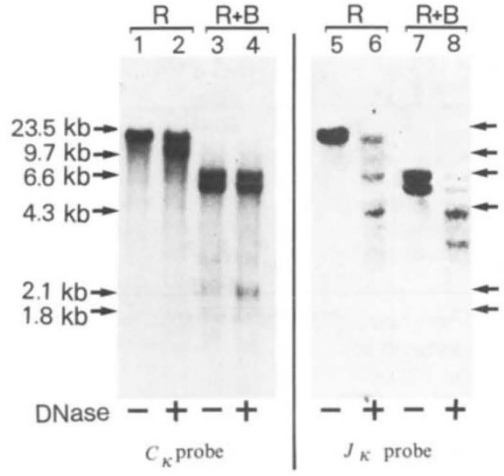

Fig. 4 Boundaries of the inducible hypersensitive site. DNA was isolated from LPS-treated cells either with or without previous DNase digestion of nuclei. After cleavage with either *Eco*RI alone (R) or a combination of *Eco*RI and *Bam*HI (R+B), the DNA was electrophoresed, transferred to nitrocellulose, and probed for either κ constant region (lanes 1–4) or J_κ (lanes 5–8) sequences. The J_κ probe was a 2.5-kb *Hin*dIII fragment of the embryonic κ locus[15], isolated from a recombinant plasmid provided by E. Max and P. Leder. DNase concentrations were 1.0 U ml^{-1} in lanes 2 and 4, and 3.0 U ml^{-1} in lanes 6 and 8. The approximate sizes of the subfragments generated by DNase digestion are: lane 2, 10 kb; lane 4, 2.1 kb; lane 6, 6.5 and 4.2 kb; lane 8, 4.2 and 3.7 kb.

of the C_κ region[15] (Fig. 3). The hypersensitive site lies inside the region which forms the large intervening sequence of a rearranged κ gene, with its 3′ boundary 0.5–0.7 kb upstream of the constant region coding sequence. To identify the 5′ boundary of the hypersensitive site, restriction digests of DNA from LPS-treated cells were probed for fragments bearing the J_κ region (Fig. 4). Mild DNase digestion always produced two distinct J_κ-bearing subfragments, corresponding to the two κ alleles. Analysis of the sizes of these subfragments indicated that DNase hypersensitivity arises at the same location in both the rearranged and germline κ alleles after exposure to LPS, and is confined to a discrete region not more than 0.5 kb long. The location of this hypersensitivity does not coincide with known sites of transcriptional initiation, DNA recombination[16,17] or RNA splicing, and serves no other recognized function. Recently, however, the sequence of a short (150-base pair) segment of DNA occupying approximately this same location, has been found to be conserved evolutionarily between the human and mouse κ genes, whereas the remaining intronic sequences on either side have undergone extensive divergence[18]. This segment is, in fact, the only conserved noncoding region in the vicinity of the C_κ locus. No additional hypersensitive sites were detected; in particular, there was no evidence of hypersensitivity near the 5′ end of the rearranged κ gene. We have detected neither constitutive nor inducible transcripts derived from the germ-line κ locus in these cells, suggesting that hypersensitivity is not simply a consequence of ongoing transcription (data not shown). The factors which prevent coordinate activation of the germ-line allele remain to be elucidated.

Thus, our data indicate that when κ light chain expression is induced by LPS treatment, a discrete DNA region closely linked to the constant region coding sequence becomes extremely sensitive to DNase digestion, presumably as a result of a localized change in chromatin structure. While this region has no identified function, its sequence has apparently been conserved during evolution, whereas adjacent sequences have not. Similar hypersensitive sites have been observed in other genes[19–25], where they are frequently (but not exclusively) found near the transcriptional initiation site at the 5′ end of the gene. Because the hypersensitivity of certain of the sites correlates with gene activity, it has been argued that these may have a role in the regulation of transcription[20–25]. By analogy with these sites, it is tempting to speculate that the LPS-inducible hypersensitive site found inside the κ gene may play a part in transcriptional activation of the gene in response to lipopolysaccharide.

We thank R. P. Perry for providing 70Z/3 cells and for helpful discussion, and J. E. Donelson for reviewing the manuscript. This research was supported by NIH grants AM20858, AM24037 and AM25295 (Diabetes and Endocrinology Research Center), and by VA research funds. T.G.P. received support from Medical Scientist Training grant GM07337. D.K.G. was a VA Medical Investigator.

