The protozoan nucleus

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Abstract

The nucleus is arguably the defining characteristic of eukaryotes, distinguishing their cell organisation from both bacteria and archaea. Though the evolutionary history of the nucleus remains the subject of debate, its emergence differs from several other eukaryotic organelles in that it appears not to have evolved through symbiosis, but by cell membrane elaboration from an archaeal ancestor. Evolution of the nucleus has been accompanied by elaboration of nuclear structures that are intimately linked with most aspects of nuclear genome function, including chromosome organisation, DNA maintenance, replication and segregation, and gene expression controls. Here we discuss the complexity of the nucleus and its substructures in protozoan eukaryotes, with a particular emphasis on divergent aspects in eukaryotic parasites, which shed light on nuclear function throughout eukaryotes and reveal specialisations that underpin pathogen biology.

1.1 Introduction

Eukaryotes are distinguished from prokaryotes by the presence of multiple subcellular organelles. The first such organelle to be described was the nucleus, by the pioneering microscopist Antonie van Leeuwenhoek in the early 18th century. During the 1800s Leeuwenhoek's 'lumen' was confirmed in mammalian, plant and sea urchin cells at which point it became clear that a nucleus is a universal feature of eukaryotes, though not all cells in a multicellular organism are nucleated. The clearest purpose of the nucleus is that the nuclear envelope serves to contain the nuclear genome, just as the (normally) smaller genomes of the mitochondrion, chloroplast and apicoplast are enclosed by a membrane. However, whereas there is compelling evidence that the above non-nuclear organelles were acquired by symbiosis, the evolutionary history of nuclear acquisition is less well understood (1, 2). As a result of this uncertainty, a number of competing theories on the origin of the nucleus

have been proposed and are still contested (3), and there is no clear evidence for a similar phagocytotic event acting as the source of the nucleus (2, 4).

At least some of the difficulty in understanding the evolutionary origins of the nucleus stems from the fact that the organelle is bounded by a nuclear envelope composed of a double cell membrane, and from the realisation that the nuclear envelope is contiguous with the endoplasmic reticulum. In this context, the increasing evidence that eukaryotes may have arisen from an archaeal ancestor (5) means that a prokaryotic cell in which the genome was, most likely, associated with the cell membrane to allow segregation during cell division (6, 7) gave rise to the more complex eukaryotic cell in which the nuclear genome is separated from the cell membrane and is enclosed by a second layer of membranes. Within this generalised framework of eukaryotic cell organisation, eukaryotes have evolved considerable complexity in nuclear organisation. Moreover, it is increasingly clear that nuclear structure is intimately associated with the machineries that direct eukaryotic chromosome function, such as transcription, replication and segregation (2, 4, 8). As a result, evolution of the nucleus has allowed eukaryotes to elaborate many aspects of core biology, including gene expression, chromosome structure and organisation, and the generation of subnuclear structures and domains. Protozoan organisms, including parasites, have provided key insights into the extent of variation in these features of nuclear biology.

1.2 Multiple nuclei and mitosis

Early studies of eukaryotic cells concentrated on those that have a single nucleus within the cytoplasm, though a few examples of specialised cells (such as red blood cells) were described that lack a nucleus. It remains the case that most eukaryotic cells have a single nucleus, and this includes all cell types that have been described during the life cycle of many protozoan parasites, such as *Trypanosoma* and *Leishmania*. However, cell biological analysis of protozoans reveals that in some cases multiple nuclei are the norm. In common with all diplomonads, *Giardia* trophozoite cells possess not one nucleus, but two. Each nucleus contains a complete, actively transcribed copy of the

diploid *Giardia* genome and, indeed, the two nuclei appear to exist independently, being segregated by individual spindles during mitosis (see below)(9). Remarkably, in the infectious cyst cells of *Giardia* the number of nuclei increases to four, through mitosis without cytokinesis. During excystation cytokinesis is completed, returning *Giardia* to having two nuclei per cell, but not before some genetic exchange occurs. Most ciliates, including *Tetrahymena*, share nuclear dualism with diplomonads, but the organisation of the genome within the two nuclei is radically different and driven by spectacular genome rearrangements. The two nuclei in a *Tetrahymena* cell are called the macronucleus and micronucleus (10). The micronucleus is diploid and is inherited by mitosis, but is not transcribed during vegetative growth. However, in times of starvation the micronucleus becomes active and undergoes complex sexual genetic exchange. In contrast, the macronucleus, which does not undergo conventional mitosis, is transcribed and is polyploid, a state that arises due to genome-wide rearrangements that shatter the five chromosomes into hundreds of smaller molecules and generate amplification and loss of genes and sequences.

Nuclear dualism represents a relatively static condition of two nuclei per cell. In apicomplexan protists, even greater numbers of nuclei per cell can be found, though this appears to be a dynamic feature of the life cycle. In the parasites *Plasmodium*, *Toxoplasma* and *Eimeria*, a replication process termed schizogony has been described in which mitosis of the nucleus occurs after genome copying but without subsequent cytokinesis of the outer cell membrane, with the result that many nuclei share a single cytoplasm (11). This multi-nucleate state is transient, however, as daughter cells, each with a single nucleus, are then generated by cytokinesis. Indeed, schizogony appears related to two subtly variant processes that transiently yield cells with dual nuclei or a single polyploid nucleus (11), and all appear to be means to rapidly assemble large numbers of infective life cycle forms after the parasites invade host cells. Nonetheless, these reactions demonstrate the variable strategies that can be adopted, or must be adopted in the case of diplomonads, when eukaryotic cells undergo a critical reaction that had to evolve to tackle the greater cellular complexity presented by a nucleus: mitosis.

1.3 Genome segregation during mitosis

Segregation of the replicated genome during cell division differs radically between prokaryotes and eukaryotes. Unlike in eukaryotes, chromosome segregation in all bacteria and many archaea occurs concurrently with DNA replication (12, 13). Moreover, whereas cell biological descriptions of mitotic segregation of replicated eukaryotic DNA has been available for more than a century, and the key machinery is well known, prokaryotic chromosome segregation has been more difficult to describe, with three apparently overlapping machineries acting (of which only one group, the SMC complexes, is clearly conserved in eukaryotes)(12).

The textbook picture of eukaryotic mitosis details a number of key steps: dissolution of the nuclear envelope, alignment of condensed chromosomes in the centre of the nucleus to allow segregation of the chromosomes by 'spindle' microtubules, and reformation of the nuclear envelope around the segregated chromosomes. In this picture, two key elements are revealed, which are needed to allow the microtubules to act in chromosome segregation: the centrosome, associated with two centrioles, which acts as a microtubule organising centre outside the nucleus to direct spindle microtubules; and the centromere, which provides DNA elements on which the kinetochore complex is built to allow the spindle microtubules to attach to the chromosomes. The operation of mitosis is much more diverse than this picture, however. Mitotic condensation of chromosomes occurs to varying extents, with some compaction in *Giardia* (14) but little evidence in kinetoplastids (15). In many eukaryotes, including yeast, there is no nuclear envelope dissolution, and in apicomplexans such 'closed mitosis' is important in the generation of multinucleate cells (above) that are central to infectivity (11).

The evolutionary origin of centromeres and the kinetochore remains complex due to the absence of a mitotic spindle in prokaryotes, but broad parallels have begun to be drawn, at least in part due the contribution of SMC proteins to chromosome segregation in all cells (albeit performing potentially quite different functions in prokaryotes and eukartyotes)(2). The kinetochore is a multi-protein

assembly that has been considered conserved throughout eukaryotes, but the recent description of a potentially highly diverged kinetochore in *T. brucei* (16) calls that conservation into question and could shed light on its evolution. Indeed, the extent to which regulation of kinetochore assembly and function during segregation (mediated by the spindle assembly checkpoint) is conserved amongst eukaryotes has also been questioned in both *T. brucei* (17) and *Giardia* (18). Centrosome function is also variable. Within the apicomplexa, some organisms retain structurally variant centrioles, while in others centrioles are lacking (11), a feature shared with plants, yeast and amoeba, where microtubule spindle assembly occurs on centrosome-like structures on the nuclear envelope (1). These variations in organisation have led to the suggestion that the earliest eukaryote may have had a linked, membrane-associated centrosome and centromere (1), an organisation of chromosome segregation closer to that seen in prokaryotes. Such a scenario is consistent with a growing view that evolution of the nucleus and mitosis arose in parallel with the elaboration of internal cell membranes in eukaryotes (1, 4), a view compatible with our expanding understanding of the widespread functions provided by the proteins in the nuclear envelope.

1.4 The nuclear envelope

The envelope that surrounds the nucleus is more complex than merely being an internal cell membrane, and is perhaps the most complex structure surrounding any eukaryotic organelle (19). As mentioned above, the nucleus is surrounded by a double membrane composed of two lipid bilayers. However, this is an incomplete description, as each bilayer is not contiguous; instead the outer and inner bilayers join at thousands of locations, meaning the nuclear membrane is, in effect, composed of many flat sacs (Fig.1). In addition, the outer layer is contiguous with the ER and is thus coated in ribosomes. At the sites of outer and inner bilayer fusion are enormous nuclear pore complexes, though many other, less characterised transmembrane proteins are found in the nuclear membranes. Finally, a filamentous 'lamina' demarcates the boundary of the inner membrane and the nucleoplasm. The wealth of structure at the nuclear envelope contributes to a wide range of cellular functions.

The nuclear pore complex (NPC) is a huge entity, composed of ~30 proteins (nucleoporins), most of which are found in multicopy within the structure (20). The structure can be summarised as a series of rings that surround and provide the walls of a central channel, into which some polypetides protrude. The rings of the structure are formed by interacting groups of nucleoporins that generate structural modules, which are repeated and localise to defined positions within the NPC, such as the cytoplasmic or nucleoplasmic faces. Sequence comparisons of the nucleoporins across a wide range of eukaryotes reveals that the 'core scaffold', which interacts with the nuclear membranes, is well conserved and, interestingly, is related to membrane deforming complexes that are found in endomembrane and secretory pathways (21, 22). This observation has led to suggestions that the nucleus evolved by elaboration of the prokaryotic cell membrane through the action of protocoatomers, which have become components of the NPC (1, 4). The extent of protein sequence conservation across eukaryotes decreases as nucleoporin position within the structure moves towards the central channel, and as proteins are compared that project into the nucleoplasm or into the cytoplasm (21). Clearly, the central channel provides the major route for communication between the nucleus and cytoplasm, and the pattern of protein conservation in eukaryotic NPCs may then reflect lineage-specificity in NPC cargo or contribution of the NPC to other cell functions.

