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Figures and figure supplements

Arabidopsis plants perform arithmetic division to prevent starvation at night

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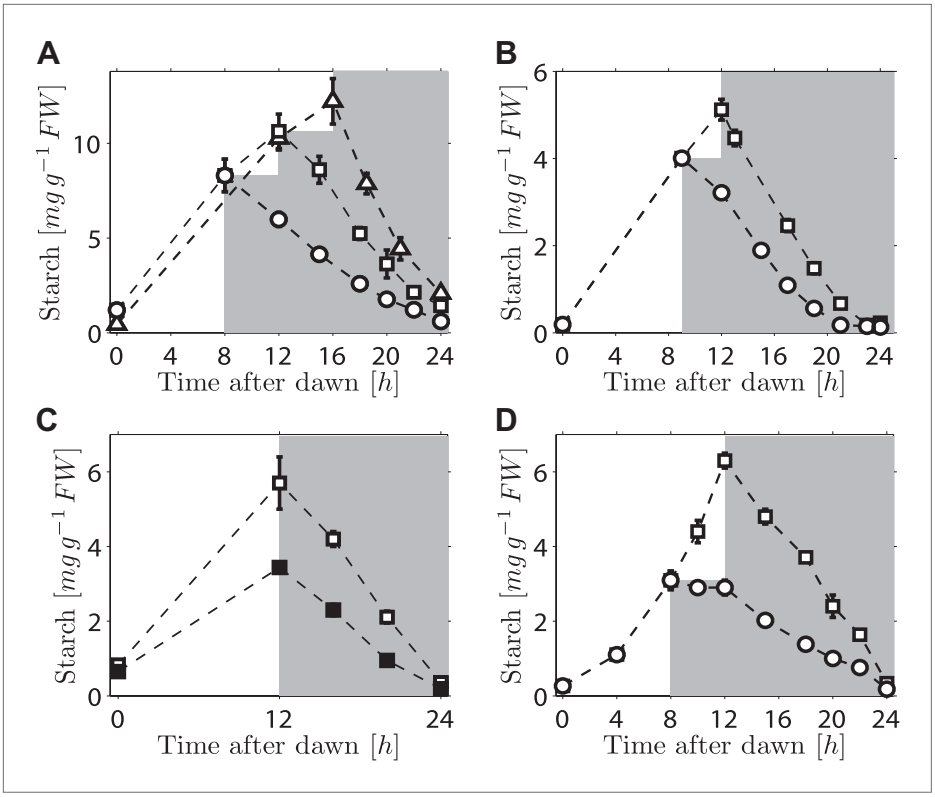

Figure 1. Starch content levels from experiments with unexpected variation in either starch content at the onset of darkness or the time of onset of darkness. (**A**) Starch turnover in Arabidopsis grown in 12-hr light/12-hr dark, then subject to unexpected early (8 hr, n = 6 individual rosettes, circles) normal (12 hr, n = 6, squares) or unexpected late (16 hr, n = 5, triangles) onset of darkness. (**B**) Starch turnover in Arabidopsis *cca1/lhy* mutant grown in 12-hr light/12-hr dark, then subject to unexpected early (9 hr, circles), or normal (12 hr, squares) onset of darkness (n = 6–10). (**C**) Starch turnover in Arabidopsis exposed to different daytime light levels: 90 μmol quanta $m^{-2}\,s^{-1}$ (open squares) or 50 μmol quanta $m^{-2}\,s^{-1}$ (filled squares) (both n = 5, previously all plants grown in 12-hr light/12-hr dark with 90 μmol quanta $m^{-2}\,s^{-1}$). (**D**) Starch turnover in *Brachypodium* grown in 12-hr light/12-hr dark, then subject to unexpected early (8 hr, circles) or normal (12 hr, squares) onset of darkness (both n = 6). Error bars are standard error of the mean throughout.