Received 15 March; accepted 13 July 1982.

1. Brack, C., Hirama, M., Lenhard-Schuller, R. & Tonegawa, S. *Cell* **15**, 1–14 (1978).
2. Seidman, J. G. & Leder, P. *Nature* **276**, 790–795 (1978).
3. Early, P. W., Davies, M. M., Kaback, D. B., Davidson, N. & Hood, L. *Proc. natn. Acad. Sci. U.S.A.* **76**, 857–861 (1979).
4. Max, E. E., Seidman, J. G. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3450–3454 (1979).
5. Perry, R. P. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 1937–1941 (1980).
6. Paige, C. J., Kincade, P. W. & Ralph, P. *J. Immun.* **121**, 641–647 (1978).
7. Paige, C. J., Kincade, P. W. & Ralph, P. *Nature* **292**, 631–633 (1981).
8. Maki, R., Kearney, J., Paige, C. J. & Tonegawa, S. *Science* **209**, 1366–1369 (1980).
9. Perry, R. P. & Kelley, D. E. *Cell* **18**, 1333–1339 (1979).
10. Parslow, T. G. & Granner, D. K. (in preparation).
11. Mather, E. L. & Perry, R. P. *Nucleic Acids Res.* **9**, 6855–6867 (1981).
12. Storb, U., Wilson, R., Selsing, E. & Walfield, A. *Biochemistry* **20**, 990–996 (1981).
13. Perry, R. P., Kelley, D. E., Coleclough, C. & Kearney, J. F. *Proc. natn. Acad. Sci. U.S.A.* **78**, 247–251 (1981).
14. Van Ness, B. G. *et al. Cell* **27**, 593–602 (1981).
15. Selsing, E. & Storb, U. *Cell* **25**, 47–58 (1981).
16. Choi, E., Kuehl, M. & Wall, R. *Nature* **286**, 776–779 (1980).
17. Seidman, J. G. & Leder, P. *Nature* **286**, 779–783 (1980).
18. Heiter, P. A., Max, E. E., Seidman, J. G., Maizel, J. V. & Leder, P. *Cell* **22**, 197–207 (1980).
19. Stalder, J. *et al. Cell* **20**, 451–460 (1980).
20. Groudine, M. & Weintraub, H. *Cell* **24**, 393–401 (1981).
21. Keene, M. A., Corces, V., Lowenhaupt, K. & Elgin, S. C. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 143–146 (1981).
22. Samal, B., Worcel, A., Louis, C. & Schedl, P. *Cell* **23**, 401–409 (1981).
23. Wu, C. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1577–1580 (1981).
24. Storb, U., Arp, B. & Wilson, R. *Nature* **294**, 90–92 (1981).
25. Elgin, S. C. R. *Cell* **27**, 413–415 (1981).
26. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).
27. Jackson, V. & Chalkley, R. *Biochemistry* **13**, 3952–3956 (1974).
28. Smith, G. E. & Summers, M. D. *Analyt. Biochem.* **109**, 123–129 (1980).
29. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).
30. Schwartz, R. C., Sonenshein, G. E., Bothwell, A. & Gefter, M. L. *J. Immun.* **126**, 2104–2108 (1981).
31. Kioussis, D. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 4370–4374 (1979).
32. Parslow, T. G., Murphy, W., Katzen, C. & Granner, D. K. (in preparation).

Genomic environment of the expression-linked extra copies of genes for surface antigens of *Trypanosoma brucei* resembles the end of a chromosome

Titia De Lange & Piet Borst

Section for Medical Enzymology and Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, PO Box 60.000, 1005 GA Amsterdam, The Netherlands

The surface coat of trypanosomes consists essentially of a single protein, the variant surface glycoprotein (VSG)[1,2]. But more than a hundred different VSGs can be produced[3]. By switching from the synthesis of one VSG to the next, trypanosomes change the antigenic nature of their surface and thus escape destruction by the host immune system[4–6]. Each VSG is encoded by a separate gene and expression of some of these genes involves the duplication and transposition of a silent basic copy (BC) into a new genomic environment[7–9], where the gene is transcribed[10,11]. The DNA segment downstream of this transposed expression-linked extra copy (ELC) of the gene has two remarkable properties. It is devoid of restriction endonuclease cutting sites for a stretch of 7 kilobases (kb) which ends abruptly in an apparent cluster of at least 17 restriction endonuclease cutting sites. Here we show that in intact DNA the 'barren' region 3′ to two active VSG genes is preferentially attacked by exonuclease *Bal*31. We conclude that the expressed copies of these genes are located adjacent to a discontinuity in the DNA, presumably the end of a chromosome.

