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Symbiosis: A duplicated host protein controlling a nascent mutualism

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Mechanistic studies on how eukaryotes ensure vertical inheritance of beneficial intracellular prokaryotes have focused mostly on highly integrated relationships. A new study by Zakharova, Tashyreva *et al.* reveals how a duplicated host gene impacts symbiont inheritance in a young mutualism.

Symbiotic relationships between eukaryotes and intracellular prokaryotes — bacteria and archaea — are widespread and have been established independently countless times[1]. Mutualistic endosymbiotic interactions that benefit both partners have long been appreciated as powerful adaptive forces in eukaryotic evolution: ancient prokaryotic symbionts were the substrates used to forge mitochondria and plastids, and diverse prokaryotic metabolic repertoires offer modern-day eukaryotes the promise of thriving in inhospitable environments[2], or on nutritionally incomplete diets[3]. Establishing stable, long-term mutualisms is, however, likely more difficult than it appears at first glance. For instance, it is advantageous to develop a mechanism that ensures the faithful transmission of symbionts to the host's progeny so that they may continue to enjoy the same benefits; but it is unclear exactly how this happens at a molecular level. And even though the evolutionary interests of the mutualistic partners become intertwined, there can be too much of a good thing: negative fitness consequences accrue to a host that is supporting too large a symbiont load, so control over 'titre' becomes essential[4]. Studies of ancient obligate symbioses and endosymbiotic organelles have shown that the host mostly solves these problems by eventually controlling symbiont growth, division[5], distribution, and metabolism[6]. But this high degree of interdependency makes it difficult to characterize the innovations that foster and stabilize obligate mutualisms. In contrast, evolutionarily young obligate relationships are potentially more 'breakable' and amenable to genetic dissections that may provide compelling new insights. A new study in this issue of *Current Biology* by Zakharova, Tashyreva *et al.*[7] does just that by characterizing a neo-functionalized, host-encoded gene duplicate that allows a unicellular eukaryote host, *Novymonas esmeraldas*, to regulate the vertical inheritance of its symbiont, *Ca. Pandoraea novymonadis*.

Novymonas belongs to the order Trypanosomatida, a group of insect-vectored obligate parasites that includes numerous medically relevant species, including those in the genera *Trypanosoma* and *Leishmania*. And although *Novymonas* is a close relative of *Leishmania*, it is not a pathogen. Rather, it is an insect-limited parasite that inhabits the hindgut of a bug, *Niesthrea vincentii* (Hemiptera: Rhopalidae), and was first isolated during a diversity survey in Ecuador[8]. *Novymonas* harbours a small but variable number of β-proteobacterial symbionts, and alternates between a

substratum-attached proliferative 'rosette' stage and an elongated motile 'swimmer' stage.

So, why is this obscure system important to understanding how obligate mutualisms work? One reason is that the symbiosis is still becoming obligate and might offer insights into the process of how mutualisms first evolve. For instance, the total symbiont titre is not tightly regulated as it is in more mature obligate symbioses[5]; however, observations of lysosomes fusing with symbiontophorous vacuoles indicates that some level of host control is exerted[9]. Also, *Novymonas* can survive without its bacterial partners — in an 'aposymbiotic' state — but it suffers from suppressed growth and metabolism in their absence[10]. Likewise, the symbiont can be cultured axenically in rich medium. But its genome has shrunken relative to free-living relatives; pseudogenes have been purged, and its metabolic repertoire has been streamlined, altogether indicating a high degree of adaptation to the host[11]. The symbiont likely benefits the host by providing essential amino acids and purines, and complementing heme synthesis, whereas the symbiont receives phospholipids and uses host carbohydrate metabolic enzymes to complement its own deficiencies[10]. But there are many other young obligate symbioses known from both microbial[12] and multicellular[13] eukaryotes, so why is *Novymonas* a good system for investigating this? The answer is that trypanosomatids offer several experimental advantages over other symbiotic systems. They can usually be grown axenically to high density on a defined medium and offer a sophisticated molecular toolkit that allows researchers to dissect gene function.

To search for host proteins associated with control of symbiont localization, inheritance, and titre, Zakharova, Tashyreva *et al.* started from a simple premise. They reasoned that if such host proteins exist, they should be most abundant in symbiont-enriched subcellular fractions. Using sensitive, tandem-mass-spectrometry analyses, the authors identified 2,879 host-encoded and 669 symbiont-encoded proteins, including many contaminants

