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Localization of the modified base J in telomeric VSG gene expression sites of Trypanosoma brucei

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African trypanosomes such as Trypanosoma brucei undergo antigenic variation in the bloodstream of their mammalian hosts by regularly changing the variant surface glycoprotein (VSG) gene expressed. The transcribed VSG gene is invariably located in a telomeric expression site. There are multiple expression sites and one way to change the VSG gene expressed is by activating a new site and inactivating the previously active one. The mechanisms that control expression site switching are unknown, but have been suggested to involve epigenetic regulation. We have found previously that VSG genes in silent (but not active) expression sites contain modified restriction endonuclease cleavage sites, and we have presented circumstantial evidence indicating that this is attributable to the presence of a novel modified base β-D-glucosyl-hydroxymethyluracil, or J. To directly test this, we have generated antisera that specifically recognize J-containing DNA and have used these to determine the precise location of this modified thymine in the telomeric VSG expression sites. By anti J-DNA immunoprecipitations, we found that J is present in telomeric VSG genes in silenced expression sites and not in actively transcribed telomeric VSG genes. J was absent from inactive chromosome-internal VSG genes. DNA modification was also found at the boundaries of expression sites. In the long 50-bp repeat arrays upstream of the promoter and in the telomeric repeat arrays downstream of the VSG gene, J was found both in silent and active expression sites. This suggests that silencing results in a gradient of modification spreading from repetitive DNA flanks into the neighboring expression site sequences. In this paper, we discuss the possible role of J in silencing of expression sites.

Key Words: DNA modification; silencing; antigenic variation; VSG; sequence repeats

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some population, and this fraction increased with the length of the associated telomeric repeat tract (Bernards et al. 1984b).

The recently identified unusual base β-D-glucosyl-hydroxymethyluracil (β-gluc-HOMeU), called J (Gommers-Ampt et al. 1991, 1993), is a good candidate for this telomere-linked DNA modification. This modified base, detected by 32P-nucleotide postlabeling combined with separation on two-dimensional thin-layer chromatography, has only been found in DNA of African trypanosomes. It is present at low levels (0.2 mole%) in BF trypanosomes and is absent from PC trypanosomes (Gommers-Ampt et al. 1991). By nucleotide postlabeling analysis of purified telomeric tracts, we have recently shown that about half of all J is concentrated in both strands of the telomeric (GGGTTA)n repeats (van Leeuwen et al. 1996). Besides J and its putative precursor HOMeU, no other DNA modifications, such as DNA methylation, have been found in T. brucei (Crozatier et al. 1988; Gommers-Ampt et al. 1991; van Leeuwen et al. 1996). We have now verified that J prevents cleavage by restriction endonuclease Pvull. With antisera specific for J-containing DNA, we have located J in and around the telomeric VSG expression sites.

Results

J prevents cleavage by restriction endonuclease Pvull

Partial cleavage of restriction sites suggested the presence of a DNA modification in silenced telomeric VSG genes in bloodstream T. brucei (see Fig. 1A). The modified base J has the properties expected for the postulated modification, as a bulky base such as J may be expected to block cleavage by restriction enzymes (Huang et al. 1982). We have tested whether J blocks cleavage by Pvull, using DNA duplexes of short oligonucleotides encoding the Pvull site and its flanking sequences of VSG gene 221 (Fig. 1B). Figure 1C shows that duplexes with a hemimodified Pvull site were not cleaved, whereas duplexes without J were digested completely. J replacing T two positions downstream of the Pvull site did not block cleavage, showing that J at a short distance does not affect the endonuclease–DNA interaction. Pvst has been shown already to be sensitive to the presence of HOM eU in the target sequence (McClelland et al. 1994) and is therefore expected to be also sensitive to J. These results confirm that J can block cleavage by restriction endonucleases and support further the correlation between J and the postulated modification in telomeric VSG genes. They do not, however, prove that J is present in VSG expression sites. Because restriction site polymorphisms and genomic sequencing (see Discussion) do not exclude the presence of other modifications, we set out to generate J-specific antisera to make it possible to detect low amounts of J in unique sequences in the genome.

