January 1997

# A Eubacterial Gene Conferring Spectinomycin Resistance on *Chlamydomonas reinhardtii*: Integration into the Nuclear Genome and Gene Expression

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*Genetics*, Vol 145, issue 1 (January 1997), pp. 97–110.


# A Eubacterial Gene Conferring Spectinomycin Resistance on *Chlamydomonas reinhardtii*: Integration into the Nuclear Genome and Gene Expression

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**Abstract**

We have constructed a dominant selectable marker for nuclear transformation of *C. reinhardtii*, composed of the coding sequence of the eubacterial aadA gene (conferring spectinomycin resistance) fused to the 5' and 3' untranslated regions of the endogenous RbcS2 gene. Spectinomycin-resistant transformants isolated by direct selection (1) contain the chimeric gene(s) stably integrated into the nuclear genome, (2) show cosegregation of the resistance phenotype with the introduced DNA, and (3) synthesize the expected mRNA and protein. Small linearized plasmids appeared to be inserted into the nuclear genome preferentially through their ends, with relatively few large deletions and/or rearrangements. Multiple copy transformants often integrated concatemers of transforming DNA. Our detailed analysis of the complex integration patterns of plasmid DNA in C. reinhardtii nuclear transformants should be useful for improving the technique of insertional mutagenesis. We also found that the spectinomycin-resistance phenotype was unstable in about half of the transformants. When maintained under nonselective conditions, neither the aadA mRNA nor the AadA protein were detected in these subclones. Moreover, since the integrated transforming DNA was not altered or lost, expression of the RbcS2::aadA::RbcS2 gene(s) appears to be repressed. Measurements of transcriptional activity, mRNA accumulation, and mRNA stability suggest that expression of this chimeric gene(s) may also be affected by rapid RNA degradation, presumably due to defects in mRNA processing and/or nuclear export. Thus, both gene silencing and transcript instability, rather than biased codon usage, may explain the difficulties encountered in the expression of foreign genes in the nuclear genome of Chlamydomonas.