

Contributions of *Oxyrrhis marina* to molecular biology, genomics and organelle evolution of dinoflagellates

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The flagellate *Oxyrrhis marina* has been the subject of numerous studies addressing diverse aspects of protist biology including feeding, motility, ecology and cell biology. In spite of the rich body of information that has been built around this organism, the molecular biology of *O. marina* has remained virtually unstudied until very recently. Studying the molecular biology and genomics of *O. marina* is not only important due to its role as a model organism and practical accessibility; current evidence shows that it occupies a basal position within the dinoflagellate lineage, making it an ideal starting point to reconstruct the evolution of several interesting characters in this diverse group and its sister group Apicomplexa. Among these features, dinoflagellates have very divergent mitochondrial genomes, a complex history of plastid evolution and unique features involving the nuclear genome and transcription. Here we review and discuss how recent findings from *O. marina* are contributing to shed light on these and other aspects of the evolutionary history of two important eukaryotic lineages, the apicomplexans and dinoflagellates.

KEYWORDS: *Oxyrrhis*; genome; molecular biology; plastid; mitochondria; nuclear; dinoflagellates; apicomplexa

INTRODUCTION

Oxyrrhis marina is a popular model for addressing aspects of protistan biology (Montagnes *et al.*, 2011). Studies conducted on this organism cover topics as diverse as mechanisms of feeding (Roberts *et al.*, 2011), factors involved in foraging behaviour (Breckels *et al.*, 2011), distribution and dispersal (Watts *et al.*, 2011), predation (Yang *et al.*, 2011), swimming behaviour (Boakes *et al.*, 2011) and various cytological aspects such as cell and nuclear division (Gao and Li, 1986; Kato *et al.*, 2000) and flagellar motility (Gagnon *et al.*, 1994; Cosson *et al.*, 1996; Gagnon *et al.*, 1996). In spite of this rich body of information, little is known about *O. marina* at the

molecular level. Until recently, DNA sequence information was restricted to a few genes used for phylogenetic inference, e.g. those encoding ribosomal RNA genes (small subunit and ITS), actin, heat-shock protein 90 and alpha- and beta-tubulin (Saldarriaga *et al.*, 2003; Cavalier-Smith and Chao, 2004; Leander and Keeling, 2004; Lowe *et al.*, 2005, 2010).

Oxyrrhis has early on been considered a dinoflagellate, although a very unusual one (Senn, 1911; Hall, 1924). Along with their sister lineage Apicomplexa (e.g. *Plasmodium*, *Toxoplasma*, *Cryptosporidium*) and Ciliates (e.g. *Paramecium*, *Tetrahymena*), dinoflagellates belong to the major eukaryotic group known as Alveolata (Fig. 1)

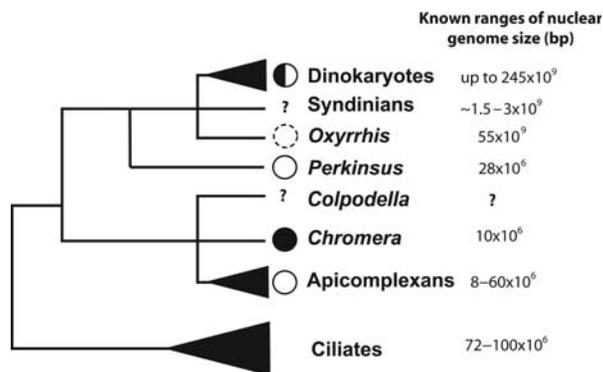


Fig. 1. A schematic representation of the phylogenetic relationships among alveolates highlighting the three main groups: Ciliates, Apicomplexans and Dinoflagellates (black triangles). Genera or groups relevant to the study of the characters described in the text are also shown. Circles represent distribution of photosynthesis and plastids. Dark circles indicate the presence of photosynthetic plastids and white circles mark non-photosynthetic groups where plastids have been found (dinoflagellates include substantial numbers of both photosynthetic and non-photosynthetic sub-groups). *Oxyrrhis marina* is non-photosynthetic, but evidence for a plastid remains inconclusive. References for ranges of nuclear genome size are (Shirley, 1994; Veldhuis *et al.*, 1997; Gardner *et al.*, 2005; LaJeunesse *et al.*, 2005; Aury *et al.*, 2006; Eisen *et al.*, 2006; Teles-Grilo *et al.*, 2007a; Obornik *et al.*, 2009; Sano and Kato, 2009).

