A general framework for modeling mammalian cell growth & division

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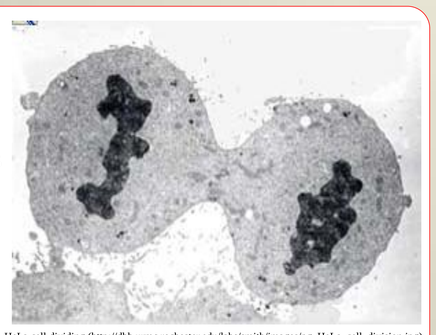
Abstract — A framework for modeling complex cell-biologic processes is presented, based on two constructs: one describing the entire lifecycle of a molecule and the second describing the basic cellular machinery. Use of these constructs allows models to be built in a straightforward manner that fosters rigor and completeness. To demonstrate the framework, an example model of the mammalian cell cycle is presented that consists of several hundred differential equations of simple mass action kinetics. The additional complexity afforded by the framework allows the example model to be calibrated to both large-scale and small-scale observations and allows predictions to be made at both the systems level and the molecular level.

Why?

A **standard model** can be a bridge between experimentalists and theorists (as it is in Physics).

But it must be…

1. Explanatory
2. Alterable
3. Rigorous
4. Useful



HeLa cell dividing (http://dbb.urmc.rochester.edu/labs/smith/images/pg_HeLa_cell_division.jpg)

And to be useful, a standard model should generate testable hypotheses.

PURPOSE

- Modeling of the eukaryote cell cycle, has been practiced for many decades[1-3],
- These have produced descriptions of cell-cycle subprocesses[4] and predictions[5,6,7],
- Beyond the realm of the mental models used by many researchers,
- These models typically involve a small set of regulating enzymes (<ten) and are often independent of the functioning of the entire cell.

We have developed a general framework that allows an increase in modeling complexity and tying of pathways to the basic cellular machinery. The framework is demonstrated in a cell-cycle model that allows several novel predictions. Application of the model to viral entry and in predicting host-virus interactions is outlined and discussed.

How?

Use templates based on System Dynamics to describe molecule lifecycles.

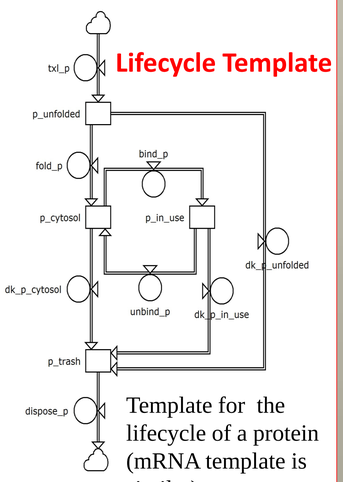
The squares represent states, ODEs typically of the form:

$$\frac{dC}{dt} = R_{in} - R_{out}$$

The circles represent rates, typically of the form:

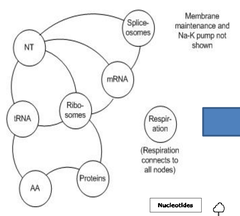
$$R = kC_1C_2\ldots$$

Connect the templates using a relationship diagram. (Modeling to date has been only of these networks.[1,2])



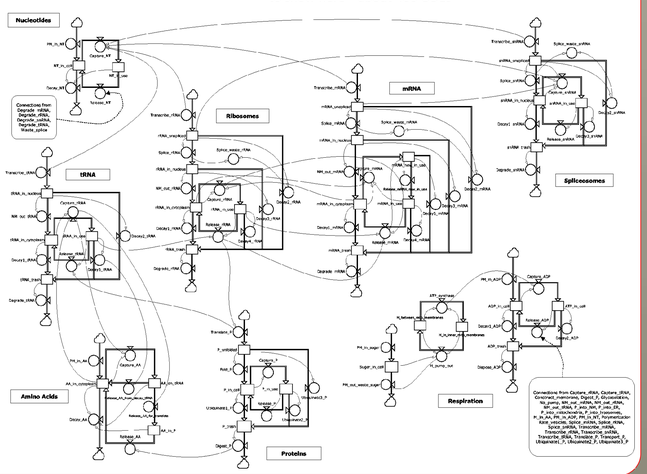
Template for the lifecycle of a protein (mRNA template is similar).

