MINIREVIEW

Biosynthesis of Glycolipid Anchors in Trypanosoma brucei

Trypanosoma bruceiにおける糖脂質アンカーの生合成

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Introduction

Addition of a glycosylphosphatidylinositol (GPI) membrane anchor to a protein represents both a novel means for holding proteins to lipid bilayers and for attaching carbohydrate to a polypeptide (Cross 1990). For these reasons there has been considerable interest in this recently characterized post-translational modification. Since the first description of the structure of the GPI-anchor from Trypanosoma brucei variant surface glycoprotein (VSG) (Ferguson et al. 1988), detailed structural data for several other protein anchors and related molecules have been reported from species ranging from protozoan parasites to mammals (reviewed most recently by Thomas et al. 1990). The wide range of species shown to make GPI-anchored proteins demonstrates that this post translational modification probably appeared early in eukaryote evolution. Using the African trypanosome as an experimental model system, a pathway for the biosynthesis of the VSG GPI-anchor has been proposed. In this review we shall briefly discuss the simplest model consistent with the data available and highlight some alternative models and undocumented aspects of the biosynthesis of the GPI-anchor in trypanosomes. Also we shall describe some recent data on the developmental regulation of GPI-anchor biosynthesis in different life stages of T. brucei.

The Simplest Model

The common core of all GPI-anchors sequenced to date has the structure EthN-P-Man,GlcN-Inositol. The presence of additional substituents, e.g., α-galactose in some VSG variants (Ferguson et al. 1988), GalNAc and extra mannose and phosphoethanolamine in rat brain Thy-1 (Homsans et al. 1988), has been reported but the function of these moieties remains unclear. Anchors with the minimal core structure have been identified in VSG from T. brucei (Ferguson et al. 1988) and gp63 from Leishmania (Schneider et al. 1990). Kinetic evidence suggests that the GPI-anchor is acquired in an early post-translational event involving replacement of a short carboxyl-terminal sequence in the target protein with a preformed glycolipid (Bangs et al. 1985, Ferguson et al. 1986). Preformed glycolipid precursors containing GPI-anchor components (e.g. mannose, ethanolamine) have been detected in bloodstream...
trypanosomes (Lipids A and C, Krakow et al. 1986, Lipids P2 and P3, Menon et al. 1988) and characterized in detail (Mayor et al. 1990a, b, Fig. 1). P2 and P3 have a structure equivalent to a free GPI-anchor, with the amine of the ethanolamine underivatised. P3 differs from P2 solely by the presence of one or more fatty acid substituents on the inositol ring (Mayor et al. 1990b, see below). From these structural data the most obvious mechanism for the construction of the precursors would be by sequential glycosylation of phosphatidylinositol (PI), i.e. the addition of one glucosamine and three mannose residues followed by the addition of ethanolamine phosphate to the terminal mannose residue (Fig. 2). Glycolipids with the expected structures of intermediates on the pathway to construction of P2 and P3 were found in radiolabelling studies using crude lysates of T. brucei (Masterson et al. 1989, Menon et al. 1990a). These glycolipids contain glucosamine and zero to three mannose residues, apparently in the same linkages as seen in the VSG GPI-anchor and in P2 and P3 (Menon et al. 1990a). The glucosamine is derived from UDP-GlcNAc, and the first potential GPI-precursor is GlcNAc-PI, which is de-N-acetylated to GlcN-PI (Doering et al. 1989). All three of the mannose residues are derived from dolichol-P-mannose (dol-P-man, Menon et al. 1990b). The addition of ethanolamine phosphate to the Man, GPI-lipid appears to involve another lipid-linked donor, phosphatidyl-ethanolamine (AKM, S. Mayor, R.T. Schwarz, submitted). In addition to the glycosylation of the inositol, the fatty acids on the glycerol are altered (see below).

