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Cytoplasmic localization of transcripts of a complex G+C-rich crab satellite DNA

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Abstract. The primary sequence and higher order structures of a G+C-rich satellite DNA of the Bermuda land crab *Gecarcinus lateralis* have been described previously. The repeat unit of the satellite is approximately 2.1 kb. In exploring a possible function for this satellite, we asked whether it is transcribed. As a probe for transcripts, we used a segment of DNA amplified from a 368 bp *Eco*RI fragment from the very highly conserved 3′ end of the satellite DNA. During polymerase chain reaction (PCR) amplification, the probe was simultaneously either radiolabeled or biotinylated. Tissue- and stage-specific transcripts were observed when blots of poly(A)$^+$ mRNAs recovered from polysomes isolated from crab tissues [including midgut gland (hepatopancreas), limb bud, and claw muscle] were probed with the satellite DNA fragment. The presence of satellite transcripts in polysomal mRNAs is strong evidence that the transcripts had reached the cytoplasm. To corroborate the presence of transcripts in the cytoplasm, we investigated in situ hybridization of satellite probes with RNAs in tissue sections. Biotinylated satellite DNA probes were applied to sections of midgut gland, limb bud papilla, ovary, or testis of anecdysial crabs. Retention of RNAs in tissue sections was improved by UV-irradiation prior to hybridization. Transcripts were abundant in the cytoplasm of all tissues except testis. Sections of crab midgut gland treated with RNase A prior to hybridization and sections of mouse pancreatic tumor served as controls; neither showed any signals with the probe.

Introduction

The simple repetitive nature of the sequences of many, but not all, satellite DNAs and their localization in heterochromatic regions of chromosomes led to the belief that satellites are not transcribed (Walker 1971; Skinner 1977; Brutlag 1980). Subsequently, however, transcripts of satellite DNAs have been reported in the RNAs of a number of organisms, both vertebrates and invertebrates. These include amphibians, both newts (*Triturus cristatus carnifex*, Varley et al. 1980; *Notophthalmus viridescens*, Diaz et al. 1981) and frogs (*Xenopus laevis*, Jamrich et al. 1983; *Rana catesbeiana*, Wu et al. 1986). Satellite transcripts have also been described in a mammal (mouse, *Mus musculus*, Singh et al. 1984) and in arthropods (*Chironomus thummi*, Baumlein et al. 1982a, b; *Drosophila hydei*, reviewed in Hennig 1985, 1993; *Drosophila melanogaster*, Singh et al. 1984; Bonaccorsi et al. 1990; a blowfly, *Chrysoma rufifacies*, Kirchhoff 1988). These include several brief reports of transcripts of the complex G+C-rich satellite of the Bermuda land crab (*Gecarcinus lateralis*, Wang et al. 1988; Varadaraj et al. 1990). Here we provide further information about transcripts of the land crab satellite. In particular, we provide evidence for the presence of satellite transcripts in the cytoplasm of a number of different tissues.

Approximately 3% of the genome of *G. lateralis* is composed of a G+C-rich satellite DNA (Skinner 1967) with sequences that are conserved in some other Crustacea (Graham and Skinner 1973). There are 16,000 copies per genome of the satellite, which has a repeat unit of approximately 2.1 kb (LaMarca et al. 1981). Satellite repeats are characterized by six domains ranging from 50 to 560 bp that are 85%–98% conserved among different variants of the repeat unit; conserved domains are interspersed with highly divergent domains that contain simple sequences typical of other satellites (Fowler et al. 1985, 1988). In the present study, we describe analyses of blots of polysomal mRNAs and in situ hybridization experiments on sections of several tissues of *G. lateralis*. The segment of the satellite used as a probe is recovered as a 368 bp fragment in *Eco*RI digests of purified satellite DNA; it represents the 3′ end of satellite variants that are truncated in such digests because of the presence of an extra *Eco*RI site and contains segments of two of the highly conserved domains (domains 5 and 6; Fowler et

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al. 1985, 1988). The satellite segment has been subcloned, first into pUC19 (Stringfellow et al. 1985), and subsequently into pGEM-3 (Wang et al. 1988).

Materials and methods

Animals. Land crabs were obtained from the Bermuda Biological Station. The stage of the animal in the intermolt cycle was determined from the length of regenerating limbs and the size of gastroliths (Skinner 1962). Crustacea regenerate missing appendages only during proecdysis and precocious molts in *G. lateralis* can be induced by autotomy of five or more walking legs (pereopods; Skinner and Graham 1970). Proecdysial crabs used in experiments described here were induced to molt by autotomy of six pereopods.

Polysomal mRNA preparations. Midgut gland, regenerating limbs or claw muscle were removed from crabs at different stages of the intermolt cycle and stored in liquid nitrogen. Polysomal mRNAs were isolated by magnesium precipitation as described (Clemens 1984), further enriched for poly(A)$^+$ mRNAs by two rounds of selection on Hybond-mAP (messenger RNA affinity paper; Amersham), and ethanol precipitated. They were solubilized in diethyl pyrocarbonate (DEPC)-treated distilled water, treated with DNase I (Promega) at 37° C for 30 min, and loaded directly on agarose gels (see RNA blot analyses, below).

