Pancreatic Cancer Research and HMGB1 Signaling Pathway

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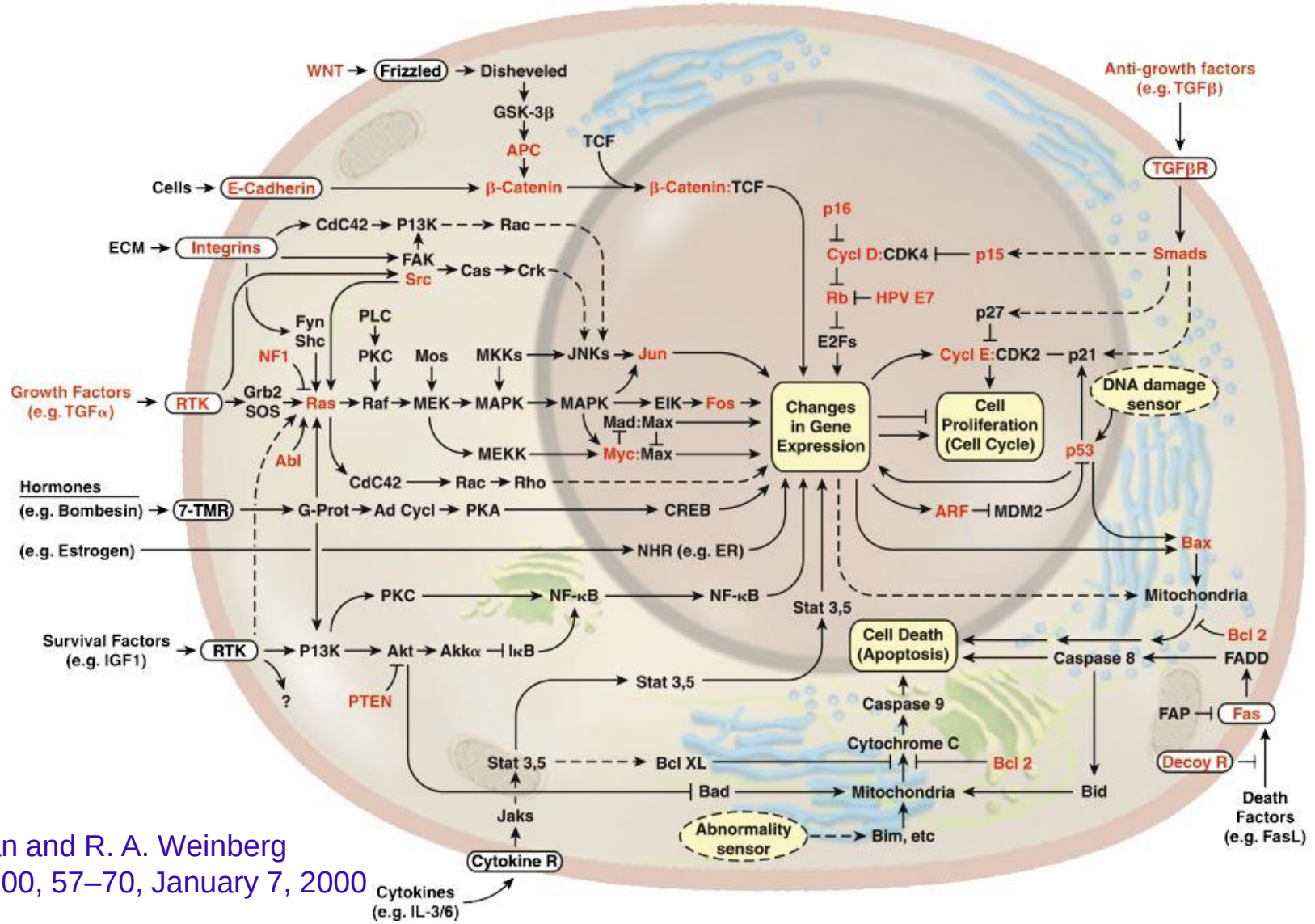
The Hallmarks of Cancer

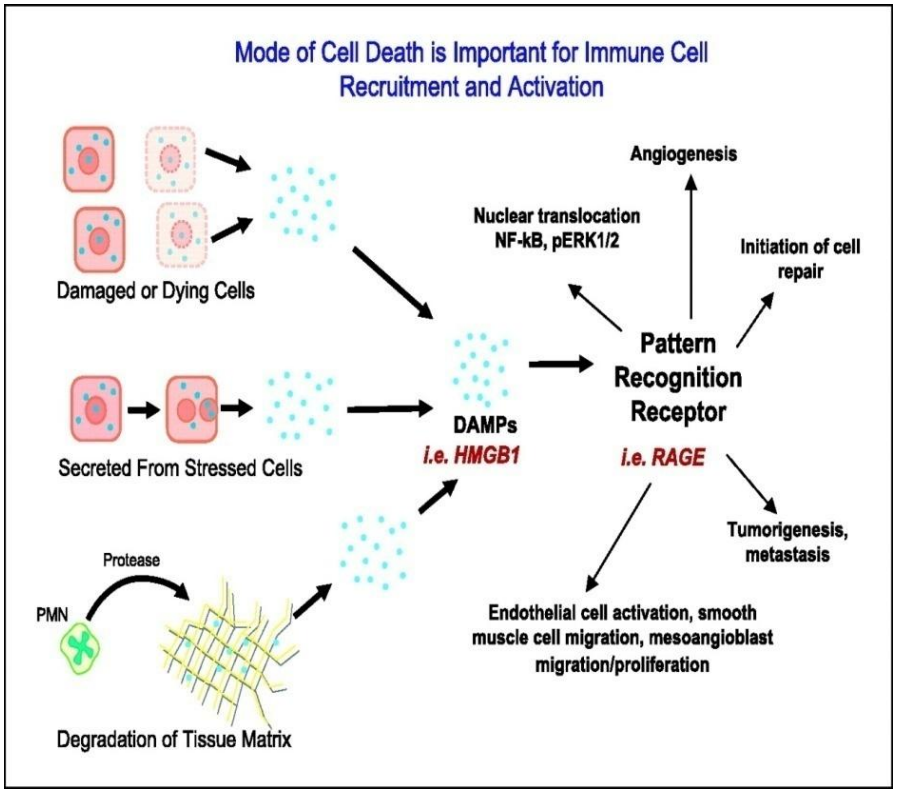


D. Hanahan and R. A. Weinberg
Cell, Vol. 100, 57–70, January 7, 2000

Outline

1. Introduction
   - HMGB1 Protein
   - Important Signaling Pathways
2. Model Building
   - BioNetGen Model
   - Simulation Results
3. Model Checking
   - Statistical Model Checking
   - Verification of HMGB1 model
4. Conclusions

The Protein HMGB1



- High-Mobility Group Protein 1 (HMGB1):
  - DNA-binding protein and regulates gene transcription
  - released from damaged or stressed cells, etc.
- HMGB1 activates RAGE or TLR2/4
  - RAGE: Receptor for Advanced Glycation End products.
  - TLR: Toll-like receptor

- RAGE/TLR activation can activate NFκB and RAS signaling pathways which causes inflammation or tumorigenesis.

HMGB1 and Pancreatic Cancer (Lotze *et al.*, UPMC)

Experiments with pancreatic cancer cells:

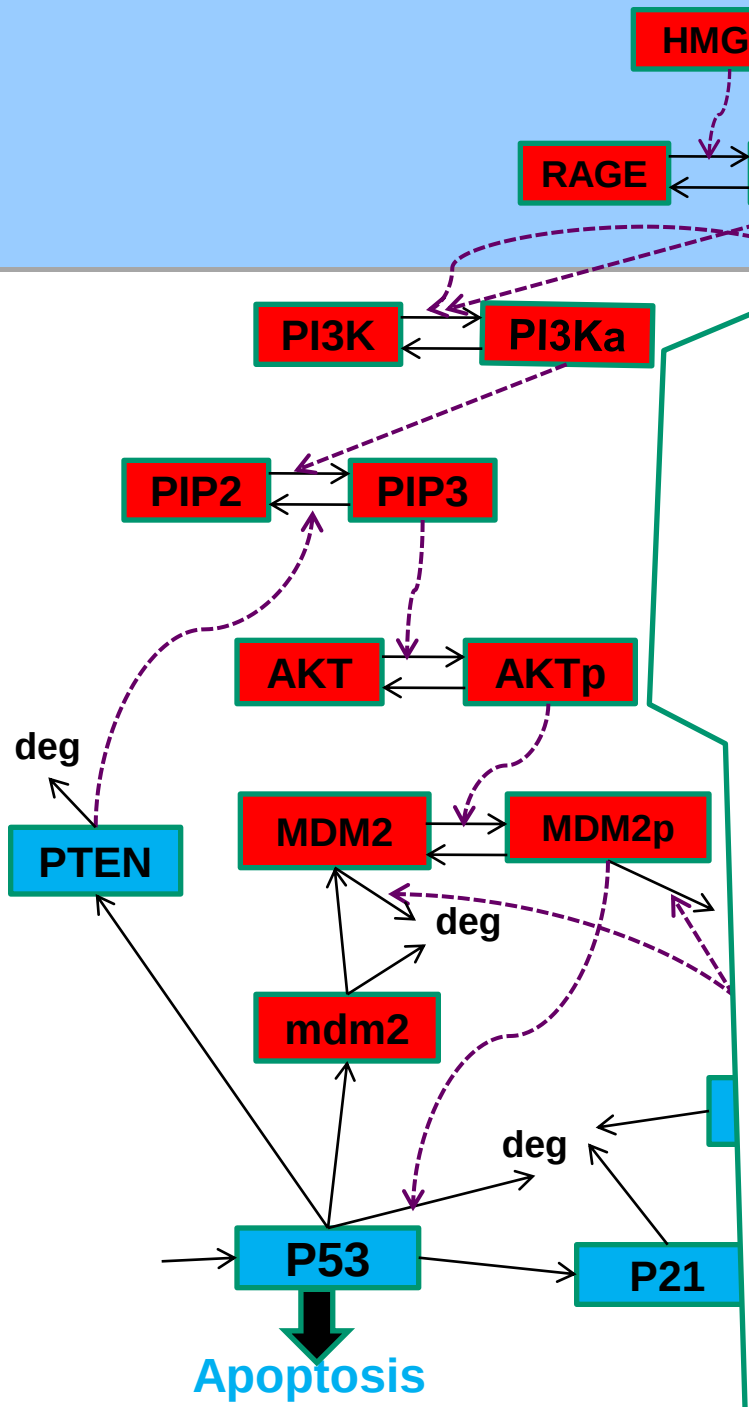
- Overexpression of HMGB1/RAGE is associated with diminished apoptosis, and longer cancer cell survival time.
- Knockout of HMGB1/RAGE leads to increased apoptosis, and decreased cancer cell survival.

Our Goals

- We use the BioNetGen language (*http://bionetgen.org*) to describe the crosstalk of important signaling pathways activated by HMGB1.
  - We focus on the p53, RAS, NFkB & RB-E2F signaling pathways.
  - How the expression level of HMGB1 influences the cell's fate.
- We use statistical model checking to formally verify behavioral properties expressed in temporal logic:
  - Can express quantitative properties of systems
  - Scalable, can deal with large models

P53-RAS-RB Crosstalk Model

- First computational model of HMGB1 signal transduction in tumorigenesis.
- Focus on the crosstalk of p53, RAS, & RB signaling pathways.
- More details in the paper "***Analysis and Verification of the HMGB1 Signaling Pathway***" published in *BMC Bioinformatics 11 (Suppl 7)* (2010)



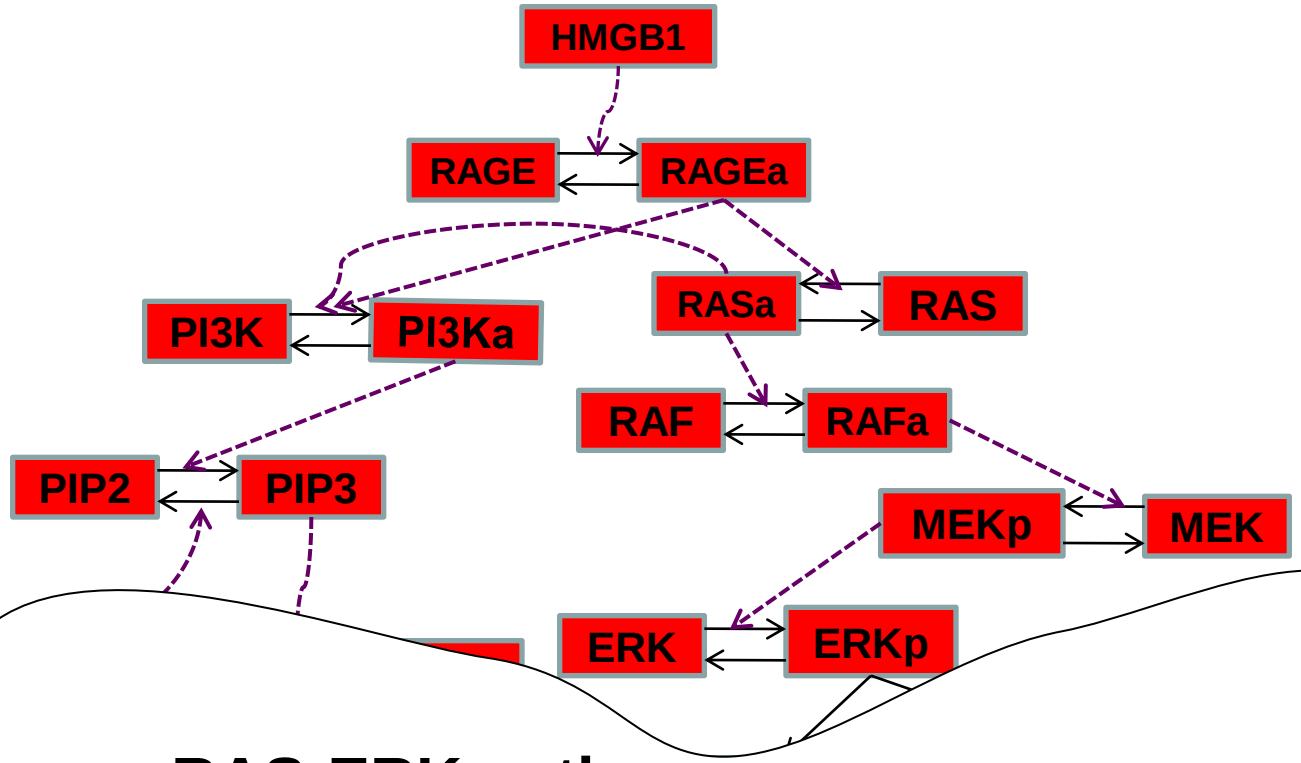
PI3K-p53 pathway

P53 is a tumor suppressor, is mutated in more than **50% of cancers**

Functions of P53:

1. **Induces cell cycle arrest**: P21, etc.
2. **DNA repair**
3. **Initiates apoptosis** – Programmed Cell Death: Bax, etc.

- Negative feedback loop:
  PI3K → PIP3 → AKT → MDM2 ⊣ p53 → MDM2
- Positive feedback loop:
  p53 → PTEN ⊣ PIP3 → AKT → MDM2 ⊣ p53



RAS-ERK pathway

1. Activation of RAS signaling causes cell growth and survival.
2. RAS family has three members: HRAS, KRAS, NRAS.
3. KRAS mutations are found in more than 90% of pancreatic cancers

- RAGE → RAS → RAF → MEK → ERK1/2 → TFs → Cyclin D → Cell-cycle progression
- RAS → PI3K → PIP3 → AKT → MDM2 ⊣ Apoptosis

