GENOME WATCH

A new piece of the eukaryotic puzzle

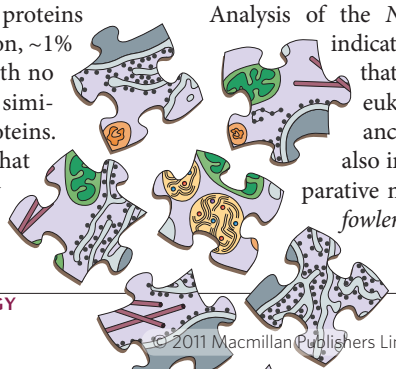
Alejandro Sanchez-Flores

This month's Genome Watch discusses the genome of the free-living amoeboflagellate protist *Naegleria gruberi*.

In the eukaryotic tree, the free-living protist *Naegleria gruberi* belongs to the class Heterolobosea, which is a major eukaryotic lineage and part of the ancient JEH (Jakobida–Euglenoza–Heterolobosea) clade. This clade was not resolved until the *N. gruberi* genome became available, and its resolution contributed to the re-rooting of the eukaryotic tree with all six major eukaryotic groups — Opisthokonta, Ameobozoa, Viridiplantae, Alveolata, POD (Parabasalia–Oxymonadida–Diplomonadida) and JEH. This provided a wider window on early eukaryotic evolution[1].

The genome of *N. gruberi* comprises 41 million bp, distributed across 12 chromosomes. In addition, a 14 kb extrachromosomal plasmid and a 50 kb mitochondrial genome were sequenced[2]. The nuclear genome has 15,727 protein-coding genes (57.8% of the total), so it is compact but gene rich. The authors compared these proteins with 17 proteomes from across the six major eukaryotic groups. They constructed 4,133 protein groups containing a minimum of one *N. gruberi* protein and two orthologues, requiring at least one of the orthologues to be from another major eukaryotic group. These proteins are inferred to have been present in the common ancestor of all extant eukaryotes, an assumption supported by the fact that 92% and ~50% are present in three and five major eukaryotic groups, respectively. Of the 4,133 proteins, 40% belong exclusively to eukaryotes, and most are hypothetical proteins of unknown function. In addition, ~1% of the proteins in *N. gruberi* with no eukaryotic homologues are similar to bacterial or archaeal proteins. Phylogenetic analysis suggests that these genes were acquired by lateral gene transfer[2].

The *N. gruberi* genome revealed several interesting cellular features[2,3]. For example, there is no cytological evidence of a Golgi complex in this species, but genes relevant for membrane trafficking in the Golgi complex were detected. The genome also contains a large superfamily of tubulin genes spanning almost all known tubulin subfamilies. This includes a group of divergent α-tubulin proteins that are probably involved in the assembly of the atypical mitotic spindle in *N. gruberi*. The mitochondrial genome is contained in a single circular chromosome[3] instead of many circular DNA molecules, thus resembling a bacterial genome. In contrast to the mammalian mitochondrial genome, which encodes only 13 proteins and a few RNA-coding genes, the *N. gruberi* mitochondrial genome encodes 69 genes, including 43 that are protein coding. A complete set of tRNAs is present, suggesting independent mitochondrial-gene translation. Some of the protein-coding genes correspond to cytochrome units that are lost in other JEH organisms, and their presence results in a flexible metabolism in both aerobic and anaerobic conditions. Genomic information on the metabolism of *N. gruberi*[4] is important, as experimental data are scarce. In addition to mitochondrial metabolism, important anaerobic traits were predicted. For example, from the carbohydrate metabolic pathway it can be inferred that the final products of aerobic metabolism are carbon dioxide and water. In anaerobic conditions, metabolites such as succinate, acetate and probably also ethanol and D-lactate can be produced[4].



Analysis of the *N. gruberi* genome indicates ancestral features that are indicative of the eukaryotic common ancestor. *N. gruberi* is also important as a comparative model for *Naegleria fowleri*, a closely related pathogenic species responsible for primary amoebic meningoencephalitis in humans. The authors propose that some potential *N. fowleri* drug targets can be elucidated from the *N. gruberi* genome[4], including the enzymes involved in the hydroxymethylglutaryl CoA (HMG-CoA) pathway, a bacterial-like mitochondrial nitroreductase (NTR1) and the alternative oxidase (AOX). The HMG-CoA pathway generates the precursor for isoprenoids and sterols, which are components of the plasma membrane. Current treatment[5] for primary amoebic meningoencephalitis includes amphotericin B, which associates with ergosterol and disrupts the membrane. Shutting down the HMG-CoA pathway would generate the same effect. NTR1 has been detected only in trypanosomatids, in which it activates drugs into parasiticidal metabolites. AOX is absent in humans but is a key metabolic enzyme for *N. fowleri* proliferation in the human brain.

The knowledge base provided by the *N. gruberi* genome not only reveals ancestral eukaryotic features and the diversity of the eukaryotes but also provides a comparative model to study the rare fulminant pathogen *N. fowleri*.

Alejandro Sanchez-Flores is at the Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.

e-mail: microbes@sanger.ac.uk

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Competing interests statement
The author declares no competing financial interests.