

Genetic manipulation indicates that *ARD1* is an essential N^{α} -acetyltransferase in *Trypanosoma brucei*[☆]

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Abstract

N^{α} -acetylation, the most common protein modification, involves the transfer of an acetyl group from acetyl-coenzyme A to the N -terminus of a protein or peptide. The major N^{α} -acetyltransferase in *Saccharomyces cerevisiae* is the *ARD1*-*NAT1* complex. To investigate N^{α} -acetylation in *Trypanosoma brucei* we have cloned and characterised genes encoding putative homologues of *ARD1* and *NAT1*. Both genes are single copy and *ARD1*, the putative catalytic component, is expressed in both bloodstream-form and insect-stage cells. In either of these life-cycle stages, disruption of both *ARD1* alleles was only possible when another copy was generated via gene duplication or when *ARD1* was expressed from elsewhere in the genome. These genetic manipulations demonstrate that, unlike the situation in *S. cerevisiae*, *ARD1* is an essential gene in *T. brucei*. We propose that protein modification by *ARD1* is essential for viability in mammalian and insect-stage *T. brucei* cells. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

N^{α} -acetylation, mediated by N^{α} -acetyltransferases, is the most common protein modification. N^{α} -acetylation occurs during protein synthesis and involves the transfer of an acetyl group from acetyl-coenzyme A to the protein α -NH₂ group

(reviewed in [1]). Acetylation neutralises a positive charge and, like protein phosphorylation, may influence protein function by altering stability or interactions with other molecules [2].

ARD1 and *NAT1* are sub-units of an N^{α} -terminal protein acetyltransferase in the yeast *Saccharomyces cerevisiae*. The *ARD1*-*NAT1* complex, both sub-units of which are required for enzyme activity, is the major N^{α} -acetyltransferase in these cells [3]. *ARD1*-*NAT1* modifies about 20% of all yeast proteins [4,5] including 24 ribosomal proteins [6] and six 20-S proteasome sub-units [7]. Up to 80% of proteins are thought to be N^{α} -acetylated in other eukaryotes. N^{α} -acetyltrans-

[☆] Note: Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the accession numbers AJ277476 (*ARD1*) and AJ277477 (*NAT1*).

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ferase activity is lost in *ard1* or *nat1* mutants [5] but, despite the fact that the ARD1-NAT1 complex modifies many proteins, such mutants are viable, displaying only a few specific phenotypes. For example, *ard1* mutants are unable to enter stationary phase or sporulate. These phenotypes suggested a defect in arrest of the mitotic cell cycle and lead to the designation *ard1* for arrest defective [8]. Yeast *ard1* mutations also cause a mating defect due to de-repression of the mating-type locus, HML [9]. In addition, *ARD1* was among a number of genes shown to be necessary for gene silencing at telomeres [10]. *nat1* (*N*^α-acetyltransferase, also known as *aaa1*) mutants exhibit identical phenotypes to *ard1* mutants [5,10,11]. Since the yeast ARD1-NAT1 complex modifies a large number of proteins it is not clear how these phenotypes occur, but the mechanism certainly appears to be indirect [12]. Another yeast acetyltransferase mutant (*sas2*) displays similar phenotypes [13].

ARD1 is a member of a large and diverse super-family of acetyltransferases that includes histone acetyltransferases [14] suggesting that it is the catalytic component of the ARD1-NAT1 complex. Crystal structure and mutagenesis studies with other members of this family reveal the acetyl CoA binding site and suggest a mechanism for catalysis (reviewed in [15]). In addition to ARD1-NAT1, there are at least two other *N*^α-acetyltransferases in *S. cerevisiae* known as NAT3 and MAK3 [16]. Whether NAT2 [17] is an *N*^α-acetyltransferase is not so clear [16]. The ARD1-NAT1 or NatA substrates are acetylated on Ser, Ala, Gly and Thr termini [6,16] following removal of the initiator methionine.

