A Mathematical Model of the Regulation of AMPK Activation

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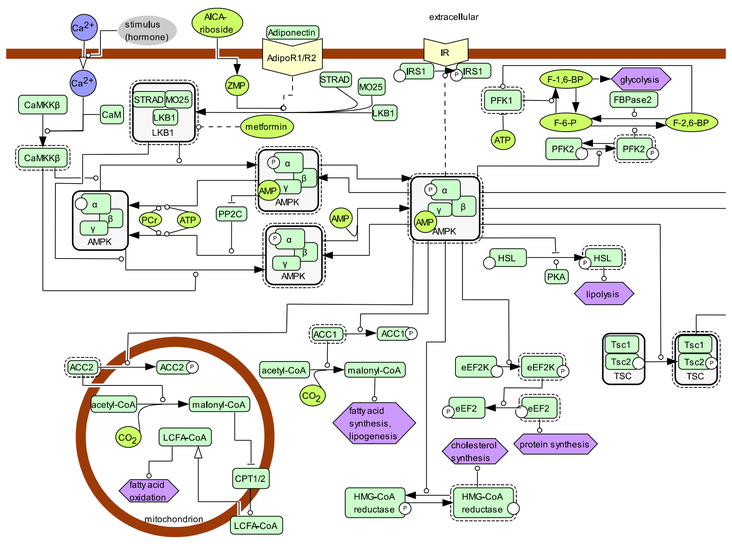
Background: AMPK

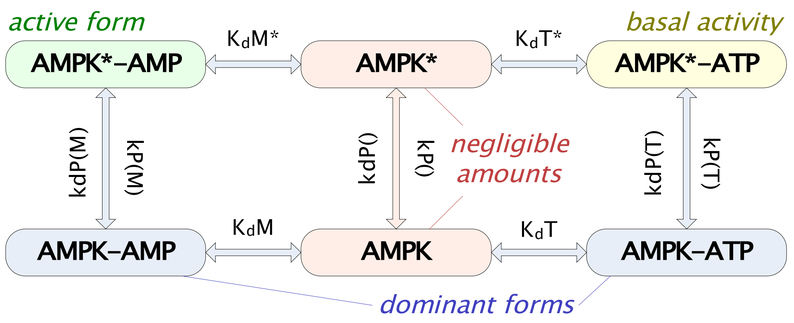
- AMP-activated protein kinase (AMPK): regulation of cell's energy metabolism
  - Role in diabetes & obesity
- Structure: 3 subunits, several isoforms
  - 1 catalytic (α1/2), 2 regulatory (β1/2,γ1-γ3)
  - Homolog to yeast SNF1-complex, also in many other species (e.g. SnRK1 in plants)



- Regulates (if active; Hardie 2007)
  - Glucose uptake, glycolysis, fatty acid oxidation, mitochondrial biogenesis ↗
  - Fatty acid, glycogen, protein synthesis ↘
- Regulated by (Carling 2004, Xiao *et al.* 2007)
  - LKB1 (main upstream kinase), CaMKKβ
  - Binding of AMP and ATP

AMPK Regulation in Detail

- 3 binding sites for AMP
  - 1 non-exchangeable, 2 also bind ATP
- AMPK* with bound AMP 2-5 times as active as AMPK* with bound ATP
- Doubling of [AMP] => doubling of AMPK activity (A_{AMPK^*})



- Thought model (Xiao *et al.* 2007):
  - observed activity ratio (high/low AMP)

$$ac = \frac{A_{AMPK^*\cdot ATP}[AMPK^* - ATP] + 2A_{AMPK^*\cdot AMP}[AMPK^* - AMP]}{A_{AMPK^*\cdot ATP}[AMPK^* - ATP] + A_{AMPK^*\cdot AMP}[AMPK^* - AMP]}$$

$$r := \frac{AMPK^* - AMP}{AMPK^* - ATP}, A_{AMPK^*\cdot ATP} = \frac{1}{3} A_{AMPK^*\cdot AMP}$$

$$ac = \frac{1/3 + 2r}{1/3 + r} \approx 2$$ … only if r is large ($\gg 1$)

Mathematical Model

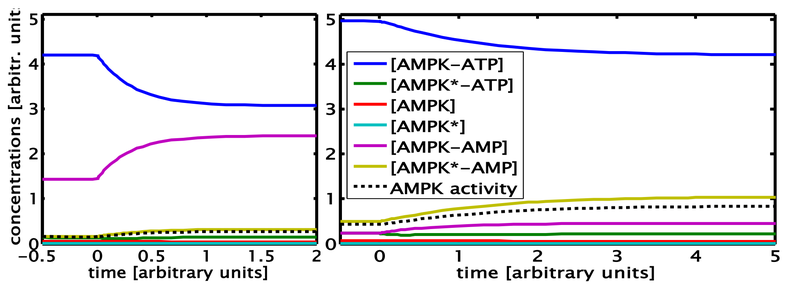
- Mass-action model
  - only 1 parameter per reaction, as opposed to Michaelis-Menten or full enzyme kinetics

$$\frac{d[AMPK - ATP]}{dt} = kdP(T) \times [AMPK^* - ATP] - kP(T) \times [AMPK - ATP] + K_dT_{-1} \times [AMPK - ATP] - K_dT_{+1} \times [AMPK] \times [ATP]$$

$$\vdots$$

$$\frac{d[AMPK^* - AMP]}{dt} = kP(M) \times [AMPK - AMP] - kdP(M) \times [AMPK^* - AMP] + K_dM_{+1} \times [AMPK] \times [AMP] - K_dM_{-1} \times [AMPK^* - AMP]$$

