Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The 75 S RNA Transcription Unit in Balbiani Ring 2 and Its Relation to Chromosome Structure

B. Daneholt, S. T. Case, M. M. Lamb, L. Nelson and L. Wieslander

Phil. Trans. R. Soc. Lond. B 1978 **283**, 383-389
doi: 10.1098/rstb.1978.0043

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1978 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B. **283**, 383–389 (1978) [383]
Printed in Great Britain

The 75 *S* RNA transcription unit in Balbiani ring 2 and its relation to chromosome structure

By B. Daneholt, S. T. Case, M. M. Lamb, L. Nelson and L. Wieslander
Department of Histology, Karolinska institutet, S-10401 *Stockholm* 60, *Sweden*

[Plate 1]

A defined transcription unit in the Balbiani ring 2 (BR 2) region of chromosome IV in the salivary glands of *Chironomus tentans* has been characterized on the basis of analysis of the corresponding primary transcript, 75*S* RNA, and its functional significance. The available information on the transcription unit and its relation to chromosome structure can be summarized in the following way:

1. The size of the 75*S* RNA transcription unit in BR 2 is on the order of 30 000 base pairs.
2. The unit is likely to contain a long coding segment (at least 6000 base pairs), probably corresponding to information for salivary polypeptides.
3. The sequences are distributed in more than one chromomere (probably in 3–5 chromomeres). Further studies are needed before it can be stated whether or not there is a simple one-to-one relation between chromomeres and transcription units in the BR 2 region.

1. Introduction

It has been recognized for several years that the individual elements of polytene chromosomes, the chromatids, display a defined superstructure (for review, see Beermann 1972). Each chromatid consists of a deoxyribonucleoprotein filament that is extended in some regions, while it is heavily coiled into bead-like structures, the chromomeres, in other regions. However, it has still not been settled whether a chromomere–interchromomere organization is also characteristic of chromatin in diploid cells, although recent studies suggest that this might be the case. By using the electron microscope it has been observed that the chromatin exists as a nucleofilament which can be wound up into helical structures (Finch & Klug 1976). It is also possible that higher orders of supercoiling may occur. When Benyajati & Worcel (1976) treated supercoiled *Drosophila* chromatin with DNase I, they found that each nick relaxed the supercoiled condition only in a restricted segment of the chromatin. Furthermore, it was estimated that such a segment corresponded in size to the larger chromomeres in polytene cells of *Drosophila*. Consequently, there are similarities between the organization of the chromosomal material in polytene and diploid cells, but the degree of homology can of course only be a matter of speculation at the present time.

When the question of the functional significance of superstructure in the chromosomal material is approached, it is evident that polytene chromosomes present certain useful properties. Most important, genetic as well as biochemical information can be related to the chromomere–interchromomere subdivision of defined chromosome segments (for discussion, see Daneholt 1974). For example, on the basis of cytological and autoradiographic studies of RNA synthesis on polytene chromosomes in the salivary glands of *Chironomus tentans*, Beermann (1964) and Pelling (1966) brought forward the idea that a chromomere corresponds to a transcription unit. Because of the limitations of the techniques used, their proposition could

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

only be tentative. During recent years we have further investigated the RNA synthesis on polytene chromosomes in *C. tentans* and in particular focused our attention on one specific chromosomal region, Balbiani ring 2 on chromosome IV. In this paper we are going to discuss the size and function of the transcription unit in BR 2. We will also summarize what can presently be stated on the relation of this transcription unit to the chromomeric organization of the chromatids in this segment of the fourth chromosome.

