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G. A. M. Cross

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Structure of the variant glycoproteins and surface coat of *Trypanosoma brucei*

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The pathogenic African trypanosomes have a unique mechanism for antigenic variation. Each cell is covered by a surface coat consisting of about seven million essentially identical glycoprotein molecules drawn from a large repertoire of variants, each encoded by an individual gene. Amino acid sequence variation extends throughout the molecule but reduces from the amino terminus to the carboxy terminus, where certain features, especially the grouping of cysteine residues, are quite conserved. The range of diversity within the thousand or so variant glycoprotein genes that exist in each cell is large. New variants may arise instantaneously by segmental gene conversion. Variant surface glycoproteins are synthesized with amino terminal signal sequences and hydrophobic carboxy terminal tails. The tails are extraordinarily conserved. After synthesis, they are replaced by a complex glycolipid structure in which myristic (dodecanoic) acid serves to anchor the polypeptide to the surface membrane. Enzymic cleavage of myristic acid releases variant glycoproteins from the surface coat.

Introduction

The Trypanosomatidae are ubiquitous. Several species of the genera *Leishmania* and *Trypanosoma* cause severe diseases in animals and man. At the turn of the century, epidemics of human sleeping sickness threatened to depopulate large areas of central Africa. Today, animal trypanosomiasis is the major problem, having a serious economic, social and nutritional impact in Africa. However, trypanosomiasis presents a serious continuing threat to human health. Certain historical landmarks give a perspective to the progress of our knowledge of the biology and pathology of the trypanosomes of mammals (Hoare 1972). The first pathogenic trypanosome (*Trypanosoma evansi*) was discovered in the blood of equines and camels in India in 1880. *Trypanosoma brucei* was implicated as the causative agent of nagana in 1894 and trypanosomes were first demonstrated in human sleeping sickness in 1902, although the disease was first described in 1803. The existence of a developmental cycle for *T. brucei*, in *Glossina*, was recognized by Kleine in 1909. Franke (1905), working in collaboration with Paul Ehrlich, described experiments from which he concluded that '... the trypanosomes must therefore have acquired other biological characters during their stay in (the monkey's) semi-immune body that rendered them resistant to the defensive substances'. Today, antigenic variation is generally recognized as the primary mechanism for the failure of an immune response to eliminate infection.

The pathogenic African trypanosomes, exemplified by *T. brucei*, are unicellular flagellated protozoa, about 20 μm long and 2 μm diameter. They live and multiply in the bloodstream and tissue fluids of the animal host. They are continously exposed to the immune responses of the host. The characteristic cyclical parasitaemia (Ross & Thomson 1910) is a consequence

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of antigenic variation. Each wave of parasites is limited by the immune response, which nevertheless fails to eliminate the infection. Death of the host is the usual outcome, if the infection is untreated.

The existence, in *T. brucei*, of surface-bound but soluble antigen was first noted by Wietz (1960*a*, *b*). The 'exoantigen' was originally believed to be actively secreted by the trypanosome. Vickerman (1969) first postulated a relation between antigenic variation and the surface coat, a novel morphological feature of the bloodstream stage of salivarian trypanosomes. It was subsequently demonstrated that the surface coat of an individual trypanosome is composed, primarily or exclusively, of about seven million molecules of a single species of glycoprotein (Cross 1975). Each serologically homogenous population of trypanosomes yields a characteristic variant surface glycoprotein (v.s.g.) which can stimulate immunity to homologous but not heterologous challenge. Knowledge of antigenic variation and v.s.g. structure (before cloning) has been adequately reviewed elsewhere (Cross 1978, 1979*a*; Gray & Luckins 1976; Doyle 1977; Vickerman & Barry 1982). Turner (1982) reviewed v.s.g. biochemistry in depth. I shall limit my discussion to a summary of recent data relating to v.s.g. structure and the surface coat.

