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Two genes in Balbiani ring 2 with metabolically different 75S transcripts

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Balbiani ring 2 (BR2) in salivary glands of *Chironomus pallidivittatus* and *C. tentans* (two sibling species of the subgenus *Camptochironomus*) is a favoured model system for studies of gene organization and transcript formation. Here we show that BR2 is more complex than hitherto believed, containing two 75S RNA-producing genes, BR2a and BR2b, present in different 35–40 kb blocks of DNA. The transcripts hybridizing to two different repeat units originating in BR2 differ in size. Further support for the presence of two genes comes from RNA studies during experimentally induced BR2 regression. The amounts of BR2a RNA per cell remain more or less constant throughout the course of the experiment, whereas the BR2b RNA decreases considerably. Under normal conditions there is 5–6 times more BR2a RNA than BR2b RNA. This ratio increases ~3-fold under experimental conditions. BR2a and BR2b, although partially homologous, contain repeat units with characteristic differences. BR2a contains a repeat unit that is much more similar to a BR1 repeat than it is to the BR2b repeat. The possibility is discussed that a Balbiani ring in general represents an integrated set of active genes rather than a singe gene.

Key words: Balbianbi ring/*Chironomus*/gene structure/giant transcript/repetitive sequences

Introduction

Balbiani ring 2 (BR2) in salivary glands of members of the subgenus *Camptochironomus* (*Chironomus tentans, C. pallidivittatus*, two sibling species) is a giant puff, usually the major seat of transcription in polytene chromosomes of salivary gland cells (Pelling, 1964; Daneholt *et al.*, 1969). A 75S transcript is found in BR2 (Daneholt, 1972) and appears in the cytoplasm (Daneholt and Hosick, 1973; Lambert, 1973) as mature mRNA (Daneholt *et al.*, 1977) not measurably different in size from the primary transcript (Case and Daneholt, 1978). Another BR, BR1, also produces 75S RNA under normal conditions. BR1 probably consists of at least two genes, since it has two origins, separated by three bands (Beermann, 1973). It is not known whether BR2 contains more than one gene. Hybridization experiments by Wieslander (1979) suggested 4–5 75S RNA transcription units per genome, making it unlikely that there are many more than one 75S RNA genes in BR2.

Two different repeat unit types have been localized in BR2 of *C. tentans* (Wieslander and Lendahl, 1983) and of *C. pallidivittatus* (Jäckle *et al.*, 1982, and present paper). In *C. tentans* one type (pCt63) hybridizes to 70% of th BR2 RNA and the other one (pCt7) to 4–15% only. On the assumption that these two repeat types represent different parts of a single gene, the more abundant type was subsequently designated the α repeat and the less abundant one β repeat (Höög and Wieslander, 1984). Alternatively, these two repeat units might represent two different genes, both localized in the BR2 region, in which case the α type gives rise to more RNA than the β type.

Here we investigated whether the α and β type of BR2 repeat units belong to two different genes. For this purpose the α unit has been isolated from *C. pallidivittatus* and used together with the previously isolated β unit (Jäckle *et al.*, 1982) in experiments designed to reveal whether they are present in one or two transcription units. The results show that the units are localized in separate blocks of DNA, that they are transcribed into RNA of somewhat different size and that they react differently during experimental regression of BR2. All evidence argues in favour of two BR2 genes which we have designed BR2a (represented by the α unit) and BR2b (represented by the β unit).

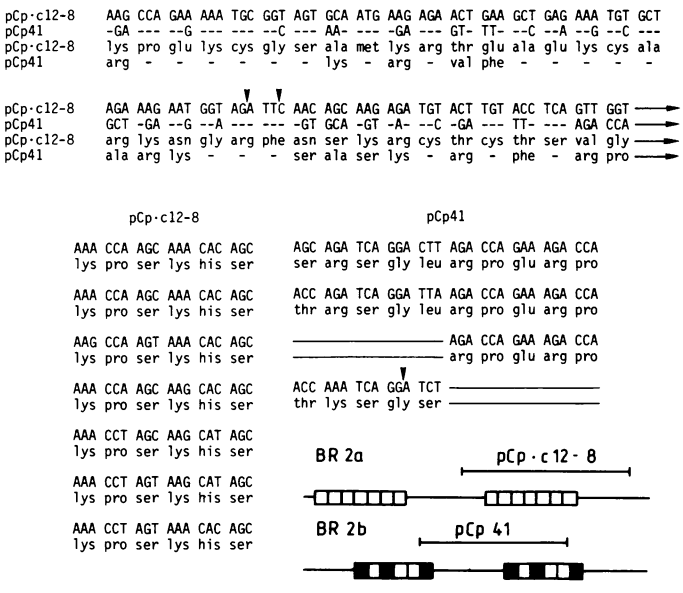

Fig. 1. Sequences of the repeat units of pCp.c12-8 (BR2a) and of pCp41 (BR2b) shown in the 5′→3′ direction. The constant parts that are partially homologous are aligned, and nucleotides or amino acids in pCp41 that are identical with those of pCp.c12-8 are indicated with dashes. The subrepeated parts, which follow in the 3′ direction, are shown separately, divided into subrepeats. Insertion sites into the vector are shown with arrowheads. Insert (lower right) shows models of parts of the genes represented by the cloned inserts. The location of the inserts in relation to the repeat structure is also shown. Subrepeats are shown as boxes (for BR2b of two different kinds) and constant parts are shown as single lines connecting the rows of boxes.