Generation of antisera specific for J-containing DNA

To obtain antinucleic acid antisera with a high specificity for DNA containing J in various sequence contexts,
we induced antibodies with nucleotide-protein immunizing conjugates (see Materials and Methods). Immuno- 
ization with J-5’-monophosphate (MP) conjugated to keyhole limpet haemocyanin (KLH) or to bovine serum albumin (BSA) resulted in polyclonal rabbit antisera 538xJ and 539xJ, respectively. The specificity and sensi- 
tivity of the antisera were tested on DNA dot-blots with 
dilution series of various DNA samples, using immuno- 
detection combined with enhanced chemiluminescence. 
With both antisera we could detect less than one J in 106 
dilution series of various DNA samples, using immuno- 
tivity of the antisera were tested on DNA dot-blots with 
HOMeC), phage T4 (HOMeC), E. coli, calf thymus, 
or PC T. brucei, showing that they do not cross- 
react with nonmodified or methylated DNA, and con- 
fiming that PC trypanosomes are devoid of J (Fig. 2B). 
The antisera weakly recognized nonglucosylated 
HOMeU because some cross-reaction was found with phage 5e DNA, in which all thymines are replaced by 
HOMeU. A stronger cross-reaction was found with β- 
glucosyl-hydroxymethylcytosine (β-gluc-HOMeC), but not with α-gluc-HOMeC, bases found in T-even phages. 
In bacteriophages T2 and T4, HOMeC replaces C (Korn- 
berg et al. 1961). In both phases, 70% of HOMeC is α- 
glucosylated, in phage T4 another 30% is β-glucosylated. 
The glucosylated cytosine variants have only been found in 
T-even phage DNA and can be distinguished from J by 
32P-nucleotide-postlabeling and combined with two-dimen- 
sional thin-layer chromatography (Gommers-Ampt et al. 1991). Partially deaminated T2 DNA, in which a fraction 
of α-gluc-HOMeC was converted into α-gluc-HOMeU 
(0.1%-11% of T) did not cross-react, showing that the 
antisera react specifically with β-glucose linked to 
HOMeU or HOMeC (data not shown).

Immunoprecipitation of modified DNA

Immunoblots with denatured DNA provide a sensitive 
tool for the detection of J in DNA, but are less useful to 
study J-modification of specific sequences in a genome. 
We therefore tested whether the antibodies would im- 
munoprecipitate J-containing double-stranded DNA 
fragments. Duplex DNA fragments of 118, 426, and 943 
bp, each with one J residue, were generated by PCR am- 
plication of part of the 221 VSG gene using one anti- 
sense primer with J and three different sense primers 
without J (Fig. 3A). As a negative control, the shortest 
fragment was amplified with two J-less primers. The 
118-bp PCR products were ligated to each other to obtain 
a ladder of fragments of different sizes but with the same 
J density. Fragments were end-labeled, incubated with αJ 
antisera, and antibody–DNA complexes were captured 
by protein-A beads. Bound DNA was released by prote- 
ase treatment and phenol extraction, separated by agar- 
ose gel-electrophoresis, and then blotted (Fig. 3B). One J 
residue was sufficient to immunoprecipitate a fraction of the 
duplex DNA molecules (Fig. 3B, lanes 1,2) and this 
fraction decreased with length. The effect of length was 
less if the density of modification was kept constant (Fig. 
3B, lanes 3,4). DNA without J was not immunoprecipi- 
tated (Fig. 3B, lane 5,6). Quantitation of the relative ef- 
ciciency of immunoprecipitation (IP) of the various frag- 
ments (Fig. 3C) showed that anti-J IP is dependent on the 
size of the target fragment and the degree of modifica-

Having the tools to select for J-containing DNA, we 
set out to analyze modified genomic restriction frag- 
ments from BF T. brucei DNA. Because we had found 
previously that the telomeric repeats contain about 4% J 
compared with 0.2% J in the total genome (van Leeuwen 
et al. 1996), we first tested the long telomeric repeat 
arrays and found that despite their length (2–26 kb) these 
were immunoprecipitated readily (see below). Further- 
more, immunoprecipitation of sonicated T. brucei DNA 
resulted in up to 20-fold enrichment for J (data not shown).