One consequence of the generation of a nucleus is that in eukaryotes, unlike in prokaryotes, transcription and translation are spatially separated in the cell. There are many stages of gene transcription, RNA processing and translation at which the level of expression can be dictated, but the NPC provides the route by which mature mRNAs are passed from the nucleus to the cytoplasm for translation. Thus, the NPC may contribute to gene expression controls, and evidence exists for at least two routes by which this occurs (23): NPC components can contribute to 'quality control' checks on mRNA integrity, and the NPC has been shown to interact with some actively transcribed genes, perhaps shortening the distance the mRNA must travel for export. In theory, the NPC could also dictate the rate of passage of different mRNAs across the nuclear envelope. In kinetoplastids gene expression is highly divergent, since virtually all nuclear genes are expressed from multigene

transcription units (24), meaning that most gene expression controls are post-transcriptional (25). Thus, it is possible that the kinetoplastid NPC may play a pronounced role in gene expression. In *T. cruzi* two factors have been described that contribute to mRNA transport across the NPC (26, 27), but whether they provide gene expression control remains unclear. Remarkably, localisation of the highly expressed *var* gene in *Plasmodium falciparum* revealed that though this occupies a perinuclear location, this is not due to association with the NPCs (28). Despite this, NPC number and localisation exhibit dramatic changes during the developmental stages of the *Plasmodium* life cycle (29), perhaps indicating a wider role for the NPC in the control of gene expression.

The above picture of a static NPC belies considerable evidence for dynamism. It is increasingly clear that several nucleoporin components of the NPC, acting in concert with nuclear transporters and GTPases, contribute to kinetochore, centrosome and mitotic spindle functions (30). Dissociation of nucleoporins from the NPC to act in mitosis is found also in *T. brucei*, where Mlp2 has been described to localise with the unconventional kinetochore, while its loss results in chromosome aneuploidy (31). A distinct form of dynamism is observed in *Tetrahymena*, where NPC composition appears to differ between the macro-and micronuclei (32), though to date the functional consequences of this variation have yet to be described.

Effects on nuclear and cellular function are not limited to the NPC, since the laminar filaments that subtend the inner nuclear membrane have profound effects on both gene expression and chromosome organisation and replication (19). However, despite the predicted important role of the lamina in providing structure to the nucleus, this key feature had, until recently, only been described within metazoan organisms, since lamin orthologues had escaped detection in other eukaryotes (33). Though it remains possible that yeast lack a nuclear lamina, it is now clear that such a structural feature is present in amoeba (34, 35) and in kinetoplastids (36), suggesting a wide distribution in eukaryotes. Understanding the evolutionary origins and distribution of the lamina remains complicated, since the laminar-like protein in kinetoplastids, termed NUP-1, shares no clear sequence conservation with lamina in metazoans and amoeba, beyond being largely composed of

repeated motifs (37). Whether lamin sequence divergence indicates functional specificity is unclear, since functional characterisation of NUP-1 in *T. brucei* suggests broad similarities with metazoan lamina activities, since loss of NUP-1 results in altered nuclear morphology, changes in NPC and chromosome distribution and increased expression of normally inactive surface coat genes (36). Nonetheless, the unusual gene expression and chromosome organisation of kinetoplastids (see below) and other protozoa may have resulted in the evolution of novel genome interactions with the nuclear envelope. In this regard, a shared feature of African trypanosomes and *Plasmodium* is the population of chromosome subtelomeres by gene families that undergo co-ordinated silencing and activation to thwart the mammalian immune response in a process termed antigenic variation (28, 38). In *T. brucei* the chromosome subtelomeres are also the targets of recombination to facilitate antigenic variation of the Variant Surface Glycoprotein (VSG) coat (39-41), while the subtelomeric *var* gene family in *P. falciparum* appears to be subject to elevated rate of mitotic recombination-driven diversification (42, 43). If and how these key survival reactions might be influenced by the nuclear envelope and the lamina has only just begun to be explored, including how widespread might be the use of nuclear actin to direct peripheral gene expression control (44).

Replication of eukaryotic chromosomes initiates at hundreds of discrete loci termed origins and displays a temporal order during S phase, with some regions replicating early and others late. In mammals, such replication timing is cell type-specific and is, at least in part, dictated by early replication of chromosome domains that are in the interior of the nucleus and in transcription-permissive chromatin, whereas late-replicating regions are in repressive chromatin, including in the lamina-associated nuclear periphery (45). How widespread the lamina is in influencing replication remains unclear, since a replication timing programme is also seen in yeast (46), whose nuclei may lack lamina. Mapping of replication origins in *T. brucei* reveals a timing programme here also, though understanding how this is established is complicated by the unusual multigenic transcription and the diverse subtelomeres (where origins could not be mapped)(47): e.g. most origins localise to promoters, but if all promoters are constitutively active what features distinguish early- and late-

acting origins; are the subtelomeres passively replicated from core origins due to their association with the lamina? However, no such replication timing programme can be found in *Leishmania* (48), meaning the influence of nuclear architecture on genome copying may vary within the kinetoplastida. Further complexity is seen when imaging replicating DNA and the replication machinery in kinetoplastid cells, since in *T. cruzi* replication appears to predominantly occur at the nuclear periphery (49), whereas there is no evidence for such localisation in *T. brucei* (50). What underlies this striking difference in closely related parasites has not been explored.

1.5 Chromosome organisation in the nucleus

In bacteria, despite a lack of clarity in our understanding of the machinery (and processes) that compacts the genome into a nucleoid, it is well established that genetic loci in the single chromosome assume specific positions within the cell (12). Such large scale organisation influences gene expression, as well as genome repair and transmission. In eukaryotes, the histones and associated factors and modifications that provide genome packing are better understood than in bacteria, and decades of analysis, first by microscopy (8) and then by next generation sequence-based 'chromosome capture' methodologies (51), make it clear that eukaryotic genomes are also not randomly distributed in the nucleus, but display considerable organisation (52). Genome folding in the nucleus is not invariant, as it can be seen to change during the cell cycle, between cell types and in quiescent relative to active cells. Nonetheless, several levels of organisation have been described (53): chromosome territories, which arise because individual chromosomes can occupy specific parts of the nucleus; localisation of active and inactive chromatin into distinct compartments, which is influenced by subnuclear structures, such as the nucleolus and the lamina at the nuclear periphery; regions within individual chromosomes can form topologically associated domains, which appear to encompass groups of genes with common expression patterns and may relate to replication timing; and, finally, on the smallest scale, chromatin loops can form as DNA binding factors interact, most obviously during control of gene expression by enhancer or silencer elements.

To date, little work has applied chromosome capture approaches to examine the layers of nuclear chromosome organisation in the nuclei of protists, though in the context of the variant organisation of their chromosomes and divergent gene expression strategies, this may be revealing. In *P. falciparum*, two studies have used chromosome capture to map the genome within the nucleus in a number of life cycle stages (54, 55). Beyond a surprising disagreement as to whether the parasite chromosomes occupy discrete nuclear territories, clustering of centromeres, telomeres and rDNA genes was found, suggesting a broadly similar design organisation to that described in, for instance, yeast and mammals. Beyond this, clustering of virulence genes was seen, as was evidence for clustering of genes that display related levels of expression, which may underlie gene expression profiles as *Plasmodium* progresses through its life cycle. By selecting for *P. falciparum* populations that express different *var* genes, it was possible for Lemieux et al (55)to show that a switch in expression does not involve large scale changes in sequence localisation, which seems to rule out a previously undetected, enhancer-like controller of *var* antigenic variation. Whether such a regulator is also absent in other pathogens that use transcription to drive antigenic variation, and how chromosome organisation might influence the dynamics of antigenic variation by recombination, awaits further analysis. Indeed, whether the predominance of multigenic transcription has a global impact on chromosome structuring in kinetoplastids is unknown. More widely, the pronounced plasticity of some protozoan genomes may warrant such analysis to reveal how variant chromosome number and ploidy is organised, tolerated and maintained.

Genome plasticity in kinetoplastids comes in a number of forms. In *T. brucei*, most of the nuclear genome is found on 11 predominantly diploid chromosomes, which range in size from ~1.0-5.0 Mb (56). However, in addition to these 22 molecules, the parasite has evolved a further ~100 aneuploid chromosomes, which are referred to as intermediate chromosomes (~1-5 copies, ~0.2-0.9 Mb) and minichromosomes (~100 copies, 50-150 Kb). The anueploid chromosomes are clearly related, sharing a core of 177 bp repeats (57), and appear to have evolved to expand the available store of VSG genes needed host immune evasion by antigenic variation (58). In evolving such an expanded

chromosome repertoire, novel strategies for transmission have emerged. Replication of each of the 22 megabase chromosomes requires multiple origins of replication (47), while segregation of each appears to involve a single kinetochore-bound centromere (16, 59). A single, bidirectional origin of replication has been suggested as sufficient for copying the minichromosomes (60), but the timing of this replication and whether it is directed by the same initiation machinery (61) remains unknown. Segregation of the aneuploidy chromosomes during mitosis appears not to involve a centromere (59) and instead the molecules are 'stacked' on the mitotic spindles such that their segregation is spatially and temporally distinct from the megabase chromosomes (62). The differing organisation of diploid megabase and aneuploid chromosome segregation appears also to extend to the machinery for chromatid cohesion (63), though transmission is remarkably efficient (64, 65).

In contrast with the chromosome-specific and localised aneuploidy (see below) seen in *T. brucei*, aneuploidy is a pervasive, potentially genome-wide feature of the nucleus in *Leishmania* and *T. cruzi*. In multiple species of *Leishmania*, both after lab-adaptation and soon after isolation from the host, multiple chromosome are found that deviate from the expected diploid configuration (66, 67); most frequently, the chromosomes are >diploid (3-5 N) but also occasionally haploid (68, 69), and fluorescent in situ hybridisation suggests that individual chromosome ploidy changes with growth (70). Very recently, similar genome-wide aneuploidy has been described in *T. cruzi* (71), indicating it is not limited to *Leishmania*. However, the relatively stable diploidy seen in the *T. brucei* megabase chromosomes, which display pronounced synteny with *T. cruzi* and *Leishmania* chromosomes (72), raises intriguing questions about how supernumery chromosomes arise and are tolerated in kinetoplastids. Whether or not aneuploidy in *Leishmania* is mechanistically related to genome-wide gene copy number variation (68, 69, 73) and gene amplification through episome formation (74), in some cases allowing adaptive changes in gene expression, remains unclear. In *T. brucei*, such copy number variation is most prominently seen in the megabase chromosomes subtelomeres, due to VSG gene rearrangements (75), perhaps indicating a differing strategy for genome maintenance. In this regard, the recent demonstration that each *Leishmania* chromosome may be copied from a

single origin of (48) reveals a pronounced difference from multi-origin chromosome replication in *T. brucei* (47), and perhaps provides an explanation for the differing pictures of genome stability in the two parasites.

1.6 Subnuclear structures

Our recent, growing understanding of the spatial organisation of chromosomes in the nucleus is related to a longer understanding that some nuclear functions are directed by detectable structures within this organelle. Though at least some of these substructures might be thought of as static, there is evidence for considerable dynamism, and the extent to which their structure is dictated by underlying chromosome sequence appears variable.