Other Aspects; Alternative Pathways and Topology of Glycosylation

The information outlined above provides evidence for a simple model for anchor biosynthesis. Except in the case of addition of GPI (P2 and P3) to polypeptide (S. Mayor, AKM, Menon et al. 1990a, b, Fig. 1), P2 and P3 are esterified by ethanolamine phosphoglycerolipids. A phosphaethanolamine derivative has also been detected (Masterson et al. 1989, Menon et al. 1990a). T. brucei are unique in having a phosphatidyl ethanolamine instead of a phosphatidyl choline, and in having ethanolamine as the terminal phosphate of phosphatidyl ethanolamine. The addition of glucose occurs on the ethanolamine phosphate, and the ethanolamine phosphate is not esterified by the fatty acids on the glycerol.

Fig. 2. Scheme for the biosynthesis of P2 and P3 GPs in bloodstream trypanosomes. In this model the GPI is translocated from the cytoplasmic face of the RER to the lumen as GlcN-PI. It is possible that the GPI in fact crosses the membrane at any point following synthesis of PI. The phospholipid is glycosylated and ethanolamine phosphate is added. Remodelling of the diglyceride and inositol acylation occur late in the sequence. PPI, the GPI in procyclines, is probably made by a similar pathway, with some alteration in the final phases.

Key: ■ GlcNAc; ○ Mannose; □ GlcN; ● Ethanolamine phosphate; ◆ Inositol; ◆ ◆ Fatty acyl inositol; ▴ ▴ Diacylglycerol.
G.A.M. Cross, submitted, and see below) and the conversion of GlcNAc-PI to GlcN-PI (Doering et al. 1989), it has not been formally demonstrated that there is a true precursor-product relationship between the various structures identified, and we are left with an essentially static picture of steady-state levels of GPI-lipids in T. brucei membranes. With only this information, it is not possible to be confident that the suggested route for addition of monosaccharides to PI is correct or that it is the sole route by which P2 and P3 are built up. For example, it is a formal possibility that the species containing two mannose residues could be trimmed by an α-mannosidase, with the subsequent addition of a dimannose saccharide. Whilst this is unlikely (most glycosylation reactions proceed by the addition of monosaccharide residues), such dol-P homopolymers have been detected in other systems (Spirto and Spirio, 1985). If mannosylation was to proceed exclusively by addition of single mannosides, it would be predicted that, under conditions of nonsteady state labelling, the specific activity of the second mannoside should be intermediate between that of the other two, as label would be diluted by pools of partially constructed GPI within the ER. However, methylation analyses show that the specific activity of the second (middle) mannoside from ethanamine-containing GPIs is lower than that of the first (linked to GlcN) (Menon et al. 1990b, MCF, AKM, G.A.M. Cross, submitted), consistent with a trimming and addition pathway rather than sequential addition. The trimming and addition model is not incompatible with the observations that dol-P-[3H]man labels all the mannose residues in P2 and P3, and therefore cannot be discounted. It has recently been shown that there exists more than one pathway for processing of glucosylated oligomannosidic glycans in mammalian cells (Moore and Spirio 1990), so the presence of more than one route for GPI-biosynthesis operating in the same cell remains a possibility.

Investigation of the biosynthesis of the ManGlcNAc2-PP-dol donor for N-linked glycans has shown that UDP-GlcNAc can cross the ER membrane but GDP-Man cannot (Hirschneg and Sniider 1987). In this case the GlcNAc residues are added to dol-P on the cytoplasmic face of the ER (Abeijon and Hirschneg 1990). The first five mannose residues are added from GDP-Man (Hirschneg and Sniider 1987), and the lipid then crosses the ER membrane to the luminal leaflet where the last four mannose residues are added from dol-P-Man. Although dol-P-Man is formed from dol-P and GDP-Man in the cytoplasmic leaflet of the ER, it has been shown to partition into the luminal side (Hirschneg and Sniider 1987).

