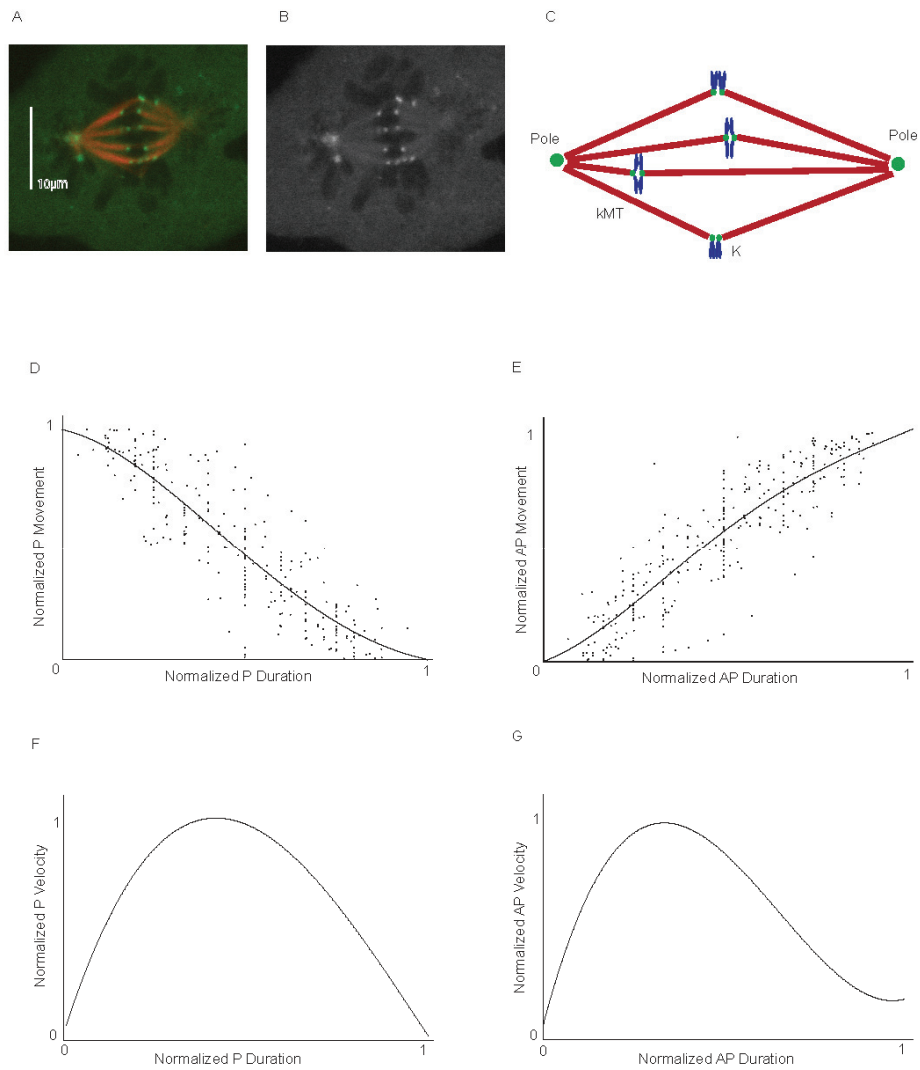


Supplemental Figure 1

### Supplemental Figure 1. Oscillation simulations

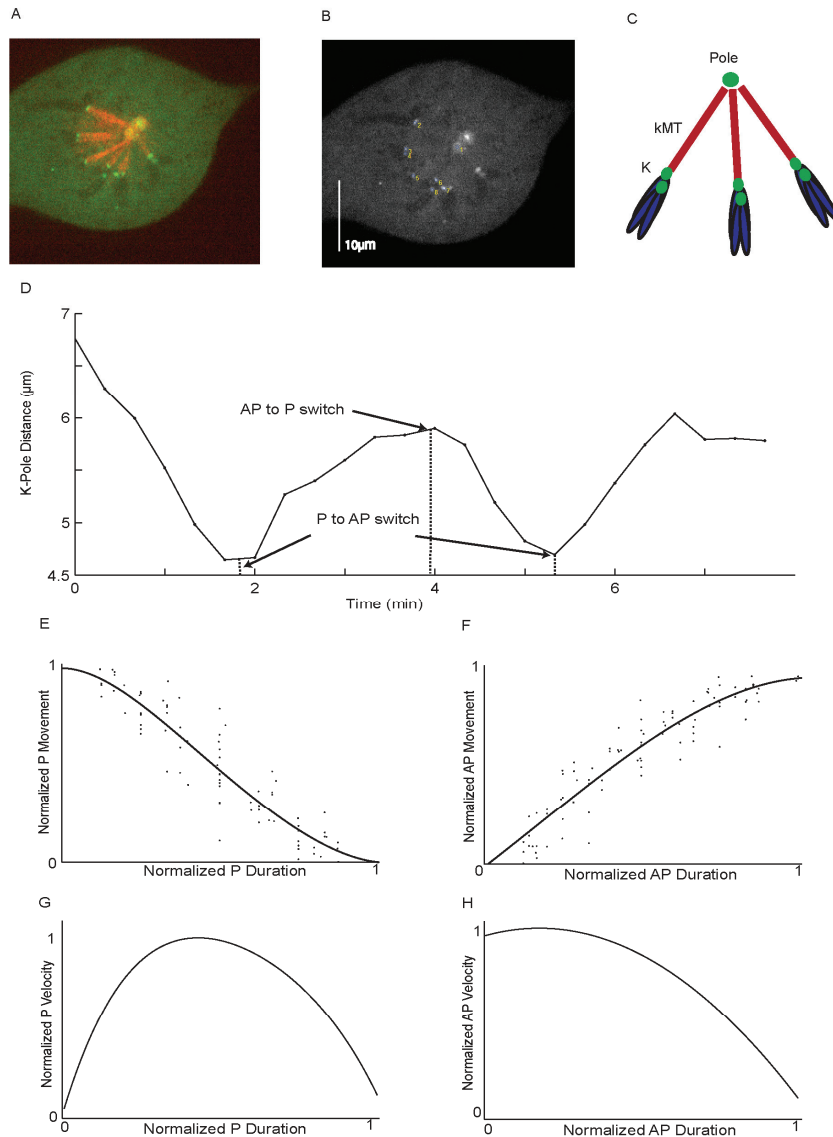
The amplitude and period of oscillation were made equal to the average value in PtK1 cells. Blue and red lines represent the distance between pole and kinetochore for a sister pair. Black lines are the K-K distance. Simulation of oscillations by sine wave (A) and symmetrical triangular wave (B). In either case, the oscillation frequencies are equal between K-P distance and K-K distance and the phase difference between the sister pair does not change the frequency of K-K distance oscillation. (C) Simulation of oscillation by asymmetric triangle wave with the two kinetochores moving out of phase by about 180 degrees as occurs in vivo. The asymmetry between P and AP movements are similar to the PtK1 oscillation in vivo. The frequency of K-K oscillation is doubled.



Supplemental Figure 2

**Supplemental Figure 2.** Measurements of kinetochore and centromere oscillations for normally bioriented chromosomes in late prometaphase spindles.