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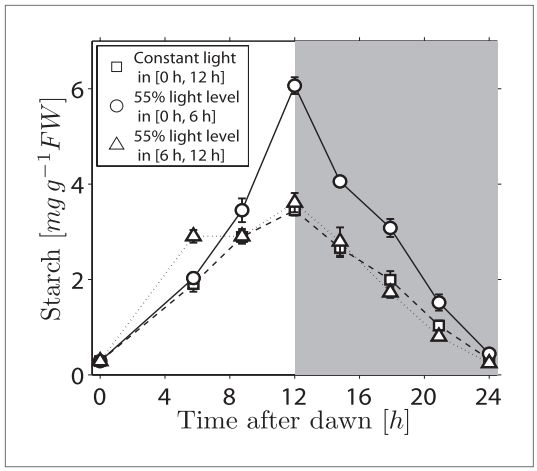

Figure 1—figure supplement 1. Starch content levels in Arabidopsis plants exposed to different regimes of varying light level over a single light period. Three sets of plants (each n = 5 individual rosettes) were grown in 12-hr light, 12-hr dark and were then subject to different light regimes during a single day. One set (squares) was exposed to normal light levels (180 μmol quanta m^{-2} s^{-1}), the other two were shaded to about 55% of normal light level (100 μmol quanta m^{-2} s^{-1}) for either the first 6 hr (circles) or the second 6 hr (triangles) of the 12-hr light period, with the normal light level for the other 6-hr period. Error bars are standard error of the mean.
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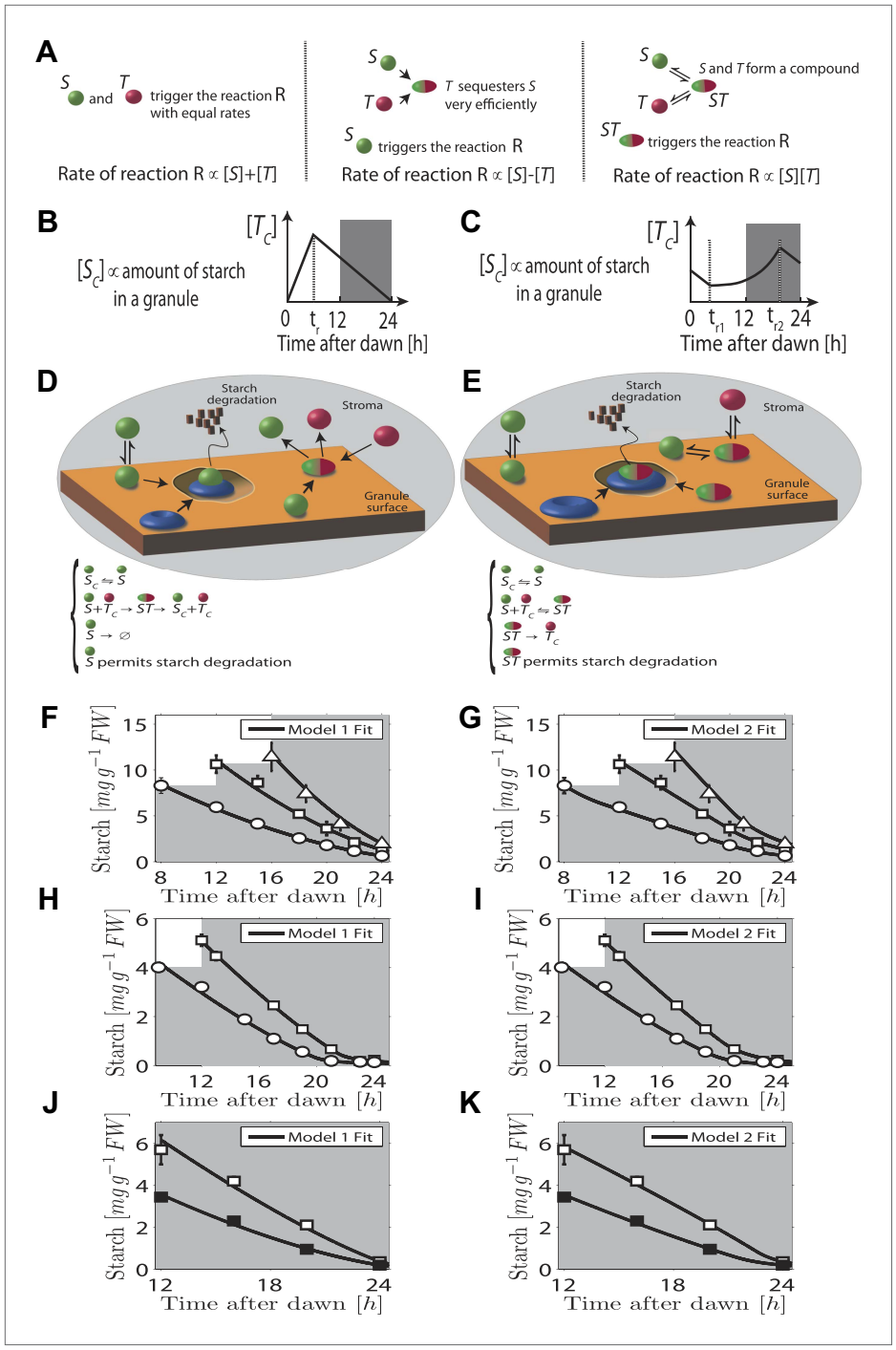

