature, it seemed likely that they were a discrete population of fragments, perhaps from regions of DNA having a common function. This consideration has prompted us to examine the DNA of another eukaryote, *Drosophila melanogaster*. *Drosophila* was chosen for study because of its small genome size and because it is amenable to detailed genetic and cytological analysis. We report that DNA from *Drosophila melanogaster* cultured cells contains a polypyrimidine component which is considerably longer than those found in L-cell or HeLa DNA. [^{3}H]Thymidine-labeled polypyrimidines have been isolated and shown to anneal very rapidly to their homologous DNA to form a reasonably stable and specific duplex.

Materials and Methods

Culture of Drosophila Cells and Isolation of DNA. Cultured cells of *D. melanogaster* were grown at 25° in spinner culture on modified Schneider's medium. The cell lines were derived from Schneider's line 2 (Schneider, 1972) and grown by Garen (Yale University, New Haven, Conn.) in shaker culture as line E. Our medium was modified by removing trehalose, organic acids, cystine, vitamins, and bactopeptone and reducing the fetal bovine serum (Gibco, heat-treated) to 5%. Karyotype analysis has shown that 30–60% of the cells have a normal or near normal diploid chromosome composition. Cells were labeled in a medium similar to that described above, but lacking yeast extract and serum. The cell suspensions (2–4 × 10^6/ml) were incubated with [*methyl*-^{3}H]thymidine (18 Ci/mol; 2 μCi/ml) for 4 hr at 25°.

Cells were harvested by centrifugation and washed twice in buffered fly Ringer's solution (0.01 *M* sodium phosphate (pH 6.8), 0.75% NaCl, 0.035% KCl, and 0.021% $CaCl_2$). Cells were suspended in this solution and lysed with Triton X-100 (1% final concentration); lysis was monitored by phase contrast microscopy. The suspension was layered over a 30% (w/v) sucrose solution containing 0.1 *M* Tris-HCl (pH 7.4) and centrifuged for 10 min at 4000*g*. For preparation of labeled DNA the nuclear pellet was suspended in 20 ml of 1 *M* LiCl, 0.1 *M* Tris-HCl (pH 8.0), 0.1 *M* EDTA, and 2% sodium dodecyl sulfate and heated at 60° for 15 min. Predigested Pronase was added to 500 μg/ml and the mixture was incubated for 1 hr at 50°; 10 ml of chloroform and 10 ml of phenol (saturated with 0.1 *M* Tris-HCl (pH 8.0)) were added and the preparation was mixed vigorously at room temperature for 30 min. The emulsion was clarified by centrifugation. The interphase was reextracted with LiCl–Tris–EDTA–dodecyl sulfate and the combined aqueous layers were deproteinized twice more with equal volumes of the chloroform–phenol mixture. [^{3}H]Thymidine-labeled DNA, recovered by alcohol precipitation, had a specific activity of 250,000 dpm/μg. Unlabeled DNA was prepared by a similar procedure. Residual RNA was removed by alkaline hydrolysis (0.33 *N* NaOH, 50°, 40 min).

The procedures for isolating L-cell [^{3}H]thymidine-labeled and unlabeled DNA have been described elsewhere (Straus and Birnboim, 1974). The specific activity of the labeled DNA was 250,000 dpm/μg.

Isolation of ^{3}H-Labeled Polypyrimidines. [^{3}H]DNA (200–1000 μg) was dissolved in 0.5 ml of water and mixed with 1.5 ml of 90% formic acid–2.7% diphenylamine. The mixture was incubated in the dark at 30° for 18 hr (Burton, 1967). Diphenylamine and formic acid were removed by a

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Table I: Recovery of ^{3}H-Labeled Polydeoxynucleotides after Ether Extraction of Formic Acid–Diphenylamine.[a]

