Dynamic modeling of Protein Kinetics

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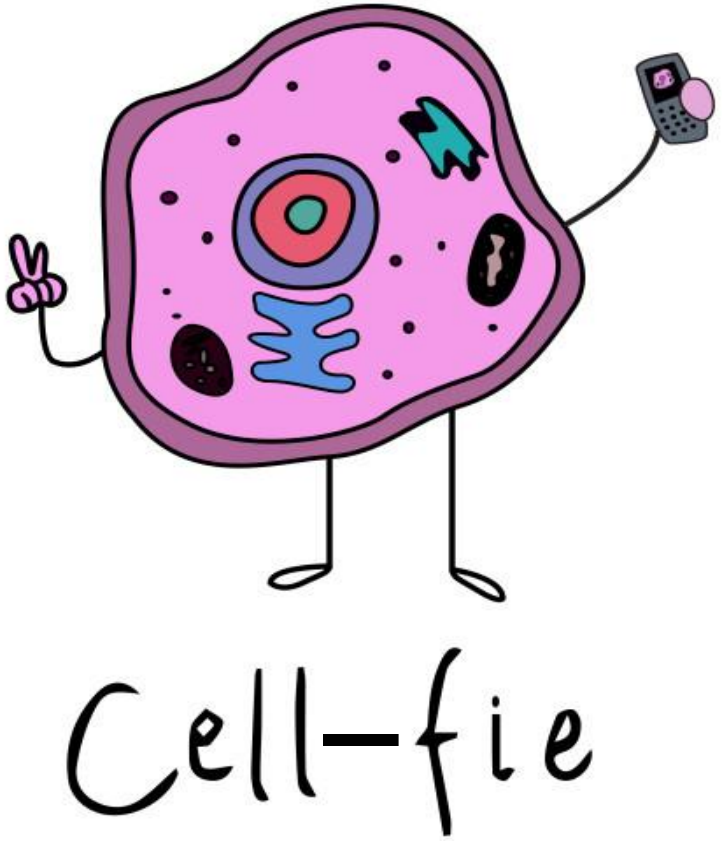
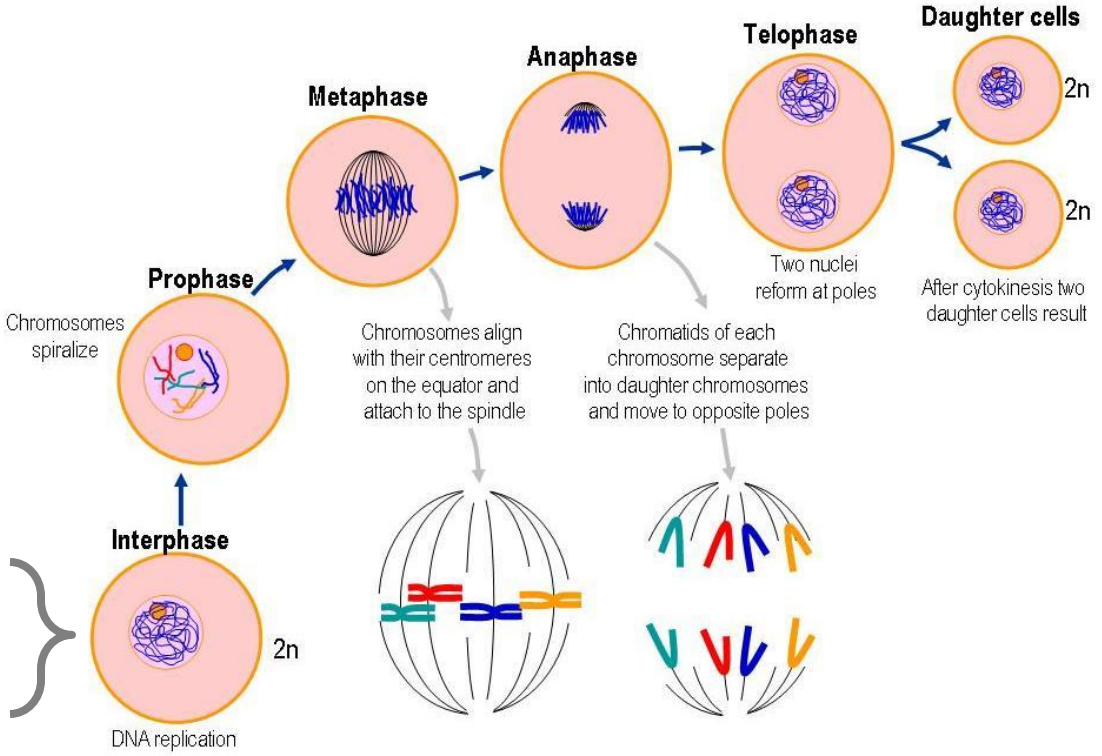
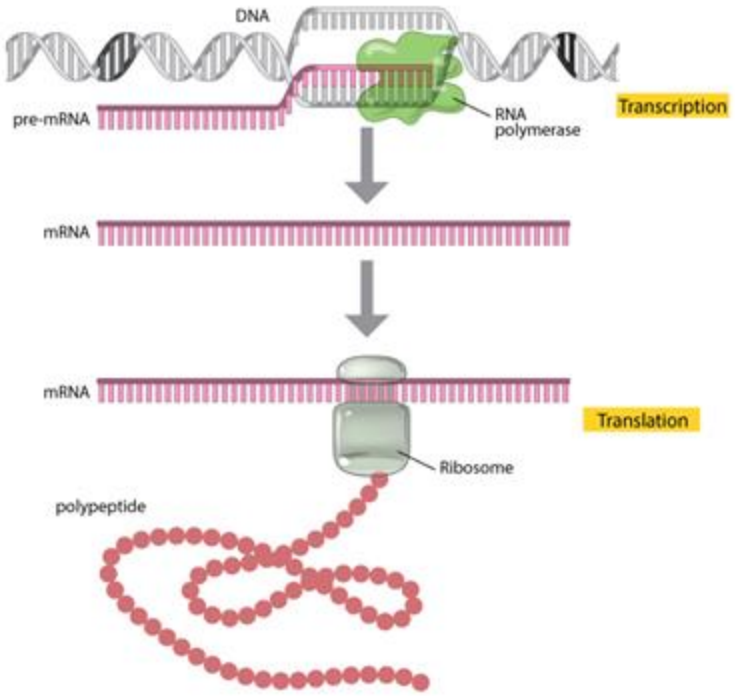
The cell reproduces by making an exact copy of itself



The cell reproduces by undergoing several processes: cell cycle



DNA replication: Quantitative description of protein expression



What determines the kinetics of mRNA and protein expression?

