The extent to which models of attention and memory can be integrated and linked to brain theta oscillations may, thus, be an interesting topic for future research.

The study of the brain electrophysiological dynamics underlying human memory has been a topic of research for the last 30 years. A resurgence of this interest seems to have been taking place over the past few years with the incorporation of machine learning techniques, which allow the uncovering of temporal fine-grained mechanistic principles by which memory representations are accounted for by the human brain. This research may also provide fundamental insights to test mechanistic predictions derived from computational and animal work, thereby contributing to establishing similarities and differences across species. While much of the research in humans remains to be done, studies such as that by Kerrén *et al.* [1] illuminate the path towards inspiring, fruitful and exciting research in the upcoming years.

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Cell Biology: Functional Conservation, Structural Divergence, and Surprising Convergence in the MICOS Complex of Trypanosomes

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The MICOS complex is conserved across eukaryotes, but little is known about it outside the group that comprises animals and fungi. A new study finds that mitochondria of trypanosomatid parasites bear a divergent MICOS with both ancestral and derived subunits, but with conserved functions in crista development and membrane contact-site formation.

If you ever need a reminder that evolution does not create perfect forms, just look at the mitochondrial genome of trypanosomatid parasites. *Trypanosoma brucei*, the cause of sleeping sickness in humans, has a dense mass of DNA inside its single mitochondrion called the kinetoplast, which is the defining feature of kinetoplastids. In the kinetoplast, the

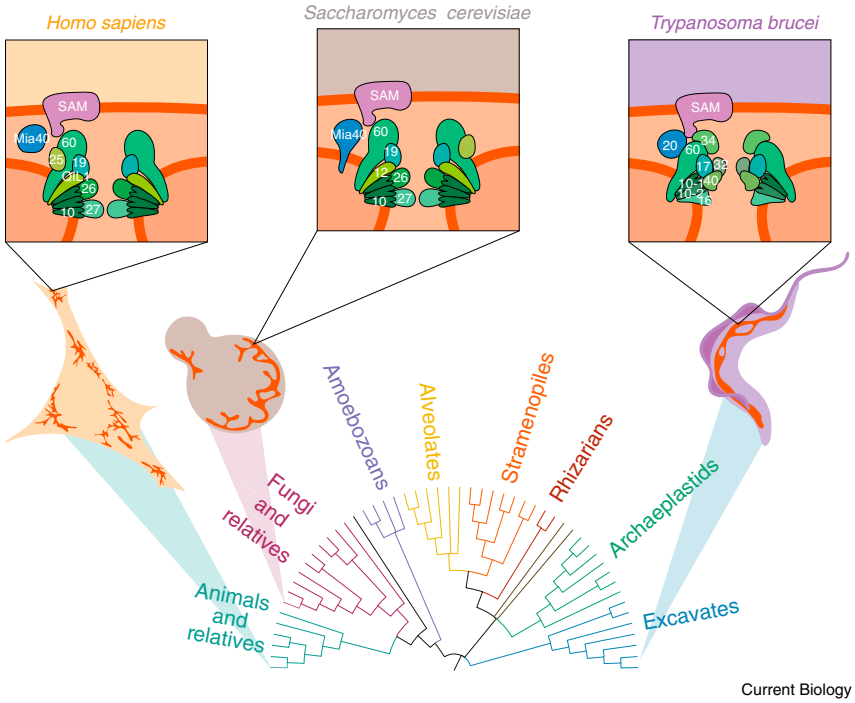

Figure 1. Experimentally characterized MICOS complexes across the eukaryote tree of life. The MICOS complex has now been experimentally well characterized in three different lineages from disparate branches of the eukaryote tree. Human and yeast MICOS are structurally and compositionally quite similar because both contain Mic60, Mic10, Mic19, Mic12/QIL1, and independent duplications resulting in Mic26 and Mic27. Mic25 resulted from a vertebrate-specific duplication of Mic19. *T. brucei* MICOS contains nine subunits including membrane-embedded *Tb*Mic10-1, *Tb*Mic10-2, *Tb*Mic16 and *Tb*Mic60 and peripheral *Tb*Mic17, *Tb*Mic20, *Tb*Mic32, *Tb*Mic34, and *Tb*Mic40. Even though *T. brucei* MICOS is structurally and compositionally divergent, its core functions in crista biogenesis and contact site formation (by interacting with *Tb*Sam50, the trypanosomatid sorting and assembly machinery) are conserved. Mia40 functions in the import of proteins into the mitochondrial intermembrane space in animals and fungi and interacts with Mic60 in yeast. In *T. brucei*, the thioredoxin-related Mic20 subunit has probably convergently evolved to function similarly to Mia40 in yeast. The number of branches per group is not proportional to diversity but to genome sampling. Part of the diagram takes inspiration from Rampelt *et al.* [20].

mitochondrial genome is found distributed in a mess of tangled mini- and maxi-circles whose cryptic genes require massive RNA editing to be decoded. Why did this extravagant mitochondrial genome and expression system evolve? It appears most probable that these unnecessarily complex features evolved neutrally in a ratchet-like manner and without providing any new selective advantage [1]. But this is not the only unusual feature of trypanosomatids and their mitochondria. In this issue of *Current Biology*, Hashimi and colleagues [2] experimentally characterize the functionally conserved but structurally divergent mitochondrial contact site and cristae organizing system (MICOS) complex in *T. brucei* and uncover a surprising convergence along the way.