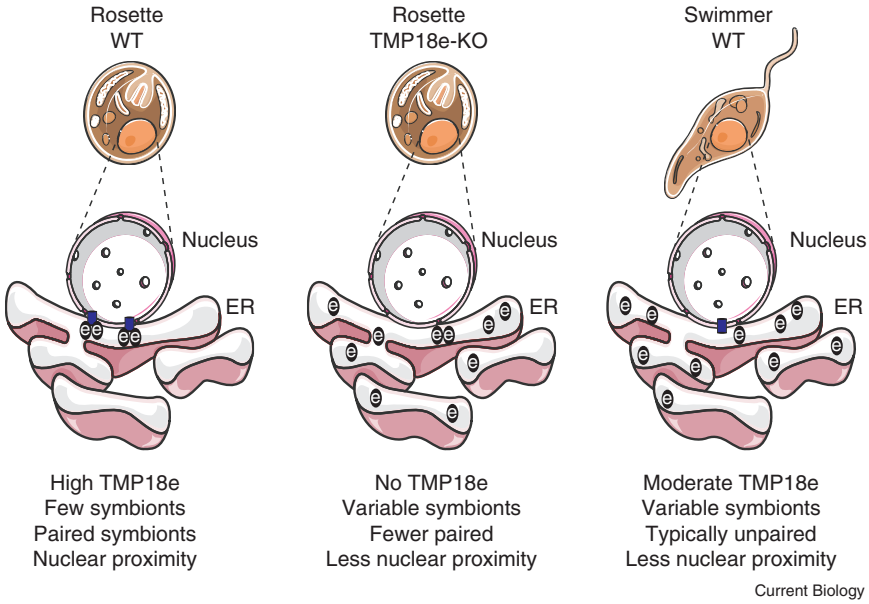

Figure 1. Distribution of *Novymonas* symbionts as a function of life cycle and TMP18e abundance.
In wild-type (WT) 'rosettes', TMP18e concentrations are relatively high, which likely sequesters ER-localized symbionts in proximity of the nuclear envelope. Symbionts in this proliferative stage are frequently paired, and in low abundance. When TMP18e expression is ablated, the tight nuclear association of symbionts is disrupted, as is consistent pairing of symbionts. Symbiont number may be higher or lower than in the wild type. In the dispersal 'swimmer' stage, TMP18e expression is lower than in rosettes, symbionts are infrequently paired, and their number is often higher. Base images for rosettes (*Leishmania* amastigote), swimmers (*Leishmania* promastigote), nuclei, and the ER are from bioicons (bioicons.com). Black circles labeled 'e' represent endosymbiont cells, and blue cylinders represent TMP18e.

from membranous host organelles, like mitochondria and peroxisomes. But among the most enriched host-encoded proteins was an intriguing candidate, TMP18e, a divergent, *Novymonas*-specific duplicate of transmembrane protein 18 (TMP18), a pan-eukaryotic protein of completely unknown function that localizes to the nuclear envelope in plants[14] and animals[15]. The potential for gene duplication and neofunctionalization in allowing cells to explore new functions has long been appreciated[16], and tantalizing differences between TMP18e and its TMP18 homologs suggest that it might play a role in symbiont inheritance. Of particular interest is a ~25 amino acid long amino-terminal extension predicted to face the lumen of the endoplasmic reticulum. Notably, complementary microscopic approaches used by Zakharova, Tashyreva *et al.* show that the endosymbionts are housed in the endoplasmic reticulum lumen, raising the possibility that the amino terminus physically interacts with symbionts.

These same microscopy experiments offer insight into the differences between symbiotic behaviour in the actively dividing 'rosette' and the dispersing swimmer stages (Figure 1). Immunofluorescence microscopy of genetically tagged TMP18e protein showed co-localization with symbionts in rosettes and swimmers, but the association was tighter in rosettes. This was supported by electron microscopy, which showed that symbionts in rosette cells — and not swimmers — surround the nucleus and connect to the host perinuclear space via short tubules. Altogether, these observations suggest that symbionts may take advantage of their tight connection to the host nucleus in proliferating rosettes so as to facilitate their passage into host progeny.

If symbiont inheritance is somehow mediated *via* TMP18e, one might expect to see it present in higher quantities in rosettes than in swimmers — so, do we? Yes: tagged TMP18e protein levels are higher in rosettes, in both

symbiont-containing and aposymbiotic lines. On the flip side, what happens when the gene encoding TMP18e is knocked out? It depends on developmental stage. In swimmers, knockout of TMP18e did not alter the number of symbionts per cell, though it did lead to more skew and a higher proportion of symbiont-lacking hosts. But rosettes lacking TMP18e had an increased symbiont load, a disruption of the nucleus-symbiont association, and a smooth continuum of symbiont number per rosette, in contrast to wild-type rosettes that have a small number of paired symbionts (Figure 1).

It remains uncertain exactly what role TMP18e plays in ensuring proper symbiont inheritance. It's unlikely to coordinate cell division between rosettes and symbionts because specific antibiotic treatments can reduce host division without impeding symbiont division. Instead, Zakharova, Tashyreva *et al.* suggest that TMP18e is important for symbiont segregation, for instance, by keeping symbionts in proximity of the host nucleus. So, how does this proposed mechanism stack up against what we know from other obligate mutualists? There are no perfect comparisons, but an emerging common theme is that host gene duplication and transfer of symbiont-derived genes to the host nucleus contribute disproportionately to controlling symbionts. In the trypanosomatid *Angomonas deanei*, duplicated host dynamin-like proteins are retargeted to the symbiont outer membrane, where they regulate symbiont division[5], much as they do for many mitochondria[17]. In contrast, mealybugs gain control over symbiont division through transfer of some peptidoglycan biosynthetic genes to the host genome[6] — a process that necessarily involves duplication of the symbiont gene prior to its loss.