(Cavalier-Smith, 1993). Most species in the crown of the dinoflagellate lineage, often referred to as dinokaryotes (and colloquially typical or true dinoflagellates), exhibit distinctive features such as the cingulum, sulcus, traverse flagellum and the peculiar birefringent chromosomes (Spector, 1984b; Taylor, 1987; Hackett *et al.*, 2004). The dinoflagellate lineage includes lesser-known organisms (e.g. Syndinians, Noctilucoids, *Parvilucifera*, *Perkinsus*), but the exact relationships among them are still matter of debate (Fensome *et al.*, 1993; Fast *et al.*, 2002; Cavalier-Smith and Chao, 2004; Leander and Keeling, 2004; Saldarriaga *et al.*, 2004). The unusual features exhibited by *Oxyrrhis* and the existence of different views of what comes under the term “dinoflagellate” complicate its high-level taxonomic treatment (Fensome *et al.*, 1993; Saldarriaga *et al.*, 2004). Some authors consider *Oxyrrhis* a basal member of the dinoflagellate lineage, branching after *Perkinsus* but before dinokaryotes (Fensome *et al.*, 1993; Saldarriaga *et al.*, 2003; Slamovits *et al.*, 2007; Zhang and Lin, 2008). A contending view considers *Oxyrrhis* a highly derived dinokaryotic dinoflagellate (Cavalier-Smith and Chao, 2004). The latter view is supported by phylogenies based on SSU rDNA sequences, but this molecule is highly divergent in *Oxyrrhis* and thus prone to artefacts that may confound the phylogenetic analysis (Saldarriaga *et al.*, 2003). The basal position, on the other side, is supported by a growing wealth of protein sequence and other molecular data (Saldarriaga *et al.*, 2003; Leander and Keeling, 2004; Zhang and Lin,

2008), which include a phylogenetic analysis with a 30-protein data set (Slamovits *et al.*, 2007; see Lowe *et al.*, 2011a for more details and references). In summary, current evidence places *Oxyrrhis* as a basal branch of the dinoflagellate lineage, leaving the exact taxonomic treatment (i.e. a Dinoflagellate) open to individual criteria.

The phylogenetic position of *O. marina*, at the base of the dinoflagellate lineage and close to the divergence of Apicomplexans makes it a potentially valuable starting point to reconstruct the evolution of a range of interesting characters within these diverse groups. These two eukaryotic lineages are of great medical, economic and environmental importance. For example, the Apicomplexa includes parasites such as *Plasmodium*, *Theileria*, *Toxoplasma*, *Babesia* and *Cryptosporidium*, which cause human and animal diseases (Perkins *et al.*, 2000). Furthermore, dinoflagellates are important and diverse group in planktonic ecosystems, having significant roles in primary productivity, nutrient cycling, harmful algal blooms, coral bleaching and parasitic diseases of crustaceans (Shields, 1994). In addition to its advantageous phylogenetic position, *O. marina* is also easy to grow and maintain features that favour its choice as a model organism (Lowe *et al.*, 2011a). We will focus on how *O. marina* has and will help in our understanding of the nature and evolution of genes and genomes along the dinoflagellate lineage.

Dinoflagellate genomes have taken many twists and turns in evolution (Raikov, 1995; Rizzo, 2003; Hackett *et al.*, 2004; Moreno Diaz de la Espina *et al.*, 2005). Because of its position in the lineage, ease of culture and free living lifestyle *O. marina* constitutes a useful model to address the origin and evolution of several of the more unusual cellular and molecular features of dinoflagellates and apicomplexans, as well as general questions about endosymbiosis and plastid evolution. To this end, we review what is known regarding the structure and content of three main regions of *O. marina* evolutionary development: (i) nuclear, (ii) mitochondrial and (iii) plastid genomes. We then related this to our understanding of genome dynamics and evolution in dinoflagellates, in general, through a range of processes including lateral (or horizontal) gene transfer (LGT) and gene expression. Thus, we illustrate how *O. marina* has and can be used as a model to assess evolutionary trends.

NUCLEAR GENOME

The nuclei of dinokaryotic dinoflagellates exhibit an impressive gallery of oddities at the structural and molecular levels, some of which appear to defy basic concepts of the eukaryotic cellular structure (Wargo and Rizzo, 2001; Hackett *et al.*, 2004; Costas and Goyanes,

2005; Moreno Diaz de la Espina *et al.*, 2005). Their chromosomes are large and numerous, remain condensed throughout the cellular cycle and exhibit a characteristic birefringent banding pattern (Livolant, 1978; Livolant and Bouligand, 1978; Herzog and Soyer, 1981; Rizzo, 1991, 2003; Moreno Diaz de la Espina *et al.*, 2005). Dinoflagellate genomes also contain large amounts of DNA, in some cases more than 100 pg, or about 100 billion base pairs (Gb): equivalent to about 30 human genomes (Rizzo, 1987; Veldhuis *et al.*, 1997; LaJeunesse *et al.*, 2005). Nucleosomes are absent in dinoflagellates, replaced by a still unclear system of basic proteins that may have originated from the histone-like proteins of prokaryotes (Bodansky *et al.*, 1979; Herzog and Soyer, 1981; Rizzo, 1981, 1991; Li, 1983; Wong *et al.*, 2003; Chan *et al.*, 2006). The profound differences in the chromatin structure between dinokaryotic dinoflagellates and other eukaryotes raise questions about how these features originated, and these transitions can be understood better by looking at organisms, such as *O. marina*, which represent basal lineages.