RB-E2F pathway

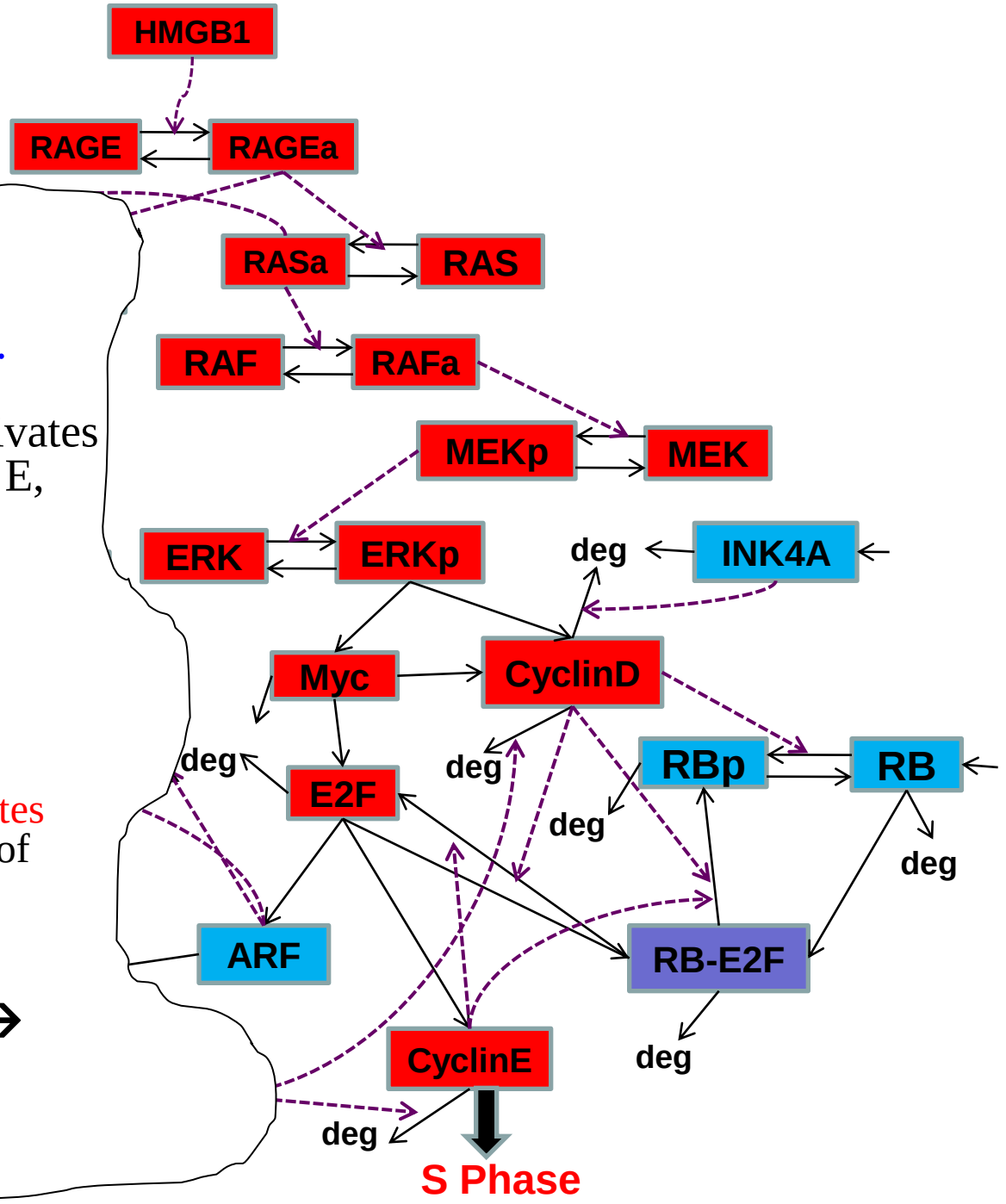
- Regulates the G1-S phase transition in the cell cycle.

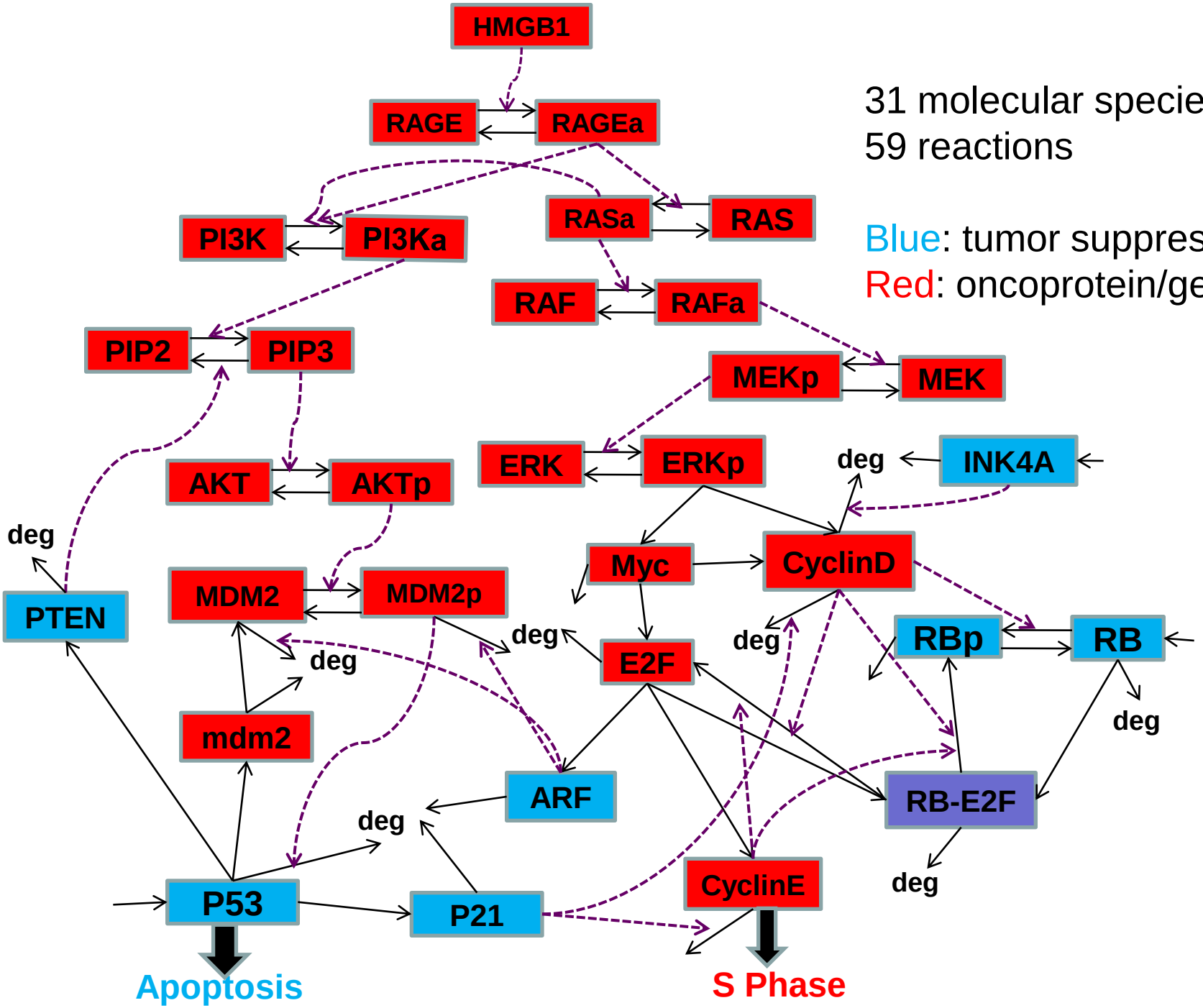
1. E2F is an oncoprotein, activates the transcription of Cyclin E, and it is modulated by RB.