How can we describe heterogeneous gene expression at the single-cell level?

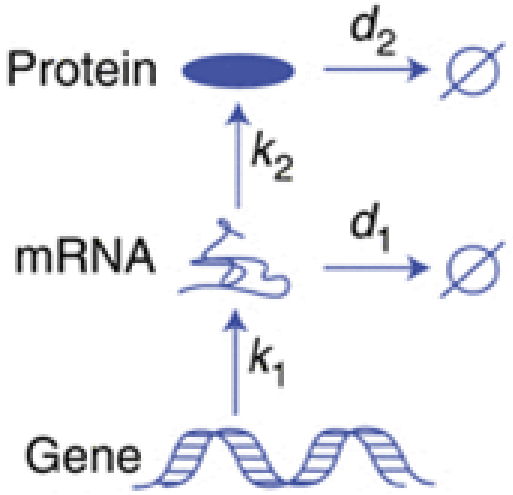
Outline

Deterministic model of gene expression

Stochastic model of gene expression captures cellular heterogeneity

Applications: Deterministic modeling of proteins controlling anaphase

A simple model for transcriptional regulation of protein expression



$$\frac{d(\text{mRNA})}{dt} = k_1 - d_1 \cdot \text{mRNA}$$

$$\frac{d(\text{Protein})}{dt} = k_2 \cdot \text{mRNA} - d_2 \cdot \text{Protein}$$

Assumptions

- Translation proportional to mRNA concentration
- First-order decay of mRNA and protein

mRNA, Protein	Variables/Concentrations
k_i, d_i	Kinetic parameters

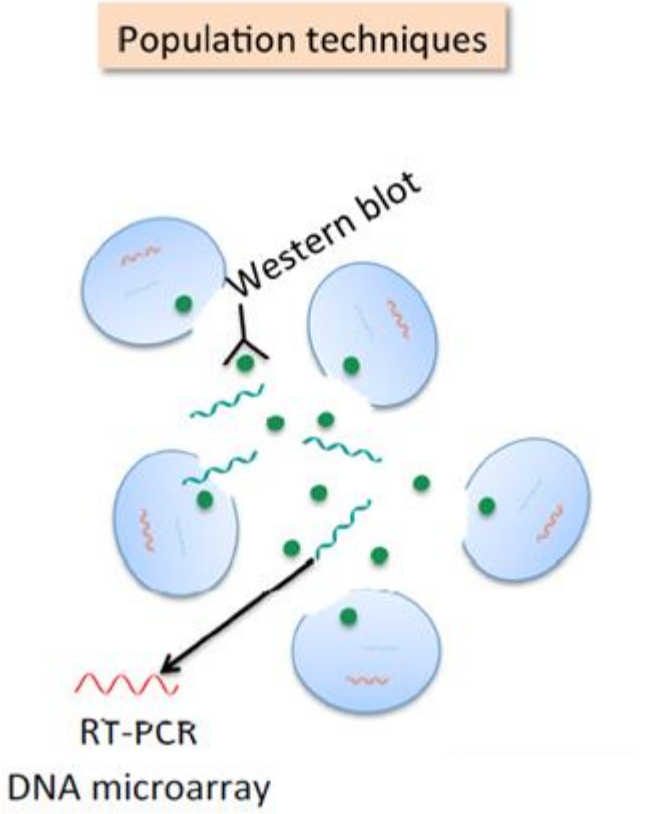
Assumptions underlying ordinary differential equation models

Continous

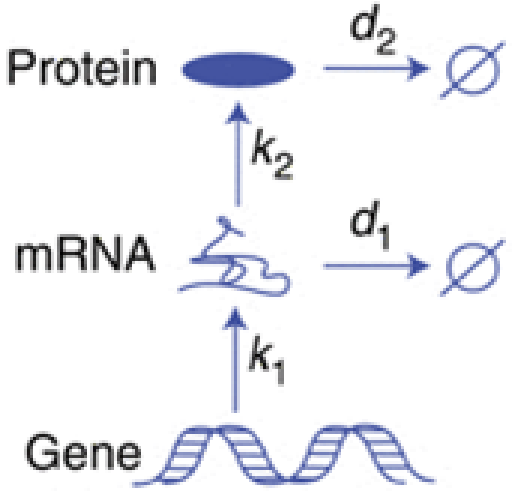
Real concentration
of mRNAs/proteins

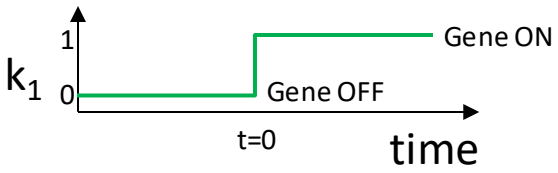
Deterministic

Average behavior of
large molecule numbers



What determines the protein dynamics in response to changes in transcription?





$$\frac{\mathrm{d(mRNA)}}{\mathrm{d}t} = k_1 - d_1 \cdot \mathrm{mRNA}$$

$$\frac{\mathrm{d(Protein)}}{\mathrm{d}t} = k_2 \cdot \mathrm{mRNA} - d_2 \cdot \mathrm{Protein}$$