Probe synthesis and labeling. A subclone containing the 3′ end of the satellite DNA inserted into the *Eco*RI site of pGEM-3 (Stringfellow et al. 1985; Wang et al. 1988; Wang and Skinner, in preparation) was linearized by cutting hybrid plasmids with *Pvu*II and purified by electrophoresis on 3% NuSieve GTG agarose followed by sorption to and elution from a SpinBind microcentrifuge cartridge using FMC BioProducts protocol. For the polymerase chain reaction (PCR), two deoxyoligonucleotide primers based on the sequence of pGEM-3 were synthesized on an Applied Biosystems, (Foster City, Calif.) model 391 DNA synthesizer. Primer 1 was a 30-mer: 5′-ATTTAGGTGA CACTATAGAA TACAAGCTTG-3′. Primer 2 was a 29-mer: 5′-CGAAATTAAT ACGACTCACT ATAGGGAGA-3′.

DNA was amplified by PCR using a GeneAmp DNA Reagent Kit (Perkin Elmer Cetus) with 15 ng of template. During PCR, DNA was simultaneously either radiolabeled with [^{32}P]dCTP or biotinylated with biotin-11-dUTP (BioRad). Reaction mixtures (100 µl) contained 5 units of AmpliTaq DNA polymerase and 5% dimethyl sulfoxide (DMSO) (Varadaraj and Skinner 1994). Radiolabeled or biotinylated DNAs were purified on Nick columns (Pharmacia) and used immediately as probes of RNA blots or in in situ hybridizations. For use as a probe in a control experiment, a 383 bp segment from the 3′ end of the land crab α1-tubulin noncoding region (Varadaraj et al. 1993) was PCR-amplified and simultaneously radiolabeled with [^{32}P]dCTP.

RNA blot analyses. RNA blot analyses were performed as described (Sambrook et al. 1989). Polysomal mRNAs were electrophoresed on gels containing 1.5% nuclease-free ultrapure agarose (BRL), 2.2 M formaldehyde, 50 mM MOPS, pH 7, 8 mM sodium acetate, and 1 mM EDTA, pH 8. After electrophoresis, gels were rinsed in DEPC-treated water to remove formaldehyde before mRNAs were transferred to Nytran nylon membranes (Schleicher and Schuell). Hybridizations were in 5 X SSPE (20 X SSPE is 2.99 M NaCl, 0.2 M NaH_2PO_4, 2 mM EDTA, pH 7.4), 5 X Denhardt's solution, 1% SDS, 50% formamide, at 42° C for 18 h. After hybridization, filters were washed twice at high stringency [0.1 X SSC (20 X SSC is 3 M NaCl and 0.3 M trisodium citrate) plus 0.1% SDS, at 68° C for 1 h]. Filters were then exposed to X-ray film.

In situ hybridization with biotinylated probes. The hybridization procedure was modified from Current Protocols (Zeller 1989). Crab tissues, including limb bud papillae (before proecdysial growth had begun), midgut gland, ovary, and testis, and a mouse pancreatic tumor, were fixed in freshly prepared 4% paraformaldehyde (PFA; pH 7.2) in phosphate-buffered saline (PBS; PBS is a mixture of 130 mM NaCl/10 mM Na_2HPO_4 and 130 mM NaCl/10 mM NaH_2PO_4, pH 7.2) at 4° C for 1 h. Sections (5 µm) were cut from paraffin blocks, transferred to positively charged slides purchased from Fisher Scientific, and stored at −80° C. Before processing, slides were brought to room temperature and incubated in toluene (three changes, 2 min each) to remove paraffin. After an initial rinse in 100% ethanol, slides were incubated in 3% H_2O_2 in 100% methanol for 30 min and rehydrated through an ethanol series. Slides were incubated in 2 X SSC at 70° C for 30 min and chilled in 1 X iced PBS. They were post-fixed for 5 min in freshly prepared 4% PFA, washed with 3 X PBS for 5 min and 1 X PBS for 1 min, acetylated, and washed in 2 X SSC for 5 min. They were covered with 2 X SSC and irradiated with a 30 W germicidal UV lamp at a dose of 300 µW/cm^2 for 8 or 16 min, dehydrated in ethanol and air dried. Prehybridization was for 2 h, after which the sections were hybridized with a biotinylated probe for 16 h at 37° C in a moist chamber. Precipitated biotinylated DNA probes were dissolved in 2 vol. of 1.2 M NaCl, 20 mM Tris-HCl, pH 7.5, 4 mM EDTA, 2 X Denhardt's solution, 1 mg/ml yeast tRNA, 200 mg/ml poly(A), and 2 vol. deionized formamide. After 1 vol. of 50% dextran sulfate was added, probes were denatured by boiling for 2 min, chilled on ice and brought to 50 mM dithiothreitol (DTT). To protect tissue sections during hybridization, siliconized coverslips were sealed in place with rubber cement. After hybridization, coverslips were floated off in 2 X SSPE and 10 mM DTT. Slides were washed in prewarmed (37° C) buffer (0.6 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 50% formamide, 0.1% β-mercaptoethanol) for 2 h with four to five changes, then in 1 X PBS (three changes, 5 min each). Slides were incubated for 10 min at room temperature in 1 X PBS containing 10% normal goat serum and 2% BSA, washed in 1 X PBS (three changes, 10 min each), incubated in ABC reagent (avidin-biotin peroxidase complex; Vector Laboratories) for 60 min, and washed twice in 1 X PBS for 10 min. After incubating in nickel-diaminobenzidine (Ni-DAB) substrate for 7 min at room temperature, slides were incubated for 5 min in Ni-DAB containing fresh 0.03% H_2O_2. Unreacted substrate was removed by washing in 2 X SSC for 5 min. Signals were intensified by incubating slides for 4 min at room temperature in developer consisting of equal volumes of stock solutions (A) 470 mM Na_2CO_3 and (B) 12 mM $AgNO_3$, 25 mM NH_4NO_3, 1.75 mM tungstosilicic acid, to which was added 7 ml 40% formaldehyde per liter (Gallyas and Merchenthaler 1988). Slides were counterstained with hematoxylin.