Relationship diagram



Base Model

Complete model is too involved to show here—about 400 ODEs



Cell Cycle Model Details

- Involves describing the lifecycle of individual molecules and describing the basic cellular machinery (the base model). [8]
- Lifecycle construct calculates the creation and destruction of a molecule as well as applicable interactions with other molecules.
- Base model calculates energy usage, amino acid and nucleotide usage, membrane transport, RNA synthesis and destruction, and overall protein synthesis and destruction.
- Cell-cycle model built on the framework calculates the quantities of 33 proteins, and their concomitant 33 mRNAs, over time.
- Exclusive of the base model, consists of 387 ODEs and 1100 rate equations.
- The model is implemented in Matlab with ODE solver, ode15s.

PROTEIN	LOC	G0	UBIQ	INDUCER	ACTIVATOR	INACTIVATOR	FUNCTION
p27	N	high	KPC, SCF(Skp2), APC(Cdc20)	constitutive			inhibits cyclins--prevent S
Rb	N	high		constitutive, E2F inhibits	Cdc14*	cycD/Cdk4or6, cycE/Cdk2, cycA/Cdk2, cycA/Cdk1, cycB/Cdk1	inhibits E2F
cycD	N	0	SCF(Skp2), APC(Cdc20)*	mitogen, B-Myb*	Cdk4or6		activates Cdk4or6 kinase; inactivates APC(Cdh1)
Cdk2	N	high		constitutive, E2F	cycE, cycA, Cdc25A		kinase--phosphorylates RC--allows DNA replication
cycE	N	0	SCF(Fbw7)--on Cdk2, SCF(Skp2)--free	E2F	Cdk2	p27	activates Cdk2 kinase
B-Myb	N	0	SCF(Skp2)--free	E2F, B-Myb	cycA/Cdk2, cycE/Cdk2*		TF for cycD, Cdk1, Plk1, DNA pol, B-Myb, TF-grow, others
NF-Y	N	0	?	constitutive, E2F	cycA/Cdk2, cycE/Cdk2*	Cdc14*	TF for cycA, cycB, Cdk1, Cdc25C, TF-grow
E2F	N	0	SCF(Skp2), APC(Cdc20)*	constitutive, E2F, B-Myb inhibits**		Rb, cycB/Cdk1, cycA/Cdk2**, cycA/Cdk1**, cycD/Cdk4or6**	TF for cycE, cycA, Rb, Cdk1, Cdk2, E2F, Cdk25A, DNA pol, others
cycA	N	0	APC(Cdh1), SCF(Skp2)**, APC(Cdc20)**	E2F, NF-Y, mitogen, adhesion**	Cdk2, Cdc25A, Cdk1, Cdc25A, Cdc25B, Cdc25C	p27 (for cycA/Cdk2), Wee1 (for cycA/Cdk1)	activates Cdk2 and Cdk1 kinases
SCF	CN	0	APC(Cdh1)	constitutive	Skp2, Btrc, Fbw7		ubiquitinase (requires subunit)
Skp2	N	0	auto (when no Emi1 or Wee1), APC(Cdh1)	constitutive	SCF		ubiquitinase subunit for p27, E2F, RC, TF-grow, B-Myb, free cycE, cycA, cycD, others
Btrc	C	0	auto (when no p27, cycE, E2F, or RC), APC(Cdh1), APC(Cdc20)	E2F	SCF		ubiquitinase subunit for Emi1, Cdc25A (sometimes), Wee1, others
Fbw7	N	0	auto (when no cycE, TF-grow**, or RC**)	E2F	SCF		ubiquitinase subunit for cycE, TF-grow, RC**
TF-grow (eg,c-myc, c-Jun, Notch)	N	0	SCF(Skp2), SCF(Fbw7) (not ubiquitinated while TF-grow is on DNA)*	mitogen, Skp2, B-Myb			TF for cell growth