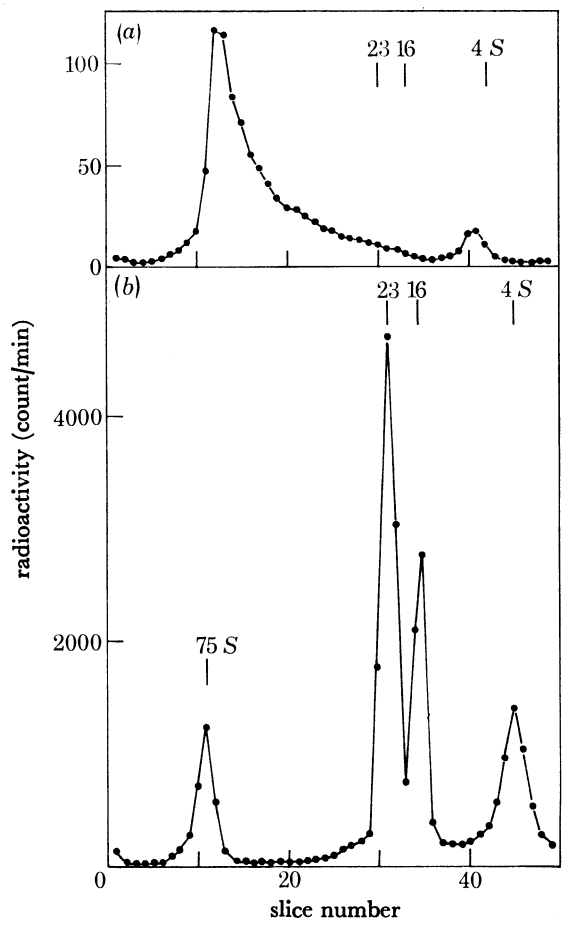

FIGURE 1. Electrophoretic analysis of Balbiani ring 2 RNA (*a*) and total salivary gland RNA (*b*). (*a*) Salivary glands of *Chironomus tentans* were incubated *in vitro* for 45 min at 18 °C in the presence of tritiated cytidine and uridine. Afterwards the salivary glands were fixed and 30 Balbiani rings isolated by microdissection. The RNA was released in a sodium dodecylsulphate–pronase solution, precipitated in ethanol in the presence of *Escherichia coli* RNA, redissolved and analysed by electrophoresis in a 1% agarose gel (for further details, see Daneholt 1972). (*b*) *Chironomus tentans* larvae were kept for 3 days at 18 °C in 20 ml culture medium provided with tritiated cytidine and uridine. The glands were then excised, and total RNA was extracted in a sodium dodecyl sulphate–pronase solution, precipitated in ethanol, redissolved and analysed in a 1% agarose gel.

2. SYNTHESIS OF BR 2 RNA AND ITS TRANSFER TO CYTOPLASM

Beermann (1952) observed that the BR 2 region of chromosome IV is heavily expanded, puffed, in the salivary glands, but not in other tissues containing polytene chromosomes such as Malpighian tubules. He interpreted the puffed condition in BR 2 to mean that BR 2 is a site for gland-specific genetic activity. This conclusion was later substantiated by Beermann

Downloaded from rstb.royalsocietypublishing.org

(1961) and Grossbach (1969, 1973) during cytogenetic studies in which it was shown that BRs in *Chironomus* salivary glands are coupled to the production of salivary polypeptides. By autoradiography Pelling (1964) could also reveal that BR 2 was exceptionally active in RNA synthesis. Subsequently the RNA products in BR 2 were characterized by biochemical techniques (Daneholt 1972). *C. tentans* salivary glands were isolated and incubated in a solution containing radioactive RNA precursors. The glands were then fixed and the BRs isolated by microdissection. The labelled RNA was extracted and analysed by agarose gel electrophoresis (figure 1*a*). One major RNA species is observed (sedimentation value of about 75*S*), but a more or less continuous distribution of molecules smaller than 75*S* is also recorded. From several lines of evidence (for discussion, see Daneholt 1975) it has been concluded that this population of molecules represent growing molecules that will ultimately attain the size of 75*S* RNA. In a kinetic analysis of the 75*S* RNA in various cellular compartments (Daneholt & Hosick 1973) it was furthermore possible to demonstrate that 75*S* RNA initially appeared in the nuclear sap, and later also in cytoplasm where it accumulated. The fact that 75*S* RNA molecules in cytoplasm do originate from BR 2 has been directly demonstrated by hybridization *in situ* (Lambert & Edström 1974). In the salivary gland cells, 75*S* RNA constitutes a major non-ribosomal and non-transfer RNA species which can be readily observed in a 3-day labelling experiment *in vivo* (figure 1*b*). The transfer of 75*S* RNA from the DNA template in BR 2 into cytoplasm hints at the possibility that BR 2 RNA acts as a messenger. Recently, this hypothesis has been further supported in studies of the salivary gland polysomes and their constituent RNA.

3. BR 2 RNA IN LARGE POLYSOMES

The polysomes of the salivary gland cells were extracted at 2–4 °C in a detergent solution and loaded on a 15–60 % sucrose gradient (Daneholt, Andersson & Fagerlind 1977). The result of a sedimentation analysis of polysomal material labelled with radioactive RNA precursors for 3 days is presented in figure 2. Apart from a monosome peak (75*S*) a broad distribution of polysomal material (200–2000*S*) can be recognized. When, as a control, the extract was treated with EDTA and subsequently sedimented in the sucrose gradient, it could be concluded that the 200–2000*S* material was sensitive to EDTA, confirming its polysomal nature (figure 2). The high sedimentation values suggested that the glands contained polysomes of very large sizes. This was confirmed when the polysomes from various sucrose gradient fractions were observed in the electron microscope. It was found that the size of the polysomes approximately corresponded to those predicted from the sedimentation values (Reisner, Askey & Aylmer 1972). On average, the 400*S* polysomes contained about 15 ribosomes per polysome while those sedimenting at 1500*S* contained about 60 ribosomes. Although infrequent, polysomes harbouring more than 100 ribosomes were also recognized in the 1500*S* fraction.