0028-0836/82/390451-02$01.00

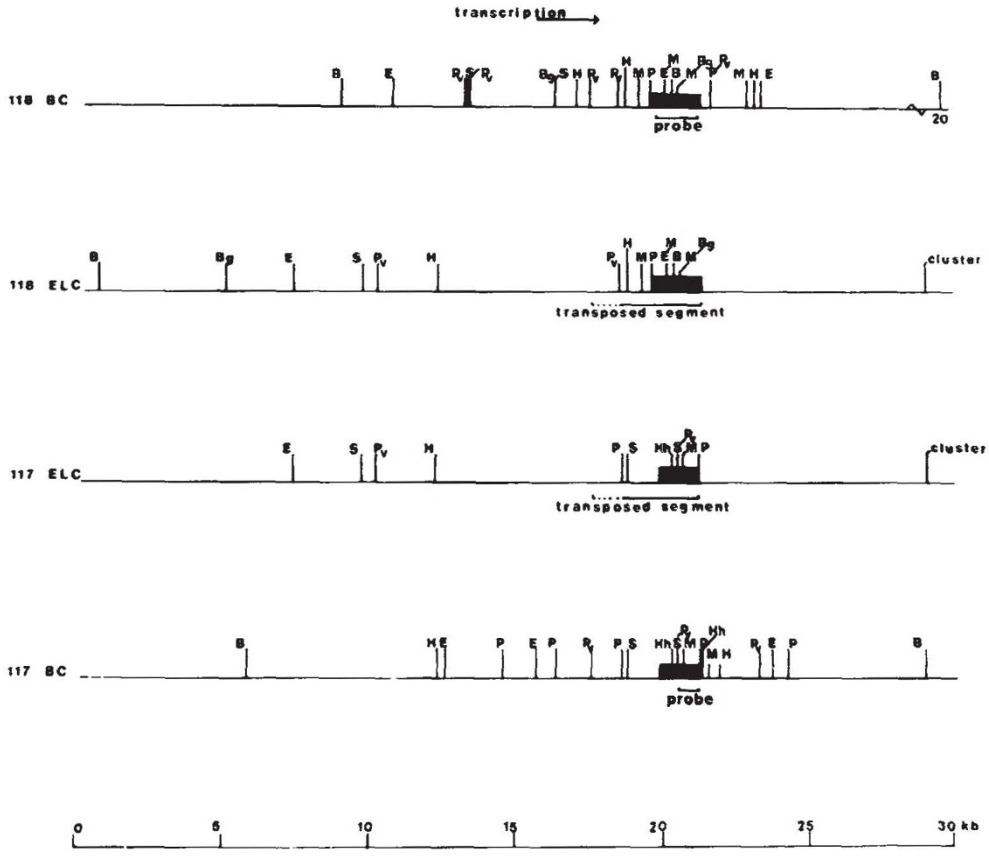

Fig. 1 Physical maps of the 117 and 118 VSG genes. Restriction endonuclease cleavage sites in and around the BC and ELC genes for VSGs 117 and 118 in *Trypanosoma brucei* (from ref. 12). The clusters at the 3′ ends of the ELC maps indicate small regions where 17 restriction enzymes appear to cut (*Eco*RI, *Bam*HI, *Pst*I, *Msp*I, *Hin*dIII, *Pvu*II, *Bgl*II, *Sal*I, *Hha*I, *Hph*I, *Taq*I, *Xba*I, *Bsp*I, *Xho*I, *Ava*I, *Kpn*I, *Mbo*I). The transposed segment of the BC genes is shown in the ELC maps. The sequences that hybridize to the probes used in the experiments shown in Fig. 2 are indicated underneath the BC genes. Abbreviations: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; Hh, *Hha*I; H, *Hin*dIII; M, *Msp*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I.

The physical maps of the BCs and ELCs of VSG genes 117 and 118 are shown in Fig. 1. The regions surrounding both ELCs are indistinguishable and both genes may have been inserted into the same expression site. This site is characterized by long stretches of DNA in which no restriction enzymes cut. These stretches may represent repetitive DNA, but this has not been verified, as all attempts to isolate this DNA as recombinant DNA have failed[11,12].