Trypanosoma brucei are evolutionary divergent [18] protozoan parasites of mammals, causing sleeping sickness in humans and Nagana in cattle. Antigenic variation, which allows *T. brucei* to persist in a mammalian host, relies upon gene silencing at telomeres (reviewed in [19]). Since the ARD1-NAT1 *N*^α-acetyltransferase is required for telomeric gene silencing in yeast [10] we chose to study *T. brucei* genes related to *ARD1* and *NAT1*. Also, although protein phosphorylation has been studied in some detail in *T. brucei*, very little is known about protein acetylation in this organism.

We have cloned and characterised *T. brucei* genes encoding homologues of both components of the ARD1-NAT1 *N*^α-acetyltransferase. We show that, unlike the situation in yeast, *ARD1* is essential for growth in both bloodstream-form and insect-stage *T. brucei* cells.

2. Materials and methods

2.1. Cells

T. brucei strain MiTat 427 bloodstream-form cells were maintained and cloned as previously described [20]. Bloodstream-form trypanosomes were differentiated to the insect-stage by transferring 1×10^7 cells into DTM with 3 mM citrate/*cis*-aconitate [21] at 27°C. Electroporation was as previously described [20] except that cells were pulsed once at 1.4 kV and 25 μ F in 2 mm gap cuvettes using a Gene pulser II (Bio-Rad). Plasmid DNA, prepared using anion-exchange columns (Qiagen), was digested with *Pst*I-*Apa*I (pARD-BLE), *Sma*I-*Apa*I (pARD-NEO) and *Sal*I (pTUB-HYG-ARD) prior to electroporation. Medium containing 2 μ g ml⁻¹ phleomycin (Cayla), 2 μ g ml⁻¹ G418 (MBI Fermentas) and 2.5 μ g ml⁻¹ hygromycin B (Sigma) were added to bloodstream-form cultures 6 h after electroporation to select for BLE, NEO or HYG expression, respectively. Following drug addition bloodstream-form cultures were distributed in 12 or 24-well plates. For insect-stage cultures, 2.5 μ g ml⁻¹ phleomycin, and/or 15 μ g ml⁻¹ G418 were added 24 h after electroporation to select for BLE and/or NEO expression, respectively, and cultures were maintained in flasks.

2.2. *ARD1* and *NAT1* cloning

Sequences of portions of the putative *ARD1* and *NAT1* genes from *T. brucei* were from the EST database [22,23]. We designed primers based on these sequences and, using the polymerase chain reaction (PCR) we amplified fragments from genomic DNA using the primer pairs, ARD-a (5'-gaattctttgacgcaatgcaggtg-3') and ARD-b (5'-gaattcgaccgtacagcagctgtgc-3') and NAT-a (5'-

gggagatttctcaagcttcg-3') and NAT-b (5'-ccctcatcgtgcacctac-3'), respectively. Briefly, temperature was cycled 25 times through 94, 58 and 72°C for 30 s each, in the presence of 100 ng *T. brucei* genomic DNA and Taq DNA polymerase (MBI-Fermentas) according to the manufacturer instructions. An ~150-bp ARD1 and an ~550-bp NAT1 product were cloned into a pGEM-T vector (Promega). A clone that hybridised with the ARD1 PCR product was isolated from a *T. brucei* genomic library, consisting of *ApaI-PstI* fragments in pBluescript (Stratagene). Clones that hybridised with the NAT1 probe were isolated from a *T. brucei* genomic library, consisting of *KpnI-BglII* fragments in pBluescript. The ARD1 clone was designated pARD and clones containing the 5' and 3'-ends of NAT1 were designated pNAT5 and pNAT3, respectively. DNA sequencing was performed using a Thermo Sequenase dye-terminator kit (Applied Biosystems) and an ABI Prism 377 automated sequencer according to the manufacturer instructions. All oligonucleotides were synthesised by Gibco-BRL.

2.3. Plasmid constructions

ARD1 targeting constructs were generated as follows. Briefly, a *SacI* site was destroyed in pARD to generate pARD-SacΔ. A *SacI* fragment was then removed from pARD-SacΔ and replaced with *BLE*, a *BamHI* fragment from pbRn5 [20], or *NEO*, a *SmaI-BamHI* fragment from pHD30neo [24], to generate pARD-BLE and pARD-NEO, respectively. Since the putative ARD1 splice acceptor site, as determined by comparing our sequence to the EST sequence, was disrupted, *BLE* and *NEO* were inserted along with actin splice acceptor signals [24].