There have been no studies of the topology of the glycosylation reactions leading to the formation of GPI. PI is synthesised from inositol and CDP-diglyceride on the cytoplasmic face of the RER (Bell et al., 1981), and clearly the completed precursor must end up on the luminal face for addition to newly translocated polypeptide to occur. As UDP-GlcNAc, dol-P-
Man and PE have access to both faces, addition of all the glycolipid headgroup substituents could occur on either side of the ER membrane. However, assuming that the enzymology of glycosylation is similar for both PI and dol-P, a single localization for the growing GPI represents the most simple mechan-ism. It is possible that synthesis begins on the cytoplasmic face, by glycosylation of PI by UDP-GlcNAc and is followed by translocation to the ER luminal face, where three α-mannose residues from dol-P-Man are then added. Until the relevant intermediates are localized to one or other face of the ER membrane, the step at which translocation takes place remains open to speculation.

Only a small fraction of the PI within the trypanosome cell is converted to GPI. The selection of specific PI for GPI assembly may be based on a structural feature (i.e., fatty acid composition), a membrane localization effect (which could correlate with a structural subpopulation of PI) or stochastic selection. The observations that GPIs in T. brucei cannot be labelled with 3H-inositol in either the bloodstream or procyclic stage, whilst PI is efficiently labelled (our unpublished observations), suggests that a specific subpopulation of PI is utilized for GPI biosynthesis. In the case of the GPI-anchor from Torpedo marmorata the lipid composition is completely different to that of cellular PI, suggesting that a subpopulation is selected for GPI biosynthesis (Bukitoe et al. 1990).

The mannosyltransferase activities within the ER lumen which are involved in construction of GlcMan,GlcNAc2-MPI and dol, are capable of synthesis of Man5C1-2,3,6 linkages, whilst the cytoplasmic transfers synthesise Man5C1-2,3,6 and Manβ1-4 linkages. In the GPI-lipid glycan, the GlcN linkage to inositol of the Man5C1-4 linkage have not been seen in other lipid- or protein-linked glycans, whilst the Man5C1-6 linkages are common to the N-linked glycan pathway. It is not known if the same enzymes catalyze addition to GPI as well as dol-P linked species, but at least some of the ER glycosyltransferase activities involved in GPI synthesis must belong exclusively to this pathway. The influence of aglycons such as GlcN-PI and GlcNAc2-MPI on the specificity of ER mannosyltransferases is uncharted at this time. In this regard, it should be borne in mind that the trypanosomatids have unique differences in their synthesis of N-linked glycans and subsequent processing of the glycans after they have become attached to protein (see Bosch et al. 1988 and references therein), so that an extrapolation from mammalian and avian systems (where most of the data on N-glycosylation have been obtained) to these organisms may not be warranted. However the core glycans of the GPI-anchors so far characterized are conserved between the protozoan parasites and mammals (e.g. VSG and Thy-1, Homans et al. 1988) so that at least some comparisons are valid, and the major features of the GPI biosynthetic pathway are probably conserved.

Further elaboration of the protein-linked GPI probably
occurs as a late event (Bangs et al. 1988), and may take place within the Golgi cistermae. This location is consistent with the types of modifications seen, e.g. addition of galactose (VSG) and GalNAc (Thy-1), as it is known that galactosyltransferases and GaINAC transferases are present within the Golgi (Kornfeld and Kornfeld 1985). In the case of VSG, a variable number of β-galactose residues are added to the core glycan at this stage. Similar to processing of N or O-linked glycans (Kornfeld and Kornfeld 1985), this results in microheterogeneity at a specific site, e.g. the major VSG 117 GPI-anchor species contains two to four β-galactose residues (Ferguson et al. 1988). In addition, different VSG variants have a different overall number of β-galactose residues, consistent with the view that the degree of processing is influenced by the polypeptide (Ferguson and Williams 1988). GPI-anchors from other proteins contain modifications other than β-galactose, so that the cell type is also important in determining the final spectrum of structures present on the mature glycoprotein. However, as the data base of full GPI-anchor glycan structures is currently small it is not possible to draw detailed conclusions with regard to the control or importance of structural microheterogeneity.