In *O. marina*, the chromatin exhibits a differentiated but seemingly intermediate state. Specifically, *O. marina* chromosomes remain permanently condensed and are weakly birefringent, but they lack the conspicuous periodic banding observed in other dinoflagellates (Cachon *et al.*, 1989; Kato *et al.*, 1997). A fundamental difference between dinoflagellate chromatin and that of other eukaryotes lies in the nature and amount of DNA-associated proteins. In typical eukaryotic chromatin, five classes of histones constitute the structural basis of the nucleosomes, and the proportion of DNA to proteins is about 1:1, whereas in the chromosomes of dinoflagellates, basic proteins are less abundant, with a DNA-basic protein ratio of 10:1 (Rizzo and Nooden, 1973). The main basic nuclear protein in *O. marina* is a single 29 kDa protein, termed Np23, which localizes only to chromosomes in dividing and non-dividing cells (Kato *et al.*, 1997). Its primary structure is unknown, but the molar amino acid composition suggests that Np23 and histone H1 have fundamental structural differences (Kato *et al.*, 1997). Given its basal position, whether genes homologous to those coding typical histone proteins exist in *O. marina* is an important question that is currently the subject of ongoing studies.

MITOCHONDRIAL GENOME

An overview

Mitochondrial genomes occur in a variety of forms, from single linear molecules to multiple chromosomes

(Gray *et al.*, 2004), but some of the most unusual states occur in alveolates: ciliates have circular mitochondrial genomes much like several other eukaryotic groups (Burger *et al.*, 2000), while apicomplexan and dinoflagellate mitochondrial genomes are reduced and derived. Apicomplexans have small (i.e. 7 kbp) linear-mapping genomes in their mitochondria that encode only three proteins, cytochrome *b*, cytochrome oxidase I and cytochrome oxidase III (*cob*, *cox1* and *cox3*, respectively); they also possess extensively fragmented ribosomal RNA genes for which many portions are unaccounted (Feagin, 1992; Feagin *et al.*, 1997). In dinoflagellates, the mitochondrial genome is a large and heterogeneous collection of sequences including complete genes, gene fragments, repeats and non-coding DNA (Chaput *et al.*, 2002; Jackson *et al.*, 2007; Nash *et al.*, 2007, 2008; Slamovits *et al.*, 2007; Waller and Jackson, 2009). As in apicomplexans, the only protein-coding genes are *cob*, *cox1* and *cox3*. Likewise, ribosomal RNA genes are fragmented. Beyond these similarities with apicomplexans, the mitochondrial genomes of dinoflagellates exhibit important differences. Dinoflagellates do not use ATG (Methionine) as a start codon, and stop codons are generally not encoded in the transcribed portion of any genes. Instead, stop codons are created by oligoadenylation in *cox3* transcripts and are not employed at all by either *cox1* or *cob*, the protein products of which appear to end in a short lysine tail encoded by the oligo-A tail (Jackson *et al.*, 2007; Slamovits *et al.*, 2007). All three protein-coding genes and several rRNA fragments undergo extensive substitutional editing, predominantly involving transitional edits, as commonly occur in other systems, but also unique substitutions occur, including five types of transversions (Jackson *et al.*, 2007; Lin *et al.*, 2008).

What *O. marina* has revealed

The *O. marina* mitochondrial genome has provided insight into the evolution of these unusual characteristics. It shares a number of features with apicomplexans and dinokaryotes, such as reduced coding content, extensive fragmentation of LSU rDNA genes and the disuse of Met (i.e. ATG) as a start codon (Jackson *et al.*, 2007; Nash *et al.*, 2007; Slamovits *et al.*, 2007).

In other ways, the *O. marina* mitochondrial genome resembles that of typical dinoflagellates. In particular, the mitochondrial genome is made of thousands of heterogeneous fragments of DNA that in some cases contain apparently intact, coding genes, but in many other cases contain single or multiple fragments of genes, all embedded in a sea of DNA of undetermined size and structure (Chaput *et al.*, 2002; Jackson *et al.*, 2007; Nash

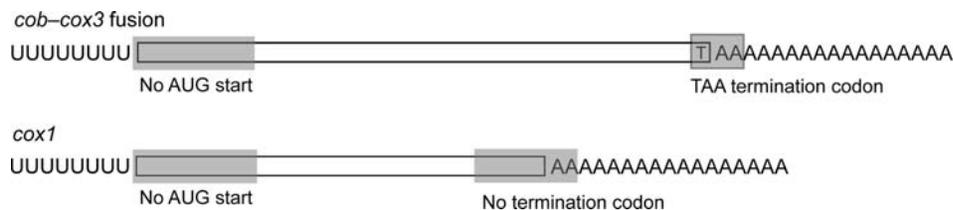


Fig. 2. Schematic representation of the mature transcripts encoded by the mitochondrial genome of *O. marina*. These transcripts have 5' terminal uridine stretches, possibly constituting a novel type of RNA processing. Stop codons are not encoded at the DNA level. Instead, in the *cob-cox3* transcript an UAA terminator is created post-transcriptionally after oligoadenylation, whereas the *cox1* transcript ends with an in-frame series of As.

et al., 2007, 2008; Slamovits *et al.*, 2007). However, unlike other dinoflagellates (Chaput *et al.*, 2002), there is no evidence for *O. marina* that any fragments contain random permutations of all three genes (Slamovits *et al.*, 2007). Also similar to other dinoflagellates, the *O. marina* mitochondrial genes lack stop codons, but one is created at the 3' end of *cob* by oligoadenylation (Fig. 2). In other ways, the *O. marina* mitochondrial genome shares some ancestral states with apicomplexans, in particular in the lack of dinoflagellate RNA editing (Slamovits *et al.*, 2007; Zhang and Lin, 2008).