The MICOS complex, experimentally known from only animals and fungi, plays an important role in the development and stability of mitochondrial cristae [3–5]. Cristae are sub-compartments that house the machinery involved in aerobic respiration, and they are formed by the regulated invagination of the mitochondrial inner membrane. In *Saccharomyces cerevisiae* and *Homo sapiens*, MICOS is composed of six and seven subunits, respectively (Figure 1). The MICOS complex makes contact sites between the mitochondrial inner and outer membranes to anchor cristae, and bends membranes to make crista junctions — the entry gates to respiratory cristae (Figure 1). MICOS is also important for the proper import of proteins into mitochondria [4] and for lipid transfer to and from mitochondrial membranes [6–8].

Evolutionary analyses revealed that the MICOS complex not only is an ancient and ancestral multi-protein complex of eukaryotes, but also had its origin in the progenitors of mitochondria, the alphaproteobacteria [9–12]. The same analyses showed that the two core MICOS subunits, Mic60 and Mic10, are widespread among eukaryotes, but trypanosomatids, surprisingly, appeared to lack Mic60. How could the MICOS of an aerobic mitochondrion work without Mic60, its functionally most important and ancient subunit? But this is not the only conundrum; trypanosomatids also appeared to lack Mia40, a protein that cooperates with Mic60 and the translocase of the outer membrane (TOM) complex, which is responsible for the oxidative import of proteins into the mitochondrial intermembrane space [13].

The apparent lack of both Mic60 and Mia40 in trypanosomes was puzzling. By experimentally characterizing the MICOS complex of *T. brucei* (*Tb*MICOS), Kaurov *et al.* [2] now provide tentative answers to this intriguing mystery. Using multiple methods, they identify at least nine *Tb*MICOS subunits and biochemically distinguish membrane-bound from peripheral subunits. Because *Tb*Mic60 is much smaller than other Mic60s, they propose that the ancestral Mic60 of kinetoplastids was split in two, the amino-terminal region becoming *Tb*Mic60 and the carboxy-terminal region becoming *Tb*Mic34. Only *Tb*Mic10-1 and *Tb*Mic10-2 (the two paralogs of Mic10), putatively *Tb*Mic60/*Tb*Mic34, and possibly *Tb*Mic17 show similarities to animal and fungal MICOS subunits. The other subunits appear to be specific to kinetoplastids.

Despite being considerably divergent in composition and structure, *Tb*MICOS appears to be functionally conserved. Kaurov *et al.* [2] show that *Tb*MICOS is associated with crista membranes, and disruption of some subunits (but not others) leads to elongated cristae and apparent loss of crista junctions, in agreement with what has been seen in fungi and animals [5,14]. Furthermore, *Tb*MICOS interacts with the sorting and assembly machinery (SAM) complex and therefore

is involved in making contact sites between the mitochondrial inner and outer membranes, as do animal and fungal MICOS complexes. Finally, similar to fungal MICOS, *Tb*MICOS aids in the oxidative import of intermembrane-space proteins. But without Mia40 how is this even possible?

The really big surprise uncovered by Kaurov *et al.* [2] was the discovery of a putative alternative mechanism for protein import into the mitochondrial intermembrane space. In most other aerobic eukaryotes, Mia40 plays a major role in the import and oxidative folding of intermembrane-space proteins with CX_3C and CX_9C motifs [13]. But Mia40 is lacking in all kinetoplastids including *T. brucei*, even though several of its substrates are readily identified (for example, the small Tims, Erv1, and Cox17), causing researchers to puzzle over how these proteins might be imported and modified [15]. In yeast, Mia40 interaction with MICOS is important for efficient import of these intermembrane-space proteins [4]. Kaurov *et al.* [2] show that depletion of the thioredoxin-like *Tb*Mic20 subunit correlates with a reduction in the steady-state levels of many intermembrane-space proteins, specifically several proteins containing CX_3C and CX_9C motifs. This observation led them to suggest that *Tb*Mic20 is a functional analogue of Mia40. If *Tb*Mic20 has truly replaced Mia40, then this is an unprecedented example of convergent replacement in the mechanism of mitochondrial protein import. Only time will tell if other lineages lacking Mia40 (such as stramenopiles, alveolates, and rhizarians) have also experienced similar convergent replacements.