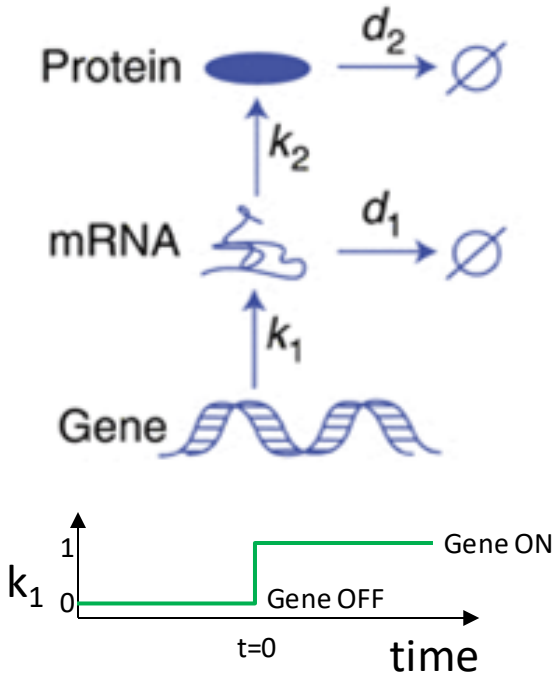
Analytical Solution (by integration)

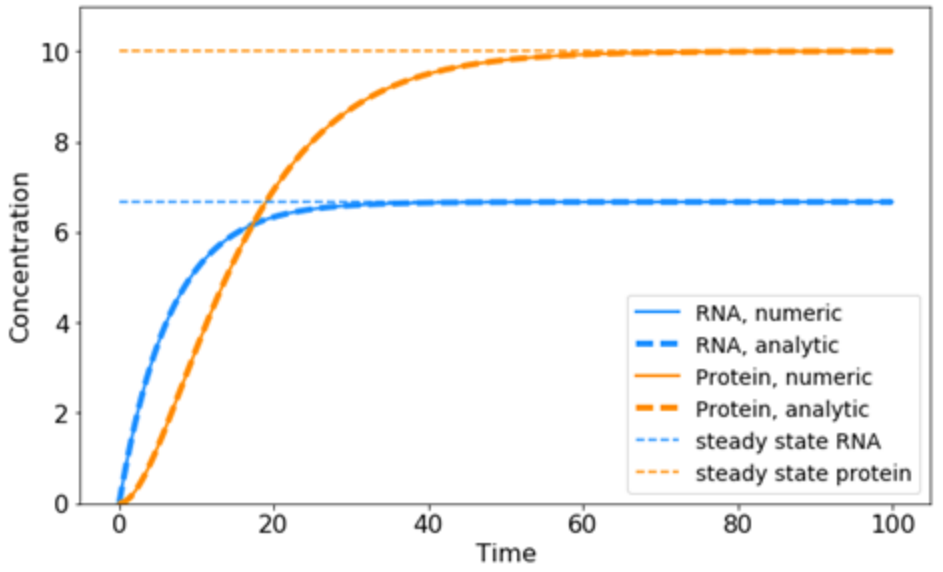
$$mRNA(t) = \overline{mRNA} \cdot (1 - e^{-d_1 t})$$

$$Protein(t) = \overline{Protein} \cdot \left(1 - \frac{d_1 e^{-d_2 t} - d_2 e^{-d_1 t}}{d_1 - d_2}\right)$$

⇒ Approximate solution using numerical integration

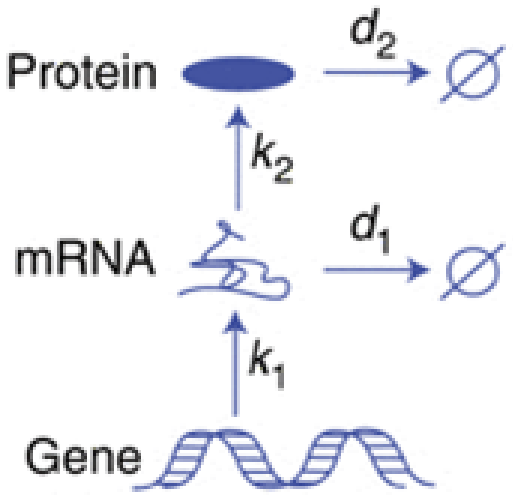
Time course of mRNA and protein in response to gene activation





System asymptotically approaches steady state
Protein dynamics has a "Response time"

What determines protein expression level at steady state?