2. RB is a tumor suppressor: prevents the replication of damaged DNA.

3. Cyclin D-CDK4 phosphorylates RB, leading to the activation of E2F.

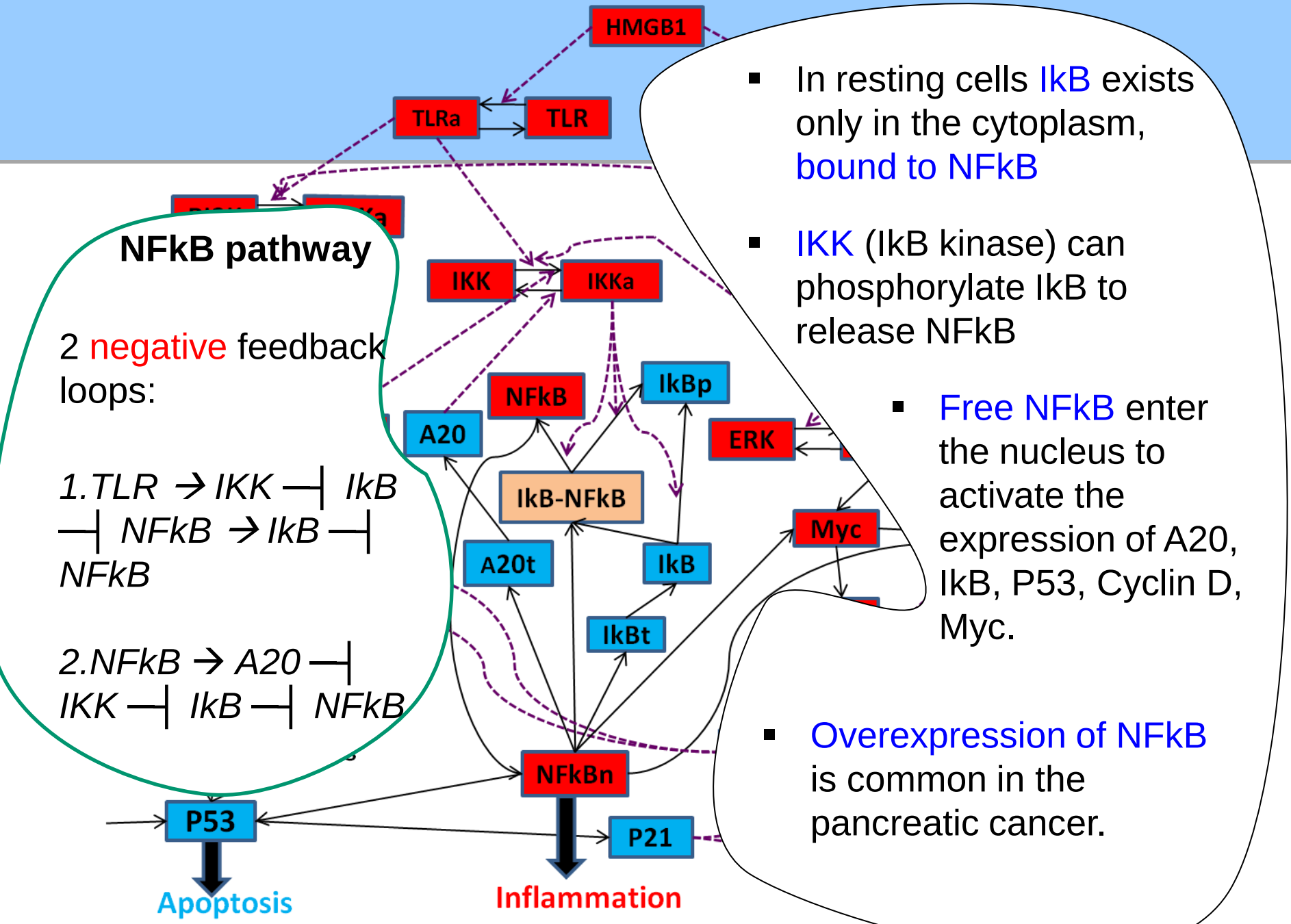
- **CyclinD ⊣ RB ⊣ E2F → CyclinE → S Phase**




HMGB1
RAGE
RAGEa
PI3K
PI3Ka
RASa
RAS
RAF
RAFa
PIP2
PIP3
MEKp
MEK
ERK
ERKp
deg
INK4A
AKT
AKTp
deg
Myc
CyclinD
PTEN
MDM2
MDM2p
deg
deg
E2F
deg
RBp
RB
deg
mdm2
ARF
deg
RB-E2F
deg
CyclinE
P53
P21
Apoptosis
S Phase
31 molecular species
59 reactions
Blue: tumor suppressor
Red: oncoprotein/gene

P53-NFkB-RAS-RB Crosstalk Model

- Crosstalk of p53, NFkB, RAS, & RB signaling pathways.
- NFkB protein is involved in inflammation, cell proliferation and apoptosis.
- NFkB is a transcription factor for the pro-apoptotic gene p53, for anti-apoptotic genes Bcl-XL and for the cell-cycle regulatory proteins Myc and Cyclin D.
- More details in the paper "***Computational Modeling and Verification of Signaling Pathways in Cancer***" published in *Algebraic and Numeric Biology Proceedings* (2010).



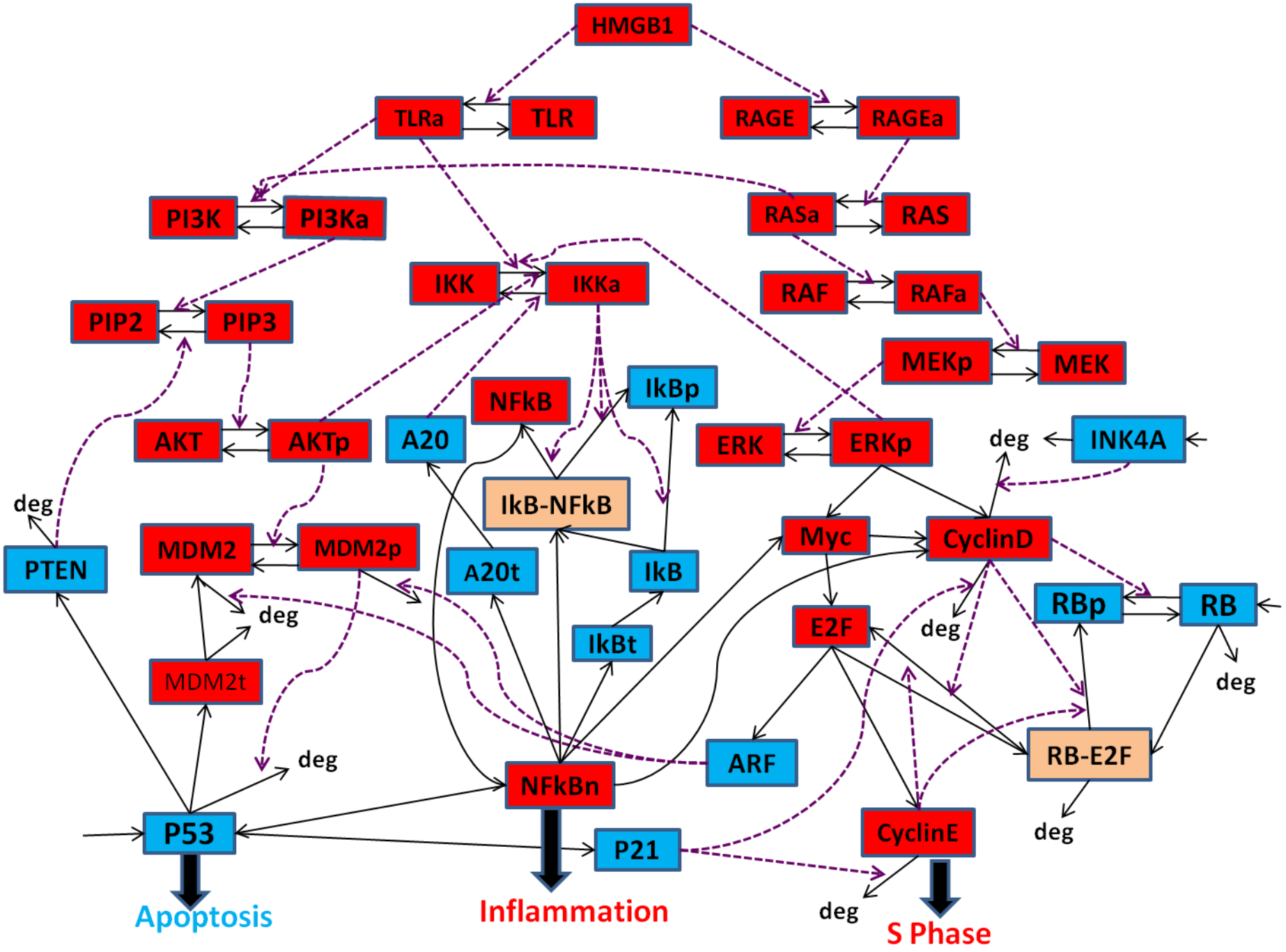
NFkB pathway

2 negative feedback loops:

1.TLR → IKK ⊣ IkB ⊣ NFkB → IkB ⊣ NFkB

2.NFkB → A20 ⊣ IKK ⊣ IkB ⊣ NFkB

- In resting cells IkB exists only in the cytoplasm, bound to NFkB
- IKK (IkB kinase) can phosphorylate IkB to release NFkB
- Free NFkB enter the nucleus to activate the expression of A20, IkB, P53, Cyclin D, Myc.
- Overexpression of NFkB is common in the pancreatic cancer.

