Am. J. Trop. Med. Hyg., 29(5) Suppl., 1980, pp. 1033–1036

THE GENES FOR VARIANT ANTIGENS IN TRYPANOSOMES

P. BORST, A. C. C. FRASCH, A. BERNARDS, J. H. J. HOEIJMAKERS,*
L. H. T. VAN DER PLOEG, AND G. A. M. CROSS

Section for Medical Enzymology and Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, P.O. Box 60.000, 1005 GA Amsterdam, The Netherlands, and The Wellcome Research Laboratories, Department of Immunology, Langley Court, Beckenham, Kent BB3 3BS, England

Abstract. We have studied the mechanism of antigenic variation by using DNA complementary to the messenger RNAs for four variant surface glycoproteins of *Trypansoma brucei*. Pure complementary DNAs were obtained by cloning as recombinant DNA in *Escherichia coli*. Using these complementary DNAs as hybridization probes, we have analyzed the genes for these variant surface glycoproteins. The results provide new information on the origin and evolution of antigenic variation, and on the mechanism involved in switching from one antigenic type to another.

A single African trypanosome can make a large series of cell surface glycoproteins, and the sequential synthesis of different variants is used to evade the immunological defense of the host. Variant surface glycoproteins (VSGs), purified from cloned variants of *Trypanosoma brucei*, have been shown to have molecular weights in the 50–60 kd range, but to differ in amino acid composition and sequence.[1] Major questions that remain are: How does one trypanosome clone make so many different VSGs, and how does the trypanosome switch from one VSG to the next?

To answer these questions, we have cloned DNA complementary to the messenger RNAs (mRNAs) for four VSGs of *T. brucei* 427, variants 117, 118, 121, and 221.[2] The initial work with these clones led to the following main conclusions:[2,3]

1. When hybridized to filters containing blots of electrophoretically-separated poly(A)$^+$ RNAs from the four *T. brucei* variants, each complementary DNA (cDNA) clone sees one prominent band only in homologous RNA. This eliminates the possibility that the expression of VSG genes is controlled at the translation level and suggests that control is transcriptional.

2. cDNA of each variant recognizes a distinct set of fragments in restriction enzyme digests of *T. brucei* nuclear DNA. Since our cDNAs cover about one-half to two-thirds of the length of the VSG mRNAs, this shows that functional VSG genes are not made like the genes for immunoglobulin chains in antibody-forming cells, i.e., by welding one of a large repertoire of genes for the variable N-terminal half onto one or two genes for a constant C-terminal half. We cannot yet rule out that the mRNAs have a smaller segment, e.g., at the 5′-terminus, in common, since our probes do not cover the whole mRNA.

3. In nuclear DNA digests of different *T. brucei* variants the cDNA probes see two kinds of VSG genes: a basic gene copy present in all variants, and an expression-linked VSG gene copy, only present in the homologous variant. We conclude from these and other results that expression of a VSG gene is accompanied by a duplication of that gene, followed by an alteration of the DNA surrounding this duplicate gene to yield the expression-linked copy.

The expression-linked copy of the VSG 118 gene

Figure 1 compares the physical maps of the basic copy and the expression-linked copy of the VSG 118 gene. The map was constructed by using oriented probes, obtained by cutting a cDNA plasmid with EcoRI and PstI and separating the 5′ and 3′ cDNA fragments. The main point of interest is that the sequences on both sides of the gene are altered in the expression-linked copy relative to the basic copy. We have no evidence for cleavage sites that could be within the gene and that differ in the basic and the expression-linked copy. Both copies could, therefore, be complete. Comparison of the cleavage sites surrounding the expression-linked copies of the 118 and the 121 gene indicates that these are not identical. Of the various models that could be considered for the origin of the alterations—nucleotide modification, inversion of sequence, transposon inserted next to

*Present address: Laboratory of Medical Microbiology, University of Amsterdam, Mauritskade 57, 1092 AD Amsterdam, The Netherlands.