1.6.1 The nucleolus.

The most prominent subnuclear structure is the nucleolus, where rDNA transcription, modification and processing of pre-rRNA, and the initial steps of ribosome assembly occur. Eukaryotic cells contain tens to thousands of ribosomal genes, which are organized in tandem arrays on one or more chromosomes. Such gene clusters are known as nucleolus organizer regions (NORs) due to their role in nucleolus nucleation after the completion of mitosis (76). Ribosome biogenesis in eukaryotes requires numerous temporally ordered reactions, including: transcription of rDNA by RNA pol I to generate pre-rRNA, association of proteins and small nucleolar RNAs (snoRNAs) with pre-rRNA, rRNA modification by pseudouridination and methylation, processing of pre-rRNA in 18S rRNA, 5.8S and 28S, and assembling of the ribosome particle by incorporating ribosomal proteins and 5S rRNA (77). In addition, it seems now clear that the nucleolus, besides being the location of rRNA synthesis, is the site of modification and assembly of other RNAs and ribonucleoproteins (RNPs) (78), including the telomerase RNP and the signal recognition particle (SRP)(79-82). In trypanosomes the SRP has been described to also assemble in the nucleolus, although it lacks Alu domain-binding proteins (83). Importantly, the nucleolus also directs cellular responses to several forms of stress, as well as regulating proteins important for cell cycle progression (78).

The temporal organization of the reactions involved in ribosome biogenesis is facilitated by sub-compartmentalisation of the nucleolus, allowing the pre-rRNA to visit various nucleolar compartments before mature pre-ribosome particles are assembled. These nucleolar subcompartments are distinguishable by transmission electron microscopy (TEM), meaning that in human cells the nucleolus can be divided into three elements: Fibrillar Centers (FCs; present in single or multicopy), which are surrounded by a dense layer of fibrous material called the Dense Fibrillar Component (DFC), and a granular region called the Granular Component (GC) in which the above structures are embedded. Flourescence microscopy has revealed that each one of these structures have different compositions, with elements involved in transcription (the rDNA loci, RNA pol I and DNA topoisomerase I) located in the FC, proteins involved in the early stages of rRNA maturation (e.g. fibrillarin and nucleolin) in the DFC, and the GC proteins (e.g. ribosomal protein S1 and ribocharina) contributing to later stages in pre-ribosome assembly (84). Although division of the nucleolus into FC, DFC and GC compartments has been taken as universal amongst eukaryotes, it has been suggested that a tripartite nucleolus only in fact appeared late in evolution, during the transition from anamniotes to amniotas (85). Thus, many organisms have a simpler, bipartite nucleolus (Thiry and Lafontaine 2005). In these organisms, the fibrillar component comprises elements of both the FC and DFC, while the GC is essentially invariant from that of a tripartite nucleolus. It is now widely accepted that both the transcriptionally active rDNA genes and the transcription reaction (measured by incorporation of BrUTP) are located in fibrillar components of both types of nucleoli (Thiry and Lafontaine 2005) and, subsequently, the rRNAs mature during their migration to the GC. This migration process can be observed by optical fluorescence microscopy using pulse-chase BrUTP RNA labeling, which shows that in mammalian cells rDNA transcription co-localizes with RNA pol I in several foci (Thiry et al. 2000). TEM analyses suggest that the nucleoli of African and American trypanosomes have a bipartite structure (86) (Ogbadoyi et al., 2000; López-Velázquez et al., 2005). However, TEM studies have not been able to clearly distinguish a fibrillar component in the nucleolus of *T. brucei*, since a homogeneous GC seems to occupy the entire

volume of this parasite's prominent nucleolus (87) (88) (89). Lack of fibrillar detection remains perplexing, since further work suggested the presence of a nucleolar zone with a subtle FC aspect (86). The explanation for this anomaly may lie in experiments that showed the proteins Nopp140 and NoLP localize to the nucleolar periphery in *T. brucei* (90), while NOG1 (a GTP-binding protein that interacts with NOPP44/46) and the CK2 kinase display a perinucleolar localisation (91, 92). These localisation data are interesting, since they correlate with observations that the rDNA locus in *T. brucei* is also found at the periphery of the nucleolus, suggesting a pronounced deviation from the expected position within the central compartment (93). Furthermore, RNA pol I displays a dotted pattern that is also clearly at the nucleolar periphery, and nascent rRNA localises in a perinucleolar horseshoe pattern (93, 94). Finally, it has been reported that the exosome complex involved in processing of rRNAs is enriched in the edge of the nucleolus in a ring pattern (95, 96). Taken as a whole, this range of observations indicates that the nucleolus structure of *T. brucei* differs from other eukaryotes, with most rRNA synthesis occurring at the periphery, meaning the fibrillar components might be positioned there (and thus explaining why they have been difficult to observe in TEM). What feature(s) of kinetoplastid biology might explain these changes in the nucleolus are only beginning to be investigated.

In mammals, entry into mitosis is accompanied by nucleolus disassembly and cessation of rDNA transcription while the chromosomes condense and the nuclear envelope dissolves (Alberts et al. 1996). However, during yeast mitosis, clear condensation of chromosomes is not apparent, the nuclear envelope remains intact and the nucleolus does not disassemble (Loidl 2003; Fuchs and Loidl 2004). In these circumstances, the distribution of the nucleolar machinery follows the segregation of the rDNA to opposite poles of the mitotic nucleus during anaphase. In *T. brucei*, both the nucleolus and the nuclear envelope also remain visible during mitosis, suggesting a nucleolar division mechanism similar to that described for yeast. This conclusion is supported by early TEM experiments (Vickerman and Preston 1970) and, more recently, has been validated by fluorescence microscopy using an uncharacterized nucleolar marker (Ogbadoyi et al. 2000). Positional mapping of

both the rDNA locus and the largest subunit of RNA pol I (RPA1) at different stages of the cell cycle, in bloodstream and the procyclic forms of the parasite, revealed that RNA pol I remains part of one nucleolus that is associated with the rDNA locus until chromatids segregate during anaphase.

Ingestion of African trypanosomes by tsetse is accompanied by differentiation from bloodstream form cells to procyclic form, which results in many structural and metabolic changes, including exchange of the highly variable VSG coat for a more invariant glycoprotein, named procyclin. Both these genes are transcribed by RNA PolI, but attempts to map the nuclear localisation and site of transcription of the procyclin locus have been the subject of a long-running controversy. In 1991 Van der Ploeg and colleagues, using RNA FISH to detect the products of a marker gene inserted in a procyclin locus, concluded that transcription occurred in the nucleolus (Rudenko et al 1991; Chung et al. 1992). However, in 1998 Borst and colleagues, using an improved RNA FISH RNA protocol, deduced that procyclin transcription occurs in the nucleoplasm, since there was no colocalization between rRNA transcripts and RNA derived from marker genes inserted into the procyclin locus (97, 98). Furthermore, detection of procyclin mRNA was not possible in either study, an observation that was explained by proposing that procyclin mRNA is rapidly transported out of the nucleus, leaving insufficient subnuclear levels for FISH RNA detection (99). Previous data suggest that rRNA maturation in *T. brucei* takes place in the nucleolus (59, 90), while the maturation of pre-mRNA occurs in the nucleoplasm (100), similar to what happens in other eukaryotes. It seems likely, therefore, that the highly unusual situation whereby RNA PolI is used to direct the expression of mRNA explains the inconsistencies in the above studies. Altogether, the results of Chaves et al. (1998) can be combined with more recent work by Landeira and Navarro (2007) to suggest an interesting model whereby nuclear architecture can explain how transcription of rRNA and mRNA can take place in the same nucleolar subcompartment (at the periphery), whilst RNA products derived from that common location are subsequently maturated by different routes. While pre-rRNA is processed during a relatively conventional migration in the nucleolus, procyclin pre-mRNA must be recognized as a different product and directed for maturation by migration to the nucleoplasm,

where the splicing machinery necessary to produce a mature mRNA is located. Such an unusual use of the nucleolus (generating both rRNA and mRNA) may provide an explanation its novel structure in *T. brucei*, since a perinucleolar position might facilitate access to the two completely different splicing machineries required for the maturation of procyclin mRNA and rRNA. Examining nucleolus structure in related kinetoplastids could provide further tests of this model.

The structure and size of the nucleolus is largely determined by the level of ribosome assembly required in the cell and is therefore reflective of cell proliferation. In most eukaryotic cells, both the nucleolus and the DFCs increase in size after stimulation, showing a clear relationship between structure and function. Such restructuring appears to operate at two levels. First, nucleolus structure depends on ribosome assembly, since inhibition of ribosomal subunit assembly leads to disorganization of the nucleolar components (101). Second, it is also known that transcriptional activity directed by RNA pol I can drastically alter the structure of the nucleolus, since transcription inhibition causes a redistribution of nucleolar components, while introduction of rDNA genes into cells on non-integrative plasmids results in the formation of micronucleoli. These effects appear to be limited to RNA PolI, since experiments in yeast and *Drosophila* have shown that rRNA production by RNA PolII is insufficient to generate a typical nucleolar structure (102), or to generate ectopic nucleoli when rDNA genes are present as RNA Pol II-transcribed transgenes (103). During its life cycle, *T. cruzi* presents a prominent nucleolus with active rDNA transcription in dividing cells (104), whereas in non-dividing developmental stages the nuclelous is either disassembled or significantly reduced in size (105, 106), suggesting that similar variations in nucleolus structure are found in kinetoplastids. FRAP (Fluorescence Recovery After Photobleaching) experiments have provided some insight into how these changes occur, since nucleolar components are in a continuous and rapid exchange with the surrounding nucleoplasm (107). The time of residence for most nucleolar proteins is in the order of tens of seconds, while non-nucleolar proteins residencies that are 10-200 times lower. It seems that residence time is determined by molecular interactions between nucleolar components, meaning the higher the affinity of one nucleolar component with another,

the greater the time of residence (78). In total, the data available to date suggest that the nucleolus is a dynamic, self-organizing body that depends on the presence of NORs and RNA pol I function for nucleation.

1.6.2 The expression site body; a subnuclear structure unique to African trypanosomes?

African trypanosomes multiply in the bloodstream of their mammalian host and escape the immune response by periodically changing the composition of their VSG surface protein coat. For antigenic variation to progress efficiently, a single VSG is expressed on the surface of a given parasite cell at any given time. Thus, out of the many thousands of *VSG* genes that are found in the *T. brucei* genome (58), the expressed VSG is subject to monoallelic transcription (108, 109). The orchestration of VSG monallelic expression is remarkably complex. First, like procyclin, VSG mRNA is not transcribed by RNA pol II, but by RNA pol I. Second, the VSG is co-transcribed with multiple non-VSG genes from a telomeric site termed the VSG expression site (VSG-ES). Finally, there are about 15 VSG-ESs that can be used to expression VSG in bloodstream from cells, each of which are very similar in structure and in their promoter sequence. How, then, does *T. brucei* achieve VSG monoallelic expression?

