Blow et al., http://www.jcb.org/cgi/content/full/jcb.200911037/DC1

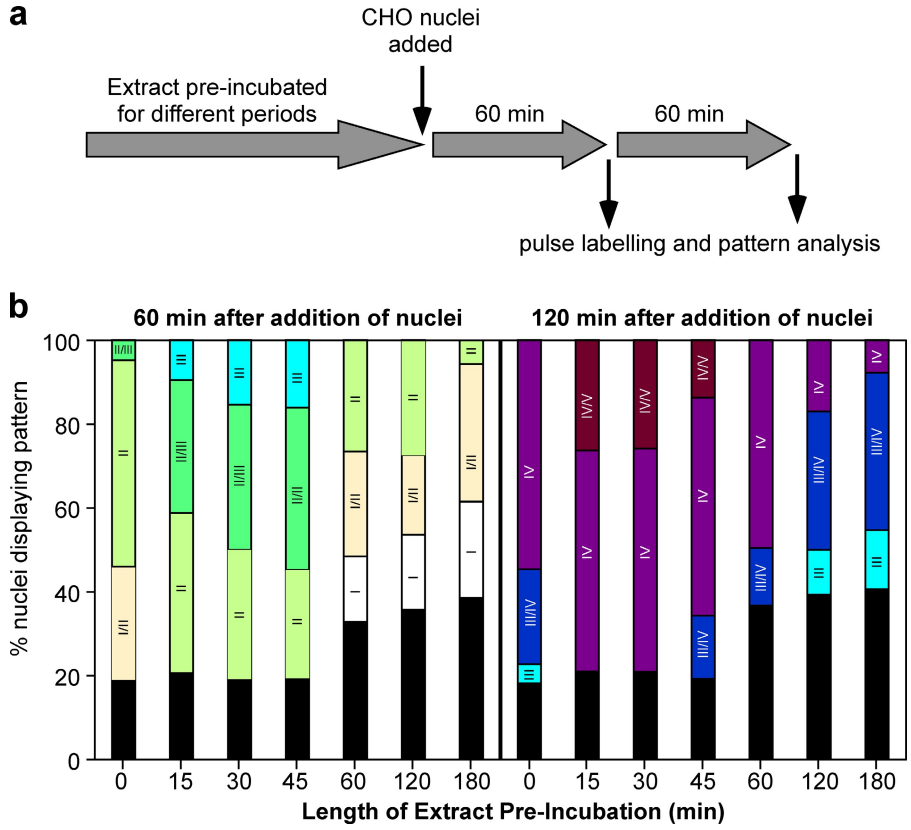

Figure S1. **Effect of preincubation of egg extract on the timing program.** (a and b) *X. laevis* egg extracts were preincubated at 23°C for different times after addition of $CaCl_2$ to promote exit from metaphase. They were supplemented with 10,000 nuclei/µl CHOC-400 nuclei and geminin. At either 55 or 115 min after nuclear addition, nascent DNA was pulse labeled for 5 min with Cy3-dUTP. At the end of the pulse, nuclei were isolated, and the percentage of nuclei showing different replication patterns was measured. (a) Cartoon of experimental protocol. (b) Percentage of replication patterns at either 60 (left) or 120 min (right) after addition of nuclei. Note that all other experiments in this study were performed with extracts preincubated for 15 min.