Specific binding of satellite probes was tested as follows. After rehydration, slides with midgut gland sections were treated with bovine pancreatic RNase A (40 µg in 10 mM Tris-HCl, pH 7.5, 5 mM EDTA, 0.3 M NaCl; 500 µl per slide) for 30 min at room temperature and rinsed in DEPC-treated water. To avoid cross-contamination, slides were processed in separate containers. They were hybridized as described above.

Results

Transcripts present in different tissues at different stages of the intermolt cycle

Blots of polysomal mRNAs (Fig. 1) that had been isolated from midgut gland (lanes 1–3), regenerating limb bud (lanes 4 and 5) and claw muscle (lanes 6–8), were probed with the 3′ end of the satellite DNA, and found to contain transcripts of many different sizes. In general, transcripts were both tissue and intermolt cycle stage specific, each tissue exhibiting transcripts of very differ-

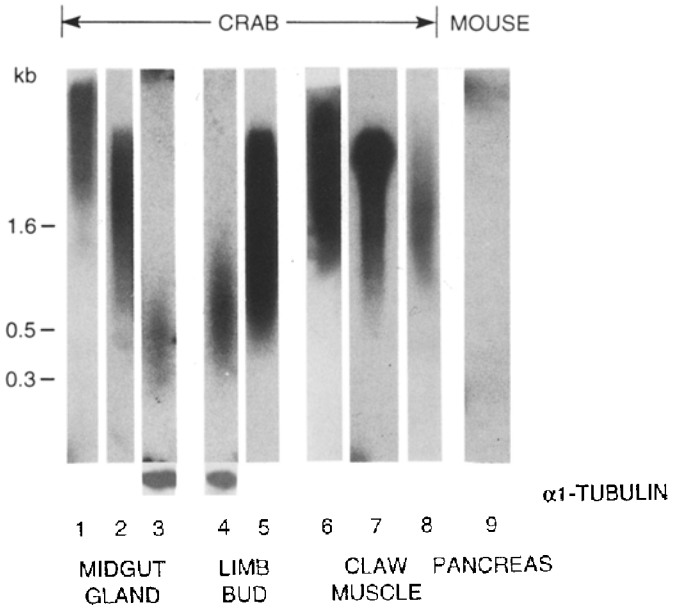

Fig. 1. Hybridization of polymerase chain reaction (PCR)-radiolabeled crab satellite DNA (3′ end) probe to blots of polysomal mRNAs isolated either from tissues of male *Gecarcinus lateralis* during different stages of the intermolt cycle or from a mouse pancreatic tumor (control). Polysomal mRNAs (3 μg per lane) were isolated from: midgut gland during anecdysis, or early or late proecdysis (lanes *1*, *2* and *3*); limb bud during early or late proecdysis (lanes *4* and *5*); claw muscle during anecdysis, or early or late proecdysis (lanes *6*, *7* and *8*); mouse pancreatic tumor (lane *9*). The sizes indicated are those of fragments from a *Hinf*I digest of pBR322 that had been heat denatured. As a control to evaluate the integrity of the mRNA, the filter containing polysomal mRNA from late proecdysial midgut gland (lane *3*) and early proecdysial limb bud (lane *4*) was stripped and hybridized with the 3′ noncoding region (383 bp) of α1-tubulin of *G. lateralis*

ent sizes at different stages of the intermolt cycle. Mouse pancreatic tumor polysomal mRNA was used as a control to ascertain the specificity of hybridization; this control RNA did not hybridize to the satellite probe.

Table 1 illustrates the size distribution of transcripts of the crab satellite. An example of specific differences is that in late proecdysis, transcripts of 2 kb or larger were detected in limb bud and claw muscle but not in midgut gland; other characteristic distribution differences are also evident. In general, there were fewer transcripts in midgut gland and claw muscle than in regenerating limb bud. There were many more transcripts in midgut gland and in claw muscle from anecdysial and early proecdysial animals than from either of those tissues recovered from late proecdysial animals. Transcripts were much more abundant in late as compared with early proecdysial limb bud mRNAs, the reverse of the pattern seen in the other two tissues.