^{3}H-Labeled Polydeoxynucleotide Added	% Recovery of ^{3}H dpm after Ether Extraction by	
	Standard Procedure of Burton (1967)	Modified Procedure
Ethanol-soluble pyrimidine oligonucleotides	86.1	89.1
Poly(dT)	13.1	92.8
L-cell polypyrimidines	31.7	78.6
Drosophila polypyrimidines	23.0	82.9

[a] Replicate samples containing 200 μg of calf thymus DNA in 0.5 ml of water and 1.5 ml of 90% formic acid–2.7% diphenylamine were incubated in screw-capped, conical glass tubes at 30° for 22 hr. A small volume (5–50 μl) of the indicated ^{3}H-labeled oligo- or polydeoxynucleotide was added and formic acid–diphenylamine removed by ether extraction in either of two ways. For the *standard* procedure (Burton, 1967), 1.0 ml of water was added and the solution was extracted three times at room temperature with 5 ml of ether. The aqueous phase was recovered and counted directly in 5 ml of PCS scintillator. For the *modified* procedure, 1.0 ml of 10 *M* urea–1 *M* sodium acetate replaced the 1.0 ml of water in the standard procedure. Counting efficiencies were estimated using [^{3}H]H_2O as an internal standard, and ranged from 27 to 33%. *Drosophila* polypyrimidines (5600 dpm) and L-cell polypyrimidines (27,000 dpm) were those described in this paper. Poly(dT) (143,000 dpm) had a specific activity of 50 Ci/mol. Ethanol-soluble oligonucleotides were prepared from [^{3}H] thymidine-labeled L-cell DNA, as described under Materials and Methods.

modified ether extraction procedure (Birnboim et al., 1973) because the method of Burton (1967) results in a selective loss of high molecular weight polynucleotides (Table I). In the modified procedure, 1.0 ml of a solution containing 10 *M* urea, 1 *M* sodium acetate, and 100 μg of tRNA was added to the hydrolysis mixture. The solution was extracted three times with 5 ml of ether. The extraction tube was rinsed with 1.0 ml of 1 *M* sodium acetate at 60° and the aqueous solutions were combined; 50 μg of tRNA and two volumes of cold ethanol were added. After 30 min at −20°, the precipitate which formed was collected by centrifugation at 30,000*g* for 15 min at 0°. It was dissolved in 1.0 ml of 0.1 *M* sodium acetate, 0.05 *M* Tris-HCl (pH 8.0), and 5 m*M* CDTA[1] and the polynucleotides were reprecipitated with ethanol. This was repeated once with 0.2 ml of the acetate–Tris–CDTA solution.

Polypyrimidines were separated by polyacrylamide gel electrophoresis or by alkaline sucrose gradient sedimentation. Electrophoresis was more satisfactory for L-cell pyrimidine tract analysis, whereas sedimentation was more favorable for *Drosophila* material. Conditions for specific experiments are given in the figure legends.

Preparative amounts of polypyrimidines for annealing experiments were isolated using a column of Sephadex G-50 fine (0.9 × 25 cm), equilibrated with 7 *M* urea, 0.1 *M* NaCl, 0.02 *M* Tris-HCl (pH 8.0) and 5×10^{-4} *M* CDTA. Alcohol-precipitated samples were dissolved in 1.0 ml of this solution, applied to the column, and eluted at a flow rate of 0.275 ml per min per cm^2. Fractions were collected at 2.5-min intervals and the amount of ^{3}H radioactivity in 10-μl aliquots of each was determined. Long pyrimidine tracts, excluded from the gel matrix, were quantitatively precipitated as follows. Pooled fractions were diluted with an equal volume of water; $ZnCl_2$ and NaOH were each added to a final concentration of 5 m*M*. Oligonucleotides and polynucleotides are quantitatively coprecipitated with zinc hydroxide which forms a heavy precipitate (Birnboim, 1972). The zinc hydroxide precipitate was washed with water and the tightly bound pyrimidine tracts were released by suspending the precipitate in 1.0 ml of a mixture containing 70% ethanol, 30% 0.1 *M* sodium acetate, 0.167 *M* CDTA, and 0.167 *M* Tris-HCl (pH 8.0). Zn^{2+} was complexed by the CDTA and polynucleotides were precipitated by the ethanol. After 30 min at −20°, the sample was centrifuged at 0° for 15 min at 30,000*g*. The precipitate was washed once more with ethanol–acetate–CDTA–Tris as before. Purified polypyrimidines were dissolved in 10 m*M* NaCl, 1 m*M* EDTA, 1 m*M* Tris-HCl (pH 7.4), and 0.2% sodium dodecyl sulfate.