To test whether the apparent restriction site cluster downstream of the ELCs really is a discontinuity in the DNA, we determined whether this site is preferentially attacked by *Bal*31 exonuclease in large DNA. DNA with a molecular weight of 50–80 kb was isolated from trypanosome variant antigen types 117 and 118 and incubated with *Bal*31 for various periods of time. The DNA was recovered and digested with appropriate restriction endonucleases to examine the effect of *Bal*31 digestion on ELC and BC fragments in Southern blots.

Figure 2*a* shows a Southern blot of 117 DNA treated with *Bal*31 and restricted with *Pvu*II. The filter-bound DNA was hybridized to the 3′ part of a cloned VSG 117 complementary DNA (cDNA) fragment. Two restriction fragments are detected: a 2.7-kb BC gene fragment and a 8.7-kb fragment that represents the ELC gene. The ELC gene fragment is progressively shortened by *Bal*31, whereas the BC gene fragment is unaffected even by the most extensive *Bal*31 treatment.

The probe used to visualize the 117 VSG gene fragments is known to cross-hybridize with a family of putative VSG genes, the 117-related genes[6–8,12]. Even at the high stringency used in this experiment (0.1×SSC at 65 °C), 117-related genes are detected. All cross-hybridizing fragments, some of which are larger than the 117 ELC gene fragment, are resistant to *Bal*31 digestion, suggesting that the shortening of the 117 ELC gene fragment by *Bal*31 nuclease is a specific event, unrelated to the size of the target.

Figure 2*b* shows the result of an analogous experiment with variant type 118 DNA. After *Bal*31 treatment, the DNA samples were cleaved with *Bgl*II and a Southern blot of this

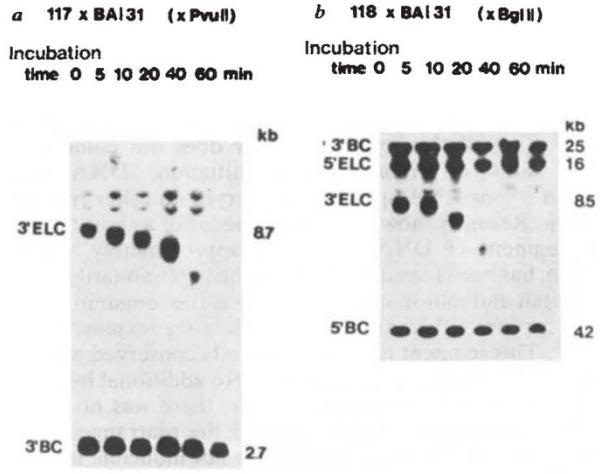

Fig. 2 Autoradiograms showing the preferential sensitivity of the 3′ flanking region of the 117 and 118 ELC genes to digestion by *Bal*31 nuclease. The DNA from trypanosome variant types 117 and 118 (MITat 1.4 and 1.5) was isolated as described in ref. 11 and digested with 1 U *Bal*31 nuclease (Biolabs) per 30 μg DNA, as described in ref. 13, for the times indicated. The DNA was collected by ethanol precipitation after phenol extraction and digested with the restriction enzymes indicated. The restricted DNA was size-fractionated on agarose slab gels, blotted onto nitrocellulose filters and hybridized to nick-translated ^{32}P-labelled VSG cDNA fragments. *a*, Southern blot of 117 DNA treated with *Bal*31, digested with *Pvu*II and hybridized to the 3′ *Sal*I–*Pst*I fragment from TcV117-5 (ref. 11). *b*, Southern blot of 118 DNA treated with *Bal*31, digested with *Bgl*II and hybridized to the *Pst*I insert fragment of TcV118-2 (ref. 11). The localization of the genomic sequences that hybridize to the cDNA fragments is shown in Fig. 1. The origin and size of the fragments detected are indicated. The extra hybridizing fragments in *a* represent 117-related genes (see text).

DNA was probed with a cloned VSG 118 cDNA fragment. One of the four *Bgl*II fragments detected is shortened by *Bal*31 nuclease. This 8.5-kb *Bgl*II fragment contains the 3′ part of the 118 ELC gene and its flanking region.