Results

The α repeat unit of C. pallidivittatus

The pCp.c12-8 clone was subcloned from a fragment of a cDNA clone, pCp.c12, which was generated from 75S RNA with oligo(dT) as primer. The sequence of pCp.c12-8 is given in Figure 1. In analogy to *C. tentans* (Höög and Wieslander, 1984) it will be called BR2α repeat. Its abundance in the genome was determined by titration as previously described (Bäumlein *et al.*, 1982) using *Hinf*I-restricted DNA fragments. The abundance was 67 (average of 54 and 80). Since the size of the repeat unit is 231 bp it should make up ~15 kb. Partial digestions with *Alu*I revealed a tandem arrangement of repeat units with up to 13 discernible ladder bands (data not shown).

The pCp.c12-8 unit hybridized to BR2 of *C. pallidivittatus* (Figure 2). Whereas cross-hybridization to BR1 occurred when slides were washed at 60°C or lower in 2 × SSC, hybridizations were exclusively to BR2 after washing at 65°C, showing that this repeat is specific for BR2 DNA.

For comparison, the BR2β repeat (Jäckle *et al.*, 1982) is given in Figure 1 and its *in situ* hybridization shown in Figure 2. Its repeat unit length is 195 bp and its abundance is 67 times, making up 17 kb (Galler *et al.*, 1984). Subrepeated parts from different repeat units have several base differences. To minimize problems in gene titration, pCp31 which contains less than half of the subrepeat region was therefore used (Jäckle *et al.*, 1982).

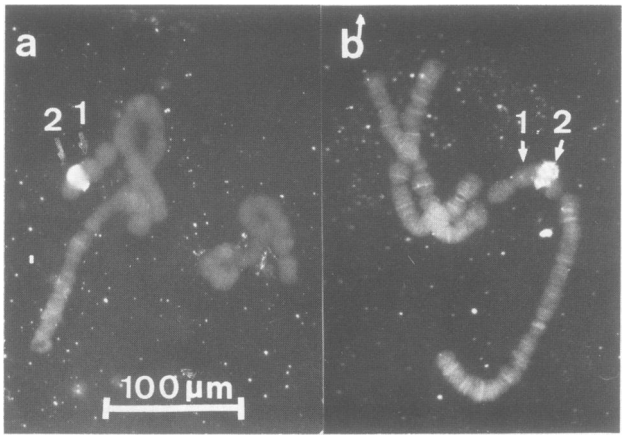

Fig. 2. *In situ* hybridization of polytene chromosomes with biotinylated pCp.c12-8 **(a)** and pCp31 **(b)** plasmid DNA. In both cases hybridization occurs to BR2. There is no cross-hybridization to BR1. Numbers 1 and 2 stand for BR1 and BR2, respectively.

Genomic arrangement of BR2 DNA

The arrangement of the two types of repeat units in the genome was studied by digesting genomic DNA with *Eco*RI or with *Eco*RI and a restriction enzyme which cuts either in the repeat unit of the α type or β type, respectively. Various genomic digests were electrophoresed on 0.4% and 1.5% agarose gels, transferred onto DBM paper and probed with cloned DNA of either type (Figure 3). The α type DNA hybridizes to an *Eco*RI fragment of ~39 kb, the β type probe to one of ~36 kb. The two bands are resolved in a co-hybridization experiment (Figure 3 BII). The additional digestion of *Eco*RI-digested DNA with either *Cfo*I or