To generate pTUB-HYG-ARD, ARD1 was linked to a hygromycin resistance gene (*HYG*) and both genes were inserted into a tubulin-targeting construct. Briefly, a *HYG* gene flanked by aldolase splice acceptor and polyadenylation signals was excised from pBEAHA5' [25] as a *SpeI-SalI* fragment and cloned into pBluescript to generate pAHA. The ARD1 coding region and processing signals were removed from pARD-

SacΔ as a *SnaBI-KpnI* fragment and cloned into a *SalI* site downstream from the HYG cassette in pBS-AHA generating pHYG-ARD. The tubulin target was a *SalI* fragment cloned into pBlue-script generating pTUB. Following the destruction of a *SalI* site in pHYG-ARD, a *XhoI-SpeI* fragment containing both *HYG* and *ARD1* was inserted into a *MluI* site in pTUB generating pTUB-HYG-ARD.

2.4. DNA and RNA analysis

Southern and northern blotting were carried out according to standard protocols. DNA and RNA were isolated using DNA-Stat-60 and RNazolB, respectively (Biogenesis). All post hybridisation washes were at 65°C in 0.2 × SSC and 0.2% SDS. The ARD1 probe was a *SacI* fragment, the 3' probe was a *KpnI* fragment and the BLE and NEO probes were the entire coding regions. For PCR assays, temperature was cycled 22 times through 94, 58 and 72°C for 30, 30 and 45 s, respectively, in the presence of 40 ng of genomic DNA, Taq DNA polymerase, a forward primer, ArdF (5'-cagcccggtccgagtg-3') and three reverse primers, ArdR (5'-gggcaggcagcgtaggtt-3'), BleR (5'-caccacctggtctctggac-3'), and NeoR (5'-tcagagcagccgattgtct-3').

3. Results and discussion

3.1. *N^z-acetyltransferase* homologues in *T. brucei*

Expressed sequence tags of putative *T. brucei* ARD1 and NAT1 homologues were identified by El-Sayed and colleagues [22,23]. Based on these sequences, we designed oligonucleotides to PCR amplify portions of the putative ARD1 and NAT1 genes from the MiTat 427 strain of *T. brucei* (see Materials and methods). The PCR products were used as probes to isolate full-length ARD1 and NAT1 genes from *T. brucei* genomic libraries (see Materials and methods). Sequencing revealed that *TbARD1* encodes a 239 amino acid polypeptide with a predicted molecular mass of ~27.5 kDa while *TbNAT1* encodes a 711 amino acid polypeptide with a predicted molecular mass of

~82.2 kDa. The predicted protein sequences are aligned for comparison with ARD1 and NAT1 from *S. cerevisiae* and the putative homologues from *Drosophila* while comparisons with putative homologues from other species are tabulated (Fig. 1).

Despite the evolutionary divergence of these organisms [18], ARD1-related proteins from hu-

man, *Drosophila*, nematode, yeast, slime-mould and trypanosome are highly conserved (Fig. 1A) suggesting that all these sequences represent N^{α} -terminal protein acetyltransferase. Of particular significance, motif A (Q/RxxGxG/A where x represents a variable amino acid), which forms part of the acetyl CoA binding loop [15], is found in all of these putative ARD1 homologues (Fig. 1A, always RxxGxA in ARD1). Comparison of the predicted amino acid sequences of NAT1-related proteins from *T. brucei*, yeast and *Drosophila* reveals significant similarity between and throughout the three proteins (Fig. 1B) suggesting that *Tb*NAT1 is an N^{α} -terminal acetyltransferase subunit.