The preferential sensitivity to *Bal*31 digestion of the 3′-flanking region of the 117 and 118 ELC genes shows that this region contains a discontinuity. As the *Bal*31 preparations also cleave single-stranded DNA[14], this discontinuity could either be in one DNA strand (nick or gap) or in both DNA strands. The fact that 17 restriction endonucleases appear to cut at this position is compatible only with a discontinuity in both strands. Whether this discontinuity is also present *in vivo* or unmasked by removal of protein or RNA during DNA isolation is not easily tested with trypanosomes, because they lack condensed or polytene chromosomes suitable for cytological (hybridization) analysis.

The ends of the linear DNA molecules found in viruses or chromosomes are known to have unusual properties. A protein may be covalently linked to the end[15,16] or the two DNA strands may be linked and form a hairpin[17–21]. The presence of a long hairpin (more than 500 base pairs) in the trypanosome DNA was rendered unlikely by running restriction digests of nuclear DNA on an alkaline gel before and after treatment with S_1 nuclease, which specifically cuts single-stranded DNA. No shortening of ELC fragments was seen (experiment not shown).

Another unusual feature of chromosome ends is the presence of tandem repeats. These were first detected at the ends of ribosomal DNA molecules, which were shown to contain 20–70 tandem repeats of the hexanucleotide CCCCAA[16]. This simple repeated sequence is probably present at most termini of *Tetrahymena* macronuclear DNA[22] and analogous short terminal repeats have been found in other ciliates[23–25], in the rDNA of the slime mould *Dictyostelium*[26] and in linear viral DNAs[21,27]. The recent finding that the ends of rDNA from *Tetrahymena* can be stably replicated in yeast nuclei as ends of linear plasmids[28] suggests that terminal repeats may indeed be a common characteristic of most stable linear nuclear DNAs. The 'barren' regions surrounding the ELCs (Fig. 1) could, therefore, represent analogous short tandem repeats marking the end of a chromosome.

This work was supported by a grant from the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO) and the World Health Organization (T16/181/T7/34).

Received 1 June; accepted 9 July 1982.

1. Vickerman, K. *J. Cell Sci.* **5**, 163–193 (1969).
2. Cross, G. A. M. *Parasitology* **71**, 393–417 (1975).
3. Capbern, A., Giroud, C., Baltz, T. & Mattern, P. *Expl Parasit.* **42**, 6–13 (1977).
4. Vickerman, K. *Nature* **273**, 613–617 (1978).
5. Marcu, K. B. & Williams, R. O. in *Genetic Engineering* Vol. 3 (eds Setlow, S. K. & Hollaender, A.) 129–155 (Plenum, New York, 1981).
6. Borst, P. & Cross, G. A. M. *Cell* **29**, 291–303 (1982).
7. Hoeijmakers, J. H. J., Frasch, A. C. C., Bernards, A., Borst, P. & Cross, G. A. M. *Nature* **284**, 78–80 (1980).
8. Borst, P. *et al. Cold Spring Harb. Symp. quant. Biol.* **45**, 935–943 (1981).
9. Pays, E., Van Meirvenne, N., Le Ray, D. & Steinert, M., *Proc. natn. Acad. Sci. U.S.A.* **78**, 2673–2677 (1981).
10. Pays, E., Lheureux, M. & Steinert, M. *Nature* **292**, 365–367 (1981).
11. Bernards, A. *et al. Cell* **27**, 497–505 (1981).
12. Van der Ploeg, L. H. T., Bernards, A., Rijsewijk, F. A. M. & Borst, P. *Nucleic Acids Res.* **10**, 593–609 (1982).
13. Yao, M. C. *Cell* **24**, 765–775 (1981).
14. Gray, H. B., Ostrander, D. A., Hodnett, J. L., Legerski, R. J. & Roberson, D. L. *Nucleic Acids Res.* **2**, 1459–1492 (1975).
15. Rekosh, D. M. K., Russel, W. C., Bellet, A. J. D. & Robinson, A. J. *Cell* **11**, 283–295 (1977).
16. Wimmer, E. *Cell* **28**, 199–201 (1982).
17. Geshelin, P. & Berns, K. I. *J. molec. Biol.* **88**, 785–796 (1974).
18. Blackburn, E. H. & Gall, J. G. *J. molec. Biol.* **120**, 33–53 (1978).
19. Cavalier-Smith, T. *Nature* **250**, 467–470 (1974).
20. Bateman, A. J. *Nature* **253**, 379 (1975).
21. Baroudy, B. M., Venkatesan, S. & Moss, B. *Cell* **28**, 315–324 (1982).
22. Yao, M. C. & Yao, C. H. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7436–7439 (1981).
23. Katzen, A. L., Cann, G. M. & Blackburn, E. H. *Cell* **24**, 313–320 (1980).
24. Oka, Y., Shiota, S., Nakai, S., Nishida, Y. & Okubo, S. *Gene* **10**, 301–306 (1980).
25. Klobutcher, L. A., Swanton, M. T., Donini, P. & Prescott, D. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3015–3019 (1981).
26. Emery, H. S. & Weiner, A. M. *Cell* **26**, 411–419 (1981).
27. Davison, A. J. & Wilkie, N. M. *J. gen. Virol.* **55**, 315–331 (1981).
28. Szostak, J. W. & Blackburn, E. H. *Cell* **29**, 245–255 (1982).