Genetic basis of v.s.g. structure and diversity

Since 1979, the application of recombinant DNA techniques has greatly enhanced our understanding of the genetic basis of antigenic variation. Each v.s.g. is essentially encoded by a single gene, except in the case of segmental DNA-conversion-generated expression linked copies (Pays *et al.* 1983*a*, *b*). The DNA sequence is uninterrupted and is colinear with the amino acid sequence of the v.s.g. Analysis of trypanosome genomic DNA libraries in cosmid vectors shows 1000 to 2000 v.s.g.-related DNA sequences that are clustered and occupy about 10% of the haploid genome (Van der Ploeg *et al.* 1982). At high hybridization stringency, DNA probes encoding predominately the amino terminal v.s.g. domain (Johnson & Cross 1979; Cross 1979) may recognize a single gene or a family of closely related genes (Williams *et al.* 1979, 1982; Hoeijmakers *et al.* 1980; Marcu & Williams 1981; Donelson *et al.* 1982; Young *et al.* 1982, 1983; Pays *et al.* 1983*c*). At lower hybridization strigency, especially with DNA probes encompassing the v.s.g. carboxy terminal domain, many related sequences are visualized (Frasch *et al.* 1980; Borst *et al.* 1981; Bernards *et al.* 1981). Such experiments exemplify the enormous range of sequence diversity within the v.s.g. gene repertoire.

It is difficult to deduce the extent to which the observed DNA sequence diversity is reflected in serological diversity expressed by v.s.g.s at the cell surface. Although closely related genes may differ in only a few codons, there are examples of proteins in which single amino acid changes can be distinguished serologically. Although several epitopes can be distinguished on purified v.s.g.s, few are visible on the surface of living trypanosomes (Hall & Esser 1984). The extent of the serological repertoire is indeterminate. Although many v.s.g. genes may mutate and evolve slowly, there are examples of entirely new sequences or hybrids of pre-existing v.s.g.s being generated by gene rearrangements associated with the regulation of v.s.g. gene expression (Pays *et al.* 1983*a*, *b*; Rice-Ficht *et al.* 1982). In contrast, the human-infective *T. gambiense* appears to express a limited antigenic repertoire (Gray 1972, 1975; Jones *et al.* 1981).

Possible mechanisms regulating the expression of v.s.g. gene diversity will be discussed by Van der Ploeg (this symposium). The availability of cloned genes has also contributed greatly to our knowledge of v.s.g. structure, especially through comparison of amino acid sequences determined directly on the v.s.g.s with those deduced from DNA sequences.

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Structure of the variant surface glycoproteins

V.s.g.s were recently shown (Cardosa de Almeida & Turner 1983) to be obtainable in two forms: the conventionally purified (Cross 1975, 1977, 1984) soluble form (s.v.s.g.) and the true membrane form (m.f.v.s.g.: see later), as it exists in the trypanosome surface coat. Hitherto, structural studies have been performed exclusively on s.v.s.g., which consists of a single polypeptide of about 450 amino acids. The entire amino acid sequence of one v.s.g. has been determined directly (Allen *et al.* 1982) and independently deduced from a cloned DNA sequence (Boothroyd *et al.* 1982). The results agreed with the standard genetic code. The disulphide linkages of this v.s.g. have been determined (Allen & Gurnett 1983). Limited cleavage of variant glycoproteins by trypsin suggested that v.s.g.s are organized in two functionally distinct domains (Johnson & Cross 1979; Cross 1979*a*). This concept has been upheld by recent studies. Amino acid sequence diversity is distributed throughout the v.s.g. molecule, in contrast to immunoglobulin structure. The DNA hydridization data, referred to above, and DNA sequence analysis in several laboratories (see legend to figure 1 for references) shows that amino acid and nucleotide sequence similarities, between v.s.g.s, are greatest in the carboxy terminal domain, comprising one quarter to one third of the polypeptide.