Moreover, *O. marina* has also evolved a few unique characteristics found in neither apicomplexans nor dinoflagellates. All apicomplexan and dinoflagellate mitochondria encode *cox1*, *cox3* and *cob*, but in *O. 2marina*, *cob* and *cox3* are fused to form a single coding unit, making it the smallest known protein-coding gene complement (two) in any genome (Slamovits *et al.*, 2007). Another characteristic that is unique to *O. marina* is the presence of a 5' oligo-U cap on its mitochondrial mRNAs (Slamovits *et al.*, 2007). Whether this is post-transcriptionally added or added by error-prone transcription is unknown. No evidence for a similar feature has been so far reported in other dinoflagellates or in apicomplexans.

PLASTID GENOME

Endosymbiosis and plastid evolution

The origin and spread of plastids in the alveolates has been a contentious issue in its own right (Williamson *et al.*, 1994; Kohler *et al.*, 1997; Cavalier-Smith, 1999; Fast *et al.*, 2001; Funes *et al.*, 2002; Bodyl *et al.*, 2009a, b). Dinoflagellates and apicomplexans include species with plastids. In apicomplexans, the plastid has evolved into a non-photosynthetic organelle of great metabolic importance known as apicoplast (Waller and McFadden, 2005), whereas dinoflagellates contain a great diversity of photosynthetic plastids of different

origins (Keeling, 2010). However, the distribution and the characteristics of the plastids in each group have made it difficult to determine if the apicoplast and any of the different types of plastids found in dinoflagellates have a common origin. In particular, non-photosynthetic organisms, such as *O. marina* itself, fall at the base of the dinoflagellate lineage (Saldarriaga *et al.*, 2003; Cavalier-Smith and Chao, 2004; Leander and Keeling, 2004). One interpretation of this distribution is that apicomplexans and dinoflagellates acquired their plastids independently and that lineages like *O. marina* represent the heterotrophic, ancestral condition (Delwiche and Palmer, 1997; Palmer and Delwiche, 1998). However, plastid distribution and the distribution of photosynthesis are two different things (Fig. 1). A closer look at several colourless members of typically photosynthetic lineages has indicated that absence of photosynthesis does not mean an absence of plastids or that a plastid may have existed in an ancestor and been lost (Williams and Keeling, 2003). Recently, evidence for such an ancestry has mounted for several of these non-photosynthetic lineages, including *O. marina*, *Perkinsus marinus* and *Cryptocodinium cohnii* (Sanchez-Puerta *et al.*, 2007; Teles-Grilo *et al.*, 2007b; Slamovits and Keeling, 2008a). Evidence for plastid ancestry in an organism can be investigated by searching for genes with similarity to known plastid or plastid-derived genes. Ideally, the structure of the organellar genome is a direct indication, as it was the case with the discovery of the nature of the apicoplast (Wilson *et al.*, 1996; McFadden and Waller, 1997; Foth and McFadden, 2003). Alternatively, one can look at nuclear genes under the assumption that if the organism derives from a plastid-containing ancestor, at least some genes could have been retained in the nucleus. Many of such genes would retain similarity to genes of plastid origin or their cyanobacterial homologues and this can be investigated by conducting phylogenetic analyses with each candidate gene (Archibald *et al.*, 2003). The phylogenetic analysis is not by itself taken as conclusive evidence for plastid ancestry as other explanations such phylogenetic

artefacts or LGT may apply (see below). The function and structural features of the encoded proteins must also be evaluated, and if consistent, add support to the hypothesis of plastid ancestry. EST surveys on *P. marinus*, *C. cohnii* and *O. marina* identified genes of algal or cyanobacterial origin encoding proteins associated with plastidic functions. The three species have homologues of IspC (also DXR), encoding 1-deoxy-D-xylulose-5-phosphate reductoisomerase (Grauvogel *et al.*, 2007; Sanchez-Puerta *et al.*, 2007; Slamovits and Keeling, 2008a), an enzyme involved in the non-mevalonate pathway (MEP) for isoprenoid biosynthesis (Hunter, 2007). This pathway is missing in all known non-photosynthetic eukaryotes and has only been described so far in bacteria and plastids of eukaryotes. The MEP pathway is present in the *Plasmodium* apicoplast and is being studied as a potential target for chemotherapy of Malaria (Waller and McFadden, 2005). The dinoflagellate *dxr* gene is closely related to the homologue from Chromists, which is thought to have red algal ancestry along with most of the genes belonging to the MEP pathway (Frommolt *et al.*, 2008).

Oxyrrhis marina and *C. cohnii* also have genes for haem biosynthesis, a process that is entirely plastidic in plants and green algae whereas in animals (the canonical type) is partitioned between the cytosol and mitochondria. The sets of enzymes that conform each type have different evolutionary origins (Obornik and Green, 2005). Apicomplexans seem to have conjoined the two types into a hybrid where the cytosolic steps of the canonical pathway were lost and substituted by the equivalent steps occurring in the apicoplast (Ralph *et al.*, 2004; Varadharajan *et al.*, 2004; van Dooren *et al.*, 2006). *Cryptocodinium cohnii* and *O. marina* have HemB and HemC, respectively (Sanchez-Puerta *et al.*, 2007; Slamovits and Keeling, 2008a), which represent the plastidial (apicoplast) steps in apicomplexans (Lim and McFadden, 2010). In addition to isoprenoid and haem, biosynthesis of certain amino acids are also plastid-associated functions in plants and algae, and some genes on these pathways have been found in *O. marina* and *C. cohnii* (Sanchez-Puerta *et al.*, 2007; Slamovits and Keeling, 2008a). Finally, genes specifically involved in photosynthesis, such as Rubisco in *C. cohnii* and Carbonic anhydrase in *O. marina*, were identified, unexpectedly since these are heterotrophic protists.