The polysomal RNA was extracted from the heavy (h.p.) as well as from the light (l.p.) polysome region (figure 2) and studied by electrophoresis in agarose gels (Daneholt *et al.* 1977). It was found that both fractions contained RNA in a broad size range (10–75*S* RNA), but the largest molecules, including 75*S* RNA, were preferentially located in the h.p. region of the gradient. Furthermore, it could be inferred from EDTA-shift experiments that most of the 75*S* RNA molecules were indeed present in polysomal structures. Finally, by hybridization *in situ* it was directly demonstrated that BR 2 sequences reside in heavy as well as in light polysomes (Wieslander & Daneholt 1977). The result of an experiment *in situ* with polysomal

Downloaded from rstb.royalsocietypublishing.org

RNA from the h.p. fraction is shown in figure 3, plate 1. Grains can be observed over both BR 2 and BR 1. Since the cytoplasmic BR sequences seem to be confined to the 75*S* RNA fraction (Lambert & Edström 1974), the *in situ* hybridization study also indicates, although indirectly, that molecules of 75*S* size reside in polysomal structures.

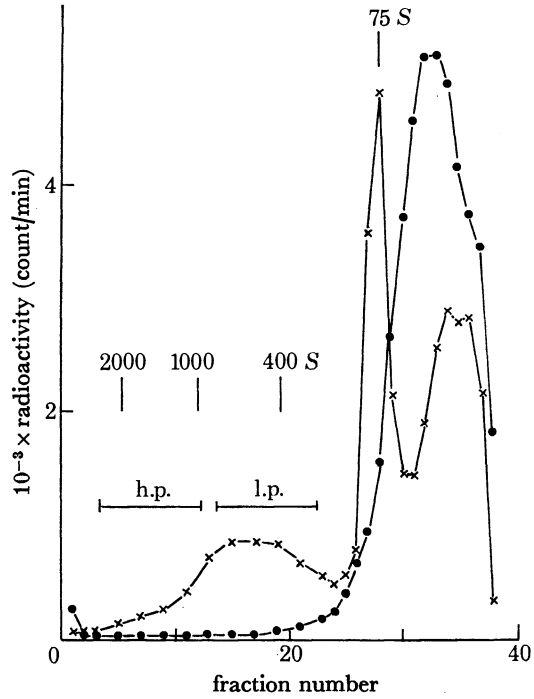

FIGURE 2. Sucrose gradient sedimentation of *Chironomus* polysomes. Eight salivary glands were labelled *in vivo* for 3 days. The polysomes were extracted at 2–4 °C in a detergent solution containing 0.5% sodium deoxycholate and 0.5% Tween 80. One half of the extract was treated with EDTA (final concentration 0.02 mol l^{-1}). Each sample was layered on a 15–60% sucrose gradient and spun at 40000 rev/min for 30 min at 4 °C. For further experimental details, see Daneholt *et al.* (1977). Untreated sample, crosses; EDTA-treated, filled circles.

The presence of 75*S* RNA in polysomes strongly suggests that 75*S* RNA is a messenger RNA molecule. Due to the exceptional sizes of the polysomes containing 75*S* RNA, it can furthermore be argued that the putative coding segment in 75*S* RNA is likely to be long. Large polysomes that are responsible for the synthesis of giant polypeptides are known from several other biological systems. Examples are those making myosin in chick muscle cells (Heywood, Dowben & Rich 1967), fibroin in the silk glands of *Bombyx mori* (McKnight, Sullivan & Miller 1976) and vitellogenin in rooster liver (Roskam, Gruber & Ab 1976). By comparison of the sizes of these polysomes to the molecular weight of their corresponding polypeptide products it can be estimated that a polysome containing 60 ribosomes could contain a segment capable of coding for a polypeptide of molecular mass 200000. This would imply a coding segment in 75*S* RNA of about 6000 bases. It should, however, be recalled that salivary gland polysomes of larger sizes than 60 ribosomes per polysome were observed and thus the 6000-base figure could be looked upon as a minimum estimate of the coding region in BR 2 RNA.