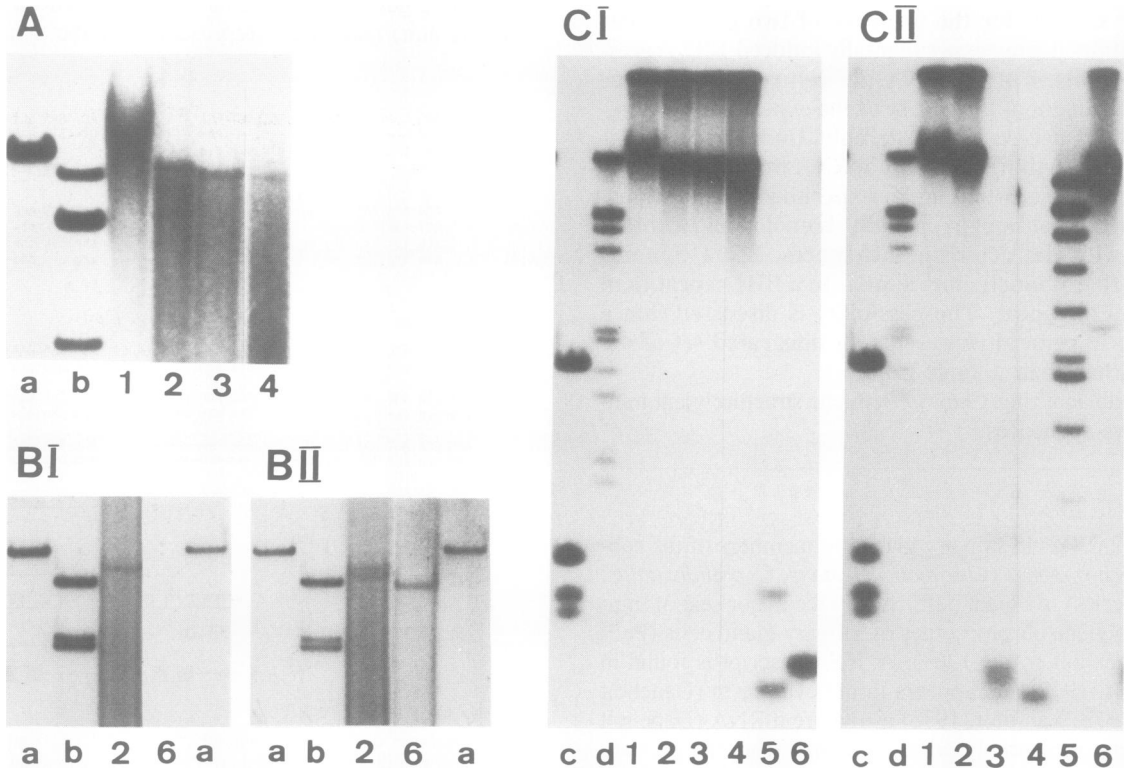

Fig. 3. Genomic Southern blot hybridizations. DNA of *C. pallidivittatus* was either digested with *Eco*RI **(2)** or with *Eco*RI and a restriction enzyme which cuts once per repeat: *Eco*RI and *Cfo*I **(3)**; *Eco*RI and *Sau*3A **(4)**; *Eco*RI and *Dde*I **(5)**; *Eco*RI and *Rsa*I **(6)**; undigested DNA **(1)**. **Lanes (a)–(d)** are markers, **(a)** λ-DNA (48.5 kb), **(b)** a mixture of λ-DNA digested with *Xho*I and of λ-DNA digested with *Xba*I (33.5, 24.7, 23.8, 15 kb), **(c)** bands of *Hinf*I-digested pBR322 which hybridize to pCp.c12-8 and pCp31 (1631, 517, 396, 344 bp), **(d)** λ-DNA digested with *Eco*RI and *Hind*III (21 221, 5150/4974, 4271, 3538, 2024, 1906, 1584, 1375, 947, 832, 564 bp). The DNA is electrophoresed on 0.4% (**A** and **B**) or 1.5% agarose gel (**C**), respectively. **Blot A** is hybridized with pCp.c12-8. **Blot B** is first hybridized with pCp.c12-8 **(BI)** and then hybridized with pCp31 without removal of the initially hybridized probe **(BII). Blot C** is first hybridized with pCp.c12-8 **(CI)**, then denatured to remove the hybridized probe and rehybridized with pCp31 **(CII)**.

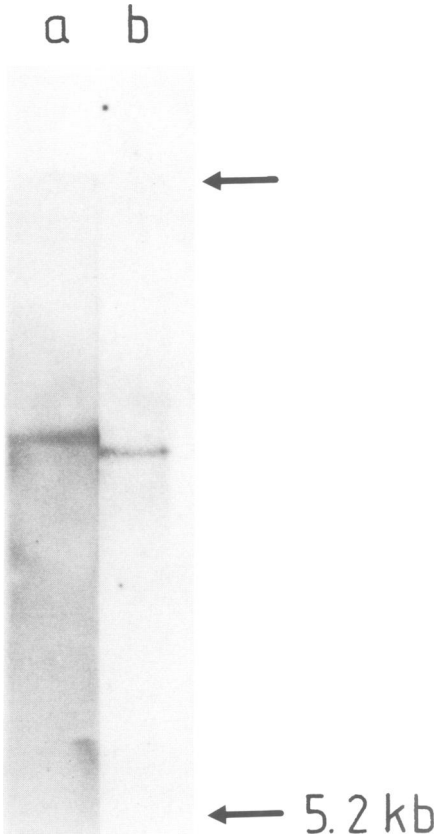

Fig. 4. Northern blot analysis. Total salivary gland cell RNA (~10 μg) from *C. pallidivittatus* was separated on a 0.75% agarose gel containing formaldehyde with a 30 mm wide slot and transferred to nitrocellulose filter. After transfer several horizontal lines were drawn on the filter which was subsequently cut into two halves which were probed with nick-translated pCp.c12-8 **(a)** and pCp31 **(b)** DNA. Autoradiograms were taken separately (**a**; 5 h exposure; **b**; 2 days exposure) and aligned accurately with the aid of the lines drawn on nitrocellulose filter pieces. Arrow shows origin. The 5.2-kb reference is from λ-*Hind*III-digested marker.