Figure 2. Chemical kinetic models capable of implementing analog arithmetic operations. (**A**) Pictorial summaries of schemes for analog implementation of addition, subtraction and multiplication between the concentrations of two molecules *S* and *T*. Square brackets indicate concentrations. (**B**) and (**C**) Schematic behavior of the stromal concentrations of *S* and *T* molecules ($[S_C]$ and $[T_C]$ respectively), in (**B**) first and (**C**) second arithmetic division models. In the first model, the *T* molecule tracks the time to expected dawn after a reset-time t_r. In the second model the *T* molecule concentration increases with time proportionally to 1/(expected time to dawn) between t_{r1} and t_{r2}. (**D**) and (**E**) Pictorial summaries of (**D**) first and (**E**) second analog arithmetic division models (not all reactions shown in pictures, for full details see 'Materials and methods'). In the reaction schemes, molecules not attached to the starch granule surface have a '*C*' subscript. The blue disk represents components of the starch degradation apparatus potentially activated by the *S* molecule in the first model, and by the *ST* complex in the second model.

Figure 2. Continued on next page

Figure 2. Continued

Best fits (full lines) of first (**F**), (**H**), and (**J**) and second (**G**), (**I**), and (**K**) arithmetic division models to Arabidopsis data from ***Figure 1A–C***.