Annealing of ^{3}H-Labeled Polypyrimidines to DNA. [^{3}H]Thymidine-labeled polypyrimidines were mixed with homologous or heterologous DNA, heated at 100° for 5 min, and then annealed to indicated C_0t values (Britten and Kohne, 1968). Hydroxylapatite chromatography was used to assess the amount and thermal stability of duplex structure which formed (Miyazawa and Thomas, 1965). Samples were diluted to 4 ml with phosphate buffer (pH 6.8) and charged onto a hydroxylapatite column containing 0.3 g of dry weight of Bio-Gel HTP (Bio-Rad Laboratories). The column was eluted with 4.0-ml portions of phosphate buffer at 5° temperature intervals to monitor thermal stability or by elution at 55 and 95° for C_0t analysis. Radioactivity bound at 55° and eluted at 95°, divided by the input radioactivity, was taken as a measure of reassociated ^{3}H-labeled polypyrimidines. Specific conditions for each experiment are given in the legend to Table II and Figure 3.

Miscellaneous. ^{3}H Radioactivity was determined by liquid scintillation counting. Fractions from hydroxylapatite chromatograms (4 ml of 0.12 *M* phosphate buffer) were mixed with 15 ml of PCS scintillation fluid (Searle Instrumentation) and counted at a ^{3}H efficiency of 24%. Fractions from alkaline sucrose gradients were neutralized with 0.5 ml of 0.1 *M* acetic acid and counted in 4 ml of Aquasol (New England Nuclear). Phosphate buffer used throughout is sodium phosphate (pH 6.8).

Poly(A) was purchased from P-L Biochemicals and [^{3}H]poly(dT) at 50 Ci/mol was purchased from General Biochemicals. Other methods dealing with the isolation and characterization of long pyrimidine tracts have been described previously (Birnboim et al., 1973).

Results

Selective Loss of Long Pyrimidine Tracts Following Formic Acid–Diphenylamine Hydrolysis. Pyrimidine tract analyses in this and earlier experiments (Birnboim et al., 1973; Straus and Birnboim, 1974) were carried out using the Burton (1967) procedure for hydrolyzing DNA. Short pyrimidine tracts represent the bulk of the nucleotide material in such hydrolysates, and they may readily be freed of formic acid and diphenylamine by ether extraction (Burton, 1967; Table I). On the other hand, ether extraction is unsatisfactory for studying long pyrimidine tracts since there were marked losses associated with this step (Table I). The mechanism of this loss has not been studied in detail, but it appears that polynucleotides bind very tightly to glass as the formic acid is extracted. The introduction of urea and

[1] CDTA, cyclohexane-1,2-diaminetetraacetic acid from Koch-Light Lab, is a chelating agent which binds metal ions more tightly than does EDTA (O'Sullivan, 1969). Also, it is more soluble in acid and ethanol solutions.

