Antigenic Variation in African Trypanosomes

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Parasites have developed clever tricks to evade the deadly immune defense of their mammalian hosts. Regularly changing surface coats, that is, antigenic variation, is one of the most appealing of these tricks. What could be simpler and more effective than to fool the immune system of the host by regularly changing surface antigens more rapidly than new antibodies can be made? But in practice, nature is never simple, and this also holds true for antigenic variation, as investigators are gradually finding out. Several bacterial pathogens, such as *Neisseria gonorrhoeae* (gonorrhea) and *Borrelia* sp. (relapsing fever) use antigenic variation, but we shall limit this Perspective to the African trypanosomes, the fly-transmitted agents of sleeping sickness in humans, which still hold center stage in molecular studies of antigenic variation (*1–3*).

Even though classical genetics and faithful in vitro transcription are not yet available for trypanosomes, these organisms are experimental model protozoa in many other respects. Some variants grow rapidly (with a doubling time of 6 hours) to high densities in rats and mice, but do not infect the investigator. Both bloodstream trypanosomes and insect-form trypanosomes can be cultured in vitro (*4*), and it is possible to convert one form into the other in vitro. Convenient transient and stable transformation systems have been developed in recent years. Stable transformation occurs by homologous recombination only, making gene replacement and disruption experiments relatively simple (*5–7*). Although not yet comparable to *Escherichia coli* or yeast, trypanosomes are easier to work with than HeLa cells and often yield more striking results.

Changing the surface coat

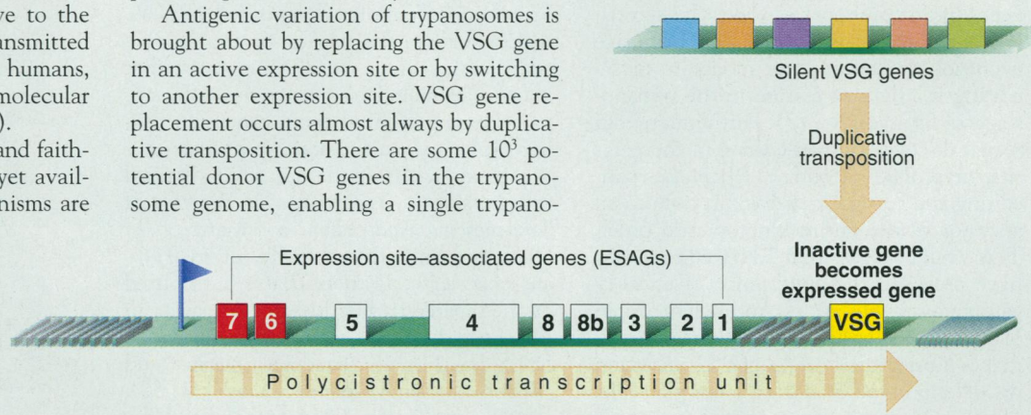
The expression sites for surface antigens are the main control points for antigenic variation of trypanosomes (see figure). The surface coat of *Trypanosoma brucei* consists of a dense layer of a single protein species, the variant surface glycoprotein (VSG). Active VSG genes are located in telomeric expression sites together with at least eight other genes, called expression site–associated genes (ESAGs) (*8, 9*). The expression site is transcribed into a long precursor RNA that is chopped up by polyadenylation and trans-splicing (*10, 11*), a process discovered in trypanosomes and discussed in another Perspective in this issue by Nilsen. This polygenic organization of transcription units is typical of trypanosomes and other Kinetoplastida. Most of the differential control of gene expression seems to occur posttranscriptionally, by regulation of pre-mRNA processing or RNA stability (*12, 13*).

Antigenic variation of trypanosomes is brought about by replacing the VSG gene in an active expression site or by switching to another expression site. VSG gene replacement occurs almost always by duplicative transposition. There are some 10^3 potential donor VSG genes in the trypanosome genome, enabling a single trypanosome and its progeny to make an inordinate number of surface coats that have no exposed antigenic determinant in common. Only part of the elongated and tightly packed VSG molecule in an intact surface coat is visible to host antibodies. Replacement of only part of the active VSG gene should therefore suffice for antigenic variation, as has been experimentally verified. Although early in a chronic trypanosome infection complete replacement of VSG genes is usually observed, later in the infection partial (segmental) replacement and even point mutations occur as well and can create a novel antigenic specificity (*14, 15*).

The mechanism of the duplicative transposition of VSG genes or gene segments is still unresolved. Comparison of the substrates and products of the transposition reaction suggest that transposition is mediated by a gene conversion (duplex break repair) mechanism. Transposition of complete VSG genes requires short blocks of sequence homology both upstream and in the 3′ ends of donor and acceptor genes. Whether site-specific nucleases are required for transposition has been difficult to establish: The rate of transposition is low, and intermediates in the process have not been detected (*16*). There are also no trypanosome mutants incapable of switching that might help to define intermediate steps. The ability to replace VSG genes by selectable marker genes has introduced a new way to tackle the problem, although this approach is not going to be simple either.

