in neocortex folding at E18.5, reminiscent of gyrfication, a hallmark of human neocortex (Fig. 4). Cortical plate area in the gyrus-like structures was increased compared with the contralateral smooth neocortex, with proper cortical lamination.

The methodology for isolation of cortical progenitor subpopulations established here can be applied to other mammalian species, including primates, opening avenues for comparative evolutionary studies. Furthermore, the present transcriptome data provide insight into molecular differences between the various types of cortical NPCs in developing mouse and human neocortex and constitute a resource for future studies.

A very recent, independent analysis of human radial glia transcriptome (39) has concentrated on genes present in both mouse and human genomes but expressed only in human cortical progenitors, identifying a role for platelet-derived growth factor signaling (16) in human radial glia. In contrast, we focus here on genes present only in the human, but not mouse, genome and highly expressed in basal radial glia.

Thus, we identify ARHGAP11B as a human-specific gene that amplifies basal progenitors and is capable of causing neocortex folding in mice (33, 34). This probably reflects a role for ARHGAP11B in development and evolutionary expansion of the human neocortex, a conclusion consistent with the finding that the gene duplication that created ARHGAP11B occurred on the human lineage after the divergence from the chimpanzee lineage but before the divergence of the human lineage after the divergence from the Neandertal whose brain size was similar to that of modern humans.

Note added in proof: In work published after online publication of this paper, Johnson et al. (35) used a complementary approach to similarly isolate and compare the transcriptomes of human and mouse apical and basal radial glia.

REFERENCES AND NOTES

PARASITOLOGY

The in vivo dynamics of antigenic variation in Trypanosoma brucei

Monica R. Mugnier, George A. M. Cross, F. Nina Papavasiliou*

Trypanosoma brucei, a causative agent of African Sleeping Sickness, constantly changes its dense variant surface glycoprotein (VSG) coat to avoid elimination by the immune system of its mammalian host, using an extensive repertoire of dedicated genes. However, the dynamics of VSG expression in T. brucei during an infection are poorly understood. We have developed a method, based on de novo assembly of VSGs, for quantitatively examining the diversity of expressed VSGs in any population of trypanosomes and monitored VSG population dynamics in vivo. Our experiments revealed unexpected diversity within parasite populations and a mechanism for diversifying the genome-encoded VSG repertoire. The interaction between T. brucei and its host is substantially more dynamic and nuanced than previously expected.

The protozoan parasite Trypanosoma brucei, a major cause of human and animal Trypanosomiasis, lives extracellularly within its mammalian host, where it is constantly exposed to the host immune system. T. brucei has evolved a mechanism for antigenic variation during infection in which the parasite can turn on and off variant surface glycoprotein (VSG)—encoding genes from a genomic repertoire of ~2000 different genes (1). Each parasite expresses one VSG at a time, from one of ~15 telomorphic expression sites (2); the rest (silent VSGs) sit in silent expression sites or in other genomic locations (2). The highly antigenic VSG is so densely packed on T. brucei’s surface that it obscures other cell-surface components from immune recognition. At any time, a few parasites in a population will stochastically switch their VSG. As previous variants are recognized by the immune system and cleared, newly switched variants emerge, giving rise to characteristic waves of parasitemia (3). These waves have long been interpreted as the sequential expression and clearance of one or a few VSGs, a notion supported by experimental evidence that relied on low-resolution approaches (4–6).

Despite attempts at modeling, little is known about the kinetics of VSG expression during infection (9–12). To assess this, we developed a targeted RNA sequencing (RNA-seq) approach, termed VSG-seq, in which VSG cDNA, amplified by using conserved sequences at the 5’ and 3’ ends of every mature VSG mRNA (fig. S1), is sequenced and then assembled de novo by a transcriptome reconstruction method called Trinity (13). We validated

s t r u c t i o n m e t h o d c a l l e d T r i n i t y ( s e c t i o n 2.2). To assess this, we developed a targeted RNA sequencing (RNA-seq) approach, termed VSG-seq, in which VSG cDNA, amplified by using conserved sequences at the 5’ and 3’ ends of every mature VSG mRNA (fig. S1), is sequenced and then assembled de novo by a transcriptome reconstruction method called Trinity (13). We validated and discussion, especially D. Sterzel for support in obtaining fetal human tissue, J. Paridaen and M. Wilsh-Bräuninger for advice, and N. Kalibic and K. Long for critical reading of the manuscript. We thank B. Höber and A. Weihmann of MPI-EVA for expert DNA sequencing; B. Habermann of Max Planck Institute of Biochemistry (MPI-B) for bioinformatics advice; and K. Kalbuchi and M. Amano (Nagoya University) for pCAGGS-myC-KK1, pCAGGS-HA, and anti-MYP11 antibody. M.F. was a member of the International Max Planck Research School for Cell, Developmental and Systems Biology and a doctoral student at the Technische Universität Dresden. W.B.H. was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) (SFB 655, A2) and the European Research Council (25097), the DFG-funded Center for Regenerative Therapies Dresden, and the Fonds der Chemischen Industrie. The supplementary materials contain additional data. RNA-seq raw data have been deposited with the Gene Expression Omnibus under accession codes GSE56000 and GSE185634.