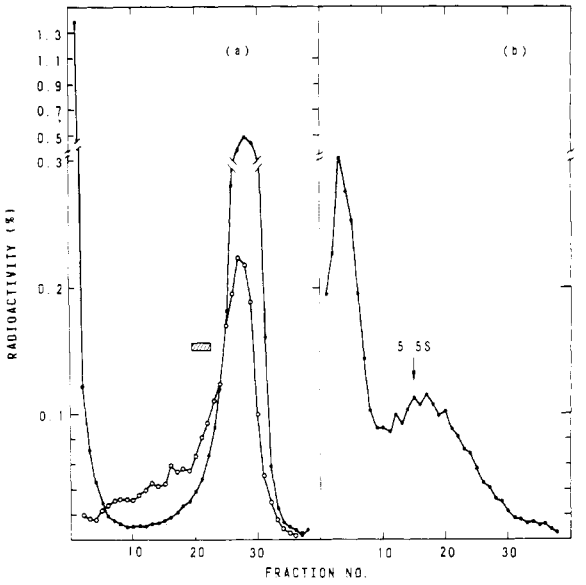

FIGURE 1: Size estimation of polypyrimidines from *Drosophila* and L-cell DNA. (a) Polyacrylamide gel electrophoresis. Alcohol-precipitable pyrimidine tracts from *Drosophila* DNA (●) and from L-cell DNA (○) were prepared as described under Materials and Methods and separated by gel electrophoresis. The shaded box indicates the position of a Bromophenol Blue dye marker, 4.0 cm from the origin. Gels were prepared, run, and counted as described previously (Birnboim et al., 1973). The total sample of *Drosophila* DNA contained 2.2×10^6 cpm. (b) Alkaline sucrose gradient sedimentation. Alcohol-precipitable pyrimidine tracts from 2.3×10^5 cpm of *Drosophila* DNA were dissolved in 200 μl of 0.2 *N* NaOH-0.05 *M* CDTA and layered over a 12.9 ml 5–20% (w/v) gradient of sucrose in 0.9 *N* NaCl-0.1 *N* NaOH. The sample was centrifuged in a Beckman SW 40 rotor at 38,000 rpm for 17 hr at 20°. Fractions were collected and counted as described under Materials and Methods. Marker DNA in this figure and in Figure 2 was sheared mouse DNA whose sedimentation coefficient in 0.9 *N* NaCl-0.1 *N* NaOH was determined using a Beckman Model E centrifuge. In both (a) and (b), the direction of migration is from left to right. The ordinate shows radioactivity in each gel or gradient fraction as a percentage of ^3H radioactivity in total DNA. These values are not corrected for losses (20–30%) of polypyrimidines from DNA hydrolysates (Figure 2).

