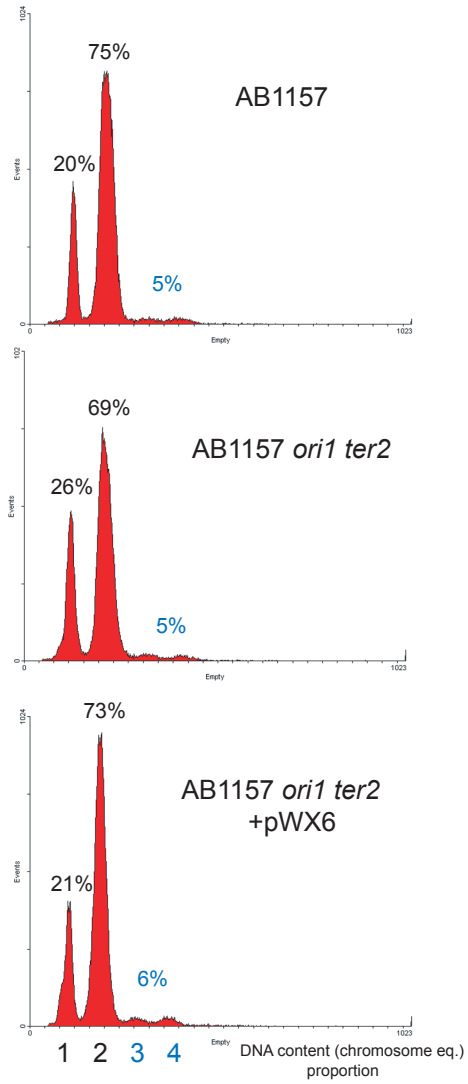
supplemental materials



Legend-Supplementary Figure

Flow cytometry. Flow cytometry was performed on a FACSCalibur flow cytometer (Becton Dickinson). Samples for flow cytometry were grown, fixed and the DNA was stained with Syto16 (Molecular Probes). Run out experiments used cultures that had been incubated in rifampicin and cephalexin for 210 min (Skarstad et al. 1985), while exponentially growing cultures were typically harvested at A_{600} = 0.15. G1 was calculated by determining the fraction of 1 chromosome cells after run out and correcting for the fact that the frequency of new borne cells in an exponential population is twice that of cells about to divide and that there is an exponential relation between cell age and fraction of DNA in a given class.

One set of replication 'run-out' experiments is shown for the parental and experimental strains. The relative fractions of each chromosome type are shown, from which the ratio of G1 to S/G2/M can be calculated (Skarstad et al. 1985). The great majority of cells had either a single chromosome (cells in G1 prior to drug addition), or two chromosomes (S/G2/M cells). A small minority of cells had three or four chromosomes in the run-out experiments, which arise when a second round of DNA replication has initiated prior to cell division. For the experimental repressor-producing strain, the computed G1 value for the curve shown is 18 min for the majority cell population in which replication initiation has occurred in the same cell as termination. Analysis of exponential cultures gave the expected G1, S and G2/M populations, although the overlap of the three populations made precise estimates of G1, S and G2/M impossible. Nevertheless, the data were in general accord with our inferences from the snapshot analysis. Multiple independent measurements for the strains used in the microscopic analysis gave results that varied significantly from day-to-day despite little variation in generation time, with G1 varying from less than 5 min to ~20 min. We suspect that the variation in G1 may result from heterogeneity within the exponentially growing population, with a variable proportion of quiescent cells that remain in G1 for extended periods. Although we are unable to precisely relate the time of visible *ori1* segregation to initiation of DNA replication at *ori*C, we are confident that *ori1* separation occurs no more that 15 min after initiation of DNA replication, a result consistent with FISH analysis of a different *E.coli* strain (Bates and Kleckner, 2005).