MICOS structure appears to be unusually divergent in *T. brucei* relative to most eukaryotes. But this should almost be expected in this lineage. In fact, kinetoplastids are well known for their weird cell biology. Their mitochondria have discoidal cristae (unlike the lamellar cristae of mitochondria from animals, plants and fungi, and tubular cristae in other eukaryotes) and a barely recognizable TOM complex [16]. As a consequence, some have thought that these and many other odd features (including divergent kinetochores, nuclear pore complexes, and peroxisomes) are the outcome of primary divergence and independent evolution from all other eukaryotes [17]. But, in reality, these structures are secondary divergences with conserved functions [16,18,19].

So, is this the end of the MICOS story in kinetoplastids? Not likely. Trypanosomes surely have a few more tricks hidden up their sleeves, or rather their flagellar pockets. Further biochemical investigation of *Tb*MICOS subunits will lead to a better understanding of the differences seen in *T. brucei* compared to animal and fungal MICOS. For instance, is it possible that the extreme divergence of some subunits and the presumptive split of Mic60 arose from neutral evolutionary processes? Or are these differences in *Tb*MICOS the result of adaptive evolutionary processes? Furthermore, because of their different life stages in different hosts, *T. brucei* mitochondria undergo drastic morphological changes. Such morphological transitions offer a great opportunity to study the *de novo* development of cristae. Other questions that remain open with regard to *Tb*MICOS are whether *Tb*Mic60/34 can bend membranes, and whether *Tb*Mic10 also associates with the F_1F_o-ATP synthase complex. With these questions yet to be answered, we believe that the findings of Kaurov *et al.* [2] are only a first glimpse of what is yet to come.

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Neuroscience: Memory Encoding in the Absence of Cell Firing

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New research suggests that rats can learn new spatial information in the absence of cell firing. A small enhancement of GABAergic inhibition with a low dose of muscimol blocked cell firing but left long-term potentiation induction intact, while behaviorally it blocked memory retrieval but left memory encoding intact.

In most situations, learning new information (i.e., memory encoding) and performance (outputting behavior, for example, based on memory retrieval or other factors) are intermingled and difficult to separate. A key insight to setting up the dissociation of encoding and retrieval comes from arranging 'everyday memory'. For example, imagine a waiter who covers a new set of tables at a restaurant each workday. One might imagine that the waiter would initially return to yesterday's old table before setting out to the newly assigned table. With experience, the waiter would shift to the newly assigned table.

As reported in this issue of *Current Biology*, Rossato and colleagues [1] have arranged for an everyday memory assessment in rats. They used a Morris water maze with a platform, which was inaccessible for the first minute of searching, after which it was raised to be just below the surface of the opaque water. In the daily matching to place procedure, the platform rises at a new, unpredictable location on the first search of each daily session. Across a small number of subsequent trials, the platform consistently rises at that day's fixed location. This protocol allows for a separation of memory retrieval and encoding. If the rat remembers yesterday's platform location, it will swim to yesterday's location. Because the platform does not rise at yesterday's location, the rat gradually searches the pool until it finds the new location. After successfully locating today's new location, rapid new learning would lead the rat to today's location on subsequent trials. Across three successive days, this protocol isolates encoding on the first session, retrieval on the first trial of the second day followed by new encoding to update information about the second day's location. On the third day, the process repeats with retrieval of yesterday's old location and encoding of today's new location, which allows for an assessment of the effectiveness of learning that may have taken place on the previous day.

Rossato and colleagues administered a drug or vehicle shortly before the second session. This allows for potential dissociations of encoding, memory retrieval, and memory updating. Consider an animal given drug infusions directly into the hippocampus on the second session, followed by an assessment of performance in a post-drug third session. A key interest is the search behavior on the *first* trial of each day. In the new study, 'silent learning' was observed using a low-dose of muscimol (which causes a small enhancement of GABAergic inhibition after intrahippocampal infusion) with searching behavior on the initial trial as follows: In the pre-drug session, the rats searched appropriately, meaning that much of the search path was directed at yesterday's location; thus, a high percentage of time was spent in the zone surrounding the previous session's location. On the second day (with muscimol present), searching behavior was unfocused all over the watermaze; correspondingly, the percent of searching in the zone surrounding yesterday's location was at the level expected based on random searching. On the third day (in the absence of drug), searching was correctly focused on yesterday's location; percent searching in yesterday's zone was again high. Apparently, although the rat could not accurately retrieve the first session's location during muscimol treatment, it was successful in learning to update the new location during the second session, as revealed in the drug-free state on the third session when the rat