3.2. N^{α} -acetyltransferase gene organisation and expression

To determine the organisation of the *ARD1* and *NAT1* genes we carried out Southern analysis. Representative digests are shown for *ARD1* (Fig. 2A) and *NAT1* (Fig. 2B). This analysis suggested that both genes are present as a single copy per haploid genome. Although both *ARD1* and *NAT1* are required for enzyme activity in yeast, *ARD1* appears to be the catalytic component of the complex (see above) so subsequent experiments focussed on the *ARD1* gene. Pulsed-field gel electrophoresis indicated that *ARD1* is

Fig. 1. ARD1 and NAT1 sequence alignments. Predicted protein sequences from *T. brucei*, *S. cerevisiae* and *Drosophila melanogaster* were aligned using ClustalW followed by manual adjustment. Residues sharing identity with the *T. brucei* sequences are white on a black background. Dashes indicate gaps introduced to optimise the alignment. (A) ARD1 alignment. Stars indicate highly conserved residues in the AcCoA binding site (see text). Thirty-eight amino acids were removed from the *Sc* sequence, Δ. Sequence identity (%) between species over the *N*-terminal two thirds of ARD1 is indicated in the box. *Tb*, *T. brucei*, this report, AJ277476; *Sc*, *S. cerevisiae* [8], M11621; *Dm*, *D. melanogaster*, AAF50178; *Hs*, *H. sapien* [27], X77588; *Ce*, *C. elegans*, AAC04428; *Dd*, *D. discoïdium*, P36416. (B) NAT1 alignment. A number preceded by the Δ symbol indicates where amino acids were removed from the *Sc* or *Dm* sequences. Sequence identity (%) between species for the entire length of NAT1 is indicated in the box. Species abbreviations are as in A. *Tb*, this report, AJ277477; *Sc* [5,11], P12945; *Dm*, AE003512; *Sp*, *S. pombe*, CAA19338.

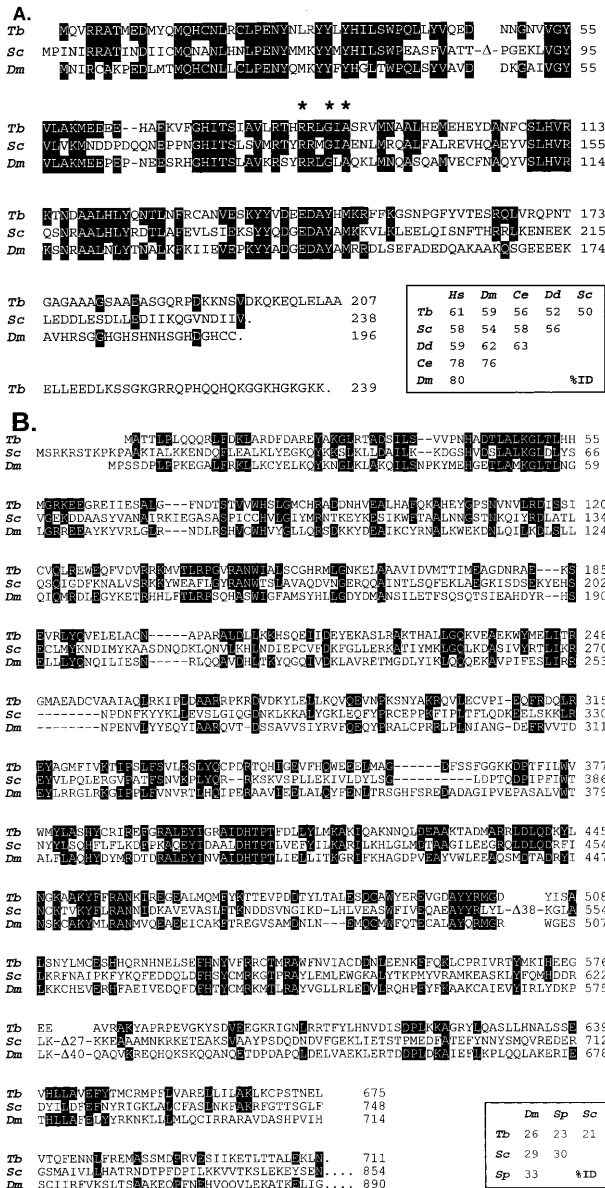


Fig. 1.