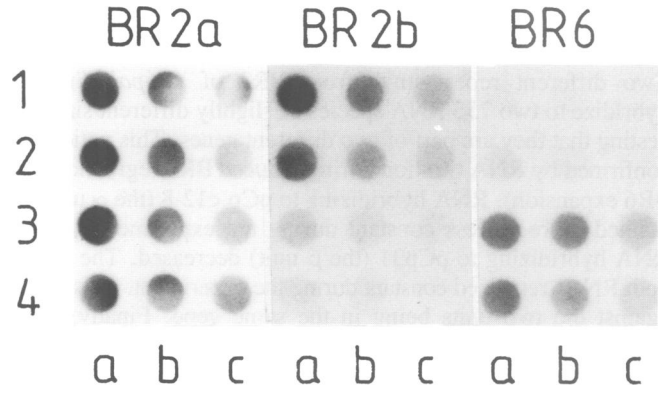

Fig. 5. Dot-blot analysis of total salivary gland RNA. Total RNA was extracted from salivary glands (five glands for each time point) and measured amounts [130 ng **(a)**; 65 ng **(b)**; 32.5 ng **(c)**] were spotted in triplicate on nitrocellulose filters. Each filter was then hybridized separately to nick-translated probes of pCp.c12-8 (BR2a), pCp31 (BR2b) and pCp16-7 (BR6), respectively. Filters were autoradiographed with screen on X-ray films for 20 h (BR2a and BR6) or 5 days (BR2b). **1,2,3** and **4** stand for controls (1), animals treated with glycerol for 3 days **(2)**, 6 days **(3)** and 9 days **(4)**.

*Sau*3A produces slightly shortened α type DNA fragments determined to be 33 kb and 34 kb (Figure 3 AI, 3,4), whereas for the β repeat only signals of the length of the basic repeat or less were obtained (Figure 3 CII, 3,4). The additional digestion of *Eco*RI-digested DNA with *Dde*I or *Rsa*I, on the other hand, produces short fragments for the α type sequence (Figure 3 CI, 5,6). For the β sequence there remains a 33 kb long *Rsa*I fragment (Figure 3 BII, 6). Digestion of β type DNA with *Dde*I produces a series of different length fragments (Figure 3 CII, 5). In agreement with earlier conclusions for *C. tentans* (Wieslander and Lendahl, 1983) these data show that the α and β repeats are not intermingled.

Northern analysis of RNA hybridizing with α and β repeat units

If the two types of repeat units are contained in the same gene the RNA to which they hybridize should be identical in size. Northern anlaysis showed, however, a small but reproducible migration difference. The β unit hybridizes to an RNA migrating slightly faster than the RNA hybridizing to the α unit (Figure 4). This result strongly suggests that the pCp31 type repeat and the pCp.c12-8 type repeat belong to different genes. Tests were also performed (data not shown) showing that the hybridization signals were entirely sensitive to ribonuclease.

Amounts of BR2 RNAs under normal conditions and during BR2 regression

Total RNA was extracted from salivary glands of animals kept under normal conditions or under conditions that cause a regression of BR2 (and an induction of BR6). The experimentals were exposed to 50 mM glycerol for up to 9 days (Edström *et al.*, 1982). Dot blot experiments showed (Figure 5) that the amount of RNA hybridizing to the α unit remains more or less constant throughout the course of the experiment. On the other hand, that of the RNA hybridizing to the β unit dropped. It can also be seen that BR6 RNA appears after treatment with glycerol for 6 days, showing the effectiveness of the treatment. These relationships were the same whether filters were washed under normal or high stringency conditions.

Using S1 nuclease analysis we titrated the RNA hybridizing to the α unit and to the BR6 repeat unit during the course of the experiments. Figure 6 shows that the RNA of α unit remains more or less unchanged in amounts, at least for 6 days, whereas BR6 RNA shows the expected rise between 3 days and 6 days of treatment. Due to the variable nature of the subrepeat region in the β unit S1 analysis was not carried out with pCp41.

A complementation of the S1 nuclease analyses for β sequences could, however, be achieved by Northern analysis. As shown in Figure 7 there is a continuous decrease in amounts of intact RNA hybridizing to the β unit. Northern analyses of α type RNA, although somewhat obscured by background smears, again indicated that this RNA remains constant during the experiment. The results in Figure 7 also confirm those shown in Figure 4, i.e., RNA hybridizing to the β unit migrates slightly faster than RNA hybridizing to the α unit (as can be seen by comparison with the reference bands).