But the molecular data not only suggest that a plastid likely existed in an ancestor of *O. marina*, they also suggest that the organelle still may exist in some form. At least four of the predicted proteins in *O. marina* have N-terminal extensions with the characteristics of a plastid-targeting signal: a clear secretory signal peptide and a region consistent with the characteristics of a

transit peptide of plastid-targeted proteins of dinoflagellates (Patron *et al.*, 2005). Briefly, plastid targeting in secondary endosymbionts is a combination of the secretory pathway and plastid translocation: a secretory signal peptide directs the protein across the outermost plastid membrane, then it is cleaved and a peptide with the characteristics of a plastid targeting leader determines the final destination (for details and references, see Cavalier-Smith, 1999; Foth *et al.*, 2003; Patron *et al.*, 2005). That these genes still encode a region with the characteristics of a targeting signal suggests that a target organelle exists in *O. marina*, because they would otherwise be secreted or targeted to the endomembrane, which is inconsistent with the functions of the proteins. Alternatively, targeting to other organelles cannot be ruled out with the existing data. For example, Fe-SOD1 and Fe-SOD2 of *P. marinus* have N-terminal peptides with similarity to plant and dinoflagellate plastid-targeting leaders but localize to mitochondria and to an unknown single-membrane compartment, respectively (Schott and Vasta, 2003; Fernandez-Robledo *et al.*, 2008). Although plastid-like bodies have never been observed in *O. marina*, despite several studies involving TEM (Gao and Li, 1986; Roberts *et al.*, 1993; Kato *et al.*, 1997, 2000; Jeong *et al.*, 2008), it is relatively easy to overlook a relict plastid, in particular when there is no other evidence to suggest one exists (Williams and Keeling, 2003). Plastid-like bodies have been observed in *Perkinsus atlanticus* using TEM (Teles-Grilo *et al.*, 2007b), but direct evidence for a plastid will have to come from techniques such as immunolabelling with antibodies against the putatively plastid-targeted proteins, and only then will it be clear whether or not an organelle persists.

Finding a plastid (or evidence for it having existed) in *O. marina* and other heterotrophic lineages around the base of apicomplexans and dinoflagellates shifts the balance of evidence in favour of their plastids having evolved from a common ancestor. The question of the origin of plastids in alveolates is a big theme *per se* and outside the scope of this work, but it has been thoroughly discussed elsewhere (Cavalier-Smith, 1999; Bodl and Moszczyński, 2006; Sanchez-Puerta and Delwiche, 2008; Keeling, 2009, 2010; Obornik *et al.*, 2009; Janouskovec *et al.*, 2010). Once the function of these organelles is better understood, we may be able to reconstruct the early evolution of plastids in these lineages and solve the persistent puzzle of why many dinoflagellates remain photosynthetic among so many non-photosynthetic relatives (Keeling, 2009). Given the ease of culturing *O. marina* under controlled conditions (Lowe *et al.*, 2011b), we support its use to pursue this fundamental question.

Plastid-derived biochemistry in *O. marina* and the evolutionary origin of its unusual nutritional biochemistry

If a plastid-derived biochemistry actually exists in *O. marina*, it may constitute the molecular basis to explain important nutritional requirements, which appear to be unusually low for a predatory protist. Plants and green algae have a robust and comprehensive set of metabolic resources that confer an ability to synthesize most or all of their required complex organic compounds. Many of these biosynthesis processes occur entirely or partially in the plastids and are largely carried out by proteins encoded by genes that originated with the ancestral cyanobacterial endosymbiont, hence the tremendous importance of primary endosymbiosis for eukaryotes. This toolset of biosynthetic pathways is generally shared by all autotrophic organisms, whereas heterotrophs rely upon feeding. *Oxyrrhis marina* gains nutrition primarily via phagotrophy; thus it is usually cultured in the presence of algae or bacteria (Lowe *et al.*, 2011a). However, *O. marina* may be cultured axenically (Droop, 1970). The medium described by Droop (Droop, 1970; but see Lowe *et al.*, 2011a) is based on a simple seawater medium such as f/2 (Guillard and Ryther, 1962) and includes ethanol or acetate as carbon source and a source of Nitrogen (e.g. Proline or Alanine) but is free of cells and complex nutrients such as amino acids (Droop, 1970), which means that *O. marina* can synthesize the full set of amino acids in addition to other compounds that heterotrophs are usually unable to synthesize. Remarkably, *O. marina* has Ketol-acid reductoisomerase (Slamovits and Keeling, 2008a) whereas *C. cohnii* has three branched-chain aminotransferases (Sanchez-Puerta *et al.*, 2007), all these proteins being involved in synthesis of the essential amino acids Valine, Leucine and Isoleucine. Indeed, Droop (Droop, 1970) noticed in general a remarkable degree of versatility and nutrient-independence of *O. marina* compared with most heterotrophic protists, to the point of referring to it as “to all intents and purposes an animal, though with many plant-like nutritional features”. The sequestration of “plant-like” biochemical pathways from a cryptic plastid offers an intriguing explanation for the nutritional versatility displayed by *O. marina*.

