
Modeling cell life and cell death in cancer

Andrei Zinovyev

“Computational Systems Biology of Cancer”

U900 Institut Curie/INSERM/Ecole des Mines Paristech, Paris, France

Institut Curie

100 years of fighting with cancer

Highlight
Most common cancer, breast cancer is a major public health issue. As a center of excellence, Institut Curie receives more than 100,000 patients a year over 6000 who are suffering from this cancer. Our research improves patient management, sets up new therapeutics and conducts research for the benefit of patients.

The Institut Curie fighting cancer
The Institut Curie continues to improve cancer treatment and research and the originality of the Curie model to support the excellence of patient management. The Institut Curie gathers scientists and nurses supporting the same ambition "Together, let's beat cancer". Accredited as a public service body since 1921, the Institut Curie profits from the generosity of the public by donation, legacy and sponsorship.

Editorial of the Director

Presentation of the Research Center

Scientific activities

- Research units
- Groups
- Technological Equipment and Platforms
- Incentive and Cooperative Programs

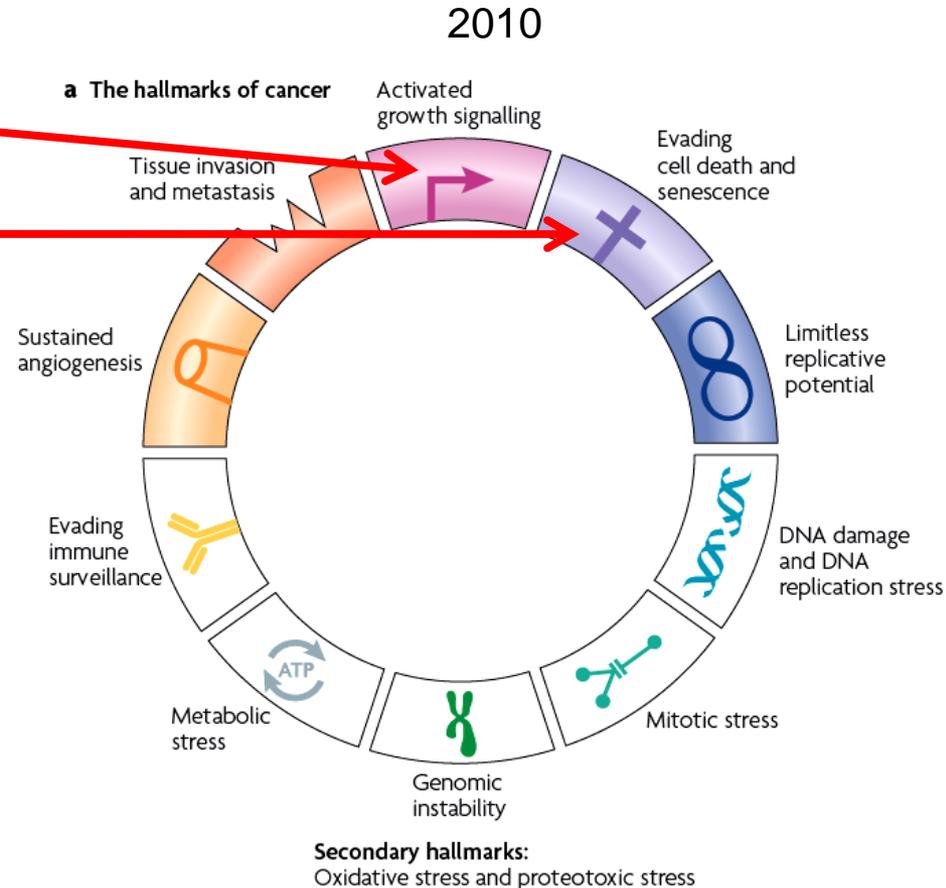
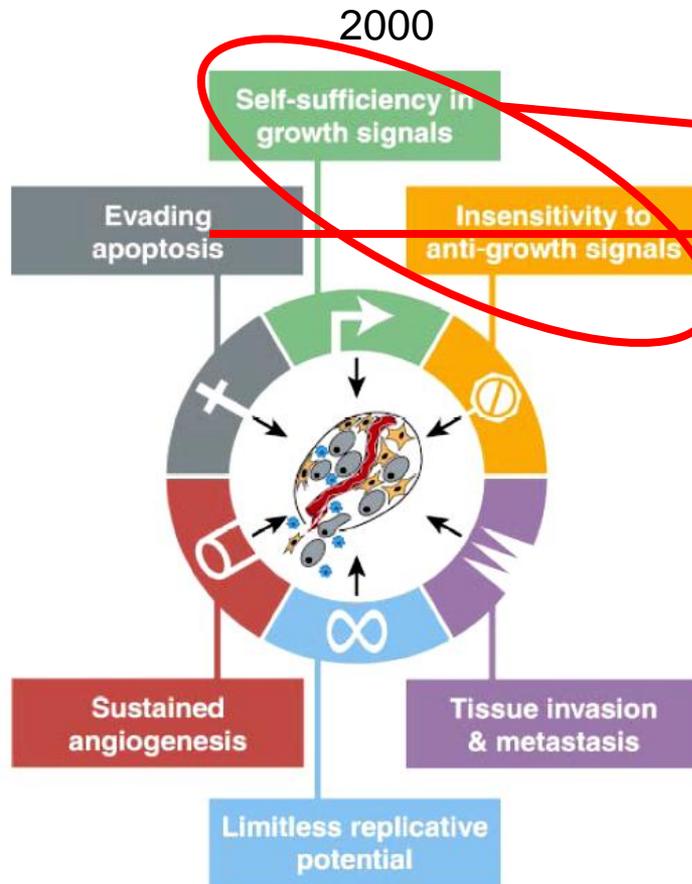
Education

Researcher's

Computational Systems Biology of Cancer

Home	People	News	Projects	Call for positions	Publications	Software	
	Emmanuel Barillot, PhD Director of the U900 Institut Curie/INSERM/Ecole de Mines ParisTech Data integration, Systems Biology of Cancer, Dynamics of network motifs		Valentina Boeva, PhD SITCON project, next generation sequencing Genomic sequence analysis		Laurence Calzone, PhD APO-SYS and CALAMAR projects Cell-cycle modeling		Gautier Stoll, PhD SITCON project Pathway qualitative modeling
	Thomas Fink, PhD Dynamics of network motifs Information theory for data analysis		Simon Fourquet, PhD APO-SYS project Systems Biology of Apoptosis		Loredana Martignetti, PhD SITCON project Regulatory sequence analysis		Antonio Cappucco, PhD ANR "Skin TSLP" project Systems immunology
	Inna Kuperstein, PhD Curie-Servier alliance on basal breast cancer Systems biology of cancer		Paola Vera-Licona, PhD Curie-Servier alliance on basal breast cancer Mathematical modeling of biological networks		Andre Zinovyev, PhD Scientific coordinator of the Systems Biology team Systems Biology of Cancer, Complexity and Model reduction		