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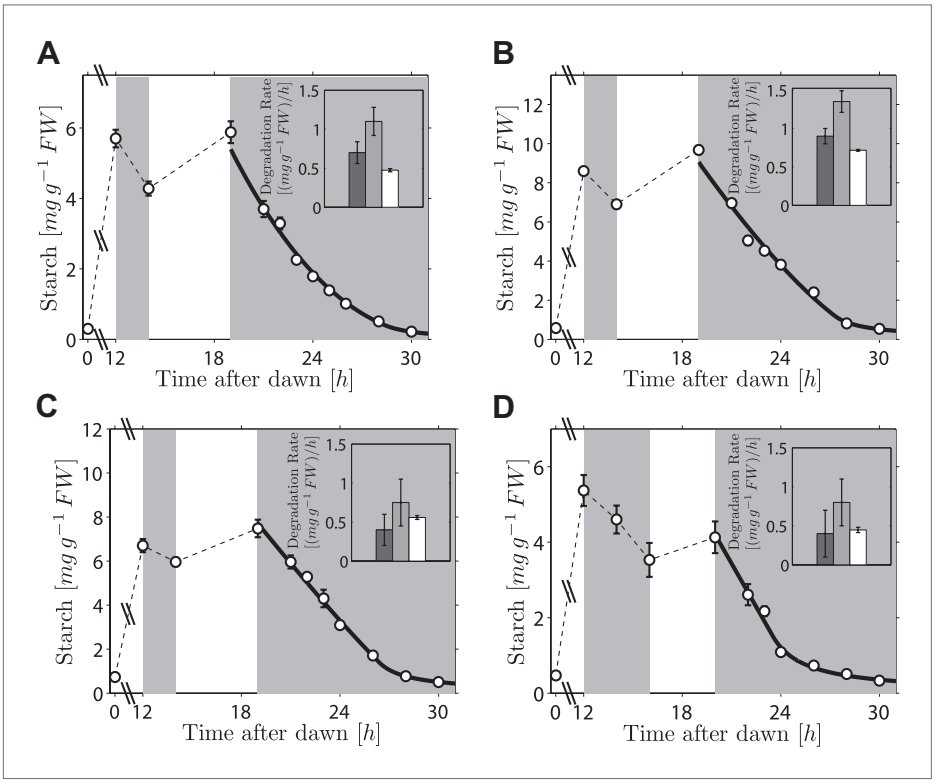

Figure 3. Starch content levels from experiments incorporating night-time light period. Arabidopsis plants grown in 12-hr light/12-hr dark were subjected to onset of darkness at 12 hr, followed by an unexpected period of light, followed by extended darkness. (**A**)–(**C**) Three data sets (n = 12 individual rosettes, except n = 10 for **C**), in which the unexpected period of light was between 14 hr and 19 hr after dawn. (**D**) In the fourth dataset (n = 12) the period of light was between 16 hr and 20 hr after dawn. Full lines are best fits to the first division model. The second model produces very similar fits (see ***Figure 3—figure supplement 2***). The insets show the respective starch degradation rates computed from the 12-hr and 14-hr experimental time points (dark grey bars) compared to those computed from the 19-hr and 21-hr experimental time points in panels (**A**–**C**) or the 20-hr and 22-hr time points in panel (**D**) (light grey bars). The white bars are the expected starch degradation rates in a normal 12 hr night, that is rates that would have ensured the complete depletion of the starch content measured at 12 hr at the time of expected dawn (24 hr). Error bars are standard error of the mean throughout.

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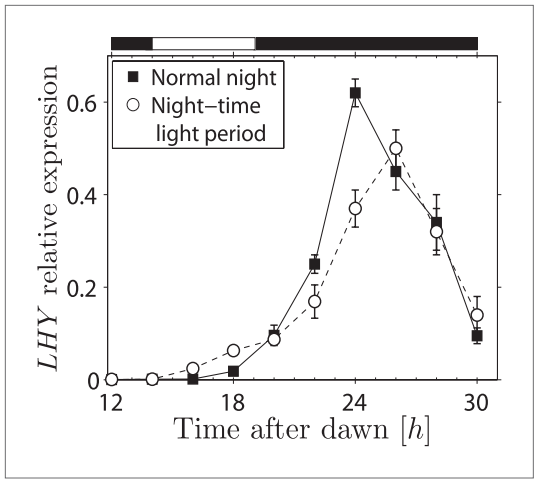

Figure 3—figure supplement 1. Transcript levels of *LHY* from experiment incorporating night-time light period. *LHY* transcript levels (relative to *ACT2*) measured in Arabidopsis plants kept in continuous darkness after a normal night (squares), or subjected to a 5-hr night-time light period between 14 hr and 19 hr after dawn, and then kept in continuous darkness (circles), as in ***Figure 3A–C***. Data for the night-time light period are from the same plants as in ***Figure 3B***. n = 5 individual rosettes, error bars are standard error of the mean. The night-time light period is shown on top of graph.