Steady state

$$k_1 - d_1 \cdot \text{mRNA} = 0$$

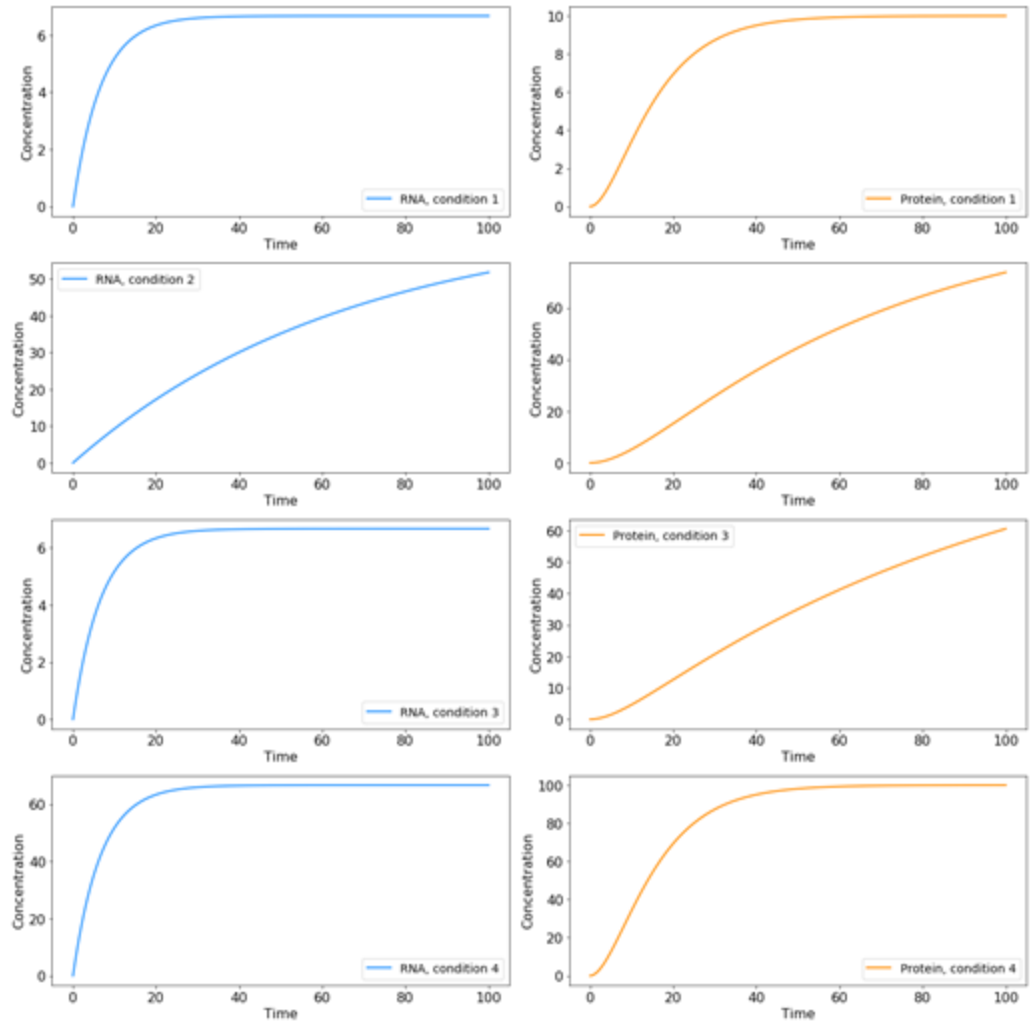
$$k_2 \cdot \text{mRNA} - d_2 \cdot \text{Protein} = 0$$

$$\overline{mRNA} = \frac{k_1}{d_1} \qquad \overline{Protein} = \frac{k_1 k_2}{d_1 d_2}$$

Steady state level set by ratio of synthesis and degradation rates

Transcription induces proportional changes in mRNA and protein levels

Protein dynamics solely determined by mRNA and protein degradation rates



$$Protein(t) = \overline{Protein} \cdot \left(1 - \frac{d_1 e^{-d_2 t} - d_2 e^{-d_1 t}}{d_1 - d_2}\right)$$

mRNA degradation rate

changes final steady state and response time

protein degradation rate

changes final steady state and response time

mRNA and protein synthesis rates

change only final steady state

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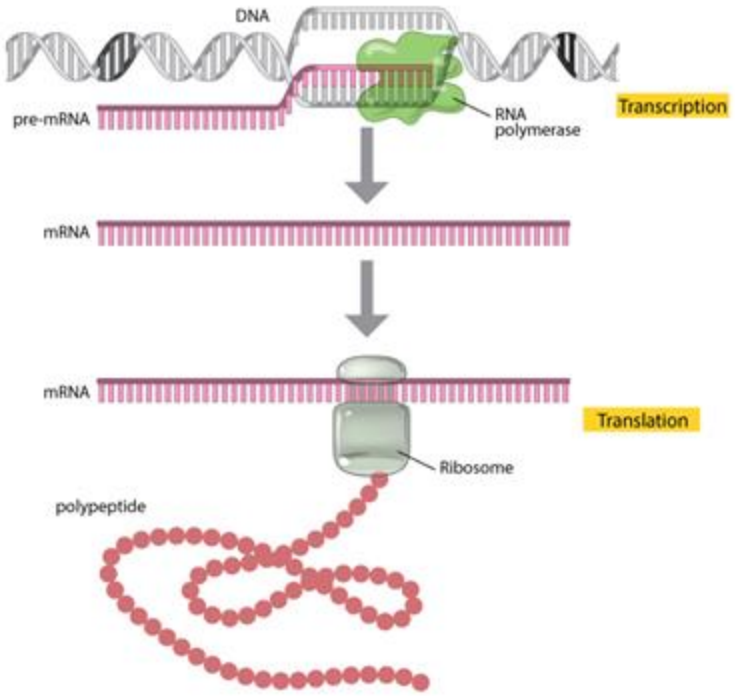

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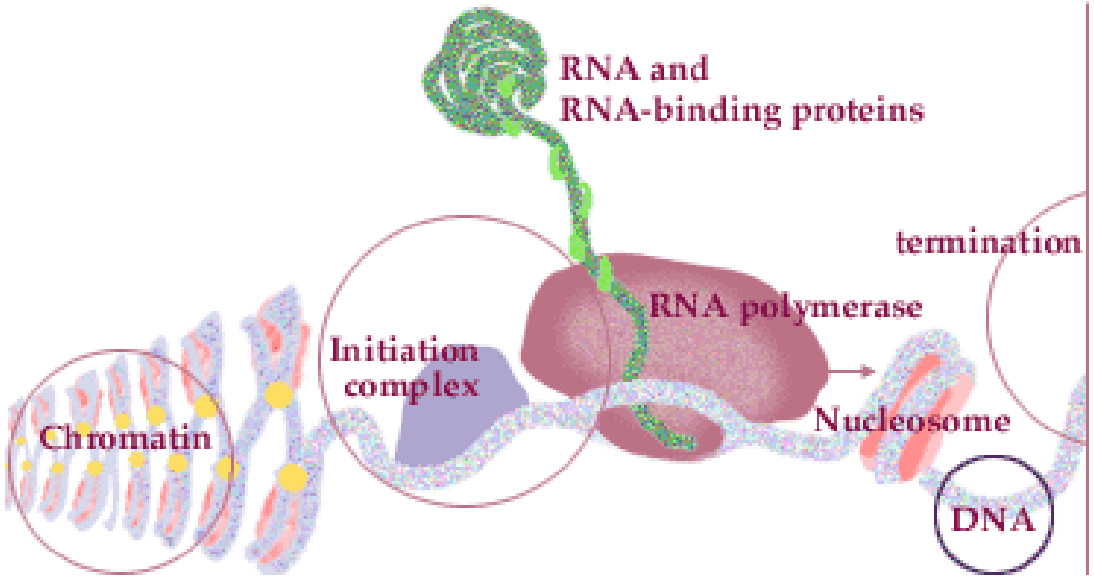
Gene expression – a stochastic process

