Preferential DNase I sensitivity of insert-free ribosomal RNA repeats of *Drosophila melanogaster*

Richard L. Wayne, Z. Dave Sharp* and James D. Procunier

Biology Department, Rice University, Houston, TX 77005, USA

Received 7 September 1984; Revised and Accepted 15 March 1985

ABSTRACT

The five predominant types of rDNA repeats in *D. melanogaster* were analyzed with respect to their DNase I sensitivity. Only the insert-free repeats showed a generalized DNase I sensitivity pattern whereas the major type I, both minor type I and type II repeats were not as extensively degraded by the nuclease. For XX and XY embryonic nuclei, where there is rapid cell division, the majority of the In^- repeats were DNase I sensitive. This indicated that these In^- repeats have the potential to be transcribed during this developmental stage. When compared to the In^- repeats, the chromatin configuration of the In^+ repeats is indicative of a higher order of chromatin folding. The paucity of In^+ primary gene transcripts observed *in vivo* could result from In^+ repeats being packaged into a more condensed form of chromatin.

INTRODUCTION

Genes active in a particular cell type are packaged into a more open chromatin configuration than inactive genes (1, 2, 3). Digestion of transcriptionally active chromatin with DNase I or micrococcal nuclease is faster than digestion of inactive chromatin. Data from many species shows that the domains of the nuclease sensitivity include the structural gene and discrete sites at or near the 5' end of the structural gene. These 5' sites are extremely sensitive to DNase I and are named the DNase I - hypersensitive sites (4, 5).

The fruit fly's rRNA transcription unit within the repeat unit consists of a 5' external transcribed spacer (ETS), the 18S coding gene, an internal transcribed spacer coding for the 5.8 and 2S rRNA species and the 3' 28S gene (6). The non-transcribed spacer (NTS) lies adjacent to the 28S gene and contains a number of tandemly repeating 'Alu I' islands (240 bp in length) upstream from the transcription initiation site (7). The promoter region for RNA polymerase I is adjacent to the 5' end of the ETS and shares sequence homologies with the upstream 'Alu I' islands (8). These islands serve as sites for infrequent transcription initiation as shown by short 'prelude' transcripts (9). In *Xenopus*, comparable upstream 'islands' contain sub repeat regions which enhance transcription of the rDNA unit (10, 11). The most

upstream 'island' is DNase I-hypersensitive (12).

The nucleolus organizer region (NO) of *D. melanogaster* contains about 206-228 ribosomal DNA repeat units on each of the X and Y chromosomes (13). About 73% of the X-NO repeats (In^+) contains an intervening sequence in the 28S rRNA gene (14). These insertion sequences vary in size and in sequence. For X-NO repeat units, the type I inserts are divided into size classes of 5.0, 1.0 and 0.50 kb (kilobases) and are present in approximate amounts of 40, 12 and 5%, respectively. Sequences homologous to the type I inserts are located outside of the X-NO region in the centric heterochromatic region where they are arranged as tandem repeats (15, 16). Type II inserts are non-homologous to type I, ranging in length from 0.50 to 3.4 kb; they are present in about 16% of the X-NO repeats. The Y-NO has type II inserts in 16% of its repeats and the remaining repeats are insert-free (In^-).

Neither the major type I (17), minor type I (18) or type II (18, 19) repeats are significantly transcribed *in vivo*. Less than one copy of major type I rRNA per nucleus is present while more than 1000 rRNA transcripts from the In^- repeats are detected. The concentration of transcripts from either the minor type I repeats or type II repeats also contributes insignificantly to the rRNA pool. Even under conditions in which there is a strong pressure to use all the available rDNA repeats, as in *bobbed* (large deletions of rDNA) flies, transcription of the In^+ repeats occurs infrequently (20). These results concur with the absence of active In^+ repeats in Miller spreads of nucleolar chromatin in *D. hydei* (21). However, In^- and In^+ repeats have identical sequences surrounding the initiation site (22) and are equally active as templates for *in vitro* transcription (23). This suggests that the In^+ repeats *in vivo* may exist in a chromatin configuration which inhibits transcription. Previous evidence indicates that the In^+ repeats of *D. melanogaster* are contained in a large block(s) distinct from the In^- repeats (14). In *C. erythrocephala*, In^+ repeats are localized to inactive heterochromatin associated with the NO (24). The *in vivo* inactivity of In^+ repeats of *D. melanogaster* may be due to their distinct chromosomal location and chromatin configuration.