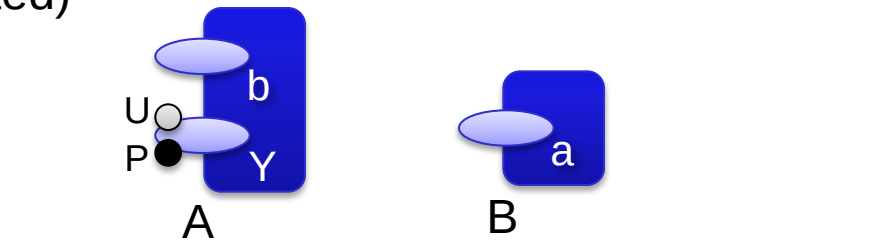

HMGB1
TLRa
TLR
RAGE
RAGEa
PI3K
PI3Ka
RASa
RAS
IKK
IKKa
RAF
RAFa
PIP2
PIP3
MEKp
MEK
NFkB
IkBp
AKT
AKTp
A20
ERK
ERKp
deg
INK4A
deg
IkB-NFkB
PTEN
MDM2
MDM2p
A20t
IkB
Myc
CyclinD
RBp
RB
E2F
deg
deg
IkBt
MDM2t
deg
ARF
RB-E2F
deg
NFkBn
P53
P21
CyclinE
deg
Apoptosis
Inflammation
S Phase

The BioNetGen Language

begin molecule types

```
A(b,Y~U~P) # A has a component Y which
      # can be labeled as U (unphosphorylated)
      # or P (phosphorylated)
B(a)
```

end molecule types

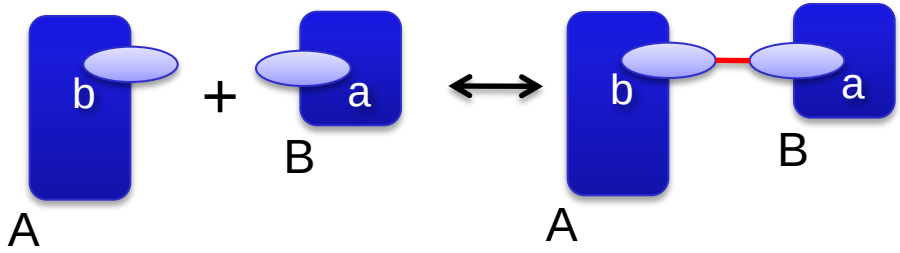


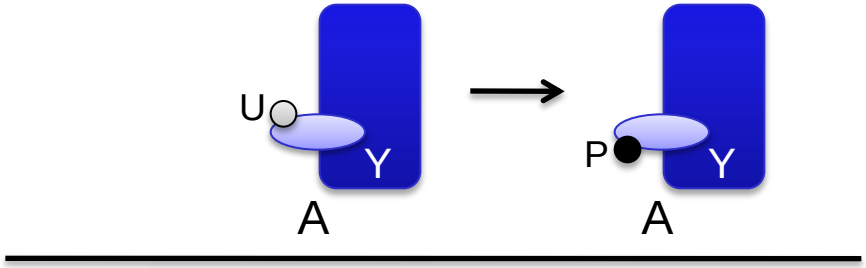
begin reaction rules

```
A(b)+ B(a)<-> A(b!1).B(a!1)

A(Y~U) -> A(Y~P)
```

end reaction rules





Ordinary Differential Equations and Stochastic simulation

Faeder JR, Blinov ML, Hlavacek WS ***Rule-Based Modeling of Biochemical Systems with BioNetGen.*** *In Methods in Molecular Biology: Systems Biology, (2009).*

BioNetGen

- ***Two Events: PIP3 phosphorylates AKT, and AKT dephosphorylates.***

begin species

```
AKT(d~U)    1e5
AKT(d~p)    0
```

end species

begin parameters

```
k    1.2e-7
d    1.2e-2
```

end parameters

begin reaction_rules

```
PIP(c~p) + AKT(d~U) → PIP(c~p) + AKT(d~p)    k
AKT(d~p) → AKT(d~U)                          d
```

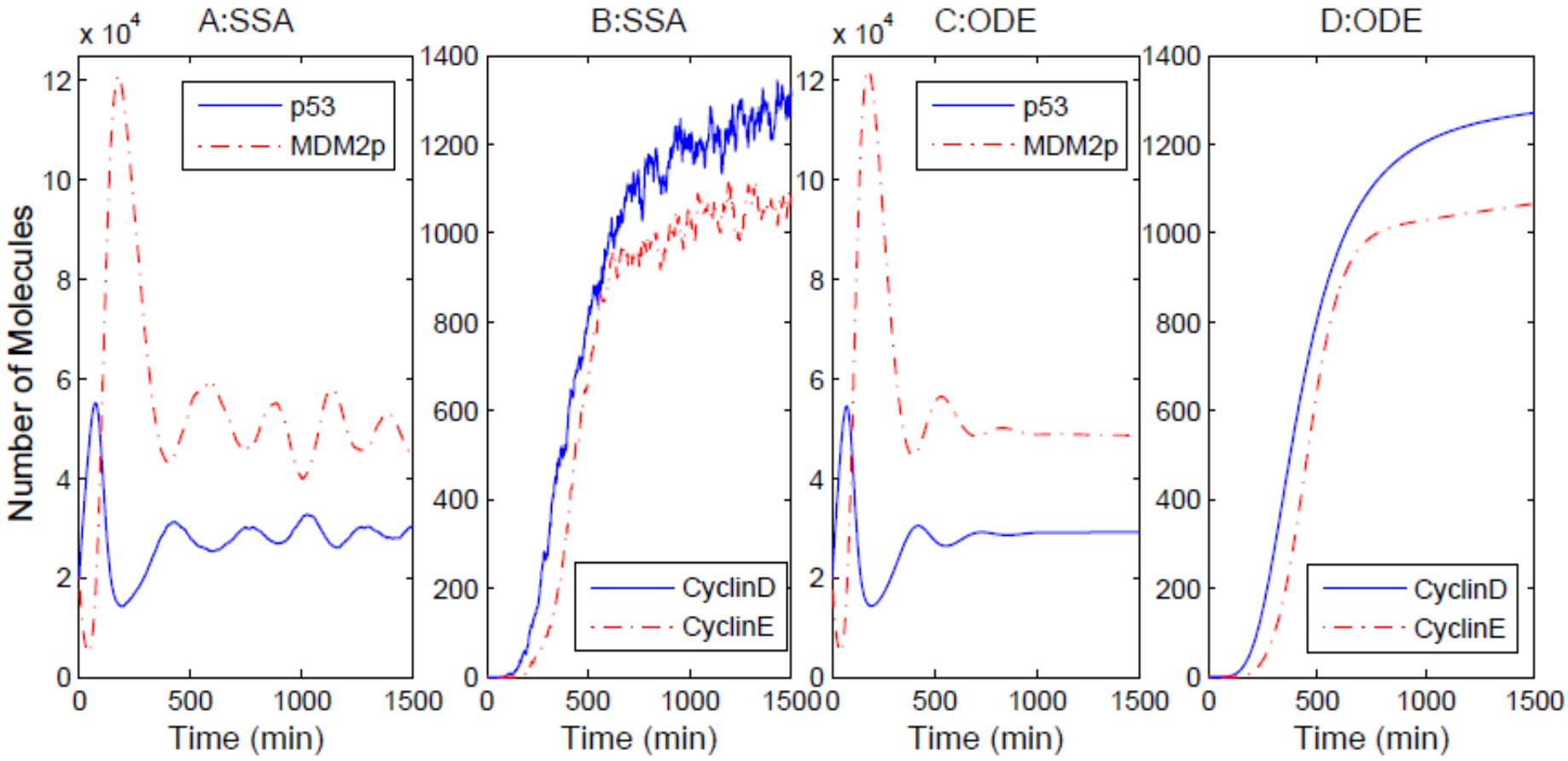
end reaction_rules

- The corresponding ODE is:

$$[AKT(d\sim p)](t)' = k\cdot[PIP(c\sim p)](t)\cdot[AKT(d\sim U)](t) - d\cdot[AKT(d\sim p)](t)$$