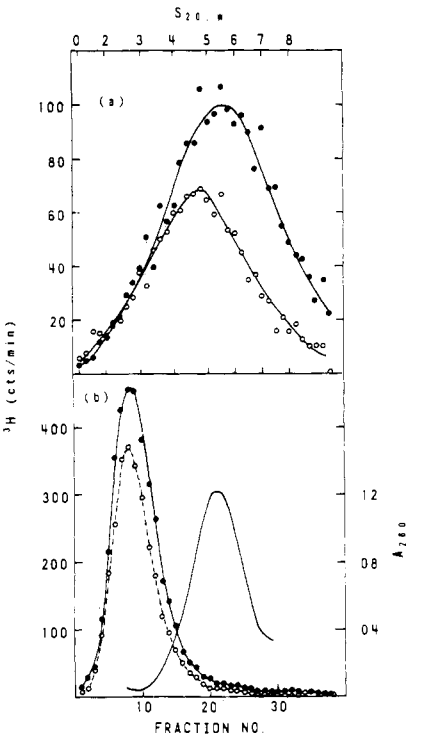

FIGURE 2: Sedimentation analysis of isolated polypyrimidines from (a) *Drosophila* DNA and (b) L-cell DNA. Polypyrimidines were isolated from hydrolysates of [^3H]thymidine-labeled DNA using Sephadex chromatography as described under Materials and Methods. A portion of each was analyzed directly (●). An equal portion was mixed with 200 μg of calf thymus DNA and retreated with formic acid–diphenylamine at 30° for 18 hr. The samples were extracted with ether and precipitated as described under Materials and Methods (○). Both samples were dissolved in 0.2 ml of 1 *N* NaOH and layered over 12.9-ml gradients containing 5–20% (w/v) sucrose in 0.9 *N* NaCl-0.1 *N* NaOH. The gradients were centrifuged in a Beckman SW 40 rotor at 38,000 rpm for 22.5 hr at 20°. The solid line in (b) is the absorbance profile of sheared mouse DNA which was added to each sample. The marker DNA had a sedimentation coefficient of 5.5 S, as determined in a Beckman Model E centrifuge. The $s_{20,w}$ values on the upper abscissa were calculated according to the method of McEwen (1967).

sodium acetate considerably improved the recovery of long tracts (polypyrimidines).

Size Estimate of Drosophila Polypyrimidines. In our previous studies, polypyrimidines were detected in HeLa DNA and L-cell DNA by hydrolyzing the DNA with formic acid–diphenylamine and analyzing the ethanol-precipitable fraction by polyacrylamide gel electrophoresis. When DNA from *D. melanogaster* cultured cells was similarly treated, the gel profile was very different than that obtained using L-cell DNA (Figure 1a). In the former, there was a pronounced peak of radioactivity at the top of the gel, as well as a lesser amount in the polypyrimidine region (fractions 2–22).[2] Additional purification of the *Drosophila* DNA did not appreciably alter this result.

Sedimentation in alkaline sucrose was tested as an alternative method for size separation since with this method (i) aggregation is less likely to be a problem than with gel electrophoresis and (ii) molecular weights can be estimated (Abelson and Thomas, 1966). Pyrimidine tracts from *Drosophila* DNA were sedimented in an alkaline sucrose gradient. The ^3H profile showed two peaks of radioactivity, one near the top of the tube (short pyrimidine tracts, fractions 1–7) and a second which represented more rapidly sedimenting polydeoxynucleotides (Figure 1b). The latter comprised approximately 1.6% of the total [^3H]thymidine in DNA and was considered to be polypyrimidines.

In order to characterize this material further, labeled DNA from *Drosophila* was hydrolyzed and polypyrimidines were isolated by Sephadex G-50 chromatography (see Materials and Methods). ^3H-labeled polynucleotides which eluted in the excluded volume showed a sedimentation profile (Figure 2a) which was similar to the second peak in Figure 1b. When this material was retreated with formic acid–diphenylamine, very little breakdown occurred (Figure 2a). Thus, the large size of these pyrimidine tracts is unlikely to be due to incomplete hydrolysis of purine residues. For comparison, the sedimentation profile of similar material prepared from L-cell DNA is shown in Figure 2b. From the sedimentation profile, polypyrimidines from *Drosophila* are estimated to range from 100 to over 1000 nucleotides long, with weight average molecular weight corresponding to 780 (Studier, 1965). For the L-cell material, the estimate is from 25 to 150 nucleotides long, with a weight average of 70. Particularly in the case of *Drosophila,* the size estimate should be considered a minimum since

[2] Differences in the recovery of shorter pyrimidine tracts (fractions 25–30) were not considered significant since they were not reproducible. This "peak" actually represents the tail end of a much larger peak of small pyrimidine oligonucleotides, and variations in recovery may depend upon slight differences in precipitation by ethanol.

