Variant-specific surface protein (VSP) gene subsets in *Giardia*

by

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Abstract

This project was concerned with the family of genes that encode variant-specific surface proteins (VSP) in the parasitic protozoon, *Giardia*. The VSP are immunodominant surface antigens on *Giardia* trophozoites and the alternative expression of different *vsp* genes underlies observations of antigenic variation within *Giardia* populations. Specific goals were to identify and compare closely related genes, which may form subsets within the larger VSP gene family. Fewer than 10 of a possible repertoire of 150-300 VSP genes had been characterised completely at the time the project was commenced.

Two novel VSP gene subfamilies were identified in the study. One, designated the *vsp*136 subfamily (for which the previously described gene, *crp*136, is the prototype), comprises genes with tandem repeat elements. The other, designated the *vsp*72 subfamily, has members that lack tandem repeat elements and includes numerous pseudogenes. Most of the genes examined were identified among 21 distinct plasmids isolated by screening *Giardia* genomic DNA libraries using hybridisation analysis. Eight genomic DNA fragments (size range, 3.5-9.5 kb) were characterised completely by nucleotide sequence determinations. Another 15 fragments (3.5-15 kb) were characterised partially by a combination of restriction fragment length polymorphism (RFLP), Southern hybridisation and polymerase chain reaction (PCR) analysis. Amplified segments (1-1.6 kb, derived from both genomic and complementary DNA) from an additional 7 *vsp* gene loci were also characterised.

Analysis of individual and aligned nucleotide sequences and the polypeptides (functional and pseudo-VSP) encoded by these various genes revealed conserved segments that allowed the identification of distinct gene subfamilies. Characterised members of the *vsp*136 subfamily contain different copy numbers of related (in some cases, identical) tandem repeat elements. These genes show clear evidence of recombination. The *vsp*72 subfamily consists of tandemly arrayed pseudogenes (of which 13 were identified in this study) as well as functional genes, all of which lack tandem repeat elements. To complement the genetic analysis, mRNA *in situ* hybridisation experiments were performed on *Giardia* trophozoites to identify cells expressing functional genes belonging to (or derived by recombination from nonfunctional members of) these subfamilies. In all, nucleotide sequences comprising a total of 43,568 bp and involving 23 loci (of which 10 were characterised completely) were determined during the course of this project.