Integrity of mRNAs used in RNA blots

To test the integrity of the polysomal mRNAs prepared for this investigation, a control experiment was conducted using as probe [^{32}P]dCTP labeled land crab α1-tubulin, which has been sequenced and is 1,935 bp long

Table 1. Tissue distribution of *G. lateralis* satellite DNA transcripts of different sizes at different stages of the intermolt cycle

Tissue	Stage of intermolt cycle	Size range of transcripts (kb)[a]
Midgut gland	Anecdysis	1.5–3
	Early proecdysis	0.6–2.5
	Late proecdysis	0.3–1.1
Limb bud	Early proecdysis	0.3–1.5
	Late proecdysis	0.4–2.5
Claw muscle	Anecdysis	1.3–2.95
	Early proecdysis	0.65–2.45
	Late proecdysis	0.9–2.4

[a] Approximate

(Varadaraj et al. 1993). The blot containing polysomal mRNAs from late proecdysial midgut gland (Fig. 1, lane 3) and early proecdysial limb bud (Fig. 1, lane 4) that had been probed initially with the 3′ end of the satellite was used. The bound probe DNA was stripped from the membrane in 0.1 X SSC, 1% SDS, by heating at 100° C for 45 min. The stripped filter was exposed to X-ray film for 3 days to confirm the complete removal of labeled probe. The filter was then probed with [^{32}P]dCTP-labeled 3′ noncoding region of the crab α1-tubulin gene. Specific hybridization of the α1-tubulin probe with a sharp band of 2.2 kb in the same blot that had been hybridized to the satellite probe indicated that the mRNAs had not been degraded. Rather, the cross-hybridizations with mRNA transcripts of a broad range of sizes observed in the initial hybridizations were presumably due to the heterogeneous nature of the satellite transcripts.

Cytoplasmic localization of satellite DNA transcripts in different tissues by in situ hybridization

In situ hybridization experiments were carried out on tissues of anecdysial crabs including midgut gland (Fig. 2A, panels 1–5), limb bud (Fig. 2B, panels 1 and 2), ovary (Fig. 2C, panels 1–4) and testis (Fig. 2D, panels 1 and 2). The probe hybridized with mRNA transcripts in the cytoplasm of all of the tissues tested, although testis contained very few transcripts (Fig. 2D, panel 2). The negative results with testis support the argument for specificity of in situ hybridization as seen in the other tissues. UV-irradiation of tissue sections for 8 min prior to hybridization significantly increased the intensity of the signal over the background (Fig. 2A, panel 3); irradiation for 16 min increased the background (Fig. 2A, panel 4).

In midgut gland, satellite transcripts were scattered throughout the cytoplasm up to the edge of the lumen in the finger-like projections characteristic of this tissue (Fig. 2A, panels 2 and 3). In limb bud papilla, transcripts were more abundant in epidermal cells than in muscle cells (Fig. 2B, panel 2). In ovary, many transcripts were observed at the periphery of the large nuclei typical of oocytes (Fig. 2C, panels 2–4); transcripts were so abun-