J is present in silenced telomeric VSG genes

To test whether J is present in the silenced telomeric 
VSG genes, we used three related BF trypanosome 
clones, each expressing a different VSG gene. One PC 
clonc, not expressing any VSG gene and devoid of any 
modification was used as a negative control. Maps of the 
VSG genes, which are present in all four clones, are de- 
picted in Figure 4A. 121a BF cells express VSG gene 121 
(expression linked copy or ELC) in the dominant expres- 
sion site (Liu et al. 1985). All four clones have three 
additional silent chromosome-internal VSG 121 genes 
(basic copies or BCS). 221a BF cells, which express the 
single-copy VSG gene 221 in the 221 expression site,
arose from clone 121a by an in situ switch. r5-1.1 BF cells express the single-copy VSG gene 1.1 and arose from clone 221a by a complex event (see Materials and Methods). The inactive 221 gene at its new location is not modified on its PvuII site and is not sensitive to Bal31 exonuclease treatment, showing that the 221 gene had been transposed to a chromosome-internal position in clone r5-1.1 (data not shown).

Figure 4B shows the results of immunoprecipitations of the three VSG genes studied. Genomic DNA of all clones was digested with various restriction enzymes to obtain small VSG gene fragments. These restriction fragments were analyzed by anti-J IP combined with Southern blot hybridization. In all BF clones, the actively transcribed VSG gene was not bound by antibodies, whereas silenced telomeric VSG genes were invariably immunoprecipitated. These results show a clear correlation between base J and telomeric gene repression.

Whereas the inactive telomeric 221 gene in 121a cells (Fig. 4B), and in 221aR12, 118a, and 118a' cells (data not shown) was efficiently bound by antibodies, the silenced chromosome-internal 221 gene in r5-1.1 cells was not detectably bound by the antisera. This indicated that J is absent from chromosome-internal VSG genes. We confirmed this by analysis of the silent chromosome-internal 121 gene copies (BC), which can be separated from each other and from the ELC by a HindIII digest. None of the 121 BC genes was immunoprecipitated, whereas the ELC, here linked to the telomeric repeats, was pulled down (Fig. 4C; see below). The same lack of immunoprecipitation was found for the chromosome-internal basic copy of VSG gene 1.8 (data not shown). Together, the results from Figure 4, B and C, show that the inverse correlation between the presence of J and VSG gene activity holds true only for telomeric VSG genes, in agreement with the distribution of blocked restriction sites.

Telomere repeat arrays associated with inactive as well as active expression sites are modified

