On differences between experimental and real-life models

- Models that describe behavior of a natural biological system
- Models that include mechanisms or variables artificially induced in a biological experiment

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Modeling of intracellular processes

• Goals:
  – To understand control mechanisms
  – Once the mechanisms are known, to control dynamics of cell populations/tissues
Modeling of intracellular processes

• General procedure:
  – Preliminary biological experiments
  – Model development and parameter estimation
  – Model analysis (properties, robustness/sensitivity, perturbations) – new findings/conclusions
  – Experimental confirmation of the above

• But
  – Different cells respond differently to the same type of stimulus
  – The dynamics may change qualitatively when the same pathway is activated by various stimuli
  – Experiential procedures may in some cases disturb the picture
Problem 1 – choice of time points for measurements

Partial conclusion:
• preliminary modeling part of research may be quite useful
Problem 2 – direct effects of experimental procedures

Transfection experiments
• Consist in introduction of so called reporter plasmids into cells
• Have been shown to heavily influence experimental results
  • no follow-up research results have been published so far (?)
• Nevertheless, are quite widely used in investigation of control mechanisms governing cell responses to external stimuli
miRNA function and mechanism of action

Experimental procedure

1. Determine promoter response element (RE) of interest.

2. Clone RE upstream of the firefly luciferase (luc) gene.

3. Transfect construct into cells.

4. Add luciferase detection reagent and measure firefly luciferase activity.

5. Add Renilla detection reagent and measure Renilla luciferase activity. 
   **Note:** Reduced luminescence in well "A" may be due to cytotoxicity.

The production of Renilla luciferase is independent of experimental modulation of the response element of interest.

Experimental procedure
Motivation

- In many cases experimental research on miRNA is based on plasmid transfection
- It has been shown that transfection experiments (in other areas) may result in misleading conclusions
- Mathematical models of miRNA activity do not take into account the nature of experiments whose results the model output should reflect
- Transfection efficiency is not high – what are the implications?
Experimental procedure

- Plasmids used in our experiments:
  - contain specific Response Elements (Binding Sites – BS), which are the same as in the target gene of interest
  - code for mRNA which is fully complementary to the miRNA of interest
Experimental procedure

- Cells in a population are transfected with variable number of plasmid copies
Questions to be answered

1. What are the direct (unintended) effects of plasmid introduction on intracellular processes?

2. What are the differences among cells, with respect to the number of plasmids that actually entered them?

3. Can we base the analysis on average results (as given, e.g. by RT-PCR), without taking into account heterogeneity of cell populations?

4. Can we draw conclusions about wild type cells behavior basing on experimental results from transfected cells?
Xu Xue, Wang Xi, Hu Wenzhong, A modeled dynamic regulatory network of NF-kappaB and IL-6 mediated by miRNA, 2013
Models to be compared

Models describing the dynamics of a signaling pathway under consideration

• Describing only the variables and processes that are subject of the analysis (labeled „predicted” in subsequent plots)

• With additional terms and variables that correspond to molecules introduced in the transfection experiment and their actions
  • After fitting model parameters to match simulation and experimental data, the plasmid concentration is set to 0 (labeled „real” in subsequent plots)
Computational procedure

• Randomize transfection efficiency
  – Generate random numbers that represent numbers of plasmids that got into each cell
    • High transfection efficiency
    • Low transfection efficiency
    • Variable transfection efficiency

• For each cell simulate its behavior
mRNA levels
What are we looking at?

Data available:

- Protein coded by plasmid (waiting for it)
- Data from experiments without plasmid

The goal is to investigate

- miRNA control actions exerted over NFkB pathway
- changes in specific miRNA concentration measured indirectly with the transfected reporter system
Conclusions

• Caution required when drawing conclusions from plasmid-based experiments

• Models whose dynamics is fitted to results of experiments performed with transfected cells should include additional terms and variables that correspond to molecules introduced in the transfection experiment

• Transfection efficiency influence qualitative system behavior
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