In bloodstream form trypanosomes, a minor population of GPIs also contain α-galactose substituents (S. Mayor, AKM, G.A.M. Cross, manuscript in preparation). Whilst the significance of this is unclear, one possibility is that the elaboration of these glycolipids is due to the escape of GPI, that have not been transferred to protein, from the ER to the Golgi, where they may then act as acceptors for glycosyltransferases. GPI headgroups larger than the core glycan have also been seen in procyclic form T. brucei (Field et al. submitted). In this regard it is interesting to note that ceramide-based glycolipids are synthesized in the Golgi, and are transported unidirectionally to the plasma membrane, and not back to the ER (van Meer 1989). A similar vectorial transport may affect GPIs that migrate into the Golgi, i.e. a retrieval pathway such as that seen for ER proteins (Pelham 1987) may not be present for glycolipids.

Fatty Acid Substituents; Remodelling and Inositol Acylation

The VSG anchor contains exclusively dimyrystylglycerol (Ferguson et al. 1985), even though myristic acid is a rare fatty acid component of phospholipids in eukaryotic membranes. P2 and P3 (glycolipids A and C in Masterson et al. 1989) are the only GPIs to be labelled with 3H-myristate in a trypanosome in vitro system (Menon et al. 1990b), suggesting that the PI that is initially glycosylated is not dimyrystylPI. It has subsequently been reported that the fatty acid c1 of the glycerol is initially stearic acid (C18:0), but is remodelled to myristate in the mature GPI precursor P2 (Masterson et al. 1990). The original fatty acid at C2 of the glycerol has not been identified.
The mechanism for remodelling of the precursor involves the removal of the fatty acid at C2, followed by the addition of myristic acid in its place. The second fatty acid at C1, stearic acid, is similarly removed and replaced by myristate (Masterson et al. 1990). Therefore two lyso intermediates and a 1-stearoyl, 2-myristyl species are produced during this process. A complication of this model involves the presence of the second GPI-lipid, P3 (Fig. 1). Like P2, P3 also contains only myristate in the glyceride portion (Mayor et al. 1990b) and therefore is also remodelled. However, the inositol residue in P3 may be labelled with palmitate.

P3, although present at levels comparable to P2 (Mayor et al. 1990a), is not found linked to VSG in the bloodstream trypanosome, despite being capable of transfer to VSG in vitro (S. Mayor, AKM, G.A.M. Cross, submitted). P3-type GPI-anchors do occur, e.g. in human erythrocyte acetylcholine esterase (Roberts et al. 1988) and the procyelic acidic repetitive protein (PARP) (Clayton and Mowatt 1989, our unpublished observations). It has been proposed that P3 functions during the remodelling process: the monomyristoyl-P2 that must be generated during the exchange of the fatty acid at C1 would be quite polar and might not remain tightly associated with the ER membrane (Masterson et al. 1990). The inositol may then be acylated following the remodelling of the first fatty acid, and decylated after completion of the second. Only a static picture of this process is available at present, and in the absence of kinetic data any proposal must remain hypothetical. Enzyme activities necessary for the remodelling (acyl-CoA transferase and phospholipase A1) have been described in T. brucei (Samaad et al. 1988), although these enzymes have not been biochemically characterized in detail.

The acylation of the inositol could also be important in the selection of GPI molecules for subsequent glycosylation (see above). The presence of the fatty acid could alter the conformation of the PI headgroup considerably, and therefore may provide a basis for the specificity of the UDP-GlcNAc: PI transferase reaction. Alternatively the acylation may facilitate the (probably) protein-mediated flip-flop of PI species so that the headgroup then faces the ER lumen. This latter possibility would also provide selectivity for the elaboration of the GPI headgroup, especially if the glycosylation reactions are all localized in the lumen.

The function of VSG GPI-glycan elaboration and the remodelling of the fatty acid substituents of the precursor P2 is as yet unclear. In other GPI-anchors analysed to date, (Thy-1 (Homanus et al. 1988), human and bovine erythrocyte acetylcholine esterase (Roberts et al. 1988), and gp63 from Leishmania (Schneider et al. 1990)) there is no specific requirement for either a single or a highly restricted glyceride moiety in terms of alkyl or acyl substituents, and therefore the specificity seen in VSG cannot be a requirement of GPI-anchors per se. In (Menon et al. 1990b). This is due to glycosylation of PI which may be different in PI glycan-anchored VSG in vivo. The PI in VSG is therefore subject to two additional modifications: N-acetylation and O-linked glycosylation.