In this paper, we have compared the nuclease sensitivity of the chromatin of the In^- and In^+ II repeats in actively dividing embryonic tissue. By using a wide range of DNase I concentrations, the In^- repeats were preferentially sensitive to digestion which suggests that the In^+ repeats are contained in a more compact chromatin configuration than the insert-free repeats.

MATERIALS AND METHODS

Drosophila stocks.

D. melanogaster cultures were reared at 25°C on a standard cornmeal, molasses, agar and yeast medium. The wild-type Oregon-R strain originated from an

isogenic line of flies. The X nucleolus organizer region has about 228 rRNA repeats and the Y-NO about 206 repeats (13). Eggs were collected by mating XY males and XX females in a large population cage.

Isolation of nuclei and DNA.

Zero to twenty-four hr old embryos were harvested and dechorionated with a 50% Chlorox, 0.01% triton X-100 (Kodak) solution for 5 min. Nuclei were extracted by a modification of the procedure of Fisher *et al.* (25). All operations were performed on ice. One ml of distilled water washed, dechorionated, packed embryos ($4\text{--}5.0 \times 10^4$ individuals) were suspended in 10 ml of extraction buffer containing 50 mM NaCl, 5 mM MgCl_2 , 250 mM sucrose, and 50 mM Tris-HCl (pH 7.5). The suspended embryos were disrupted in a Dounce homogenizer (six strokes, pestle A) and filtered through two layers of Miracloth. The homogenized embryos were centrifuged at 1000g for 10 min. The crude nuclear pellet was resuspended in 5 ml of extraction buffer and centrifuged again at 1000g for 10 min. The pellet of purified nuclei was then suspended in 1 ml of 20 mM Tris-HCl (pH 7.5) and 5 mM MgCl_2 . The purified nuclei were either immediately used or quickly frozen in liquid nitrogen for later use. Fresh and frozen samples of nuclei showed no significant nuclear disruption when examined under phase-contrast light microscopy.

High molecular weight protein-free DNA for the control experiments was prepared by the Blin and Stafford method (26). XX and XY embryos were ground in a mortar and pestle (at -70°C) containing liquid nitrogen. The powdered eggs were suspended in 0.5% Sarkosyl, 100 $\mu\text{g}/\text{ml}$ proteinase K and 0.5 M EDTA (pH 8.0) and incubated for 3 hrs at 50°C with swirling. Three gentle chloroform extractions (equal volumes of water-saturated phenol, pH 8.0, containing 0.1% 8-hydroxyquinoline and chloroform) were carried out and the DNA solution dialyzed extensively against 0.01 M EDTA, 0.01 M NaCl and 0.05 M Tris (pH 8.0). Heat treated RNase A (100 $\mu\text{g}/\text{ml}$) was added and the RNA digested for 3 hrs at 37°C with swirling. Two additional chloroform extractions were done followed by two ether extractions. The DNA solution was then dialyzed against 10 mM sodium borate and 1 mM EDTA (pH 9.1) and stored at -70°C .

DNase I digestion.

DNase I (Boehringer-Mannheim) was used in concentrations ranging from 0 unit/ml to 10 units/ml following the procedure of Shermoen and Beckendorf (5). Appropriate concentrations of DNase I were adjusted in 25 μl of digestion buffer (60 mM KCl, 15 mM NaCl, 100 mM MgCl_2 , 0.1 mM CaCl_2 , 15 mM Tris-HCl (pH 7.4) and 0.5 mM dithiothreitol). 25 μl of purified nuclei (containing 25 μg DNA) were adjusted to a volume of 475 μl in 60 mM KCl, 15 mM NaCl, 0.05 mM CaCl_2 , 0.1 mM EDTA, 0.5 mM dithiothreitol, 0.25 mM sucrose and 15 mM Tris-HCl (pH 7.4). DNase

I in the digestion buffer was added to the nuclear suspension and digestion was allowed to proceed at 25°C for 3 min. Digestion was terminated by the addition of 20 μ l of 0.5 M EDTA. Nuclei were disrupted by adding SDS to a final concentration of 0.5% followed by gentle vortexing. The DNA was isolated by digesting overnight with 70 μ g/ml of proteinase K (Boehringer-Mannheim) at 37°C followed by extraction twice with chloroform and twice with ether. The aqueous phase was made 2.5 M in ammonium acetate, tRNA was added to a concentration of 25 μ g/ml and the DNA was precipitated in 2.5 vol of ethanol overnight at -20°C. Purified DNA was digested with both BamHI and EcoRI obtained from Boehringer-Mannheim following the manufacturer's protocol. RNA was removed prior to loading on a 0.8% gel by using heat treated RNase T1 (1 unit/ml) and RNase A (0.05 μ g/ml) and incubating at 65°C for ten min. Our size standard was Drigest III obtained through New England Biolabs.

