Supplemental Data for:
Hunter et al., Molecular Cell *11*, pp. 445–457

In order to estimate the number of MCAK binding sites per MT, the affinity of MCAK for these sites, and the speed with which the sites are filled up, we considered two models. In both models, the following assumptions were made: (1) the kinetics are the same at both ends; (2) there is one MCAK per binding site; (3) no reassembly of MTs occurs; and (4) the binding sites are independent, though this condition is relaxed at the end. In anticipation of the results, we are thinking that the binding sites correspond to the ends of the protofilaments.

Two Models

Processive Model
MCAK (M) binds to a binding site (P) at the end of a MT (Mt) (with a second order association rate constant k_{on}) and forms a depolymerizing enzyme complex (MP) which removes tubulin dimers (Tb) from the end of the MT with a rate constant equal to k'_2 while staying at the end. At some lower rate constant, k_{-1}, MCAK dissociates from the binding site

$$\mathrm{M} + \mathrm{P} \underset{k_{-1}}{\overset{k_{on}}{\leftrightarrow}} \mathrm{MP} \xrightarrow{k'_2} \mathrm{MP} + \mathrm{Tb} \qquad \frac{d[\mathrm{MP}]}{dt} = k_{on}[\mathrm{M}][\mathrm{P}] - k_{off}[\mathrm{MP}] \qquad \text{(S1)}$$

where $k_{off} = k_{-1}$.

Nonprocessive Model
MCAK binds to the end (with on-rate k_{on}), and dissociates from the protofilament end with the terminal tubulin dimer with an off-rate k''_2

$$\mathrm{M} + \mathrm{P} \underset{k_{-1}}{\overset{k_{on}}{\leftrightarrow}} \mathrm{MP} \xrightarrow{k''_2} \mathrm{M} + \mathrm{P} + \mathrm{Tb} \qquad \frac{d[\mathrm{MP}]}{dt} = k_{on}[\mathrm{M}][\mathrm{P}] - k_{off}[\mathrm{MP}] \qquad \text{(S2)}$$

where $k_{off} = k_{-1} + k''_2$. Note that the difference between the models is purely in the k_{off} term: in the nonprocessive model there are two mechanisms by which MCAK can dissociate from the MT end, whereas in the processive model dissociation with the tubulin dimer is assumed not to occur.

Solution

In both cases, the rate of loss of polymer is equal to $k_{cat}[\mathrm{MP}]$ where k_{cat} is equal to k'_2 and k''_2 for the processive and nonprocessive models, respectively (or $k'_2 + k''_2$ if there are features of both models). The amount of polymer as a function of time is therefore given by

$$[\mathrm{Poly}](t) = P_0 n_0 - k_{cat}\int_0^t [\mathrm{MP}](s)ds = [\mathrm{Tb}]_0 - k_{cat}\int_0^t [\mathrm{MP}](s)ds \qquad \text{(S3)}$$

where P_0 is the total concentration of binding sites (= protofilament ends), $[\mathrm{Tb}]_0$ is the total tubulin concentration (= initial polymerized tubulin because all the tubulin is in MTs at the start of the experiments), and n_0 is the initial length (in tubulin dimers) of half a protofilament. Thus $P_0 = [\mathrm{Tb}]_0/n_0$. Note that $n_0 = L_0/(2 \times 8\ \mathrm{nm})$, where L_0 is the initial length of the MT; because the typical MTs were 2 μm long, the typical value for n_0 is 125.

Steady-State Solution

Let M_0 be the total concentration of MCAK dimers (free and bound). Then M_0 = [M] + [MP]. Note that P_0 = [P] + [MP]. Suppose that P_0 is fixed, and let $M_{1/2}$ be the MCAK concentration required for half-maximal activity. The half-maximal activity occurs when half the sites are filled; that is when [P] = [MP] = $^1/_2P_0$. This occurs when [M] = K (Equation 1). Thus, $M_{1/2}$ = [M] + [MP] = $K_d + {}^1/_2P_0$. Equation 2 then follows.

Transient Solution

Using P_0 = [P] + [MP] and M_0 = [M] + [MP], where M_0 is the total concentration of MCAK dimers (free and bound), the differential equations associated with both models (Equations S1 and S2) have the form

$$\frac{d[\mathrm{MP}]}{dt} = k_1([\mathrm{MP}]-\alpha)([\mathrm{MP}]-\beta) \qquad \text{(S4)}$$

(Howard, 2001). The parameters are

$$\alpha = \tfrac{1}{2}\left\{\gamma - \sqrt{\gamma^2 - 4M_0P_0}\right\} \qquad \beta = \tfrac{1}{2}\left\{\gamma + \sqrt{\gamma^2 - 4M_0P_0}\right\} \qquad \gamma = K_M + M_0 + P_0$$

with $K_M = k_{off}/k_{on}$ (= K in Equation 1 in the text). The solution is

$$[\mathrm{MP}](t) = \alpha\frac{1-e^{-k_{on}(\beta-\alpha)t}}{1-(\alpha/\beta)e^{-k_{on}(\beta-\alpha)t}} \qquad (\to \alpha \text{ as } t \to \infty) \qquad \text{(S5)}$$

The delay, Δt, before the polymer concentration begins to fall appreciably is mainly determined by the time constant in the numerator of Equation S5: $\Delta t \cong [k_{on}(\beta - \alpha)]^{-1}$. Thus we can estimate k_{on} from

$$k_{on} \cong \frac{1}{\Delta t(\beta-\alpha)} = \frac{1}{\Delta t\sqrt{(K_M + M_0 + P_0)^2 - 4M_0P_0}} \cong \frac{1}{\Delta t|M_0 - P_0|}$$

($M_0 \neq P_0$, $K_M << M_0, P_0$) where the terms on the right-hand side can be estimated from the experimental data.

Cooperativity

If the binding of MCAK to protofilament ends is positively cooperative—i.e., the binding of one MCAK to one site proceeds more readily if there is MCAK bound at other sites—or if the activity of MCAK is positively cooperative, then there will be relatively fewer active MCAKs at a given total MCAK concentration (M_0) (compared to the noncooperative case) and the activity will rise sigmoidally with the MCAK concentration. The exact dependence of the depolymerase activity on M_0 will depend on the details of the interaction between the various sites; in general, the solutions are quite complex and to some extent arbitrary because the data do not have enough resolution to distinguish between the various models of cooperativity. For this reason, we model cooperativity by multiplying [MP] in Equation S5 by $(\alpha/P_0)^{n-1}$ (where n is analogous to the Hill coefficient). This approximation has the sigmoidal concentration dependence characteristic of cooperativity, and has the same limit as the noncooperative model when $M_0 >> P_0$.