Supplementary Table 2:

Equations governing the dynamics of the MAP kinase cascade

We assume mass conservation for each kinase, i.e. the sum of the non-phosphorylated, the phosphorylated and the dually phosphorylated form, during the relevant period. ATP is considered to be in excess and its concentration is included in the rate constants of the kinase reactions.

$$\frac{d}{dt}Ssk2 = -v_1^{MAP} + v_{-1}^{MAP} - Ssk2 \cdot V_{ratio} \tag{2.1}$$

$$\frac{d}{dt}Ssk2P = v_1^{MAP} - v_{-1}^{MAP} - Ssk2P \cdot V_{ratio} \tag{2.2}$$

$$\frac{d}{dt}Pbs2 = -v_2^{MAP} + v_{-2}^{MAP} - Pbs2 \cdot V_{ratio} \tag{2.3}$$

$$\frac{d}{dt}Pbs2P = v_2^{MAP} - v_{-2}^{MAP} - v_3^{MAP} + v_{-3}^{MAP} - Pbs2P \cdot V_{ratio} \tag{2.4}$$

$$\frac{d}{dt}Pbs2P_2 = v_3^{MAP} - v_{-3}^{MAP} - Pbs2P_2 \cdot V_{ratio} \tag{2.5}$$

$$\frac{d}{dt}Hog1 = -v_4^{MAP} + v_{-4}^{MAP} - v_{trans2}^{Hog1} + v_{trans1}^{Hog1} \cdot \frac{V_{nuc}}{V_{cyt}} - Hog1 \cdot V_{ratio} \tag{2.6}$$

$$\frac{d}{dt}Hog1P = v_4^{MAP} - v_{-4}^{MAP} - v_5^{MAP} + v_{-5}^{MAP} - Hog1P \cdot V_{ratio} \tag{2.7}$$

$$\frac{d}{dt}Hog1P_{2cyt} = v_5^{MAP} - v_{-5}^{MAP} - v_{trans}^{Hog1P_2} - Hog1P_{2cyt} \cdot V_{ratio} \tag{2.8}$$

$$\frac{d}{dt}Hog1P_{2nuc} = v_{trans}^{Hog1P_2} \cdot \frac{V_{cyt}}{V_{nuc}} - v_{dephos}^{Hog} \tag{2.9}$$

$$\frac{d}{dt}Hog1_{nuc} = v_{trans2}^{Hog1} \cdot \frac{V_{cyt}}{V_{nuc}} - v_{trans1}^{Hog1} + v_{dephos}^{Hog} \tag{2.10}$$

Rate equations

$$v_1^{MAP} = k_1^{MAP} \cdot Ssk2 \cdot Ssk1 \tag{2.11}$$

$$v_{-1}^{MAP} = k_{-1}^{MAP} \cdot Ssk2P \tag{2.12}$$

$$v_2^{MAP} = k_2^{MAP} \cdot Pbs2 \cdot Ssk2P \tag{2.13}$$

$$v_{-2}^{MAP} = k_{-2}^{MAP} \cdot Pbs2P \tag{2.14}$$

$$v_{3}^{MAP} = k_{3}^{MAP} \cdot Pbs2P \cdot Ssk2P \tag{2.15}$$

$$v_{-3}^{MAP} = k_{-3}^{MAP} \cdot Pbs2P_2 \tag{2.16}$$

$$v_{4}^{MAP} = k_{4}^{MAP} \cdot Hog1 \cdot Pbs2P_2 \tag{2.17}$$

$$v_{-4}^{MAP} = k_{-4}^{MAP} \cdot Hog1P \tag{2.18}$$

$$v_{5}^{MAP} = k_{5}^{MAP} \cdot Hog1P \cdot Pbs2P_2 \tag{2.19}$$

$$v_{-5}^{MAP} = k_{-5}^{MAP} \cdot Hog1P_{2cyt} \tag{2.20}$$

$$v_{trans1}^{Hog1} = k_{trans1}^{Hog1} \cdot Hog1_{nuc} \tag{2.21}$$

$$v_{trans2}^{Hog1} = k_{trans2}^{Hog1} \cdot Hog1 \tag{2.22}$$

$$v_{trans}^{Hog1P_2} = k_{trans}^{Hog1P_2} \cdot Hog1P_{2cyt} \tag{2.23}$$

$$v_{dephos}^{Hog1} = \frac{Protein_2}{Protein_2^0} k_{dephos}^{Hog1} \cdot Hog1P_{2nuc} \tag{2.24}$$

$$Hog1P_2 = Hog1P_{2cyt} \cdot \frac{V_{cyt}}{V_{total}} + Hog1P_{2nuc} \cdot \frac{V_{nuc}}{V_{total}} \tag{2.25}$$

Conservation relations

$$Ssk2_{tot} = Ssk2^0 + Ssk2P^0 \tag{2.26}$$

$$Pbs2_{tot} = Pbs2^0 + Pbs2P^0 + Pbs2P_2^0 \tag{2.27}$$

$$Hog1_{tot} = \left(Hog1^0 + Hog1P^0 + Hog1P_{2cyt}^0\right) \cdot \frac{V_{cyt}}{V_{total}} + \left(Hog1P_{2nuc}^0 + Hog1_{nuc}^0\right) \cdot \frac{V_{nuc}}{V_{total}} \tag{2.28}$$

Parameters

$k_i^{MAP} = 1.538\,(\mu\mathrm{M} \cdot \mathrm{s})^{-1}$ $\qquad$ $k_{-i}^{MAP} = 0.011\,\mathrm{s}^{-1}$ $\qquad$ for $i = 1,\ldots,5$

$k_{trans}^{Hog1P_2} = 0.029\,\mathrm{s}^{-1}$ $\qquad$ $Ssk2_{tot} = 0.0067\,\mu\mathrm{M}$ (274 molecules per cell)[1]

$k_{dephos}^{Hog1} = 0.0053\,\mathrm{s}^{-1}$

$k_{trans1}^{Hog1} = 0.110\,\mathrm{s}^{-1}$

$k_{trans2}^{Hog1} = 0.091\,\mathrm{s}^{-1}$

$Pbs2_{tot} = 0.053\,\mu\mathrm{M}$ (2160 molecules per cell)[1]

$Hog1_{tot} = 0.167\,\mu\mathrm{M}$ (6780 molecules per cell)[1]

Initial concentration values

$Ssk2^0 = 5.306 \cdot 10^{-3}\,\mu M$

$Pbs2^0 = 42.9 \cdot 10^{-3}\,\mu M$

$Pbs2P^0 = 8.38 \cdot 10^{-3}\,\mu M$

$Hog1^0 = 79 \cdot 10^{-3}\,\mu M$

$Hog1P^0 = 15.5 \cdot 10^{-3}\,\mu M$

$Hog1P_{2cyt}^0 = 0.9 \cdot 10^{-3}\,\mu M$

$Hog1P_{2nuc}^0 = 0.03\,\mu M$

$Hog1_{nuc}^0 = 0.5\,\mu M$