Southern transfers.

Electrophoresis was performed on 0.8% agarose gels and the DNA transferred to Gene Screen nylon filters (NEN) following the manufacturer's protocol. The filters were probed with 32 P-dCTP nick translated plasmids pBW 416, 423, C2 and C24 (Figure 1). The probes were used either separately or in combination. All nick translation enzymes were obtained through Boehringer-Mannheim. A specific activity of $3 - 10 \times 10^7$ cpm/ μ g DNA was obtained. Hybridizations were done as previously described (14). The filters were air-dried, sealed in plastic bags and autoradiographed at -70°C with Kodak XAR-5 film overlaid by a Du Pont Cronex Lighting Plus intensifying screen. The film was preexposed to a change of OD₅₄₀ of 0.15 (27) to ensure sensitivity in the linear response range. The autoradiographs were scanned with a Kraton SD 3000 spectrodensitometer interfaced with a Hewlett-Packard 3390A integrator. Many autoradiographic exposures per filter were scanned to allow determinations within the linear response range. The filters were later stripped by immersion in 95°C distilled water for 10 min and reprobed.

RESULTS AND DISCUSSION

Restriction endonuclease EcoRI has one cleavage site in the 18S coding region of each rDNA repeat unit and three sites in the type II 28S insertion. BamHI has two cleavage sites about one kb apart in the major type I insertion, two sites in the 1 kb minor type I insertion, one site in 0.50 kb minor type I insertion and no sites in In^- repeats or type II insertions. Digestion of rDNA with EcoRI and BamHI (Figures 1 and 2) generates a 12 kb band composed solely of In^- repeats. Thus the DNase I sensitivity of the transcriptionally active In^- repeats can be determined. In addition, the double digestion separates the various In^+ repeat types (major type I,

Figure 1. *D. melanogaster* rDNA repeat units. The three length variants of type I insertion repeat units (5.0, 1.0 and 0.50 kb inserts) and the type II repeat (3.4 kb insert) are shown. The inserts are within the 28S coding region. ITS is internal transcribed spacer and NTS is non-transcribed spacer. The external transcribed spacer is immediately 5' to the 18S gene. The 28S gene is subdivided into its two mature halves, 28S and 28S B. The sites for restriction enzymes *EcoRI* (P) and *BamHI* (Q) are shown. Fragments of each repeat unit generated by single digestion with *EcoRI* or *BamHI* and double digestion with *EcoRI/BamHI* are illustrated. Hybridization probes are shown above the repeats.

both minor type I and type II) when probed with plasmid pBW 416. *EcoRI/BamHI* digestion cleaves the major type I repeat into a 5' end of 9.0 kb and a 7.4 kb 3' end; the minor type I repeats are cleaved into 7.4 kb 3' ends and 5' ends of about 4.5 and 5.0 kb in length. The type II *In*⁺ repeats are cleaved into 8.0 and 5.4 kb bands. The *In*⁻ repeats are undigested by *BamHI* and give *EcoRI* fragments of 12 kb in length. The tandemly repeated non-nucleolar type I elements, heterogenous in length and sequence, are cleaved by *BamHI* to give fragments about 4 kb in length (15). DNA digested with *EcoRI/BamHI* and hybridized with the type I insertion plasmids C2 and C24 showed bands about 4.0 kb (unpublished data). However, these non-nucleolar insert elements do not hybridize with either the pBW416 or 423 plasmids (14). This ensures that the 5.4 and 4.5 kb bands hybridizing with pBW416 contain only the type II and minor type I (1.0 kb) repeats, respectively. The relative amount of each type of repeat from embryonic nuclei was determined by *EcoRI/BamHI* digestion of rDNA and probing with plasmid pBW 416 (Table 1). Embryonic XX and XY protein-free DNA was digested with *EcoRI/BamHI*, separated on 0.8% agarose gels, Southern transferred and probed with ³²P-pBW 416 (autoradiogram identical to Figure 2, lane 0). The results were in agreement with the published percentages of the five repeat