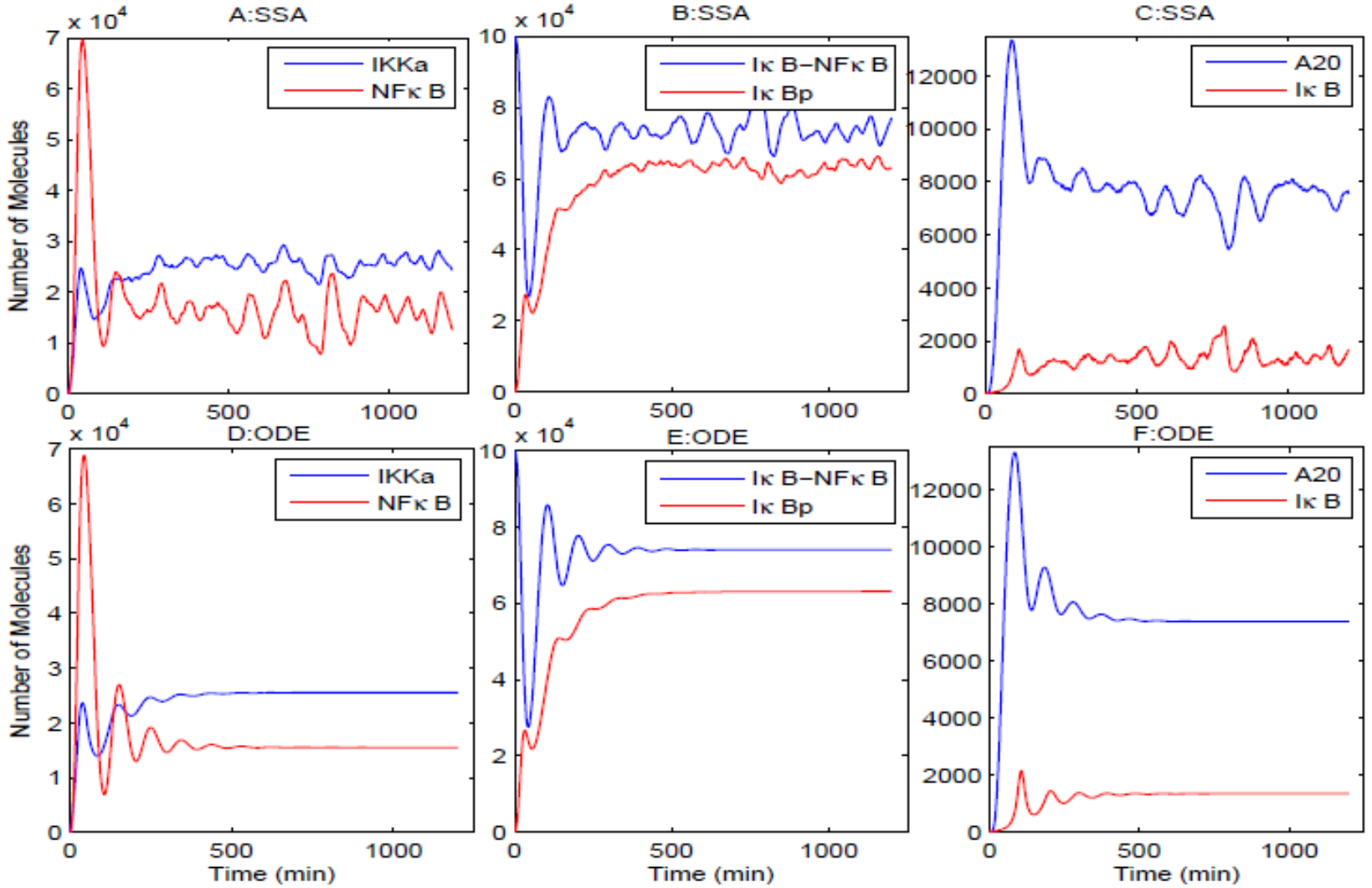
Simulations (I)

- Baseline simulation of p53, MDM2, Cyclin D/E in response to HMGB1 release: ODE vs stochastic simulation

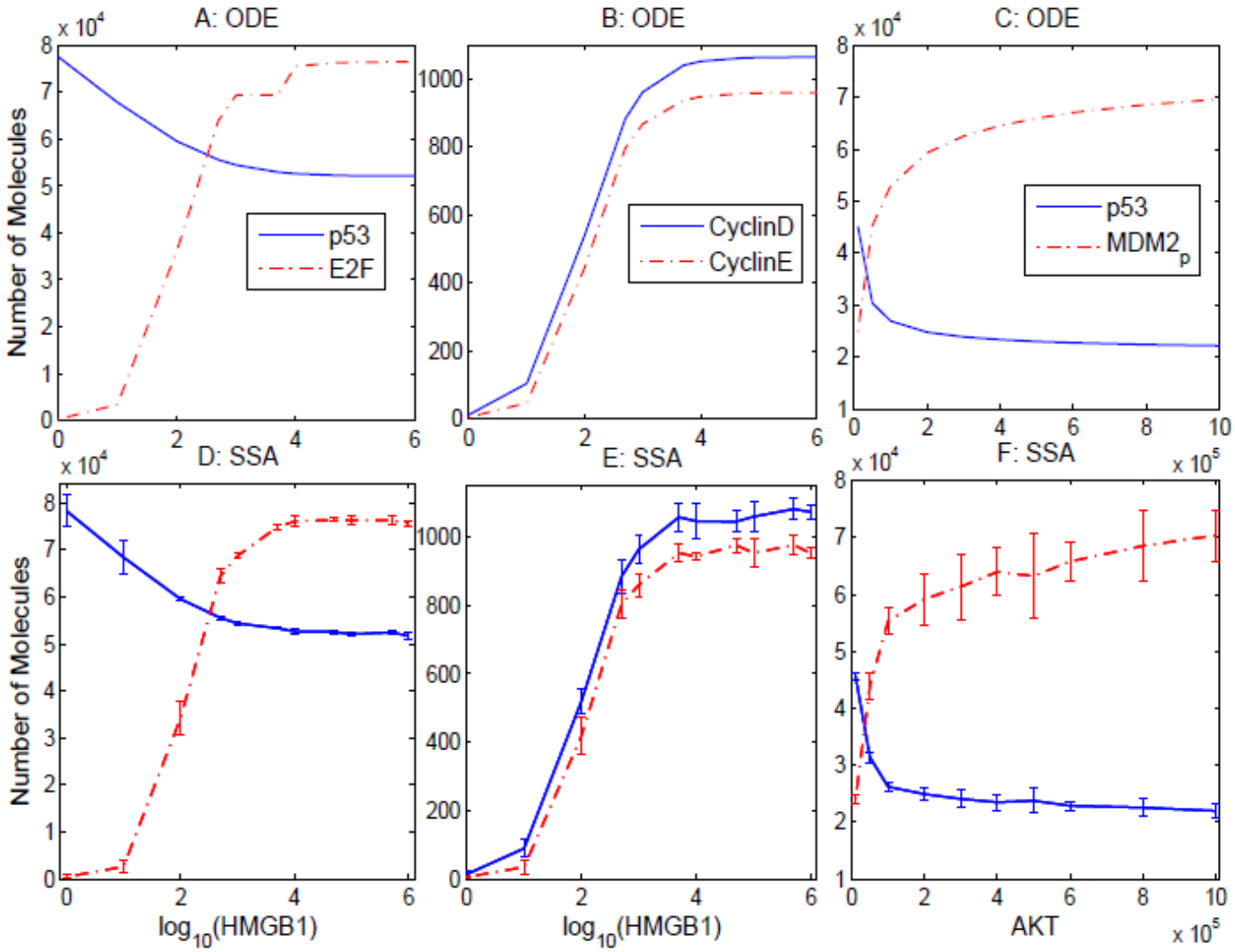


Simulations (II)

- Baseline simulation of NFkB, IkB, IKK and A20 in response to HMGB1 release.

