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The Copy Number of Chloroplast Gene Minicircles Changes Dramatically with Growth Phase in the Dinoflagellate *Amphidinium operculatum*

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The chloroplast genome of algae and plants typically comprises a circular DNA molecule of 100—200 kb, which harbours ~120 genes, and is present in 50—100 copies per chloroplast. However, in peridinin dinoflagellates, an ecologically important group of unicellular algae, the chloroplast genome is fragmented into plasmid-like 'minicircles', each of 2—3 kb. Furthermore, the chloroplast gene content of dinoflagellates is dramatically reduced. Only 14 genes have been found on dinoflagellate minicircles, and recent evidence from EST studies suggests that most of the genes typically located in the chloroplast in other algae and plants are located in the nucleus. In this study, Southern blot analysis was used to estimate the copy number per cell of a variety of minicircles during different growth stages in the dinoflagellate *Amphidinium operculatum*. It was found that minicircle copy number is low during the exponential growth stage but increases during the later growth phase to resemble the situation seen in other plants and algae. The control of minicircle replication is discussed in the light of these findings.
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Key words: organelle; plastid; Dinophyceae; plasmid; biogenesis; photosystem.

Introduction

Plastid genomes of land plants and most algae usually contain about 120 genes on a 120—150 kb circular DNA molecule. Many of the genes encode subunits of the complexes that carry out the light reactions of photosynthesis (photosystems I and II, the cytochrome b_6f complex and the ATP synthase) as well as ribosomal proteins, rRNAs and tRNAs (Sugiura 1992). Despite the broad range of plastids, and the complex evolutionary history they have followed, the plastid genome is remarkably well conserved, with many genes in a similar order across plant and algal groups (Martin et al. 1998; Stoebe and Kowallik 1999).

However, in recent years it has become apparent that many species of peridinin-containing dinoflagellate algae have a unique chloroplast genome organisation. Genes for many plastid proteins are arranged on small plasmid-like molecules of 2—3 kb, instead of a plastid genome of more conventional length. Each minicircle contains up to three genes, as well as a highly conserved 'core' region, which is thought to be

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responsible for minicircle replication and transcription. As well as coding minicircles, there are also ‘empty’ minicircles with no obvious coding region. The minicircles are thought to be located in the chloroplast based on various lines of indirect evidence, including sequence analysis (Howe et al. 2003; Koumandou et al. 2004), the presence of *psbA* mRNA in the dinoflagellate chloroplast (Takishita et al. 2003), and the fact that PsbA protein synthesis is inhibited by chloramphenicol (Wang et al. 2005), which is known to block protein synthesis in plastids in other organisms. However, it has been argued on the basis of hybridisation data that, at least in some species, the minicircles are outside the chloroplast (Laatsch et al. 2004). Only 14 genes have been identified so far on dinoflagellate minicircles, indicating a severely depleted chloroplast gene complement, while expressed sequence tag (EST) studies indicate that many genes typically retained in the chloroplast in plants and algae have been transferred to the nucleus in dinoflagellates (Bachvaroff et al. 2004; Green 2004; Hackett et al. 2004).

This unique organisation raises questions about how the multiple minicircles are maintained and whether certain genes are present at higher copy number than others. It has also been suggested that dinoflagellates may have a conventional master chloroplast genome but that this has escaped detection because the minicircles are present at a much higher copy number (Koumandou et al. 2004). Usually, photosynthetic eukaryotes maintain 50—100 copies of their chloroplast genome per chloroplast. During certain developmental stages, however, e.g. during leaf development in plants, there can be up to 250—500 genome copies per chloroplast. This increase in chloroplast DNA abundance may be needed to meet the increased demand for certain gene products of the chloroplast genome, for example the chloroplast-encoded ribosomal RNAs, to support high expression of chloroplast genes (Bendich 1987). Rarely, if ever, do plastid genome numbers per cell, or per plastid, fall below a multiplicity of 5 (Maguire et al. 1995). Low chloroplast genome copy number is often associated with stages of rapid growth (Maguire et al. 1995; Coleman and Nerozzi 1999) but recent studies have also indicated that the chloroplast DNA content in various plant species decreases dramatically as leaves mature, even to the point where mature chloroplasts contain no detectable DNA (Oldenburg and Bendich 2004; Rowan et al. 2004; Shaver et al. 2006).

Nothing is known about the copy number of minicircles in dinoflagellates. We therefore set out to examine whether dinoflagellates maintain multiple copies of each minicircle, whether all minicircles are present at a similar copy number, and whether the minicircle copy number varies with growth stage. The peridinin dinoflagellate *Amphidinium operculatum*, whose chloroplast gene minicircles have been studied in detail (Barbrook and Howe 2000; Barbrook et al. 2001; Nisbet et al. 2004), was used for all analyses. We used Southern blot analysis as it is likely to be more sensitive and more specific than DNA-specific DAPI (4′, 6-diamidino-2-phenylindole) staining, which is often used to study the increase in chloroplast genome copy number in the developing leaves of plants (Lawrence and Possingham 1986; Nakamura et al. 1986). Southern blot analysis is also likely to be more reliable than real-time (quantitative) PCR for dinoflagellates, as modified bases present in dinoflagellate DNA (Steele and Rae 1980) as well as the circular nature of minicircle DNA and the presence of aberrant minicircles (Barbrook et al. 2001; Nisbet et al. 2004) would complicate the interpretation of PCR results.

Results

Amphidinium operculatum Growth Curves and DNA Content per Cell

The growth of seven 1 l cultures was followed over 1—2 months by counting fixed cells under the microscope. All cultures showed exponential growth until a cell concentration of about 100,000 cells/ml was reached, after which the cultures entered into a period of slower growth (Fig. 1). When the growth of a culture was followed for longer than 2 months, growth eventually stopped at a cell concentration of about 500,000 cells/ml. The average growth rate during exponential growth (before reaching 100,000 cells/ml cell density) was 0.25/day, corresponding to a doubling time of 4 days. The average growth rate during slow growth (after reaching 100,000 cells/ml cell density) was 0.08/day, corresponding to a doubling time of approximately 13 days.

DNA was extracted from four different cultures at different stages of growth. Prefixes ‘e’ and ‘s’ refer to DNA extracted from the exponential and the slower growth stages, respectively (Fig. 1). DNA ‘es1’ was extracted after the culture had reached a concentration of 100,000 cells/ml but before the growth rate had slowed down

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