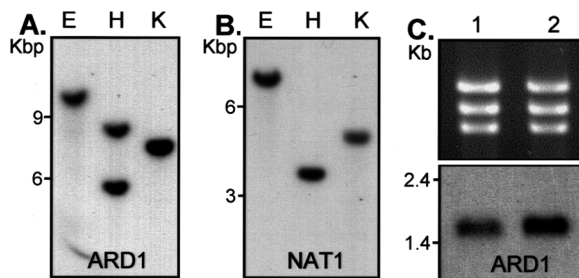


Fig. 2. *N*-acetyltransferase gene organisation and expression. Southern analysis for *ARD1* (A) and *NAT1* (B). Genomic DNA from wild type cells was digested with a variety of restriction enzymes: E, *Eco*RI; H, *Hind*III; K, *Kpn*I and Southern blots were hybridised with the probes indicated. (C) Northern analysis. Equal quantities of RNA from bloodstream-form (lane 1) and insect-stage (lane 2) cells were separated in a 1% denaturing agarose gel in the presence of ethidium bromide (upper panel) and the blot was hybridised with an *ARD1* probe (lower panel).

located on one of the largest (~6 Mbp) chromosomes in the MITat 427 strain (data not shown). To investigate *ARD1* expression we extracted RNA from bloodstream-form and insect-stage cells. A northern blot was hybridised with an *ARD1* probe (Fig. 2C) revealing *ARD1* mRNA in both cell types. *ARD1* mRNA appears to be slightly more abundant in insect-stage cells.

3.3. Attempts to disrupt *ARD1* in bloodstream-form *T. brucei*

S. cerevisiae ard1 and *nat1* null mutants are viable and display similar phenotypes [5,9–11]. We wanted to address the role of *ARD1* in *T. brucei* and therefore attempted targeted disruption of the *ARD1* gene. *T. brucei* is diploid and specific loci can be targeted by homologous recombination [26] so two constructs, designed to replace both *ARD1* alleles, were created using the resistance markers *BLE* and *NEO*, coding for the *Sh*BLE protein and neomycin phosphotransferase, respectively. Our approach should result in the removal of all but the last 71 nucleotides of the *ARD1* coding region. This remaining segment will lie immediately downstream of the marker gene stop codon (see Fig. 3A). The constructs do not contain promoters but rely upon endogenous

polycistronic transcription for selectable marker expression.

We transformed bloodstream-form *T. brucei* cells with each construct. Of approximately 60 drug-resistant clones obtained, four were selected for further manipulation (two BLE-resistant clones and two NEO-resistant clones). We attempted to generate NEO and BLE double drug-resistant cells from these four clones. Double drug-selection was applied to eliminate cells in which the first selectable marker had been replaced by the second. Only two clones survived NEO and BLE selection. These results suggested that, relative to replacement of the first allele, cells survived replacement of a second *ARD1* allele at a 30-fold lower frequency.

Genomic DNA from single and double drug-resistant clones was analysed by Southern blotting and in a quantitative, competitive PCR assay (see Materials and methods). Some of the Southern blots are shown in Fig. 3B. Wild-type cells (Fig. 3B, lane 1) contain both *ARD1* alleles, while cells

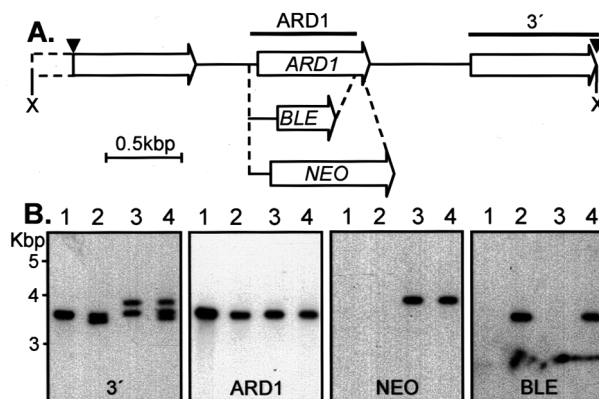


Fig. 3. Targeting *ARD1* in bloodstream-form *T. brucei*. (A) Schematic representation of the *ARD1* locus and the gene disruption strategy. Arrows flanking *ARD1* indicate other open reading frames. The map is extended to the left beyond the sequenced region to indicate a relevant *Xho*I site, X. Probes used for Southern analysis are indicated as bars above the map and arrowheads above the map indicate the extremities of the targeting fragments present in the constructs. (B) Southern analysis. DNA from wild type (lane 1), BLE-resistant (lane 2), NEO-resistant (lane 3) and BLE-NEO-resistant cells (lane 4) was digested with *Xho*I. The blot was sequentially hybridised with the probes indicated.