RC(eg, hORC1 hCdc6)	N	0	SCF(Fbw7)**, SCF(Skp2), APC(Cdh1)	E2F	cycE/Cdk2, cycA/Cdk2, cycD/Cdk4or6	cycA/Cdk1, p27	DNA replication complex
DNA poly	N	0		E2F, B-Myb, NF-Y*, inhibits itself*	RC		DNA polymerase
Wee1	C	high	SCF(Btrc)	constituitive	Cdc14	cycA/Cdk2**, cycA/Cdk1**, cycB/Cdk1**, Plk1	kinase--prevents Cdk1 activation
cycB	CN	0	APC(Cdc20), APC(Cdh1)	E2F, B-Myb, NF-Y	Cdk1		activate Cdk1 kinase
Cdk1	CN	high*		E2F, B-Myb, NF-Y, constitutive	cycA, cycB, B-Myb, NF-Y, Plk1, Cdc25A, Cdc25B, Cdc25C	Wee1, Cdc14	kinase--activates APC(Cdc20)
Cdc25C	CN	high		constitutive, NF-Y**	cycB/Cdk1, Plk1, cycA/Cdk1*	Cdc14	phosphatase--activates cycB/Cdk1
Plk1	C	0	APC(Cdh1)	E2F, TF-grow	cycB/Cdk1, cycA/Cdk1*		kinase--activates Cdc25C; deactivates Emi1, Wee1; translocates cycA/Cdk1, cycB/Cdk1, Cdc25C, and Plk1 to nucleus
Emi1	CN	0	SCF(Btrc), SCF(Skp2)*	E2F		cycB/Cdk1, cycA/Cdk1*	inhibits Cdh1, Cdc20
APC	N	high		constitutive	Cdh1, Cdc20		ubiquitinase (requires subunit)
Cdh1	N	high	auto (when no Skp2, cycA, cycB, Cdc25A, Plk1, RC, Cdc20, SCF*, p27**)	constitutive	APC, Cdc14	Emi1, cycA/Cdk2, cycB/Cdk1, cycD/Cdk4or6, cycE/Cdk2	ubiquitinase subunit--maintains G0, G1--ubiq Cdc20, cycA (free), cycB (free), Cdc25A, RC, Plk1, Skp2, others
Cdc20	N	0	APC(Cdh1)	constitutive	APC, cycB/Cdk1, cycA/Cdk1*	Emi1, Cdc14	ubiquitinase subunit for Securin, cycB, cycA
Cdc14 (reprsnts MEN pathw)	N	high		constitutive	Plk1*	Securin	phosphatase--ends M--activates p27, Wee1, Cdh1; deactivates Cdc25A, Cdc25B, Cdc25C
Cdc25A	N	0	APC(Cdh1), SCF(Btrc)*	constitutive, E2F, TF-grow	cycE/Cdk2, cycA/Cdk2, cycA/Cdk1*, cycB/Cdk1*	Cdc14	phosphatase--activates cycE/Cdk2, cycA/Cdk2, cycA/Cdk1*, cycB/Cdk1*
Cdc25B	C	0	APC(Cdh1)*, SCF(Btrc)*	E2F*, TF-grow*,	TF-grow**, cycA/Cdk1*, cycB/Cdk1*	Cdc14	phosphatase--activates cycA/Cdk1, cycB/Cdk1*
Securin	N	0	APC(Cdc20)	E2F			keeps separase from destroying chromotin cohesion proteins
cycC	N	high		constitutive	Cdk8, Cdc14*	mitogen*	inhibits RNA pol
KPC	N			constitutive	mitogen		ubiquitinase for p27
RNA poly	N	high		constitutive, TF_grow*	mitogen*	cycC/Cdk8, APC(Cdc20)*	txs
eIF-4	C	high		constitutive	mRNA		initiates txl

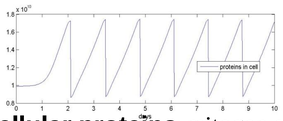
The proteins represent different layers of abstraction (see Additional file 1): **LOC** = location, **N** = nucleus, **C** = cytoplasm, **G0** = steady-state concentration, **UBIQ** = ubiquitinating molecules, auto = autoubiquitination, **INDUCER** = molecule effecting transcription, **ACTIVATOR** = molecule promoting activity, **TF** = transcription factor, txs = transcription, txl = translation, ? = unknown, * = assumed, ** = ignored.

