Supporting Information

Dauvillée et al. 10.1073/pnas.0907424106

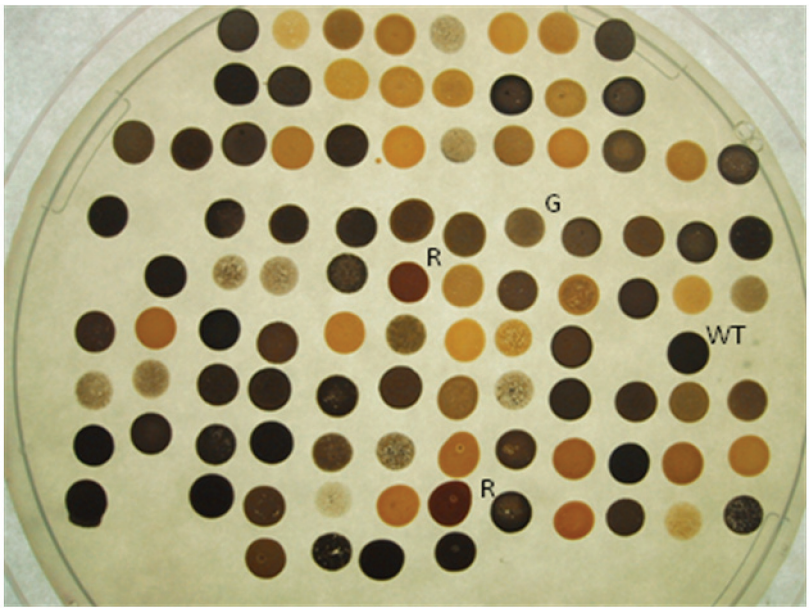

Fig. S1. Iodine screening of the *C. cohnii* mutant bank. Each single colony was grown on rich-medium agar plates then vaporized with iodine. Mutants subclones were then purified and retested. Cell patches were inoculated and incubated for 1 week at room temperature. The 97 mutants selected are displayed on this large size petri dish. The yellow mutants contained <20% of the normal starch amount. The wild-type strain (WT), the strains suspected to be deficient in amylose (red, R) and amylopectin (greenish, G) synthesis are highlighted.

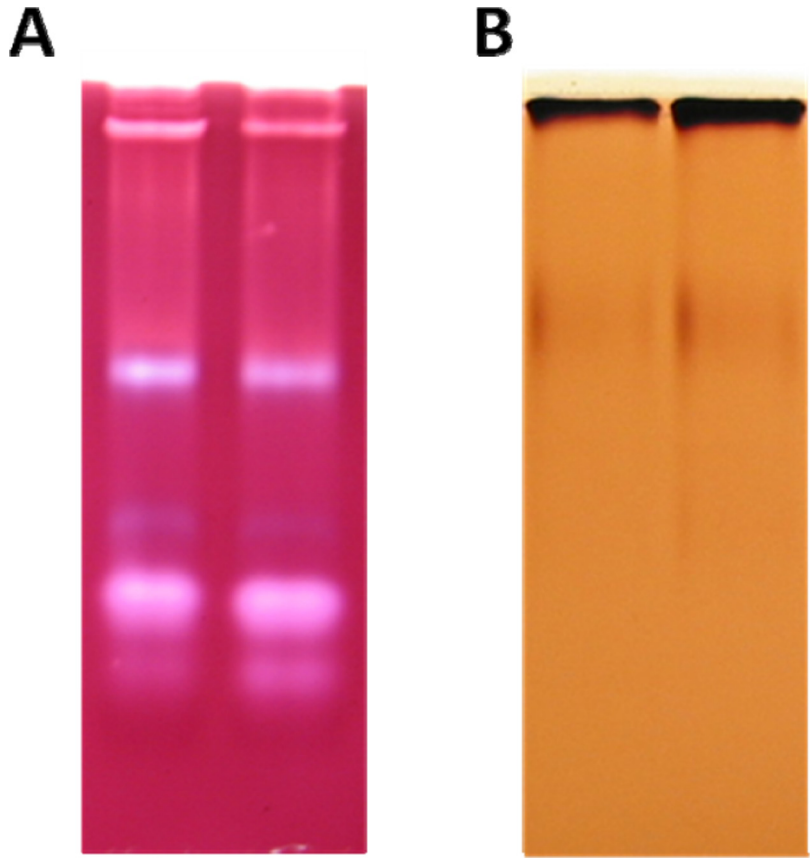

Fig. S2. Detection of starch metabolism enzymes by zymograms. (*A*) A starch-containing gel incubated in buffer without glycosyl nucleotides or hexose phosphates enables to visualize all types of starch hydrolases. The blue band could correspond to a starch debranching enzyme; the red and the pink bands are consistent with the presence of distinct branching enzymes, while the lower white band could be due to an amylase. However, these stains are at best suggestive of the corresponding enzyme activities. (*B*) A glycogen-containing zymogram gel incubated with glucose-1-phosphate and stained with iodine. The brown bands correspond to starch phosphorylase activities. For each gel, the left lane corresponds to the wild-type and the right lane to the *sta1-1* mutant. None of the detectable starch hydrolases or phosphorylases seem significantly affected by the presence of *sta1-1*.