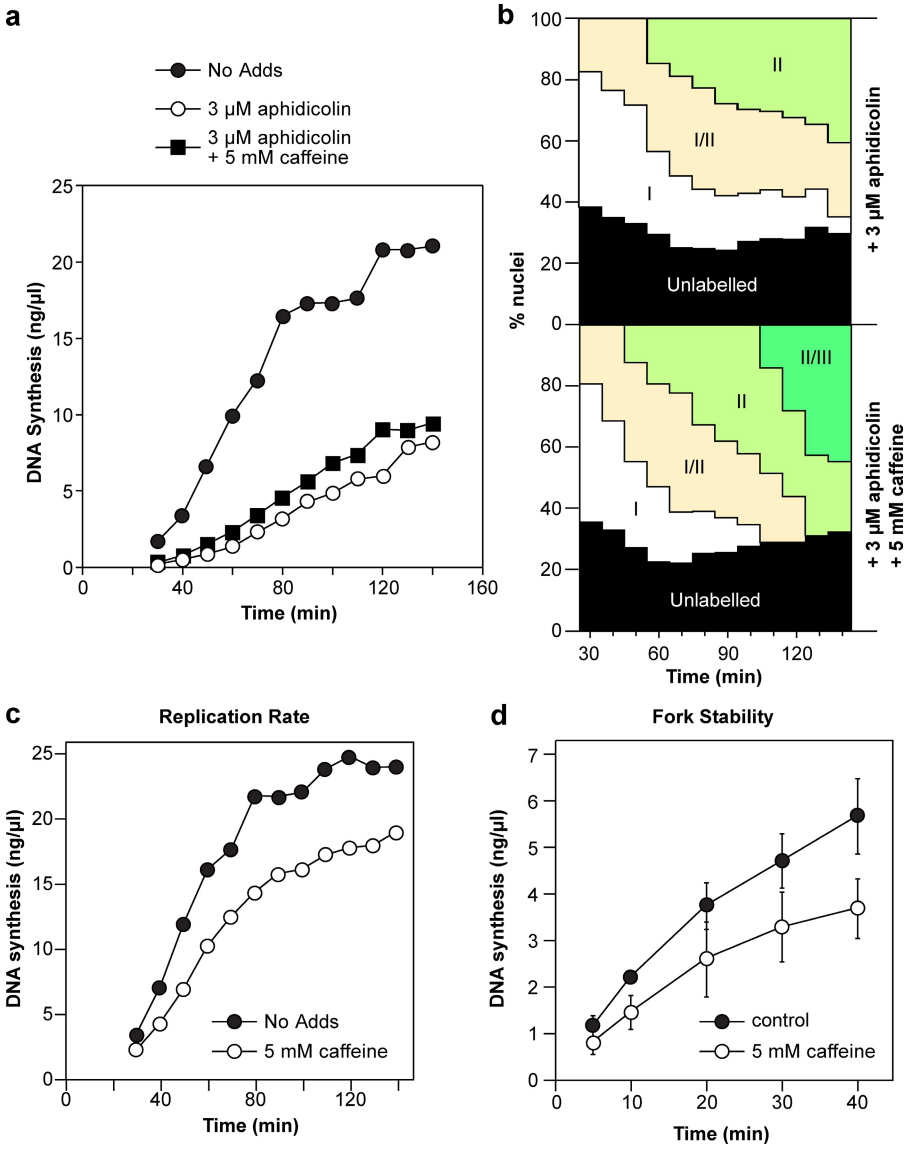

Figure S2. **Aphidicolin slows the timing program and caffeine causes fork instability.** (a–c) CHOC-400 nuclei were incubated at 10,000 nuclei/μl in *X. laevis* egg extracts supplemented with geminin ± 3 μM aphidicolin or 5 mM caffeine. (a and c) Extract was also supplemented with α-[^{32}P]dATP. At different times, DNA was TCA precipitated, and the amount of total DNA synthesis was assessed by scintillation counting. (b) At different times, nascent DNA was pulse labeled with Cy3-dUTP. Nuclei were isolated, and the percentage of nuclei showing different replication patterns was assessed. (d) CHOC-400 nuclei were incubated at 10,000 nuclei/μl in *X. laevis* egg extracts supplemented with geminin. After 35 min, extracts were supplemented with 100 μM aphidicolin, 1 mM roscovitine ± 5 mM caffeine, and the incubation was continued for an additional 60 min. Nuclei were isolated and incubated in fresh extract supplemented with α-[^{32}P]dATP and roscovitine. At different times, DNA was TCA precipitated, and the amount of total DNA synthesis was assessed by scintillation counting. The rate of synthesis provides an indication of the number of active forks that had remained after the incubation with aphidicolin plus or minus caffeine.