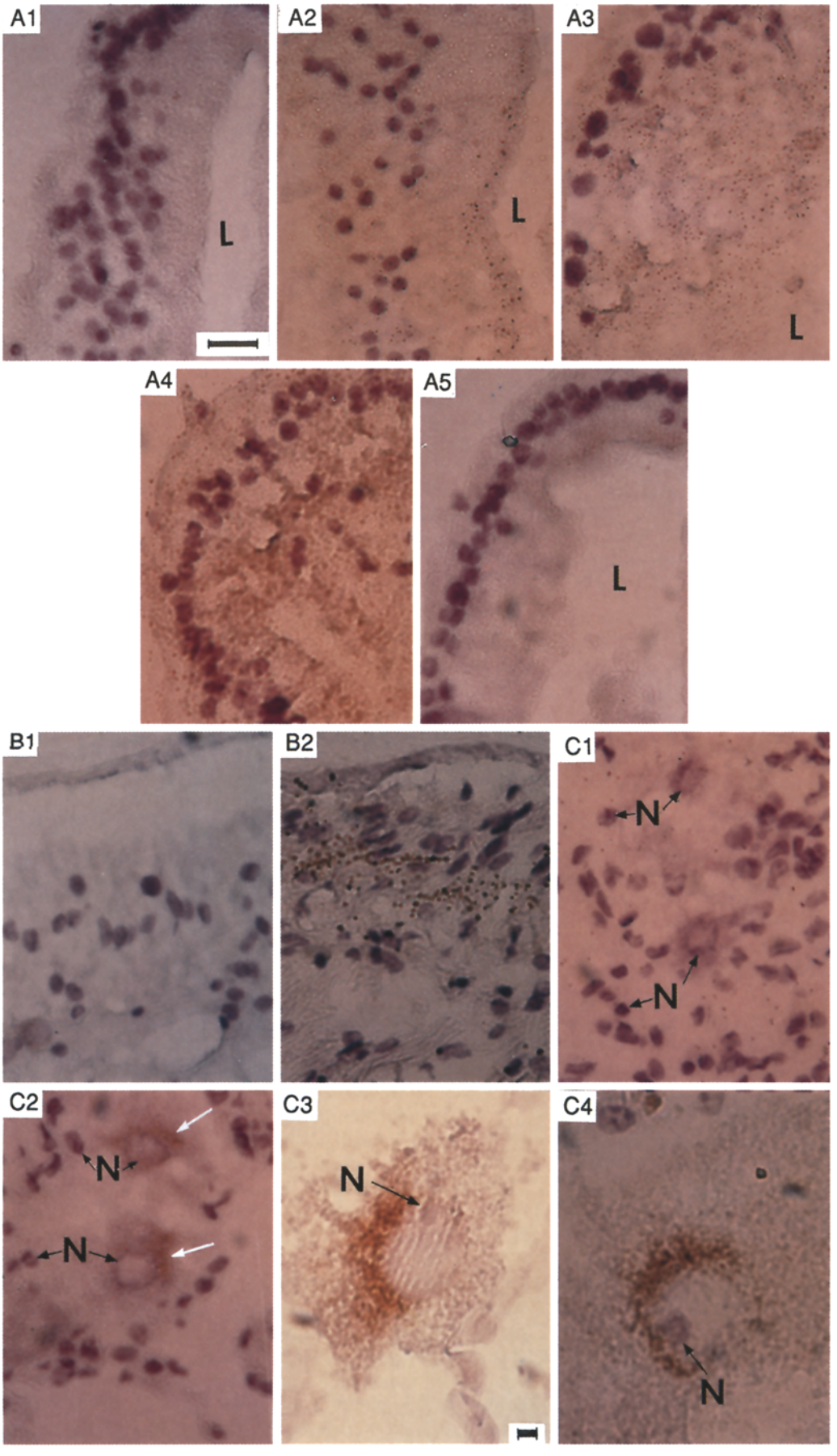

Fig. 2 A–C

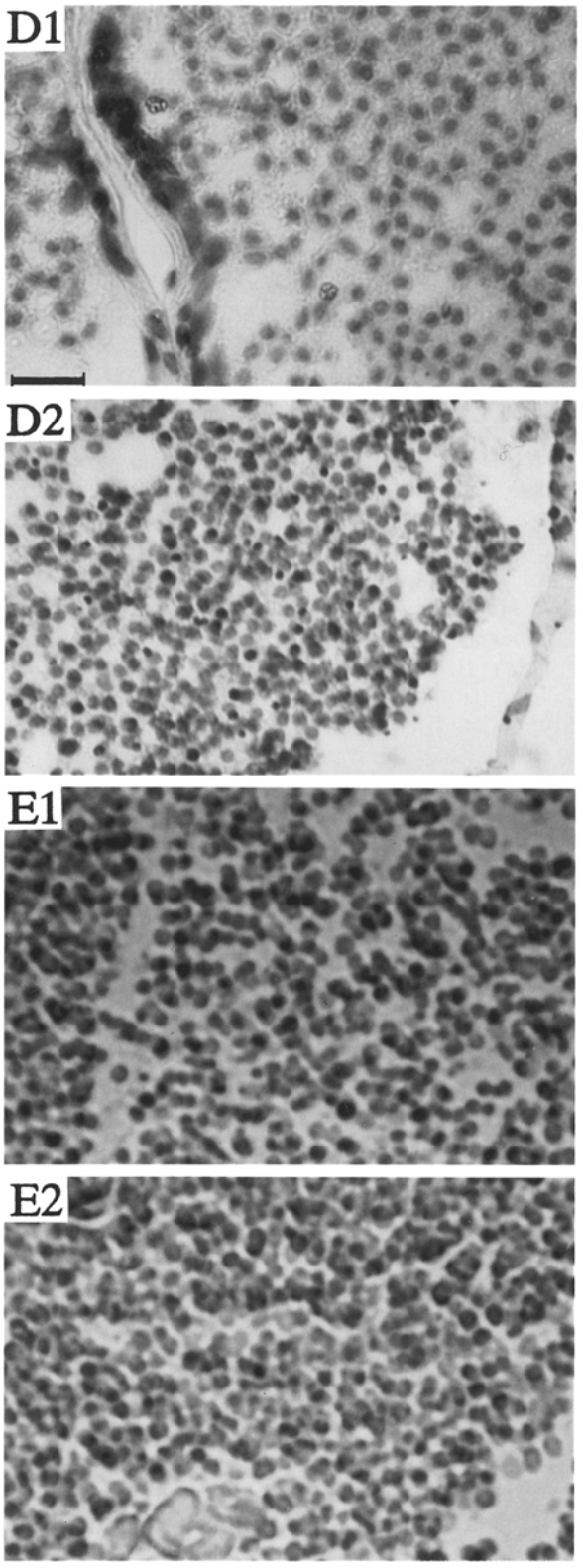

Fig. 2A–E. In situ localization of transcripts in four tissues of an anecdysial crab by hybridization with a biotinylated 3′ end probe of crab satellite DNA. Paraffin sections were 5 μm thick. Except where indicated, tissue sections were UV-irradiated for 8 min and were not treated with RNase A. **A** Midgut gland; *L* lumen: *panel 1* control, not exposed to the probe; *2* no UV-irradiation before prehybridization, then hybridized to probe; *3* UV-irradiated for 8 min before prehybridization, then hybridized to probe; *4* UV-irradiated for 16 min before prehybridization, then hybridized to probe; *5* treated with RNase A, then hybridized to probe. **B** Limb bud papilla: *panel 1* control, not exposed to the probe; 2 hybridized to probe. **C** ovary; *N* nucleus of oocyte or follicle cell; the latter are densely stained: *panel 1* control, not exposed to the probe; *2* hybridized to probe; *white arrows* point to labeled regions. *Panels 3* and *4* to visualize the signals more clearly, higher magnifications of oocytes that had been hybridized to the probe are shown. **D** testis: *panel 1* control, not exposed to the probe; *2* hybridized to probe. **E** Mouse pancreatic tumor; *panel 1* control, not exposed to the probe; *2* hybridized to probe. Panels **C3** and **C4** are the same magnification, which is indicated by the bar representing 3.3 μm. In all other panels, the bar represents 17 μm (see panel **A1**)

dant that individual silver grains could not be discerned. In contrast to what had been observed in midgut gland and limb bud, few grains were observed scattered throughout the cytoplasm of oocytes. Follicle cells, characterized by densely staining nuclei, were devoid of transcripts.