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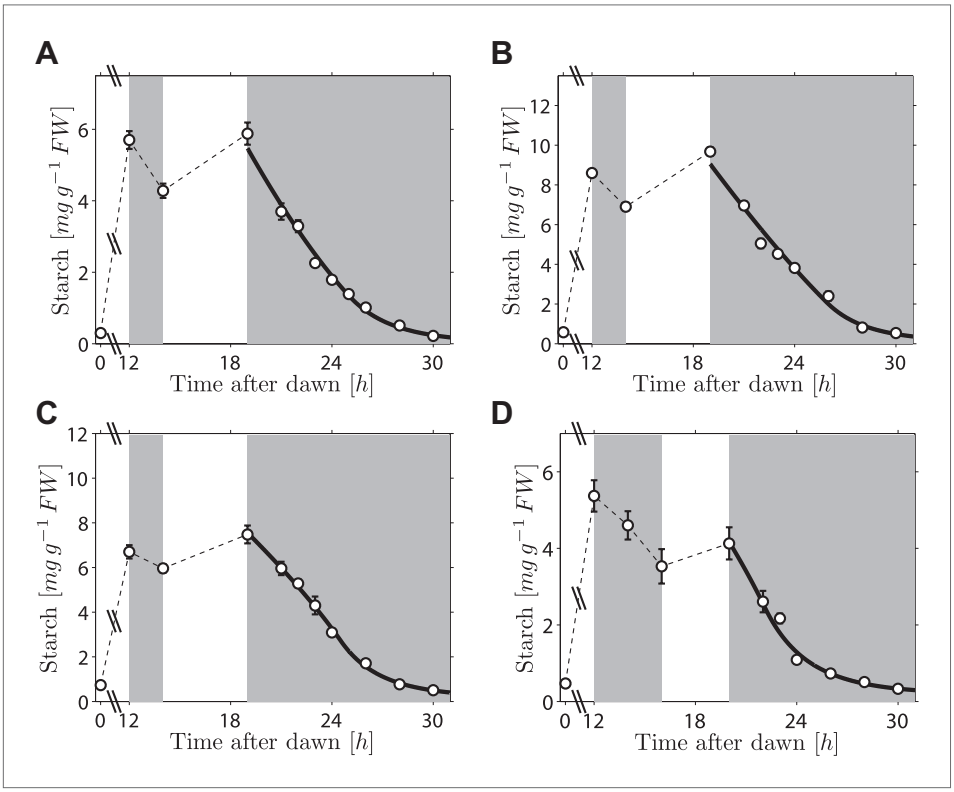