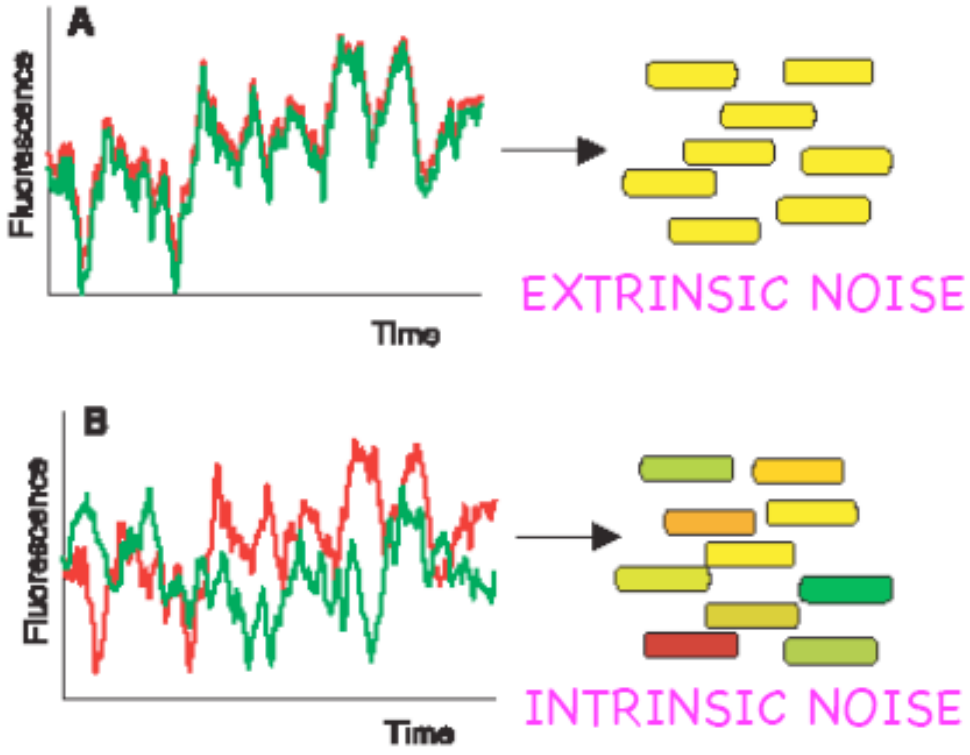


Stochastic dynamics shown for transcription initiation and elongation

Randomness arises from low molecule numbers!

- each cell contains few copies of each gene
- transcription factors often present in low amounts

Intrinsic and extrinsic sources of gene expression variability



Fluctuations in biosynthetic machinery (polymerases, ribosomes)

Eukaryotes

Random binding of transcription machinery to each promoter (low copy numbers!)

Bacteria

Eukaryotes

Elowitz et al., Science 2002

Stochastic models account for event probabilities at low molecule numbers

Deterministic ODE model	vs.	Stochastic model
Average behavior of large molecule numbers		Probabilistic behavior (randomness) at the single-molecule level
Continuous: Concentration of mRNAs/proteins		Discrete: Absolute molecule counts

Stochastic version of simple protein expression model

1. **RNA production:** $RNA \xrightarrow{\beta} RNA + 1$
2. **RNA decay:** $RNA \xrightarrow{\gamma_m \cdot RNA} RNA - 1$
3. **Protein production:** $protein \xrightarrow{k \cdot RNA} protein + 1$
4. **Protein decay:** $protein \xrightarrow{\gamma_p \cdot protein} protein - 1$

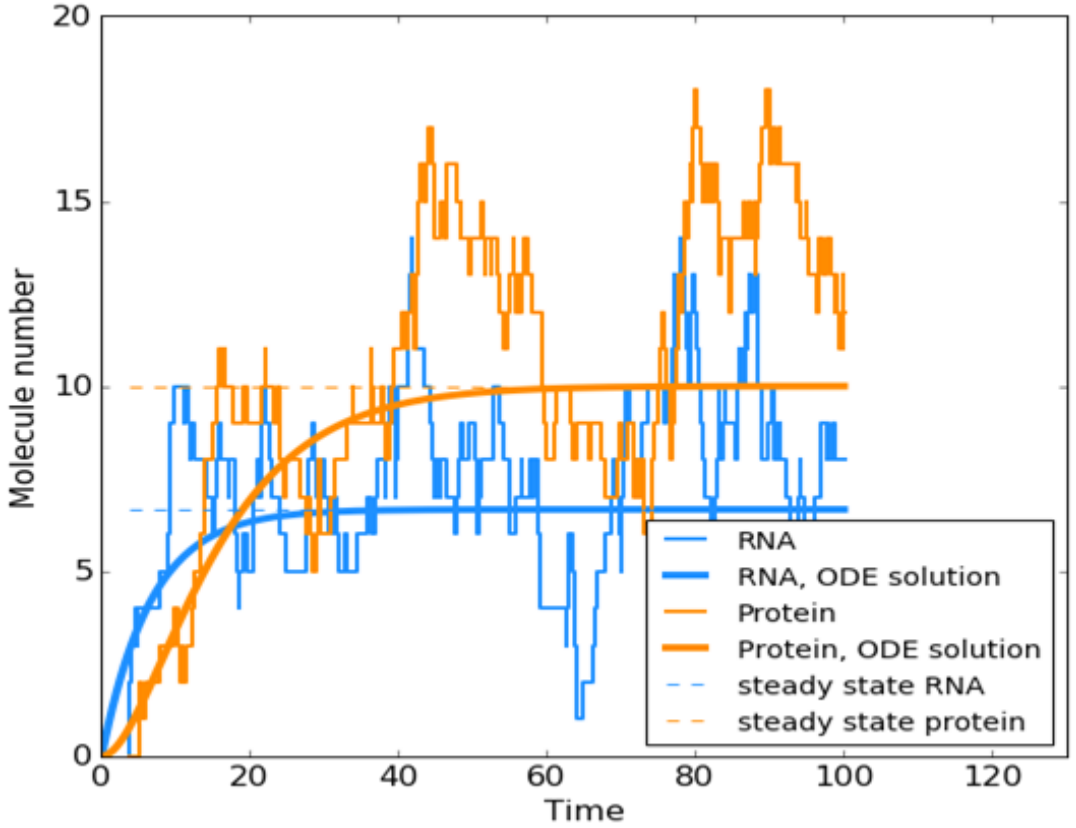
Reactions occur with certain probabilities