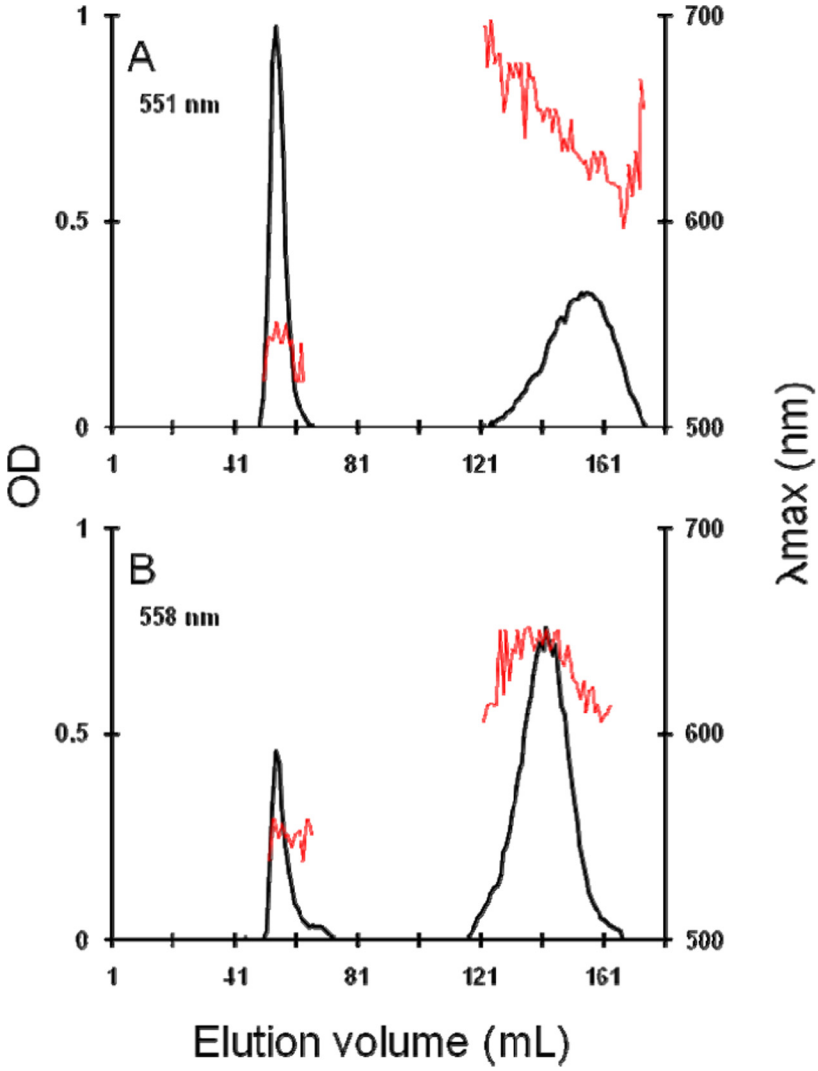

Fig. S3. Separation of amylopectin and amylose by size exclusion chromatography. Fifteen milligrams of starch purified from midlog phase cultures of the wild-type (*A*) and the *sta1-1* mutant (*B*) strains in rich liquid medium were subjected to Sepharose CL-2B chromatography. For each fraction, the optical density (OD; unbroken black line) of the iodine-polysaccharide complex was measured at the wavelength of the maximal absorbance of the iodine-polysaccharide complex [λmax (red line)]. Due to its very high molecular weight, the amylopectin is excluded as a single sharp peak from the Sepharose column. The second peak displays the population of amylose molecules with a typical green color (λmax >600 nm). The values of the λmax for each amylopectin is indicated on the graphs. The left *y*-axis corresponds to the OD values, while the right *y*-axis corresponds to the value of λmax in nanometers. The *x*-axis corresponds to the elution volume in milliliters. Precise quantifications of amylose and amylopectin are performed by pooling the corresponding fractions and assaying glucose amounts within each pool by the amyloglucosidase assay.