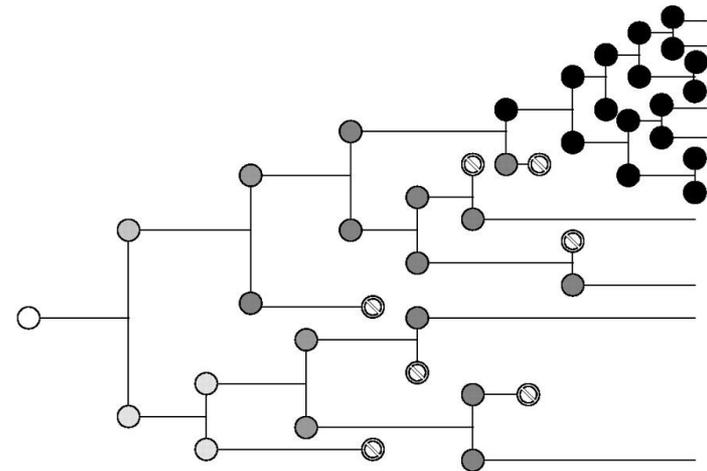
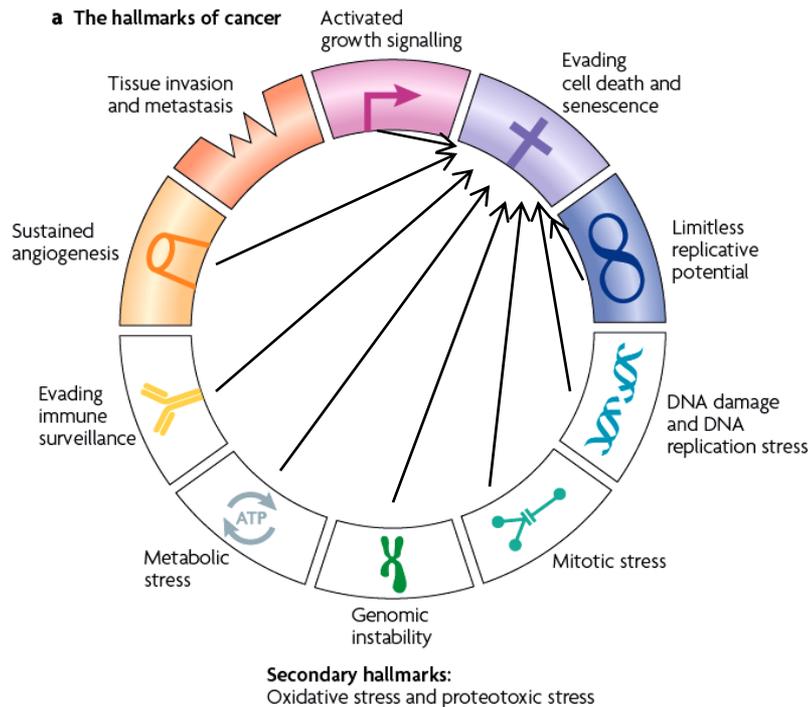
Hallmarks of cancer



Hanahan and Weinberg, 2000, Cell

Negrini et al, 2010, Nat Rev Mol Cell Bio

Cell life/death decisions in cancer



APO-SYS: First EU FP7 large-scale project on systems biology of cancer

The image shows a screenshot of the APO-SYS Consortium website. The browser address bar displays 'apo-sys.eu'. The page title is 'APO-SYS Consortium'. The main content area features the text: '... The principal objectives of APO-SYS consortium are to understand the basic cell biology of apoptosis and to transform this knowledge into computer models of the relevant biological processes...'. Below this text, two boxes are overlaid: a green box labeled 'Experimentalists' and a red box labeled 'Computational teams'. A map of Europe is shown with various institutions marked by colored boxes: green for experimentalists and red for computational teams. The institutions marked include: RCS (UK), VIT (Finland), DCS (Denmark), SDU (Denmark), EMBL (Germany), MPG (Germany), VIB (Belgium), CERSM (Belgium), DKFZ (Germany), UUM (Germany), INSERM (France), UNIL (Switzerland), UCBL (France), UNIMED (Italy), IMB (Italy), INMI (Italy), SUNAP (Italy), and WIS (Israel). The map also shows other countries like Norway, Sweden, Finland, Poland, Czech Republic, Slovakia, Hungary, Romania, Bulgaria, Greece, and Turkey. The scale is 1:19,500,000. The page footer includes 'AIDS' and 'Cancer' logos, and the 'MINES PARIS' logo.

Apo-SYS Consortium

Apoptosis Systems Biology

An integrated approach of e system engineering and mo

Home Objectives Members Anno

Home

The APO-SYS Consortium

The APO-SYS team is the extension of a 2006 D Kroemer) that involves the coordinator of the pr Piacentini, Josef Penninger and Klaus-Michael De uniting a critical mass of investigators specialized in solve important, disease-relevant problems by a syst theoretical part (Emmanuel Barillot, Ralf Herwig/P ("European Systems Biology Initiative for combatin APO-SYS consortium involves a BioTech company t The APO-SYS consortium aims at obtaining major p a series of systems biology approaches, in alico, in t acquired on tissue samples from patients suffering

... The principal objectives of APO-SYS consortium are to understand the basic cell biology of apoptosis and to transform this knowledge into computer models of the relevant biological processes...