Since the different approaches showed similar results we could use the dot-blot analyses to determine the ratios in amounts of the two types of RNA. For this purpose dots were cut out and the radioactivity determined. Corrections were made for probe specific activity and the size of the probes. Under normal conditions the ratio between α type RNA and β type RNA is 5–6. During the course of the experiment this ratio increases to 15 as a result of decreasing amounts of β type RNA (Table I). Since

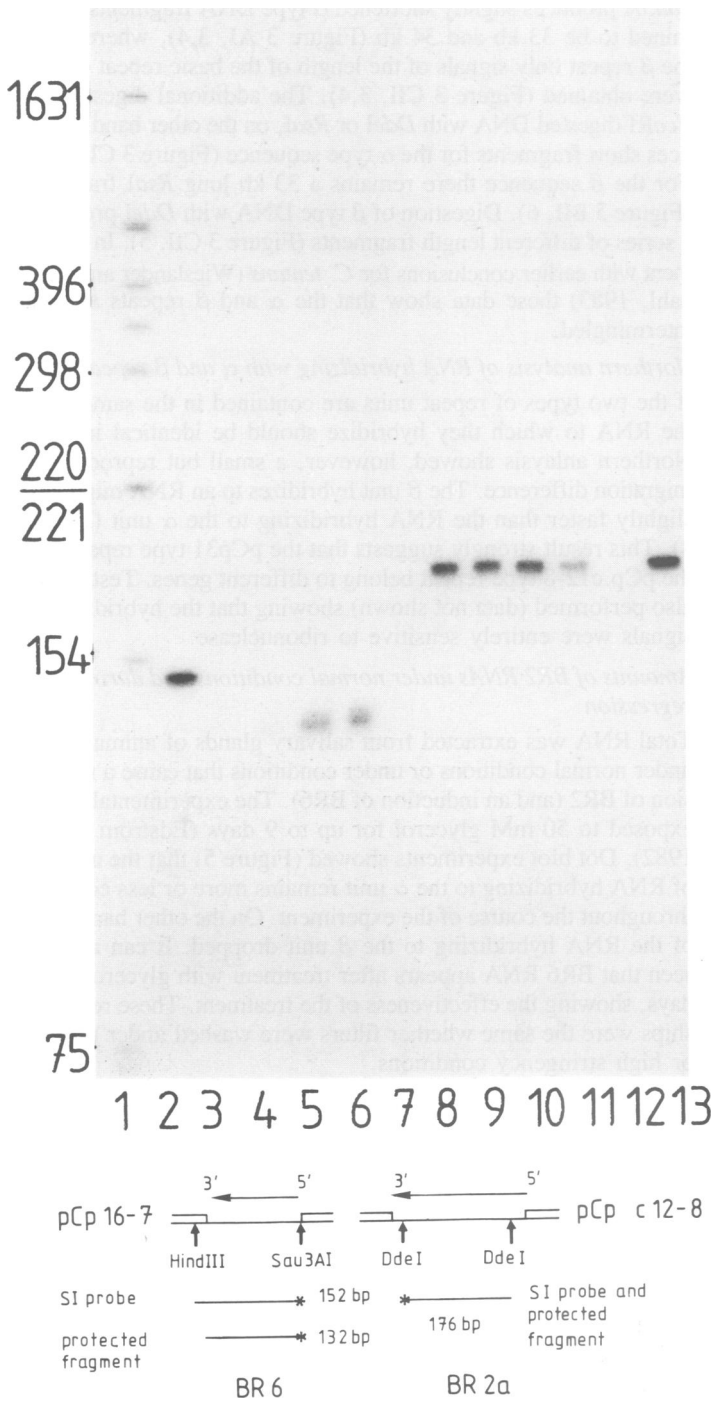

Fig. 6. S1 nuclease analysis of total salivary gland RNA. Hybridization was performed with pCp.c12-8 and pCp16-7 as probes. The size of the probes and the expected protected fragments are shown in the lower part of the figure. Single-stranded probes (10 ng each) were hybridized to 80 ng of total RNA from each RNA batch (cc; g3; g6; g9; i.e., controls and animals treated with glycerol for 3, 6 and 9 days, respectively). After S1 nuclease digestion, resistant products were separated on 6% polyacrylamide gels containing urea. **Lane 1** shows end-labelled, pBr322-*Hinf*I fragments and **lanes 2** and **13** show the probes only. **Lanes 7** and **12** are controls in which *E. coli* tRNA in equivalent amounts to the total salivary gland RNA were used for hybridization. **Lanes 3+8, 4+9, 5+10** and **6+11** show RNA from cc, g3, g6 and g9 animals, respectively.

the two repeat units make up a similar fraction in each of the two genes and since the two genes are similar in size these ratios should be representative for the actual RNA ratios.