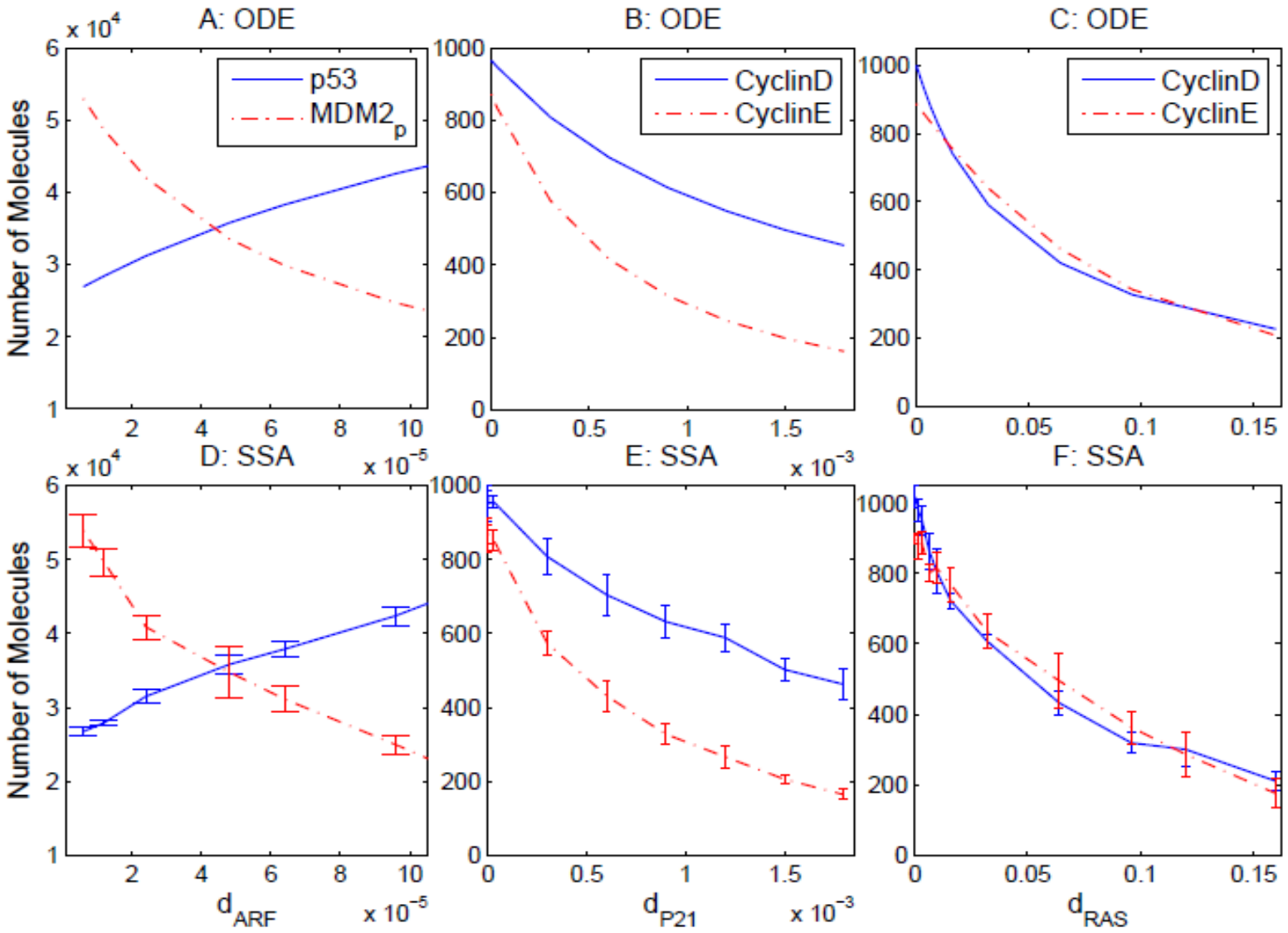


Simulations (III)



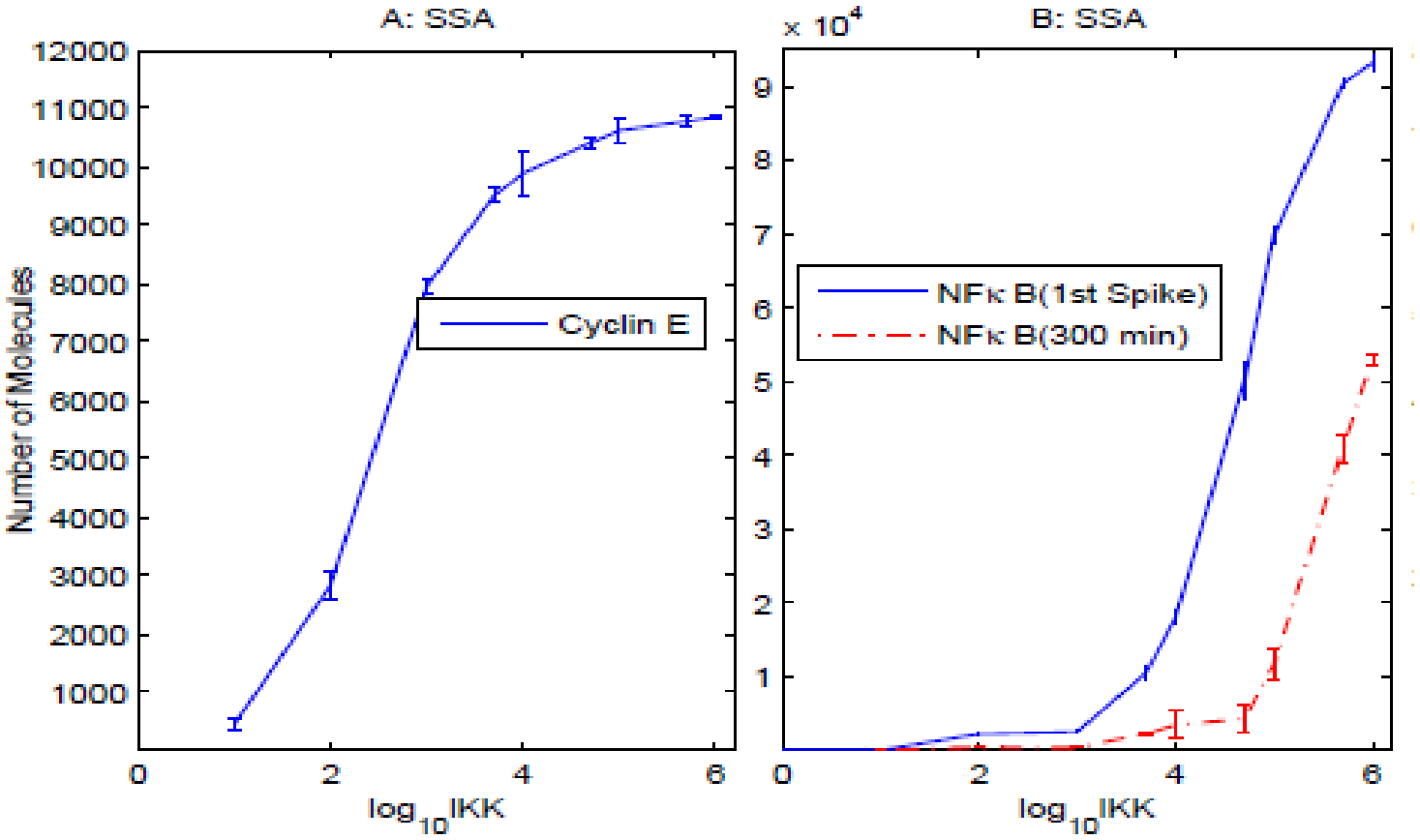
- Overexpression of HMGB1 leads to increase of E2F and Cyclin D/E, decrease of p53.
- Overexpression of AKT represses p53 level

Simulations (IV)



Simulations (V)

- IKK overexpress in many cancer cells, it promotes NFkB's transcription activity and accelerate cell proliferation.
- Overexpression of NFkB is common in pancreatic cancer.



Bounded Linear Temporal Logic

- Bounded Linear Temporal Logic (BLTL): Extension of LTL with time bounds on temporal operators.
- $\mathbf{F}^{t}$ a – "a will be true in the Future *within time t* "
- $\mathbf{G}^{t}$ a – "a will be Globally true *between time 0 and t* "
- For example: "# of AKTp reach 4,000 within 20 minutes?" – $\mathbf{F}^{20}$ (AKTp ≥ 4,000)
- Let $\sigma = (s_0, t_0), (s_1, t_1), \ldots$ be an execution of the model
  - along states $s_0, s_1, \ldots$
  - the system stays in state s_i *for time* t_i
- σ^i: Execution trace starting at state *i*.

Verification of BioNetGen Models

- Given a stochastic BioNetGen model $\mathcal{M}$, Temporal property Φ, and a fixed $0<\theta<1$, we ask whether $P_{\geq\theta}(\Phi)$ or $P_{<\theta}(\Phi)$.
- For example: "could AKTp reach 4,000 within 20 minutes, with probability at least 0.99?" : $P_{\geq 0.99}(\mathbf{F}^{20}(\text{AKTp} \geq 4{,}000))$
- Does $\mathcal{M}$ satisfy ϕ with probability at least θ?