expressing either *NEO* or *BLE* have a single *ARD1* allele (Fig. 3B, lane 2 and 3 and data not shown) and *NEO* or *BLE* genes integrated as expected (Fig. 3B, lanes 2 and 3). The double drug-resistant cells, however, have all three genes intact (Fig. 3B, lane 4). One possible explanation to account for the persistence of *ARD1* in double-drug-resistant cells is that constructs designed to target the second *ARD1* allele had in fact integrated elsewhere in the genome leaving the native *ARD1* allele intact. This does not appear to be the case however since the fragments detected by the 3' probe display the expected change in size brought about by integration of the constructs at the *ARD1* locus (Fig. 3B, lane 4). Our results indicate that the *ARD1* locus has been duplicated in these cells, presumably a relatively rare event (~1 in every 30 integration events), which explains why these cells were obtained at a reduced frequency (see above). An additional copy of the locus may have been generated during parental clone expansion or may have been induced by the second integration event. Our data are more consistent with a tandem duplication induced during integrative recombination (data not shown). The results of this study are consistent with disruption of the *ARD1* gene being a lethal event in these cells.

3.4. *ARD1* is essential for growth in bloodstream-form cells

The possibility that our genetic manipulations disrupted some other *ARD1*-proximal, essential gene had to be ruled out to demonstrate that this phenotype is a direct consequence of *ARD1* disruption. To determine whether *ARD1* is essential we chose to attempt disruption of both wild-type alleles while expressing *ARD1* from an independent locus. *ARD1* was linked to a gene (*HYG*) conferring hygromycin B resistance and the entire cassette was targeted to the tubulin (*TUB*) array (see Fig. 4A). This construct was introduced into cells maintaining a single copy of *ARD1*. Of over 100 *HYG*-resistant clones obtained we chose two for further analysis. These two clones were subsequently transformed with a second *ARD1*-

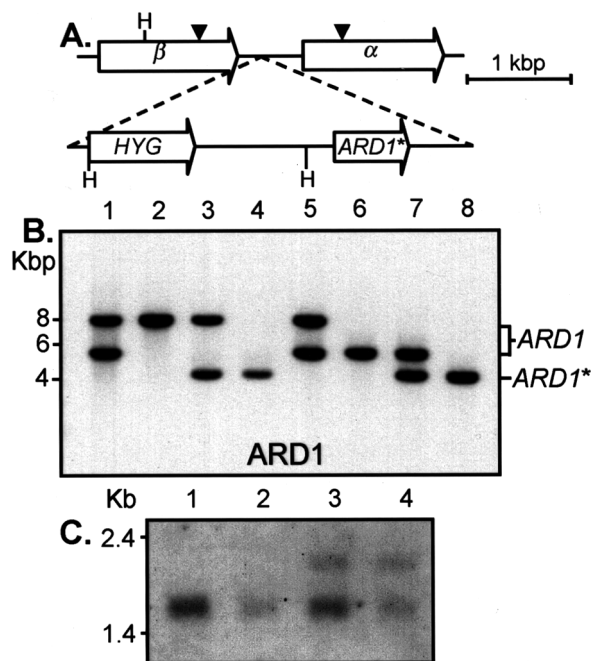


Fig. 4. Targeting *ARD1* in the presence of an ectopic copy of the gene. (A) Schematic representation of a *T. brucei* tubulin-repeat and the construct containing *ARD1* indicating the expected integration site. Arrowheads above the map indicate the extremities of the targeting fragments present in the construct. H, *HindIII*. (B) Southern analysis. DNA from bloodstream-form cells of wild type (lanes 1 and 5), BLE-resistant (lane 2), BLE/*HYG*-resistant (lane 3), BLE/*HYG*/*NEO*-resistant (lanes 4 and 8), *NEO*-resistant (lane 6) and *NEO*/*HYG*-resistant (lane 7) was digested with *HindIII*. The blot was hybridised with an *ARD1* probe. The native *ARD1* alleles (*ARD1*) and the ectopic copy (*ARD1**) are indicated. Native *ARD1* targeting constructs are illustrated in Fig. 3A. (C) Northern analysis. Equal quantities of RNA from bloodstream-form cells were separated in a 1% denaturing agarose gel and the blot was hybridised with the *ARD1* probe. Lanes 1–4 are as in B.