Sections of midgut gland treated with pancreatic RNase A before incubation with the biotinylated probe showed no signals (Fig. 2A, panel 5), nor did sections of mouse pancreatic tumor that had not been treated with RNase prior to hybridization (Fig. 2E, panel 2).

Discussion

Intermolt cycle correlated polysomal mRNA transcripts of the crab satellite

RNA blots revealed that sequences in conserved segments 5 and 6, at the 3′ end of *Eco*RI-cut repeat units of the G+C-rich crab satellite, are transcribed abundantly in midgut gland, regenerating limb bud, and claw muscle. Transcripts of the satellite are both stage specific and tissue specific. The broad range of sizes of transcripts (Fig. 1, lanes 1–8) suggests that satellite repeats that are scattered throughout the genome (Biesiot et al. 1987) are transcribed in preference to the repeat units arranged in tandem (Skinner 1967). The interspersed repeats may be segments of repeat units, full-length repeat units, multiples of full-length repeat units (Stringfellow et al. 1985) or repeat units that contain multiple copies of a 142 bp segment that is amplified from 5-fold (Bonnewell et al. 1983) to as much as 60-fold in some of the satellite variants (Biesiot et al. 1987). Transcription of the many different sized interspersed repeats could result in a population of transcripts as was observed (Fig. 1).

Transcripts of the *G. lateralis* satellite were more abundant in polysomal mRNAs of anecdysial and early proecdysial midgut gland than in late proecdysial midgut gland. This may be related to the degeneration that the midgut gland undergoes during proecdysis. Similarly, transcripts were more abundant in claw muscle from anecdysial and early proecdysial animals than late proecdysial animals. In muscle, as with the degenerating midgut gland, reduced transcription of the land crab satellite during proecdysis may be associated with a specific degradative physiological event: claw muscle under-

goes atrophy prior to ecdysis (Skinner 1966; Mykles and Skinner 1982). The marked increase in the number of different sized transcripts in limb buds in late as compared with early proecdysis may reflect the more rapid rate of growth of the several tissues being regenerated at the later of these intermolt cycle stages.

Cytoplasmic localization of satellite DNA transcripts

In the in situ hybridizations, transcripts of the *G. lateralis* satellite were observed in the cytoplasm of midgut gland, limb bud papilla and ovary; they were particularly dense at the periphery of the large nuclei of oocytes. Probes would be expected to detect transcripts with or without poly(A) tails. When sections of midgut gland were pretreated with RNase A prior to in situ hybridization, no signals were detected (Fig. 2A, panel 5), suggesting that hybridization was specific. Further evidence of specificity was the absence of signals in sections of mouse tissue (Fig. 2E, panel 2).

UV-irradiation is routinely used to fix DNA (Church and Gilbert 1984) or RNA (Sambrook et al. 1989) to nylon membranes prior to hybridization. In the present paper we used UV-irradiation as a simple and efficient method to improve retention of RNAs in tissue sections; such treatment markedly increased the intensity of the signals (compare Fig. 2A, panels 2 and 3). Given the presence of large unlabeled areas in the sections, we conclude that the hybridization following irradiation is not non-specific.

The increase in signal intensity in the UV-irradiated crab tissue sections may be the result of a more efficient immobilization of RNAs by covalent cross-linking to the amine groups of other macromolecules in the sections as well as to the positively charged slides. The higher background in tissue sections that had been UV-irradiated for 16 min (Fig. 2A, panel 4) may be due to networks formed by heavily cross-linked macromolecules interfering with washing procedures.

Transcription of satellite DNAs in arthropods

Transcripts of several other satellite or satellite-like DNAs have been detected in other arthropods. For example, transcripts of a satellite with a 5 bp repeat unit were detected in blots of total testis RNA of *D. melanogaster*; they were similar to those seen in *G. lateralis* in their highly heterogeneous sizes. They ranged from "large" ("migration at limiting mobility", no maximum size indicated), to less than 1 kb; no discrete bands were visible (Bonaccorsi et al. 1990). Transcripts of that *D. melanogaster* satellite were seen only in the loops of *Y* chromosomes of primary spermatocytes; they did not accumulate in the cytoplasm (Bonaccorsi et al. 1990). It has been suggested that, rather than being translated, the AAGAC transcripts may bind specific proteins during the formation of the very long sperm tail characteristic of this species (Bonaccorsi et al. 1988; Pisano et al. 1993).

