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NOTE

The NIT1 Promoter Allows Inducible and Reversible Silencing of Centrin in Chlamydomonas reinhardtii

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An inverted repeat corresponding to parts of the centrin gene of Chlamydomonas reinhardtii was placed downstream of the NIT1 promoter, which is induced by ammonium starvation. After induction, transformants developed centrin deficiency as assayed by immunofluorescence, Western blotting, and Northern blotting. The effect was reversible, demonstrating that the NIT1 promoter allowed controlled RNA interference in Chlamydomonas reinhardtii.

Sequencing of the genome of the unicellular green alga Chlamydomonas reinhardtii has led to the discovery of numerous functionally uncharacterized genes (JGI website: http://genome.jgi-psf.org/chlre2/chlre2.home.html). Because it allows targeting of specific genes, RNA interference (RNAi) is a useful technique to investigate the role of gene products (1). In C. reinhardtii, a variety of genes have been silenced using vectors that contain constitutive promoters and inverted repeats (IR) corresponding to the gene of interest (4, 7, 9). To achieve temporal and spatial control of gene silencing, vectors based on inducible promoters have been developed for animals, plants, and some protists, e.g., Trypanosoma brucei (2, 6, 11). Here we report a vector for inducible RNAi in Chlamydomonas based on the NIT1 promoter.

The NIT1 structural gene encodes nitrate reductase (NR; EC1.6.6.2); its expression is repressed by ammonium and induced by ammonium starvation (3, 8). An IR consisting of parts of the vfl2 gene encoding the cytoskeletal protein centrin (7) was fused between the NIT1 promoter and the RbcS terminator (Fig. 1A) and transformed into C. reinhardtii CC3395 (arg7-8cwdmt−). Strains nitRNAi1 and nitRNAi2 were chosen for further analysis. When maintained in Tris-acetate-phosphate (TAP) medium containing NH4Cl (5), most nitRNAi1 cells (98.2%, n = 211, Fig. 1Ba) had a wild-type centrin cytoskeleton and wild-type levels of centrin transcripts and protein (Fig. 1D and E). A control strain transformed with the selectable marker alone displayed a wild-type centrin cytoskeleton and wild-type amounts of centrin under both conditions (Fig. 1D). As previously reported for transformants expressing the centrin IR from the strong constitutive HSP70A/RbcS2 fusion promoter, nitRNAi1 lost the centrin-deficient phenotype after 15 to 20 weeks of constant induction (not shown). PCR and Southern blotting revealed that the NIT1-centrin-IR transgene was still present (not shown). Stock cultures of nitRNAi1 maintained permanently in medium supplemented with NH4Cl were still inducible at this time point, suggesting that inactivation of the transgene initially required expression of the construct.

After transfer to NH4Cl-free medium, it took several days for the cells to respond, e.g., 91.6% of cells retained wild-type centrin fibers 2 days after induction, but more than 90% of the cells had lost centrin fibers after 6 days (Fig. 2A and B). Similarly, the mutant phenotype was lost gradually after repression of the transgene (Fig. 2C). The NIT1 promoter was previously used in reporter constructs and the gene products were detected 2 hours after induction and plateaued within 24 hours (8, 10, 14). To explain the slow development of the centrin deficient phenotype, we assume that some centrin is carried over from the mother into the daughter cells each

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division and that several days were required to turn the degradation of mRNAs on and off.

Loppes et al. (8) showed that the NIT1 promoter is activated by light and repressed by active NR, which is inhibited by tungstate. Indeed, the centrin-deficient phenotype developed faster in constant bright light than with a light/dark cycle of 14 h/10 h and when tungstate was substituted for molybdate in NH₄Cl-free TAP. However, in the presence of NH₄Cl, constant bright light induced defective centrin fibers (up to 43.9%, n = 214), whereas wild-type fibers were present in a light-dark
cycle of 14 h/10 h (98%, n = 232). Tungstate had no effect on cells maintained in NH₄Cl medium and did not enhance the phenotype when added to cells fully induced by ammonium starvation and constant light. In general, we used constant light and NH₄Cl-free medium for induction and a light-dark cycle of 14 h/10 h in NH₄Cl medium for repression.

Analysis of several transformants showed that the phenotypes were weaker than those observed when the same centrin IR was expressed from the strong constitutive HSP70A/RbcS2 fusion promoter (fully induced nitRNAi2 had 1.1 basal bodies/cell, compared to 0.53 basal bodies/cell for the fusion promoter) (7). Using a NIT1::ARS reporter construct, the transgene was induced up to 30-fold higher when expressed in an NR/ strain compared to NR/ strains (8). Here, an NR/ strain was used; we expect that better expression of the NIT1-centrin-IR transgene could be obtained in an NR/ background.

In summary, the NIT1-centrin-IR construct allowed inducible and reversible generation of a centrin-deficient phenotype simply by transferring cells from NH₄Cl-containing to NH₄Cl-free medium. Inducible RNAi of centrin enabled us to analyze the phenotype during the course of development. The NIT1 promoter is controlled tightly enough to maintain the wild-type phenotype under repressive conditions. This feature might be especially useful for the analysis of essential genes and others involved in cell growth and division which, when constitutively repressed, would affect cell viability. Controlled RNAi using the NIT1 promoter can facilitate the functional characterization of genes in C. reinhardtii.

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