There are no *direct* experimental data as to the type of genetic information present in 75*S* RNA from BR 2. However, if it is accepted that 75*S* RNA is an mRNA species, it can be

Downloaded from rstb.royalsocietypublishing.org

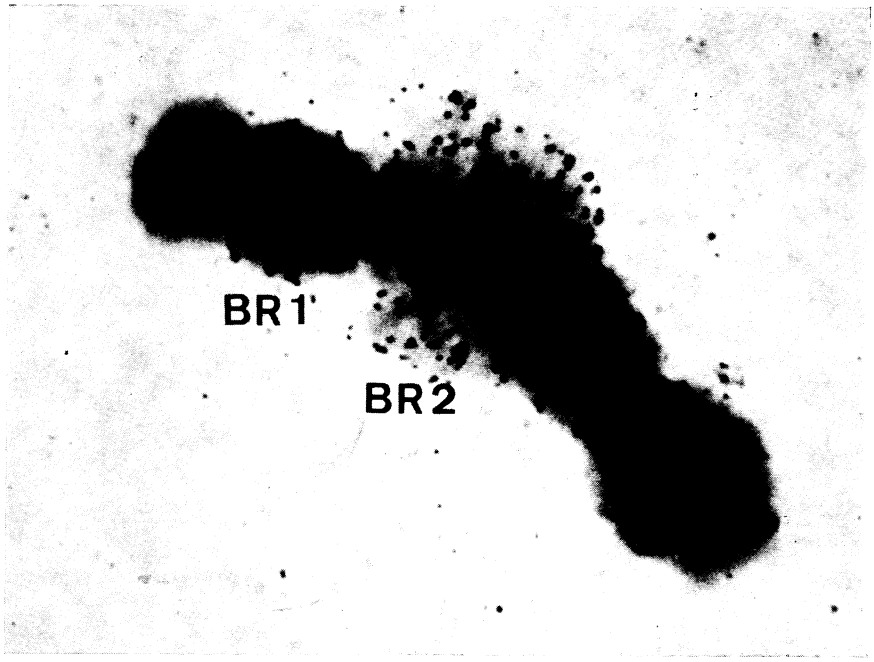

FIGURE 3. Hybridization *in situ* of labelled RNA from the heavy polysome region (h.p.) of the sucrose gradient depicted in figure 2. Each second fraction in the h.p. region (4, 6...12) was pooled, and the RNA was released by addition of Sarkosyl and pronase. The sample was dialysed twice against SSC (SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) and the RNA was precipitated in ethanol, redissolved and hybridized *in situ* (Wieslander & Daneholt 1977).

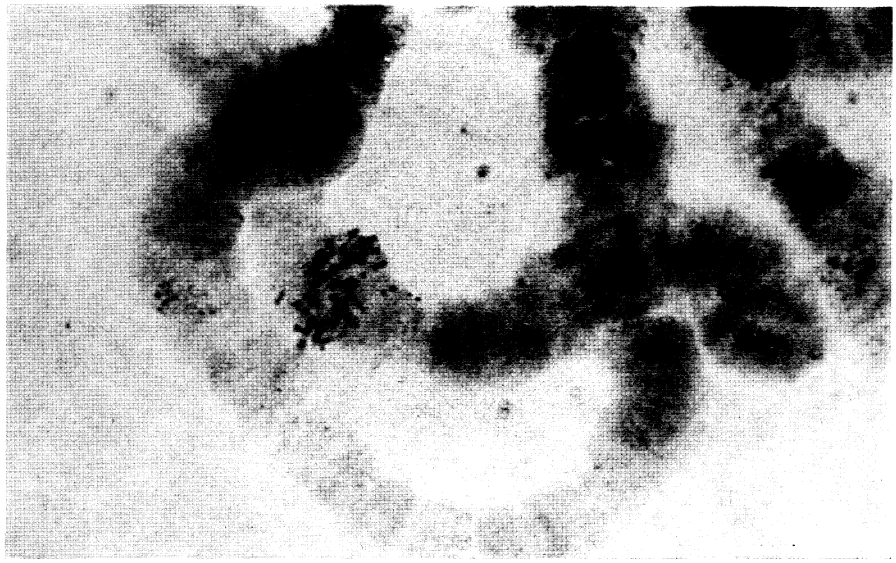

FIGURE 5. Hybridization *in situ* of BR 2 RNA to rectum chromosomes of *Chironomus tentans*. The grains are located over region 3 B of chromosome IV (see figure 6). For further details, see Lambert (1975).

(*Facing p.* 386)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

argued that 75*S* RNA, being the most abundant mRNA in these cells, is likely to code for the predominant protein product, the salivary polypeptides. Such a view is in good agreement with the cytogenic data (Beermann 1961; Grossbach 1969, 1973) coupling the BRs to the production of the salivary polypeptides. One of the most important tasks in further analysis of the BR 2 system will be to read the genetic information in 75*S* RNA in a suitable translational system in order to find out the nature of the coding information and whether this large transcription unit corresponds to one or several translational units.