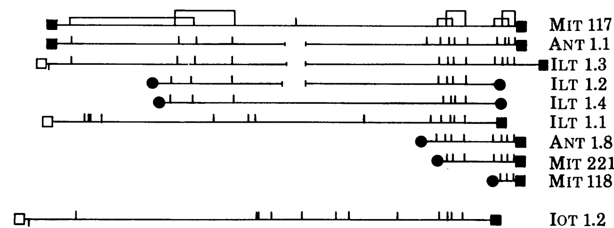

FIGURE 1. Alignment of v.s.g. sequences illustrating conservation of cysteine residues. Disulphide bonds are shown for v.s.g. 117. Vertical bars in other sequences indicate cysteine residues. ⊣ ⊢, Gap introduced to maximize cysteine alignments; ■, known termini of mature v.s.g.; □, predicted initiation methionine of prev.s.g.; ●, termini of incomplete sequences. All sequences where known to be functional (that is, were derived, in the critical regions, from cDNA recombinants or v.s.g.s themselves). Sources of the data are Allen & Gurnett 1983; Allen *et al.* 1982; Boothroyd *et al.* 1981, 1982; Majumder *et al.* 1981; Rice-Ficht *et al.* 1981, 1982; Donelson *et al.* 1982; Matthyssens *et al.* 1981; Holder & Cross 1981; Liu *et al.* 1983; Michiels *et al.* 1983; Pays *et al.* 1983*a*, *b*; J. E. Donelson, personal communication.

Several complete and partial v.s.g. DNA sequences have been aligned in figure 1 to illustrate structural relations. As in other protein families, including immunoglobulins, most cysteine residues are strikingly conserved in v.s.g.s. In determining disulphide linkages within the carboxy terminal domain and despite the existence of many potential cleavage sites, Allen & Gurnett (1983) encountered severe protease resistance, implying a tightly folded structure. It is interesting that, in all the sequences illustrated and despite a few quite dramatic diversions from common patterns of cysteine residues elsewhere in these v.s.g.s, at least one and generally both of the two groups of four carboxy domain cysteine residues are highly conserved. Three of the illustrated sequences are incomplete in this region. Interestingly, one v.s.g. whose sequence is complete (IITat 1.1) appears to have survived a deletion of one group of otherwise conserved carboxy domain cysteine residues. We can be quite confident that the sequence is correct and complete. It was determined from both cDNA and genomic recombinants (Rice-Ficht *et al.* 1982). Although the authors reported nucleotide and amino acid differences, between cDNA and genomic recombinants, in some regions of the sequence, the carboxy

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terminus and its downstream sequences appear authentic. Thus, it appears that whatever function requires conservation of carboxy terminal domain conformation can be satisfied with one cysteine segment. There is an odd group of cysteine residues closely spaced towards the amino terminus of the IITat 1.1 sequence, and further inspection of the nucleotide sequences suggests that the carboxy terminal deletion might have resulted from intragenic recombination.

Closer inspection of amino acid and nucleotide sequences shows other similarities between v.s.g. carboxy terminal domains. The two cysteine segments may have arisen by duplication. Recent support for the domain structure of v.s.g.s has come from preliminary X-ray diffraction data (Freymann *et al.* 1984). Stable crystals, suitable for high resolution X-ray analysis, contain only the amino terminal v.s.g. domain, apparently a consequence of proteolytic cleavage during crystallization. This behaviour mirrors that of immunoglobulins, where flexibility of the 'hinge' region prevents crystallization of the intact molecule.

Amino acid sequence homology is most striking at the carboxy termini of mature v.s.g.s (table 1). Although many carboxy termini have been deduced by homology with the six proven sequences (Holder & Cross 1981), it seems likely that all v.s.g.s terminate with aspartic acid or serine, with the exception of one v.s.g. that terminates in asparagine. The sequences compiled in table 1 confirm and extend the homology first detected by Rice-Ficht *et al.* (1981).

Table 1. Carboxy termini of mature v.s.g.s

(Asterisked sequences were determined by amino acid (Holder & Cross 1981) sequencing. Other alignments were deduced, by homology from DNA sequences. Sources cited in legend to figure 1 plus De Lange *et al.* 1983; Merritt *et al.* 1983.)

```
Mit  1.1000BC   G K T G D K H N C A F R K G K D G K E E P E K E K C C D
     118A       T P G K S A D C G F R K G K D G E T D E P D K E K C R N    ***
     121                                                W E G E T C K D    ***
     117A           D K C K G K L E D T C K K E S N C K W E N N A C K D    ***
Ant  1.1        P A E K C T G K K K D D C K D G -.- C K W E A E T C K D
     1.8        T T D K C K G K L E D T C K K E S N C K W E G E T C K D
Ilt  1.3              K C K D K K K D D C K S P - D C K W E G E T C K D

Mit  1.1006BC                             R D R E K M E T T N T T A   S
     221                                                N T N T T G S S    ***
     055                                              A E T N T T G   S    ***
     060                                                  A N T T G   S    ***
Ilt  1.1        A K R V A E Q A A T N Q E T E G K D G K T T N T T G   S
IoT  1.2        D K E E A K K L E E K T E Q N D S K T V T T N T T G   S
Txt  1                        E E A A E N Q E G K K E K T S N T T A   S
Txt  5.28                                               T E K K D E K S
```