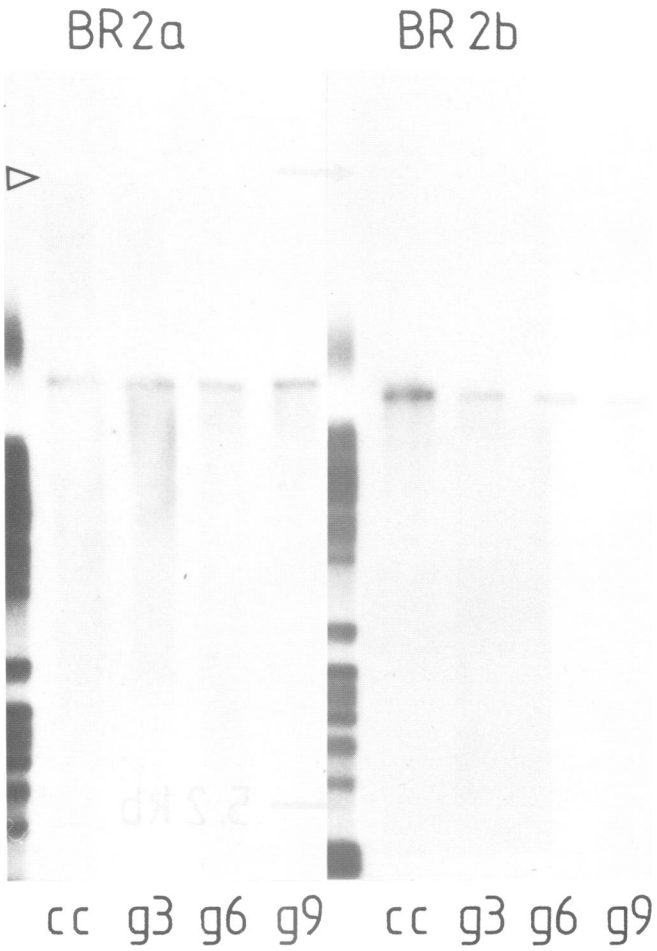

Fig. 7. Northern blot hybridization of salivary gland cell RNA from *C. pallidivittatus* separated on 0.75% agarose gels containing formaldehyde. Identical amounts of total RNA (1 μg) were applied from cc, g3, g6 and g9 glands and hybridized to pCp.c12-8 DNA (BR2a) and pCp31 DNA (BR2b). (The markers are end-labelled *Hind*III-restricted λ fragments.) Arrow shows origin.

Discussion

Two genes in BR2

Two different repeat units from BR2 of *C. pallidivittatus* hybridize to two 75S RNA species of slightly different size suggesting that they are part of two different genes. This notion was confirmed by RNA titration during induced BR2 regression (and BR6 expansion). RNA hybridizing to pCp.c12-8 (the α unit) remained more or less constant during the experiment whereas RNA hybridizing to pCp31 (the β unit) decreased. The size of both RNAs remained constant during the experiment. This argues against the two units being in the same gene. Finally, DNA hybridizing to the two repeats was localized in different 35–40 kb fragments.

The present conclusions should be applicable to the sibling species *C. tentans* where closely similar repeat units have been isolated (Sümegi *et al.*, 1982; Case *et al.*, 1983; Wieslander and Lendahl, 1983). In *C. tentans* the α and β units are also localized in two different fragments of 35–40 kb on the genomic blots (Widmer, unpublished). We suggest that the BR2 gene containing α repeat units is designated BR2a and the gene containing β units BR2b.

Derksen *et al.* (1980) using 75S RNA as probe (mainly BR2a) found most *in situ* hybridization in the band IV-3B10 in the poly-

Table I. Determination of relation between BR2α (pCp.c12-8) and BR2β (pCp31) sequences in total salivary gland RNA during glycerol treatment

Clone	Type of RNA	c.p.m. recovered on filter	ng total RNA	α/β[a]
α	cc[b]	1451	130	5.8
β	cc	169	130	
α	cc	767	65	5.4
β	cc	96	65	
α	g3	1509	130	6.7
β	g3	161	130	
α	g6	1730	130	15.2
β	g6	82	130	
α	g9	1466	130	15.3
β	g9	69	130	

[a]The calculations take into account: (i) the copy number of each repeat unit, α = 67, β = 89; (ii) probe length, α = 234 bp, β = 151 bp; (iii) specific activity: α = 4 × 10^8 c.p.m./μg, β = 3.2 × 10^8 c.p.m./μg.
[b]cc, g3, g6 and g9 refer to controls taken directly from the cultures (zero time controls), animals exposed to 50 mM glycerol for 3, 6 and 9 days, respectively.

tene chromosomes, whereas Sass (1984) using pCp31 (BR2b) obtained hybridization over the adjoining interband. The difference in the results might thus have been due to portrayal of two different genes.