We have found previously that about half of J is present in telomeric repeats. Approximately 80% of the telomeres are part of minichromosomes (Van der Ploeg et al. 1984) and the analysis of telomeric repeats is therefore dominated by these minichromosomes, which do not contain functional VSG gene expression sites (Zomerdijk et al. 1990). To test whether the telomeric repeat arrays associated with expression sites were also modified, we analyzed restriction digests of genomic DNA in which the expression-linked VSG genes were still associated with the telomeric repeat arrays. This allowed the unique VSG sequences to be used as specific probes for individual telomere tracts. The length of these fragments varies between different clones because telomeres in trypanosomes grow and contract on cell division, resulting in clonal variation and heterogeneity of the size of the telomere repeat arrays (Bernards et al. 1983). The results in Figure 4D show that VSG genes linked to telomeric repeats were immunoprecipitated efficiently. Unexpectedly, however, this occurred irrespective of the transcriptional state of the upstream expression site. Because transcribed VSG genes alone were not modified (Fig. 4B), these results show that the hexameric repeat tracts flanking active expression sites are modified. The efficient antibody binding of the long telomeric tracts of active and inactive sites shows that both must contain a similarly high degree of modification. This is in agreement with the 2%–4% J found in purified telomeric repeat arrays (van Leeuwen et al. 1996). The lack of IP of the PC 1.1 telomere in a mix of PC DNA and BF 221a DNA excludes the possibility that unmodified telomeres nonspecifically coimmunoprecipitated with modified DNA (Fig. 4D). It has been suggested that tran-
scripion of the expression site reads through into the downstream telomeric repeats (Rudenko and Van der Ploeg 1989), and it is therefore possible that the first part of the repeat array is not modified. Transcribed repeats are thought to be sensitive to nucleases, resulting in formation of shorter telomeres during clonal propagation (Pays et al. 1983). The 12-kb smear associated with the 121 telomeric band in clone 121a (Fig. 4D, lane 1) could be an example of such an event and the absence of detectable immunoprecipitation of this smear might be caused by a higher proportion of transcribed repeats compared with the longer band.

Immunoprecipitation of VSG genes linked to telomeric repeats was always more efficient than that of (inactive) VSG genes alone. A silent VSG gene that is still linked to telomeric repeats because of partial cleavage caused by DNA modification will therefore be enriched by immunoprecipitation. This has allowed us to identify two additional restriction enzymes that yield partial cleavage products with silenced telomeric VSG genes—NcoI and DraI showed partial cleavage of VSG gene 121 and 1.1, respectively (data not shown; see Discussion).

J in and around expression site promoters

The experiments described above show that J is present in silenced telomeric VSG genes and in expression site-associated telomeric tracts, the most distal sequences in the expression site. The analysis of expression site sequences other than VSG genes is complicated by the high degree of homology between expression sites (Pays et al. 1989a; Kooter et al. 1987). To study the promoter region of expression sites, we used cell lines in which the
221 expression site was tagged with a unique sequence, the hygromycin resistance (HYG) gene (Rudenko et al. 1995; Blundell et al. 1996). In anti-J IP experiments, a small 0.6-kb segment of the HYG gene alone, either active (ES2) or silent (ES2-R1), did not bind to the antibodies (Fig. 5). A longer 1.1-kb fragment spanning the promoter element was also negative. The upstream part of the HYG gene linked to the long 50-bp repeat array, however, was immunoprecipitated, both from an inactive and an active expression site (Fig. 5). The absence of IP of the nonmodified HYG-marked 50-bp repeat fragment from PC cells (PCES2) mixed with BF 221a DNA (which does not contain a HYG gene) excludes nonspecific co-IP of these very long restriction fragments (~45 kb). Cell lines in which a HYG gene was integrated in a 221 expression site, in which the expression site promoter was replaced by a ribosomal RNA promoter, gave the same results (data not shown). J is therefore present in the long repetitive DNA stretches closely upstream of the promoter, regardless of expression site activity. The function of the 50-bp repeat arrays is not known, but hybridization studies have shown that 50-bp repeats are invariably associated with expression site promoter sequences (Zomerdijk et al. 1990, 1991; G. Rudenko and P. Borst, unpubl.).

The presence of J at the borders of expression sites prompted us to test whether sequences in between the VSG gene and the promoter are modified in inactive expression sites. The lack of probes specific for individual expression sites in this region only allowed global analysis of the total pool of expression sites. Therefore, genomic DNA was sonicated and analyzed by J-immunoprecipitation combined with dot-blot hybridization. Because all sequences are sonicated to the same size range (0.5–3 kb), the relative efficiency of immunoprecipitation could be used as a measure for the density of modification (see also Fig. 3). Telomeric repeats, 50-bp repeats, inactive VSG genes, and also 70-bp repeats, which are just upstream of VSG genes, were immunoprecipitated efficiently (Fig. 6). Expression site promoter and ESAG 1 fragments bound inefficiently, and other ESAGs bound even more inefficiently to the antibodies, albeit three to four times more than chromosome-internal DNA, such as tubulin genes or ribosomal 18S DNA (Fig. 6). These results show that expression site sequences are only sparsely modified outside the VSG gene and the repeats. Whether expression site promoter and ESAG 1 genes are really modified more densely than the other ESAGs, or whether the greater immunoselection is caused by linkage to modified 50- or 70-bp repeats is uncertain. We could not use sonicated DNA fragments shorter than 500 bp because this resulted in higher background IP. It should also be noted that 70-bp repeats and copies of some ESAGs (but not all) are also present outside of expression sites. Whether these outsiders are also modified and contribute to the immunoprecipitated fraction is not known.