Among several current models to explain trypanosome monoallelic expression (109), each must take into account a unique subnuclear body (94) that is associated with VSG expression; indeed, this body may yet provide the most compelling model for singular VSG expression. In other eukaryotes RNA pol I is exclusively located in the nucleolus. In *T. brucei*, the single transcribed *VSG* gene is not transcribed by RNA pol I in the nucleolus, but rather in the nucleoplasm (97, 98). Later, this extranucleolar site was shown by Navarro and Gull (2001) to be a coherent nuclear structure and was termed the Expression Site Body (ESB; Fig.2). Antibodies against RPA1, the largest RNA pol I subunit, colocalise with the active VSG-ES locus (Fig.2) and with nascent RNA labelled with BrUTP in the presence of α-amanitin, which inhibits RNA pols II and III (98). Moreover, the ESB can still be detected in absence of DNA, suggesting it is more than merely an assembly of the transcription

machinery on the VSG-ES. In contrast to the active VSG-ES, the inactive VSG-ESs are distributed in the nucleoplasm, rather than being positioned in a common domain, such as at the nuclear envelope or any other putative silencing compartment of the nucleus (97, 98). However, silent VSG-ESs are not completely inactive, since the promoter proximal region of the multigene unit is transcribed to some extent (110). These data suggest a model for VSG expression whereby one VSG-ES is recruited to the single ESB, which contains all the machinery for transcription initiation, elongation and mRNA splicing. Thus, the model predicts that only the VSG-ES within the ESB can be fully transcribed from the RNA PolI promoter to the telomere, where the *VSG* gene is located, and only here are the VSG-ES transcripts efficiently processed as mature mRNAs. Moreover, inactive but to some extent transcriptionally-competent VSG-ESs compete for the single ESB, allowing a new VSG-ES to displace the active VSG-ES, leading to a transcriptional switch to a different VSG. The activity of the ESB is tied to the action of RNA Pol I, since replacement of the endogenous VSG RNA Pol I promoter by an rDNA promoter results in extra-nucleolar VSG transcription (111), which is presumably located in a ESB since the VSG is transcribed in a stable manner and the chimera VSG-ES is able to switch off and on (111). In contrast, replacement by a RNA T7 promoter does not produce VSG transcripts and induces transcriptional switches to a different VSG-ES (112). These data suggest that RNA pol I transcription is central to the functioning of the ESB, whose wider molecular composition remains to be investigated.

A key question is how the transcriptional status of the active VSG-ES is inherited from one generation to the next (Fig.3). More frequently than not, the transcription status of the single active VSG-ES is passed on during cell division, as is VSG-ES association with the ESB (98). Thus, it is necessary to investigate the relationship between the active VSG-ES locus and the ESB during the cell cycle, particularly during the phases of DNA synthesis and chromosome segregation. One can envisage two hypotheses to explain how the transcriptional state of the VSG-ES can be inherited: (1) after replication of the active VSG-ES, the ESB is not duplicated but the newly synthesized chromatin is epigenetically marked so that it has the capability of recruiting or forming a new ESB after the

segregation of chromosomes; (2) after replication of the active VSG-ES, both sister chromatids are associated with a single ESB, which is separated between the two daughter cells after chromosome segregation, as seems to occurs with the nucleolus in trypanosomes (86). Dynamic analysis of both the active VSG-ES locus (tagged with the GFP at the promoter region) and the ESB (113) allowed the above hypotheses to be tested. 3D microscopy suggests that the ESB is not generated de novo on the newly replicated VSG-ES locus, since two VSG-ES/ESB complexes were not detected in any phase of the cell cycle. Neither has it been possible to detect two separated VSG-ES chromatids tagged with GFP, one associated with the ESB and the other not, except when the cohesin complex is depleted (see below). Nonetheless, two VSG-ES/ESB complexes are clearly detectable very early after mitosis, in a cell containing two nuclei that have not yet been separated by cytokinesis (98, 113). The same analysis also revealed a significant difference between the active VSG-ES and other loci, with the separation of sister chromatids significantly delayed for the active VSG-ES. This delay is dependent on the cohesin complex, as partial depletion of the SCC1 subunit leads to premature separation of sister-chromatids from each other and the absence of an ESB on one of the chromatids (113). Most importantly, premature separation of the active VSG-ES chromatids in these circumstances leads to a significant increase in VSG switching. These results suggest that the chromatid that is no longer attached to the ESB is then capable of a transcriptional switch to another VSG-ES, and reinforces the single VSG-ES/ESB occupancy model as a mechanism to ensure transcriptional inheritance. Moreover, the ESB structure is often found to be 'stretched out' in pre-mitotic cells (113). Thus, it has been proposed that the cohesin complex functions to ensure inheritance of the active transcriptional state of a single VSG-ES by forming a complex containing the two chromatids of the active VSG-ES associated with a single ESB (113). Therefore, the cell-cycle persistence of the ESB, the detection of an elongated ESB form associated with both sister chromatids, as well as with the highly SUMOylated focus (HSF)(113, 114), suggests that the ESB separates into two bodies by splitting in a similar manner to that described for the separation of human Cajal bodies (115) (Figs.2, 3). Thus, contrary to what had been suggested (116), the ESB

appears not to be generated de novo on epigenetically marked chromatin in pre-mitotic cells, because the unique dynamics of sister chromatid separation on the active VSG-ES suggest these sites stay together after S phase until the onset of mitosis (in contrast to other loci) and remain associated with a single ESB. Notwithstanding, the splitting of a single ESB associated with the two sister chromatids may serve to transfer epigenetic marks to the daughter chromatid and thus maintain the active transcriptional state. The existence of a mechanism which delays sister chromatid separation specifically on the active VSG-ES is possible, since a delay is not detected in either the inactive VSG-ES subtelomeric loci or in the highly transcribed rDNA (76)(as previously described in yeast) (117). A biphasic dissociation of cohesin that is locus-dependent, as described in mammalian chromosomes, remains possible, whereby most of the cohesin complex discharges from chromosomes in prophase while a fraction remains on some chromosomes until anaphase (118, 119). Determining whether the active VSG-ES imposes variant regulation on the cohesin complex, perhaps through epigenetic signals, relative to other loci is also worth considering.

Some studies have described conditions in *T. brucei* that alter nuclear structure, including the ESB or nucleolus. One example is the depletion of the telomeric protein RAP1, which induces a dispersed pattern of RNA Pol I in the nucleus, which could be interpreted as multiple ESBs (120). Recently, development of the stumpy form (a quiescent stage of the parasite, preadapted to life in the tseste) was also shown to be accompanied with a delocalization of RNA Pol I into many small nuclear foci (121), similar to what can be observed after cells are treated with a transcription inhibitor (J.M. Bart and M. Navarro, unpublished results). Dispersal of RNA Pol I most likely results from lack of activity, such as has been shown for the nucleolus in other eukaryotes, or from aberrant control of RNA PolI transcription. In either case, the link between the ESB and the nucleolus deserves greater analysis, especially because other alterations, such as cohesin depletion (which alters VSG switching frequency), does not detectably change ESB structure (113).

Other subnuclear structures are found in eukaryotes, but have not to date been the subject of as extensive analysis as the nucleolus or ESB in kinetoplastids or other protozoa. Some nuclear bodies

can regulate protein post-translational modification (PTM), such as SUMOylation and phosphorylation. For example, PML body formation requires both a SUMOylation site and noncovalent binding by the SUMOylation Interative Motive (SIM) of PML for nucleation events to subsequently recruit other SUMOylated proteins and/or proteins containing SIM to the PML body (122). Recently, data has emerged that suggests the *T. brucei* ESB can convey post- translational SUMO modifications into associated proteins, perhaps facilitating protein-protein interactions that serve as a nucleation mechanism or maybe allowing epigenetic marks to be transmitted to daughter chromatin (114). Thus, a highly SUMOylated focus (HSF) has been shown to co-localize with the nuclear body ESB (Fig.2) and the VSG-ES locus tagged with the GFP (114). Using ChIP analysis, chromatin SUMOylation was detected across the entire active VSG-ES locus, including the upstream promoter region, in contrast to other RNA pol I-transcribed loci. SUMOylation of chromatin-associated proteins is required for the active transcriptional state of the VSG-ES and essential for efficient recruitment of RNA pol I to the VSG-ES promoter. The mechanism employs a novel SIZ/PIAS SUMO E3 ligase responsible for the VSG-ES chromatin SUMOylation. Furthermore, the largest subunit of RNA pol I is SUMOylated in a TbSIZ1-dependent manner, suggesting a positive PTM mechanism via SUMOylation to regulate VSG transcription. Unfortunately, partial depletion of either the SUMO E3 ligase or the actual TbSUMO, did not affected the ESB stability (114).

Speckles are discrete nuclear bodies found in mammalian cells implicated in the retention and maturation of mRNAs. In *T. brucei*, no such nuclear body has been clearly described, although splicing components, as well as Sm proteins and U2 snRNA, have been found to localize in discrete nuclear structures that may be related to speckles (123).

A wider range of functional subnuclear localisation is also evident, though whether it relates to discrete structures is less clear. One prominent example of this relates to telomeres. The telomeric position of the VSG-ES locus and, in particular, telomere proximity of the *VSG* gene, has led to suggestions that VSG regulation in *T. brucei* (124) (125) might act by a mechanism similar to telomere position effect (TPE); reviewed in (126). In *P. falciparum* antigenic variation also occurs by

transcription switching, and here it is clear that silent *var* genes (most of which are telomeric) cluster at the nuclear periphery, while the single active gene escapes this clustering (127). However, whether any structures dictate the silent clusters, or drive expression of the active gene at the nuclear periphery (perhaps akin to an ESB), is unknown. In *T. brucei*, the ESB is no longer detectable in procyclic form cells, where all VSG-ES are downregulated (93), and these changes are associated with all VSG-ES being relocated to the nuclear periphery (Fig.1). This is relevant, because it suggests that nuclear peripheral localisation may elicit silencing of RNA PolI, meaning such a mechanism is not restricted to RNA pol II-mediated transcription, as has been described in yeast. This is important because models for how nuclear periphery and gene expression silencing are linked are under debate (128). In *T. brucei*, nuclear-repositioning of the active VSG-ES is rapid during cell differentiation and is followed by later chromatin condensation. In yeast, Gasser and colleagues have demonstrated that repositioning is not necessary for SIR-dependent silencing (128). However, these results cannot rule out the possibility that establishment of silencing in yeast requires a transient perinuclear localization. In another study (129), performed on different cell types isolated from mice at several differentiation stages, it has been shown that the immunoglobulin locus is located at the nuclear periphery in hematopoietic progenitors and pro-T cells. Interestingly, in *T. brucei*, silencing by nuclear repositioning may target the VSG-ES promoter region, rather than telomeric sequences (located ~60 kb downstream), which may correlate with studies in yeast where the need for a telomere in nuclear peripheral silencing appears to be under discussion (128). Most importantly, the rapid nuclear repositioning in *T. brucei* exclusively affects the VSG-ES promoter that was active in bloodstream form cells and not the inactive VSG-ES promoters, which share highly homologous sequences (93). Nonetheless, RNAi that targets NUP-1, the major laminar component in the nuclear periphery, results in increased expression of silent VSGs (36). However , in bloodstream form cells this effect could be a consequence increased VSG antigenic switching, since in TbNUP-1 depleted cells a significant increase in cells expressing other VSGs on the surface was observed. Interestingly, cells that are negative for detectable VSG types were seen, suggesting NUP1 RNAi can

induce switching to a wide range of VSGs. In addition, NUP-1 knockdown represses differentiation-induced VSG-ES repositioning to the nuclear periphery in procyclic form cells, where non VSG is expressed (36). Clearly, we still have much to learn, but these data indicate that NUP-1 has an important role in maintaining *T. brucei* nuclear architecture, which, in turn, has a key role in VSG expression. It seems highly likely that nuclear architecture has a wide role in genome biology in all protozoan parasites.