Experimentalists

Computational teams

RCS

VIT

DCS

SDU

EMBL

MPG

VIB

CERSM

DKFZ

UUM

INSERM

UNIL

UCBL

UNIMED

IMB

INMI

SUNAP

WIS

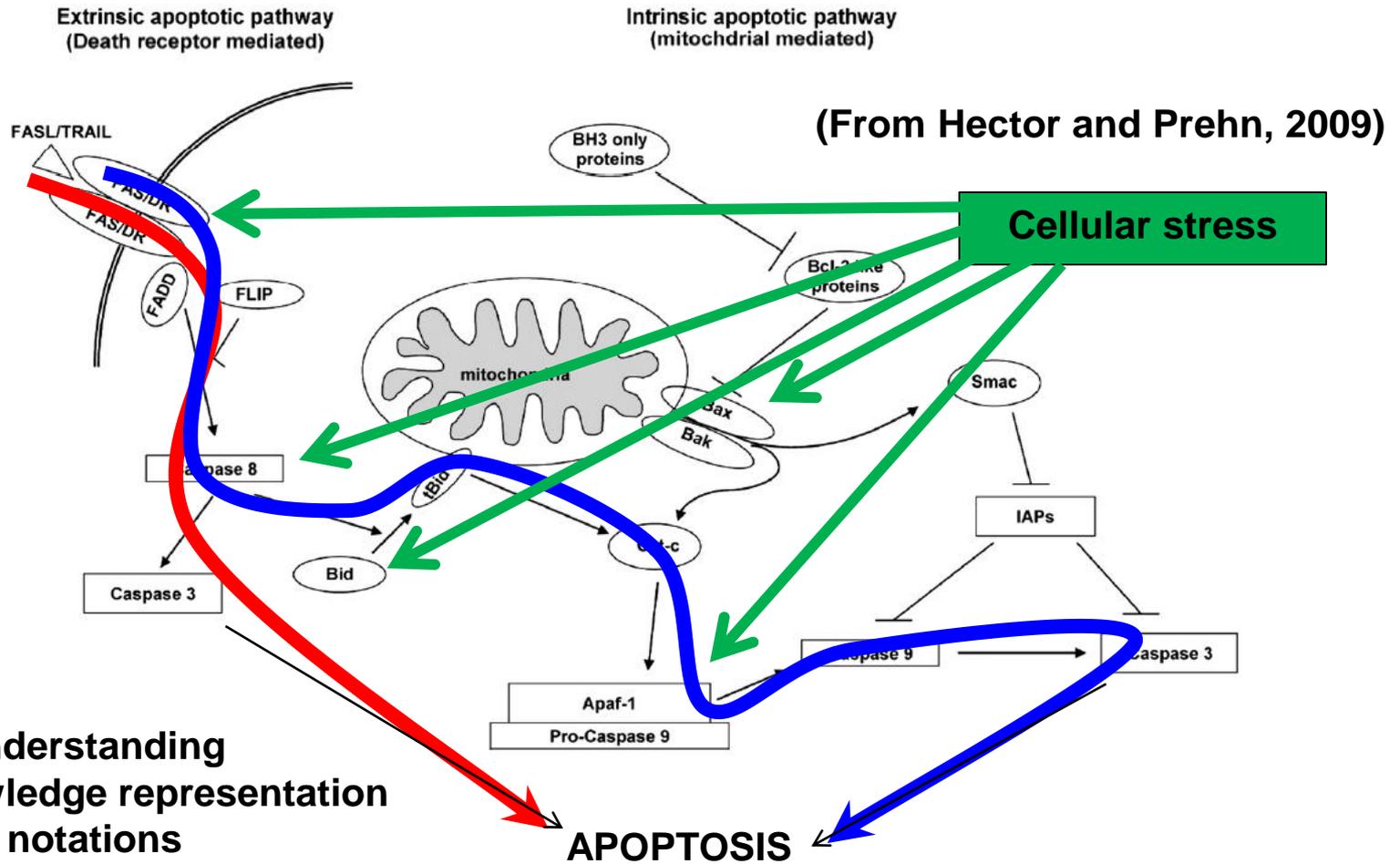
TAU

Scale 1:19,500,000
Lambert Conformal Conic Projection
standard parallels 40°N and 56°N

AIDS Cancer

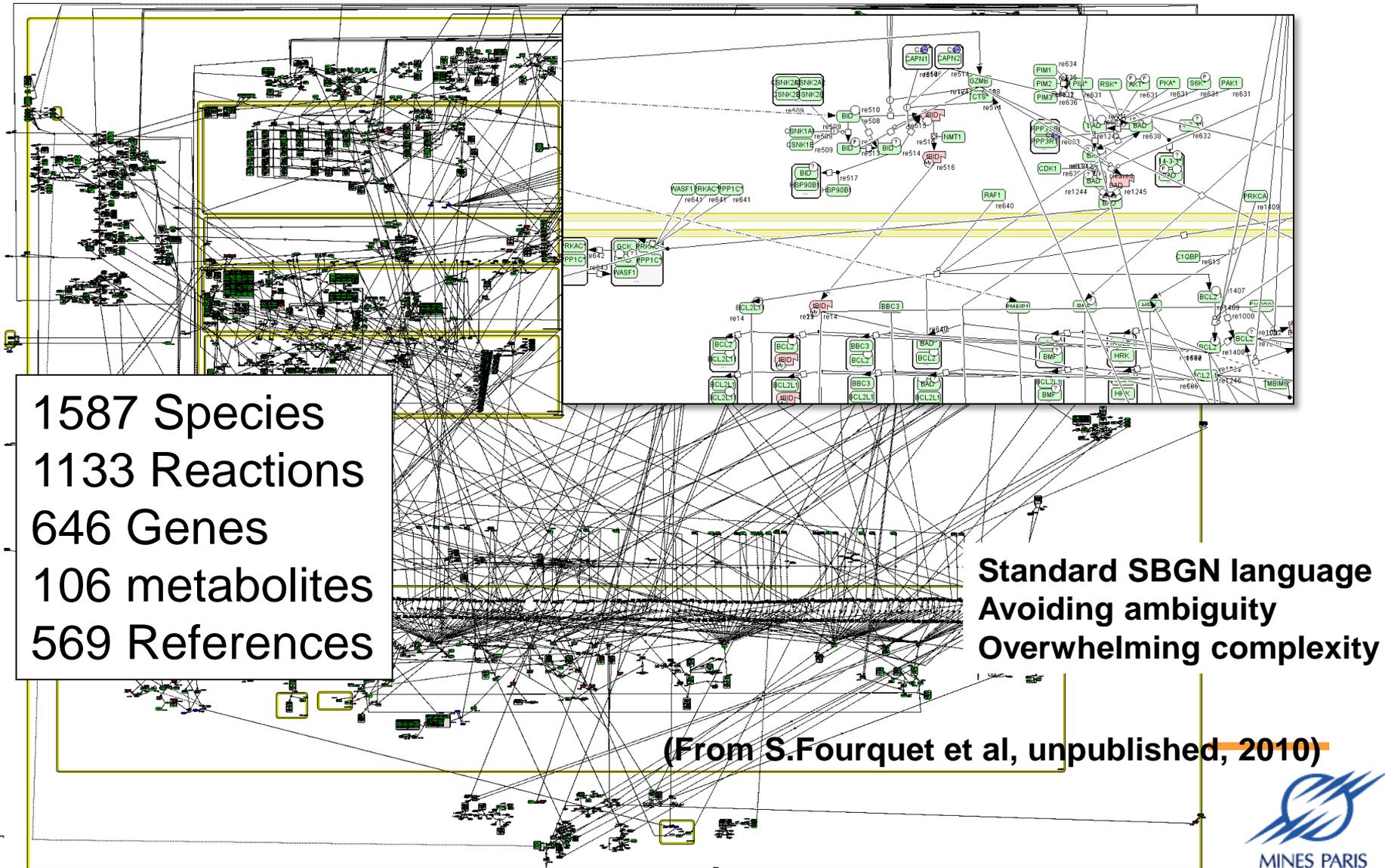
MINES PARIS

A textbook view on apoptosis



Illusion of understanding
Art-like knowledge representation
Ambiguity in notations

A systems biologist's view on apoptosis



1587 Species
1133 Reactions
646 Genes
106 metabolites
569 References

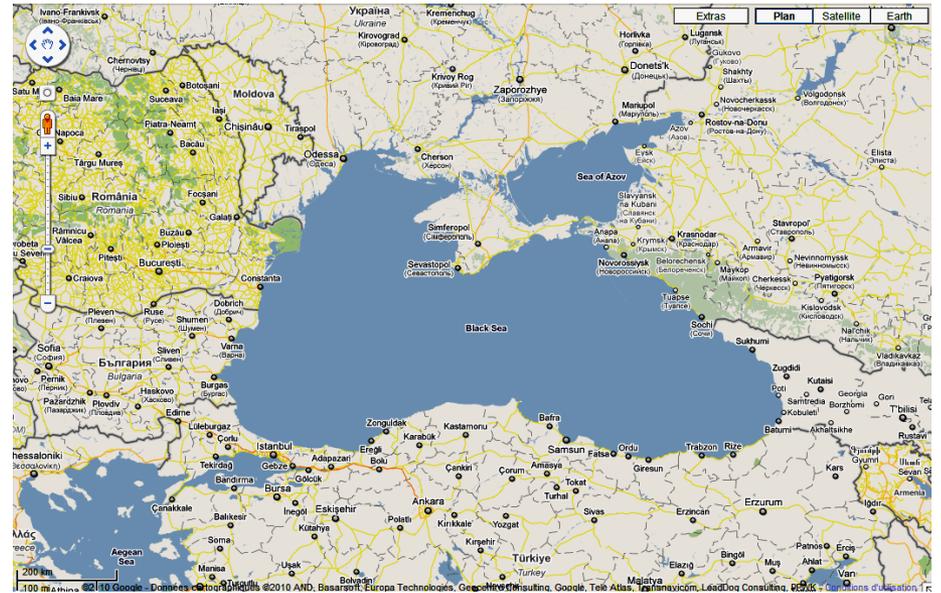
Standard SBGN language
Avoiding ambiguity
Overwhelming complexity

(From S.Fourquet et al, unpublished, 2010)

Map and Map



1559



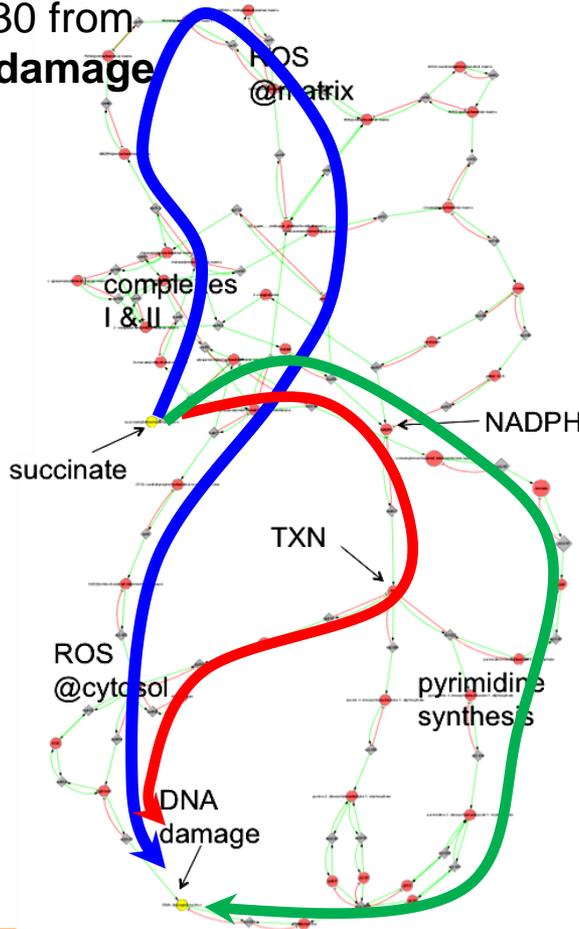
2000

Role of comprehensive map

- It is a territory map: all that is possible
- It is an interactive encyclopedia of the domain
- It is a formal knowledge representation
- It is connected to ~600 most significant publications
- It is accessible to computer analysis
- It allows to formulate hypotheses
- It allows to focus on specific problems and **make mathematical models**

Using the cell death map: listing hypotheses

All path of length <30 from
succinate to DNA damage



Through ROS formation by the
respiratory chain

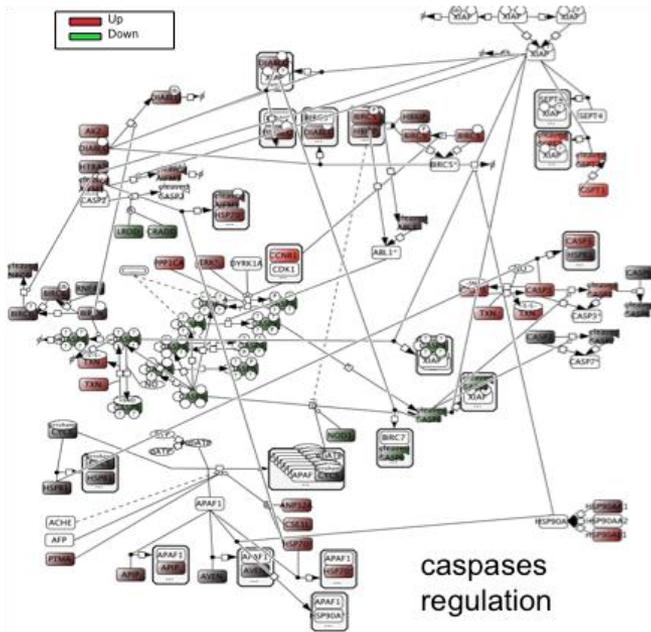
Through transfer of the reductive
equivalents of succinate to NADPH and
thioredoxin, then ROS detoxification
or RNR activity and DNA repair

Through reduction of ubiquinone, the
oxidative equivalents of which are
necessary for pyrimidine biosynthesis
and DNA repair

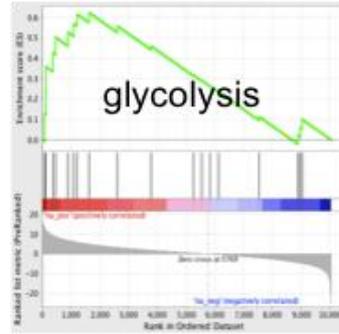
(see Khutornenko AA et al., PNAS, 2010,107,12828)

Using the cell death map: map high-throughput data

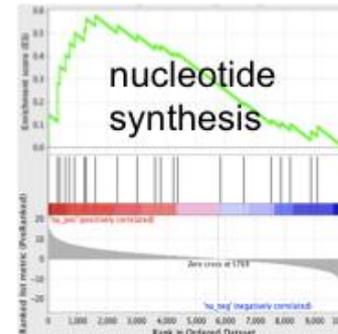
Basal breast cancer gene expression compared to healthy adipocytes



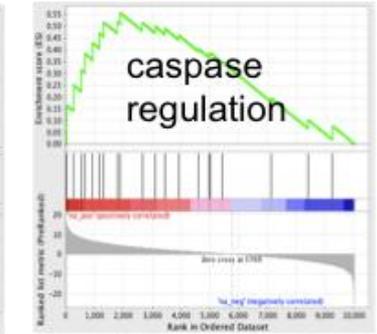
Map high-throughput data and infer “differentially deregulated subnetworks”



Enrichment Score (ES)	0.62411755
Normalized Enrichment Score (NES)	1.7380258
Nominal p-value	0.0045731706
FDR q-value	0.0372127
FWER p-Value	0.026



Enrichment Score (ES)	0.5772682
Normalized Enrichment Score (NES)	1.6615664
Nominal p-value	0.009009009
FDR q-value	0.04496614
FWER p-Value	0.06

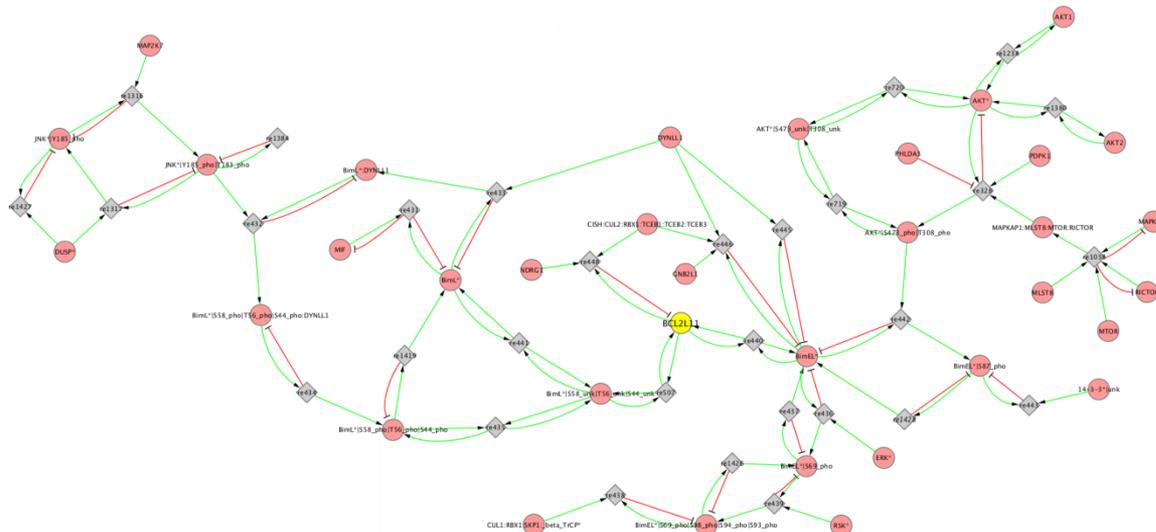
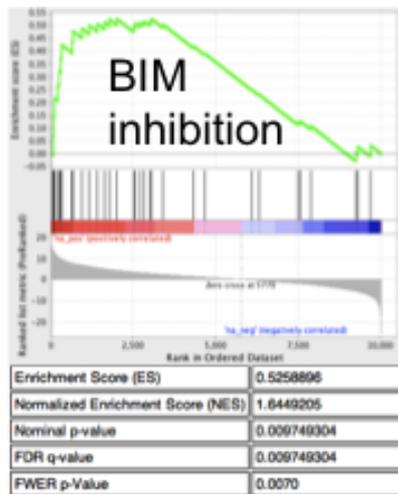


Enrichment Score (ES)	0.5580466
Normalized Enrichment Score (NES)	1.6066436
Nominal p-value	0.010324484
FDR q-value	0.055682277
FWER p-Value	0.104

➤ Glycolysis and nucleotide synthesis positive enrichment : signature of cancer metabolic adaptation – **Warburg effect**

➤ Caspase regulation : the gene set contains more inhibitors than activators of caspases – **escape from apoptosis**

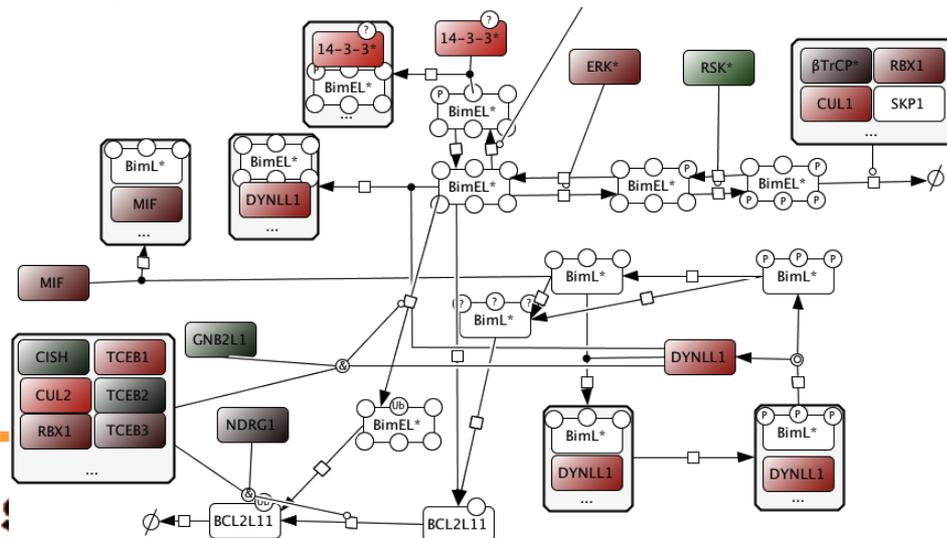
Using the cell death map: detecting hot spots of activity



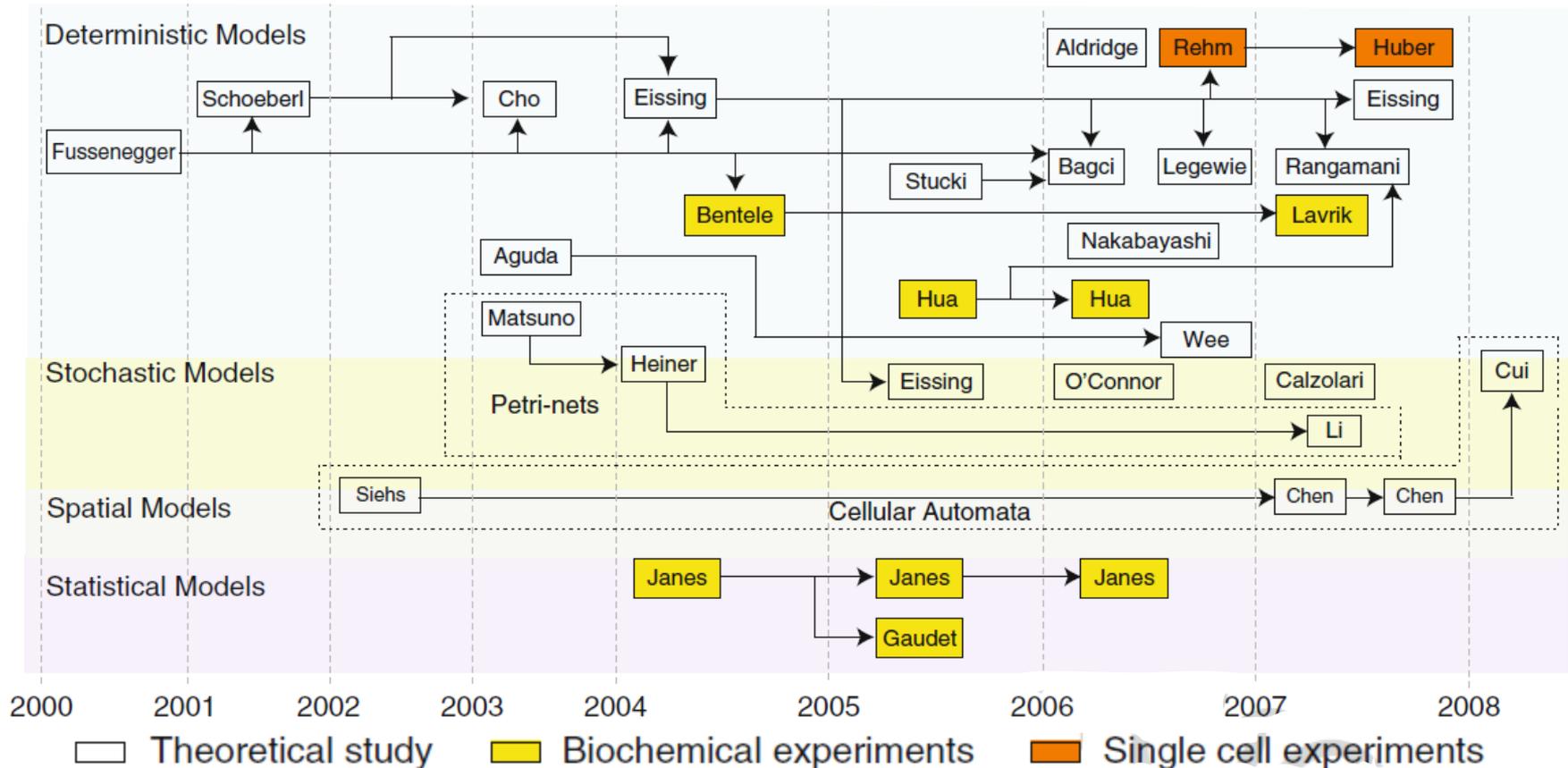
Extract of all path of length ≤ 10 ending at BIM

Identify BIM regulators and classify them as activators or inhibitors

Perform enrichment analysis taking this information into account



Systems Biology of Apoptosis



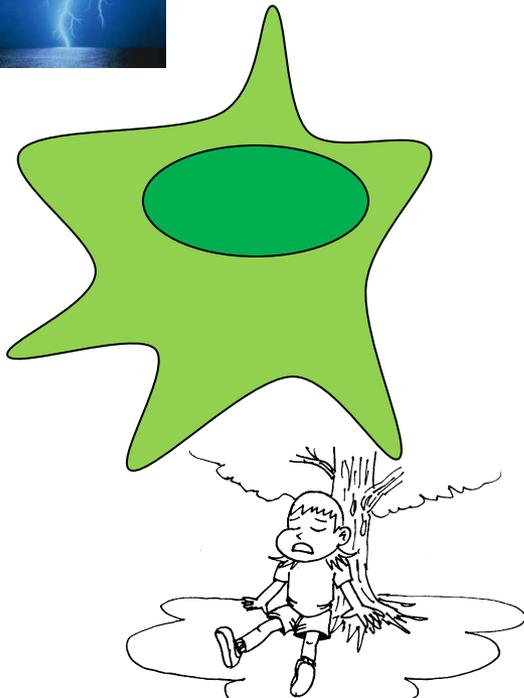
(From Huber, Bullinger and Rehm, Systems Biology Approaches to the Study of Apoptosis 2009)

“Passive” vs “active” survival



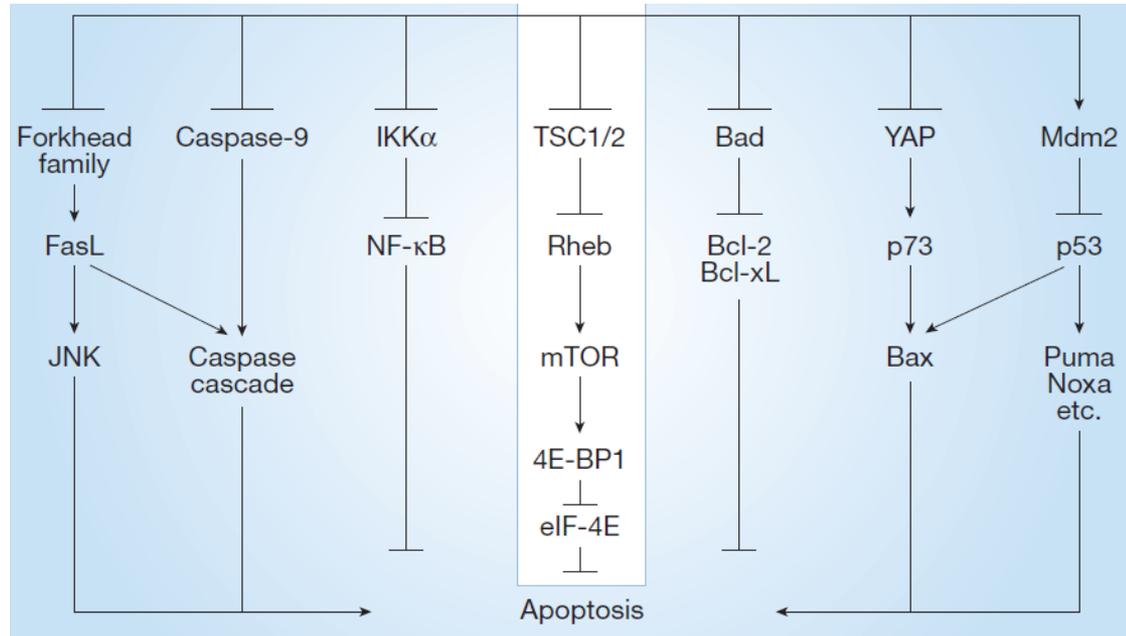
Stress

Toxic stress
DNA damage
Nutrient deprivation



Naïve resting cell

AKT Survival signalling pathways

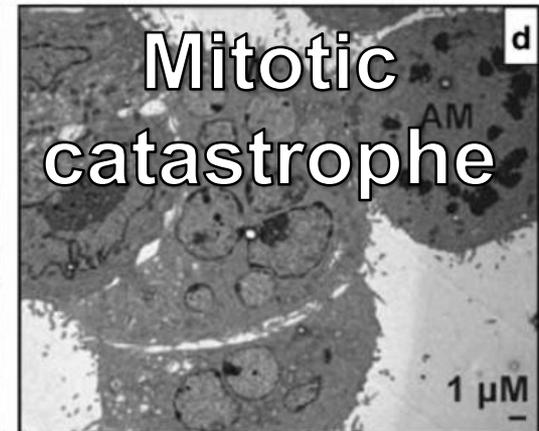
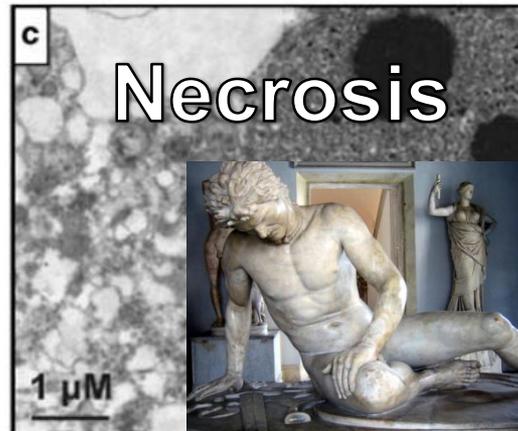
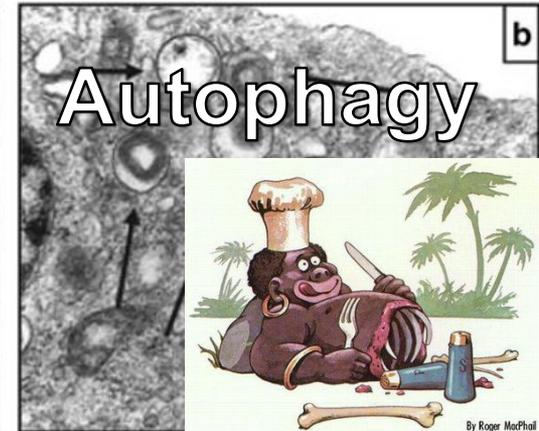
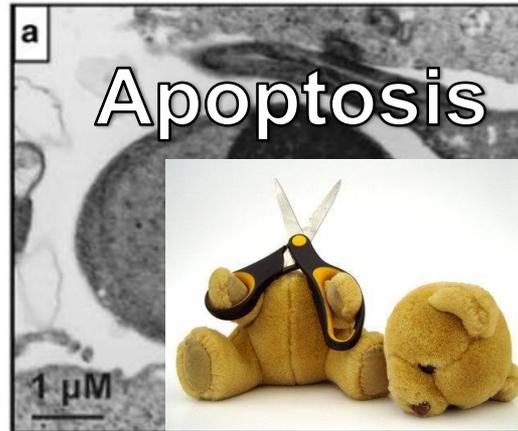


(From McCormick, Nature, 2004)

Four Faces of Cell Death

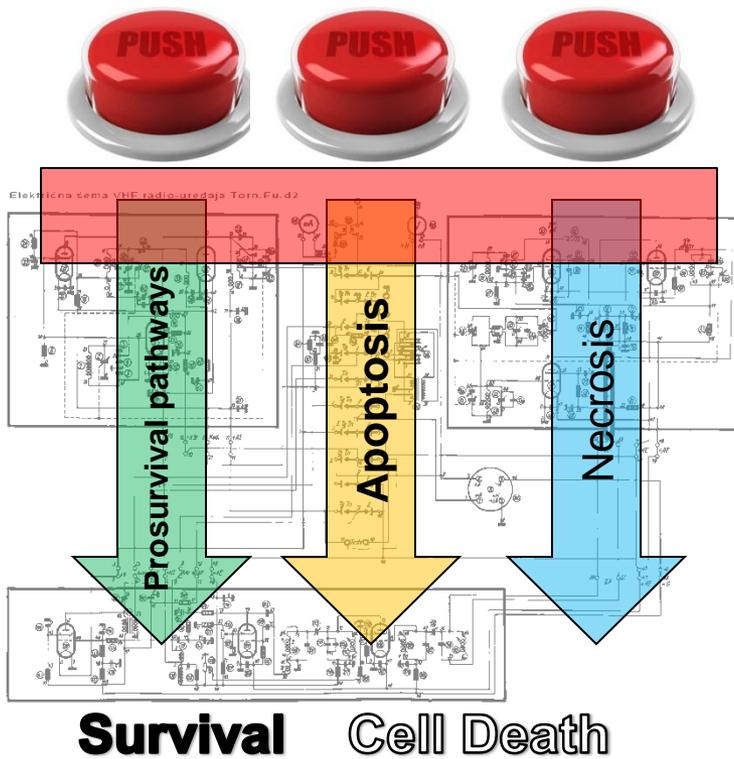
(From Galuzzi et al, Cell Death and Diff, 2007)