The TMP18e knockout phenotypes fit well with the proposed roles of rosette and swimmer stages in the *Novymonas* life cycle. Typically, rosettes bear an even number of symbionts, because they pass on an equal number of symbionts to each daughter cell upon division. In contrast, swimmers are a dispersal stage that grow, rather than divide, so they exercise less strict control over symbiont number. But if a high symbiont load can have negative impacts on the host, why does *Novymonas* permit swimmers to accumulate symbionts rather than degrading most *via* lysosomes[9]? This matter is far from settled; the authors suggest here and elsewhere[18] that *Novymonas* might maintain more symbionts than necessary so that they can eat them in times of exogenous prey scarcity, much like the proposed 'farming' of β-proteobacterial endosymbionts by the amoeba *Dictyostelium*[19]. However, it may also be the case that the additional symbionts are not overly burdensome, and that this simply represents acceptable sloppiness in a still-evolving relationship. Ultimately, it will be important to carry out experiments to understand how symbiont load impacts the fitness of microbial eukaryotic hosts.

In summary, Zakharova, Tashyreva *et al.* have identified in *Novymonas* a neofunctionalized gene duplicate, TMP18e, that co-localizes with its endosymbionts and, in a stage-specific manner, facilitates symbiont vertical inheritance through an incompletely understood mechanism. Although this work opens as many new questions as it answers, it represents among the first ever molecular characterizations of how symbiont vertical inheritance functions and evolves, and reiterates the importance of gene duplication in eukaryotic innovation.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Human genetics: Rich genomic history of two isolated Indigenous peoples of South America

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Genome-wide data from two Indigenous South American groups reveal their dynamic population history. The Mapuche from Southern Chile and the Ashaninka from Amazonian Peru remained largely isolated over time. Yet, both groups interacted with other South American peoples sporadically.

The genomic history of Indigenous Americans is understudied, particularly in comparison to other regions, such as Europe[1], where genomic data are available for thousands of ancient and present-day individuals. Nevertheless, large-scale genomic studies, together with archaeological, anthropological, geological and linguistic analyses, have created a broad-strokes painting. It is widely accepted that the ancestors of the first Americans originated in Siberia or northeast Asia during the last Ice Age, approximately 20–30,000 years ago. Afterwards, they migrated east through the Bering land bridge and south through the Pacific coast, as it became possible to pass the two vast ice sheets that covered northern North America more than 14,000 years ago[2]. Once south of the ice, humans expanded through the continent at a blazing speed. Over a few millennia, they settled in contrasting environments, such as the arid Great Basin of North America and the Amazon rainforest in South America. Due to this quick radiation, the genetic differentiation between the first hunter-gatherer populations across the continent was not substantial[3]. The marked population structure observed in more recent and present-day Indigenous Americans arose later during the Holocene as their ancestors adapted to local environments and, in many regions, developed complex societies supported by agriculture.

In South America, three major branches of genomic ancestry characterise peoples in the Andes, the Amazon and the Southern Cone (which roughly includes Chile, Argentina and Uruguay)[4]. These branches established early during the peopling of South America and mixed with later population expansions from the north during the Holocene[3,4]. Due to the population decline caused by colonial practices and the introduction of new diseases after the European invasion[5], our knowledge of the demographic processes that gave rise to specific Indigenous groups is limited. In addition to the significant loss of cultural and genomic diversity, population relocations reshuffled pre-colonial population structure patterns[6]. A few studies with a regional focus have provided reconstructions of specific population histories (for example, see[7–9]). However, the continent is filled with a rich diversity of Indigenous groups with living cultures whose genomic history remains to be explored. Two studies published in *Current Biology* by Arango-Isaza *et al.*[10] (in this issue) and Capodiferro *et al.*[11] (in a recent issue) look into the genomic history of the Mapuche and the Huilliche in Southern Chile, and the Ashaninka in Amazonian Peru, respectively (Figure 1). Whereas these peoples have a long account of isolation in their region, both studies reveal interesting aspects of Indigenous South American demographic history, including marked local population structure and interregional contacts.

According to the 2017 national census, Chile is home to ~2 million people who self-identify as members of an Indigenous group[12]. Among them, ~79% belong to the Mapuche, a group mainly present in Southern Chile in the 'Araucanía' region. Despite their numbers and seeming regional continuity across the last two millennia, different competing hypotheses explaining their origins have been put forward. These disparate hypotheses place the origin of the Mapuche all across South America, including the central Andes, the Gran Chaco region and even the Amazonian rainforest[10]. To settle this debate, Arango-Isaza *et al.*[10] generated genome-wide data for 64 individuals from three Indigenous groups from Southern Chile: the Pehuenche and the Lafkenche from the mountains and the coast of Araucanía, and the Huilliche from the island of Chiloé. Like many of the Indigenous groups in the region, the Pehuenche and