SUPPLEMENTARY MATERIALS

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this approach using mixtures of *T. brucei* cell lines expressing specific VSGs in known proportions (Fig. 1 and fig. S1). We compared measured expression of each VSG in the control populations with the known input and found that we could accurately assemble a VSG sequence expressed in as few as nine cells in the control mixture. VSG-seq is capable of reliably detecting variants present on 0.01% of parasites and quantifying a variant’s presence within the population, for variants present above 0.1% of the population (Fig. 1B and fig. S1). The apparent overestimation of minor VSGs in this control experiment is likely a result of low-level switching in the more abundant components of the mixture or low-level transcription of silent VSGs. The limits of detection and quantification for VSG-seq appear to be independent of starting cell number because control mixtures made from 10^6 or 10^7 cells show similar results (Fig. 1B and fig. S1).

To measure VSG expression within populations of *T. brucei*, we infected four mice with ~5 EATRO1125 parasites—originally expressing VSG AnTat1.1 (14, 15) but now heterogeneous and each expressing a distinct VSG—and tracked VSG expression dynamics for 30 days. A few variants made up the majority of the population at each time, but surprisingly, each sample also contained many rare variants that would have been undetectable by using previous approaches (Fig. 2A and fig. S2). Infections showed great diversity even within parasitemic valleys. VSG-seq identified an average of 28 variants at each time point during the first 30 days of infection (Fig. 2C).

One mouse (mouse 3) survived much longer than the other three (106 days, compared with 41 to 72 days). VSG identity has not been shown to affect growth rate or induction of the immune response (16, 17), so the increased survival and lower diversity in this mouse are more likely due to the polyclonal germline B cell repertoire, which is unique to each mouse (18), rather than the initiating VSG. Although in the later stages of this infection, VSG dynamics did appear qualitatively different—with variants persisting longer before clearance (Fig. 2B), possibly owing to immune system exhaustion—parasite populations remained diverse, with 30 to 66 variants detectable at each sampling (Fig. 2C).
To see whether these infections showed any bias or hierarchy in VSG expression (6, 8, 19, 20), we compared the VSG repertoires of all four mice. During the first 30 days of infection, 192 VSGs were expressed. Although each infection initiated with a different major VSG, the majority of variants (86%) appeared in more than one infection, and nearly half (46%) appeared in all four infections (Fig. 3C). Ninety-seven VSGs were expressed in mouse 3 from days 96 to 105. We compared the later occurring VSGs with those expressed early in mice 1, 2, and 4 and found none in common, even though early variants from mouse 3 also appeared frequently in mice 1, 2, and 4 (Fig. 3D).

Our experiments revealed striking diversity within each infection, but surprisingly frequent occurrence of the same VSGs in different infections. Within these diverse populations, many variants appeared transiently. We have termed these “minor” variants. By examining the fate of every variant, we found that at any time during the first 30 days of infection, about half (53%) of the variants present will never reach 1% of the population (Fig. 3A). Of the 48 VSGs that appeared in all four infections, few were consistently dominant, and few were only ever expressed as a minor variant (Fig. 3B). This implies that variant success is not determined only by the expressed VSG. Instead, variant success is likely to be determined by interactions between the parasite and the humoral immune response in each animal. Because of antigenic similarity among some VSGs and their consequent elimination by cross-reacting antibodies, the effective VSG repertoire will be smaller than the repertoire that the genome is capable of generating.

Besides losing variants to cross-reactivity, T. brucei’s genomic VSG repertoire consists of a high proportion of incomplete VSG genes or pseudogenes (1, 21). Indeed, the 289 VSGs observed in our infections may represent more than half of the complete VSG repertoire (~400

Fig. 3. Variant emergence during infection. (A) Minor variants present at each time point (mean ± SD). A minor variant is arbitrarily defined as any VSG that never exceeds 1% of the population during the course of infection in a single mouse. Major variants are any variant that exceeds 1% of the population at some point during infection. (B) Venn diagram comparing the fates of VSGs appearing in all four infections. (C) Intersection of sets of VSGs expressed during early infection (days 6 to 30). The total number of VSGs is listed in parentheses below the mouse number. (D) Venn diagrams showing intersection of VSGs expressed early in infection (VSGs from mouse 1, 2, or 4 versus VSGs from mouse 3, days 7 to 30) and intersection of VSGs expressed early in infection with VSGs expressed late in infection (VSGs from mouse 1, 2, or 4 versus VSGs from mouse 3, days 96 to 105).