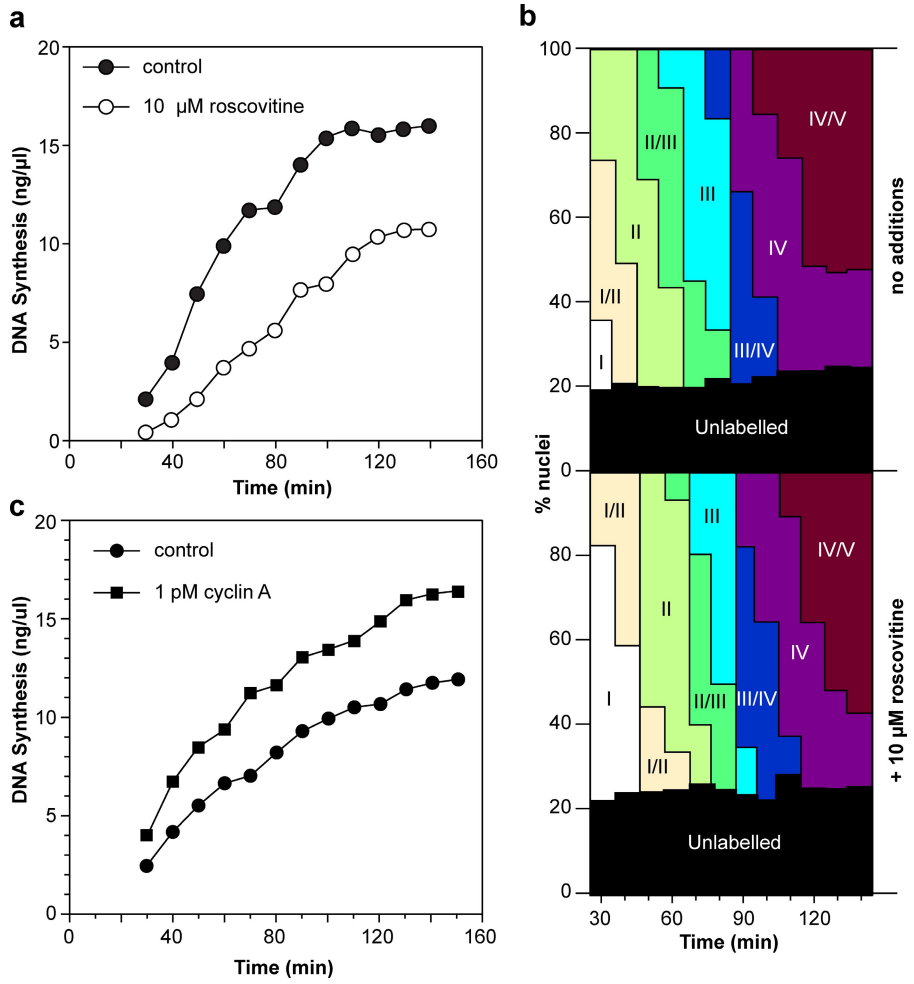

Figure S3. **Effect of roscovitine and cyclin A on the timing program.** (a–c) CHOC-400 nuclei were incubated at 10,000 nuclei/μl in *X. laevis* egg extracts supplemented with geminin ± 10 μM roscovitine (a and b) or 1 pM cyclin A (c). (a and c) Extract was also supplemented with α-[^{32}P]dATP. At different times, DNA was TCA precipitated, and the amount of total DNA synthesis was assessed by scintillation counting. (b) At different times, nascent DNA was pulse labeled with Cy3-dUTP. Nuclei were isolated, and the percentage of nuclei showing different replication patterns was assessed.