Correct?

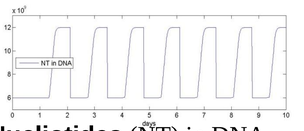
- The model is calibrated to quantitative data in the literature[9-11].
- G0 is calibrated to # proteins (10^{10}), # ATP (10^9), ATP usage (10^7 ATP/s), Na^+-K^+ ATPase energy (32% of total), elongation rates (txs ~30 NT/s, DNA replication ~50 NT/s, txl ~20 AA/s), etc.
- Cell cycle is calibrated to HeLa duration (1-1.5 days, G1 ~12 hr, S ~8 hr, G2 ~10 hr, M ~6 hr), # RNA polymerases (~30,000), # rRNA (~5×10^6), fractions of various RNAs (hnRNA ~7%, mRNA ~3%, rRNA precursor ~4%, rRNA ~71%, tRNA ~15%, RNA in nucleus ~14%), etc.
- The most difficult characteristics to match are (1) using the same reaction rates for G0 as for cell cycle, (2) transitioning from G0 to cell cycle in a reasonable time, and (3) setting a robust G2/M transition time.
- **NOTE**: Quantitative data do not exist for most individual mRNAs and proteins. Many concentrations in this model are estimated. However, reactions are dependent on the concentration and the reaction rate; when a concentration is known, rates can be adjusted (often linearly) hence.

	Quantity	Lit Value	Model Value		Quantity	Lit Value	Model Value
Cell Cycle	Cell-cycle duration (HeLa)	25 hr	33 hr	G0	# proteins	$10^{\wedge10}$	$10^{\wedge10}$
	G1 duration	12 hr	15 hr		# amino acids (AA) unbound	6×10^{10}	5.7×10^{10}
	S duration	8hr	7 hr		# nucleotides (NT) unbound	0.4wt% ~ 2×10^{10}	2×10^{10}
	G2	4 hr	7 hr		# ATP	10^9	10^9
	M	4 hr	4 hr		# ADP	10^8	10^8
	# nucleotides in RNA	5×10^{10}	1.6×10^{10} (ave)		ATP usage	10^7 ATP/s	10^7 ATP/s
	# rRNA (no pre-rRNA)	3.6×10^6	1.5×10^6		Transcription elongation rate	30 NT/s	30 NT/s
	# tRNA	9×10^7	2.8×10^7		Translation elongation rate	20 AA/s	20 AA/s
	# mRNA (no hnRNA)	3.6×10^5	2.7×10^5		DNA replication elongation rate	50 NT/s	50 NT/s
	# snRNA	1 or 2 /transcript	9.7×10^5 (~0.25\transcript)		Na -K ATPase energy (fraction of total)	33%	32%
	# RNA polymerases	$2\text{-}4\times10^4$	3×10^4 (ave)		Translation energy fraction	Most	51%
	hnRNA fraction	7%	8% (ave)		Same reaction rates in G0 and during the cell cycle		
	mRNA (cytosol) fraction	3%	3% (ave)	Misc.	G0/cell-cycle energy ratio	20%	~7%
	rRNA precursors fraction	4%	8% (ave)		G0 translation energy fraction	Most	51%
	rRNA fraction	71%	67% (ave)				
	tRNA fraction	15%	14% (ave)				
	RNA fraction in nucleus	14%	16% (ave)				

Some Results

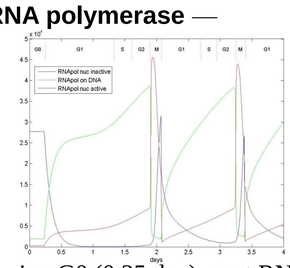


Cellular proteins-mitogen starts the cell cycle at 0.25 days; abrupt decrease indicates cell division