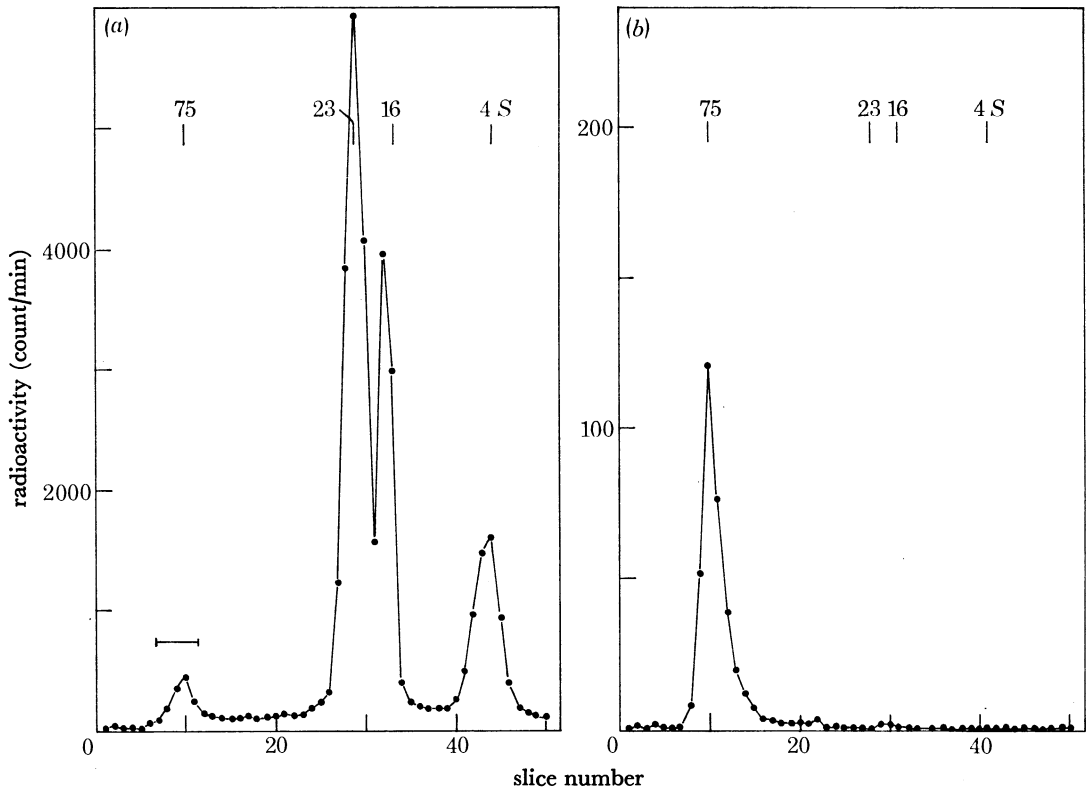

FIGURE 4. (*a*) Electrophoretic analysis of total salivary gland RNA labelled for 3 days *in vivo* and (*b*) re-electrophoresis of 75*S* RNA obtained by electrophoretic elution of the 75*S* region indicated in (*a*). For further experimental details, see Case & Daneholt (1976).

4. ISOLATION OF 75*S* RNA AND DETERMINATION OF ITS SIZE

Since a primary transcript reflects the structure of the corresponding transcription unit, 75*S* RNA can be used as a tool in the further analysis of the transcription unit in BR 2. It is therefore important that it has recently been possible to isolate undegraded 75*S* RNA in larger quantities (Case & Daneholt 1976). After fractionation of total gland RNA in agarose gels (figure 4*a*), the 75*S* RNA region is cut out and the RNA electrophoresed into a microchamber. Upon re-electrophoresis, this purified RNA has the same migration properties as in the preparative gel (figure 4*b*). At present this RNA is being used to estimate the size of 75*S* RNA in the electron microscope (S. T. Case, unpublished). Concurrently the molecular mass of 75*S* RNA is being estimated by electrophoresis in formaldehyde-containing agarose gels as described by

Downloaded from rstb.royalsocietypublishing.org

Lehrach, Diamond, Wozney & Boedtker (1977). Initial results of these two procedures imply a molecular mass for 75*S* RNA of about 10×10^6. The size of the 75*S* RNA transcription unit in BR 2 should therefore be on the order of 30000 base pairs.

5. Chromosomal distribution of BR 2 sequences

The detailed morphology of the polytene chromosomes should make it feasible to relate biochemical information to the chromomore–interchromomere organization of the chromosome. The first problem to be approached is the chromosomal location of the BR 2 sequences. Lambert (1975) has recently applied the *in situ* hybridization technique to obtain information on this point. He hybridized BR 2 RNA to rectum chromosomes, in which the unpuffed BR 2 region of chromosome IV has a well defined banding pattern (Beermann 1952). The BR 2 sequences were detected only in the BR 2 region of chromosome IV, but across a broad segment (figure 5, plate 1). It was concluded that the BR 2 sequences reside in more than one band, probably in the order of 3–5 bands. The detailed structure of the segment covered by the grains is presented in figure 6. The BR 2 sequences are most likely to be present in two or more of the thin bands in the centre of the segment. One of them (indicated by a bar in figure 6) has been designated as the origin of the giant puff BR 2 (Beermann 1952). However, at this time it can not be excluded that the two thicker, double-bands surrounding the thin bands also contain BR 2 sequences.