Figure 3—figure supplement 2. Best fits (full lines) of the second division model to starch content data from experiments incorporating night-time light period. Error bars are standard error of the mean throughout.
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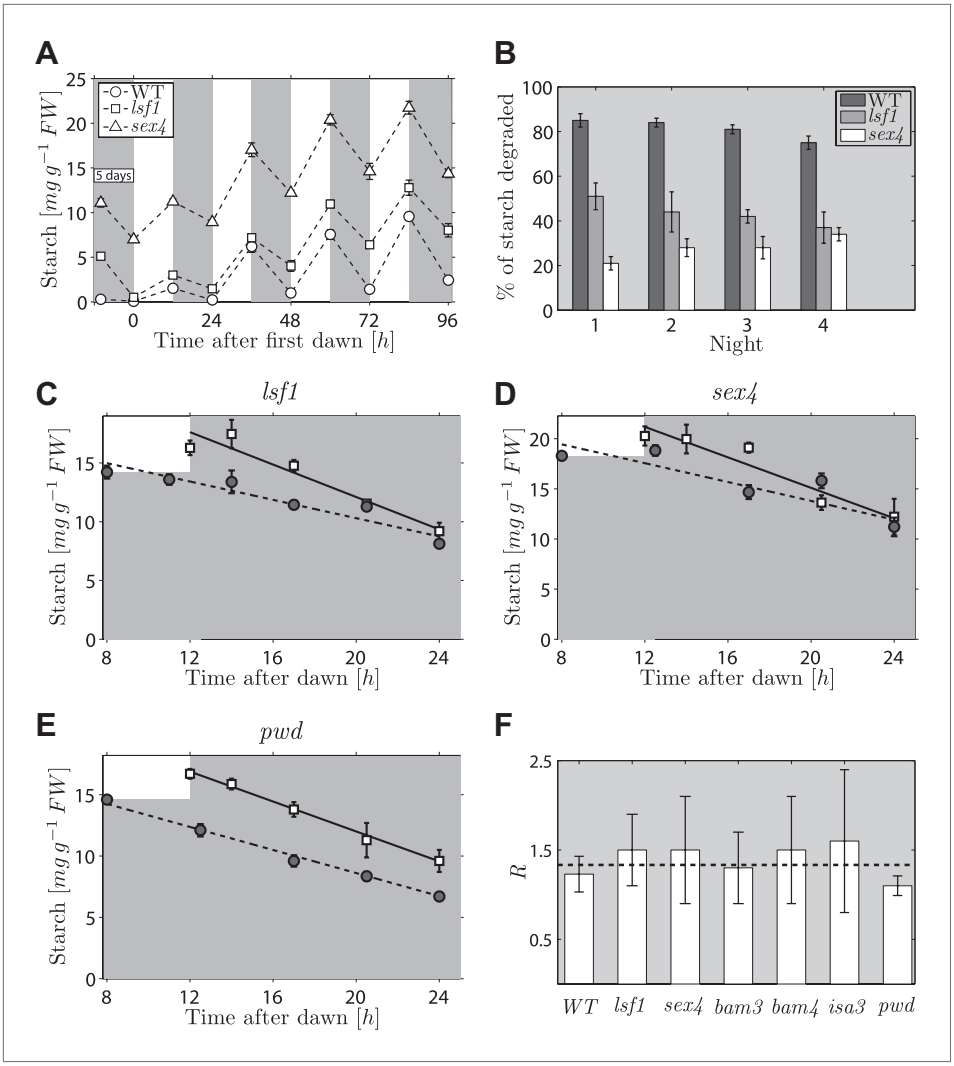

Figure 4. Starch content levels in mutant Arabidopsis plants defective in components of the starch degradation apparatus. (**A**) Starch content in wild-type (WT) plants and *lsf1* and *sex4* mutant plants during four days of 12-hr light/12-hr dark following 5 days of continuous darkness, where plants were transferred back into the light (at time 0 hr on the x-axis) 132 hr after the end of the previous light period (n = 6 individual rosettes). Data for wild-type and *lsf1* plants are from (***Comparot-Moss et al., 2010***). (**B**) The percentage of starch degraded during each of the four nights in (**A**). (**C**)–(**E**) Starch content in *lsf1*, *sex4* and *pwd* mutant plants grown in 12-hr light/12-hr dark cycles then subject to unexpected early (8 hr, circles) or normal (12 hr, squares) onset of darkness (n = 5). The continuous and dashed lines are linear fits to the normal and early night datasets respectively. (**F**) For each of the labeled genotypes, *R* is the ratio between the starch degradation rates (each normalized by their respective end-of-light period starch content and as determined from the linear fits) during the normal and early nights. The dashed line shows the expected value of *R* for wild-type (WT) plants, that is, ratio of rates that would ensure the complete depletion of the starch content in all cases at the time of expected dawn (24 hr). See 'Materials and methods' for details about the linear fitting and the calculation of *R*. Error bars are standard error of the mean throughout. ***Figure 4—figure supplement 1*** shows the datasets used to calculate *R* for WT, *bam3*, *bam4* and *isa3*.