Figure 2. DNase I sensitivity of In^- and In^+ repeats from XX and XY embryonic nuclei probed with pBW 416. Embryonic nuclei were treated with DNase I at the various concentrations shown above (units/ml). The DNA was isolated, double digested to completion with EcoRI/BamHI and electrophoresed on 0.8% agarose gels. Equivalent amounts of DNA were applied to each lane. The DNA fragments were Southern transferred to Gene Screen nylon filters and probed with ^{32}P - pBW 416. The fragment sizes was verified by Drigest III standards and are indicated on the left in kilobases.

types for equal amounts of XX and XY rDNA (13, 14). Upon DNase I digestion, protein-free DNA showed ^{32}P -pBW 416 hybridizing material as a smear with the largest fragment being no greater than one kb in length (data not shown).

Table 1 Relative percentage area of each fragment in the EcoRI/BamHI autoradiographic pattern of XX and XY embryonic DNA probed with pBW 416^a.

Repeat type	Fragment (kb)	Percentage
In^-	12.0	31.2 $\pm$.03
major type I	9.0	27.2 $\pm$.05
type II	5.4	20.6 $\pm$.02
minor type I (12.5 kb)	5.0	7.9 $\pm$.05
minor type I (13.0 kb)	4.5	13.1 $\pm$.07

^a The means and standard errors are given for at least four independent autoradiographs. Measurements were obtained from autoradiographs identical to the one in Figure 2, lane 0.

Table 2 The relative percentages of rDNA repeats from DNase I treated nuclei.^a

DNase I (units/ml)	In ⁻ (12 kb)	Major type I (9.0 kb)	Type II (5.4 kb)
0	39.1 ± .03	34.5 ± .05	26.4 ± .01
1	24.9 ± .52	42.2 ± .07	32.9 ± .44
3	11.0 ± .91	46.3 ± .18	42.7 ± 1.1
4	18.2 ± .48	51.7 ± .49	30.1 ± .98
5	11.3 ± .12	51.5 ± .54	37.2 ± .66
6	16.3 ± .36	44.6 ± .64	39.1 ± 1.00
8	5.8 ± .01	47.9 ± .36	46.3 ± .35
10	7.7 ± .07	52.9 ± .53	39.4 ± .46

^a Measurements were obtained from at least four separate autoradiographs represented by Figure 2. The means and standard errors are given. The autoradiographs were scanned with a Kraton SD 3000 spectrodensitometer interfaced with Hewlett-Packard 3390A integrator. Only the 12, 9 and 5.4 kb bands were quantitated due to the high background levels present in the smaller size range. The numbers represent the relative percentages of the three repeat types.

D. melanogaster XX and XY nuclei were treated with concentrations of DNase I ranging from 0 to 10 units/ml, the DNA extracted, double digested with EcoRI and BamHI, separated through a 0.8% agarose gel, transferred to Gene Screen and the fragments probed with ³²P-pBW 416 (Figure 2). An equivalent amount of DNA was applied to each lane. Since the autoradiographs showed less intense signals for all the bands at the higher DNase I concentrations, this indicated that each repeat type demonstrated some sensitivity to the enzyme. However, the In⁻ repeats (12 kb fragment) demonstrated a preferential sensitivity to DNase I digestion when compared to the In⁺ repeats. At the higher DNase I concentrations, densitometer scans indicated a decrease of the 12 kb In⁻ fragment of about 85 percent relative to the fragments of the major type I and type II In⁺ repeats (Figure 2, lanes 8 and 10; Table 2; Figure 3). The percentages given in the table represent only the In⁻, major type I and type II repeats. Both the minor type I repeats (5.0 and 4.5 kb bands) were difficult to quantitate due to the high levels of background at the higher DNase I concentrations. However, both of these minor type I repeat types were seen in the lower range of DNase I concentrations where there was a substantial loss of In⁻ repeats. When the rDNA fragments were reprobbed with both pBW 416 and pBW 423,

Figure 3. The ratio of In^- to In^+ repeats with increasing DNase I concentrations. The ratios of In^- (12 kb)/major type I (9.0 kb) and In^- /type II (5.4 kb) were calculated from the densitometer scan data of table 2 and plotted against DNase I concentration ranging from 0 to 10 units/ml (●-●) are the 12 kb/9 kb and (○-○) the 12 kb/5.4 kb values.

the 7.4 kb band was present at the highest DNase I concentrations used (Figure 4). This band represents the 3' ends of both the major and minor type I repeats. This supports the results shown in Figure 2 that the type I insert bearing repeats are less sensitive to DNase I digestion than the In^- repeats.