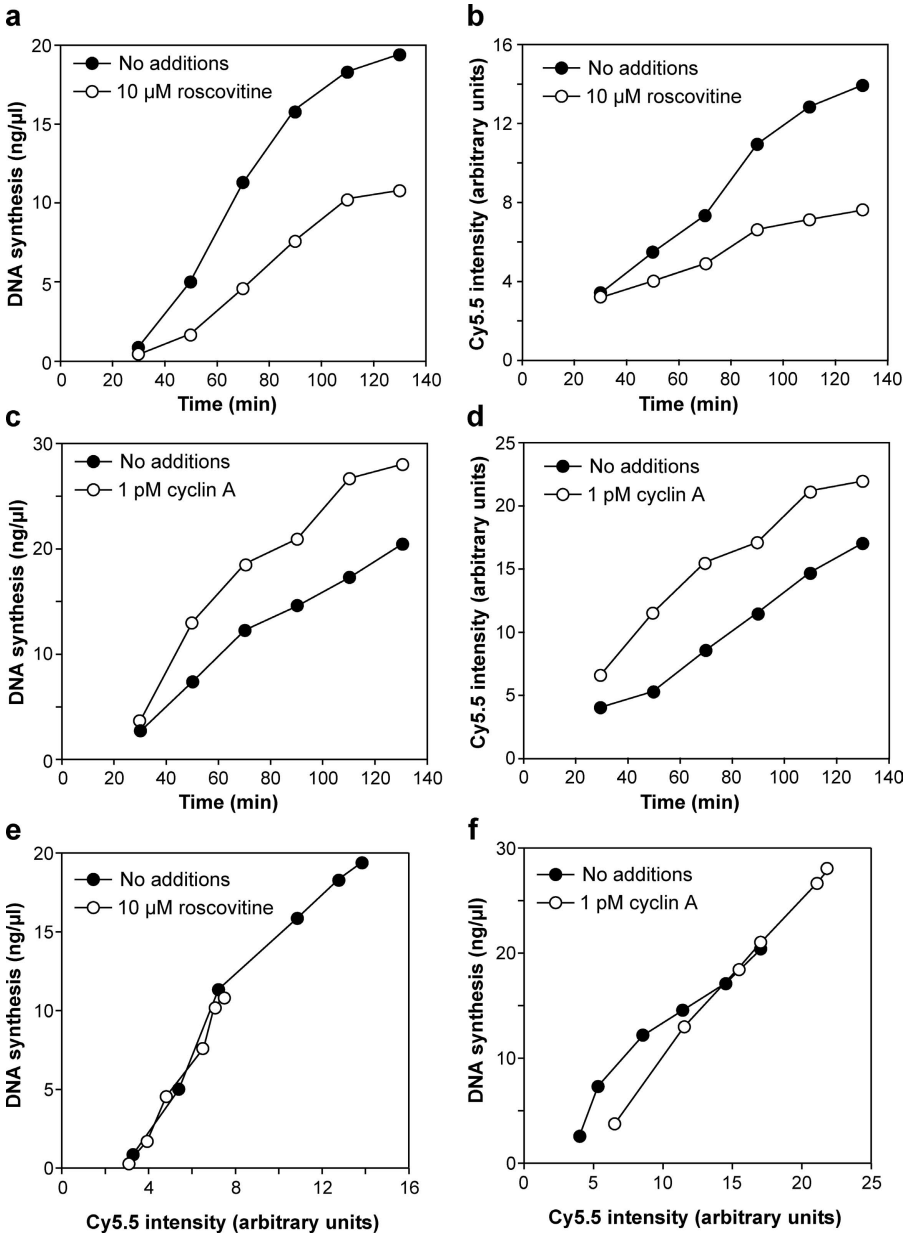

Figure S4. **Measurement of DNA synthesis in individual nuclei ± 10 μM roscovitine or 1 pM cyclin A.** (a–f) CHOC-400 nuclei were incubated at 10,000 nuclei/μl in *X. laevis* egg extracts supplemented with geminin ± 10 μM roscovitine (a, b, and e) or 1 pM cyclin A (c, d, and f). (a and c) Extract was also supplemented with α-[^{32}P]dATP. At different times, DNA was TCA precipitated, and the amount of total DNA synthesis was assessed by scintillation counting. (b and d) Extract was also supplemented with Cy5.5-dCTP. At different times, nuclei were isolated, the total Cy5.5 fluorescence of 20 randomly selected nuclei was determined by microscopy, and the mean value was determined. (e and f) For each time point in a–d, DNA synthesis as derived by α-[^{32}P]dATP incorporation was plotted against mean nuclear replication as derived from Cy5.5 intensity.