V.s.g.s that terminate with serine have a conserved Asn–Thr–Thr sequence immediately upstream. The asparagine residue is frequently glycosylated (Holder & Cross 1981; Holder 1983*a*). V.s.g.s vary in their carbohydrate content (7–17% by mass), composition (Johnson & Cross 1977) and location (Johnson & Cross 1979). The carboxy terminal amino acid is always glycosylated, as part of a complex substitution (see below). V.s.g.s that contain the least amount of carbohydrate have a single carbohydrate substituent, additional to that at the carboxy terminus, that is situated about 50 amino acids upstream from the carboxy terminus (Holder & Cross 1981; Allen *et al.* 1982). More heavily glycosylated v.s.g.s (MiTat '118', for example) are also substituted in their amino terminal domain.

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Leaders and tails

Comparison of amino acid sequences derived directly from v.s.g.s with those deduced from DNA sequences, predicted that v.s.g.s are synthesized with amino acid sequence extensions, at both ends, that are absent from purified v.s.g.s (Boothroyd *et al.* 1981). An amino terminal extension, or 'signal sequence', is a feature of almost all proteins and glycoproteins that are synthesized for export to the cell surface (Davis & Tai 1980; Perlman & Halvorson 1983; Sabatini *et al.* 1982). The v.s.g. amino terminal leader sequence is processed *in vivo* and *in vitro* in an analogous manner to other signal peptides (McConnell *et al.* 1981, 1982). As with other signal peptides, neither the length nor the amino acid sequence of v.s.g. signal peptides is

Table 2. Length of v.s.g. leader, tail and mature polypeptides and total number of cysteine residues

(Values in brackets in the Cys column indicate residues that might be in the signal peptide.)

	length of v.s.g. polypeptides				
variant	total	leader	mature	tail	cys
Mit 117	526	33	470	23	13 (+1)
Ant 1.1	503	29	451	23	13 (0)
Ant 1.10	503	29	451	23	13 (+1)
Ilt 1.3	504	?	?	23	13 (−1?)
1.1	471	?	?	17	13 (−1?)
Iot 1.2	563	?	?	17	12 (+1)

Table 3. V.s.g. signal sequences and mature amino termini

(Asterisked amino termini were determined by amino acid sequencing. Sources as previously cited plus Bridgen *et al.* 1976; Johnson & Cross 1979.)

v.s.g. leader sequences and mature N-termini

```
leader
MIT 118                      M I H S N K V A T V V L A L I S S W P A D G
    117        M D C H T K E T L G V T Q W R R S T M L T L S L L Y A I T P A D G
ANT 1.1                M V T K E R N A A L K I V M L V A S A L T L H P Q Q A L A
ANT 1.10               - - A K K C S A A L K I V M L V G A A L T L H Q Q Q A L A
MIT 221                      M P S N Q E A R L F L A V L V L A Q V L P I L V D S
ILT 1.1        M V K A I A S L M L L H I W A I L E E I K A D R A P S V S R T E C ?
ILT 1.3        M T K A Y E N R M L L Q A L V L A A V L C T T H A E G T A K A P L ?
IOT 1.2        M Q F F C L S S Q G K A P K Q L T I A L A F L V A A S L S Q G E N ?
mature
MIT 118   ***  T N N H G L K L Q K A Q A I C K M S K E
    117   ***  A K E A L E Y K T W T N H C G L A A T L
ANT 1.1   ***  Q T A G R P L A D V V G K T L C T Y S
ANT 1.10       Q T A G R P L A D A V G K A L C T Y S
MIT 221   ***  A A E K G F K Q A F W Q P L C Q V S E E L D D Q P K G A L F T L Q
    121   ***  T D K G A I K F E T W E P L Q L L T Q D F G N L Y N K A L
    055   ***  A E A K S D T A S G S V N S P Q T E A T Y ? A Q L A K T L Q R A L
```