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Figure legends

Figure 1. The nuclear envelope. *Main figure* shows a highly simplified diagram of nuclear features influenced by the nuclear envelope, which is formed by two lipid bilayers that adopt the shape of flat sacs between the Nuclear Pore Complexes (NPCs; blue box), which are found where the outer and inner nuclear envelope join. NPCs have crucial roles in import and export of cargo between the cell cytoplasm and nucleoplasm; a crucial export cargo is mature mRNA, which is translated on ribosomes found in the endoplasmic reticulum (not shown) that is contiguous with the nuclear membrane. Laminar filaments (dark grey lines) subtend the inner membrane, contact the NPCs and provide nuclear structure. Inactive genes (pink arrows) located in hetechromatin (and often at the telomeres of chromosomes) are frequently at the periphery of the nucleus, within the lamina, whereas active genes (brown arrows) are frequently found in the nuclear interior. Some highly active genes (red arrow) can associate with the NPCs, however. Nucleoporin components of the NPCs contribute to centromere and centrosome (blue circles) function by allowing chromosome segregation during mitosis through attachment to spindle microtubulues (not shown). *Insert diagram* shows the telomeric *VSG* gene within the active expression site (VSG-ES) in *T. brucei*,

illustrating that while this locus is found centrally within the nucleus (and associated with an expression site body; not shown, but see Fig.2) in bloodstream form (BSF) cells it moves to the nuclear periphery as the parasites differentiate to procyclic form (PCF) cells, at which time all VSG-ES are transcriptionally silenced.

Figure 2. Expression of Variant Surface Glycoprotein in *T. brucei*. *The upper diagrams* show sister chromatids of the active VSG-ES (aES; green) in *T. brucei* bloodstream form cells labelled with GFP-LacI,showing they remain associated together in a single expression site body (ESB) until chromosome segregation. To conduct this experiment, a cells were used with 256 Lac operator repeats inserted upstream of the active VSG-ES, and were analyzed by double indirect 3D-immunofluorescnce using anti-TbRPA1 antiserum (detecting RNA PolI; left panel, red), monoclonal anti-GFP antibodies (aES, middle) and DAPI staining (right panels, blue). The analysis (as described in Landeira et al, 2007) was conducted in early (upper panels) and late (lower panels) pre-mitotic phases, as defined by DAPI staining. Arrows indicate the ESB, which can be seen to be distinct from the larger nucleolus. The *lower diagram* showns a Highly SUMOylated Focus (HSF) (middle panel, green) that can be detected with anti-TbSUMO monoclonal antibody (Lopez-Farfan et al, 2014) and associates with the ESB. As above, images are shown as double indirect 3D-immunofluorescnce in bloodstream form *T. brucei* cells using anti-TbRPA1 antiserum (left panel; red) and DAPI staining (right panel; blue); images acquired and generated by Jean M. Bart. The splitting ESB and the HSF are indicated by arrows.

Figure 3. Model for inheritance of monoallelic VSG expression in bloodstream form *Trypanosoma brucei*. A diagrammatic depiction is shown of the inheritance of the nucleolus and expression site body (ESB), the two major RNA Pol I-associated subnuclear structures seen in bloodstream form *T. brucei* cells. Only the nucleus of a cell is shown, which is surrounded by the nuclear envelope, containing nuclear pores (pink box). The nucleolus and ESB (large and small red circles, with SUMO (green circle) associated with the latter) are shown first in G2 and pre-mitotic cells, where newly replicated sister chromatids (wavy lines) are held together by cohesin (blue double horseshoe) and chromosomes begin to line up for mitosis. For simplicity, telomeric VSGs (inactive, blue dot; active, black dot) are shown at the nuclear periphery at one end of the chromosomes; VSGs are also located at telomeres on the other end of the chromosomes (where they would also be peripheral), and chromosome-internal rRNA genes are present in the nucleolus, but neither of these features are indicated (dotted lines). Division (splitting) of the nucleolus occurs as sister chromatids, including inactive VSGs, separate and begin to be segregated during mitosis. In contrast, division of the ESB and separation of active VSGs on sister chromatids occurs later; it is not clear if the same delay occurs throughout the ESB-containing chromosome, including the silent VSG at the other telomere. After mitosis, each cell contains a single nucleolus and ESB, which normally remains associated with the previously active VSG, though switching can occur (not shown).

References

1. Devos DP, Graf R, Field MC. Evolution of the nucleus. Current opinion in cell biology. 2014;28:8-15.

2. Cavalier-Smith T. Origin of the cell nucleus, mitosis and sex: roles of intracellular coevolution. Biology direct. 2010;5:7.

3. Pennisi E. Evolutionary biology. The birth of the nucleus. Science. 2004;305(5685):766-8.

4. Wilson KL, Dawson SC. Evolution: functional evolution of nuclear structure. The Journal of cell biology. 2011;195(2):171-81.

5. Williams TA, Foster PG, Cox CJ, Embley TM. An archaeal origin of eukaryotes supports only two primary domains of life. Nature. 2013;504(7479):231-6.

6. Iyer LM, Makarova KS, Koonin EV, Aravind L. Comparative genomics of the FtsK-HerA superfamily of pumping ATPases: implications for the origins of chromosome segregation, cell division and viral capsid packaging. Nucleic Acids Res. 2004;32(17):5260-79.

7. Kalliomaa-Sanford AK, Rodriguez-Castaneda FA, McLeod BN, Latorre-Rosello V, Smith JH, Reimann J, et al. Chromosome segregation in Archaea mediated by a hybrid DNA partition machine. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(10):3754-9.

8. Foster HA, Bridger JM. The genome and the nucleus: a marriage made by evolution. Genome organisation and nuclear architecture. Chromosoma. 2005;114(4):212-29.

9. Carpenter ML, Assaf ZJ, Gourguechon S, Cande WZ. Nuclear inheritance and genetic exchange without meiosis in the binucleate parasite Giardia intestinalis. Journal of cell science. 2012;125(Pt 10):2523-32.

10. Yao MC, Chao JL, Cheng CY. Programmed Genome Rearrangements in Tetrahymena. Microbiology spectrum. 2014;2(6).

11. Francia ME, Striepen B. Cell division in apicomplexan parasites. Nature reviews Microbiology. 2014;12(2):125-36.

12. Reyes-Lamothe R, Nicolas E, Sherratt DJ. Chromosome replication and segregation in bacteria. Annual review of genetics. 2012;46:121-43.

13. Samson RY, Bell SD. Archaeal chromosome biology. Journal of molecular microbiology and biotechnology. 2014;24(5-6):420-7.

14. Tumova P, Uzlikova M, Wanner G, Nohynkova E. Structural organization of very small chromosomes: study on a single-celled evolutionary distant eukaryote Giardia intestinalis. Chromosoma. 2015;124(1):81-94.

15. Hecker H, Betschart B, Bender K, Burri M, Schlimme W. The chromatin of trypanosomes. International journal for parasitology. 1994;24(6):809-19.

16. Akiyoshi B, Gull K. Discovery of unconventional kinetochores in kinetoplastids. Cell. 2014;156(6):1247-58.

17. Akiyoshi B, Gull K. Evolutionary cell biology of chromosome segregation: insights from trypanosomes. Open biology. 2013;3(5):130023.

18. Vicente JJ, Cande WZ. Mad2, Bub3, and Mps1 regulate chromosome segregation and mitotic synchrony in Giardia intestinalis, a binucleate protist lacking an anaphase-promoting complex. Molecular biology of the cell. 2014;25(18):2774-87.

19. Wilson KL, Berk JM. The nuclear envelope at a glance. Journal of cell science. 2010;123(Pt 12):1973-8.

20. Hurt E, Beck M. Towards understanding nuclear pore complex architecture and dynamics in the age of integrative structural analysis. Current opinion in cell biology. 2015;34:31-8.

21. Holden JM, Koreny L, Kelly S, Chait BT, Rout MP, Field MC, et al. Touching from a distance. Nucleus. 2014;5(4):304-10.

22. Devos D, Dokudovskaya S, Alber F, Williams R, Chait BT, Sali A, et al. Components of coated vesicles and nuclear pore complexes share a common molecular architecture. PLoS biology. 2004;2(12):e380.

23. Oeffinger M, Zenklusen D. To the pore and through the pore: a story of mRNA export kinetics. Biochimica et biophysica acta. 2012;1819(6):494-506.

24. Daniels JP, Gull K, Wickstead B. Cell biology of the trypanosome genome. Microbiology and molecular biology reviews : MMBR. 2010;74(4):552-69.

25. Clayton CE. Networks of gene expression regulation in Trypanosoma brucei. Molecular and biochemical parasitology. 2014;195(2):96-106.

26. Inoue AH, Serpeloni M, Hiraiwa PM, Yamada-Ogatta SF, Muniz JR, Motta MC, et al. Identification of a novel nucleocytoplasmic shuttling RNA helicase of trypanosomes. PLoS One. 2014;9(10):e109521.

27. Cassola A, Frasch AC. An RNA recognition motif mediates the nucleocytoplasmic transport of a trypanosome RNA-binding protein. The Journal of biological chemistry. 2009;284(50):35015-28.

28. Guizetti J, Martins RM, Guadagnini S, Claes A, Scherf A. Nuclear pores and perinuclear expression sites of var and ribosomal DNA genes correspond to physically distinct regions in Plasmodium falciparum. Eukaryotic cell. 2013;12(5):697-702.

29. Weiner A, Dahan-Pasternak N, Shimoni E, Shinder V, von Huth P, Elbaum M, et al. 3D nuclear architecture reveals coupled cell cycle dynamics of chromatin and nuclear pores in the malaria parasite Plasmodium falciparum. Cellular microbiology. 2011;13(7):967-77.

30. Forbes DJ, Travesa A, Nord MS, Bernis C. Nuclear transport factors: global regulation of mitosis. Current opinion in cell biology. 2015;35:78-90.

31. Morelle C, Sterkers Y, Crobu L, DE MB-B, Kuk N, Portales P, et al. The nucleoporin Mlp2 is involved in chromosomal distribution during mitosis in trypanosomatids. Nucleic Acids Res. 2015;43(8):4013-27.

32. Malone CD, Falkowska KA, Li AY, Galanti SE, Kanuru RC, LaMont EG, et al. Nucleus-specific importin alpha proteins and nucleoporins regulate protein import and nuclear division in the binucleate Tetrahymena thermophila. Eukaryotic cell. 2008;7(9):1487-99.

