Modelling trafficking of proteins within the mammalian cell using Bio-PEPA

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The biology

Background

Src: An oncoprotein, implicated in tumour formation and motility of cells; specifically, a non-receptor tyrosine kinase (Src, aSrc).

FGF: A growth factor that is involved in signalling between cells.

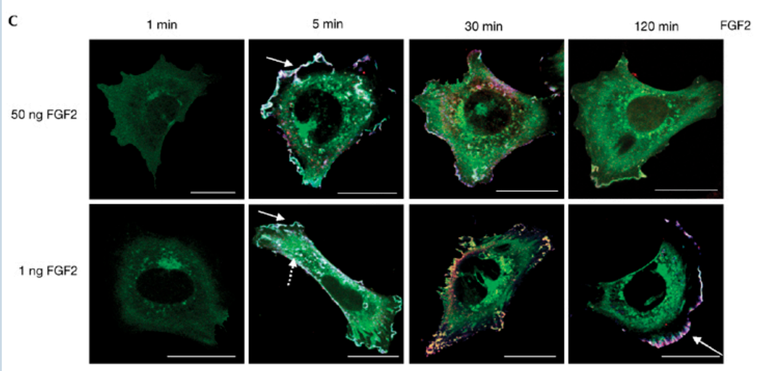
FGF receptor: A protein that sit across the membrane of the cell and to which FGF can bind. It transfers signals outside the cell to signalling pathways within the cell (FGFR).

FGFR complex: A complex consisting of a dimer of FGF and FGFR, together with active Src and other species (FGFRc).

Endosome: A membrane-bound compartment that transports molecules around the cell, moving along microtubules.

Inactive: A protein in a conformation that makes it unable to interact with other molecules. Both phosphorylation and dephosphorylation can cause activation.

Experiment: addition of FGF at high and low concentration



(Sandilands *et al*, EMBO Reports 8, 2007)

Results and hypothesis

- After stimulation with FGF,
  - Src is found in endosomes,
  - Src near the nucleus is inactive,
  - Src at the membrane is active,
  - activation takes place in endosomes, and
  - there is a gradient of inactive to active Src from nucleus to membrane (Sandilands *et al*, 2004).
- Comparing stimulation with FGF at different concentrations,
  - a high concentration leads to high levels of active Src at the membrane for a short period, and
  - a low concentration leads to high levels of active Src at the membrane for a much longer period (Sandilands *et al*, 2007).
- Hypothesis: a short recycling loop that manages active Src at basal level and a long one that traffics FGFR complex and inactive Src.

The model

Bio-PEPA

- Bio-PEPA is a stochastic process algebra for biological modelling.
- It allows multiple forms of analysis, including simulation and ordinary differential equations.
- Species are defined as follows, with reaction names, α_i, stoichiometric coefficients, κ_i, and roles, $\mathrm{op}_i \in \{\downarrow, \uparrow, \oplus, \ominus, \odot\}$.

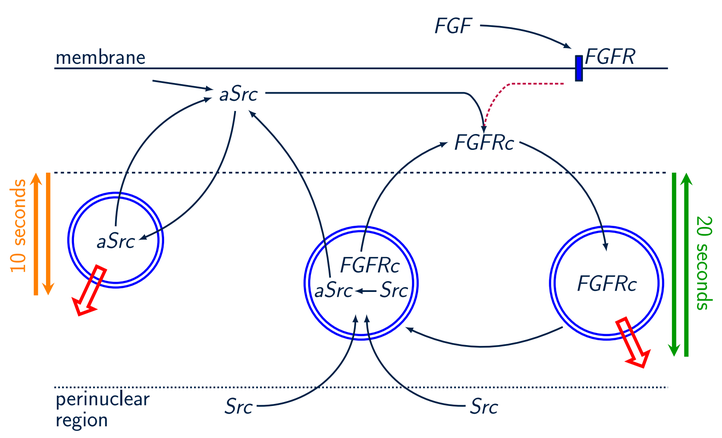
$$S \stackrel{def}{=} (\alpha_1, \kappa_1)\ \mathrm{op}_1\ S\ +\ \ldots\ +\ (\alpha_n, \kappa_n)\ \mathrm{op}_n\ S$$

- Each species has a quantity ℓ_S and they are put together using

$$P \stackrel{def}{=} S_1(\ell_{S_1}) \underset{*}{\bowtie} \ldots \underset{*}{\bowtie} S_m(\ell_{S_m})$$

- Information about cell size, compartments, species, constants and rate definitions are provided by the sets $\mathcal{L}$, $\mathcal{N}$, $\mathcal{K}$, and $\mathcal{F}$.
- Structured operational semantics define the behaviour of each species and how they interact.