- No experimental data
  - Only in-vitro values for K_dT and K_dM
  - Unknown actual concentrations
    - [ATP] > [AMP] > [AMPK] (all forms together)
  - [AMPK]+[AMPK*] probably very low
- System underdetermined
  - "Reality-consistent" behaviour achieved with many different parameter sets



Exploring the Parameter Space

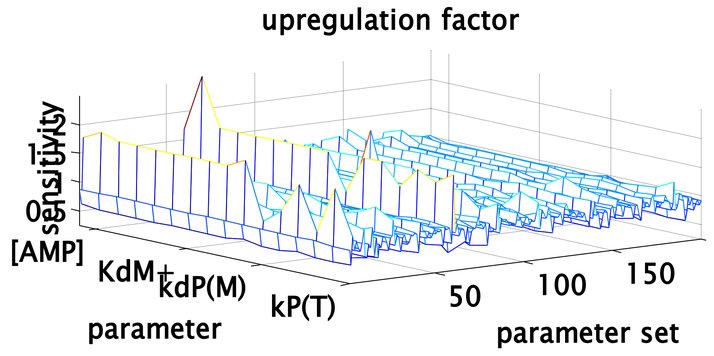
- Hypothesis: evolutionary advantage by optimising energy-regulating system for
  - fast response to signal (changes in [AMP])
  - high upregulation of total AMPK activity
  - low persistent effort to keep some AMPK phosphorylated (otherwise: waste of energy)
- No fitting to experimental data possible
- Instead: fit to presumed system behaviour

1. Choose system parameters (randomly) and determine steady state
2. Double [AMP], find new steady state and compare to old one with respect to
   - Upregulation factor
   - Response time
   - Phosphorylation turnover at steady state
3. Generate optimised parameter sets
   - Genetic algorithm with variable weights of the criteria, emphasis on upregulation

- Parameter reduction (now: 14)
  - same dis-/association rates (10 parameters)
  - same (de-)phosphorylation rates (11)
    - $kdP(M)$ left independent
  - both (7 parameters)

Analyses and Results

- 1000 optimised parameter sets analysed
- Optimisation aspects (upreg., response time, phosphorylations) independent
  - not correlated, but not conflicting either
- Sensitivity analysis & correlation coeff.
  - No aspect sensitive to any single parameter



  - Many robust parameter sets, some less so
  - No significant correlation between any pair of parameters, no pairwise sensitivities
- Principal component analysis (full model)
  - First component comprises many param.s
  - 13th component consists mostly of $K_dM_{-1}^*$
  - Last component consists mostly of $kdP(M)$
- Unsystematic parameter reduction

  i.e. run new optimisations with same settings
  - Models exhibit worse behaviour, e.g. max. upregulation with 14/11/10/7 parameters: 2.0/1.8/1.6/1.3, respectively (rounded)
- Systematic parameter reduction

  i.e. assign either value or mean from original optimised parameter sets to new parameter covering several old ones
  - Models fit expected behaviour better, e.g. max. upregulation 2.0/1.8/2.4/2.4

Conclusion

- System analysis possible even without detailed knowledge of behaviour
- Model properties:
  - System is robust w.r.t. parameter changes
  - Many local optima in parameter space (optimisation tricky)
- System properties:
  - Upregulation factor ≥2 possible, but only with extreme (i.e. unrealistic) parameters
  - $kdP(M) \approx 0$ in most good parameter sets (i.e. strong evidence for inhibited dephosphorylation of AMPK* when AMP is bound)
- Not all 14 parameters necessary to describe expected system behaviour
  - e.g. no loss of generality when AMP/ATP binding independent of phosphorylations

References

GD Hardie. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nature Reviews Molecular Cell Biology*, 8(10):774-785, October 2007.

D Carling. The AMP-activated protein kinase cascade - a unifying system for energy control. *Trends in Biochemical Sciences*, 29(1):18-24, January 2004.

B Xiao *et al.* Structural basis for AMP binding to mammalian AMP-activated protein kinase. *Nature* 449:496-500, September 2007.

Acknowledgements

The AMPK pathway map (left column, upper part) was created by Simone Frey (University of Rostock). We would like to thank for the permission to use it here.

Funding:
EC FP6 - AMPKIN

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