33. Field MC, Horn D, Alsford S, Koreny L, Rout MP. Telomeres, tethers and trypanosomes. Nucleus. 2012;3(6):478-86.

34. Batsios P, Peter T, Baumann O, Stick R, Meyer I, Graf R. A lamin in lower eukaryotes? Nucleus. 2012;3(3):237-43.

35. Kruger A, Batsios P, Baumann O, Luckert E, Schwarz H, Stick R, et al. Characterization of NE81, the first lamin-like nucleoskeleton protein in a unicellular organism. Molecular biology of the cell. 2012;23(2):360-70.

36. Dubois KN, Alsford S, Holden JM, Buisson J, Swiderski M, Bart JM, et al. NUP-1 Is a Large Coiled-Coil Nucleoskeletal Protein in Trypanosomes with Lamin-Like Functions. PLoS biology. 2012;10(3):e1001287.

37. Baxter J, Aragon L. A model for chromosome condensation based on the interplay between condensin and topoisomerase II. Trends in genetics : TIG. 2012;28(3):110-7.

38. Glover L, Hutchinson S, Alsford S, McCulloch R, Field MC, Horn D. Antigenic variation in African trypanosomes: the importance of chromosomal and nuclear context in VSG expression control. Cellular microbiology. 2013;15(12):1984-93.

39. McCulloch R, Morrison LJ, Hall JP. DNA Recombination Strategies During Antigenic Variation in the African Trypanosome. Microbiology spectrum. 2015;3(2):MDNA3-0016-2014.

40. Mugnier MR, Cross GA, Papavasiliou FN. The in vivo dynamics of antigenic variation in Trypanosoma brucei. Science. 2015;347(6229):1470-3.

41. Hall JP, Wang H, Barry JD. Mosaic VSGs and the scale of Trypanosoma brucei antigenic variation. PLoS pathogens. 2013;9(7):e1003502.

42. Bopp SE, Manary MJ, Bright AT, Johnston GL, Dharia NV, Luna FL, et al. Mitotic evolution of Plasmodium falciparum shows a stable core genome but recombination in antigen families. PLoS genetics. 2013;9(2):e1003293.

43. Claessens A, Hamilton WL, Kekre M, Otto TD, Faizullabhoy A, Rayner JC, et al. Generation of antigenic diversity in Plasmodium falciparum by structured rearrangement of Var genes during mitosis. PLoS genetics. 2014;10(12):e1004812.

44. Zhang Q, Huang Y, Zhang Y, Fang X, Claes A, Duchateau M, et al. A critical role of perinuclear filamentous actin in spatial repositioning and mutually exclusive expression of virulence genes in malaria parasites. Cell host & microbe. 2011;10(5):451-63.

45. Pope BD, Ryba T, Dileep V, Yue F, Wu W, Denas O, et al. Topologically associating domains are stable units of replication-timing regulation. Nature. 2014;515(7527):402-5.

46. Renard-Guillet C, Kanoh Y, Shirahige K, Masai H. Temporal and spatial regulation of eukaryotic DNA replication: from regulated initiation to genome-scale timing program. Seminars in cell & developmental biology. 2014;30:110-20.

47. Tiengwe C, Marcello L, Farr H, Dickens N, Kelly S, Swiderski M, et al. Genome-wide analysis reveals extensive functional interaction between DNA replication initiation and transcription in the genome of Trypanosoma brucei. Cell reports. 2012;2(1):185-97.

48. Marques CA, Dickens NJ, Paape D, Campbell SJ, McCulloch R. Genome-wide mapping reveals single-origin chromosome replication in Leishmania, a eukaryotic microbe. Genome biology. 2015;16(1):230.

49. Calderano SG, Melo Godoy PD, Motta MC, Mortara RA, Schenkman S, Elias MC. Trypanosoma cruzi DNA replication includes the sequential recruitment of pre-replication and replication machineries close to nuclear periphery. Nucleus. 2011;2(2):136-45.

50. Kaufmann D, Gassen A, Maiser A, Leonhardt H, Janzen CJ. Regulation and spatial organization of PCNA in Trypanosoma brucei. BiochemBiophysResCommun. 2012.

51. Dekker J, Marti-Renom MA, Mirny LA. Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. Nature reviews Genetics. 2013;14(6):390-403.

52. Belmont AS. Large-scale chromatin organization: the good, the surprising, and the still perplexing. Current opinion in cell biology. 2014;26:69-78.

53. Dekker J. Two ways to fold the genome during the cell cycle: insights obtained with chromosome conformation capture. Epigenetics & chromatin. 2014;7(1):25.

54. Ay F, Bunnik EM, Varoquaux N, Bol SM, Prudhomme J, Vert JP, et al. Three-dimensional modeling of the P. falciparum genome during the erythrocytic cycle reveals a strong connection between genome architecture and gene expression. Genome research. 2014;24(6):974-88.

55. Lemieux JE, Kyes SA, Otto TD, Feller AI, Eastman RT, Pinches RA, et al. Genome-wide profiling of chromosome interactions in Plasmodium falciparum characterizes nuclear architecture and reconfigurations associated with antigenic variation. Molecular microbiology. 2013;90(3):519-37.

56. El Sayed NM, Hegde P, Quackenbush J, Melville SE, Donelson JE. The African trypanosome genome. IntJParasitol. 2000;30(4):329-45.

57. Wickstead B, Ersfeld K, Gull K. The small chromosomes of Trypanosoma brucei involved in antigenic variation are constructed around repetitive palindromes. Genome research. 2004;14(6):1014-24.

58. Cross GA, Kim HS, Wickstead B. Capturing the variant surface glycoprotein repertoire (the VSGnome) of Trypanosoma brucei Lister 427. Molecular and biochemical parasitology. 2014;195(1):59-73.

59. Obado SO, Bot C, Nilsson D, Andersson B, Kelly JM. Repetitive DNA is associated with centromeric domains in Trypanosoma brucei but not Trypanosoma cruzi. Genome Biol. 2007;8(3):R37.

60. Weiden M, Osheim YN, Beyer AL, Van der Ploeg LH. Chromosome structure: DNA nucleotide sequence elements of a subset of the minichromosomes of the protozoan Trypanosoma brucei. MolCell Biol. 1991;11(8):3823-34.

61. Tiengwe C, Marques CA, McCulloch R. Nuclear DNA replication initiation in kinetoplastid parasites: new insights into an ancient process. Trends in parasitology. 2014;30(1):27-36.

62. Ersfeld K, Gull K. Partitioning of large and minichromosomes in *Trypanosoma brucei*. Science. 1997;276(5312):611-4.

63. Bessat M, Ersfeld K. Functional characterization of cohesin SMC3 and separase and their roles in the segregation of large and minichromosomes in Trypanosoma brucei. Molecular microbiology. 2009;71(6):1371-85.

64. Alsford S, Wickstead B, Ersfeld K, Gull K. Diversity and dynamics of the minichromosomal karyotype in Trypanosoma brucei. Molecular and biochemical parasitology. 2001;113(1):79-88.

65. Wickstead B, Ersfeld K, Gull K. The mitotic stability of the minichromosomes of Trypanosoma brucei. Molecular and biochemical parasitology. 2003;132(2):97-100.

66. Dujardin JC, Mannaert A, Durrant C, Cotton JA. Mosaic aneuploidy in Leishmania: the perspective of whole genome sequencing. Trends in parasitology. 2014;30(12):554-5.

67. Mannaert A, Downing T, Imamura H, Dujardin JC. Adaptive mechanisms in pathogens: universal aneuploidy in Leishmania. Trends Parasitol. 2012;28(9):370-6.

68. Rogers MB, Hilley JD, Dickens NJ, Wilkes J, Bates PA, Depledge DP, et al. Chromosome and gene copy number variation allow major structural change between species and strains of Leishmania. Genome Res. 2011;21(12):2129-42.

69. Downing T, Imamura H, Decuypere S, Clark TG, Coombs GH, Cotton JA, et al. Whole genome sequencing of multiple Leishmania donovani clinical isolates provides insights into population structure and mechanisms of drug resistance. Genome Res. 2011;21(12):2143-56.

70. Sterkers Y, Lachaud L, Crobu L, Bastien P, Pages M. FISH analysis reveals aneuploidy and continual generation of chromosomal mosaicism in Leishmania major. Cellular microbiology. 2011;13(2):274-83.

71. Reis-Cunha JL, Rodrigues-Luiz GF, Valdivia HO, Baptista RP, Mendes TAO, de Morais GL, et al. Chromosomal copy number variation reveals differential levels of genomic plasticity in distinct Trypanosoma cruzi strains. Bmc Genomics. 2015;16.

72. El Sayed NM, Myler PJ, Blandin G, Berriman M, Crabtree J, Aggarwal G, et al. Comparative genomics of trypanosomatid parasitic protozoa. Science. 2005;309(5733):404-9.

73. Valdivia HO, Reis-Cunha JL, Rodrigues-Luiz GF, Baptista RP, Baldeviano GC, Gerbasi RV, et al. Comparative genomic analysis of Leishmania (Viannia) peruviana and Leishmania (Viannia) braziliensis. BMC Genomics. 2015;16(1):715.

74. Ubeda JM, Raymond F, Mukherjee A, Plourde M, Gingras H, Roy G, et al. Genome-wide stochastic adaptive DNA amplification at direct and inverted DNA repeats in the parasite Leishmania. PLoS biology. 2014;12(5):e1001868.

75. Callejas S, Leech V, Reitter C, Melville S. Hemizygous subtelomeres of an African trypanosome chromosome may account for over 75% of chromosome length. Genome research. 2006;16(9):1109-18.

76. Raska I, Shaw PJ, Cmarko D. Structure and function of the nucleolus in the spotlight. Current opinion in cell biology. 2006.

77. Scheer U, Hock R. Structure and function of the nucleolus. Current opinion in cell biology. 1999;11(3):385-90.

78. Pederson T. The nucleolus. Cold Spring Harbor perspectives in biology. 2011;3(3).

79. Jacobson MR, Pederson T. Localization of signal recognition particle RNA in the nucleolus of mammalian cells. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(14):7981-6.

80. Politz JC, Yarovoi S, Kilroy SM, Gowda K, Zwieb C, Pederson T. Signal recognition particle components in the nucleolus. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(1):55-60.

81. Gerbi SA, Borovjagin AV, Lange TS. The nucleolus: a site of ribonucleoprotein maturation. Current opinion in cell biology. 2003;15(3):318-25.

82. Pederson T. The plurifunctional nucleolus. Nucleic acids research. 1998;26(17):3871-6.

83. Lustig Y, Goldshmidt H, Uliel S, Michaeli S. The Trypanosoma brucei signal recognition particle lacks the Alu-domain-binding proteins: purification and functional analysis of its binding proteins by RNAi. Journal of cell science. 2005;118(Pt 19):4551-62.