mRNA and protein given as *absolute* molecule count (discrete)

Simulation by Gillespie algorithm

- selects most probable next reaction
- updates molecule counts

Simulated temporal evolution of mRNA and protein in a stochastic model



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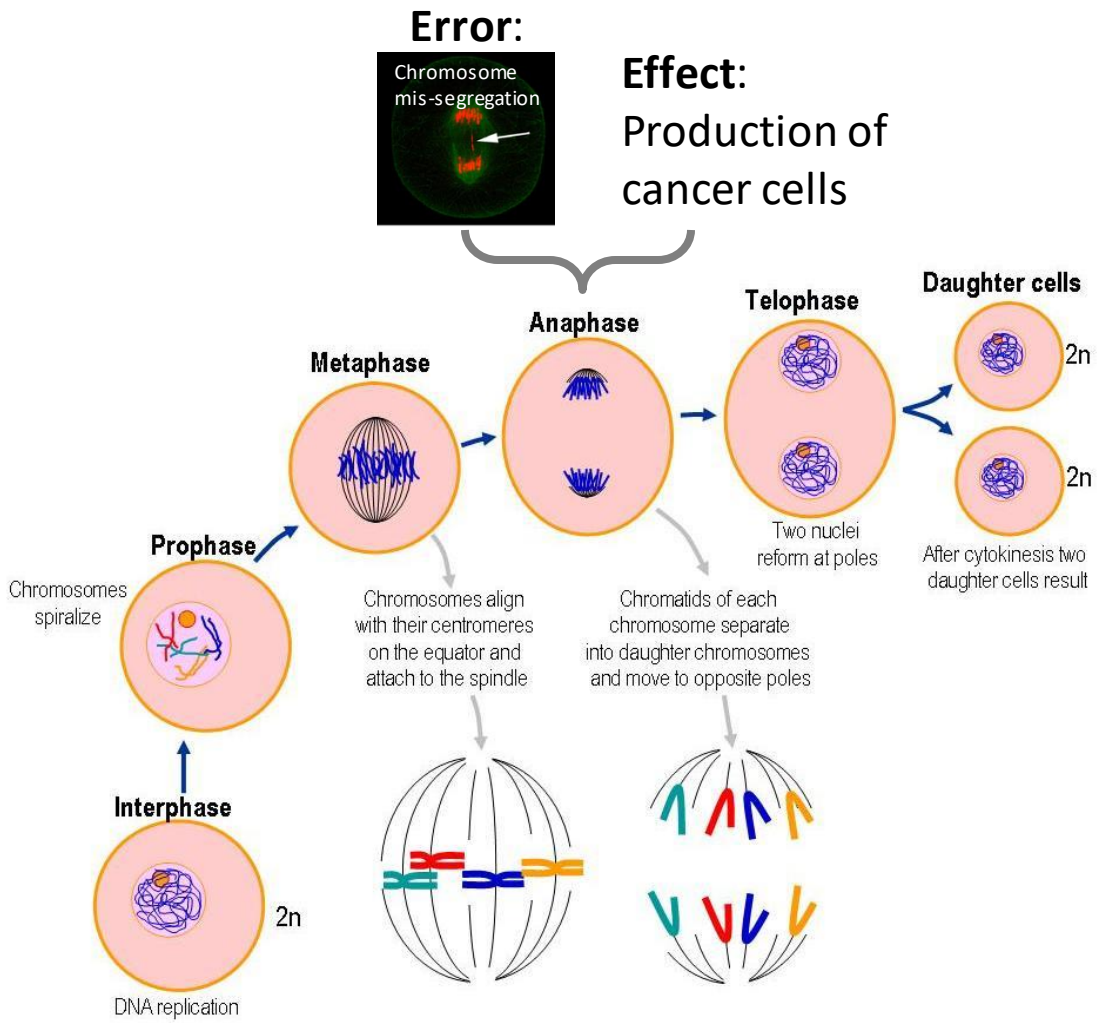
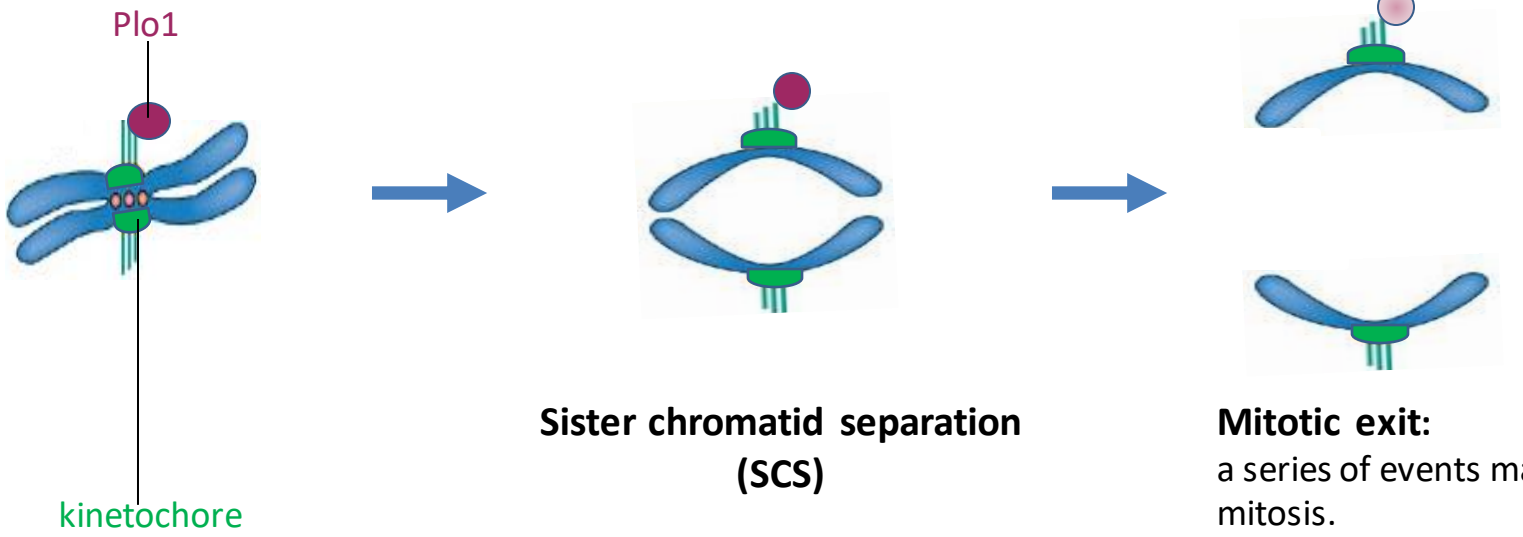
Anaphase is characterized by chromosome segregation