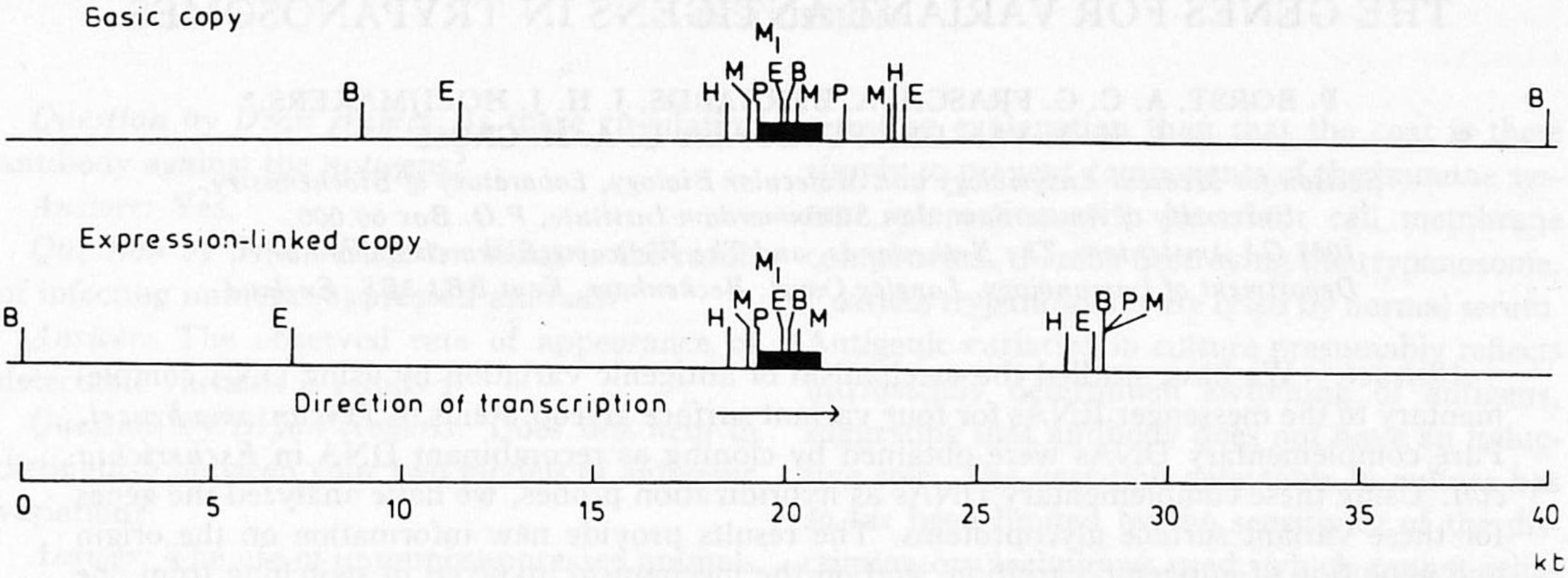

FIGURE 1. The physical maps of VSG 118 genes. Cleavage sites in and around the two copies were mapped by hybridizing nuclear DNAs from variant 118 and 221 with oriented 118 cDNA probes. The probes were obtained by digesting the TcV118-2 plasmid with EcoRI and PstI and separating the two fragments by agarose gel electrophoresis. The fragments were labelled by nick-translation (see ref. 3). The black box is the sequence cloned as cDNA. There are several MspI sites within the cDNA segment, but only two are indicated. B, BamHI; E, EcoRI; H, HindIII; M, MspI; P, PstI.

gene, gene transposition—these (and previous[3]) results agree best with a gene transposition model.

Are there intervening sequences in VSG genes?

In the 118 map the relative distance of cleavage sites within the gene is the same as in the cDNA. Moreover, we have not found additional sites in the gene not present in the cDNA. With the 221 gene we have also found that the 825 bp Hind III fragment present in the cDNA, is present in nuclear DNA. These results show that VSG genes are not riddled with introns, at least not in the segment of the gene that we can analyze with our cDNAs.

VSG genes are arranged in families

When cDNA probes are hybridized to restriction enzyme digests of *T. brucei* nuclear DNA, three types of results are obtained (disregarding the expression-linked copies):

1. cDNAs of VSG 118 and 121 mainly hybridize with one or a few fragments. In the case of VSG 118 these are derived from a single basic copy gene; with 121 this is likely, but not yet shown by mapping. In addition, these probes weakly hybridize to a limited number of other bands. This weak hybridization can be removed by washing with 0.1 × SSC at 65°C, and we attribute it to cross-hybridization to other VSG genes which have partial sequence homology with the probe used.

2. cDNAs of VSG 117 hybridize with 20–30 fragments;[4] under stringent conditions major hybridization is reduced to one or a few fragments, but weak hybridization to some other fragments remains. We infer that the 117 gene family is a larger and more closely knit family than the 118 and 121 families.

3. The 221 cDNA fragments from the middle of the cDNA give results as sub 1. Fragments corresponding to the 3′-end of 221 mRNA, however, hybridize to very many nuclear DNA fragments. Hybridization is washed off at 0.1 × SSC. Since the 3′-terminal cDNA fragments do not hybridize with the mRNAs from 117, 118, and 121, we conclude that the 221 gene contains a sequence which is repeated at least 100 times in other locations in nuclear DNA but which is not an essential element of VSG mRNAs in general. The nature and length of this sequence and the repetition precision are not yet known.

