Supplemental Information

Cell Cycle Control by Nuclear Sequestration of \textit{CDC20} and \textit{CDH1} mRNA in Plant Stem Cells

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Figure S1. Mitosis-Specific Expression of Cell Cycle Genes and Rapid CYCB1;1 Protein Degradation at Metaphase-to-Anaphase Transition. Related to Figure 1.

(A) Expression patterns of G2/M cell cycle genes. Scale bar, 50 µm.

(B) Quantification of the number of cells expressing cell cycle genes at different mitotic stages.

(C) RNA FISH to show the accumulation of CYCB1;1 transcripts at different stages of mitosis. Scale bar, 5 µm.
(D) CYCB1;1 mRNA levels at different stages of mitosis, as calculated from the fluorescence intensity of RNA FISH images.

(E) CYCB1;1-GFP protein expression at different stages of the cell cycle. H2B-RFP is used to monitor chromosome alignment and segregation. Scale bar, 5 µm.

(F and G) Protein dynamics of CYCB1;1-GFP during mitosis. GFP fluorescence intensity is shown in (G).
Figure S2. Expression Pattern of CDC20 and CCS52B. Related to Figure 2.

(A) Double RNA FISH to show the expression patterns of CDC20 and CCS52B in the same meristem. Scale bar, 50 µm.

(B) Co-expression of CDC20 and CCS52B at different mitotic stages. The anaphase and late telophase cells shown are those only expressing CCS52B. Scale bar, 5 µm.

(C) Quantification of the number of cells that express CDC20 and CCS52B.

(D) Expression pattern of CDC20 in root cells. Scale bar, 50 µm.

(E) Root cells at different stages of mitosis. Note that CDC20 mRNA is sequestered inside the nucleus at prophase. Scale bar, 5 µm.

(F) Nuclear localization of CDC20 mRNA in shoot prophase cells. Scale bars, 50 µm for shoot overview (top panels) and 5 µm for cells (bottom panels).
Figure S3. 3-D Projection of Confocal Images to Show CDC20 Expression Patterns with CYCBs and HIS4 in the Same Meristems. Related to Figure 3.

(A) Nucleocytoplasmic separation of CDC20 and CYCB1 transcripts in prophase cells.

(B) CDC20 does not co-express with S-phase marker HIS4 gene.
Figure S4. Protein Dynamics of CDC20 and CCS52B during the Cell Cycle. Related to Figure 4.

(A and B) Co-localization of GFP mRNA with CDC20 or CCS52B mRNA in pCDC20::GFP-CDC20 and pCCS52B::GFP-CCS52B transgenic plants. Note that fusion of GFP does not
affect *CDC20* or *CCS52B* mRNA nuclear localization. Scale bars, 50 µm for SAM overview in (A) and 5 µm for single cells in (B).

(C and D) Time-lapse imaging of GFP-CDC20 (C) and GFP-CCS52B (D) protein expression in the same cell as it undergoes division. Arrowheads indicate the cells analysed. Scale bars, 5 µm.

(E) MG132 treatment does not affect GFP-CCS52B protein abundance. Scale bar, 50 µm.

(F) The amount of GFP-CDC20 proteins in both SAM (left) and root (right) can be increased by MG132 treatment. Scale bar, 50 µm.
Figure S5. 5’UTR Affects CDC20 mRNA Nuclear Localization. Related to Figures 5 and 6.

(A) The expression patterns of full length GFP-CDC20 mRNAs transcribed from genomic DNA or cDNA in the shoot apex. Shown are representative meristems from one of the independent transgenic lines. Scale bar, 50 µm for SAM overview and 5 µm for single cells.

(B) The expression patterns of GFP-CDC20 truncated mRNAs.

(C) The expression patterns of GFP chimeric mRNAs.
Figure S6. Model for Cell Cycle Control by mRNA Nuclear Sequestration. Related to Figures 5 and 6.

(A) Subcellular distribution of CYCB, CDC20 and CCS52B mRNAs during cell cycle progression in plant stem cells.

(B) CYCB, CDC20 and CCS52B protein dynamics. Nuclear sequestration of CDC20 and CCS52B mRNAs in prophase prevents their translation to protein. Nuclear envelope breakdown at prometaphase enables redistribution of the mRNAs into the cytoplasm and subsequent protein synthesis, following which the proteins activate APC/C to destroy cyclin B proteins and other substrates.