Supplementary data:

Neurospora crassa ncs-1, *mid-1* and *nca-2* double-mutant phenotypes suggest diverse interaction among three Ca^{2+}-regulating gene products

Rekha Deka and Ranjan Tamuli

J. Genet. **92**, 559–563

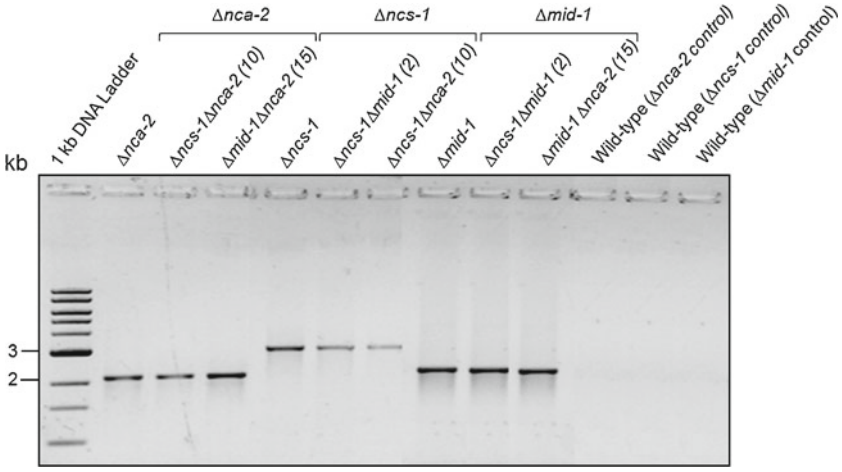

Figure 1. Confirmation of the double mutants by PCR analysis. The Δ*ncs-1*Δ*nca-2*, Δ*ncs-1*Δ*mid-1* and Δ*mid-1*Δ*nca-2* double mutants were verified by using the forward primers HI-NCS-1-F 5′-GTCTCAGCATGAAAGTCGTC-3′, HI-NCU06703-F 5′-CCAAGGCTTATGCCGTCATC-3′, and HI-NCU04736-F 5′-GTCCCATGGATTCCATACCA-3′ specific for upstream of the open reading frame of genes *ncs-1*, *mid-1*, and *nca-2*, respectively, and with the common reverse primer 5HPHR 5′-ATCCACTTAACGTTACTGAAATC-3′ that is specific for the *hph* cassette used to generate the knockout mutants (Colot *et al.* 2006; Deka *et al.* 2011). Amplification of PCR products of size ~2.089, 2.89 and 2.023 kb, respectively, indicate presence of the Δ*nca-2*, Δ*ncs-1* and Δ*mid-1* alleles in the mutants (for each of the three knockout alleles, only a set of three PCR products is shown). For the double mutants, the number in the parenthesis indicates the laboratory strain reference number. The wild-type was used as negative controls for all the three knockout alleles (indicated in the parenthesis) using the allele specific primer pairs. The single knockout mutants were generated by the *N. crassa* genome project (http://www.dartmouth.edu/~neurosporagenome/proj_overview.html) and also verified in independent works (Colot *et al.* 2006; Lew *et al.* 2008; Bowman *et al.* 2011; Deka *et al.* 2011).