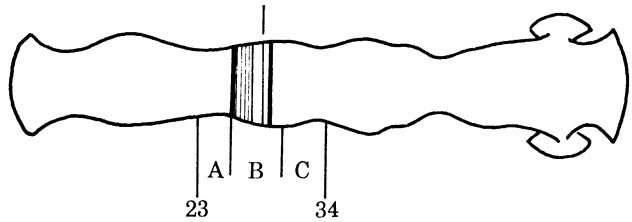

Figure 6. Balbiani ring 2 region of chromosome IV in *Chironomus tentans* (modification from Beermann 1952). The bar denotes the chromosomal band described by Beermann (1952) as the origin of the giant puff Balbiani ring 2.

It is unfortunate that the actual DNA distribution within the BR 2 region is not known. The only information available is the size of an average chromomere in *C. tentans*, which amounts to about 100000 base pairs (Daneholt & Edström 1967). It seems likely that the chromomeres of the thin bands in the BR 2 region contain less DNA than an average chromomere, but how much less is difficult to assess. It might therefore well be that each chromomere corresponds to a single transcription unit (30000 base pairs); two or more transcription units would then exist in adjacent chromomeres. However, we cannot exclude other alternatives. For example, it is still possible that there is only one BR 2 transcription unit per chromatid extending through two or more chromomeres.

Before more conclusive statements can be made on the relation of the 75*S* RNA transcription unit to the chromosome structure, further information is needed on the number and organization of the 75*S* RNA transcription units in BR 2 DNA as well as on the content of DNA and BR 2 sequences in the various chromosome bands (chromomeres) depicted in figure 6. One observation by Beermann (1973) might prove helpful: BR 2 regresses upon galactose treatment and the banding pattern is restored in the BR 2 region. This process is accompanied by a

Downloaded from rstb.royalsocietypublishing.org

dramatic repression of the synthesis of 75*S* RNA in BR 2 (L. G. Nelson, unpublished). Because the salivary gland chromosomes have a higher degree of polytenization than chromosomes from other tissues (for example rectum chromosomes), such chromosomes should be particularly suitable for studies of the location of BR 2 sequences as well as for the determination of DNA amounts within the BR 2 region. Moreover, preliminary microdissection experiments have demonstrated that it is feasible to isolate well defined chromosome segments for further analysis of BR 2 DNA in the electron microscope.

The technical assistance of Miss Eva Mårtenzon, Miss Jeanette Nilsson and Mrs Sigrid Sahlén is gratefully acknowledged. We are also indebted to Dr B. Lambert and Professor W. Beermann for allowing us to reproduce figures 5 and 6, respectively. The present work was supported by the Swedish Cancer Society, Magnus Bergvalls Stiftelse and Karolinska Institutet (Reservationsanslaget). S.T.C. is a recipient of a National Research Service Award from the National Institutes of Health (U.S.A.) and M.M.L. is supported by a postdoctoral fellowship from the American Cancer Society.

References (Daneholt *et al.*)

Beermann, W. 1952 *Chromosoma* **5**, 139–198.
Beermann, W. 1961 *Chromosoma* **12**, 1–25.
Beermann, W. 1964 In *Genetics today*, pp. 375–384. Oxford: Pergamon Press.
Beermann, W. 1972 In *Results and problems in cell differentiation* (eds W. Beermann, J. Reinart & H. Ursprung), vol. 4, pp. 1–33. Berlin: Springer-Verlag.
Beermann, W. 1973 *Chromosoma* **41**, 297–326.
Benyajati, C. & Worcel, A. 1976 *Cell* **9**, 393–407.
Case, S. T. & Daneholt, B. 1976 *Anal. Biochem.* **74**, 198–206.
Daneholt, B. 1972 *Nature, new Biol.* **240**, 229–232.
Daneholt, B. 1974 *Int. Rev. Cytol.*, suppl. **4**, 417–462.
Daneholt, B. 1975 *Cell* **4**, 1–9.
Daneholt, B., Andersson, K. & Fagerlind. M. 1977 *J. Cell Biol.* **73**, 149–160.
Daneholt, B. & Edström, J.-E. 1967 *Cytogenetics* **6**, 350–356.
Daneholt, B. & Hosick, H. 1973 *Proc. natn. Acad. Sci. U.S.A.* **70**, 442–446.
Finch, J. T. & Klug, A. 1976 *Proc. natn. Acad. Sci. U.S.A.* **73**, 1897–1901.
Grossbach, U. 1969 *Chromosoma* **28**, 136–187.
Grossbach, U. 1973 *Cold Spring Harb. Symp. quant. Biol.* **38**, 619–627.
Heywood, S. M., Dowben, R. M. & Rich, A. 1967 *Proc. natn. Acad. Sci. U.S.A.* **57**, 1002–1009.
Lambert, B. 1975 *Chromosoma* **50**, 193–200.
Lambert, B. & Edström, J.-E. 1974 *Molec. Biol. Rep.* **1**, 457–464.
Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. 1977 *Biochemistry, N.Y.* **16**, 4743–4758.
McKnight, S. L., Sullivan, N. & Miller, O. L. Jr 1976 *Progr. nucl. Acid Res. molec. Biol.* **19**, 313–318.
Pelling, C. 1964 *Chromosoma* **15**, 71–122.
Pelling, C. 1966 *Proc. R. Soc. Lond.* B **164**, 279–289.
Reisner, A. H., Askey, C. & Aylmer, C. 1972 *Anal. Biochem.* **46**, 365–373.
Roskam, W. G., Gruber, M. & Ab, G. 1976 *Biochim. biophys. Acta* **435**, 91–94.
Wieslander, L. & Daneholt, B. 1977 *J. Cell Biol.* **73**, 260–264.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