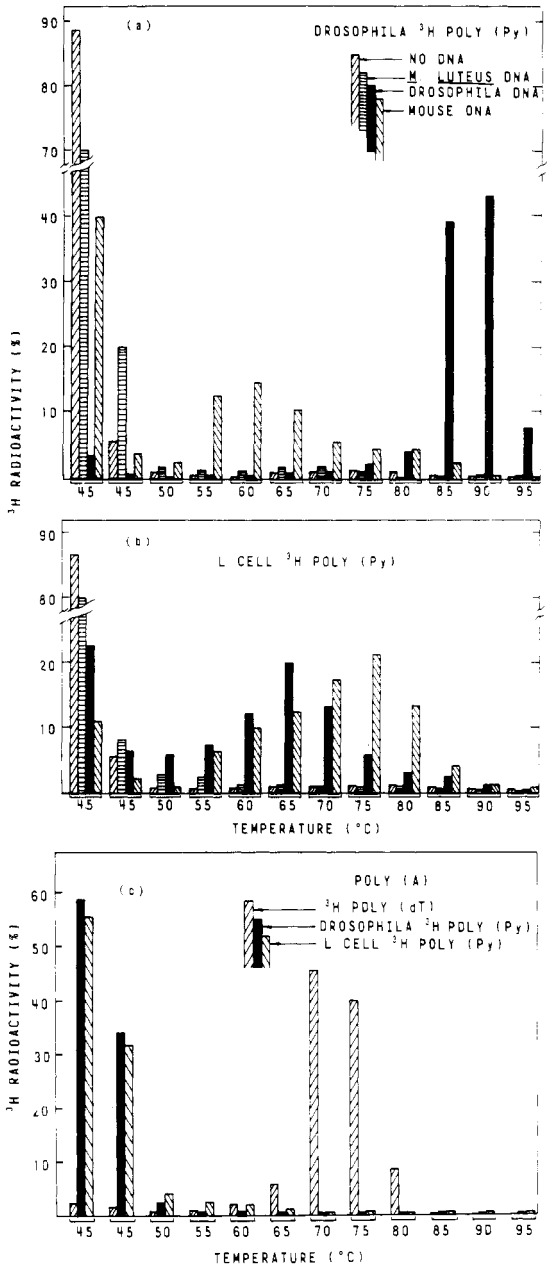

FIGURE 3: Thermal chromatography on hydroxylapatite of hybrids formed between [^{3}H]pyrimidine polynucleotides and unlabeled DNA or poly(A). The labeled species in (a) was *Drosophila* polypyrimidines (2000 cpm) and in (b) it was L-cell polypyrimidines (3200 cpm). Each was annealed in 0.05 ml of 0.14 *M* phosphate buffer at 50° either without DNA, or with *Micrococcus luteus* DNA, *Drosophila* DNA, or mouse DNA. The DNA concentration was adjusted in the last three cases to give C_0t values of 50, 40, and 80, respectively, after 16 hr of incubation. The samples were diluted into 1 ml of 0.1 *M* phosphate buffer–0.03 *M* NaCl and charged onto a column of hydroxylapatite (0.3 g) equilibrated with 0.1 *M* phosphate buffer–0.03 *M* NaCl. Each column was washed with 3 ml of phosphate–NaCl at 45°, and then with 4-ml volumes at increasing temperatures. The amount of ^{3}H radioactivity eluted at each temperature as a percentage of the total amount of radioactivity recovered is plotted. In (c), [^{3}H]poly(dT) and *Drosophila* and L-cell polypyrimidines were each mixed with unlabeled poly(A) (100 μg/ml) in 0.5 ml of 0.1 *M* phosphate buffer–0.03 *M* NaCl, heated to 100° for 3 min, then incubated at 45° for 30 min. The samples were analyzed by thermal chromatography as in (a) and (b).

some degradation of polypyrimidines may have occurred in formic acid–diphenylamine and because there may have been some selective loss of the largest molecules.

Annealing Properties of Isolated Polypyrimidines. Polypyrimidines from both *Drosophila* and L-cell DNA are capable of annealing with homologous DNA, as assessed by binding to hydroxylapatite at 55° in 0.12 *M* phosphate buffer. A preliminary estimate of the kinetics of this association is possible, although the analysis differs from standard conditions (Britten and Kohne, 1968; Wetmur and Davidson, 1968). The data of Table II show that in both cases the apparent $C_0t_{1/2}$ values are low, less than 0.001 for *Drosophila* and less than 0.04 for the L-cell material. The C_0t values are calculated with respect to total DNA, of which only 1–2% constitutes homologous sites.