Figure 4. DNase I sensitivity of In^- and In^+ repeats probed with both pBW 416 and pBW 423. The filters corresponding to the autoradiograms of figure 2 were stripped and reprobed with pBW 416 and pBW 423. The pBW 423 plasmid hybridizes to the NTS portion of the major and both minor type I repeats (7.4 kb). The NTS portion of the type II repeats (8.0 kb) are difficult to visualize in this gel system, sandwiched between the 7.4 and 9.0 kb bands. The DNase I concentrations (units/ml) are shown above and band sizes in kilobases.

The finding that the In^- repeats demonstrated a preferential sensitivity to DNase I digestion agrees with numerous *in vivo* transcriptional studies. Both type I and II insert-bearing repeats contributed insignificantly to the embryonic rRNA pool (17, 18, 19). Our data suggests that the transcriptional inactivity of the In^+ repeats is correlated with their relative resistance to DNase I digestion. In addition, rapid rRNA splicing need not be envisioned in order to explain the absence of In^+ transcripts. Previous reports (22) on the DNase I sensitivity of *D. melanogaster* rDNA repeats indicated no substantial sensitivity differences between the rRNA coding sequences, type I and type II insertion sequences in cell cultures (unpublished data). We find that insert-free repeats are significantly more sensitive to DNase I digestion than their insert-bearing counterparts. Our results show that the 28S coding region of all In^+ repeats and the major type I 5.0 kb insert are relatively insensitive to DNase I digestion. The 18S and NTS components of the major type I and both minor type I insert-bearing repeats also indicate a preferential resistance to DNase I as illustrated by the presence of a prominent 7.4 kb at the highest DNase I concentrations. Since the 7.4 kb band represents the three classes of type I In^+ repeats, the relative sensitivities of each of these classes cannot be resolved.

These studies show a generalized DNase I sensitivity pattern for the In^- repeats in *D. melanogaster*. The concentrations of DNA and DNase I used in these experiments were equivalent to those used on other rDNA systems (28, 29) where there was a generalized DNase I sensitivity pattern of transcriptionally active rRNA repeats. In *X. laevis* and *X. borealis*, the transcriptionally active rDNA repeats also have a DNase I hypersensitive site (12). By utilizing the indirect end-labeling technique, the site mapped at the most upstream copy of the numerous transcription enhancer elements in the non-transcribed spacer. The experiments in this report do not determine if such DNase I hypersensitive sites exist for the *D. melanogaster* In^- repeats.

Inactive rRNA genes are more resistant to DNase I digestion than active genes and demonstrate the typical nucleosomal ladder (29, 33). Nucleosomes, the fundamental units of chromatin, are either rarely visualized or absent in the 18S and 28S sequences of actively transcribing genes (30). The repeats are either activated for transcription and loaded at a high density with RNA polymerase molecules or they are transcriptionally silent, since repeats having a few RNA polymerase molecules are rare (31). Nuclease digestion of active genes did not show a regular nucleosome ladder for the transcribing regions (29, 32, 33). Rather, a diffuse smear of fragments was seen suggesting that the nucleosomes are either absent or occupy different positions along the DNA. In contrast, the non-transcribed spacer region in

transcriptionally active nucleoli in *Xenopus* showed nucleosomes packaged into a higher order chromatin form, the supranucleosomal structures (34).

Our results indicate that the In^+ repeats of *D. melanogaster* are significantly less sensitive to DNase I digestion than the In^- repeats. This supports the concept that the In^+ repeats are folded into a higher order chromatin configuration. Restriction enzyme and genetic data suggest that the In^+ repeats are arranged as a large tandem linkage group(s) in the nucleolus organizer region (14). The In^- and In^+ repeats share a high degree of sequence homology between their 18S, 28S and NTS components, yet only the In^- repeats are preferentially DNase I insensitive. Either the two types of inserts act in some way to confer folding into a higher order chromatin configuration or these repeats occupy a different chromosomal location and environment than the In^- repeats. In support of the latter, genetic evidence (14) suggests that a large block of major type I repeats are localized in the proximal portion of the X NO region.