In the literature BR2 has sometimes been considered to contain a single gene although there is no real evidence for this view. Lamb and Daneholt (1979) showed that BR2 transcription units are surrounded by at least 14 – 16 μm of chromatin free from other transcription units. This would correspond to 85 kb on each side assuming a packing ratio of 1.9 for inactive chromatin (Lamb and Daneholt, 1979). Together with one 37-kb gene this accounts for ~200 kb and hence does not exclude the presence of a second gene within 'the BR2 chromomere', which is estimated to be 400 kb (Derksen *et al.*, 1980). Also, nucleic acid reassociation work by Wieslander (1979) is compatible with more than one 75S RNA-producing gene in BR2.

Representation of α and β units in the BR genes

The two repeats are present in different 36 – 39 kb fragments delimited by *Eco*RI sites. The units could be trimmed further with restriction enzymes with four base recognition sequences, to 33 – 34 kb, an upper limit for the amount of these units.

Titrations, on the other hand, indicated 15 kb for the α unit (67 repeats) and 17 kb for the β units (89 repeats) per genome. These results are similar to the value of 70 repeats for the β unit in *C. tentans* (Case *et al.*, 1983) and 76 repeats for a unit in BRc of *C. thummi* (Bäumlein *et al.*, 1982). At the moment there is consequently an apparent discrepancy between the two approaches. It is not excluded, however, that the blocks delimited by restriction enzymes contain DNA of unknown character, e.g., other repeat units. In any case the α and β repeat units should represent a considerable portion of the genes.

Comparison of BR1 and BR2 repeat units

The repeat units specific for BR2a and BR2b, although partially homologous, show distinct differences in overall design. The constant parts (Figure 1) are 60% homologous at the nucleotide level. On the other hand, the subrepeats differ markedly. The α unit contains seven hexapeptide repeats, the β unit two pentapeptide repeats in a complex order.

Interestingly a unit from BR1 in *C. pallidivittatus*, the pCp90 repeat, has an overall construction very similar to BR2 α with the same length, 231 bp (Galler *et al.*, 1984). The constant parts are 75% homologous, and the pCp90 repeat also codes for seven hexapeptides: Arg(Lys)-Pro-Ser-Lys(Arg)-Pro-Ser(Thr), obviously very similar to the Lys-Pro-Ser-Lys-His-Ser arrangement in BR2 α.

It is thus striking that the α unit in BR2 is much more similar to a BR1 repeat unit than it is to another gene within the same BR. Is it characteristic for BR to consist of different genes with a defined relation to each other? We now have found BR1 analogues to the BR2b repeat unit (Saiga *et al.*, unpublished data), supporting the view that BRs may contain such integrated sets of genes rather than a single gene.

Transcript metabolism during BR2 regression

During treatment of *Chironomus* larvae with monosaccharides BR1 expands, BR2 regresses and a new BR, BR6, is induced (Beerman, 1973). The same effects are obtained with glycerol and ethanol which, like the monosaccharides, decrease the pools of inorganic phosphate in the haemolymph (Edström *et al.*, 1982). The phosphate starvation in its turn leads to marked changes in production of sp-I giant secretory proteins coded by the large BR. The normal components sp-Ia and sp-Ib, which are coded by BR1 and BR2 and are heavily phosphorylated, decrease or disappear, respectively (Galler *et al.*, 1984). In parallel with the induction of BR6 a new giant protein appears, sp-Ic, which is partially homologous with the others but is non-phosphorylated (Galler *et al.*, 1984). It is believed that sp-Ia is a product of BR1 and sp-Ib a product of BR2 (Edström *et al.*, 1980). Obviously the relationship cannot be so simple since BR2 contains at least two genes and this is likely to apply to BR1 as well. (However, since the size difference between the BR2a and BR2b transcripts is minute, translation products could co-migrate.) Considering the disappearance of sp-Ib and the considerable decrease in sp-Ia during glycerol treatment (Galler *et al.*, 1984), which was also confirmed in the present experiments (data not shown), it is however remarkable that the main BR2 transcript remained in unchanged quantities during the induction. This suggests that the BR2a transcript is maintained in a largely or entirely inactivated form during glycerol treatment, suggesting post-transcriptional control during treatment with glycerol.

Materials and methods

Animals

Mid-fourth instar *C. pallidivittatus* larvae were used, raised as described by Rydlander and Edström (1980).