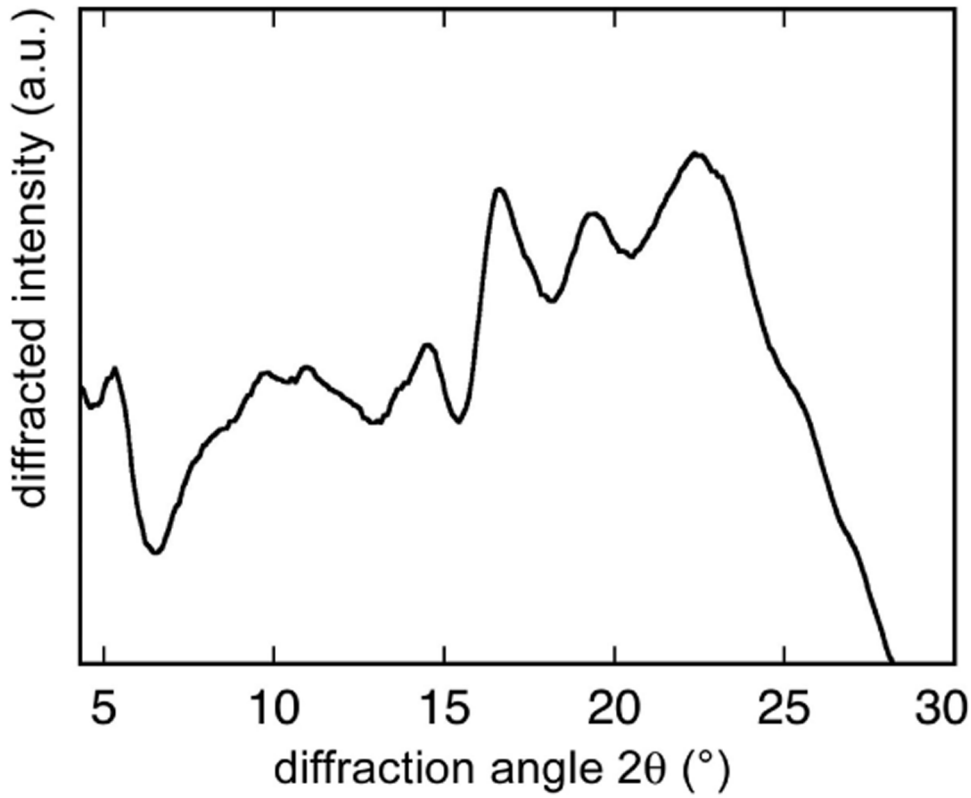

Fig. S4. WAXD profile recorded from a slurry of PP314 mutant starch granules. The profile is typical of the C-type (i.e., a mixture of A- and B-type patterns). The contribution of the excess water has not been subtracted. A typical ''signature'' of B-starch can be seen from the presence of the 100 peak at 5.3 °, which does not exist in the profile of pure A-starch.