conserved (tables 2 and 3). They do contain characteristically hydrophobic regions. The lack of sequence similarity and the length variation of known signal sequences make it impossible to spot the junction between signal peptide and mature v.s.g. in cases where the amino acid sequence was deduced solely from the nucleotide sequence. The leader of v.s.g. 117 is the longest known signal sequence.

More interesting to us was the predicted carboxy terminal tail (table 4). The amino acid sequences of the tails show similarities that are amazing, in comparison to the variability elsewhere in molecule, including the leader sequence. We do not know the significance of this

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Table 4. Pre-v.s.g. carboxy terminal tails aligned to maximize homology

(Sources as previously cited.)

Mit	1.1000BC	G S F L V N K K F A L - M V Y D F V S L L A F
	118BC,B,C	G S F L T S K Q F A L - M V S A A F V T L L F
	118D	G S F L T S K Q F A L - M V S S A F A A L L F
	118A	G S F L T S K Q F A F S V V S A A F V A L L F
	121	S S I L V T K K F A L T V V S A A F V A L L F
Mit	117BC	S S I L V T K K F A L S A - - A A F A A L L F
	117A	S S I L V T K K F A L T V V S A A F V A L L F
	117B	S S I L V T K K F A L S M V S A A F V T L L F
Ant	1.1	S S I L L T K N F A L S V V S A A L V A L L F
	1.1B,1.10	S S I L V T K K F A L S L V S A A F A S L L F
	1.8	S S I L V N K Q L A L S V V S A A F A A L L F
Ilt	1.3	S S F I L N K Q F A L S V V S A A F A A L L F
Mit	1.1006BC	N S F V I H K - - - A P L L - L A F - - L L F
	221A	N S F V I S K - - - T P L W - L A V - - L L F
Ilt	1.1BC	N S F L I N K - - - A P V L - L A F - - L L L
	1.1	N S F V I H K - - - A P L F - L A F - - L L F
Iot	1.2ELC	H S F V I N K - - - T P P L - L A F - - L L F
Txt	1	N S F V I N K - - - A P L L - L G F - - L L F
Txt	5.28	F S - - G K L R V S V P Q V F A A L V L A A F

homology, which is evident at both the amino acid and nucleotide levels. The tail sequences are very hydrophobic. With the exception of two amino acid deletions, in part of the 117 basic copy sequence not present in the corresponding mRNAs (117a or 117b), one amino acid deletion found in v.s.g. 118 mRNAs, and one bizarre *T. brucei gambiense* sequence (TxTat 5.28), which may be erroneous (Merritt *et al.* 1983), all the tails contain either 23 or 17 amino acids. Excluding TxTat 5.28, the last three amino acids are almost invariant and the serine and lysine residues at positions 2 and 7 are constant. The alignments mainly concur with those of Liu *et al.* (1983) and take account of the corresponding optimum nucleotide alignments. In all cases, the mature v.s.g.s that terminate in serine have a 17 amino acid tail and those terminating in aspartic acid (or, in one case, asparagine) have a 23 amino acid tail. Although the function of the tail is unknown, some of the homologies on each side of the junction between mature terminus and tail presumably play a role in recognition by the post-translational modification enzymes (see below).

V.s.g. organization in the surface coat

The function of the trypanosome surface coat is clearly related to the avoidance of the host's immune system, but what exactly is the protective mechanism? The v.s.g. coat itself is highly immunogenic, but perhaps only after differentiation of long slender dividing trypomastigotes to short broad non-dividing forms (Sendashonga & Black 1982; Morrison *et al.* 1982; Black *et al.* 1982, 1983). On the basis of experiments suggesting that the surface coat is impenetrable by molecules as large as concanavalin A (102000 Da molecular mass, as a tetramer), it was suggested (Cross & Johnson 1976; Cross 1977, 1978) that the primary function of the surface coat is to act as a physical barrier, preventing immunoglobulins or other components of the immune system from binding to components of the underlying plasma membrane, thereby initiating cell destruction. This concept has received additional experimental support (Ferrante & Allison 1983*a*, *b*). It is also clear that a limited range of the epitopes present on free v.s.g. are accessible to antibodies when v.s.g. is on the trypanosome surface (Hall & Esser 1984).