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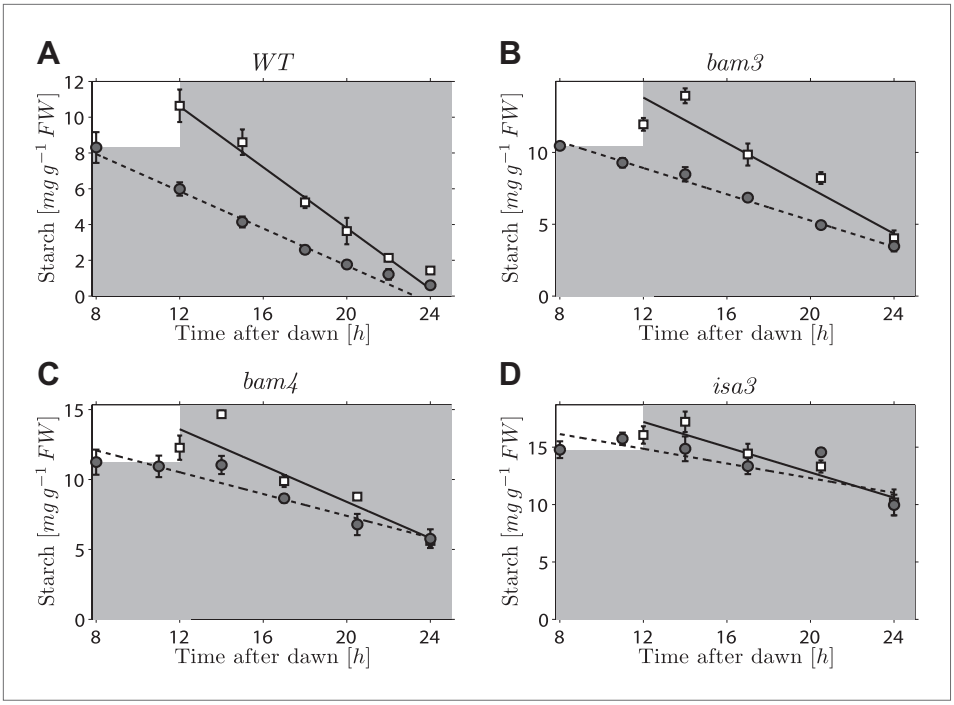

Figure 4—figure supplement 1. Starch content levels during unexpectedly early night in wild-type, *bam3, bam4, isa3* mutant plants. Starch content in wild-type (WT), *bam3, bam4, isa3* mutant Arabidopsis plants grown in 12-hr light, 12-hr dark cycles then subject to unexpected early (8 hr, circles) or normal (12 hr, squares) onset of darkness (n = 6 individual rosettes for WT, n = 5 for mutants; the WT dataset analyzed here is the one already shown in ***Figure 1A***). The continuous and dashed lines are linear fits to the normal and early night datasets respectively. Error bars are standard error of the mean throughout.

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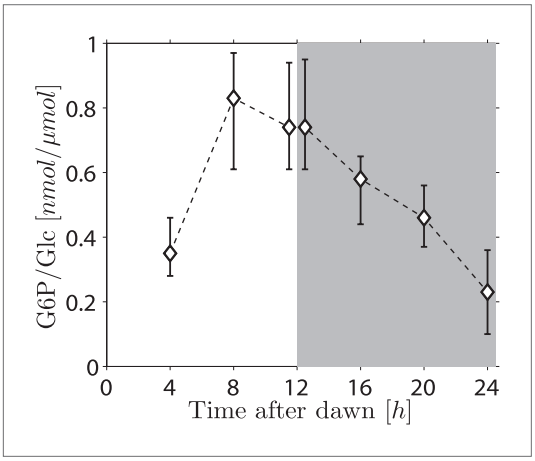

Figure 5. Daily change in starch phosphate content (measured as glucose 6-phosphate, G6P) in Arabidopsis leaves. Results are normalized by total amount of glucose (Glc) in starch at each time point. Starch was extracted from rosettes of 26-day-old plants. n = 3 pools of 10 rosettes except at 24 hr time point, with n = 2 pools of 15 rosettes. Error bars represent the range (i.e., error bar edges correspond to highest and lowest values measured).

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