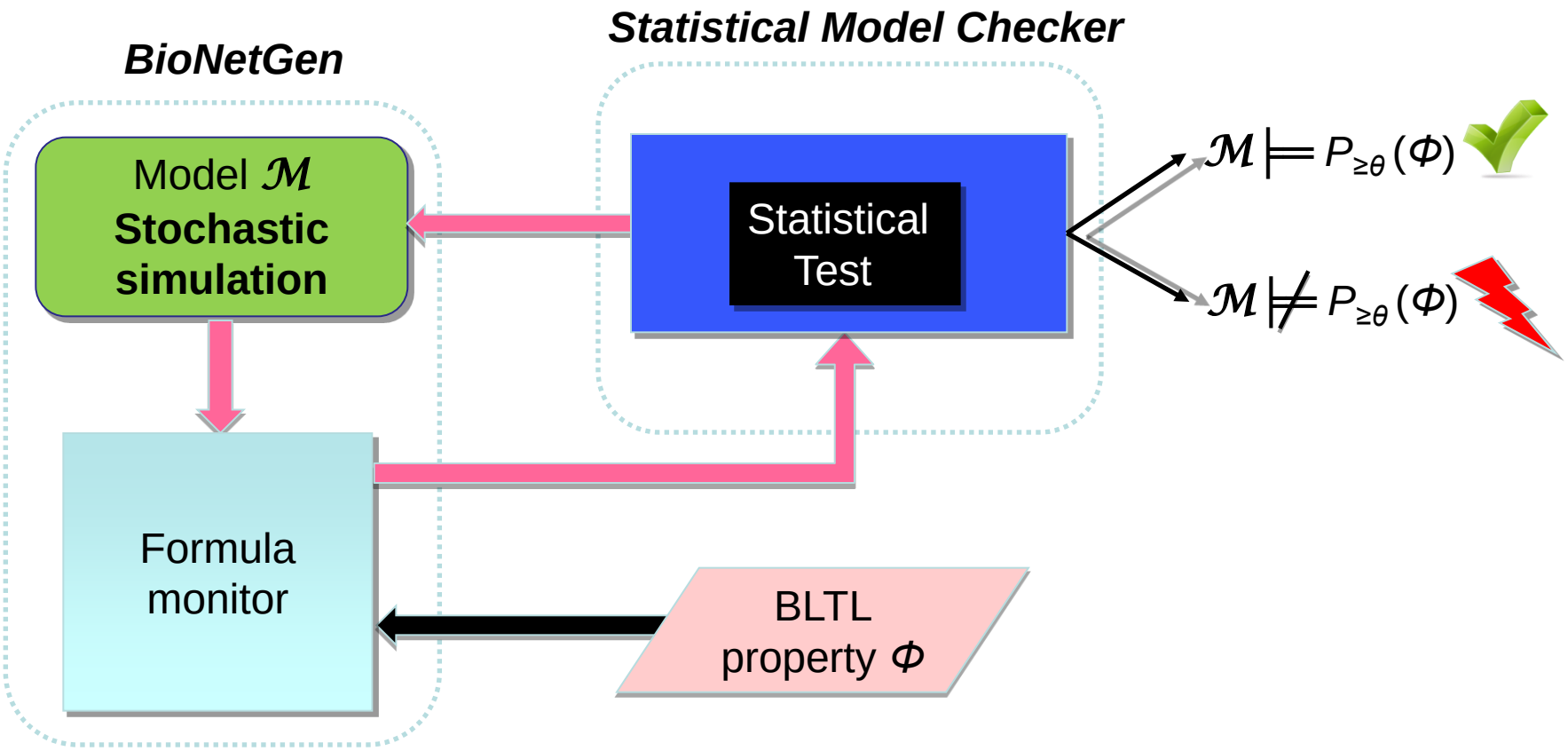
$$\mathcal{M} \models P_{\geqslant\theta}(\phi)$$

- Draw a sample of system simulations and use Statistical Hypothesis Testing: Null vs. Alternative hypothesis

$$H_0 : \mathcal{M} \models P_{\geqslant\theta}(\phi) \qquad H_1 : \mathcal{M} \models P_{<\theta}(\phi)$$

Statistical Model Checking

Statistical Model Checking of biochemical models: $\mathcal{M} \models P_{\geq\theta}(\Phi)$?



Bayes Factor

- $X = (x_1, \ldots, x_n)$ a sample of Bernoulli random variables
- Prior probabilities $P(H_0)$, $P(H_1)$ strictly positive, sum to 1
- Ratio of Posterior Probabilities:

$$\frac{P(H_0|X)}{P(H_1|X)} = \frac{P(X|H_0)}{P(X|H_1)} \cdot \frac{P(H_0)}{P(H_1)}$$

Bayes Factor B

- Fix threshold $T \geq 1$ and prior probabilities $P(H_0)$, $P(H_1)$. Continue sampling until
  - Bayes Factor B > T: Accept H_0
  - Bayes Factor B < $1/T$: Reject H_0

SMC Algorithm

Require: ***Property*** $P_{\geq\theta}(\Phi)$***, Threshold*** $T \geq 1$***, Prior density*** g

$n := 0$ {*number of traces drawn so far*}

$x := 0$ {*number of traces satisfying* Φ *so far*}

repeat

  σ := draw a sample trace from BioNetGen (iid)

  $n := n + 1$

  if $\sigma \models \Phi$ **then**

    $x := x + 1$

  endif

  $\mathcal{B} := BayesFactor(n, x, g)$

until $(\mathcal{B} > T \ \vee \mathcal{B} < 1/T)$

if $(\mathcal{B} > T)$ **then**

  return "H_0 *accepted*"

else

  return "H_0 *rejected*"

endif

Verification (I)

- Overexpression of HMGB1 will induce the expression of cell regulatory protein CyclinE.
- We model checked the formula with different initial values of HMGB1, the probability error is 0.001.

$P_{\geq 0.9}\ \mathbf{F}^{600}$ (CyclinE > 900)

HMGB1	# samples	# Success	Result
10^2	9	0	False
10^3	55	16	False
10^6	22	22	True

Verification (II)

- *P53 is expressed at low levels in normal human cells.*
- $P_{\geq 0.9}\ \mathbf{F}^{t}\ (\ \mathbf{G}^{900}\ (\ p53 < 3.3 \times 10^{4}\ )\ )$

t(min)	# Samples	# Success	Result	Time (s)
400	53	49	True	597.59
500	23	22	True	271.76
600	22	22	True	263.79

Verification (III)

- Expression level of HMGB1 influence the 1st peak of p53's level.

$$P_{\geq 0.9}\ \mathbf{F}^{100}\ (\ p53 \geq a\ \&\ \mathbf{F}^{100}\ (\ p53 \leq 4 \times 10^4\ )\ )$$

HMGB1	a (x 10^4)	# Samples	# Success	Result	Time (s)
10^3	5.5	20	3	False	29.02
10^2	5.5	22	22	True	19.65
10^2	6.0	45	12	False	56.27
10	6.0	38	37	True	41.50

Verification (IV)

- HMGB1 can activate PI3K, RAS and AKT in large quantities
- Let PI3Kr, RASr, and IKKr be the fraction of activated molecules of PI3K, RAS, and IKK, respectively
- We model checked the formula:

$$P_{\geq 0.9}\ \mathbf{F}^{t}\ \mathbf{G}^{180}\ (\text{PI3Kr} > 0.9\ \&\ \text{RASr} > 0.8\ \&\ \text{IKKr} > 0.6\ )$$

t (min)	# Samples	# Success	Result	Time (s)
90	9	0	False	21.27
110	38	37	True	362.19
120	22	22	True	214.38

Verification (V)

- Coding oscillations of NFkB in temporal logic
- Let R be the fraction of NFkB molecules in the nucleus

$P_{\geq 0.9}\ \mathbf{F}^{t}(R \geq 0.65\ \&\ \mathbf{F}^{t}(R < 0.2\ \&\ \mathbf{F}^{t}(R \geq 0.2\ \&\ \mathbf{F}^{t}(R < 0.2))))$

HMGB1	t (min)	# Samples	# Success	Result	Time (s)
10^2	45	13	1	False	76.77
10^2	60	22	22	True	111.76
10^2	75	104	98	True	728.65
10^5	30	4	0	False	5.76

Conclusions

- Computational model qualitatively confirmed the previous HMGB1 experimental phenomena.
- Our simulations predict a dose-dependent p53, CyclinE, and NFkB response curve to increasing HMGB1stimulus.
- Statistical Model Checking automatically validate our model with respect to known experimental results.

Future Work

- Parameter estimation
- Combine Machine Learning (Bayesian Network) and Model Checking to infer Gene Regulatory Network
- Multi-cellular systems
- Pancreatic stellate cells

Acknowledgments

- This work supported by the NSF Expeditions in Computing program

- Thanks to Michael T. Lotze (University of Pittsburgh) for calling our attention to HMGB1

- Thanks to Marco E. Bianchi (Università San Raffaele) for discussions on HMGB1

Thank you!

Questions?