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To form an effective barrier would presumably require close packing of v.s.g. molecules in the surface coat. From the known amount of v.s.g. some experimental attempts to estimate the likely surface area of a *T. brucei* and an assumed but sensible estimate of the possible dimensions of a v.s.g. molecule, it was estimated that the available v.s.g. could form a closely packed monolayer covering the entire trypanosome surface (Cross 1975). As pointed out by Turner (1982), this hypothesis has sometimes been regarded as dogma! However, developing knowledge of v.s.g. structure is providing support rather than opposition to the monolayer model (see below). The normal functional v.s.g. unit is probably a dimer (Auffret & Turner 1981; Freymann *et al.* 1984), which in some cases is stabilized by disulphide links (Cross 1977) or by asparagine-linked sugars (Strickler & Patton 1982).

For many years, the metastable nature of the v.s.g.-membrane linkage was an enigma. Although there is some disagreement (Diffley *et al.* 1980), it is generally thought that v.s.g. is not continually shed from healthy viable trypanosomes *in vivo* (Black *et al.* 1982). For some time it was thought that the carboxy terminal hydrophobic tail might serve to anchor v.s.g. to the membrane, as with several other eukaryotic, prokaryotic and viral glycoproteins, and that solubilization was mediated by a specific peptidase that could not be inhibited by known protease inhibitors. The demonstration of the carboxy terminal ethanolamine linkage, in purified s.v.s.g. (Holder 1983*b*), essentially discounted this possibility and prompted us to consider whether an alternative lipophilic moiety was responsible for membrane linkage. Two alternatives could not be eliminated. First, that a small proportion of v.s.g. molecules retained the polypeptide tail and that this, together with weak intermolecular interactions elsewhere in the v.s.g. molecules, was sufficient to stabilize the surface coat. Second, that other hydrophobic regions of the polypeptide looped into the membrane bilayer. This concept was supported by experimental data on the interaction of s.v.s.g. with cholesterol-containing lipid monolayers (Turner & Cordingley 1981) but not by emerging amino acid sequence data.

A major breakthrough, concerning the mechanism of v.s.g. attachment to the plasma membrane, came from experiments showing that v.s.g. extracted from trypanosomes with detergent at 100 °C could be distinguished, from conventionally purified s.v.s.g., by charge-shift electrophoresis (Cardosa de Almeida & Turner 1983). Conversion of the membrane form v.s.g. (m.f.v.s.g.) to s.v.s.g. was enzyme mediated. Only s.v.s.g. reacted with heterologous antisera, suggesting that the cross-reactive epitope (Barbet & McGuire 1978; Cross 1979*a*, *b*; Holder & Cross 1981) was masked in m.f.v.s.g. or formed concomitantly with conversion to s.v.s.g. Both forms were phosphorylated in the carboxy terminal domain, but the precise localization and stoichiometry of phosphorylation was not determined. The s.v.s.g. of *T. equiperdum* has recently been shown to contain a phosphorylated immunologically cross-reacting ethanolamine-containing glycopeptide (Baltz *et al.* 1982, 1983).

Recent studies (Ferguson & Cross 1984) show that m.f.v.s.g. contains one or two residues of myristic (tetradecanoic) acid, which is responsible for membrane attachment and is cleaved concomitantly with release s.v.s.g. Acylation of proteins and glycoproteins, in bacteria, viruses and mammalian cells, is becoming a well documented post-translational modification (Schmidt 1983). Myristic acid is attached to the amino termini of cyclic AMP-dependent protein kinase from bovine cardiac muscle (Carr *et al.* 1982) and a murine retrovirus protein (Henderson *et al.* 1983). Other examples of acylation involve ester linkage between fatty acids and hydroxy-amino acids and as diglyceride, thioester linked to cysteine (Schmidt 1983). The precise structure of the trypanosome v.s.g. carboxy terminal lipophilic moiety has to be

