CO-4 A SUMMARY OF THE MOLECULAR BIOLOGY OF ANTIGENIC VARIATION IN AFRICAN TRYPANOSOMES.

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African trypanosomes are protozoan parasites that belong to the same phylogenetic family as *Trypanosoma cruzi*. Many features of their metabolism and morphology are similar to *T. cruzi*. However, their life cycle and the way in which they interact with their mammalian hosts are quite different. The most distinctive difference is that African trypanosomes do not invade cells of the mammalian host like *T. cruzi* does, i.e., they are not intracellular parasites. Instead, during a chronic mammalian infection they mulpiply in the bloodstream and are in constant confrontation with the immune system. They evade the immune response by periodically switching the major protein on their surface, a phenomenon not found in *T. cruzi*. This switch process is called antigenic variation. This summary will describe the molecular events responsible for antigentic variation and contrast it with mechanisms of immune evasion by *T. cruzi*

African trypanosomes are transmitted to the mammalian bloodstream during the bite of a tsetse fly. While in the bloodstream each trypanosome is covered with 10^7 copies of a single surface protein called the variant surface glycoprotein (VSG). The major function of the VSG is to serve as a barrier that protects other constituents of

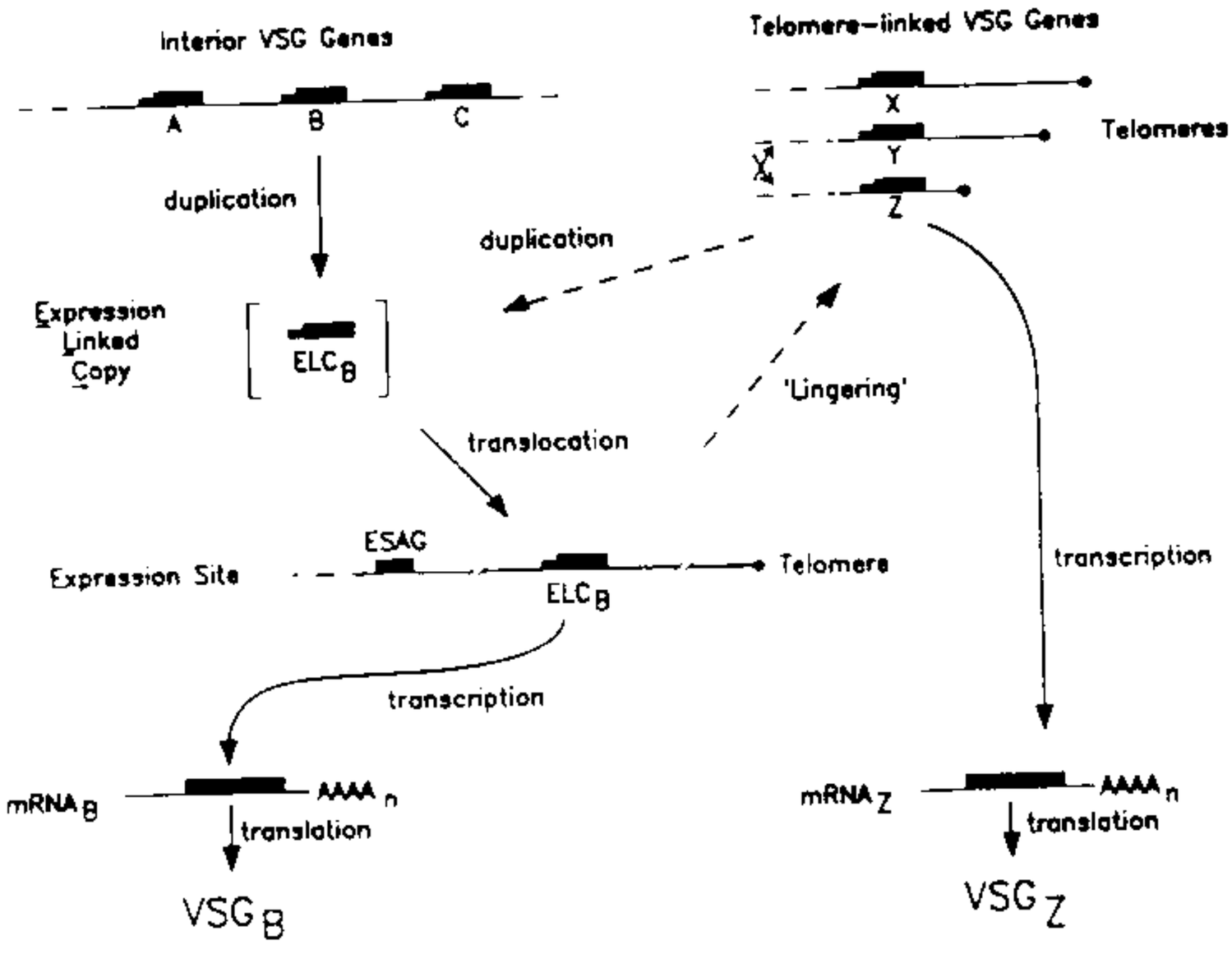
the outer membrane from the assault of the immune system. It is the switch from synthesis of one VSG to another that enable the trypanosome to keep "one step ahead" of the immune response. In the laboratoty it has been shown that a given trypanosome can sequentially express over 100 immunologically-distinct VSGs and may have the potential to produce a virtually limitless number. Each of the different VSGs is about 450 amino acids in length. The N-terminal two-thirds of the proteins are quite variable in sequence and provides the unique antigenic determinants. The C-terminal portions of the proteins have some sequence similarities which probably contribute to a common structure involved in the interaction of individual molecules on the cell surface. The C-terminal amino acid of each VSG is covalently linked through carbohydrate moieties to phosphotidylinositol in the membrane.