The *Y* chromosome of *D. hydei* has several fertility genes that are very large (1,000–4,000 kb; Hackstein et al. 1982). During meiotic prophase in primary spermatocytes, the fertility genes develop lampbrush loops that have characteristic morphological properties (Nooses, Clubs, Tubular Ribbons, Pseudonucleolus and Threads) and consist of repeated sequences arranged in tandem (Hennig 1985, 1993; Huijser and Hennig 1987; Trapitz et al. 1988, 1992; Hochstenbach et al. 1993a, b). Small tandem repeats of satellite-like DNAs (e.g. CA/GT_n) are present in the loops of the *Y* chromosome; these are interspersed with members of other repetitive DNA sequence families. Transcripts that vary in size from 10S to 60S accumulate in the loops; they are confined to the nuclei of primary spermatocytes and disappear along with the loops prior to the first meiotic metaphase (Vogt et al. 1982; Lifschytz et al. 1983). Noose and Thread have several megabase clusters of tandemly repeated DNA sequences. Repeated sequences specific to Noose are the ay1 family and the Y_s1 family; there are also sequences associated with Noose that are seen on other chromosomes as well. Thread-specific repeated sequences are Y_L1-Y_L3 and rally (ribosomal and lampbrush loop *Y* chromosome) (Huijser and Hennig 1987). These tandemly repeated sequences are transcribed in a strand-specific manner and the transcripts are of heterogeneous lengths (Vogt et al. 1982; Lifschytz et al. 1983; Vogt and Hennig 1986a, b; Huijser et al. 1988; Trapitz et al. 1988, 1992; Hochstenbach et al. 1993a, b). Despite extensive research, there is no evidence that these transcripts are translated (Hennig 1985, 1993; Trapitz et al. 1992). It has been suggested that the transcripts may be involved in the compartmentalization of proteins during sperm development.

Another satellite-like DNA in an arthropod is an approximately 37 kb gene in Balbiani ring c that codes for a high molecular weight salivary gland secretory protein that is a component of the protective tube of one of the larval stages of *C. thummi* (Baumlein et al. 1982a, b). Though there is only a single copy per genome in *C. thummi*, the gene behaves like a repeated DNA because it contains as many as 80 to 100 copies of a 249 bp repeat arranged in tandem (Baumlein et al. 1982b). The 249 bp repeat contains a subpopulation of shorter repeats that, in turn, have a set of even shorter repeats. The authors point out that the organization of the salivary gland gene, with families of repeats and subrepeats, is similar to that found in some satellite DNAs. If one accepts that interpretation, this is the first satellite for which a translation product has been detected. Genes of the same size class that are also transcribed into very large RNAs occur in Balbiani rings of salivary gland chromosomes of other chironomids, such as *Chironomus tentans* (Degelmann and Hollenberg 1981).

Transcription of satellite DNAs in other organisms

DNA segments ranging from 50 to 200 kb containing tandem repeats of satellite 1 DNA (222 bp repeat unit) of the newt *N. viridescens* are present in pericentric het-

Table 2. Characteristics of repeated DNAs that are transcribed

Organism(s)	Name of the satellite DNA	Repeat unit (bp)	Distribution in the genome	Transcripts			Localization of transcripts	Reference[e]
				Oc	Sp	So		
G. lateralis	RU (G+C-rich)	2,100, 1, 2; present study	Dispersed	+		+	Cytoplasm[a,b]	Present study
Chironomus thummi	BRc repeat	249[c]	SC	+			Cytoplasm	3
Chironomus tentans	BR1 repeat	240[c]	SC			+	Cytoplasm	3
Drosophila melanogaster	Bkm (GATA)	4	Dispersed	+		+	Cytoplasm[d]	4
	AAGAC	5	Centromeric		+		Nucleus	5
Drosophila hydei	$(CA/GT)_n$	2	Dispersed		+		Nucleus[a]	6
	ay1	400	Dispersed		+		Nucleus	7
	Y-associated sequences	24 or 35	Dispersed		+	+	Nucleus	7, 8
	Y_SI	600	Dispersed		+		Nucleus	8
	Y_LI	693	Dispersed		+		Nucleus	9
	Y_LII	77	Dispersed		+		Nucleus	9
	Y_LIII	4	Dispersed		+		Nucleus	9
	rally	200	Dispersed		+		Nucleus	10
Chrysoma rufifacies	Bkm (GATA)	4	Dispersed	+		+	?	11
Triturus cristatus carnifex	TcS1	330	Dispersed	+		+	Cytoplasm	12
	TcS2	275	Dispersed	+		+	Cytoplasm	12
Notophthalmus viridescens	Satellite 1	222	Centromeric		+		Nucleus[a]	13, 14
	Satellite 2	330	Dispersed		+	+	Cytoplasm[a]	15
Triturus cristatus karelinii	TkS1	33	Centromeric	+			Nucleus	16, 17
	TkS2	68	Centromeric		+		Nucleus	18
Triturus vulgaris meridionalis	Tvm1	330	Pericentric	+		+	Nucleus	19
Xenopus laevis	X-132A	77–79	Dispersed	+		+	Cytoplasm[a]	20, 21
	X-132B ?		Dispersed			+	Cytoplasm	20
	X1-741	741	Dispersed	+			Nucleus	21
	X-132C ?		Dispersed	+		+	Cytoplasm[a]	20, 21
Rana catesbeiana	Satellite 1	360	Dispersed	+			Nucleus[a]	22
Rana pipiens	Satellite 1	360	Dispersed	+			Nucleus[a]	22
Bungarus fasciatus	Bkm	4	Dispersed			+	Cytoplasm[a]	4
Mus musculus	Bkm	4	Dispersed			+	Cytoplasm[a,b]	4