In theory, antigenic variation could work with a single VSG gene expression site, but in practice there are more sites—at least 6 and probably as many as 20. This allows trypanosomes to switch coats by changing the expression site that is active (see figure). This switch occurs by altering transcription initiation, but how is unclear. Different expression sites appear to be activated and inactivated independently, and this regulation does not occur by DNA rearrangements close to the promoter. DNA rearrangements far upstream of the promoter have been seen after switching; such distant stochastic events may control the activity of an expression site (*17–19*).

Regulation of antigenic variation might also involve DNA modification, however. An unusual DNA base, called J, is found in small amounts in trypanosome DNA and is enriched in telomeres. When a telomeric expression site is activated, DNA modification is lost. Silencing of the site is associated with reappearance of modification.



VSG gene switching. The active VSG gene is transcribed in an expression site located at a chromosome end. The expressed VSG gene is indicated as a yellow box, and the cotranscribed expression site–associated genes (ESAGs) are indicated upstream with ESAG 6 and 7 highlighted in red. The promoter is shown by a flag, and the polycistronic transcription unit is indicated below with an arrow. Repeat sequences characteristic of expression sites are indicated with gray stripes. Switching the active VSG gene usually occurs by duplicative transposition, whereby a silent VSG gene (often located in a chromosome-internal position and indicated with colored boxes above) is copied into the active expression site, replacing the previous VSG gene.

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The modification could control the activity of expression sites or could serve only to tighten the shut-off of silent expression sites, much in the way envisaged for methylated cytosine in animal cells. J was recently shown to be β-D-glucosyl-hydroxymethyluracil (*20*). With the structure known, it should now be possible to determine the function of this novel base.

After the trypanosome switches from VSG gene A to VSG gene B, it initially makes a mixed coat containing VSG-A with an increasing amount of B. For antigenic variation to work, B must therefore not only be different in exposed epitopes but must also be able to make a tight heteromeric coat with A. The three-dimensional structures of two different VSG molecules illustrate how this is accomplished: Even molecules with very different amino acid sequences fold in very similar elongated structures that can snugly fit together in a tight surface layer (*21*).

Mutated VSG gene copies

The duplicative transposition of VSG genes requires DNA copying, and sloppy copying could introduce additional diversity in the coat repertoire. Donelson and co-workers have indeed found sequence differences between expressed VSG gene copies and the template apparently used for their generation (*22*). In three independent trypanosome isolates expressing the same VSG gene, the transposed gene copy differed in 35, 11, and 28 positions from the template VSG gene (*23*). It is unlikely that these mutations play a major role in the generation of antigenic variation, but the mechanism of their introduction is certainly intriguing. Mutations have only been found during the copying of telomeric VSGs and not of VSGs within chromosomes, and the mutations introduced display an unusual strand bias. This association with telomeric DNA has raised the question of whether the mutations are somehow related to the modified base, known to be present in silent telomeric VSG genes but not in the silent genes located in a chromosome-internal position (*23*). Copying modified DNA might somehow trigger a bout of infidelity of the duplex break repair system thought to be responsible for the duplicative transposition of VSG genes. Other mechanisms of sloppy copying cannot be ruled out at this stage, however.

An unusual transferrin receptor

A problem that has long intrigued investigators working on antigenic variation is the ability of trypanosomes to combine antigenic variation with uptake of host macromolecules. For instance, trypanosomes efficiently take up host transferrin and low density lipoprotein (LDL) in a process that has the characteristics of receptor-mediated endocytosis (*24*). How does the trypanosome manage to specifically bind host macromolecules without being hit by the antibodies that the host generates against the trypanosome receptors required for macromolecule binding? Part of the answer is that the receptors are hidden by their location in an invagination of the surface, called the flagellar pocket. This is where endocytosis of host macromolecules occurs and where the transferrin receptor is located. Of course, if LDL can get into this pocket, antibodies can get in as well, but in this location the antibodies are less effective in killing the parasite, as they cannot be accompanied by the deadly host macrophages that are far too large to enter the pocket.

Nevertheless, the gradual accumulation of antibody to the transferrin and LDL receptors in chronic infections should eventually cause trouble for the trypanosome. The antibodies might clog the receptor or induce complement-mediated lysis. A possible escape from these antibodies is suggested by the recent discovery that the transferrin receptor of *T. brucei* is encoded by ESAGs (*25*, *26*) (see figure) and that the trypanosome might be able to change receptor composition by switching to another VSG gene expression site. The transferrin binding protein (TFBP) present in the flagellar pocket of trypanosomes has an unusual structure: It consists of a subunit encoded by ESAG 6 attached to the membrane by a glycosylphosphatidylinositol (GPI) anchor, and a subunit encoded by ESAG 7 without a membrane anchor but held in place by association with the ESAG 6 protein. No transmembrane subunit is required, as demonstrated by reconstitution of transferrin binding by transfection of ESAG 6 and 7 in insect-form trypanosomes (that normally do not make TFBP) (*27*), in insect cells (*28*), and in *Xenopus* oocytes (*29*).