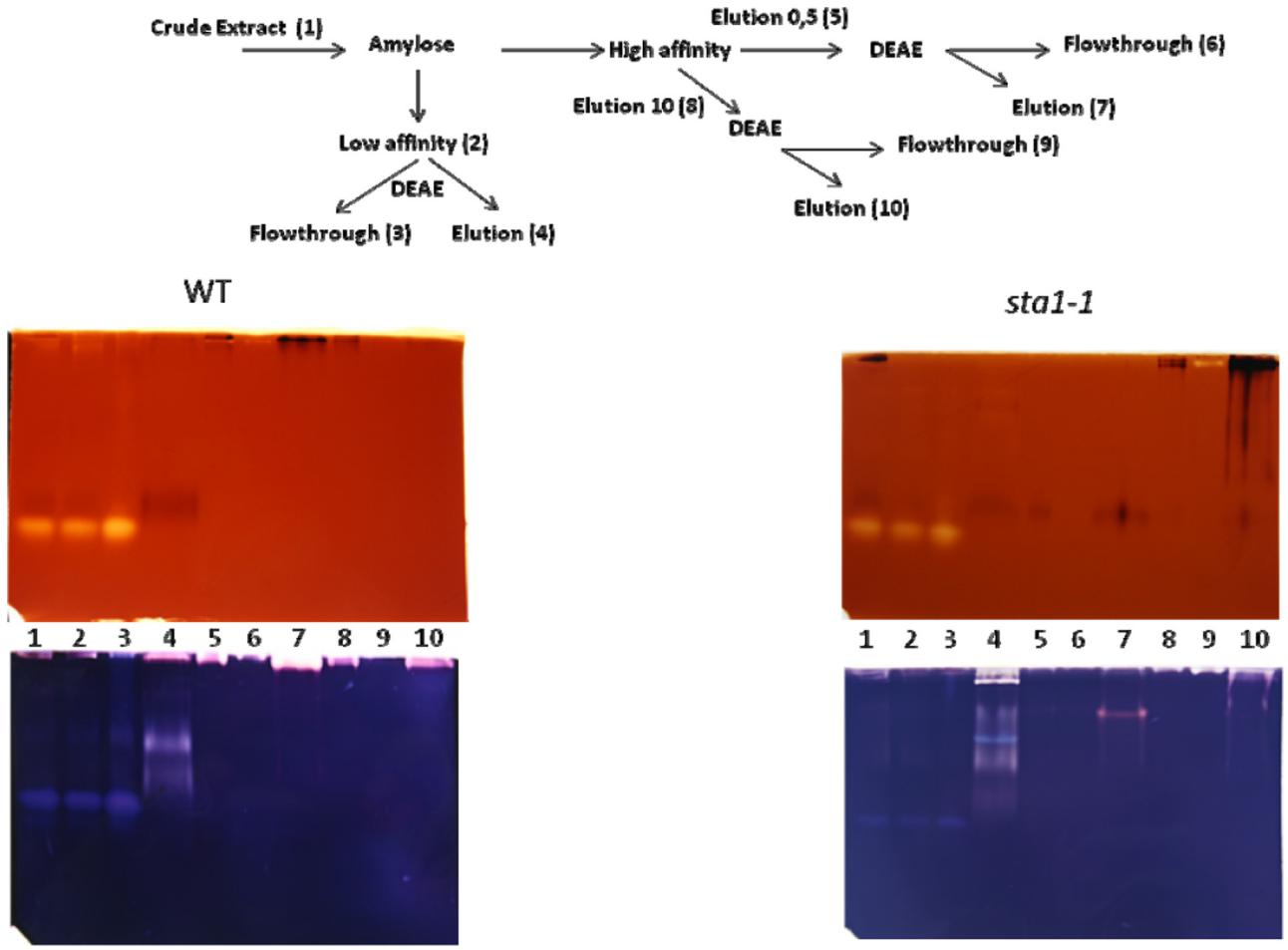

Fig. S5. Partial purification of starch synthases activities in the wild-type and the PP314 mutant strains. The glycogen-containing gels show the synthase activities detected during the different purification steps following a procedure already described for the wild-type (1). Fifty microliters of each semipurified fraction were analyzed to detect the starch synthase activities in the wild-type (*Left*) and the mutant (*Right*) strains. The crude extract of each strain (1) was, in a first step, applied on an affinity amylose column. The unretained fractions containing the low affinity starch synthase isoform obtained (2) were precipitated with glycogen (2.5% final concentration) and polyethylene glycol 8,000 (7.5% final concentration). The glycogen pellet obtained was then resuspended in purification buffer and loaded on a Fractogel TSK DEAE 650 (*M*) anion exchange column. The elution was performed with 5 mL extraction buffer plus 1 M NaCl (4), while the hydrolytic activity that did not interact with the anion exchange resin is observed in the wash fraction (3). The amylose resin was in parallel washed with purification buffer, and the proteins were eluted subsequently using 10 mL extraction buffer plus 0.5 mg/mL Glucidex 19 (5) (Roquette Frères), then 10 mL extraction buffer plus 10 mg/mL Glucidex 19 (6). The lower glucidex concentration was sufficient to elute most of the high affinity isoform of starch synthase in the wild-type, while the high concentration was required to do it in the mutant background. The fraction obtained with the low glucidex concentration was applied on the Fractogel TSK DEAE 650 (*M*) anion exchange column, which was washed with 20 mL purification buffer (12) and eluted with 5 mL purification buffer plus 1 M NaCl (13). The same procedure was applied for the eluted fractions obtained with the high glucidex concentration. The unretained fractions and the elution with NaCl are displayed in (7) and (8), respectively. The same fractions were analyzed on starch-containing gels, allowing the detection of hydrolytic activities (bottom gels).

1. Deschamps P, et al. (2008) The heterotrophic dinoflagellate *Crypthecodinium cohnii* defines a model genetic system to investigate cytoplasmic starch synthesis. *Eukaryot Cell* 7:247–257.

Table S1. Enzyme activities in wild-type and mutant *sta1–1* strains

Enzyme	Wild-type	Mutant strain
Starch synthase	3.2 ± 0.4	1.9 ± 0.3
Starch phosphorylase	11.4 ± 5.0	8.5 ± 3.9
D-enzyme	10.3 ± 1.3	8.4 ± 1.0
α-Glucosidase	0.36 ± 0.01	0.33 ± 0.05
Hydrolytic activities	28.9 ± 3.4	23.3 ± 3.5

Starch synthase activities are expressed in nmol UDP-glucose incorporated into polysaccharide min^{-1} mg^{-1} protein. Starch phosphorylase activity is expressed in nmol of Glucose-1-Phosphate produced min^{-1} mg^{-1} protein. D-enzyme is expressed in nmol glucose formed from maltotriose min^{-1} mg^{-1} protein. α-Glucosidase activities are expressed in nmol Glc formed from maltose min^{-1} mg^{-1} protein. Crude hydrolytic activities are expressed in nmol of reducing end equivalents formed from glycogen min^{-1} mg^{-1} protein. All assays were performed in triplicate and the values presented are mean ± SED.