Fig. 4. Mosaic VSGs can be identified throughout infection. (A) Transient expression of a mosaic VSG in the population. PCR confirmation of the mosaic is shown below. The black line represents total parasitemia at each day after infection, and the green line represents the number of parasites expressing the mosaic VSG. “n.q.” indicates that the VSG is detectable within the population, but not quantifiable. “n.d.” indicates that the VSG is not detectable within the population. Below the graph are products from PCR of gDNA at each time point, by using either primers specific for the mosaic VSG or the control gene, ura3. This VSG could not be amplified when first detected with VSG-seq, likely because of low cell numbers in the DNA sample (probably less than 10 cells). (B) Mosaic from late infection, with PCR confirmation of the mosaic shown below.
complete and predicted to be functional VSGs for the Lister427 strain (1), although the VSG repertoire for the EATRO1125 strain has not been fully elucidated. The 65 to 135 VSGs observed before day 30 could represent up to 35% of the preexisting repertoire. Given the sampling frequency in our experiment, these values almost certainly underestimate the expressed VSG diversity in vivo. Therefore, much of the intact VSG repertoire is likely to have been expended early in an infection, as a result of expression and subsequent recognition by the immune system. As a result, the preexisting repertoire of complete VSGs would appear to be insufficient to support the sometimes-years-long infections observed in the field. Although parasitemia is much lower in natural hosts, preexisting immunity is common in native mammals (22), requiring constant VSG diversification to sustain infection.

Segmental gene conversion events have been demonstrated in both Trypanosoma equiperdum and T. brucei infections (7, 23, 24) generating “mosaic” VSGs that were not previously encoded in the genome. Previous studies had noted that mosaics tend to arise later in infection but have not determined when these variants are formed within the genome, or how. It is unknown whether mosaic VSGs form at the active expression site or within the silent repertoire before expression. To identify possible mosaics, we compared expressed VSG sequences to two independently assembled genomes for this parasite strain. Because of limitations in the amount of material available at each time point, we could choose only a few candidates for validation. To test that these were true mosaics and to determine when they formed within the genome, we used VSG-specific primers to confirm their absence from the genome of the parental strain and presence within genomic DNA (gDNA) collected during infection. We identified three mosaic VSGs using this approach. In each case, the mosaic VSG was only detectable by means of polymerase chain reaction (PCR) when it was also being expressed within the parasite population. This suggests that mosaic formation may be a mechanism within the active expression site (Fig. 4 and fig. S3). Mosaic formation may be a mechanism for increasing repertoire diversity as infection progresses, at least in these cases, shortly after expression, with subsequent transposition occurring battery. In each case, the mosaic VSG was only detectable by means of polymerase chain reaction (PCR) when it was also being expressed within the parasite population. This suggests that mosaic formation may be a mechanism within the active expression site (Fig. 4 and fig. S3). Mosaic formation may be a mechanism within the active expression site (Fig. 4 and fig. S3). Mosaic formation may be a mechanism within the active expression site (Fig. 4 and fig. S3).

REFERENCES AND NOTES

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SUPPLEMENTARY MATERIALS
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Materials and Methods
Figs. S1 to S3
References (26–30)
Databases S1 to S5
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GEOMICROBIOLOGY

Redox cycling of Fe(II) and Fe(III) in magnetite by Fe-metabolizing bacteria

James M. Byrne,1,‡ Nicole Klugelein,1,‡ Carolyn Pearce,2,3 Kevin M. Rosso,3 Erwin Appel,4 Andreas Kappler1

Microorganisms are a primary control on the redox-induced cycling of iron in the environment. Despite the ability of bacteria to grow using both Fe(II) and Fe(III) bound in solid-phase iron minerals, it is currently unknown whether changing environmental conditions enable the sharing of electrons in mixed-valent iron oxides between bacteria with different metabolisms. We show through magnetic and spectroscopic measurements that the phototrophic Fe(II)-oxidizing bacterium Rhodopseudomonas palustris TIE1 oxidizes magnetite (Fe3O4) nanoparticles using light energy. This process is reversible in co-cultures by the anaerobic Fe(III)-reducing bacterium Geobacter sulfurreducens. These results demonstrate that Fe ions bound in the highly crystalline mineral magnetite are bioavailable as electron sinks and electron sources under varying environmental conditions, effectively rendering magnetite a naturally occurring battery.

Iron is critical to all living organisms, with many bacteria having developed pathways to access iron either as a nutrient or as an electron acceptor or donor, depending on its mobility, oxidation state, and bioavailability (1). Fe(III)-reducing bacteria, including Geobacter sulfurreducens, combine reduction of Fe(III) with oxidation of organic matter or H2 for energy conservation (2), whereas phototrophic Fe(II)-oxidizing bacteria such as Rhodopseudomonas palustris TIE-1 grow in light with Fe(II) or H2 as the electron donor (3). Bacteria of the Geobacter genus and photoferrrotrophs have previously been shown to simultaneously occur in sediments (4, 5). The mixed-valent magnetic mineral magnetite (Fe3O4), which contains both Fe(II) and Fe(III) in a 1:2 ratio, is often a byproduct of these Fe-metabolization
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