A specific replication origin in the chromosomal rDNA of *Lytechinus variegatus*

Peter M. Botchan* & Andrew I. Dayton*†

* The Wistar Institute of Anatomy and Biology,
36th Street at Spruce, Philadelphia, Pennsylvania 19104, USA
† Department of Biochemistry and Biophysics,
University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Specific replication origins have been well characterized in prokaryotes[1,2], and have been identified in eukaryotic extrachromosomal DNAs[3–8]. The best candidate, until now, for a specific eukaryotic chromosomal replication origin has been found in yeast[9], but the general existence of such origins is challenged[10] by the finding that SV40 and polyoma DNA fragments lacking the viral replication origin can replicate after injection into *Xenopus* eggs. It has thus been suggested that the origins found on extrachromosomal DNAs exist solely to circumvent the cells' strict requirement for chromosomal DNA to replicate once per cell division. Clearly the disparate views can only be reconciled after the replication of specific chromosomal genes has been studied. To this end, we have studied the replication of the ribosomal genes (rDNA) of the sea urchin *Lytechinus variegatus*. The visualization of replicating rDNA, isolated from rapidly dividing sea urchin gastrula cells, after restriction endonuclease digestion demonstrates that the initiation of replication in these chromosomal genes is sequence-specific, and is most probably confined to a region within the non-transcribed spacer.

After fertilization *L. variegatus* undergoes a rapid cleavage stage during which the embryos divide approximately every half hour. The rDNA genes of *L. variegatus* are present in about 200 copies per genome, and are isolatable as a dense satellite after equilibrium density centrifugation in CsCl[11,12]. DNA was isolated from sea urchin embryos about 4 h after fertilization. Following the first CsCl equilibrium density gradient, the ribosomal DNA peak was located by hybridization with a cloned ribosomal gene (pUNC220, provided by Dr D. Stafford). The rDNA was then recentrifuged in a second CsCl gradient and found to band at a density of 1.723 g cm^{-3}. The rDNA peak formed a dense satellite well separated from the main band DNA, which has a mean density of about 1.695 g cm^{-3} (ref. 11).

Table 1 Purity of rDNA fractions

Hours	% Chromosomal DNA bound at equilibrium Expt 1	Expt 2
24	17.5	18.4
48	15.7	14.6
72	14.0	14.3

The purity of ribosomal fraction was determined by saturation hybridization: three plasmids with inserts spanning the ribosomal repeat (see Fig. 1) were pooled on an equal weight basis, simultaneously nicked and denatured by treatment with 0.2 M NaOH for 10 min at 65 °C, neutralized and 10 μg applied to each 2.5-cm filter. Filters were cut into four identical pieces and after 1 h at 75 °C in 0.2 × SSC, 0.1% SDS, each was added to 56% (v/v) formamide, 4.6 × SSC, 9.2% dextran sulphate, 0.1% SDS containing about 2 ng ^{32}P-labelled chromosomal DNA and 0.5 ng ^{3}H-labelled nick-translated 'pooled plasmid' as an internal control. Hybridization was at 42 °C for the indicated times. Binding of the chromosomal probe and the pooled plasmid was found to be invariant over a 40- and 14-fold range, respectively. Results are normalized to the fraction of the ^{3}H-labelled pooled plasmid internal control at each time point. In both experiments, the value at 4 h was 20% but this time is omitted because it is pre-equilibrium. Maximum hybridization of the control was 57% of input for expt 1, 58% of input for expt 2.

0028-0836/82/390453-02$01.00