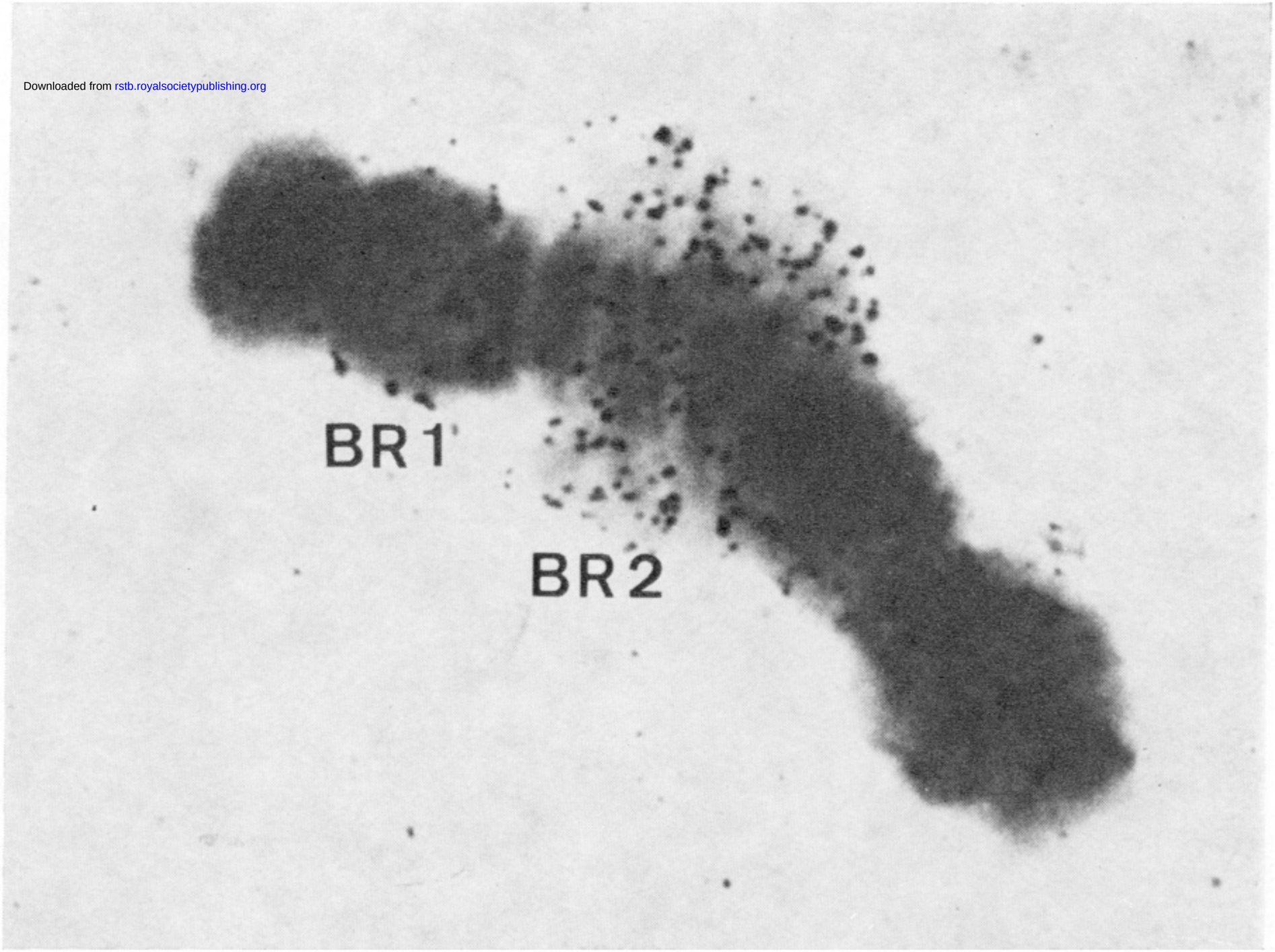

FIGURE 3. Hybridization *in situ* of labelled RNA from the heavy polysome region (h.p.) of the sucrose gradient depicted in figure 2. Each second fraction in the h.p. region (4, 6...12) was pooled, and the RNA was released by addition of Sarkosyl and pronase. The sample was dialysed twice against SSC (SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) and the RNA was precipitated in ethanol, redissolved and hybridized *in situ* (Wieslander & Daneholt 1977).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