We also analyzed the 70-bp repeats in a specific expression site using their linkage to the unique VSG pseudogene (Ψ), which is embedded in the 70-bp repeats in the 221 expression site (Bernards et al. 1985; Cornelissen et al. 1985). With restriction fragments of ~9.5 kb (HindIII) and 6.5 kb (BglII, Ncol) containing the 70-bp repeat array and (part of) the pseudo gene, we found efficient immunoprecipitation (~5%) with the inactive 221 expression site from clone 121a and no antibody binding of the transcribed fragment from clone 221a (data not shown). These results show that J is absent from VSG genes and 70-bp repeats in active expression sites and suggest that transcribed ESAGs also lack J.

**Figure 5.** Detection of J in the 50-bp repeats upstream of inactive and active expression sites. Cell lines with a HYG gene in an active (ES2) or inactive (ES2-R1) 221 expression site were used for anti-J immunoprecipitation of sequences in and around the expression site promoter (flag). (N) Ncol; (C) Cial; (P) Hpal; (X) XbaI. The 5' part of the HYG gene (line underneath the map) was used as a probe to specifically detect the tagged expression site sequences. The fragments analyzed are indicated on the left of each panel and include from top to bottom HYG gene alone, HYG gene linked to expression site promoter sequences, and HYG gene linked to the 50-bp repeat array. DNA of PC cells with a HYG gene downstream of the expression site promoter (PCES2) was mixed with wild-type BF 221a DNA (BF) as a negative control for nonspecific coimmunoprecipitation of nonmodified DNA. The solid box indicates the HYG-coding sequence, stippled boxes RNA-processing signals, and the striped box 50-bp repeats.

**Discussion**

Partial cleavage by PstI, PvuII, and other restriction enzymes suggested previously the presence of DNA modifications in silenced telomeric VSG genes in BF T. brucei (Bernards et al. 1984b; Pays et al. 1984). To test whether this is caused by J, we first verified that the presence of J in a PvuII restriction site blocks cleavage by PvuII enzyme (Fig. 1). Interestingly, a J replacing T only two positions away from the PvuII site did not block cleavage, showing that J does not affect cleavage at a distance. By anti-J DNA immunoprecipitations, we subsequently found that J correlates with silencing of telomeric VSG genes. An inverse correlation between DNA modification and transcription of specific genes, as has been
found for 5MeC in complex eukaryotes, has not been found before in simple eukaryotes (Rae and Steele 1978; Blackburn et al. 1983; Capowski et al. 1989; Bird 1995; Jablonka and Regev 1995; Tweedie et al. 1997).

By anti-J immunoprecipitations, J was also found in expression site sequences in which DNA modification previously remained undetected. The boundaries of expression sites, marked by long upstream 50-bp repeat arrays and long downstream telomeric repeat arrays, were modified substantially, regardless of expression site activity. By studying the total pool of expression sites with immunoprecipitations of sonicated DNA, we found low levels of J around the expression site promoter and in ESAGs. No J was detected in a promoter fragment derived from a silent expression site and tagged with a HYG gene, but specific modification of a single thymine would not have been detected in these experiments.

