SUPPLEMENTARY INFORMATION

Mathematical derivation Kinetochore microtubule (kinetochore-microtubule) dynamics.

Calculating the detachment rates and the mean lifetime of kinetochore-microtubules from measured kinetochore-microtubule half-lives

After photoactivating a ~2μm^2 area on the spindle the fluorescence decays at a double exponential decay rate (Supp. Figure 1A, B) that we can fit to the following equation:

$$F(t) = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \quad \text{(Eq. 1)},$$

where *F(t)* is the observed fluorescence at time t, A_1 and A_2 represent less (non-kinetochore-microtubule) and more (kinetochore-microtubule) stable microtubule populations with decay constants of k_1 and k_2, respectively [1-3].

Therefore, we can calculate the probability that a single microtubule will detach from a kinetochore within time (*T*) to be

$$\int_0^T k_2 e^{-k_2} \partial t \quad \text{(Eq. 2)},$$

from the definition of the half-life $t^{1/2}$ for an exponential decay process therefore,

$$\frac{1}{2} = \int_0^{t^{1/2}} k_2 e^{-k_2} \partial t \quad \text{(Eq. 3)},$$

where $t^{1/2}$ is experimentally estimated as the time taken for half of the kinetochore-microtubules with a photoactivated fluorescent label to detach from the kinetochore

From equation 3,

$$k_2 = \frac{\ln(2)}{t^{1/2}} \text{ (Eq. 4).}$$

We can then calculate the detachment rate (r_d) of microtubules from kinetochores, which is directly proportional to the kinetochore-microtubule occupancy (n). Thus,

$$r_d = n\frac{\ln(2)}{t^{1/2}} \text{ (Eq. 5)}$$

The mean-lifetime (τ) of an individual microtubule attached to kinetochore is

$$\tau = \frac{n}{r_d} = \frac{t^{1/2}}{\ln(2)} \text{ (Eq. 6)}$$

Calculating kinetochore-microtubule occupancy from kinetochore-microtubule half-lives

In order to calculate kinetochore-microtubule occupancy for a given experimentally measured half-life of a kinetochore-microtubule, high resolution information detailing kinetochore-microtubule occupancy is required. The most well-studied kinetochore microtubule interface is in PtK_1 cells where serial section electron microscopy was used to measure the kinetochore-microtubule occupancy during different phases of mitosis (at metaphase, n = 24.3, ref. 4). Moreover, in these cells, kinetochore-microtubule half-lives have also been measured at the different stages of mitosis (at metaphase, $t^{1/2}$ = 7.4 min., ref. 2). It was also shown that kinetochores are saturated at a *saturation occupancy* (S) of 35 microtubules [4].

We assume that, at metaphase, kinetochore-microtubule occupancy is at a steady-state so that the attachment rate (r_a) and detachment rate (r_d) of microtubules from kinetochores are equal:

$$r_a = r_d \text{ (Eq. 7),}$$

We also assume that kinetochore microtubule attachment r_a is directly proportional to the number of unoccupied sites at the kinetochore. Therefore,

$$r_a = K_a(S - n) \text{ (Eq. 8),}$$

where K_a is the rate of kinetochore-microtubule attachment if there was only one single unoccupied site at the kinetochore and we assume that it remains constant throughout mitosis.

From equations 7 and 8,

$$K_a(S - n) = r_d = n\frac{\ln(2)}{t^{1/2}} \text{ (Eq. 9)}$$

Therefore, from equations 5 and 9, the relationship between n and $t^{1/2}$ is governed by the following equation:

$$n = \frac{K_a t^{1/2} S}{K_a t^{1/2} + \ln(2)} = S\left(1 - \frac{\ln(2)}{K_a t^{1/2} + \ln(2)}\right) \text{ (Eq. 10)}$$

From previous data, if S = 35 (ref. 4), and during metaphase $t^{1/2}$ = 444 s. (ref. 2), and n = 24.3 (ref. 4), therefore

$K_a \approx 3.53 \times 10^{-3}\ s^{-1}$

SUPPLEMENTARY REFERENCES

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4. McEwen, B.F., Heagle, A.B., Cassels, G.O., Buttle, K.F., and Rieder, C.L. (1997). Kinetochore fiber maturation in PtK1 cells and its implications for the mechanisms of chromosome congression and anaphase onset. The Journal of cell biology *137*, 1567-1580.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Cancer cell lines have a functional SAC. Mitotic indices in cell lines that were untreated (blue bars) or treated for 16 hours in the presence of nocodazole. Bars represent mean ± s.e.m., n > 500 cells, 5 experiments.

Supplementary Figure 2. Kinetochore-microtubule dynamics. (A) Microtubule-release rates, r_d (s^{-1}), from kinetochores as a function of kinetochore-microtubule half-life (min.). r_d-values from several cancer cell lines at metaphase are denoted as empty circles. Prometaphase values have been omitted for clarity. (B) The mean lifetime, τ (min.), of an individual microtubule attachment at the kinetochore as a function of kinetochore-microtubule half-life (min.). τ-values from several cancer cell lines at metaphase are denoted as empty circles.

Supplementary Figure 3. RNAi depletion. (A) Total cell lysates from untreated and MCAK-depleted cells were immunoblotted with antibodies specific for MCAK, tubulin and actin as indicated. (B) Total cell lysates from untreated or APC-depleted RPE-1 cells were immunoblotted with antibodies specific for APC and actin as indicated.