ACKNOWLEDGEMENTS

We would like to thank Liebe Wetzel, Amy Howell and Jackie Lentz for their excellent technical assistance and Jim Curran for many helpful suggestions. Support to R.L.W. was provided by a NIH predoctorate training grant. This work was supported by NIH grant GM 28008.

*Present address: Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA

REFERENCES

1. Weintraub, H. and Groudine, M. (1976) *Science* 193, 848-856.
2. Garel, A. and Axel, R. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 3966-3970.
3. Wu, C., Bingham, P., Livak, K.J., Holmgren, R. and Elgin, S.C.R. (1979) *Cell* 16, 797-806.
4. Elgin, S.C.R. (1980) *Cell* 27, 413-415.
5. Shermoen, A.W. and Beckendorf, S.K. (1982) *Cell* 29, 601-607.
6. Beckingham, K. (1982) *The Cell Nucleus* 10, 205-269.
7. Long, E.O. and Dawid, I.B. (1979) *Nucleic Acid Res.* 7, 205-215.
8. Kohorn, B.D. and Rae, P.M.M. (1982) *Nucleic Acid Res.* 10, 6879-6886.
9. Miller, S.R., Hayward, D.C. and Glover, D.M. (1983) *Nucleic Acid Res.* 11, 11-19.
10. Busby, S.J. and Reeder, R.H. (1983) *Cell* 34, 989-996.
11. Reeder, R.H., Roan, J.G. and Dunaway, M. (1983) *Cell* 35, 449-456.
12. La Volpe, A.L., Taggart, M., McStay, B. and Bird, A. (1983) *Nucleic Acid Res.* 11, 5361-5380.
13. Gandhi, V.V., Sharp, Z.D. and Procunier, J.D. (1982) *Biochem. Biophys. Res. Commun.* 104, 778-784.
14. Sharp, Z.D., Gandhi, V.V. and Procunier, J.D. (1983) *Mol. Gen. Genet.* 190, 438-443.
15. Kidd, S.J. and Glover, D.M. (1980) *Cell* 19, 103-119.

16. Peacock, W.J., Appels, R., Endow, S. and Glover, D.M. (1981) *Genet. Res.* 37, 209-214.
17. Long, E.O. and Dawid, I.B. (1979) *Cell* 18, 1185-1196.
18. Labella, T., Vicari, L., Manzi, A. and Graziani, F. (1983) *Mol. Gen. Genet.* 190, 487-493.
19. Kidd, S.J. and Glover, D.M. (1981) *J. Mol. Biol.* 151, 645-662.
20. Long, E.O., Collin, M., Kiefer, B.I. and Dawid, I.B. (1981) *Mol. Gen. Genet.* 182, 377-384.
21. Glatzer, K.H. (1979) *Chromosoma* 75, 161-175.
22. Long, E.O., Rebbert, M.L. and Dawid, I.B. (1981) *Proc. Natl. Acad. Sci. U.S.A.* 78, 1513-1517.
23. Kohorn, B.D. and Rae, P.M.M. (1982) *Proc. Natl. Acad. Sci. U.S.A.* 79, 1501-1505.
24. Beckingham, K. and Rubacha, A. (1984) *Chromosoma*, in press.
25. Fisher, P.A., Berrios, M. and Blobel, G. (1982) *J. Cell Biol.* 92, 664-686.
26. Blin, N. and Stafford, D.W. (1976) *Nucleic Acid Res.* 3, 2303-2308.
27. Laskey, R.A. and Mills, R.D. (1975) *Eur. J. Biochem.* 56, 335-341.
28. Macleod, D. and Bird, A. (1982) *Cell* 29, 211-218.
29. Gottschling, D.E., Palen, T.E. and Cech, T.R. (1983) *Nucl. Acids Res.* 11, 2093-2108.
30. Franke, W.W., Scheer, U., Trendelenburg, M.F., Spring, H. and Zentgat, H. (1976) *Cytobiologie* 13, 401-434.
31. Scheer, I. (1978) *Cell* 13, 535-549.
32. Udvardy, A., Louis, C., Han, S. and Schedl, P. (1984) *J. Mol. Biol.* 175, 113-130.
33. Davis, A.H., Ruedelhuber, T.L. and Garrard, W.T. (1983) *J. Mol. Biol.* 167, 133-155.
34. Pruitt, S.C. and Grainger, R.M. (1981) *Cell* 23, 711-720.