We conclude that J has all the properties of the modification in Pvull and PstI restriction sites detected by Bernards et al. (1984b) and Pays et al. (1984)—both are developmentally regulated, that is, are present in BF trypanosomes and are absent from PC trypanosomes, both are found in silent telomeric VSG genes and not in active VSG genes or chromosome-internal VSG genes. The gradient of modification from telomere to chromosome-internal found for PstI and Pvull sites in VSG genes (Bernards et al. 1984b) correlates with the gradient of J found from telomeric repeats (high, to VSG genes, 70-bp repeats, ESAG 1, and other ESAGs (low). With the expression site telomeres studied here, no correlation was found between the length of the telomeric repeat array and the levels of J in VSG genes. Possibly the size difference of the individual telomeres studied was not great enough to cause a difference in immunoprecipitation.

The abundance of J in different repetitive DNA sequences, such as the telomeric, 50- and 70-bp repeats, VSG genes, and promoter regions were also studied in individual expression sites using restriction digests. % IP shows a quantitation of the IP efficiency (immunoprecipitated fraction of the input) of expression site sequences using sonicated DNA (average with standard deviation of two independent clones 221a and 221aR12). The numbers correspond to the expression site sequences shown above. IP of silent VSG genes in expression sites varied from 1 (±0.1) to 16.2 (±3.4) depending on the VSG gene studied. The absence of J in actively transcribed VSG genes and 70-bp repeats strongly suggest that J is also absent from active ESAGs, but this has not been tested directly.

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Figure 6. The modified base J in and around telomeric VSG expression sites. Schematic representation of the distribution of J in active and inactive VSG expression sites determined by a J immunoprecipitation of sonicated DNA combined with dot-blot hybridizations (expression site adapted from Revelard et al. 1990). ES probes are described in Materials and Methods. ESAGs were studied as the cumulative signal of all copies in the genome using sonicated DNA. Telomeric repeats, 50-bp repeats, 70-bp repeats, VSG genes, and promoter regions were also studied in individual expression sites using restriction digests. % IP shows a quantitation of the IP efficiency (immunoprecipitated fraction of the input) of expression site sequences using sonicated DNA (average with standard deviation of two independent clones 221a and 221aR12). The numbers correspond to the expression site sequences shown above. IP of silent VSG genes in expression sites varied from 1 (±0.1) to 16.2 (±3.4) depending on the VSG gene studied. The absence of J in actively transcribed VSG genes and 70-bp repeats strongly suggest that J is also absent from active ESAGs, but this has not been tested directly.
Materials and methods

Trypanosome clones and DNA

BF trypanosome clones 221a or MiTat 1.2 (Bernards et al. 1984a), 121a or MiTat 1.6a, 118a or MiTat 1.5a (Cross 1975), 221ar12 (Zomerdijk et al. 1990), or T. brucei strain 427 (Cross and Manning 1973) were grown and isolated as described (Gomers-Ampt and P. Borst, unpubl.). Trypanosome clones and DNA were used to generate standard probes (Sambrook et al. 1989). Probes were labeled with [γ-32P]dATP by random priming. A 5′-32P-labeled oligomer consisting of 5 telomeric GGGTTA repeats was used to probe for telomeric repeats. Probe fragments were separated by 20% native polyacrylamide gel electrophoresis.

Endonuclease digestion of duplex oligonucleotides

Oligonucleotides encompassing the upper and the lower strand of the PvuII site (underlined) of the VSG 221 gene were used to generate non- or hemimodified duplex molecules. Oligos were end-labeled with [γ-32P]ATP, purified by exclusion chromatography, and annealed to their nonlabeled J-lacking complementary strand by gradually cooling down from 90°C to room temperature in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 100 mM NaCl, upper-T (CAGAAGAGCGCTGCCAAAG) or upper-J (CAGAAGGCAGJCJGCAACAAG) was annealed to lower-T (CTTGTGCACTGGCTTCTG), lower-J (CTTTGTGACGCJGCCTTCTT), or lower-J* (CTTTJJCAGCTGCTTCTT). The duplex oligos were incubated for 2 hr at 37°C in the appropriate restriction buffer with or without 10 units of PvuII. The products were separated by 20% native polyacrylamide gel electrophoresis (19:1, 1 x TBE).