The fatty acid of the precursor is predominantly stearic acid (C18:0), although a small amount of linoleic acid (C18:2) is also present (Mayor et al. 1990a). The fatty acid of the mature VSG is predominantly stearic acid, with a small amount of linoleic acid. The fatty acid of the mature VSG is therefore subject to two additional modifications: N-acetylation and O-linked glycosylation.

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fact, such a restriction cannot even be extended to other GPI-anchored proteins in T. brucei as different fatty acids are present on PARP (MCF and M.A.I. Ferguson, unpublished observation, see below), and therefore this peculiarity is restricted to VSG and its precursors and suggests functional importance.

The picture is made more complex in the bloodstream trypanosome by the presence of other GPI-species with acyl
inositol, at comparatively low levels, that are equivalent to the di- and trimannosylated species probably involved in the
sythesis of P2 (Mastersen et al. 1989, Menon et al. 1990, Fig. 2). The presence of these species may indicate that the synthesis of P2 and P3 is separate, perhaps diverging at the monomannosyl
species or even earlier. Alternatively the acyl group on the inositol may be capable of being added and removed so that P2 and P3, and their respective precursors, are in equilibrium. It is also plausible that the presence of the smaller P3-type GPIs (see also a recent report of GlcN-acyl P1 in Saccharomyces cerevisiae (Orlean 1990)) represents the promiscuity of the inositol acylase, and is not physiologically relevant. The control of the level of the P2 and P3 type of GPI precursors may be under development regulatory, and is discussed in the next section.

Developmental Regulation

During the spread of infection T. brucei are taken from the blood of an infected mammal by Tsetse flies (Glossina spp.). The parasite undergoes significant biochemical and morphological alterations in the insect host. PARP and VSG are developmentally restricted in their expression, the former only seen on procyclic cells, and the latter only on bloodstream form (Roditi et al. 1989). It is important to realise that the environment that the trypanosome encounters in the insect host is vastly different from that in the mammalian bloodstream, not least of which is a 10°C drop in temperature.

The GPI-anchor of PARP is P1-PLC resistant, suggesting a P3-type anchor structure (Clayton and Mowatt 1989). It is possible that an alteration in GPI biosynthesis accompanies the bloodstream to procyclic form transformation, but the presence of both P2 and P3 in bloodstream form trypanosomes is consistent with the alternative hypothesis that the specificity of the transferase responsible for adding the GPI to protein may be altered, so that P3 anchors are transferred in procyclic cells.

A GPI-lipid, PP1, with a structure similar to P3, has been identified in cultured procyclic (insect stage) cells (Field et al. submitted). PP1 contains an ethanalamine-phosphoglycan and a palmitic acid substituent on the inositol ring identical to P3. However, the glyceride structure is very different to P3. PP1 lacks a fatty acid at the C2 of the glycerol, and has stearic acid at C1 (Fig. 1). Also procyclic cells are devoid of P1-PLC sensitive GPI species, so that a P2-type of GPI is not made by these cells (Field et al. submitted).

The phospholipid moiety in the GPI-anchor of PARP is

マニアのgp63(Schneider et al. 1990)におけるアールキルまたは
アールキル置換基といった部分に関しては、単一または高度に制
定されたグリセロイド分子への特異性要望は見当たっていな
い。それゆえに、VSGにおいて観察される特異性は、GP1-アン
カーそれ自体の特性とはいいえない。事実、そのような限定
は、PARPには異なった脂肪酸が存在すること
(MCF & M.A.I. Ferguson 未発表データ、以下を見よ)を証明することも
できる。T. bruceiの他のGP1-アンカー蛋白質にさえ当ては
めることができない。それゆえに、この特徴はVSG及びその
前駆体のみに限定されており、その機能における重要性を示唆
している。