In summary, the role of plastid-derived biochemistry in heterotrophic protists is an interesting question that has just begun to be explored, and *O. marina* represents an appropriate model to study this issue. To this end, we suggest that increasing the amount of expressed sequence data will be an effective way to understand the contributions of past endosymbiotic events to the biology and adaptation of these organisms. In addition,

an important question that needs to be resolved is whether a plastid-derived organelle actually exists in *O. marina*, as also indicated in the previous section.

LATERAL GENE TRANSFER

LGT has played a significant role in eukaryotic evolution (Keeling and Palmer, 2008; Andersson, 2009). One factor that will influence the likelihood of gene transfer is the frequency and intimacy of inter-organism contacts and molecular and cellular properties that affect the ability to take up and integrate foreign DNA (Gogarten *et al.*, 2008; Keeling and Palmer, 2008). Clearly, one such factor is feeding (Doolittle, 1998), and thus protistan predators such as *O. marina* will be prone to undergo gene exchange with their prey.

The prevalence of LGT has not been examined in detail in *O. marina*, but one case of LGT in dinoflagellates has recently been described in which *O. marina* plays a key role. In dinoflagellates, two genes, the shikimate biosynthesis gene *aroB* and an *O*-methyltransferase (*omt*), have been acquired via LGT from a lineage of cyanobacteria (Waller *et al.*, 2006). The two genes fused in dinoflagellates (and later split back into the two individual genes in one species of dinoflagellate). The timing of the transfer could also be pinpointed because *P. marinus* (another basal alveolate; Saldarriaga *et al.*, 2003) lacks both genes, whereas *O. marina* contains a copy of the AroB–OMT fusion, placing the acquisition of these genes at the base of the dinoflagellate lineage, but after the divergence of *P. marinus*. Alternatively, the gene could have been acquired earlier but lost in the lineage leading to *P. marinus* but resolving this will require thorough sampling of related organisms. The data from *O. marina* also provided another critical piece of information on the timing of the fusion. The *aroB* and *omt* genes are adjacent (but separate) in the genomes of the donor cyanobacteria. However, within the dinoflagellates, they are fused in the genus *Heterocapsa* but separated in the genus *Karlodinium*. Without the *O. marina* sequence, it would be impossible to distinguish between a recent fusion in *Heterocapsa* versus an earlier fusion in the donor bacterium, or an ancient dinoflagellate ancestor, with a later split in the *Karlodinium* lineage. This is yet another good example of how *O. marina* may act as a model to unravel evolutionary issues.

GENE EXPRESSION AND GENOME DYNAMICS

As noted earlier, dinoflagellate genomes are remarkable for their physical properties. Recent work from genome

Table I: Analysis of nucleotide substitutions between copies of three *O. marina* genes

Gene	Gene copies	Size (bp)	$d_S \pm SD$	$d_N \pm SD$	$d_S/d_N \pm SD$
Adenosylhomocysteinase	6	705	0.088 ± 0.042	$0.0018 \pm \sim 0$	47.624 ± 0.048
HSP70	5	1301	0.247 ± 0.071	0.004 ± 0.001	60.372 ± 0.097
Actin	12	446	0.304 ± 0.152	0.024 ± 0.010	13.033 ± 4.227

Each copy results from the assembly of several ESTs into clusters (unigenes). Size is the length in base pairs of each set of aligned sequences. Synonymous (d_S) and non-synonymous (d_N) substitution rates and the ratio are averaged for each alignment.

sequencing indicates that the unusual nature of these genomes is even deeper than their form, and once again *O. marina* is well situated to help us understand when and how these traits evolved. First, it is now emerging that many genes occur in multiple copies in dinoflagellate genomes (Lee *et al.*, 1993; Machabee *et al.*, 1994; Le *et al.*, 1997; Reichman *et al.*, 2003; Bachvaroff and Place, 2008; Zhang *et al.*, 2010), and it now appears that these are sometimes found arranged as clusters of tandem repeats (Bachvaroff and Place, 2008). For example, the genes encoding the light-harvesting complex protein *Pcp* in the dinoflagellate *Lingulodinium polyedra* are organized in tandem repeats with a copy number of $\sim 5 \times 10^3$ with little or no sequence variation, at least as estimated by restriction patterns and Southern blotting (Le *et al.*, 1997). In contrast, the same gene in symbiotic dinoflagellates of the genus *Symbiodinium* also form clusters but copies show significantly more nucleotide diversity than in *L. polyedra* (Reichman *et al.*, 2003). Actin is also present in tandem clusters in *Amphidinium carterae*, where a comprehensive sampling of expressed and genomic copies found great sequence diversity as well as complex patterns between expression levels of different copies (Bachvaroff and Place, 2008). In the early diverging *P. marinus*, the *pca* gene is present in at least 10 copies with high levels of synonymous substitutions. These are not tandemly organized, but the *pmacyclin2* gene occurs as a unit of four tandem repeats (Zhang *et al.*, 2010), but the extent of gene duplications in *P. marinus* is unknown (Joseph *et al.*, 2010). Duplications also occur in ciliates (Eisen *et al.*, 2006) and to a lesser extent in apicomplexans (Ribacke *et al.*, 2007), but unlike dinoflagellates, large numbers of similar copies were not observed.