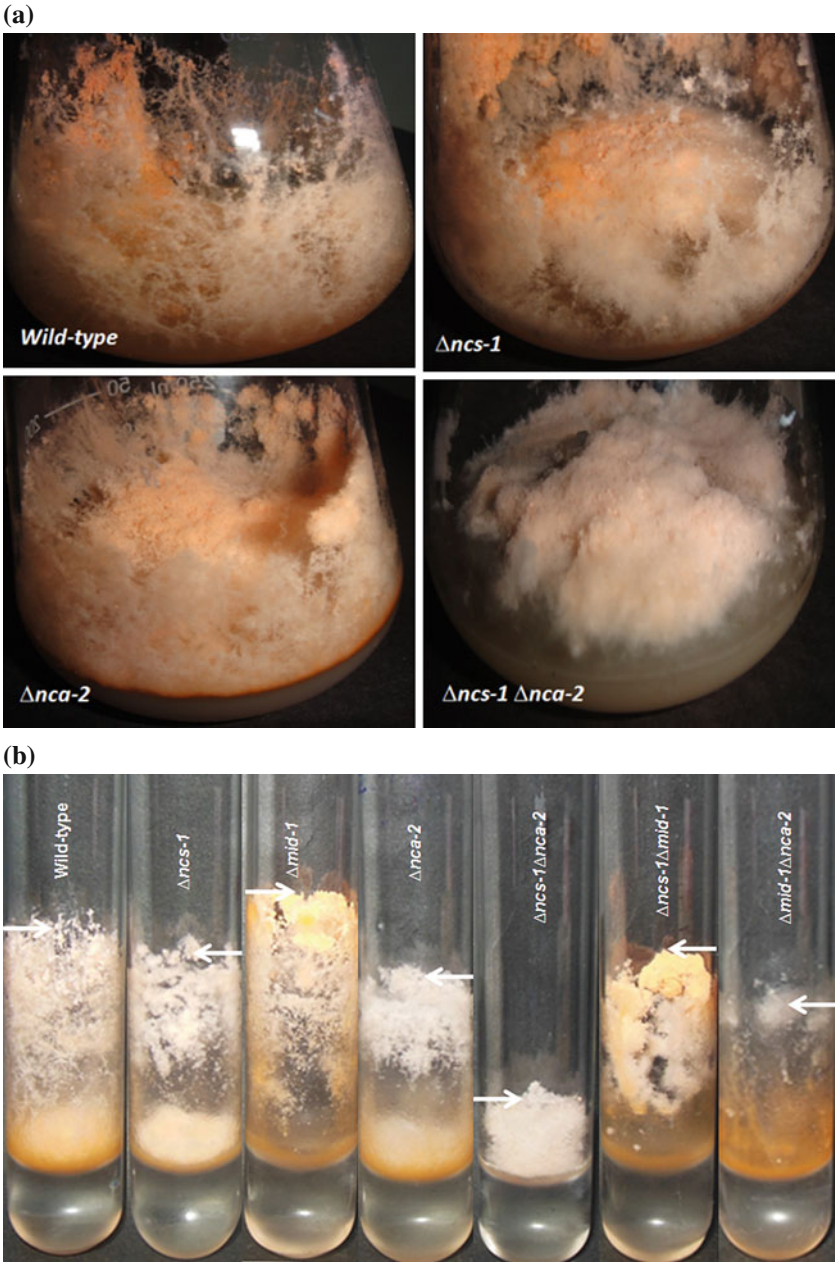

Figure 2. Colony morphology and aerial hyphae. (a) The Δ*ncs-1*Δ*nca-2* double mutant displayed novel colony morphology. The *N. crassa* strains were grown at 30°C for 5 days. (b) Growth of aerial hyphae. The *N. crassa* strains were cultured in standing Vogel's liquid medium at 30°C in darkness for 3 days, tubes were then illuminated for 24 h and photographed. The edges of aerial hyphae are indicated by white arrows.

Table 1. Average growth rates of the *N. crassa* strains in race tubes.

Strains	Average growth rates (cm h^{-1})
Wild-type	0.327 ± 0.099
Δ*ncs-1*	0.239 ± 0.067
Δ*nca-2*	0.276 ± 0.072
Δ*mid-1*	0.137 ± 0.022
Δ*ncs-1*Δ*nca-2*	0.185 ± 0.058
Δ*ncs-1*Δ*mid-1*	0.117 ± 0.022
Δ*mid-1*Δ*nca-2*	0.142 ± 0.039

Table 2. Average growth rates of the *N. crassa* strains in the medium supplemented with various concentrations of $CaCl_2$.

	Growth rates (cm h^{-1}) in various concentrations of $CaCl_2$ (M)			
Strains	0	0.2	0.3	0.4
Wild-type	0.359 ± 0.009	0.327 ± 0.009	0.288 ± 0.018	0.241 ± 0.013
Δ*ncs-1*	0.289 ± 0.018	0.218 ± 0.017	0.122 ± 0.022	0.074 ± 0.029
Δ*nca-2*	0.309 ± 0.028	0.239 ± 0.007	0.187 ± 0.024	0.095 ± 0.013
Δ*mid-1*	0.216 ± 0.011	0.397 ± 0.010	0.385 ± 0.009	0.335 ± 0.016
Δ*ncs-1*Δ*nca-2*	0.213 ± 0.007	0.153 ± 0.009	0.091 ± 0.012	0.05 ± 0.020
Δ*ncs-1*Δ*mid-1*	0.187 ± 0.013	0.251 ± 0.019	0.156 ± 0.015	0.134 ± 0.013
Δ*mid-1*Δ*nca-2*	0.227 ± 0.013	0.180 ± 0.018	0.046 ± 0.010	0.028 ± 0.000

References

Bowman B. J., Abreu S., Margolles-Clark E., Draskovic M. and Bowman E. J. 2011 Role of four calcium transport proteins, encoded by *nca-1*, *nca-2*, *nca-3*, and *cax*, in maintaining intracellular calcium jevels in *Neurospora crassa*. *Eukaryot. Cell.* **10**, 654–661.

Colot H. V., Park G., Turner G. E., Ringelberg C., Crew C. M., Litvinkova L. *et al.* 2006 A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 10352–10357.

Deka R., Kumar R. and Tamuli R. 2011 *Neurospora crassa* homologue of Neuronal Calcium Sensor-1 has a role in growth, calcium stress tolerance, and ultraviolet survival. *Genetica* **139**, 885–894.

Lew R. R., Abbas Z., Anderca M. I. and Free S. J. 2008 Phenotype of a mechanosensitive channel mutant, *mid-1*, in a filamentous fungus, *Neurospora crassa*. *Eukaryot. Cell.* **7**, 647–655.