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determined. Current data indicate that its structure, location and substitution for a precursor peptide tail are unique features. However, the carboxy terminal tryptic peptide of rat brain Thy-1 antigen contains ethanolamine, glucosamine and galactosamine, and has solubility properties that are consistent with the presence of covalently attached lipid (Campbell *et al.* 1981; A. F. Williams, personal communication). The nucleotide sequence of Thy-1 antigen cDNA (J. Silver, personal communication) predicts that the initial translated protein contains a hydrophobic tail that may be cleaved and replaced by lipid, in a similar manner to v.s.g. processing. Additional data are required to resolve this point.

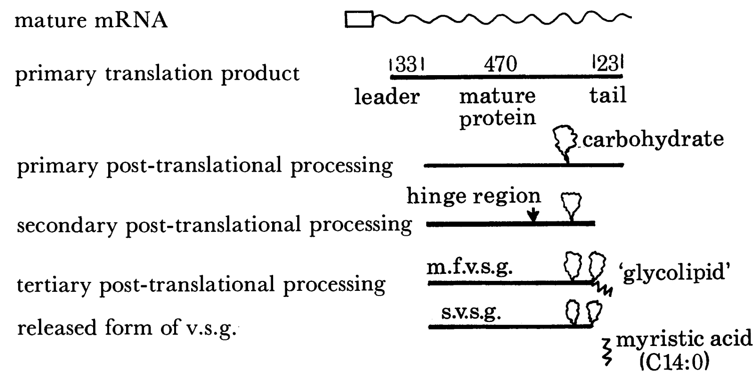

FIGURE 2. Pathway of v.s.g. biosynthesis and processing. The secondary and tertiary steps may occur as contiguous reactions.

Figure 2 summarizes the steps of v.s.g. biosynthesis and processing, excluding folding of the molecule and dimerization. Re-interpretation of previous biosynthetic data (McConnell *et al.* 1983; P. Overath, M. A. J. Ferguson and G. A. M. Cross, unpublished results), in the light of this new information on myristic acid and m.f.v.s.g. to s.v.s.g. conversion, suggests that the polypeptide tail is replaced by the lipid moiety soon after synthesis and long before v.s.g. reaches the cell surface (20–40 min after translation). Only when v.s.g. reaches the surface does the myristic acid become susceptible to cleavage (Ferguson & Cross 1984). The only detected structural difference between m.f.v.s.g. and s.v.s.g. is the presence or absence of myristic acid. Release of v.s.g. from the trypanosome surface occurs, following membrane disruption, through an enzymic reaction that can be prevented by zinc but not by protease inhibitors (Voorheis *et al.* 1982; Cardosa de Almeida & Turner 1983; Cross 1984). The physiological significance of the m.f.v.s.g. to s.v.s.g. conversion that occurs during cell disruption is unknown. The half-time for conversion at temperatures between 20 and 40 °C is about 1 min (Cross 1984). The speed of this reaction, which occurs even at 0 °C, and the difficulty in detecting the change by regular dodecyl sulphate polyacrylamide gel electrophoresis techniques (Cardosa de Almedia & Turner 1983) combined to prevent earlier recognition of m.f.v.s.g.

Although v.s.g. can be detected in the plasma of acutely infected rodents (Diffley *et al.* 1980) it is generally believed that v.s.g. is not continuously shed from healthy viable trypanosomes, *in vivo* or in culture. On the other hand, the question of whether v.s.g. is turned over by intracellular recycling has never been addressed. Presumably, some mechanism exists for replacement of the surface coat, following v.s.g. gene switching. S.v.s.g. is shed and partly proteolytically cleaved during transformation from bloodstream to procyclic forms in culture

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(Overath *et al.* 1983), and presumably also in *Glossina*. Assuming a physiological role for the v.s.g.-releasing enzyme and the likelihood of novel steps in the biosynthesis of the carboxy terminal lipopolysaccharide-like moiety, activation of the former process or inhibition of the latter could provide new targets for therapeutic exploitation.

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