Sano and Kato (Sano and Kato, 2009) used fluorescence *in situ* hybridization (FISH) and real-time PCR to investigate the localization and copy number of actin, alpha-tubulin and hsp90 genes in *O. marina*. FISH signals for each probe appeared at 3, 4 and 5 foci, respectively, whereas copy number was estimated at 33, 10 and 5 copies, respectively (Sano and Kato, 2009), indicating that clusters of multiple copies of these genes are spread at multiple locations. To gain more insight into the prevalence of multiple copies in this organism, we

examined EST data from *O. marina* and analysed sequences of actin and two other highly expressed genes: Adenosylhomocysteinase (Ahc) and Hsp70. Individual ESTs were assembled and clustered into contigs, each representing a distinct sequence variant (unigenes) totaling 5, 6 and 12, respectively, for Actin, Ahc and Hsp70. The three sets exhibited nucleotide substitutions (less in Ahc and more in Actin) evenly distributed (Supplementary Fig. 1). Moreover, in all cases, the number of synonymous substitutions greatly outnumbered the non-synonymous changes (Table I). This pattern confirms the multi-copy nature of *O. marina* Actin genes and shows that Ahc and Hsp70 are also multi-copy. Our approach cannot estimate the number of copies since ESTs transcribed from different loci sharing identical or very similar sequences will be grouped into the same contig. In addition, the representation in our sample of a given gene “family” depends on the level of expression of each member. Altogether, the present evidence strongly suggests that trends towards increased gene copy number are a widespread characteristic of the nuclear genomes among dinoflagellates, and that whatever led to the high copy number of genes was present early in the evolution of the dinoflagellate lineage. These examples also indicate that dinoflagellate genomes exhibit contrasting patterns of gene arrangements, but understanding these patterns and their implications will require extensive expressed and genomic sequencing.

The evolution of nuclear genome size in dinoflagellates represents another intriguing question, and again *O. marina* can shed light on this issue by virtue of its phylogenetic placement. Genome sizes within dinokaryotic dinoflagellates are conspicuously variable and consistently large, ranging from ~ 3 to more than 200 Gb (Spector, 1984a; Veldhuis *et al.*, 1997; LaJeunesse *et al.*, 2005). Surprisingly, the size of the nuclear genome of *O. marina* remained unknown until very recently when Sano and Kato (Sano and Kato, 2009) estimated the amount of cellular DNA in *O. marina* at about 56 pg cell^{-1} , corresponding to about 55 Gb (Sano and Kato, 2009). That *O. marina* possesses a genome that is within the range of the “large” dinoflagellate genomes has an important implication: it means that the expansion of the genome occurred

prior to the split of *Oxyrrhis* but after *Perkinsus*, which possesses a genome 2×10^3 times smaller than that of *O. marina* (i.e. 28 Mb in *P. atlanticus*; Teles-Grilo *et al.*, 2007a). Parasitic and symbiotic dinoflagellates (e.g. syndinians) have relatively small genomes of about 1.5–3 Gb (LaJeunesse *et al.*, 2005). They are also known to represent basal lineages (Saldarriaga *et al.*, 2004), but the exact branching order between these and *Oxyrrhis* is not yet resolved, so whether their smaller genome sizes represent a transitional state between the small genomes of the more basal lineages (i.e. *Perkinsus*) or have been secondarily reduced remains to be found.

It is important to note that the large genome size of *O. marina* presents prospective genome biologists with a significant challenge, severely limiting the potential for comprehensive genome scale analyses. However, given the current rapid development of powerful new sequencing technologies (Shendure and Ji, 2008), large-scale analysis of exceptionally large genomes (like those of the dinoflagellates) is likely to represent a realistic goal in the near future. Indeed, studies employing high throughput

sequencing technologies are currently underway that will explore the characteristics of the genomic landscape and intraspecific genomic variability in *O. marina*.

Lastly, recent studies have also started to indicate how molecular characteristics may explain aspects of the genomic architecture of the dinoflagellate genome. Protein-coding transcripts of a wide array of dinoflagellate species, including *O. marina* and *P. marinus*, are subject to a type of post-transcriptional RNA processing known as trans-splicing (Lidie and van Dolah, 2007; Zhang *et al.*, 2007). This process consists of the addition of a short, 22 bp fragment of RNA known as spliced leader (SL) to the 5' end of maturing transcripts (Lidie and van Dolah, 2007; Zhang *et al.*, 2007). Based on this activity, one would predict that all dinoflagellate mRNAs would initiate with the same 22 bp SL sequence, but in many cases, there are additional elements resembling truncated and degenerated copies of the SL immediately adjacent to the 5'-terminal SL (Slamovits and Keeling, 2008b). The origin of these elements has not been demonstrated, but we postulate that they result from a