Table II: Annealing of ^{3}H-Labeled Polypyrimidines to Homologous DNA.[a]

C_0t of DNA	Total Amount of DNA (μg)	Polypyrimidines Bound to Hydroxylapatite (%)
(a) *Drosophila*		
4	7.8	98.0
0.4	3.9	82.7
0.04	3.9	84.6
0.008	6.0	85.8
0.004	3.9	76.8
0.004	0.31	42.1
0.001	6.0	77.6
0.0001	0.31	28.9
(b) L-cell		
10	250	54.4
4	200	56.2
0.4	26.0	51.7
0.04	26.0	32.2

[a] Drosophila and L-cell polypyrimidines (2700 and 5000 cpm, respectively) were mixed with homologous DNA in 0.12 *M* phosphate buffer and heated at 100° for 5 min. The samples were subsequently incubated at 55° to the indicated C_0t values. Analysis of ^{3}H-labeled polypyrimidine–DNA duplex was performed using hydroxylapatite (0.3 g). Unbound ^{3}H-labeled polypyrimidines were removed at 55° and bound ^{3}H was recovered at 95° (8.0 ml of 0.12 *M* phosphate buffer at each temperature). The DNA used was sheared lightly by passage through a No. 26 needle.

The duplex structures which form can be tested with respect to their thermal stability (Figure 3). *Drosophila* and L-cell polypyrimidines were incubated with homologous DNA to C_0t 40 and 80, respectively, at 50° in 0.1 *M* phosphate buffer–0.03 *M* NaCl. The elution of ^{3}H-labeled polypyrimidines is shown as a function of increasing temperature. *Drosophila* polypyrimidines were eluted as a fairly sharp peak at 85–90° (Figure 3a). Similar results were observed when the unlabeled *Drosophila* DNA was obtained from adult flies, indicating that these sequences are not limited to cultured cells. L-cell polypyrimidines eluted as a broader peak with a midpoint of 70–75° (Figure 3b).

Cross-reaction of ^{3}H-labeled polypyrimidines with heterologous DNA was also assessed. Considerable annealing was detected between L-cell ^{3}H-labeled material and unlabeled *Drosophila* DNA (Figure 3b); the product was eluted from the column within 10° of the homologous L-cell reaction. Almost no "self-reaction" or reaction with *Micrococcus luteus* DNA was detectable under these conditions. *Drosophila* ^{3}H-labeled polypyrimidines also cross-reacted with L-cell DNA but the product had a much lower melting temperature, 60–65° (Figure 3a). No reaction with *M. luteus* DNA or "self-reaction" was observed. The cross-reaction of L-cell polypyrimidines with other DNAs has recently been studied (N. A. Straus and H. C. Birnboim, in preparation).

Polypyrimidines from both *Drosophila* and L-cells were

tested for the presence of long runs of thymidine residues by annealing with poly(A). [^{3}H]Poly(dT), about 100–150 nucleotides average length, was tested for comparison. ^{3}H-labeled samples were incubated with poly(A) (100 μg/ml) at 45° for 30 min, then loaded onto hydroxylapatite at 45° and eluted with 0.1 *M* phosphate buffer–0.03 *M* NaCl as described. [^{3}H]Poly(dT) formed a stable hybrid which eluted at 70–75°, but neither L-cell or *Drosophila* polypyrimidines showed any reaction (Figure 3c).