Oc, Oocytes; Sp, Spermatocytes; So, Somatic cells; RU, Repeat Unit; BRc, Balbiani ring c; BR1, Balbiani ring 1; SC, one copy per genome; Bkm, Banded krait minor satellite; ?Information not available

[a] Both strands transcribed
[b] Developmentally regulated
[c] 80–100 repeats in each 40 kb gene
[d] One strand transcribed

[e] 1, Skinner (1967); 2, LaMarca et al. (1981); 3, Baumlein et al. (1982a, b); 4, Singh et al. (1984); 5, Bonaccorsi et al. (1990); 6, Huijser et al. (1987, 1990); 7, Huijser et al. (1988); 8, Hochstenbach et al. (1993a, b); 9, Trapitz et al. (1988, 1992); 10, Huijser and Hennig (1987); 11, Kirchhoff (1988); 12, Varley et al. (1980); 13, Diaz et al. (1981); 14, Bromley and Gall (1987); 15, Epstein et al. (1986); 16, Baldwin and Macgregor (1985); 17, Varley et al. (1990); 18, Macgregor and Sessions (1986); 19, Barsacchi-Pilone et al. (1986); 20, Spohr et al. (1981); 21, Jamrich et al. (1983); 22, Wu et al. (1986)

erochromatin; satellite 1 repeats are also interspersed among clusters of histone genes. Transcripts of the satellite, which were observed only during the lampbrush chromosome stage and only in the interspersed repeats, did not accumulate in the cytoplasm (Epstein et al. 1986). Satellite 2 repeats (330 bp repeat unit) are dispersed uniformly throughout the genome. Both strands of satellite 2 were transcribed during oogenesis, although transcripts were strand specific in other tissues and satellite 2 transcripts accumulated in the cytoplasm (Epstein et al. 1986). Originally, it was postulated that transcription of satellite 1 might occur because of readthrough, i.e., the failure of transcription to terminate at the 3′ ends of neighboring histone genes (Diaz et al. 1981). Satellite 1 (360 bp repeat unit) of *R. catesbeiana* is scattered throughout the genome and is therefore assumed to be downstream from the promoters of a number of genes. Its transcription was also attributed to readthrough (Wu et al. 1986). More recent evidence appears to be in conflict with the so-called "readthrough" model; in *N. viridescens*, transcription frequently initiated upstream of the first histone gene promoter (Bromley and Gall 1987).

Transcripts of the so-called "Bkm" satellite (banded krait, minor satellite; repeat unit primarily GATA) have been detected in several species of snakes and in oocytes and other tissues of *D. melanogaster* (Singh et al. 1984), *C. rufifacies* (Kirchhoff 1988) and male *M. musculus* (Singh et al. 1984). Early evidence suggested that the Bkm satellite might play a role in sex determination (Singh et al. 1984). Transcripts accumulated in the proximal region of the *X* chromosome of *D. melanogaster*, the W chromosome of snake, and the sex-determining region of the Y chromosome of mouse. Transcripts were sex specific and developmentally regulated. More recent data, however, do not support their playing a role in sex

determination (Singh and Jones 1986). Genomic localizations of Bkm sequences in *C. rufifacies* and another blowfly, *Calliphora erythrocephala* (Kirchhoff 1988), the rainbow trout (*Salmo gairdneri*, Lloyd et al. 1989), sheep or cows (Miklos et al. 1989), *Alligator mississippiensis* (Demas et al. 1990) and channel catfish (*Ictalurus punctatus*, Tiersch et al. 1992) showed no sex-specific differences.

Table 2 encapsulates some of the characteristics of satellites that are transcribed and of the transcripts produced. The satellite repeat units range from 2 bp to 2 kb. Some are dispersed throughout the genome; others are to a large extent arranged in blocks. Transcription is strand specific for some, not for others.

To summarize our data, blot analyses of polysomal mRNAs and results of in situ hybridizations of biotinylated probes to cytoplasmic transcripts (in all likelihood, both polyadenylated and those that have been processed), showed that a 368 bp highly conserved domain of the G+C-rich satellite DNA of the land crab was transcribed. Absence of signals following treatment of tissue sections with RNase A and failure of probes to hybridize to a tissue from another organism (mouse pancreatic tumor) attest to the specificity of the hybrids formed.

Three sets of these observations suggest that transcripts of the crab satellite may be translated: (1) the transcripts analyzed in RNA blots had been recovered from polysomes, following which (2) the transcripts were subjected to another selection process dependent on their being polyadenylated, and (3) the transcripts had migrated to the cytoplasm as shown by in situ hybridizations. The satellite DNA has been sequenced and searched for start and stop codons that define open reading frames. One strand contains three open reading frames, one of which is bounded by splice junction sequences. The longest of the three open reading frames is 651 nucleotides. Nevertheless, although these findings are suggestive, there is no definitive evidence for a translation product of the satellite.

The tissue and stage distributions of transcripts of the crab satellite DNA suggest that they may be correlated with cell growth and development.

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