The evidence that trypanosomes can vary the antigenic properties of TFBP is still very speculative and is based on a comparison of ESAG 6 and 7 genes from different expression sites (*30*). This has yielded a remarkable pattern: The genes are highly conserved, especially the 5'-half, but contain a variable stretch of 32 nucleotides in which up to 12 nucleotides may differ. Most of these nucleotide differences result in amino acid substitutions, as if there is active selection for variable protein products. This has led to speculation that the variable stretches encode immunodominant surface loops. As the ligand-binding face of the TFBP cannot be substantially altered without losing transferrin binding, the trypanosome could have added an attractive surface loop to the molecule to distract the immune system from the invariant business end of the molecule. Whether this potential for antigenic variation of receptors is really used in vivo remains to be tested.

Vaccines?

There are eight different ESAGs (see figure), of which two encode a transferrin binding complex and one an adenylate cyclase of unknown function (*31*), leaving five ESAGs unaccounted for. It is possible that these also specify proteins required for interaction of trypanosomes with the host, such as other receptors, but they might also code for secreted molecules. Obviously, the surface coat of trypanosomes is not a good target for immune intervention, as the coat repertoire is too large. However, the minor proteins required for food uptake or influencing the host may be better candidates. Although these minor proteins are poor antigens in chronic natural infections, it might be possible to present them in a more active form to the host immune system, resulting in protective antibodies. In this way the flagellar pocket, where the trypanosome carries out its transactions with the host, may become the Achilles heel whereby we can get a grip on this slippery parasite.

References

1. G. A. M. Cross, *Annu. Rev. Immunol.* **8**, 83 (1990).
2. P. Borst, *Immunoparasitol. Today* (combined issue March), A29 (1991).
3. E. Pays, in *The Eukaryotic Genome—Organisation and Regulation*, P. M. A. Broda, S. G. Oliver, P. F. G. Sims, Eds., vol. 50 of Society for General Microbiology Symposium (Cambridge Univ. Press, Cambridge, 1993), pp. 127–160.
4. V. B. Carruthers and G. A. M. Cross, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8818 (1992).
5. A. ten Asbroek, M. Ouellette, P. Borst, *Nature* **348**, 174 (1990).
6. J. Eid and B. Sollner-Webb, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2118 (1991).
7. M. G.-S. Lee and L. H. T. van der Ploeg, *Science* **258**, 1583 (1990).
8. E. Pays *et al.*, *Cell* **57**, 835 (1989).
9. S. Lips, P. Revelard, E. Pays, *Mol. Biochem. Parasitol.* **62**, 135 (1993).
10. J. M. Kooter *et al.*, *Cell* **51**, 261 (1987).
11. P. J. Johnson, J. M. Kooter, P. Borst, *ibid.*, p. 273.
12. P. Borst, *Annu. Rev. Biochem.* **55**, 701 (1986).
13. C. Clayton, in *Progress in Nucleic Acid Research and Molecular Biology*, K. Moldave and W. Cohn, Eds. (Academic Press, San Diego, 1992), vol. 43, pp. 37–66.
14. T. Baltz *et al.*, *EMBO J.* **10**, 1653 (1991).
15. S. M. Kamper and A. F. Barbet, *Mol. Biochem. Parasitol.* **53**, 33 (1992).
16. J. D. Barry and M. R. Turner, *Parasitol. Today* **7**, 207 (1991).
17. K. M. Gottesdiener, L. Goriparthi, J. P. Masucci, L. H. T. van der Ploeg, *Mol. Cell. Biol.* **12**, 4784 (1992).
18. J. C. B. M. Zomerdijk, R. Kieft, M. Duyndam, P. G. Shiels, P. Borst, *Nucleic Acids Res.* **19**, 1359 (1991).
19. M. J. Lodes *et al.*, *Mol. Cell. Biol.* **13**, 7036 (1993).
20. J. Gommers-Ampt *et al.*, *Cell* **75**, 1129 (1993).
21. M. Blum *et al.*, *Nature* **362**, 603 (1993).
22. Y. Lu *et al.*, *Cell* **72**, 397 (1993).
23. Y. Lu *et al.*, *Mol. Cell. Biol.*, in press.
24. I. Coppens *et al.*, *J. Protozool.* **34**, 465 (1987).
25. D. Schell *et al.*, *EMBO J.* **10**, 1061 (1991).
26. D. Steverding *et al.*, *Eur. J. Cell Biol.*, in press.
27. M. Ligtenberg *et al.*, *EMBO J.*, in press.
28. M. Chaudhri *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
29. D. Salmon *et al.*, *Cell*, in press.
30. P. Borst, *Trends Genet.* **7**, 307 (1991).
31. P. Paindavoine *et al.*, *Mol. Cell. Biol.* **12**, 1218 (1992).