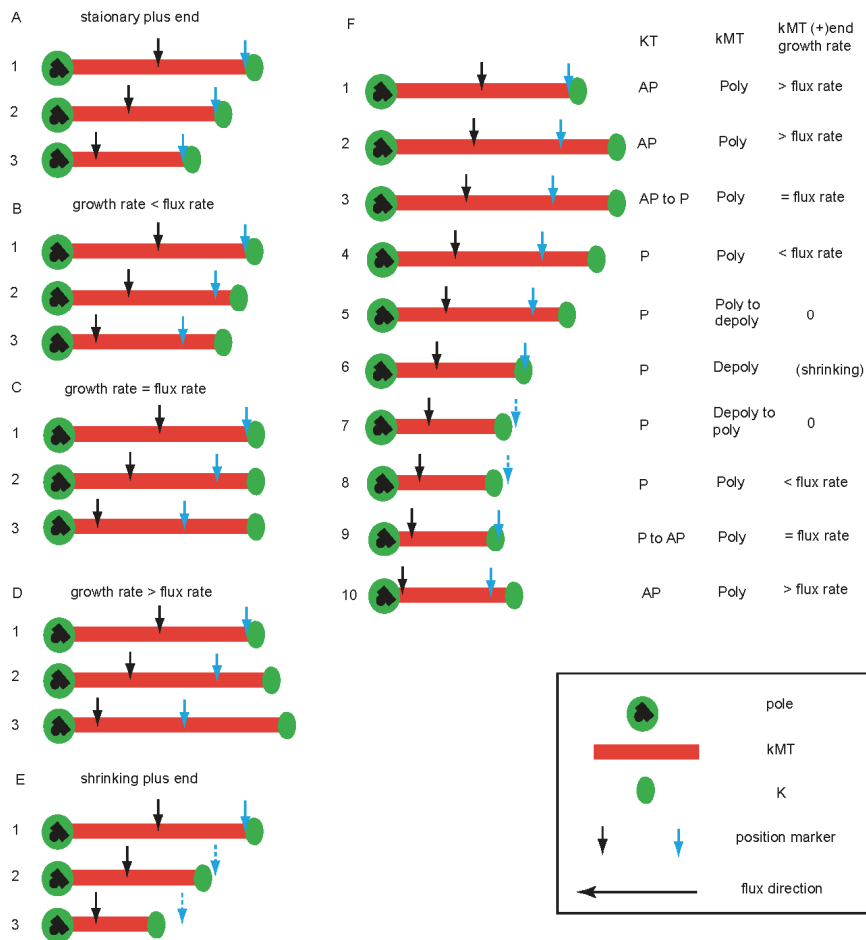
A-C as in Figure 1 and D-G as described in Figure 4 legend for normally bioriented chromosomes in metaphase spindles. (number of cells: 6; number of sister kinetochore pairs: 12)



Supplemental Figure 3

**Supplemental Figure 3.** Oscillations of mono-oriented chromosomes in monopolar spindles.

A-D as in Figure 1 and D-G as described in Figure 4 legend for normally bioriented chromosomes in metaphase spindles. In these studies, cells assembled monopolar spindles as they entered mitosis in the presence of 100 - 200  $\mu\text{M}$  monastrol, which inhibits the kinesin-5 motor that normally functions to push the poles apart. The average kMT flux in these monopoles, is slightly reduced (0.5  $\mu\text{m}/\text{min}$ ) in comparison to the rate (0.65  $\mu\text{m}/\text{min}$ ) typical of metaphase bipolar spindles (Cameron *et al.*, 2006). We did not assay chromosomes with both kinetochores attached to MTs from the pole (syntelic attachments) because asynchrony that is typical near the end of P and AP movements could complicate the analysis (e.g., one moving P while the other is moving AP). (number of cells: 6; number kinetochores: 8)



Supplemental Figure 4

**Supplemental Figure 4.** Schematic diagrams of kMT plus-end dynamic instability and kinetochore dynamic instability with poleward flux.

(A) When there is no kMT plus-end activity, the kinetochore moves poleward with flux. (B) When the plus-end polymerization rate is smaller than the flux rate, the kinetochore is in P motion, P movement velocity equals to the flux rate minus the growth rate. (C) When the polymerization rate is equal to the flux rate, the kinetochore remains stationary. (D) When the polymerization rate is greater than the flux rate, the kinetochore is in AP motion; the AP movement velocity is equal to the polymerization rate minus the flux rate. (E) When the kMT plus-end is depolymerizing, the kinetochore is in P motion; the P movement velocity is equal to the depolymerization rate plus the flux rate. (F) Kinetochore staying in P or AP state is influenced by the kMT plus-end polymerization rate and the flux rate. Black and blue arrows mark the same spots on kMT at different time points; they move with the flux. Labels 1 – 10 represent the same time points indicated in Figure 5.