Mechanisms for maintenance, replication, and repair of the chloroplast genome in plants

Brent L. Nielsen*, John D. Cupp and Jeffrey Brammer

Department of Microbiology and Molecular Biology, Brigham Young University, Provo, Utah 84602, USA

* E-mail: brentnielsen@byu.edu

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Photosynthesis is a complex process that occurs in chloroplasts of higher plants, and requires a large number of plastid proteins to assemble the photosynthetic machinery. Many chloroplast-localized proteins are nuclear-encoded and must be imported into the chloroplasts from the cytoplasm. A considerable number of genes for photosynthesis and other plastid functions, including transcription and translation, are encoded in the chloroplast genome (ctDNA), which ranges in size from about 130–160 kbp in most higher plants. CtDNA replication is not linked with the plant cell cycle and the chloroplast genome can be amplified to a very high copy number per cell in rapidly dividing leaf tissue. Later in leaf development and plant growth, the ctDNA levels reduce to very low levels (Oldenburg and Bendich, 2004b). The controls that regulate ctDNA replication initiation, replication, and copy number are not understood. From earlier publications on a number of plant species it appears that ctDNA may replicate by more than one mechanism, including a recombination-dependent replication mechanism (Rowan et al., 2010, this issue; Oldenburg and Bendich, 2004b; Marechal and Brisson, 2010), a double D-loop mechanism (Chiu and Sears, 1992; Kunnimalaiyaan and Nielsen, 1997a, b), and rolling circle replication (Kolodner and Tewari, 1975).

In this issue, Rowan et al. (2010) report on the role of chloroplast-targeted RecA (cpRecA) in the maintenance of ctDNA in Arabidopsis. Previously published reports provide evidence that some ctDNA molecules may be recombination intermediates as shown by the presence of branched DNA molecules in some DNA preparations (Oldenburg and Bendich, 2004a, b; Scharff and Koop, 2007). As summarized in a review by Marechal and Brisson (2010), recombination has been shown to be involved in the repair of double-strand breaks and point mutations in ctDNA. It has been known for some time that a plant homologue of bacterial RecA is localized in chloroplasts (Cerutti et al., 1992), but, to date, little is known about the role of DNA recombination in the maintenance of ctDNA.

Rowan et al. (2010) show clear evidence that cpRecA is involved in the maintenance of the chloroplast genome copy number in plants, as T-DNA insertions (from the Agrobacterium Ti plasmid) in the nuclear gene encoding this protein led to a reduction in ctDNA copy number in the mutant plants relative to wild-type plants and to a change in the structure of the ctDNA. The levels of detectable single-stranded DNA increased in the mutants, which is compatible with the decreased amount of cpRecA which would normally coat the single-stranded DNA regions and thus block its detection. After a few generations the mutants began to show significant signs of distress and reduced chloroplast function, including variegation and necrosis. These findings represent a significant advance in our understanding of the mechanisms involved in the maintenance of ctDNA integrity. The authors suggest that the role of cpRecA is primarily in DNA repair, as supported by the analysis of wild-type plants that have been treated with ciprofloxacin, which induces double-strand DNA breaks. In these plants, altered ctDNA structures were observed as in the cpRecA plants. Similar experiments with insertions in the DRT 100 homologue, which has only very weak homology to bacterial RecA but can partially complement E. coli recA mutants showed no effect, suggesting that DRT 100 may not be directly involved in the repair of ctDNA. The role of cpRecA in DNA repair is clearly supported by these experiments; it is also possible that cpRecA may be involved in recombination-mediated replication of the chloroplast genome.

CpRecA and DRT 100 are not the only RecA homologues localized to chloroplasts. A dual-targeted (to both chloroplasts and mitochondria) RecA (distinguished from the others as RecA2) has been identified in the Arabidopsis nuclear genome (Christensen et al., 2005). T-DNA insertions in this gene lead to non-viable plants (BL Nielsen, JD Cupp, unpublished observations; Shedge et al., 2007), suggesting that RecA2 may be essential for ctDNA and/or mtDNA maintenance and plant development. However, at this point in time there are no data to determine whether the lethal phenotype is due to the disruption of chloroplast or mitochondrial DNA maintenance mechanisms, or both. The RecA2 gene was not included in the current study by Rowan et al. (2010, this issue) but its role in ctDNA replication should be evaluated. The observation that T-DNA insertions in cpRecA were not lethal may be due to functional (at least partial) complementation by RecA2.
But because RecA2 insertions appear to be lethal, RecA2 may play some specific and essential role in ctDNA and/or mtDNA maintenance. It will be important to obtain and analyse mutants of RecA2, which may need to be generated by other approaches such as inducible RNAi, microRNA or some other technique. If such mutants can be obtained, then similar approaches to those used by Rowan et al. should be used to analyse the role of RecA2 in maintaining ctDNA copy number.