84. Raska I, Koberna K, Malinsky J, Fidlerova H, Masata M. The nucleolus and transcription of ribosomal genes. Biology of the cell / under the auspices of the European Cell Biology Organization. 2004;96(8):579-94.

85. Thiry M, Lafontaine DL. Birth of a nucleolus: the evolution of nucleolar compartments. Trends in cell biology. 2005;15(4):194-9.

86. Ogbadoyi E, Ersfeld K, Robinson D, Sherwin T, Gull K. Architecture of the Trypanosoma brucei nucleus during interphase and mitosis. Chromosoma. 2000;108(8):501-13.

87. Das A, Peterson GC, Kanner SB, Frevert U, Parsons M. A major tyrosine-phosphorylated protein of Trypanosoma brucei is a nucleolar RNA-binding protein. The Journal of biological chemistry. 1996;271(26):15675-81.

88. Rout MP, Field MC. Isolation and characterization of subnuclear compartments from Trypanosoma brucei. Identification of a major repetitive nuclear lamina component. The Journal of biological chemistry. 2001;276(41):38261-71.

89. Devaux S, Kelly S, Lecordier L, Wickstead B, Perez-Morga D, Pays E, et al. Diversification of function by different isoforms of conventionally shared RNA polymerase subunits. Molecular biology of the cell. 2007;18(4):1293-301.

90. Kelly S, Singleton W, Wickstead B, Ersfeld K, Gull K. Characterization and differential nuclear localization of Nopp140 and a novel Nopp140-like protein in trypanosomes. Eukaryotic cell. 2006;5(5):876-9.

91. Park JS, Matano Y, Doi RH. Cohesin-dockerin interactions of cellulosomal subunits of Clostridium cellulovorans. Journal of bacteriology. 2001;183(18):5431-5.

92. Jensen BC, Kifer CT, Brekken DL, Randall AC, Wang Q, Drees BL, et al. Characterization of protein kinase CK2 from Trypanosoma brucei. Molecular and biochemical parasitology. 2007;151(1):28-40.

93. Landeira D, Navarro M. Nuclear repositioning of the VSG promoter during developmental silencing in Trypanosoma brucei. The Journal of cell biology. 2007;176(2):133-9.

94. Navarro M, Penate X, Landeira D. Nuclear architecture underlying gene expression in Trypanosoma brucei. Trends Microbiol. 2007;15(6):263-70.

95. Estevez AM, Kempf T, Clayton C. The exosome of Trypanosoma brucei. Embo J. 2001;20(14):3831-9.

96. Haile S, Cristodero M, Clayton C, Estevez AM. The subcellular localisation of trypanosome RRP6 and its association with the exosome. Molecular and biochemical parasitology. 2006.

97. Chaves I, Zomerdijk J, Dirks-Mulder A, Dirks RW, Raap AK, Borst P. Subnuclear localization of the active variant surface glycoprotein gene expression site in Trypanosoma brucei. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(21):12328-33.

98. Navarro M, Gull K. A pol I transcriptional body associated with VSG mono-allelic expression in Trypanosoma brucei. Nature. 2001;414(6865):759-63.

99. Chung H-MM, Shea C, Fields S, Taub RN, Van der Ploeg LHT, Tse DB. Architectural organization in the interphase nucleus of the protozoan Trypanosoma brucei: Location of telomeres and mini-chromosomes. EMBO J. 1990;9(8):2611-9.

100. Liang XH, Liu Q, Liu L, Tschudi C, Michaeli S. Analysis of spliceosomal complexes in Trypanosoma brucei and silencing of two splicing factors Prp31 and Prp43. Molecular and biochemical parasitology. 2006;145(1):29-39.

101. Olson MO, Dundr M. The moving parts of the nucleolus. Histochemistry and cell biology. 2005;123(3):203-16.

102. Oakes M, Aris JP, Brockenbrough JS, Wai H, Vu L, Nomura M. Mutational analysis of the structure and localization of the nucleolus in the yeast Saccharomyces cerevisiae. The Journal of cell biology. 1998;143(1):23-34.

103. Karpen GH, Schaefer JE, Laird CD. A Drosophila rRNA gene located in euchromatin is active in transcription and nucleolus formation. Genes & development. 1988;2(12B):1745-63.

104. Elias MC, Marques-Porto R, Freymuller E, Schenkman S. Transcription rate modulation through the Trypanosoma cruzi life cycle occurs in parallel with changes in nuclear organisation. Molecular and biochemical parasitology. 2001;112(1):79-90.

105. Nepomuceno-Mejia T, Lara-Martinez R, Cevallos AM, Lopez-Villasenor I, Jimenez-Garcia LF, Hernandez R. The Trypanosoma cruzi nucleolus: a morphometrical analysis of cultured epimastigotes in the exponential and stationary phases. FEMS microbiology letters. 2010;313(1):41-6.

106. Lopez-Velazquez G, Hernandez R, Lopez-Villasenor I, Reyes-Vivas H, Segura-Valdez Mde L, Jimenez-Garcia LF. Electron microscopy analysis of the nucleolus of Trypanosoma cruzi. Microscopy and microanalysis : the official journal of Microscopy Society of America, Microbeam Analysis Society, Microscopical Society of Canada. 2005;11(4):293-9.

107. Phair RD, Misteli T. High mobility of proteins in the mammalian cell nucleus. Nature. 2000;404(6778):604-9.

108. McCulloch R. Antigenic variation in African trypanosomes: monitoring progress. Trends in parasitology. 2004;20(3):117-21.

109. Horn D. Antigenic variation in African trypanosomes. Molecular and biochemical parasitology. 2014;195(2):123-9.

110. Kassem A, Pays E, Vanhamme L. Transcription is initiated on silent variant surface glycoprotein expression sites despite monoallelic expression in Trypanosoma brucei. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(24):8943-8.

111. Chaves I, Rudenko G, Dirks-Mulder A, Cross M, Borst P. Control of variant surface glycoprotein gene-expression sites in Trypanosoma brucei. Embo J. 1999;18(17):4846-55.

112. Navarro M, Cross GA, Wirtz E. Trypanosoma brucei variant surface glycoprotein regulation involves coupled activation/inactivation and chromatin remodeling of expression sites. Embo J. 1999;18(8):2265-72.

113. Landeira D, Bart JM, Van Tyne D, Navarro M. Cohesin regulates VSG monoallelic expression in trypanosomes. The Journal of cell biology. 2009;186(2):243-54.

114. Lopez-Farfan D, Bart JM, Rojas-Barros DI, Navarro M. SUMOylation by the E3 Ligase TbSIZ1/PIAS1 Positively Regulates VSG Expression in Trypanosoma brucei. PLoS pathogens. 2014;10(12):e1004545.

115. Platani M, Goldberg I, Swedlow JR, Lamond AI. In vivo analysis of Cajal body movement, separation, and joining in live human cells. The Journal of cell biology. 2000;151(7):1561-74.

116. Daniels JP, Gull K, Wickstead B. Cell biology of the trypanosome genome. MicrobiolMolBiolRev. 2010;74(4):552-69.

117. Remeseiro S, Losada A. Cohesin, a chromatin engagement ring. Current opinion in cell biology. 2013;25(1):63-71.

118. Waizenegger IC, Hauf S, Meinke A, Peters JM. Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. Cell. 2000;103(3):399-410.

119. Peters JM, Nishiyama T. Sister chromatid cohesion. Cold Spring Harbor perspectives in biology. 2012;4(11).

120. Yang X, Figueiredo LM, Espinal A, Okubo E, Li B. RAP1 is essential for silencing telomeric variant surface glycoprotein genes in Trypanosoma brucei. Cell. 2009;137(1):99-109.

121. Barquilla A, Saldivia M, Diaz R, Bart JM, Vidal I, Calvo E, et al. Third target of rapamycin complex negatively regulates development of quiescence in Trypanosoma brucei. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(36):14399-404.

122. Shen TH, Lin HK, Scaglioni PP, Yung TM, Pandolfi PP. The mechanisms of PML-nuclear body formation. Molecular cell. 2006;24(3):331-9.

123. Tkacz ID, Lustig Y, Stern MZ, Biton M, Salmon-Divon M, Das A, et al. Identification of novel snRNA-specific Sm proteins that bind selectively to U2 and U4 snRNAs in Trypanosoma brucei. RNA. 2007;13(1):30-43.

124. Gottschling DE, Aparicio OM, Billington BL, Zakian VA. Position effect at S. cerevisiae telomeres: reversible repression of pol II transcription. Cell. 1990;63:751-62.

125. Horn D, Cross GAM. A developmentally regulated position effect at a Telomeric locus in Trypanosoma brucei. Cell. 1995;83(4):555-61.

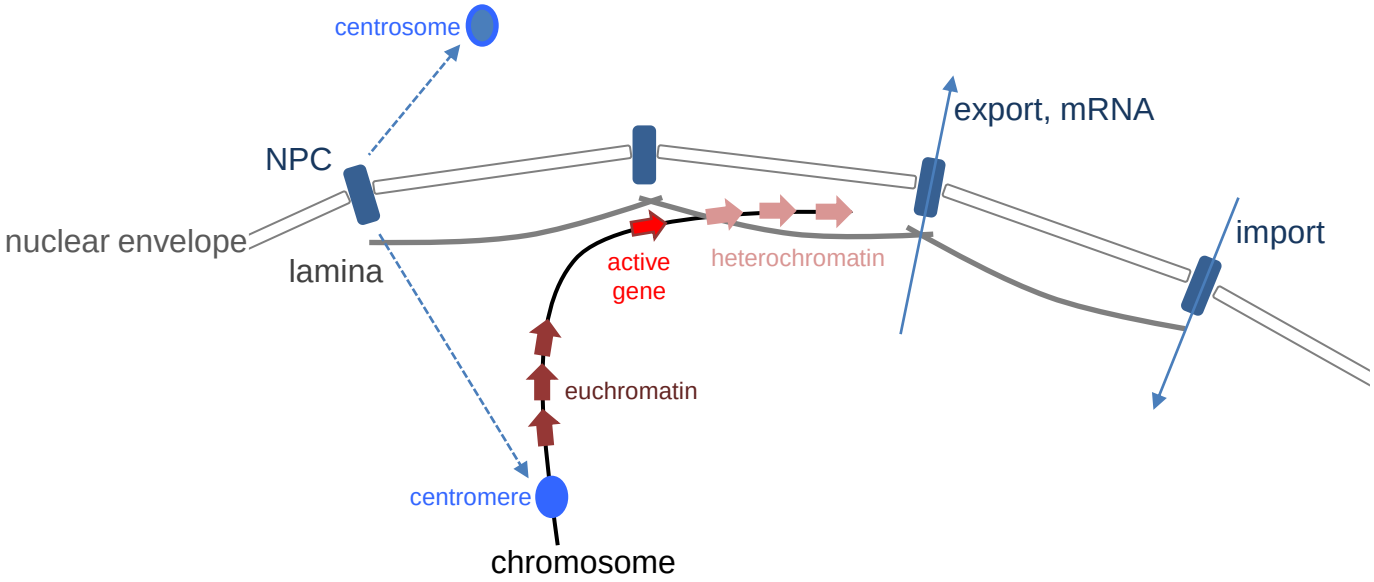
126. Taddei A, Hediger F, Neumann FR, Gasser SM. The function of nuclear architecture: a genetic approach. Annual review of genetics. 2004;38:305-45.