The trypanosome genome contains several hundred genes for different VSGs. The selection of which of these genes to express occurs at the level of transcription. Usually only one VSG gene is transcribed at a time while all of the other VSG genes are silent. DNA rearrangements contribute to the selection of which VSG gene is transcribed. These rearrangements maneuver VSG genes into, and out of, locations in the DNA that are called " expression sites". Curiously, these expression sites are always adjacent to chromosomal teleomeres - the physical ends of the DNA molecules in chromosomes. The switch from

transcription of one VSG gene to another occurs when a new VSG gene is activated in an expression site. The process is complicated by the fact that several, and perhaps many, potencial telomere-linked expression sites exist in the genome, yet only one is normally activated at a time. This extraordinary, and seemingly rather chaotic, mechanism for sequential VSG expression is summarized in the diagram below which is taken from earlier review (Donelson, J.E. (1988) Unsolved mysteries of trypanosome antigenic variation. In: The Biology of Parasitism (P. Englund & A. Sher, eds.) Alan R. Liss, Inc.).

Many of the several hundred VSG genes are located at interior chromosomal locations as represented by basic copy genes A, B and C in the diagram. These interior genes are never transcribed in these sites. To be transcribed, one of them must be duplicated (gene B) and the duplicated copy translocated to a telomere-linked expression site. This duplicative translocation is sometimes called a gene conversion event and the duplicated gene copy is referred to as an expression linked copy (ELC) gene. Once in the expression site, the ELC gene can be transcribed and the VSG synthesized. Other basic copy VSG genes, represented by genes X, Y and Z are already situated near telomeres. A gene conversion event is not essential for their expression, although ELCs of telomere-linked basic copy genes sometimes occur, as indicated by the dashed arrows pointed to the left. ELC genes usually disappear from the genome after the

switch to the expression of another VSG gene, although they accasionally "linger" to become nonexpressed, telomere-linked, basic copy genes (dashed arrow to the right). Expression of telomere-linked genes has also been correlated with exchange of telomeres between two chromosomes and with displacement of one telomere by a duplicated copy of another telomere region. In addition, sometimes a telomere-linked basic copy gene is activated without any apparent DNA rearrangement. In a few cases, DNA rearrangements have been detected that create new VSG genes composed of segments of two or more existing VSG genes. The VSGs synthesized from these composite genes can be immunologically distinct from those synthesized from the original basic copy genes. The steps in the formation of such composite genes are not known but they do provide a mechanism for the generation of a larger number of different VSGs than there are VSG in the genome.



Much of the research on VSG gene expression now focuses on the precise molecular event that triggers a switch to the transcription of a new ELC or to another telomere- linked gene. Our laboratory's approach to this question is to examine a subset of VSG genes that are expressed at the final developmental stage of the parasites in the tsetse fly. This metacyclic stage, as it is called, is the first stage of the life cycle at which a VSG is synthesized. It turns out that only about 15 different VSG genes, out of the several hundred in the genome, are ever expressed at the metacyclic stage. Thus, these 15 VSG are developmentally regulated in a manner that is different from the other VSG genes that are expressed in the bloodstream. Our cloning and sequencing of several of these metacyclic VSG genes have provided some clues about the regulation of this stage-specific expression.

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