It is possible that early ctDNA replication during germination and seedling development may be initiated by one mechanism, such as the double D-loop mechanism, and high level amplification of ctDNA may occur by rolling circle replication. Alternatively, perhaps rolling circle replication occurs initially, maybe in combination with recombination-dependent replication, and the double D-loop mechanism is then used as a way of maintaining ctDNA later in plant development. Rolling circle replication is used by many bacteriophages to produce large numbers of new DNA molecules for progeny phage very quickly. Some bacteriophages, including lambda phage, initiate replication bidirectionally from a specific origin, similar to bacterial and eukaryotic chromosomal DNA replication, but after completion of a unit circle shift to rolling circle DNA replication. Other phages such as T4 and T7 also have multiple replication mechanisms, including replication from a specific origin for both, and a recombination-dependent mechanism for T4 (Mosig, 1998) and replication as concatamers for T7 (as described in Oldenberg and Bendich, 2004a; Scharff and Koop, 2006). Thus there is ample evidence from other organisms and bacteriophages for multiple replication mechanisms for individual genomes. This possibility should be seriously considered for ctDNA, as many aspects of chloroplast genome structure, transcription and replication share similarities with bacteriophage mechanisms.

A double D-loop mechanism for ctDNA replication was reported more than 30 years ago for *Pisum sativum* (Kolodner and Tewari, 1975), which lacks the large inverted repeat common to most higher plant chloroplast genomes. In *P. sativum*, the two origins map within the rRNA spacer region and just downstream of the 5S rRNA gene, about 6 kbp apart. The rRNA operon is present in the large inverted repeat, so in species that have the inverted repeat the two replication origins are duplicated. For example, in tobacco ctDNA there are two identical pairs (one pair in each of the large inverted repeats) of replication origins implicated by both in vivo and in vitro analysis (Kunnimalaiyaan and Nielsen, 1997a, b). However, there is growing evidence that these are not the only replication origins in ctDNA.

Support for the involvement of more than one mechanism for ctDNA replication and/or additional replication origins can be inferred from the results of the Koop laboratory on insertions in the oriA and oriB replication origins in tobacco ctDNA (Scharff and Koop, 2006, 2007). Targeted inactivation of either or both of these origins was not lethal, although some deletions resulted in reduced growth rate of the plants and reduced ctDNA copy number, particularly later in leaf development (Scharff and Koop, 2007). This may suggest that the double D-loop mechanism involving these origins is involved in ctDNA replication during the transition from rapidly dividing cells to maturing cells. Scharff and Koop (2006) reported the presence of a significant amount of linear ctDNA molecules with defined ends in tobacco, a substantial portion of which mapped to previously reported ctDNA replication origins, and some of which mapped to novel specific locations. Similar results were reported earlier for maize ctDNA by Oldenberg and Bendich (2004a). The Bendich laboratory reported that the majority of ctDNA in maize is linear, and that the structure and copy number of ctDNA molecules change during development (Oldenberg and Bendich, 2004b). The earlier published work of Kolodner and Tewari (1975) suggested that a rolling circle replication initiation site may be present at a different location from the D-loop origins in the chloroplast genome. This rolling circle replication site would be in the single-copy region in species with the inverted repeat.

Another factor to consider in the control of ctDNA maintenance is the presence of two nuclear-encoded DNA polymerases that are both dual targeted to chloroplasts and mitochondria (Christensen et al., 2005; Carrie et al., 2009). These plant DNA polymerases share significant homology with bacterial DNA polymerase I rather than with the animal mitochondrial DNA polymerase γ (Ono et al., 2007). In *Arabidopsis* and tobacco the coding regions for these genes are very highly conserved, suggesting that they may be functionally redundant. However, the upstream promoter regions share no homology (BL Nielsen, J Brammer, unpublished results), raising the possibility that the two genes are differentially regulated and may be expressed at different times and have different roles in organelle DNA replication and maintenance. Indeed, our preliminary data suggest that the two enzymes are not expressed at equal levels or at the same time during plant development (J Brammer, BL Nielsen, unpublished results). One or both of these DNA polymerases would be essential for any of the above-mentioned replication mechanisms. Future work should examine the involvement of these two DNA polymerases with cpRecA in the maintenance of ctDNA.

An origin-binding protein or specificity factor, similar to dnaA for the bacterial chromosome or rep proteins involved in plasmid DNA replication initiation, or an enzyme that nick the DNA to initiate rolling circle replication, is also likely to be required for ctDNA replication. While an origin-binding activity has been characterized for ctDNA replication in *Chlamydomonas* (Wu et al., 1989), which shares some similarity with ctDNA replication in higher plants, no such protein has been identified in higher plants. Given the wide range in ctDNA levels in different tissues during plant development, it seems clear that one or more protein(s) involved in controlling initiation of ctDNA replication must be present in plants.

From this new report and previous work from a number of laboratories, there is strong support for the presence of...
multiple replication origins and/or replication mechanisms, suggesting that the maintenance of ctDNA is more complex than in bacteria, the endosymbiotic ancestor (Scharff and Koop, 2007). There is growing evidence that more than one mechanism is involved in replication of the chloroplast genome, and each may function at different times during chloroplast development and on the different forms of ctDNA (linear and circular DNA). The majority of ctDNA in most plant tissues is linear, with a varying proportion of branched and/or circular molecules, providing support for the premise that a recombination-mediated replication mechanism may be involved in ctDNA replication, compatible with the results reported by Rowan et al. (2010, this issue). It is exciting that some new progress is being made on ctDNA repair and replication, but there is still much to be learned about the process of ctDNA maintenance in plants during the various stages of plant development.

References


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