127. Frischknecht F, Mota M, Scherf A, Waters A. Biology of the malaria parasite--on the special issue for the 10th BioMalPar conference. Cellular microbiology. 2014;16(5):599-601.

128. Gartenberg MR, Neumann FR, Laroche T, Blaszczyk M, Gasser SM. Sir-mediated repression can occur independently of chromosomal and subnuclear contexts. Cell. 2004;119(7):955-67.

129. Kosak ST, Skok JA, Medina KL, Riblet R, Le Beau MM, Fisher AG, et al. Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. Science. 2002;296(5565):158-62.

Figure1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active
gene
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chromosome

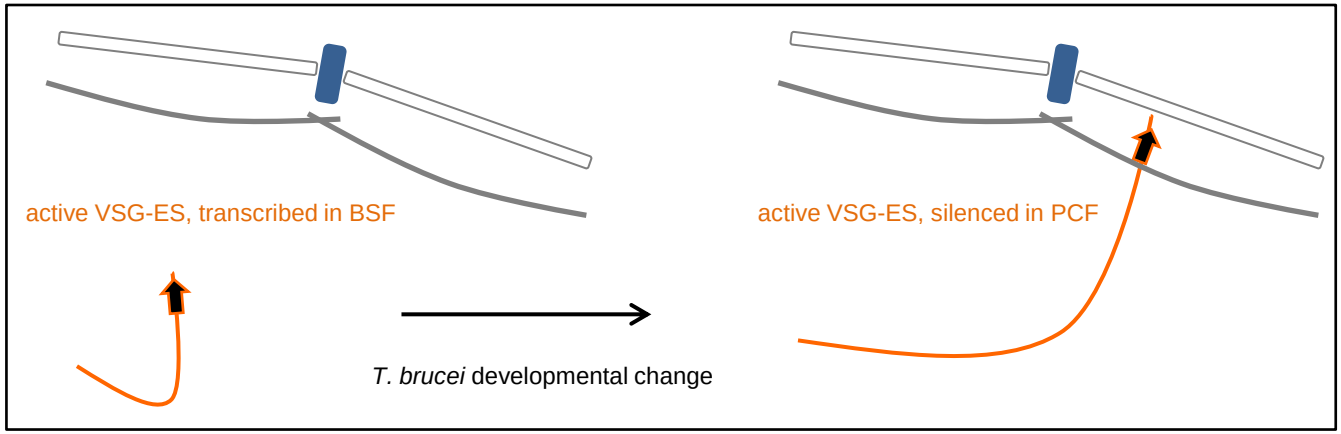

active VSG-ES, transcribed in BSF
active VSG-ES, silenced in PCF
T. brucei developmental change

Figure2

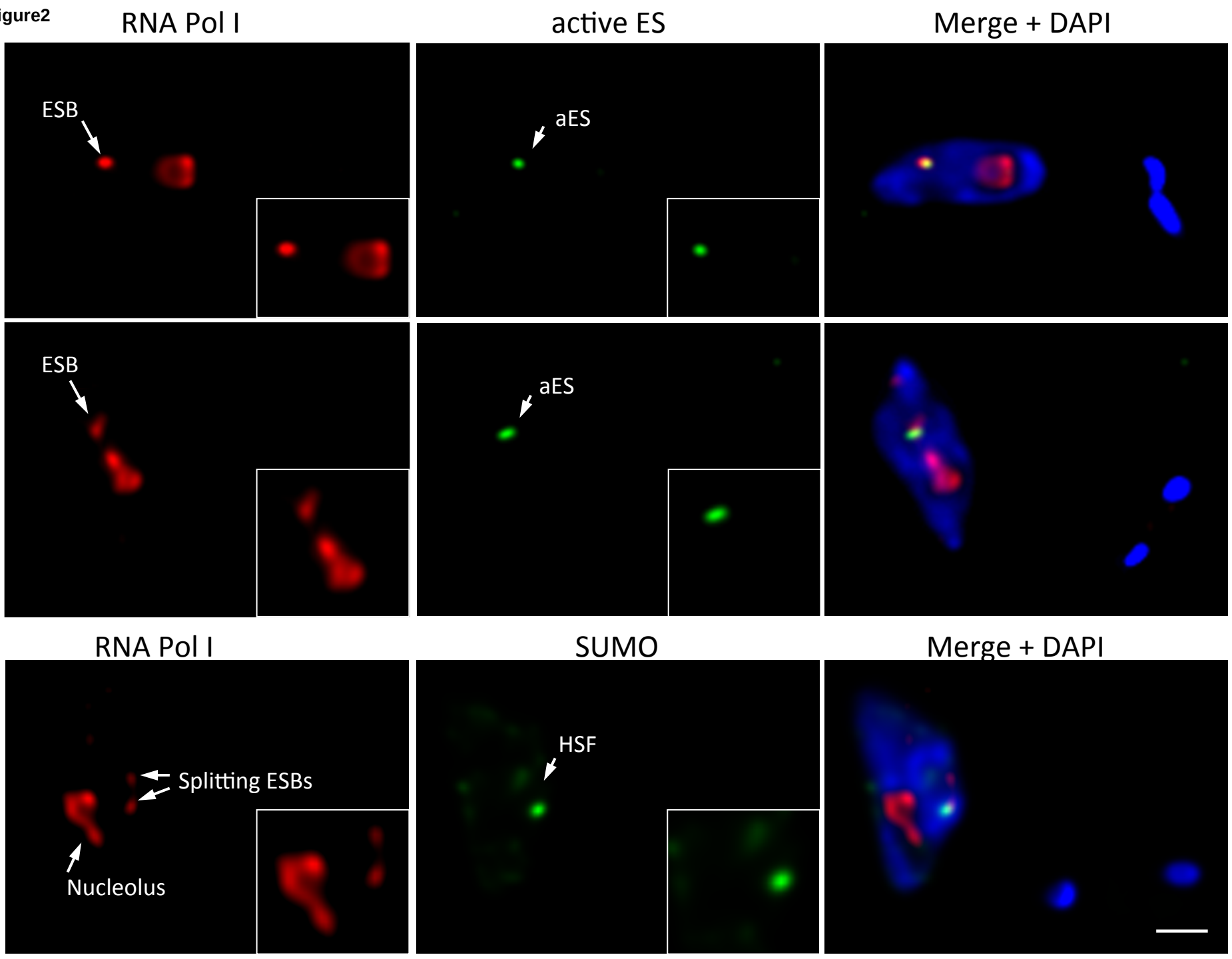
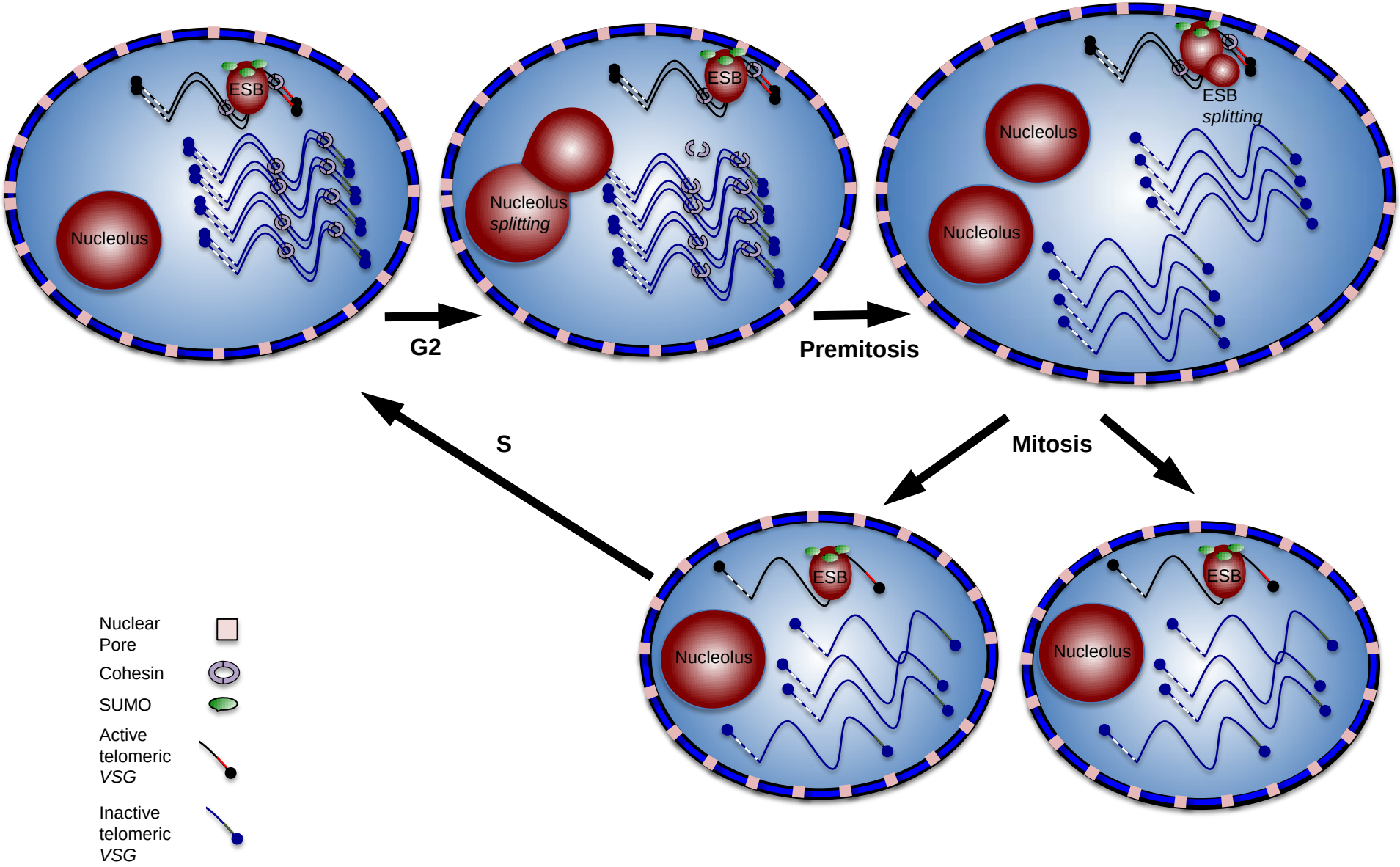

RNA Pol I
active ES
Merge + DAPI
ESB
aES
ESB
aES
RNA Pol I
SUMO
Merge + DAPI
Splitting ESBs
Nucleolus
HSF

Figure3


ESB
Nucleolus
G2
Nucleolus
splitting
Premitosis
ESB
splitting
S
Mitosis
Nuclear
Pore
Cohesin
SUMO
Active
telomeric
VSG
Inactive
telomeric
VSG