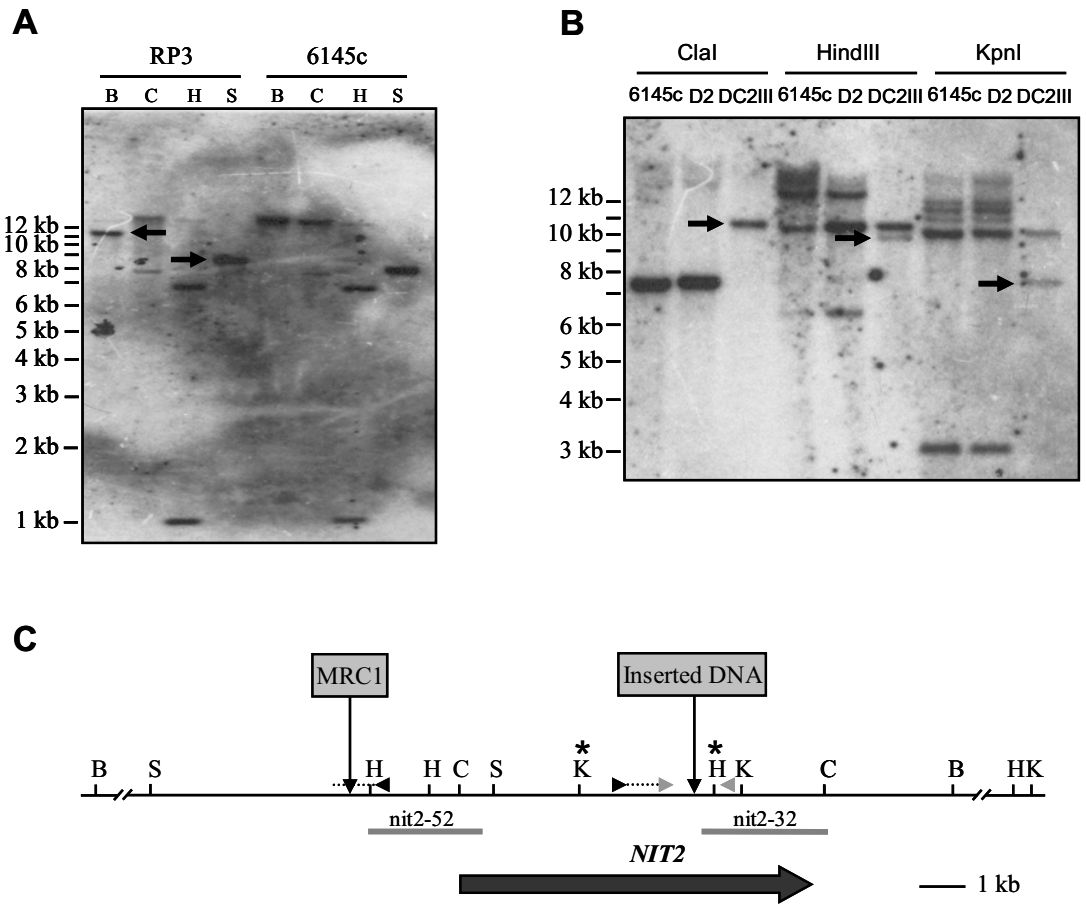

Supplemental Figure 1. Analsis of the NIT2 genomic region in mutants RP3 and DC2III.

(A) Genomic DNA (2 microg) from mutant RP3 and the wild type strain 6145c was digested with BamHI, B; ClaI, C; HindIII, H; and SalI, S, and analysed by Southern blot using the nit2-52 probe. **(B)** Southern blot conditions are as in A, but using genomic DNA from strains 6145c, D2, and DC2III and the nit2-32 probe. **(C)** Scheme of the NIT2 genomic region in mutants RP3 and DC2III from details given in the text. Stars indicate the restriction sites between which a DNA reorganization occurred in strain DC2III. Dark triangles and dotted lines represent localization of specific primers and amplified bands by RESDA-PCR (see text for details), respectively. Grey triangles indicate oligonucleotides BasInsUpper, BasInsLower for amplification of a 4 kb band in strain DC2III.

Supplemental Figure 1 Notes

Strain RP3, derived from the wild type 6145c, was proposed to be affected either at NIT2 or at a closely linked gene (Prieto and Fernández, 1993), and DC2III, derived from mutant D2, was proposed to be defective at a new locus NIT9 closely linked to NIT2 (Rexach et al., 1999; Navarro et al., 2005). To understand the nature of these mutations, an analysis for possible polymorphisms within the NIT2 region was undertaken by Southern blots using appropriate probes. Only some of these probes gave polymorphic bands for these mutants. As shown in Suplemental Figure 1A, probe nit2-52 at the 5' end of NIT2 hybridized to polymorphic bands for BamHI and SalI DNA digests of RP3 and 6145c strains. The probe nit2-32 at the 3' end of NIT2 hybridized to polymorphic bands in ClaI, HindIII and KpnI DNA digests from DC2III with respect to strains D2 and 6154c (Supplemental Figure 1B). The 18 kb band in D2 and 6154c HindIII digest corresponds to the size of a larger band in a partial DNA digestion. The polymorphic bands in both mutants suggest that some sort of rearrangement within the NIT2 genomic region occurred. Specific primers (T1RP and T2RP) at the 5´and (T1Bas and T2Bas) at the 3' regions of NIT2 (Supplemental Figure 1C) were used for RESDA-PCR (González-Ballester et al., 2005b) to get DNA from the possible regions affected. This technique allows amplification from primers of known sequence into adjacent regions by using degenerate primers containing restriction enzyme sites sequences frequent in the Chlamydomonas genome (AluI, PstI, SacII and TaqI). Bands of about 0.8 and 1.4 kb were amplified from genomic DNA of RP3 and DC2III, respectively, which were subsequently sequenced. The band from RP3 contained 324 bp from the NIT2 locus 5' region followed by 483 bp of a new sequence. Comparison of this sequence to the NCBI genebank database gave a full identity to the Chlamydomonas high copy number transposon MRC1 (Accession no. DQ446210). Thus, RP3 strain has a MRC1 insertion at -2270 bp from the NIT2 putative transcript initiation site (Supplemental Figure 1C).

In strain DC2III, sequence obtained for the RESDA-PCR fragment using T1Bas and T2Bas primers corresponded to the NIT2 gene. Thus, specific primers shown in Supplemental Figure 1C (BasInsUpper and BasInsLower) were designed. A DNA fragment of 4 kb was amplified from mutant DC2III, in contrast to the 845 bp from the parental strain. Sequence of both ends of the 4 kb fragment revealed the NIT2 sequence joined to a Chlamydomonas DNA that shows either identity or conservation to a high number of sequences in the genome. This sequence has been deposited in the NCBI GeneBank with the Accession no. DQ835537. All the above data indicate that mutants RP3 and DC2III are allelic to other nit2 mutants. They are affected within the promoter and the transcript NIT2 region, respectively (Supplemental Figure 1C).