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Nuclear Chromatin Interactions Underlie Epigenetic Regulation of Antigenic Variation

J. M. Bart, D. López-Farfan, D. Landeira, D. Van Tyne, M. Navarro*

Instituto de Parasitologia y Biomedicina Lopez-Neyra Consejo Superior de Investigaciones Cientificas CSIC. (Spanish National Research Council) Avda. del Conocimiento s/n 18100 GRANADA, Spain

In the mammalian host, bloodstream trypanosomes elude the immune response by periodically changing their main surface antigen, the Variant Surface Glycoprotein (VSG). To achieve the expression of a single type of VSG on the surface, only one out of ~15 possible subtelomeric *VSG* expression sites (ESs) is transcribed at a given time. Unusually, the active *VSG*-ES is transcribed by RNA polymerase I and is located in an extra-nucleolar nuclear body named the expression site body (ESB)[1]. It has been suggested that maintenance of the epigenetic state of monoallelic expression of the active *VSG*-ES locus could be mediated by exclusive association to the nuclear ESB[1,2,3].

Using GFP tagging of chromosomes in trypanosomes[4], we have recently shown that during the cell cycle sister chromatids of the active *VSG*-ES remain held together longer than other loci. Interestingly, sister chromatids remain associated to the unique ESB present in the nucleus until chromosome segregation. The cohesin complex is important in preserving *VSG*-ES activity, as the knockdown of cohesin subunits lead to premature separation of sister chromatids of the active *VSG*-ES and promotes a transcriptional switch from the active *VSG*-ES to a previously inactive one. These results suggest that cohesins play a crucial role in ensuring proper epigenetic inheritance of the *VSG* transcriptional state[5].

The DNA-binding protein CTCF operates as a transcriptional insulator and functionally associates with cohesins[6,7]. Removal of cohesins could then hamper the proper insulation of the active *VSG*-ES. A trypanosome CTCF functional orthologue was identified together with its binding sequences, which loop upstream of the VSG-ES promoter, as suggest by EMSA and 3C experiments. Knockdown of TbCTCF results in 15 fold down-regulation of active 221 *VSG*-ES transcription. In sum, we have identified a transcriptional insulator in *T. brucei* and described a functional association between cohesins and TbCTCF in the regulation of *VSG* monoallelic expression.

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