

## Article Addendum

# Two distinct Omp85 paralogs in the chloroplast outer envelope membrane are essential for embryogenesis in *Arabidopsis thaliana*

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Homologs of a bacterial  $\beta$ -barrel protein, Omp85, ubiquitously exist in the outer membranes of Gram-negative bacteria, mitochondria and chloroplasts. Those in non-photosynthetic bacteria and mitochondria are responsible for  $\beta$ -barrel protein sorting to the outer membranes, and thus are essential for viability of the organisms. There are two distinct Omp85 homologs in chloroplasts of the model plant, *Arabidopsis thaliana*. One of them, Toc75, functions as the main protein import translocation channel, and was shown to be indispensable from a very early stage of embryogenesis. By contrast, the role of another homolog, OEP80, remains elusive. Recently, we showed that disruption of the *OEP80* gene causes embryo abortion in *A. thaliana* at a stage later than that affected by *TOC75* knockout. This indicates that the two chloroplastic Omp85 homologs are both essential for viability of plants from very early stages of development, but may have distinct functions. Defining the functional and evolutionary relationships of Toc75 and OEP80 by further studies should advance our understanding of the importance of plastids during embryogenesis, as well as that of the molecular details of plastid biogenesis.

Chloroplasts evolved through endosymbiosis from an ancestral cyanobacterium, and now perform versatile, essential functions in photosynthetic eukaryotic cells, such as light capture, carbon fixation, and the biosynthesis of fatty acids, amino acids, and various growth regulators.<sup>1</sup> During evolution, the endosymbiotic organelle established systems to import proteins from the cytoplasm,<sup>2,3</sup> and eventually transferred most (but not all) of its genes to the host nucleus. Consequently, the chloroplast now closely coordinates its activities, including the expression of its reduced number of endogenous genes, with those of the host cell.<sup>4</sup> In a recent genetic study, we showed that a chloroplast outer envelope protein, OEP80 (Outer

Envelope Protein 80)—which is paralogous to the protein import channel, Toc75 (Translocon at the outer-envelope-membrane of chloroplasts 75)—is essential for embryogenesis in *Arabidopsis thaliana*.<sup>5</sup> Thus, a new member was added to the list of components that are indispensable for viability of this model plant from its embryonic stage.<sup>6</sup>

The two chloroplastic proteins, OEP80 and Toc75, belong to the Omp85 (Outer membrane protein 85) superfamily, a group of  $\beta$ -barrel proteins found in the outer membranes of Gram-negative bacteria, mitochondria and chloroplasts.<sup>7</sup> Prominent Omp85 homologs in bacteria and mitochondria are responsible for sorting  $\beta$ -barrel proteins to the outer membranes, and consequently are indispensable for viability of the organisms.<sup>8-11</sup> By contrast, neither of the chloroplastic Omp85 homologs has been directly implicated in  $\beta$ -barrel protein sorting so far. Nonetheless, knockout of the principal *TOC75* gene in *A. thaliana* caused a lethal phenotype that could not pass the two-cell stage of embryogenesis, confirming the essential function of Toc75 for plastid biogenesis.<sup>12,13</sup> A previous biochemical fractionation study suggested that an OEP80 homolog is not part of the known protein import complex in pea chloroplasts.<sup>14</sup> Thus, one attractive hypothesis is that OEP80 maintains the original function of the prokaryotic Omp85 by assisting  $\beta$ -barrel protein sorting into the outer membrane, while Toc75 has been adapted to gain a new function as a protein import channel.<sup>15</sup>

As an initial step to gain insight into the function of OEP80, we identified and characterized three independent T-DNA mutagenized plants with insertions in the *A. thaliana* *OEP80* (*AtOEP80*) gene. Viable homozygous plants for two of these, *oep80-1* and *oep80-2*, were never recovered. Instead, heterozygous mutants produced about 25% aborted seeds upon self-pollination, suggesting that each homozygous genotype is embryo-lethal. This possibility was further supported by Nomarski optics microscopy, which revealed embryo arrest of the *oep80-1* mutant at the globular embryo-proper stage. In contrast with these two lethal *oep80* mutants, plants homozygous for the third mutation (*oep80-3*), which carry a T-DNA insertion between the first and second ATG codons of the originally-annotated open reading frame, were phenotypically indistinguishable from wild type, and accumulated an AtOEP80 protein of the normal size. Furthermore, in vitro import assays showed that an AtOEP80 protein translated from the second AUG codon was able to insert into chloroplast membranes, and this protein displayed similar electrophoretic mobility to native AtOEP80. Altogether, these results indicate that:

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(i) OEP80 is required for embryogenesis in *A. thaliana*; and, (ii) the N-terminal region of OEP80 is not essential for its targeting, insertion and/or functionality. While these findings have not defined the molecular function of OEP80, they do facilitate a certain amount of reasonable speculation.

Disruption of the *OEP80* and *TOC75* genes caused embryo arrest at different stages. This suggests that the two chloroplastic Omp85 homologs may perform distinct tasks, which become vital at different stages of embryogenesis. Alternatively, OEP80 and Toc75 may play similar roles but do so at distinct time points. This second possibility, however, seems less likely because according to publicly available microarray data, the *OEP80* and *TOC75* genes are expressed consistently in the embryo proper at different developmental stages [gene expression omnibus (<http://www.ncbi.nlm.nih.gov/geo/>) accession numbers GSE11262, GSE12403 and GSE12404], and display similar expression profiles throughout the life cycle of *A. thaliana*.<sup>5</sup> Nonetheless, this scenario cannot be entirely dismissed, because the level of the functional protein may depend not only on accumulation of transcripts, but also on posttranscriptional regulation.

If OEP80's function is indeed distinct from that of Toc75, what might it be? Similar to its prokaryotic and mitochondrial homologs, OEP80 may mediate the sorting and insertion of other outer membrane  $\beta$ -barrel proteins.<sup>16</sup> In this scenario, OEP80 might well assist the insertion of the major  $\beta$ -barrel protein in the outer membrane, Toc75. Thus, knockout of *OEP80* would disrupt accumulation of Toc75, and might therefore be expected to cause a phenotypic defect similar to that of the *TOC75* knockout. However, *TOC75* knockout embryos could not even pass the proglobular stage.<sup>12,13</sup> This apparent discrepancy might be explained by: (i) the presence of a residual amount of OEP80 inherited from the maternal cells that is sufficient to assist Toc75 insertion at the proglobular stage; and/or, (ii) the possibility that OEP80 is partially substituted in its function by Toc75 during the earliest stages of embryogenesis. It is also possible that Toc75 insertion may not depend on OEP80.

Elucidating the functional and evolutionary relationships of the two chloroplastic Omp85 homologs will facilitate a better understanding of the roles of plastids during embryogenesis, and yield insights into the molecular processes that are essential for the biogenesis of plastids. To define the function of OEP80, we are employing different reverse-genetic and biochemical strategies to address various questions, such as the identity of its substrates and whether it forms a hetero-oligomeric complex similar to other Omp85 homologs.<sup>3,17,18</sup>

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