Evolution of VSG genes is marked by gene deletions

Our work thus far has suggested that antigenic diversity in trypanosomes is genomic and finite. To assess the possibility of developing a vaccine against African trypanosomes, it is also necessary to get information on the variability of VSG genes within the *T. brucei* population. To this end we have hybridized our cDNA clones to restriction digests of DNAs from a series of *T. brucei* strains, two *Trypanosoma evansi* strains and one *Try-*

panosoma equiperdum strain. Four points of interest have emerged thus far:

1. The non-repeated part of the 221 gene is only present in *T. brucei* 427 and *T. evansi* SAK. The 3′-terminal repetitive sequence is also present in repeated form in the other strains.

2. The 118 gene is largely absent in some strains, but not in others.

3. The 121 gene and the 117 gene family are largely conserved in all strains. Differences found include minor shifts in mobility and intensity of fragments and the apparent loss or gain of restriction sites.

4. From the analysis of sites in and around these genes the two *T. evansi* strains fall in the range of fragment patterns observed for the *T. brucei* strains; *T. equiperdum* is clearly different. *T. brucei* strains, known to be similar from maxicircle sequence analysis (Borst, P., Fase-Fowler, F., and Hoeijmakers, J. H. J., unpublished), are also similar in nuclear VSG genes.

It looks, therefore, as if VSG genes evolve by duplication followed by divergence of duplicated copies. In a genome of constant size, such a process must be accompanied by deletions of genes and this is what we have observed.

ACKNOWLEDGMENTS

We are indebted to Prof. Ch. Weissmann (Institut für Molekularbiologie, Universität Zürich, Switzerland) for his help in obtaining cDNA clones and to Mrs. J. Van den Burg and Mrs. F. Fase-Fowler for expert technical assistance. This work was supported in part by a grant to PB from the Foundation for Fundamental Biological Research (BION), which is subsidized by The Netherlands Organization for the Advancement of Pure Research (ZWO). ACCF acknowledges the Research Training Grant from the World Health Organization (M8/181/4/F.78).

REFERENCES

1. Cross, G. A. M., Holder, A. A., Allen, G., and Boothroyd, J. C., 1980. An introduction to antigenic variation in trypanosomes. *Am. J. Trop. Med. Hyg., 29:* 1027–1032.
2. Hoeijmakers, J. H. J., Borst, P., Van den Burg, J., Weissmann, E., and Cross, G. A. M., 1980. The isolation of plasmids containing DNA complementary to messenger RNA for variant surface glycoproteins of *Trypansoma brucei. Gene, 8:* 391–417.
3. Hoeijmakers, J. H. J., Frasch, A. C. C., Bernards, A., Borst, P., and Cross, G. A. M., 1980. Novel expression-linked copies of genes for variant surface antigens in trypanosomes. *Nature, 284:* 78–80.
4. Borst, P., Fase-Fowler, F., Frasch, A. C. C., Hoeymakers, J. H. J., and Weijers, P. J., 1980. Characterization of DNA from *Trypanosoma brucei* and related trypanosomes by restriction endonuclease digestion. *Mol. Biochem. Parasitol.* (In press.)

DISCUSSION

Question by Nina Agabian: Please explain your choice of VSG in terms of development in the organisms and lineage.

Answer: These particular antigenic variants were taken because they happened to be the clones for which the VSG variant surface glycoprotein had been studied already by Cross, thus permitting the comparison of amino acid sequence and DNA sequence.

Comment by George Cross: Three clones were derived from populations within a few days of each other in an infected rabbit. The fourth variant arose when one of the three clones was cultivated in vitro. The in vitro population was cloned in mice.

Question by Richard Williams: Do all the 427 trypanosome clones used originate from a single cell?

Answer: Yes, they do.

Question by Nina Agabian: What was the difference between the other *T. brucei* strains used?

Answer: The five *T. brucei* strains used came from different laboratories and have no known relation to our 427 strain. One of the strains is identical to that used by Williams.

Question by Kenneth Stuart: Do the clones that lack the VSG cDNA sequences also not express the VSG?

Answer: These are other strains of *T. brucei* and not other clones of the 427 *T. brucei* from which our cDNA derives. Strains that do not have a gene should not be able to produce the corre-

sponding gene product, but this has not been verified.

Question by Larry Simpson: Do you find a correlation between the ethidium banding patterns of the nDNA and the variable antigen genes?

Answer: The facts are the following: restriction digests of nuclear DNA electrophoresed through agarose gels give a highly complex pattern of bands when stained with ethidium. This pattern is strain-specific, but different antigenic variants of the same strain yield identical patterns. Under the conditions of these experiments, we cannot see fragments derived from single copy genes, and we attribute these bands therefore to the 30% middle and highly repetitive DNA present in *T. brucei* (see Borst et al., *Mol. Biochem. Parasitol.*, in press). Since some of the VSG genes belong to a fairly large and closely related family of genes (e.g., the 117 family), they will probably make a contribution to the middle repetitive DNA, and hence to the bands seen in ethidium-stained gels. The size of this contribution remains to be determined.