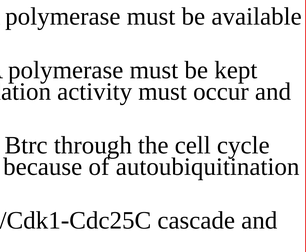
Nucliotides (NT) in DNA — increasing slope is S phase; for a time cell has twice normal DNA

RNA polymerase —

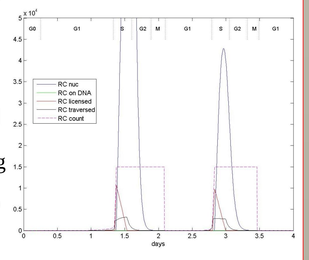


during G0 (0.25 day) most RNA poly is held inactive by cycC/Cdk8; during cell cycle most is actively traversing DNA; during M, most is active, but detached from DNA

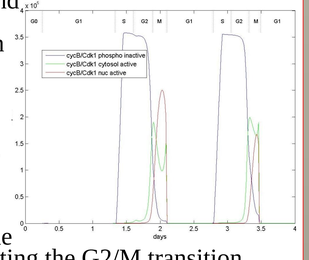
SCF bound to Fbw7, Skp2, and Btrc subunits - All molecules are present at same time. First, SCF preferentially binds Fbw7 and ubiquitinates Fbw7 substrates, at which point Fbw7 autoubiquitinates. Then SCF preferentially binds Skp2 and ubiquitinates Skp2 substrates (in particular, replication complexes RC) at which point Skp2 autoubiquitinates. Finally, SCF binds Btrc, concluding the sequence.



Replication Complexes (RC)—ORC1 and Cdc6 are induced by E2F and bind DNA at RC origins; phosphorylation by cycE/Cdk2 or cycA/Cdk2 allows traversal and release by DNA poly; SCF(Skp2) ubiquitinates released RC, marking the end of S phase (RC-count is a modeling Construct to keep track of the 15,000 RC origins)



Number of cycB/Cdk1 — cycB and Cdk1 are induced and enhanced by E2F, B-Myb, and NF-Y. Cdk1 also has constitutive expression. cycB/Cdk1 accumulates during S phase but is phosphorylated and inactivated by Wee1. Loss of Wee1 and the presence of active Cdc25B (activated by cycA/Cdk1) begin the dephosphorylation and activation of cycB/Cdk1 and thus trigger the cascade between cycB/Cdk1 and constitutive Cdc25C. Active cycB/Cdk1 further activates Plk1, which promotes the translocation of cycB/Cdk1 to the nucleus, completing the G2/M transition.



Predictions

- When redundant pathways can activate a time-critical process, one preferred pathway must inactivate the other redundant pathway(s).
- Explicit growth monitoring is unnecessary during the mammalian cell cycle.
- Pre-translation mRNA regulation (other than splicing and the number of RNA polymerase) is unnecessary during the cell cycle.
- Transcription/translation (i.e., growth) continue during S phase at unimpeded rates.
- For cells capable of division, a ready supply of RNA polymerase must be available during G0.
- For cells capable of division, either a supply of DNA polymerase must be kept inactive during G0 or intense transcription and translation activity must occur and be controlled at a saturation level.
- SCF progressively complexes with Fbw7, Skp2, and Btrc through the cell cycle because of their differences in affinity with SCF and because of autoubiquitination in the absence of substrates.
- Cdc25A and Cdc25B cooperate to instigate the cycB/Cdk1-Cdc25C cascade and the G2/M transition.
- Transcription factors act multiplicatively.
- p27 is important for maintaining G0 and for timing the duration of G1 in the first cell cycle after exiting G0.
- p27 does not inhibit cycD/Cdk4 or cycD/Cdk6 activity
- cycD/Cdk4or6 does not activate DNA replication complexes.
- Plk1 is the primary inactivator of Wee1
- Not all RNA polymerase are removed from condensed DNA during mitosis

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