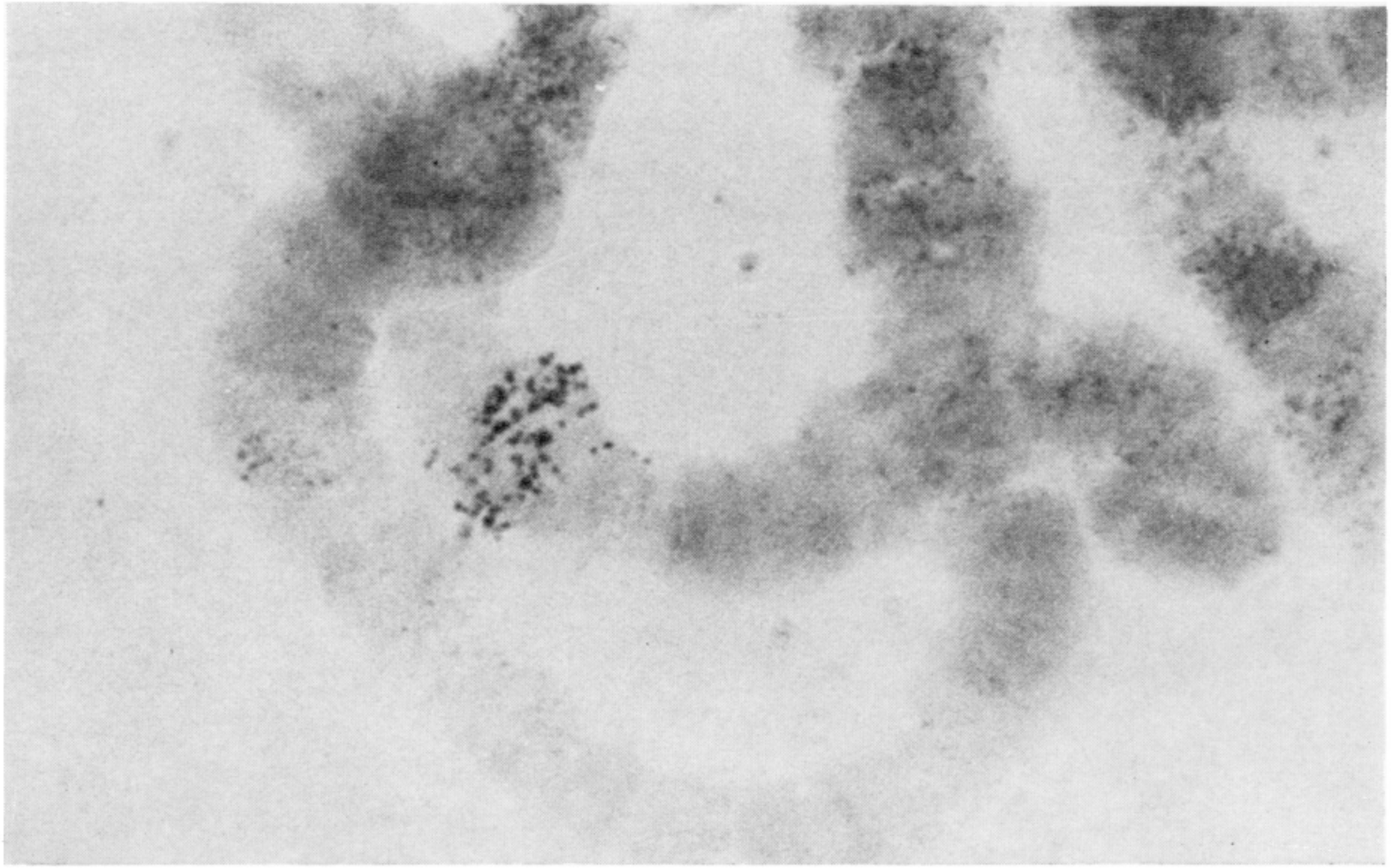

FIGURE 5. Hybridization *in situ* of BR 2 RNA to rectum chromosomes of *Chironomus tentans*. The grains are located over region 3 B of chromosome IV (see figure 6). For further details, see Lambert (1975).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES