Model Checking and Pancreatic Cancer Research

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Joint work with

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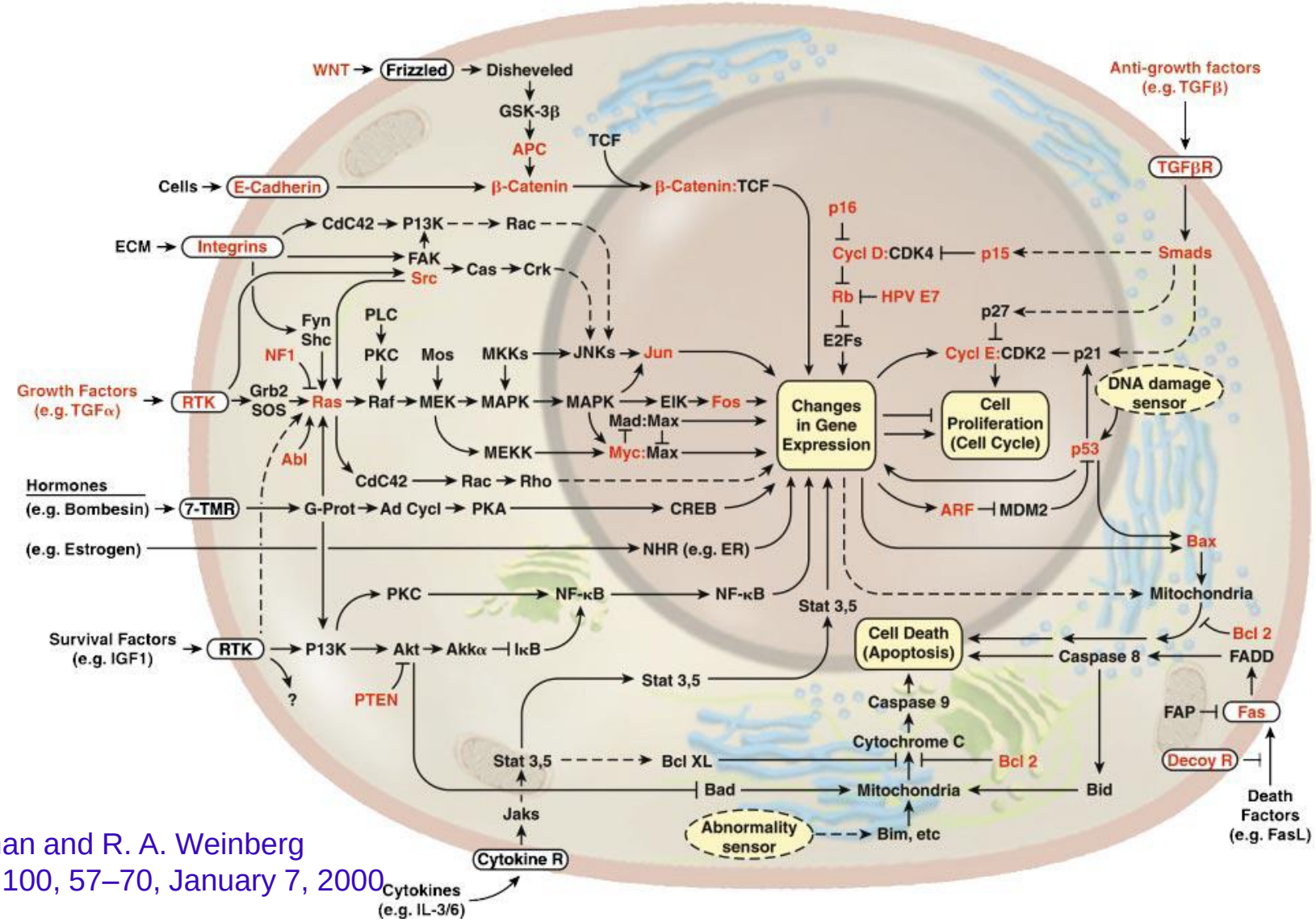
The Hallmarks of Cancer



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

Contents

1. **Statistical** Model Checking of Pancreatic Cancer Models (*2 published papers*)
   - HMGB1 Signaling Pathway Model

2. **Symbolic** Model Checking of Pancreatic Cancer Models (*2 published papers and 1 submitted paper*)
   a) HMGB1 Model (Inflammation/Necrosis)
   b) Diabetes-Cancer Model
   c) Frequently Mutated Pathways Model

HMGB1 and Pancreatic Cancer Model

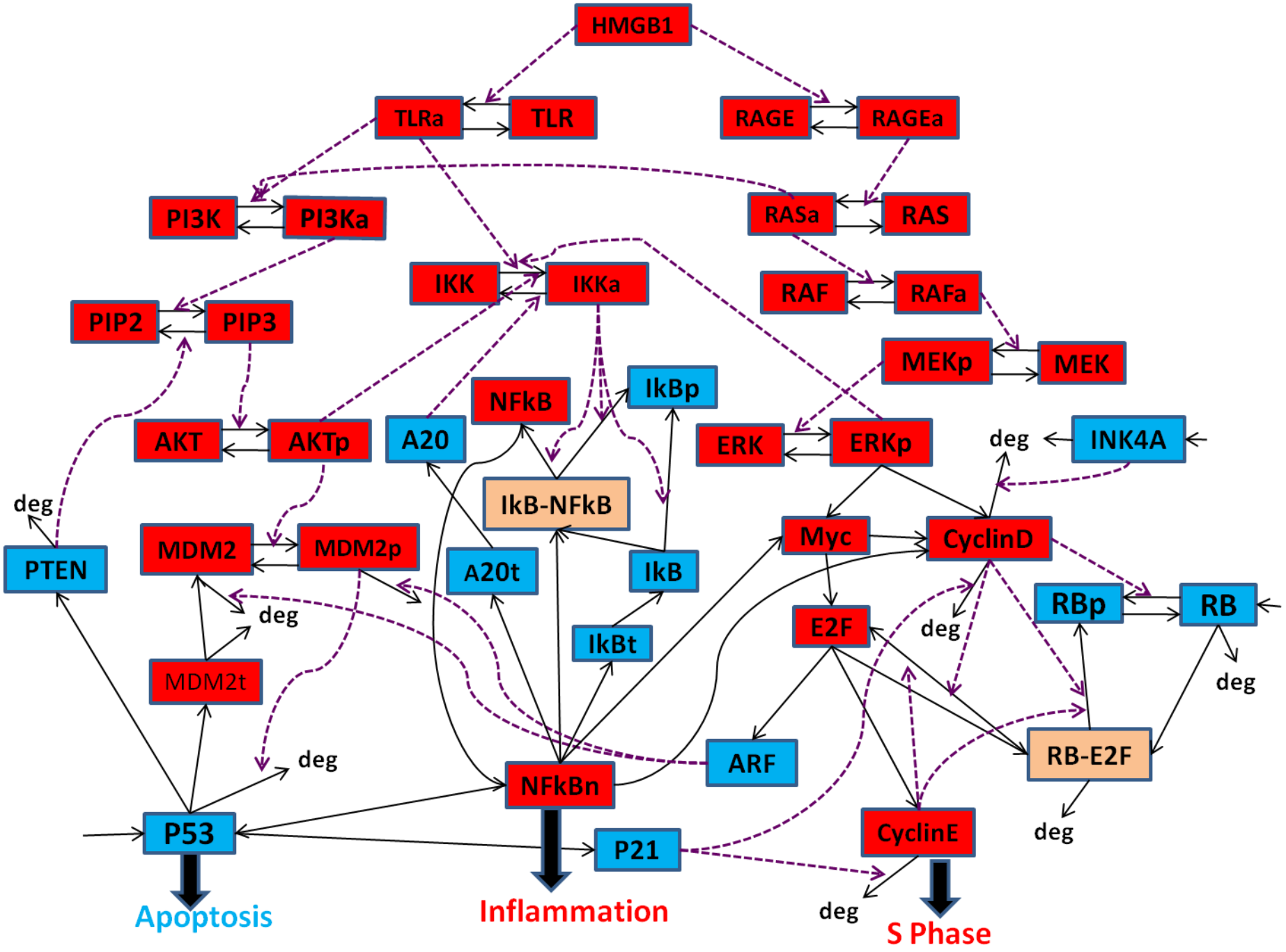
- The first complete computational model of HMGB1 signal transduction in tumorigenesis.
- Crosstalk of p53, RAS, NFkB & RB signaling pathways.
- More details in "***Analysis and Verification of the HMGB1 Signaling Pathway***". *BMC Bioinformatics 11 (Suppl 7)* (2010);
- *Best Paper Award* at the ***International Conference on Bioinformatics***, Tokyo, Japan (2010).
- "***Computational Modeling and Verification of Signaling Pathways in Cancer***". *In Algebraic and Numeric Biology* (2010).

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

- High-Mobility Group Protein 1 (HMGB1):
  - DNA-binding protein and regulates gene transcription
  - released from damaged or stressed cells, etc.

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.

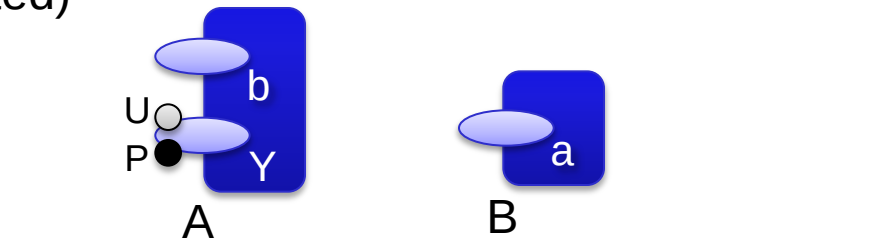

HMGB1
TLRa
TLR
RAGE
RAGEa
PI3K
PI3Ka
RASa
RAS
IKK
IKKa
RAF
RAFa
PIP2
PIP3
MEKp
MEK
NFkB
IkBp
AKT
AKTp
A20
ERK
ERKp
deg
INK4A
deg
IkB-NFkB
PTEN
MDM2
MDM2p
A20t
IkB
Myc
CyclinD
RBp
RB
E2F
deg
deg
IkBt
MDM2t
deg
ARF
RB-E2F
deg
NFkBn
P53
P21
CyclinE
deg
Apoptosis
Inflammation
S Phase

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

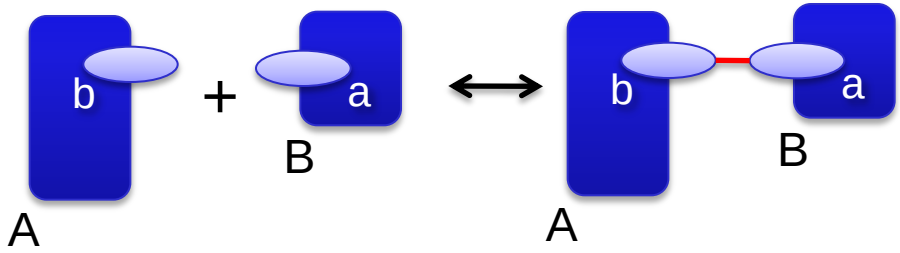


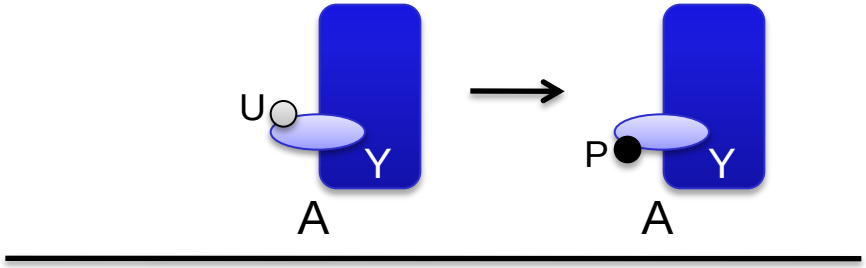
begin reaction rules

```
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 (Note: PIP(c~p) = PIP3)

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

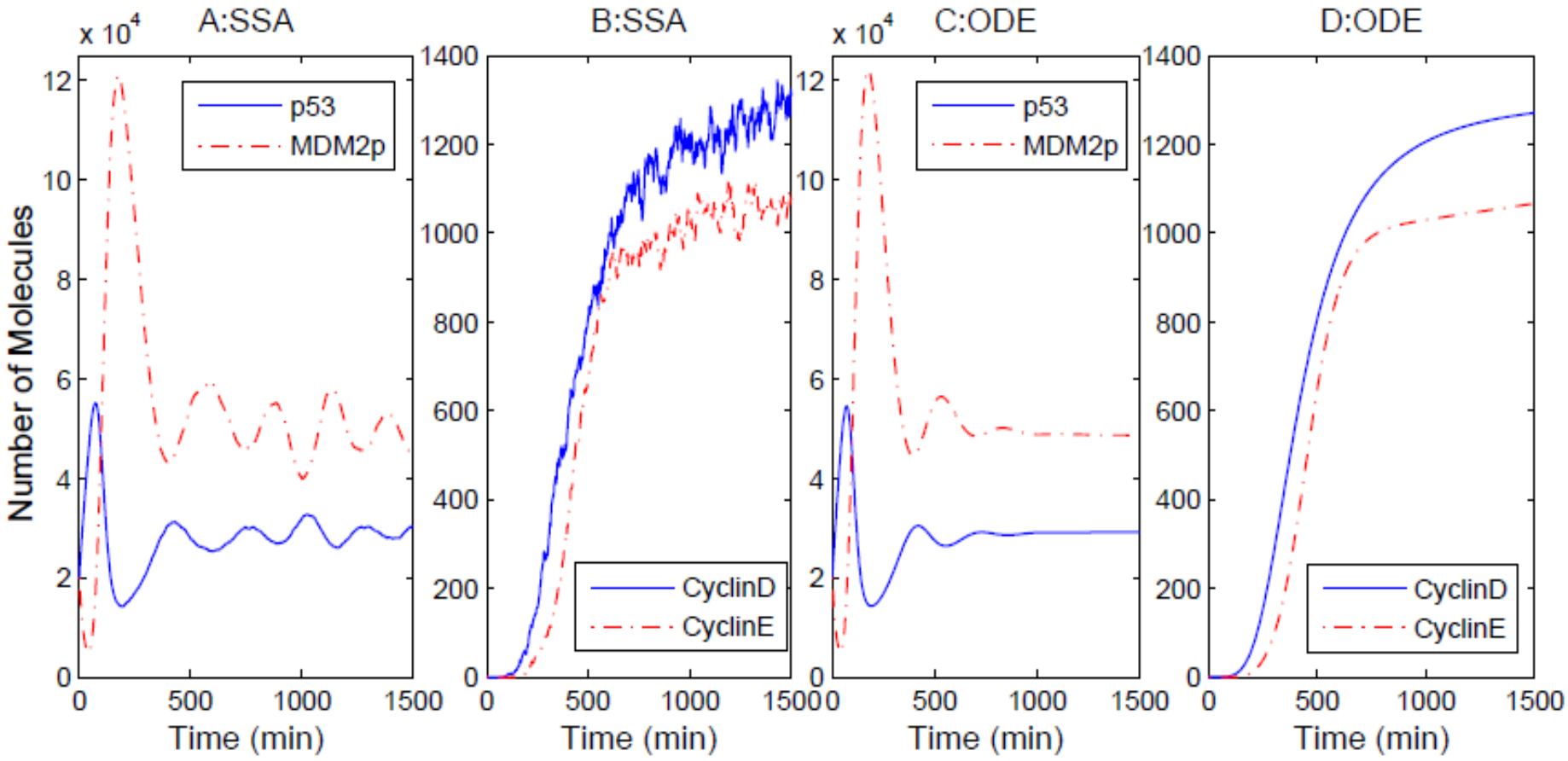
end reaction_rules

- The corresponding ODE is:

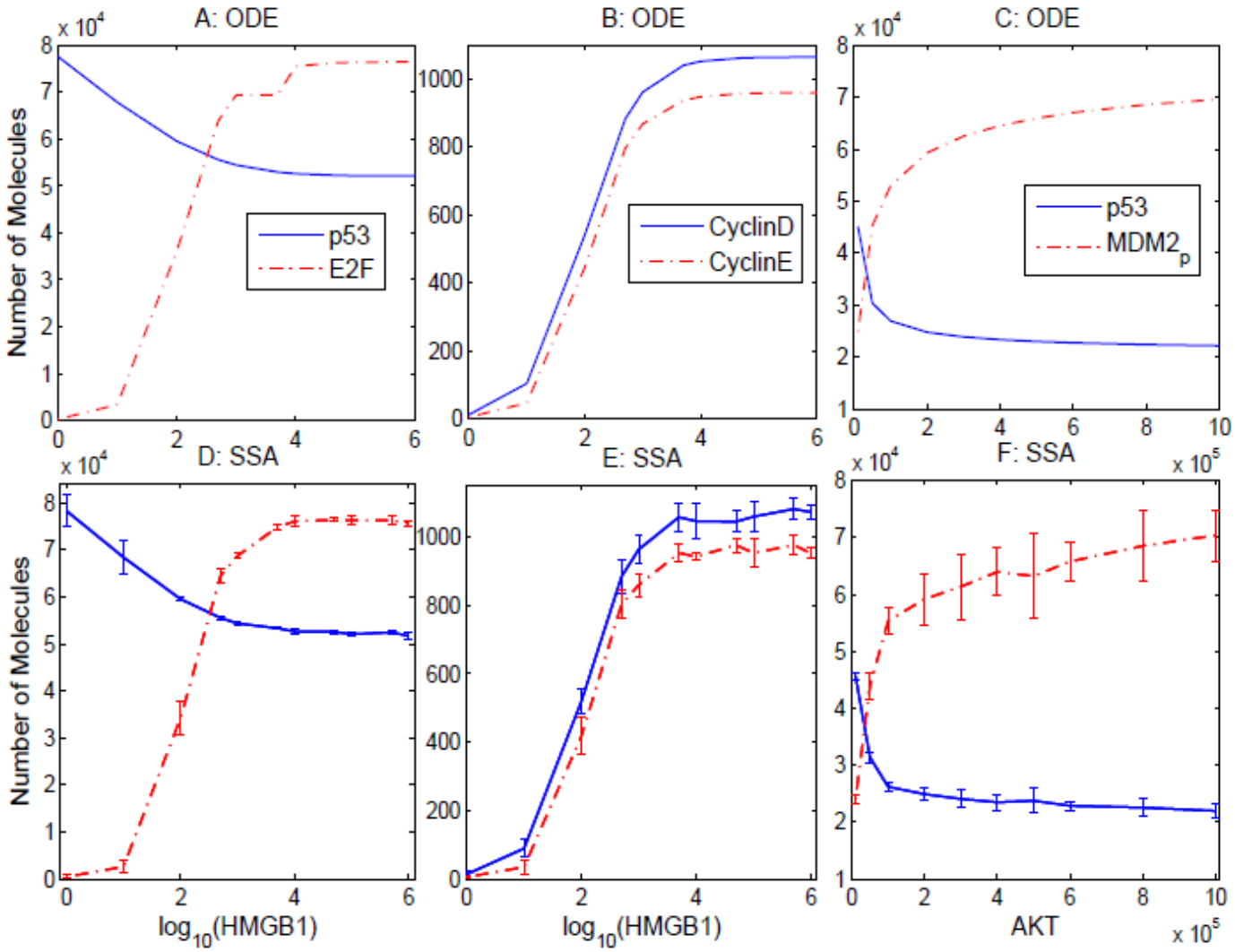
$$\frac{d[\mathrm{AKT}(d \sim p)](t)}{dt} = k\cdot[\mathrm{PIP}(c\sim p)](t)\cdot[\mathrm{AKT}(d\sim U)](t) - d\cdot[\mathrm{AKT}(d\sim p)](t)$$

Simulations (I)

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



Simulations (II)



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

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* "

- Example: "does the number of AKTp molecules reaches 4,000 within 20 minutes"

$$\mathbf{F}^{20}\ (\text{AKTp} \geq 4{,}000)$$

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

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

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)

- Coding oscillations of NFkB in temporal logic
- R is 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

Contribution I

- First computational model for investigating HMGB1 and tumorigenesis; it agrees well with HMGB1 experiments.
- Our model suggests a dose-dependent p53, CyclinD/E, NFkB response curve to increasing HMGB1 stimulus:
  - this could be tested by future experiments
- The model can provide a guideline for cancer researchers to design new *in vitro* experiments
- Statistical Model Checking automatically validates our model with respect to known experimental results.

Part II: Symbolic Model Checking of Pancreatic Cancer Models

1. Boolean Network Model

2. Applications of Symbolic Model Checking
   I. HMGB1 Model
   II. Diabetes-Cancer Model
   III. Frequently Mutated Pathways Model

3. Contribution II

Boolean Network Model

1. **Boolean network:** a **graph,** *a* ***Boolean transfer function***

2. The **state** of each node is either **ON(1)** or **OFF(0).**

3. The Boolean transfer function describes the **transformation of the state of a node *from time t to t + 1*.**

4. Nodes are classified as ***activators*** *or* ***inhibitors*.**

5. Activators can change the state of a node *n* ***if and only if no inhibitor acting* on node *n is in the ON state.***

$$n(t+1) = \{n(t) \vee \bigvee_{a \in A(n)} a(t)\} \wedge \neg\left(\bigvee_{i \in I(n)} i(t)\right),$$

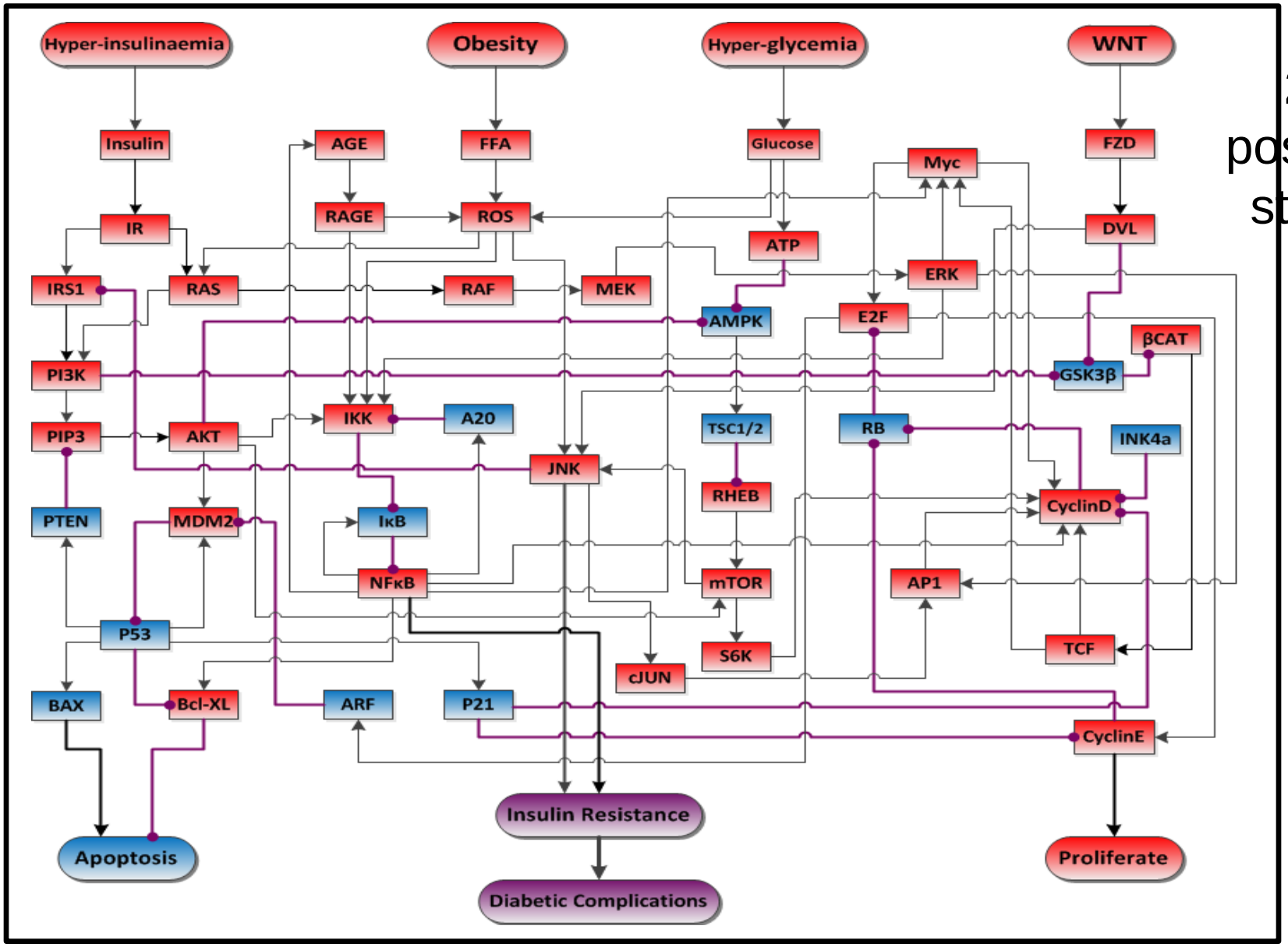
Diabetes and Pancreatic Cancer

- Diabetes: **two** major subtypes, **Type 1**, and **Type 2** (over **90%** of the diabetes population)

- Type 2 diabetes is characterized by
  - **hyperglycemia**,
  - **hyper-insulinaemia** caused by **insulin resistance** or treatment
  - activation of the **WNT pathway**.

- In **Type 2** diabetes patients the **risk for pancreatic**, colon, and breast cancer **grows by 50%,** 30%, and 20%.

Diabetes-Cancer Model



2^{49} possible states

Question 1 and Answer

- ***Question 1****: Do diabetes risk factors influence the risk of cancer or cancer prognosis?*

Property 1 : AF(Proliferate); ***Property 1' : EF(Proliferate);***

Property 2 : AF(Apoptosis); ***Property 2' : EF(Apoptosis);***

Property 3 : AF(Resistance); ***Property 3' : EF(Resistance);***

- ***Normal Cell****:* Properties 3 and 2'-3' are true. **Diabetes risk factors** can augment insulin resistance, but cell growth is still regulated by the tumor suppressor proteins. **Cancer risk might not increase.**

- ***Precancerous/cancerous cells*** *(INK4a, ARF =0):* all but Property 2 are true. **Diabetes risk factors promote growth in precancerous or cancerous cells** and augment insulin resistance.

Question 2 and Answer

- **Question 2**: *Which signaling components are common and critical to both diabetes and cancer? That is, which proteins' mutation/ knockout will promote/inhibit both cancer cell growth and insulin resistance in diabetic cancer patients?*

*AG{**RAS** → AF(Resistance & Proliferate & !Apoptosis)}*

*AG{**AKT** → AF(Resistance & Proliferate & !Apoptosis)}*

*AG{**NFkB** → AF(Resistance & Proliferate & !Apoptosis)}*

*AG{**ROS** → AF(Resistance & Proliferate & !Apoptosis)}*

*See "**Model Checking of a Diabetes-Cancer Model**", **accepted** at the 3rd International Symposium on Computational Models for Life Sciences, 2011*

Contribution II

- *"Symbolic Model Checking of Signaling Pathways in Pancreatic Cancer",* *Proceedings of the 3rd International Conference on Bioinformatics & Computational Biology, 2011*

- *"Model Checking of a Diabetes-Cancer Model",* ***accepted*** *at the 3rd International Symposium on Computational Models for Life Sciences, 2011*

- *"Formal Analysis for Logical Models of Pancreatic Cancer"*, **invited submission** to *the 50th IEEE Conference on Decision and Control and European Control Conference, 2011*

Conclusions & Future Work

- Our computational models and model checking verifications have and will continue to provide guidelines for experimental biologists to design new *in vitro* experiments in the future pancreatic cancer studies.

- The microenvironment of pancreatic cancer cells (PCC): interaction between pancreatic stellate cell and PCC (UPMC, in progress).

- Collaborated with Prof. Tongtong Wu at UMD, we have identified an **8-gene signature** for **pancreatic cancer survival** (in progress).

- Collaborated with TGEN, we are working on the EGFR pathway in pancreatic cancer. (in progress)

- Possible collaboration with UCSF Diabetes institute director, Matthias Hebrok, to study the association between diabetes & pancreatic cancer.

Acknowledgments

- This work supported by the NSF Expeditions in Computing program

- Thanks to *Marco E. Bianchi* (Università San Raffaele), *Barry Hudson* (University of Miami, Columbia University) for discussions on HMGB1

Thank you!

Questions?