targeting construct. In these experiments *HYG*-selection would not be expected to prevent replacement of the *ARD1* ectopic copy (Fig. 4A) so only BLE and *NEO* drug-selection were applied at this point. We obtained more than 50 clones from these experiments. In twice the number of experiments using cells lacking an ectopic copy of *ARD1* we had previously obtained only two clones.

A quantitative, competitive PCR assay (see Materials and methods) was used to assess the location of *ARD1* genes and selectable-markers in ten of these drug-resistant clones (data not shown). Although the *ARD1* targeting construct could have replaced the ectopic copy, the native allele presented a longer target sequence at one end. Indeed, all ten clones appeared to have lost the second native *ARD1* allele, indicating a dramatic increase (~ 50 -fold) in the efficiency of native *ARD1* disruption in the presence of an ectopic copy of the gene. Southern blot analysis of two of these clones is shown in Fig. 4B. A polymorphic *Hind*III site exists at the *ARD1* locus so Southern analysis of *Hind*III digested genomic DNA with an *ARD1* probe produces two fragments corresponding to the *ARD1* alleles (Fig. 4, lanes 1 and 5). In the single drug-resistant clones one allele of *ARD1* has been disrupted (Fig. 4B, lanes 2 and 6). Clones with an ectopic copy of *ARD1* produce an additional band showing the construct had integrated as expected (Fig. 4B, lanes 3 and 7). Finally, transformation with the second *ARD1* targeting construct results in the disruption of the remaining native *ARD1* allele while the ectopic copy remains intact (Fig. 4B, lanes 4 and 8). In combination with the results shown in Fig. 3, these results strongly suggest that *ARD1* is indeed an essential gene in bloodstream-form *T. brucei*.

To determine whether the ectopic copy of *ARD1* was expressed, total cellular RNA was isolated from bloodstream-form cells and analysed by northern blotting (Fig. 4C). Relative to wild-type cells (lane 1), *ARD1* mRNA levels appear to drop in cells which contain only one native copy of the gene (lane 2) while *ARD1* mRNA expression is restored following integration of the ectopic copy (lane 3). The ectopic copy of *ARD1* produces a larger mRNA transcript, possibly due to the use of alternative mRNA processing signals at the tubulin locus (lanes 3 and 4). Finally, the smaller of the two transcripts, derived from the native *ARD1* allele, is specifically depleted, when the last native *ARD1* allele is disrupted (lane 4). The signal in lane 4 represents mRNA derived from the *ARD1* ectopic copy.

3.5. *ARD1* is essential for growth in insect-stage *T. brucei*

ARD1 is expressed in bloodstream-form and insect-stage *T. brucei* cells. We therefore sought to determine if the gene is also essential in the insect-stage. Differentiation of bloodstream-forms to insect-stage cells can be efficiently achieved in vitro if bloodstream-form trypanosomes are subjected to a temperature drop to 27°C in a medium containing glycerol, citrate and *cis*-aconitate [21]. For this experiment wild-type *T. brucei* bloodstream-form cells and clones with a single *ARD1* allele or with one allele plus an ectopic copy of *ARD1* were differentiated. *ARD1* was targeted for disruption in all three cultures. Once again, drug-resistant cells were obtained at a reduced frequency in cells with only one copy of *ARD1* remaining. This result suggests that, similar to the situation in bloodstream-form cells, *ARD1* is essential for viability in insect-stage cells. To survive drug selection, we predict that the *ARD1*