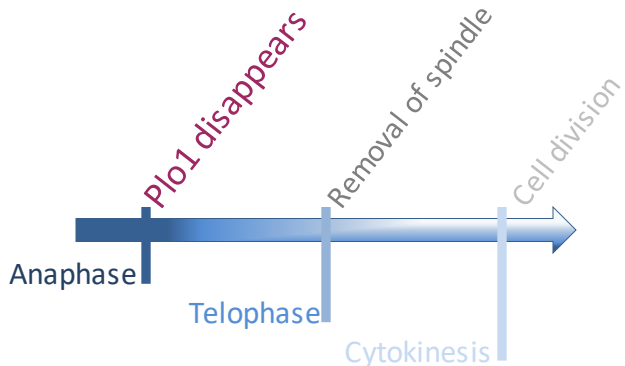
Anaphase events are temporally ordered

Anaphase:
the stage of the cell cycle characterized by chromosome segregation.

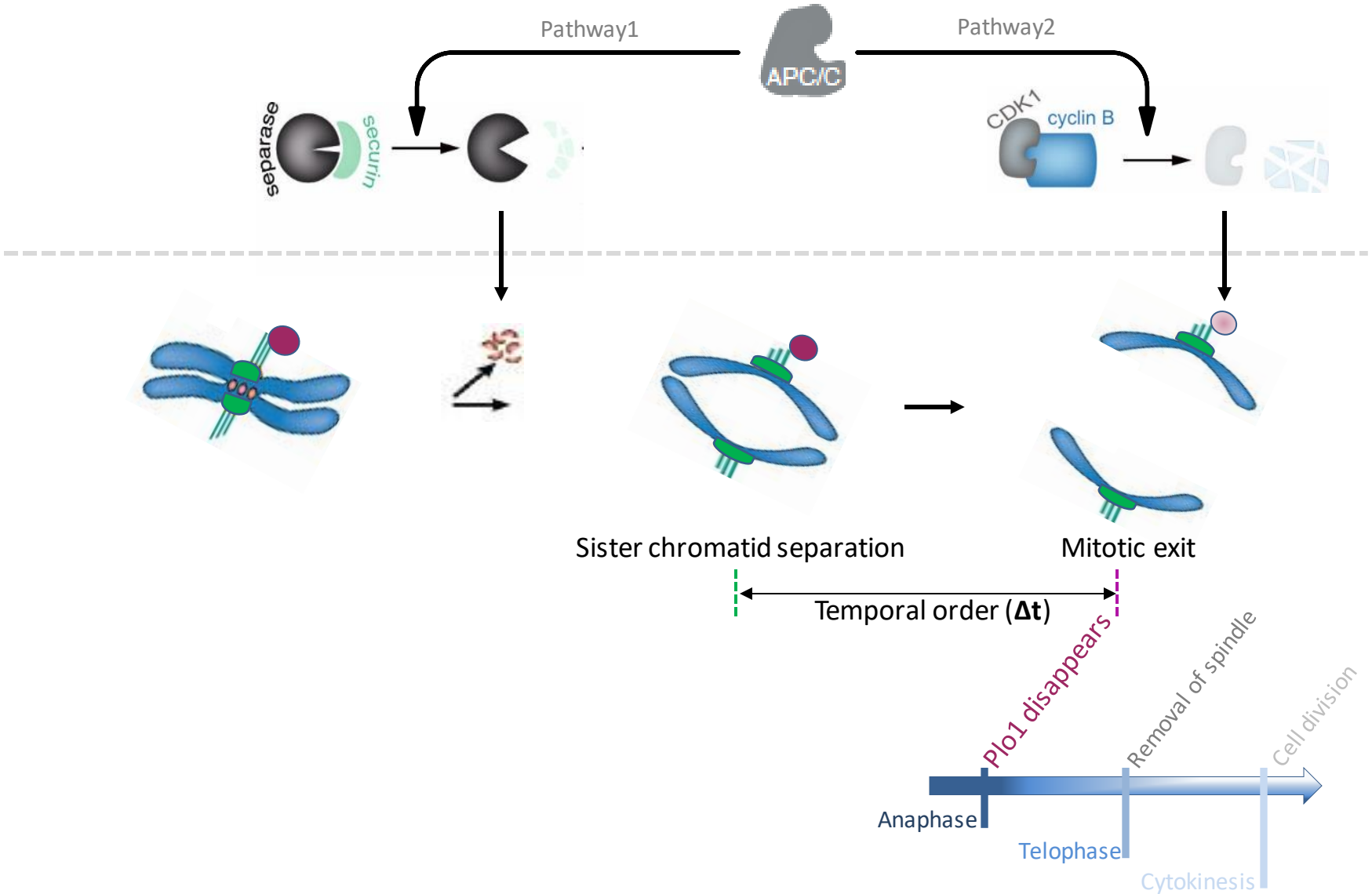


Sister chromatid separation (SCS)