Isolation of nucleic acids

Genomic DNA was usually prepared as described by Saiga and Edström (1985). For studies of genomic structure, the preparation followed, with some modifications, the protocol of Jäckle *et al.* (1982). Total RNA was prepared from isolated salivary gland cells as described by Edström *et al.* (1982) with minor modifications. 75S RNA was prepared as described by Rydlander *et al.* (1980).

cDNA cloning

cDNA cloning with 75S RNA as starting material was carried out essentially according to Maniatis *et al.* (1982). cDNAs were tailed by terminal transferase and dCTP, reannealed with dG-tailed pBR322 DNA at the *Pst*I site, and introduced into *Escherichia coli* HB101. About 300 tetracycline-sensitive and ampicillin-resistant colonies were obtained from 10 μg 75S RNA.

Subcloning and sequencing of pCp.c12-8

A 231-bp fragment was cut out with *Hinf*I from the insert of pCp.c12, filled up at the ends with Klenow fragment and subcloned into the *Sma*I site of pUC8. The nucleotide sequence was determined according to Maxam and Gilbert (1980).

In situ hybridization

In situ hybridization was performed essentially as described by Langer-Safer *et al.* (1982). DNA from the plasmid pCp31 and pCp.c12-8 was nick-translated

with biotinylated UTP (Enzo) and chromosome squashes were hybridized at 56°C overnight in 0.6 M NaCl, 0.1 M Na_2HPO_4-NaH_2PO_4 pH 7.2, 0.005 M $MgCl_2$ and 1 × Denhardt's solution. Washing was done for 2 × 20 min at 52°C in 2 × SSC followed by a 20-min wash at 65°C. Primary antibody, used at 1:500 dilution, was a goat anti-biotin IgG fraction (Enzo, EBP-807) and secondary antibody, used at 1:100 dilution, a Rhodamine-conjugated rabbit anti-goat IgG serum (Miles-Yeda 61-269).

Analysis of restriction fragments of genomic DNA

Electrophoresis on 1.5% or 0.4% agarose gel, blotting onto DBM-paper, hybridization with excised [^{32}P]nick-translated insert DNA and denaturing of hybridized filters was carried out as described by Widmer *et al.* (1984).

Dot-blotting of total salivary gland RNA

Dot-blots were made essentially as described by White and Bancroft (1982). Total salivary gland RNA precipitate (five glands from five animals) dissolved in 400 μl H_2O were diluted serially, denatured by heating at 65°C for 6 min and cooled on ice. 25 μl of each sample was added to 450 μl of 10 × SSC-2.2 M formaldehyde. Samples were then blotted onto nitrocellulose filters with the Minifold apparatus. Each dot was washed with 0.5 ml 10 × SSC twice before and after sample application at room temperature and filters were baked at 80°C for 90 min. Hybridization was carried out in 50% formamide, 5 × SSC, 20 mM potassium phosphate buffer, pH 7.0, 5 × Denhardt's solution, 0.5% SDS with 10^6 c.p.m./ml at 42°C overnight. Filters were washed at room temperature for 1 h in 2 × SSC-0.2% SDS with four buffer changes (250 ml each). Stringent washings were performed in 0.1 × SSC-0.2% SDS at 62°C for 1 h.

S1 nuclease anlaysis

This was done according to Berk and Sharp (1977). Total salivary gland cell RNAs were prepared from animals at various stages of induction and co-precipitated through ethanol with single-stranded end-labelled probes with 5 × 10^5 c.p.m. in a 3–6 × weight excess to 75S RNA, assuming a content of 3% BR-RNA in total RNA (Case and Daneholt, 1978; Edström *et al.*, 1978). Nucleic acids were precipitated in sterile Eppendorf tubes. Pellets were dissolved in 30 μl of 80% formamide, 0.4 M NaCl, 0.04 M Pipes, pH 6.4, 1 mM EDTA and denatured at 85°C for 5 min, followed by incubation at 46°C (determined as optimal temperature for both probes used) overnight. Reactions were stopped by addition of 0.3 ml ice-cold 0.3 M NaCl, 0.03 M sodium acetate, pH 4.6, 1 mM $ZnSO_4$. The mixture was incubated with 3000 U/ml S1 nuclease (Boehringer, Mannheim) for 1 h at 37°C. Resistant products were recovered by ethanol precipitation (with 1 μg *E. coli* tRNA as carrier), washed with 80% ethanol, dissolved in 2 μl H_2O plus 5 μl sequencing dye and analyzed on 1 mm thick 6% polyacrylamide gels containing urea (Maxam and Gilbert, 1980).

Northern blot hybridization

Total RNA was separated on 0.75% agarose gel containing 2.2 M formaldehyde (Lehrach *et al.*, 1977). After electrophoresis the gel was treated with 50 mM NaOH, 10 mM NaCl at room temperature for 45 min and neutralized with 0.1 M Tris-HCl (pH 8.0). RNA was transferred to nitrocellulose by the Southern method. Conditions for hybridization and washing were the same as those for dot-blots.

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On agreement at the 2nd Balbiani Ring Workshop in 'Windenhütte', Harz Mountains, September 1985, the terminology BR2a and BR2b was changed to BR2.1 and BR2.2, respectively.