Generation of J-specific polyclonal antisera

Chemically synthesized JMP (Wijisman et al. 1994) was coupled to carrier proteins with a water-soluble carbodiimide [1-ethyl-3-(dimethylamino)propyl]carbodiimide HCl, or EDC, Sigma according to a protocol modified from (Halloran and Parker 1966; Stollar 1980). Three micrograms of JMP and 400 mg of EDC were mixed with 4 mg of BSA (imject BSA, Pierce) or 4 mg of KLH (Calbiochem) in 1 ml of H2O, and incubated for 20 hr in the dark at room temperature or 37°C, respectively. This resulted in formation of phosphoramidate conjugates through the 5′-phosphate of JMP and the amino groups of the carrier proteins (Halloran and Parker 1966; Stollar 1980). The samples were dialyzed three times against 1000 volumes of PBS to remove the free JMP and EDC, monitored by UV absorbance (263/280 nm) to confirm crosslinking, and subsequently stored in 10% glycerol at −70°C. Twelve percent of the protein–nucleo-
tide complex was injected into rabbits. Antisera were obtained against BSA-JMP (539aJ) and KLH-JMP (538aJ).

Ant J-DNA immunoblot

DNA was denatured for 20 min on ice in 0.4 N NaOH, neutralized by adding one volume of ice-cold 2.5 M ammonium acetate, and blotted onto nitrocellulose using a manifold dot-blotted apparatus. The filters were baked for 2 hr at 80°C and blocked for 2 hr in TBST (10 mM Tris-HCl at pH 8.0, 150 mM NaCl, 0.02% Tween-20) with 5% milk powder. After three washes with TBST, the blots were incubated for 2 hr with anti-antiserum 539aJ, diluted 1:10,000-fold in TBST with 2% milk powder, and then washed three times with TBST. Immunodetection was performed using a horseradish peroxidase (HRP) conjugated secondary antibody (CLB, The Netherlands) in 2% milk powder in TBST, in combination with enhanced chemiluminescence (ECL, Amersham).

Generation and ligation of J-containing PCR fragments

One antisense primer with or without one J residue and three sense primers without J were used to generate J-containing and J-less 221 VSG gene PCR fragments of different sizes with Pwo polymerase. The antisense primers used were CTAGT-TCGACGCGTCTCTGTG and CTGGTGACGCTGCTTCTGTG (221as1247), the sense primers used were 221s1129 (CCACCTATAGCGCTAGCTG), and 221s304 (CCACCACTATGCGCATCA). PCR fragments were purified by QIAEX gel extraction (QiaGen) and the presence or absence of J was confirmed by 32P-nucleotide postlabeling combined with two-dimensional TLC. For detection of anti-J immunoprecipitation the fragments were radiolabeled and purified by exclusion chromatography. Part of the phosphorylated fragments were ligated for 16 hr at 16°C to generate ladders of fragments with a constant ratio of J/bp.

J-DNA immunoprecipitation

Digested or sonicated DNA (2–5 µg) was added to 5 µl antisera 538aJ in a final volume of 500 µl IP buffer (TBST with 2 mM EDTA (TBSTE), 0.1 mg/ml of TRNA, and 1 mg/ml of BSA) and incubated for 2 hr at room temperature. ProtA beads (20–30 µl, Repligen) were washed twice with TBSTE, preblocked for 30 min in 100 µl IP buffer, and incubated for 1 hr with the IP reaction. Ten to 20% of the supernatant was taken and used as a control for the DNA input. The bead-antibody-DNA complexes were washed four times with TBSTE and finally proteinase K-treated at 58°C to release the bound DNA, which was phenol-extracted and ethanol-precipitated with 20 µg glycogen.

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DNA modification in trypanosomes


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DNA modification in trypanosomes