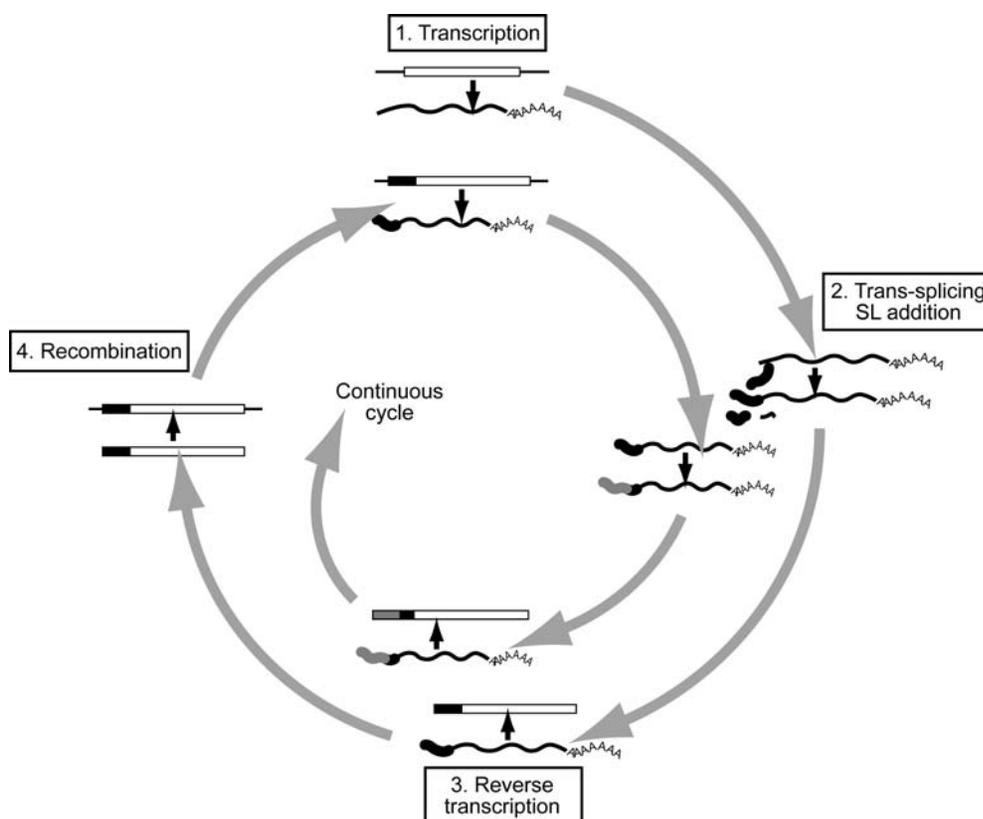


Fig. 3. A proposed model describing the cyclical process for progressive addition of SLs to dinoflagellate genes. Transcription (1) is followed by trans-splicing (2, SLs represented in thick black). Reverse transcription (3) and insertion of a processed cDNA into the genome by recombination results in a gene with a relict SL (4). Transcription of this gene (1, lower) leads to the addition of a second SL (2, SL in thick grey) at the AG dinucleotide at position 6–7 of the relict SL. Relict SL sequences decay over time, but new relict SLs are also continuously added, refreshing the pool of genes with recognizable SL relicts.

“recycling” of mRNAs, where mature transcripts are reverse-transcribed into cDNA and then integrated into the genome. If placed next to a functional promoter element, the copy becomes a new copy of the original gene (Slamovits and Keeling, 2008b). Normal trans-splicing of the transcripts of these new gene copies adds a new SL at a splice-acceptor point within the now-gene encoded “relict” SL. Some transcripts appear to have multiple relicts suggesting that this recycling process would be occurring at a high frequency, resulting in many genes being continuously recycled through the RNA stage, which probably plays some role in the expansion of gene copy number in this lineage (Fig. 3). Trans-splicing appeared early in the evolution of dinoflagellates, as evidence for this mechanism can be found in *Perkinsus* (Joseph *et al.*, 2010; Zhang *et al.*, 2010) and *O. marina* (Zhang *et al.*, 2007). The genome of *Perkinsus* is small, and lacks the characteristics of the dinokaryotic chromatin (Teles-Grilo *et al.*, 2007a). Very little is known about the genome organization of *O. marina*, although recent data suggest that it is very large and a trend towards multigenic structure is already in place. It will be interesting to see if more evidence for mRNA recycling is found in *Perkinsus* and *Oxyrrhis* and what, if any, is the relationship between this process and the unusual nature of the dinoflagellate genomes.

CONCLUDING REMARKS

Oxyrrhis marina has clearly emerged as an important model to address questions of molecular biology, genomics and evolution. This is because it is not just an advantageous model as a result of practical accessibility and ease of culturing; it also occupies a key evolutionary junction between the apicomplexans and dinoflagellates. Greater understanding of the evolutionary biology and genomics of *O. marina* could, therefore, contribute substantially towards our understanding of the biology and evolution of the alveolates as a whole. This review shows how sequence data from *O. marina* have confirmed its value in addressing various questions about genomics and organelle evolution, and as this database continues to expand in the near future, including comprehensive nuclear and organelle genomic data along with deep surveys of expressed genes, it will constitute invaluable resource to address the many fascinating questions waiting to be explored.

SUPPLEMENTARY DATA

Supplementary data can be found online at <http://plankt.oxfordjournals.org>.

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