血液中のトリパノゾーマの場合は比較的低レベルである
が、アールキル化されたイノシトールをもつ他のGP1-タイプの存在によ
り、事情はもっと複雑である。それは異なれないパック2の合成に関与す
る2つまたは3つのマンノース残基をもつ種に対応している(Mas
tersen 1989, Menon 1990, 図2)。これらの種が存在すると
という事実は、P2とP2の合成が、多分モノサルファニル型の時点ま
たはもっと早い時点で分解した、別の機能である可能性を示し
ている。一方、イノシトールのアルキル基は、P3型またはそれ
らの前駆体が平衡になるように付加されたり、除去されること
ができるかもしれない。また、小さなP3タイプの
GPI(Saccharomyces cerevisiaeにおけるGlcN-acyl P1 閉近の線文を参照)(Orlean 1990)が、イノシトールアシル化の無
差別的な作用を反映しており、生理的な寄生者は少ないと、という
説もうもっとらしい話ではある。GPI前駆体におけるP2および
P3タイプの量的な制御は発生制御のもとにおいて危急に含まれている可
能性もあり、次項で述べたい。

発生の制御

感染の拡大においては、ツェツエバエ (Glossina属)によ
る、感染した哺乳動物の血液からT. bruceiの遊走がおこる。寄
生虫は昆虫宿主の体内で有機の生化学的、形態学の変化をお
こなう。PARPとVSGは発生段階においてその発現が制限さ
れている。前者はprocyclic細胞にのみ存在するが、後者は血液型
にのみ存在する(Roditi et al. 1989)。昆虫宿主体内中のトリパノゾー
マでは、哺乳動物血液中のものは、むしろ欠乏した環境、すな
わち少なくとも10°Cも低い環境におかれていることを理解して
おくことが必要である。

PARPのGPI-アンカーはP1-PLC抵抗性であり、このことは
P3タイプのアンカー構造であることを示している。血清型から
procyclic型への形態変化にもって、GPI生合成の変化が起こ
るかということも考えられる。しかし、血清型のトリパノゾーマ
においてP2とP3の両方が存在するということは、GPIを蛋白質
に付加する転移酵素の特異性が、例えばP3アンカーはprocyclic
細胞において転移されるということに変化する、というもう一
つの仮説と合致している。

GPI亜種であるPP1はP3と類似した構造を持つており、塩
基(procyclic細胞(昆虫段階)中)で同定されている(Field et al. 投稿中)PP1はP3と同様にイノシトールアミノ酸化酵素およびイノ
structurally similar to the phospholipid in PP1. Identical lipid fragments are released from PARP and PP1 by GPI-PLD and nitrous acid treatment, and the fatty acid composition at the two sites of fatty acylation are also the same (Field et al. submitted, and our unpublished observations). This evidence strongly suggests that PP1 is the precursor of the PARP anchor and that this anchor is very different from that on VSG.

Thus in contrast to the bloodstream form trypanosome, the procyclic cell produces GPI-lipids that are exclusively of the acyl-inositol (P3) type. The absence of myristic acid in the glyceride of PP1 shows that the remodelling process seen for P2 and P3 does not occur in the procyclic, and therefore is developmentally regulated. In this regard it is interesting to note that the fatty acid identified at the C1 of the glycerol in both PP1 and unremodelled P2/P3 is the same, i.e. stearate. PP1 resembles an aborted remodelling intermediate, in that the fatty acid at C2 is not present. There is currently no information about biosynthesis of PP1, and it is not known if PP1 is synthesised from a diacyl-P1 or if the lyso structure is produced early or late in the pathway. The enzyme responsible for the putative deacylation of PP1 may or may not be the same as that acting on P2 and P3 during remodelling.

A further complication, with respect to the presence of both P2 and P3 in bloodstream trypanosomes, is the observation that P2, P3 and PP1 can be transferred to VSG in an in vitro system (S. Mayor, AKM, G.A.M. Cross, submitted). Whilst PP1 is not strictly relevant in this context as it is not present in bloodstream ER membranes, its transfer competence demonstrates that there is no requirement for dimyristoylglycerol-containing GPIs for addition to VSG. The ability of P3 to be transferred, despite the observation that no such GPI-anchor is seen on VSG in vivo is an enigma. Explanations for this could include either the loss of a cofactor in the in vitro system, which restricts transfer to P2, the sequestration of P3 in vivo in a compartment that is separate to that where transfer occurs, or the rapid inositol deacylation of a P3-type GPI-anchor following addition to protein. At present, it remains to be shown whether P2 and P3 are in equilibrium in vivo. In conclusion we suggest that the control of inositol deacylation is under developmental regulation.

