Synthetic Gene Networks That Count

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Review + Motivation

- What do we need for a counting?
  Memory + Mechanism

- Natural memory:
  - DNA sequence.
  - Level of protein concentration. (Temporary, but could be not very short in time)

- There are a lot of stable mechanisms in a cell.

- Steps to biocomputers:
    - Logical operation AND and OR.
    - An idea of digital computations with NOT, AND, NAND and N-IMPLY expression logic in single mammalian cells.

- Why can’t cells count? They can!
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• Why can’t cells count? They can!
Counting mechanism

I’m a cell. I’ve got a memory. Now I remember number 0!
Counting mechanism

Add one, please.
Counting mechanism

Ok.

Good.

1
Counting mechanism

Add one, please.
Counting mechanism

“Memory” : concentration of some protein
Regulation : extrinsic chemical stimulus
Model 1: Riboregulated transcriptional cascade (RTC), two-counter

- The cis-repressor sequence (cr) is complimentary with ribosome binding site (RBS).
- A stem-loop in a secondary structure of RNA prevents binding of ribosome (30S).
- A short noncoding taRNA binds to the cr; the stem-loop can’t form; translation is allowed.
- Only T7 polymerase, which is coded by T7RNAP, can bind to promoter PT7.

- A transcription of taRNA depends on concentration pulses of arabinose.
Model 1: Riboregulated transcriptional cascade (RTC), two-counter
Model 1: Riboregulated transcriptional cascade (RTC), three-counter
Experimental results of RTC counters

Two-counter

Three-counter

Shaded areas represent arabinose pulse

• Experimental results demonstrate that fluorescence increases only when all two (or three) arabinose pulses are delivered.
• Based on this results, Ari E. Friedland at el. constructed and analyzed a mathematical model.
Prediction of the mathematical model

Two-counter

Maximum of the predicted concentration.

Three-counter: searching of optimal combination (interval-length).
Points: the difference between model and experiment.

Three-counter

The absolute difference in fluorescence after three pulses and two
Model 2: Single Invertase Memory Module (SIMM), three-counter

- After the first arabinose pulse only Flpe protein can express.
- Flpe is a site-specific recombinase. (Site FRT)
Model 2: Single Invertase Memory Module (SIMM), three-counter

![Diagram](image_url)
Model 2: Single Invertase Memory Module (SIMM), three-counter

- After the second arabinose pulse only Cre protein can express.
- Cre is a site-specific recombinase. (Site lox)
Model 2: Single Invertase Memory Module (SIMM), three-counter

Counting cascade. Result: GFP expresses after 3 Ara pulses.

The same cascade was built for two-counter
Experimental results and Modelling of SIMM counters

Fluorescence in SIMM three-counter after 3 pulses.

GFP fluorescence ratios between the single-inducer DIC three-counter exposed to three pulses of arabinose (N) versus two pulses of arabinose (N – 1) with varying arabinose pulse lengths and intervals; experimental results are represented by black dots.
Conclusion + Future

• Imitation of computer ticks.
• Temporary effect.
• Unfortunately, cell mechanisms have got mistakes and this counters too.
• There is a dubious future for biocomputers which are based on this counters.
Thank for your attention!