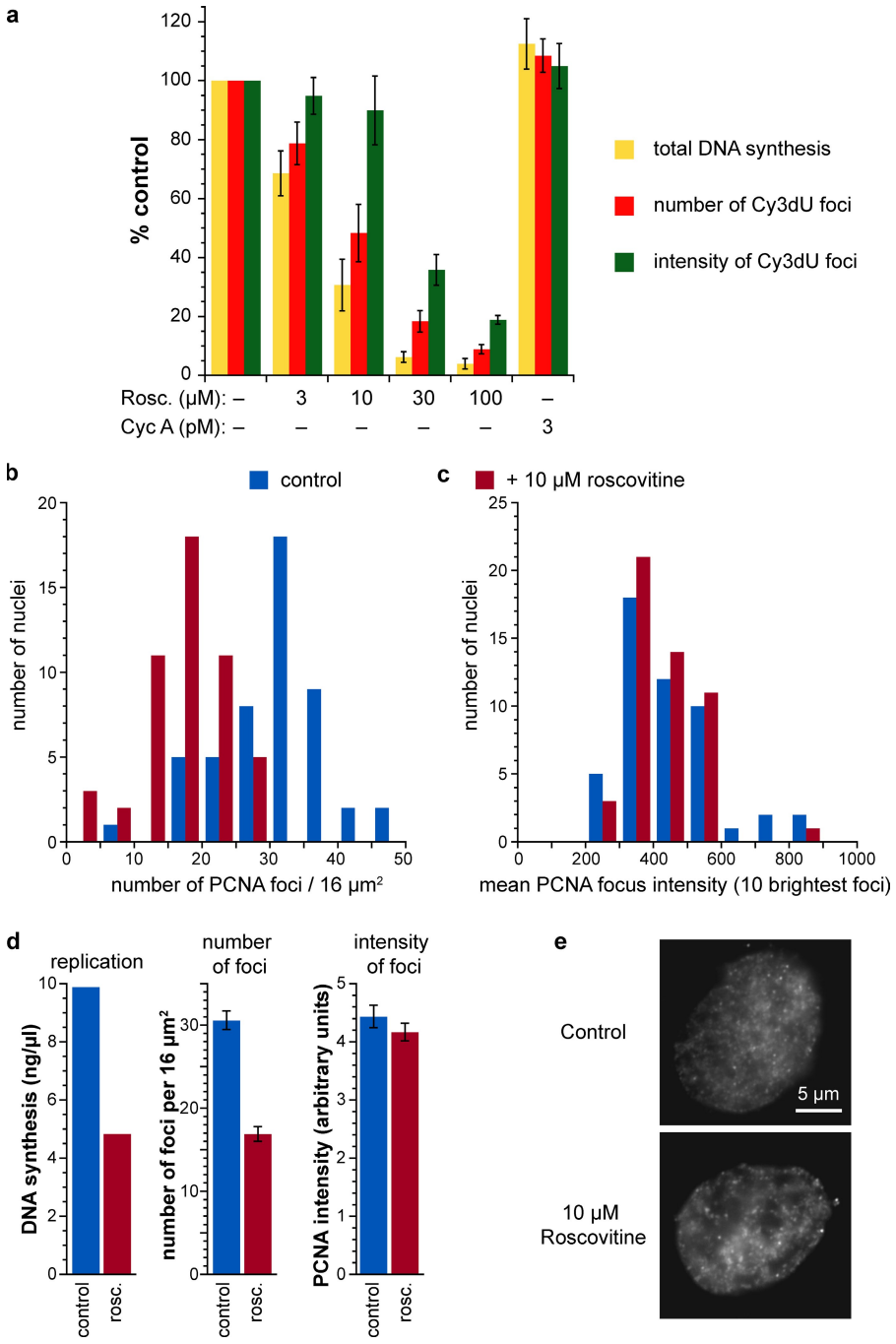

Figure S5. **Effect of roscovitine and cyclin A on replication foci.** CHO nuclei were incubated in *X. laevis* egg extract plus or minus the indicated concentrations of roscovitine (rosc) or cyclin A (cyc A). Parallel incubations were supplemented with α-[^{32}P]dATP to measure total DNA synthesis. (a) At 50 min, extract was pulsed for 5 min with Cy3-dUTP. The number and intensity of Cy3-labeled foci was measured. Results from three independent experiments were normalized to values obtained in untreated extract and combined. Error bars indicate SEM. (b–e) At 50 min, nuclei were isolated, stained with anti-PCNA antibody, and examined for the presence of replication foci. (b) The number of foci in a 4 × 4–μm square in the center of 50 nuclei was determined. (c) The mean intensity of the 10 brightest foci in 50 nuclei was determined. Mean values of total DNA synthesis, number of PCNA foci, and PCNA content of foci shown in d. (e) Representative images are shown.