Short and long recycling loops



Double blue circles are endosomes and large red arrrows indicate degradation

Techniques

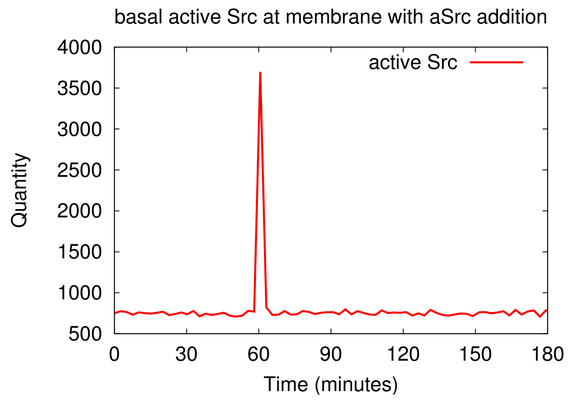
Use of species: Both proteins such as Src and endosomes containing Src are modelled as species, using stoichiometric constants for the decrease in Src and increase in endosomes and *vice versa*.

$$\begin{aligned} aSrc@mb &= \ldots \\ &\quad + (into\_endosome, 150) \downarrow aSrc@mb \\ &\quad + (outof\_endosome, 100) \uparrow aSrc@mb \\ endo@cy &= \ldots \\ &\quad + (into\_endosome, 1) \uparrow endo@cy \\ &\quad + (outof\_endosome, 1) \downarrow endo@cy \end{aligned}$$

Pseudo-diffusion: Different concentrations that surround the cell are described by different amounts of FGF and different speeds of release. High concentrations have more molecules and faster release than low ones. This mimics the diffusion that happens as FGF binds with receptors.

The results

Without and with addition of FGF



(Graphs are the average of 10 simulations)

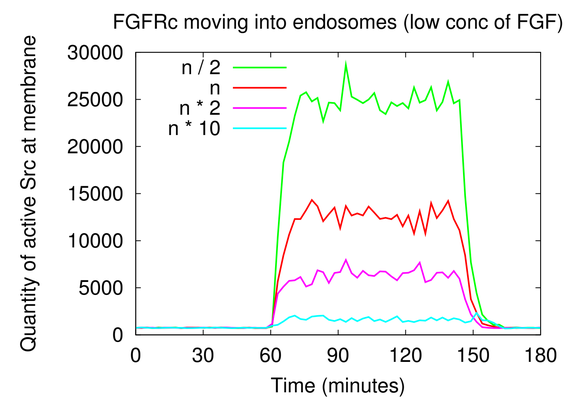
Discussion of results

- The short loop can remove large amounts of additional active Src at the membrane and return to a balanced state (top left graph).
- In the case of high concentration, the return to basal state occurs when there are no more FGFR molecules (bottom left graph).
- In the case of low concentration, the return to basal state occurs when there are no more FGF molecules (bottom left graph).
- The amount and release rate of the FGF, together with quantity of receptor cells determine the maximum amount and persistence of the increase in active Src at the membrane.
- The model is insensitive to differences in the relative speed of the long loop with respect to the short loop.
- Effect of competition for endosomes on active Src after stimulation.
  - Modifying the amount of FGF complex that can go into one endosome, affects the maximum amount of active Src at the membrane, but has much less effect on the persistence of active Src (top right graph).
  - Modifying the rate at which endosomes take up FGF complex, affects the persistence of the increase of active Src at the membrane, as well as the slope and the maximum amount of active Src (bottom right graph).

Parameters

- The data is very limited, hence quasi-quantitative, and relative quantities and parameters become important.
- Cell size and normal endosome movement speed are used to determine time taken for short and long recycling loops.
- Short loop balanced in terms of creation and degradation in basal state.
- Active Src at membrane in basal state, much less than in stimulated state

Competition for endosomes



(Graphs are the average of 10 simulations)

References

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EPSRC Engineering and Physical Sciences Research Council

BBSRC bioscience for the future

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