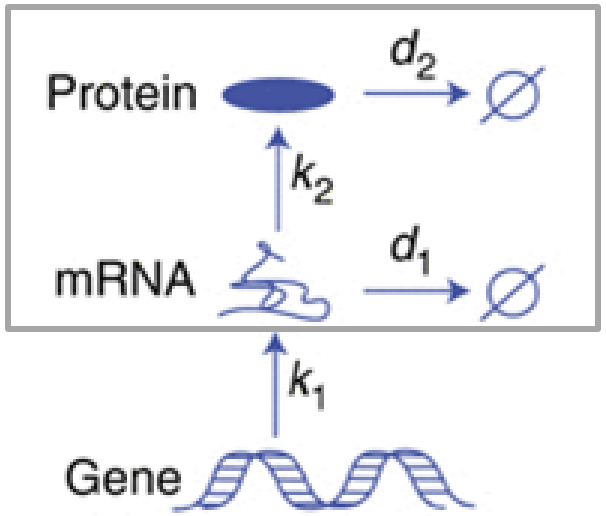
Mitotic exit:
a series of events marking the end of mitosis.

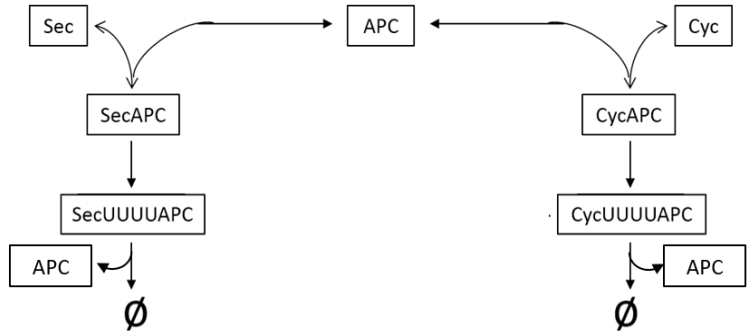


Two independent pathways control anaphase events



Deterministic modeling of protein-protein interaction (competition for substrate)





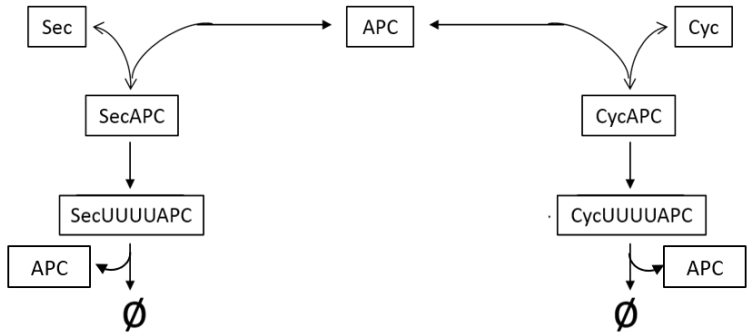
$$\frac{d(mRNA)}{dt} = k_1 - d_1 \cdot mRNA$$

$$\frac{d(Protein)}{dt} = k_2 \cdot mRNA - d_2 \cdot Protein$$

species	rate equations
'Sec'	'SecApc*koff_sec - Apc*Sec*kon_sec'
'Cyc'	'CycApc*koff_cyc - Apc*Cyc*kon_cyc'

Simulation of protein degradation kinetics (rate equations)

A



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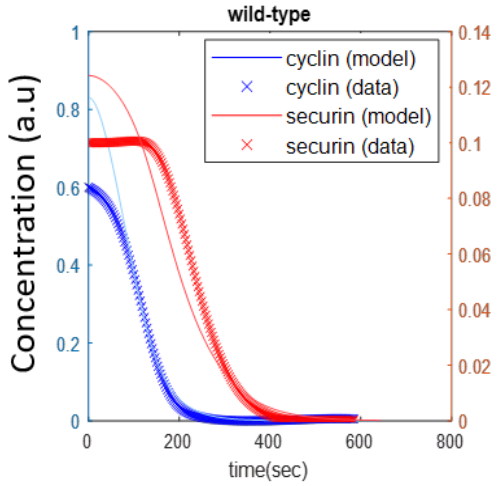
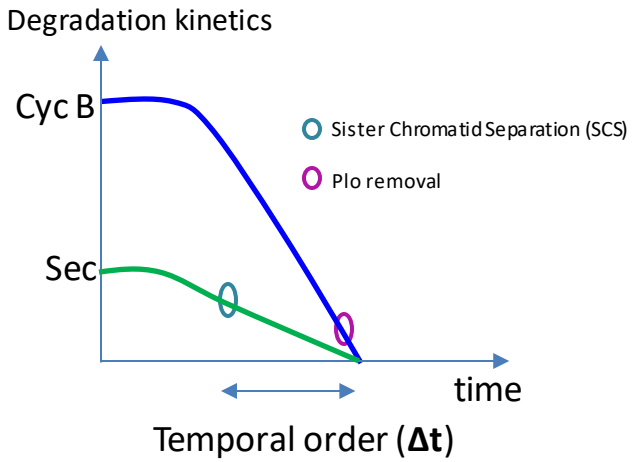


Kinetic model can be used to predict temporal order



What do we learn from the calibrated model?

- Mechanism of temporal order maintenance
- Experimental Conditions for reversal of temporal order
- Which proteins drive temporal order variability?

Double perturbation: a method for terminating cancerous cells in humans