Concluding Remarks

The African trypanosome has provided an excellent system for the study of GPI-anchor biosynthesis. Investigations using the bloodstream form have detailed the structures of a mature GPI-anchor and its biosynthetic precursors, and in the procyclic form evidence for developmental regulation of GPI biosynthesis has emerged. At least two reactions in the pathway of GPI assembly involve lipid donors of GPI components, with implications for the membrane topology of the assembly process. However, descriptions of the subcellular membrane

 Toledo 注においてパルミチン酸残基を持っている。しかしながら、グリセリドの構造はP3と非常に異なっている。PP1はグリセリドのC2位の脂肪酸が欠けており、C1にはステアリン酸を持っている（図1）。また、procyclic細胞はPI-PLC感受性のGPI種を持っていない。そのため、P2タイプのGPIはこれらの細胞では合成されない（Field et al. 投稿中）。

PARPのGPIアンカーのリン脂肪酸分は構造的にはPP1のリン脂肪酸と同じである。PARPとPAPIからGPI-PLDやPGDGase処理により構造の脂肪酸成分が遊離される。そして、脂肪酸アシル化の2つの部位の脂肪酸組成もまた同じである（Field et al. 投稿中、及び我々の未発表データ）。この事実は、PP1およびPARPアンカーの質感体であること、このアンカーはVSGのそれとは非常に異なっていることを強く示唆している。

このように、血液型トリパノソーマーに比べて、procyclic細胞は、例外なくアシルイノシトール(P3)タイプのGPI脂質を合成する。PP1のグリセリドにミリスト酸が欠けていることは、P2およびP3へのremodellingはprocyclicではなくないと考えると、そのために発生的に制限されていることを示唆している。この事に関して、P1とremodellingされていないP2/P3におけ るグリセリドのC1位の同定された脂肪酸は、同じステアリン酸であるということは興味深い。PP1はC2位における脂肪酸が存在しない型の、remodellingの中間体と似ている。PP1の生合成については、現在はどんどの報告もされていない。また、PP1がジアシルP1から生成するのかどうか、そしてリビパ体の生合成が過程の早い時点で起こるのか、遅くに起こるのかについても分かっていない。PP1の予想される脱アシル化に関する酵素が、P2とP3のremodellingに作用する酵素と同じかどうかは今後の研究で検討する必要がある。

より複雑な事象は、血液型トリパノソーマーにP2およびP3両方が存在することに関して、P2、P3及びPP1がin vitroシステムでVSGに転移されるという事実である（S.Mayorら 投稿中）。PP1は、それが血液型ER膜に存在しないという点においては厳密ではなかったかもしれないが、その動的な転移はVSGへの付加においてジアシルグリセリドを含むGPIに対応する要求性がないことを示唆している。P3が転移される能力は、in vivoではそのようなGPIアンカーは見られないことからすれば、難である。これに対する説明には、in vitroシステムでP2に対する限定的な転移を行わせるような補助因子が失われたためであるか、P3はin vivoでは、転移が起こるような場所から隔離されて別の場所に押し込まれているためであるか、蛋白質に付加され、速やかにP3タイプのGPIアンカーのインソトールの脱アシル化が起こるような理由が考えられる。

現実、P2とP3がin vivoで平衡状態にあるかどうかはまだ明らかにされていない。結論として、われわれは、インソトールの脱アシル化のコントロールは発生制御のものとされているということを示唆し得た。

おりに

アフリカ産トリパノソーマーはGPIアンカーとその生合成の
localization of individual biosynthetic steps and the distribution of lipid intermediates still need to be obtained, and questions concerning the role of inositol acylation and the enzymology and control of GPI biosynthesis remain to be explored.

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