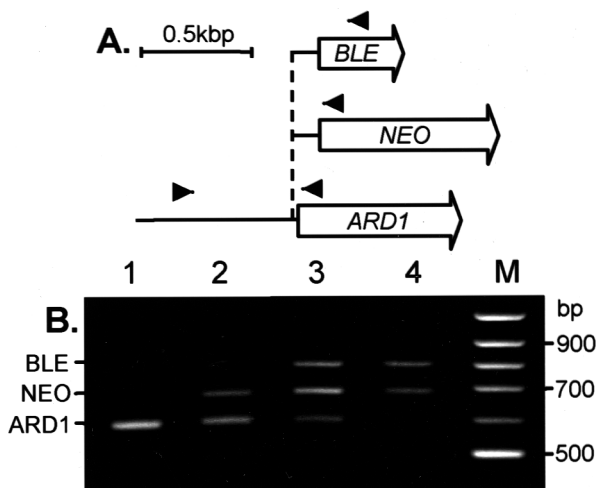


Fig. 5. Targeting *ARD1* in insect-stage *T. brucei*. (A) Schematic representation of a PCR assay used to detect native and modified *ARD1* alleles. Arrowheads indicate the position of primers used in the assay. (B) Genomic DNA from wild type (lane 1), NEO-resistant (lane 2), NEO/BLE-resistant (lane 3) and NEO/BLE/HYG-resistant (lane 4) insect-stage cells was used as PCR template. PCR products were separated in a 1.2% agarose gel. Native *ARD1* targeting constructs and the *ARD1* ectopic copy construct are illustrated in Fig. 3A and Fig. 4A.

locus was rearranged in these cells, such that the selectable marker is integrated but the *ARD1* gene remained intact. To test this we used the quantitative, competitive PCR assay (Fig. 5) in which primers specific for native *ARD1* alleles, *BLE* or *NEO* compete for an *ARD1*-flanking primer (see Fig. 5A). As predicted, this assay shows that cells lacking an ectopic copy of *ARD1* always maintain a native copy (lanes 2 and 3) even when that copy is targeted for replacement (lane 3). It was only possible to disrupt both native copies of *ARD1* in cells with an ectopic copy of the gene (lane 4). These results indicate that *ARD1* is essential in insect-stage *T. brucei*.

3.6. The role of *ARD1* in *T. brucei*

N^{α} -acetylation is the most common protein modification and the *ARD1*–*NAT1* complex is the major N^{α} -acetyltransferase in *S. cerevisiae*. Here we have identified and characterised putative *ARD1* and *NAT1* homologues from *T. brucei*, an evolutionary divergent [18] protozoan parasite. *ARD1*, the putative catalytic component of the complex, is conserved between trypanosomes and humans and database searches reveal *ARD1* and *NAT1* homologues in a variety of other organisms. Similar to the situation in yeast [5], *ARD1* is likely to be responsible for N^{α} -acetylation of a large number of proteins in *T. brucei*, many of which are common to bloodstream and insect-stage cells. Consistent with this, *ARD1* is expressed in both cell types. In contrast to the situation in yeast, however, where *ard1* mutants are viable [8], *ARD1* appears to be essential in bloodstream-form and insect-stage *T. brucei*. In fact, *S. cerevisiae* strains lacking any of the three confirmed N^{α} -acetyltransferases (*ARD1*–*NAT1*, *NAT3* and *MAK3*) are viable [16]. Although telomeric gene silencing in yeast, which requires the *ARD1*–*NAT1* complex, is not thought to affect native genes, many telomere-proximal genes involved in antigenic variation in *T. brucei* are transcriptionally repressed. All but one telomere appears to be active in bloodstream-form cells while none appear to be active in insect-stage cells. If telomeric gene silencing mechanisms are conserved between yeast and *T. brucei*, then dis-

ruption of *ARD1* in *T. brucei* may activate telomeric genes, with lethal consequences. Whatever the mechanism, our results strongly suggest that *ARD1* is an essential gene in *T. brucei*, so *ARD1* may provide a target for novel chemotherapy agents against these parasites. Further studies on *ARD1* and its substrates should reveal the role of N^{α} -acetylation in evolutionary divergent organisms. Since *ARD1*–*NAT1* is known to modify a large number of proteins in yeast, however, it may be difficult to determine which *ARD1* substrate(s) influence specific cellular functions. From our results, we propose that N^{α} -terminal protein acetylation by *ARD1* is essential for viability in mammalian and insect-stage *T. brucei*.

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