Discussion

The present experiments have documented that very long pyrimidine tracts (polypyrimidines) are present in *Drosophila* DNA and that somewhat shorter ones are present in L-cell DNA. Their resistance to alkali (during sucrose gradient sedimentation) and to repeated treatment with formic acid–diphenylamine supports the contention that they are bona fide long pyrimidine polydeoxynucleotides. Another line of evidence is their ability to anneal to homologous DNA (Figure 3), but not to poly(A) or to bacterial DNA. Additional arguments concerning polypyrimidines from HeLa have been presented (Birnboim et al., 1973). An important technical point to be noted is that there can be selective losses of polypyrimidines *after* acid hydrolysis (Table I), and this could be confused with nonspecific breakdown. The mechanism of the loss is uncertain but it is possible that this may be the cause of the discrepancy between the low rate of breakdown we observed (Figure 2) and that reported by Tate and Peterson (1974).

Since polypyrimidines from both *Drosophila* and L-cell DNA are able to anneal to DNA, as assayed by hydroxylapatite binding, we have been able to study the rate of the reaction and the thermal stability of the product. However, it is important to note that interpretation of the results is not straightforward; this reaction differs from standard DNA reassociation (Britten and Kohne, 1968; Wetmur and Davidson, 1968) in that equimolar amounts of the two reacting strands are not present. In this sense the reaction resembles RNA–DNA hybridization, and saturation of the DNA was observed (Table IIa, C_0t 0.004). Secondly, the size range of L-cell polypyrimidines is such that an appreciable effect of duplex length on T_m and hydroxylapatite binding would be expected (Wilson and Thomas, 1973). This is reflected in the decreased binding at temperatures below 55° of L-cell polypyrimidine–DNA hybrid in 0.12 *M* phosphate buffer (Table II) as compared to 0.10 *M* phosphate buffer (Figure 3). Thus, we have tended to view these results as demonstrating that reassociation of ^{3}H-labeled polypyrimidines with homologous DNA is possible, but that the rate of the reaction and the thermal stability of product are relatvely imprecise indicators of the sequence complexity and fidelity of base pairing of the hybrids. Bearing these limitations in mind, a rough estimate of the maximum sequence complexity of *Drosophila* polypyrimidines can be derived. If the $C_0t_{1/2}$ is taken to be $<10^{-3}$ with respect to total DNA, then the $C_0t_{1/2}$ of the reacting sequences (about 10^{-2} of the total DNA) would be $<10^{-5}$. Sequence complexity is directly related to $C_0t_{1/2}$ (Britten and Kohne, 1968). *Drosophila* DNA has a sequence complexity of approximately 1.4×10^8 and a $C_0t_{1/2}$ of 65 (Laird, 1971). The expected sequence complexity of polypyrimidines should be $<10^{-5}/65 \times 1.4 \times 10^8 \leq 20$. Direct analysis of complementary RNA indicates a simple base sequence (Sederoff, Lowenstein and Birnboim, in preparation). Similar calculations have not been carried out for L-cell polypyrimidines, but the data of Table II suggest that the sequence complexity is also quite low. This experiment extends our earlier observations about L-cell polypyrimidines (Straus and Birnboim, 1974). Previously we showed that when sheared, denatured DNA was allowed to reassociate, polypyrimidines were recovered from the rapidly reassociating fragments. We were unable to distinguish whether it was the polypyrimidines or some adjoining sequence(s) which were responsible for the rapid rate of reassociation. The present experiment suggests that polypyrimidines themselves have a high repetition frequency.

At present we can only speculate about the function(s) of polypyrimidines. Although they have been directly detected in DNA from cultured cells, complementary sequences have been detected in DNA from whole animals (both *Drosophila* and mouse). The size of polypyrimidine (or complementary polypurine) sequences in DNA from whole animals has not been determined. The very large pyrimidine tracts seen in *Drosophila* are likely localized to a satellite DNA component (H. C. Birnboim and R. R. Sederoff, in preparation). Somewhat smaller tracts, such as those seen in HeLa and L-cell DNA, have also been seen in DNA from several eukaryotes (H. C. Birnboim and N. A. Straus, in press). We are studying cellular RNA to determine if these sequences are transcribed.

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