Cell Death Modalities

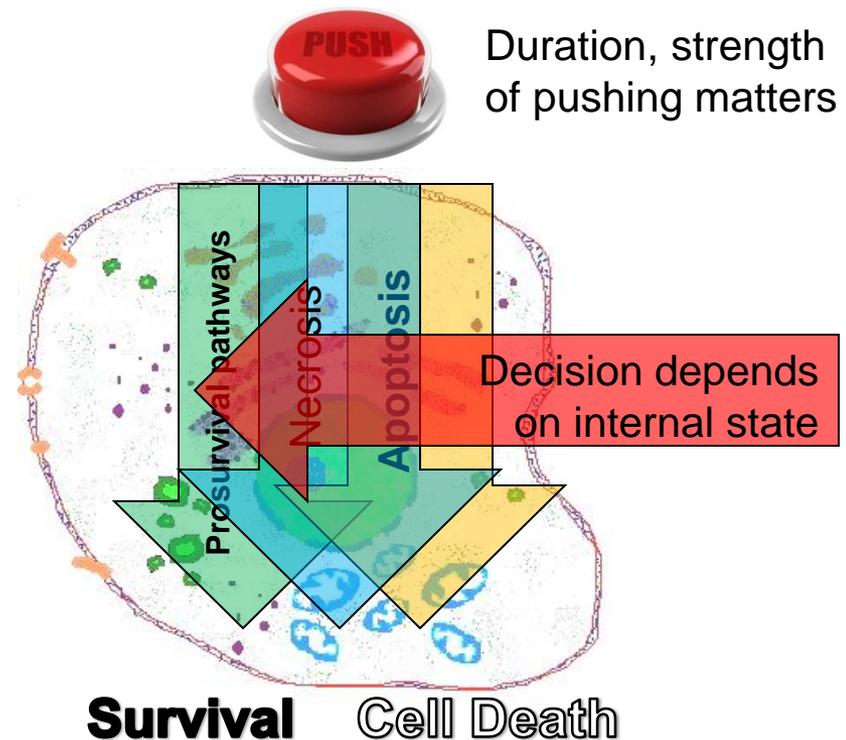


Engineering vs Biology

Engineering solution

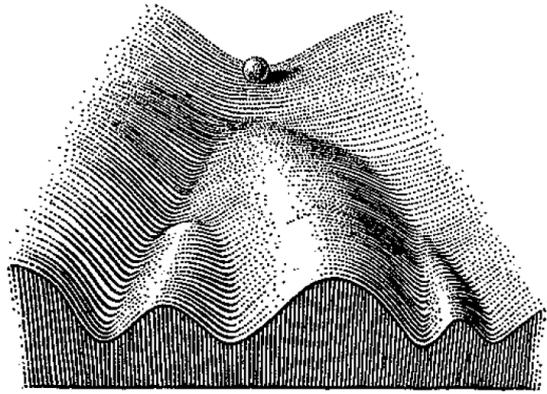


Biological solution



Cell fate decisions

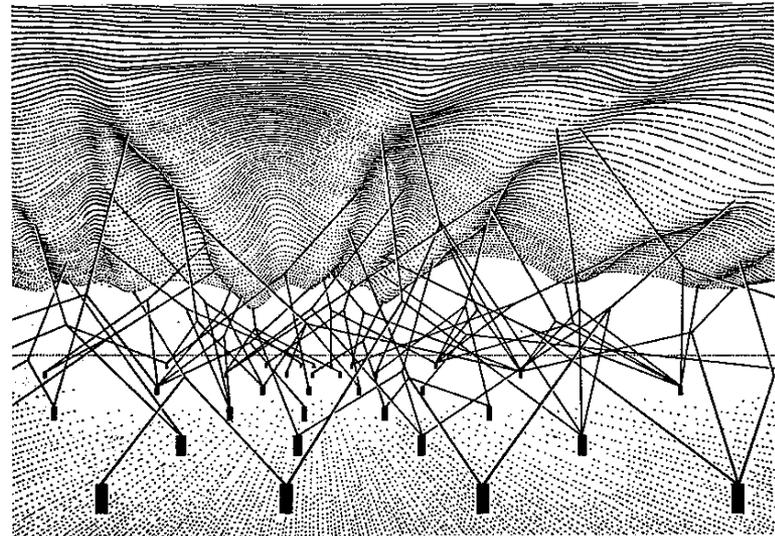
Conrad Hal Waddington, Professor of Animal Genetics at the University of Edinburgh, 1957.



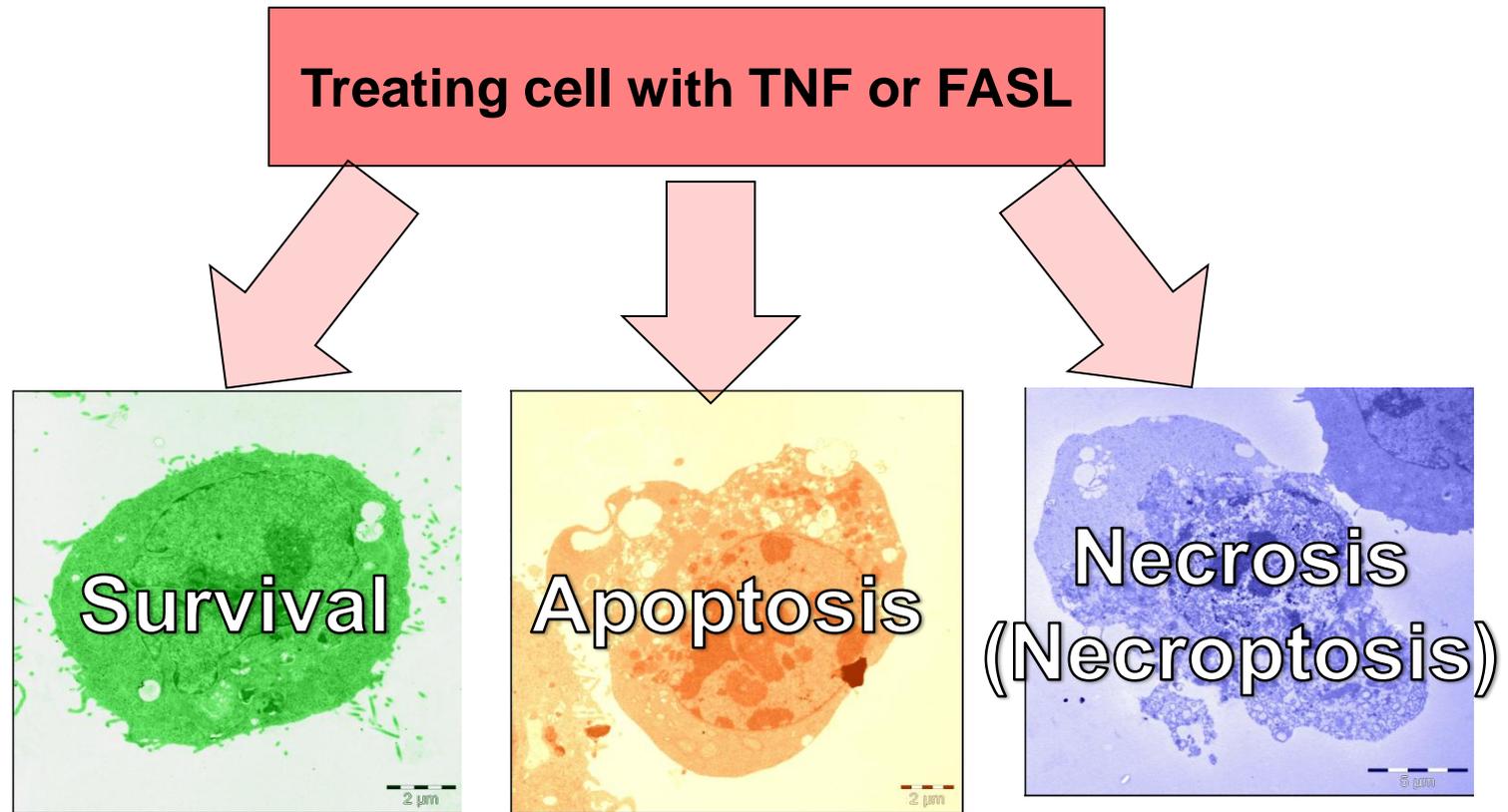
4. "Part of an Epigenetic Landscape. The path followed by the ball, as it rolls down towards the spectator, corresponds to the developmental history of a particular pa

Epigenetic landscape,
canalization

Complex system of genes,
underlying the landscape



Apoptosis vs Necrosis vs Survival



OPEN ACCESS Freely available online

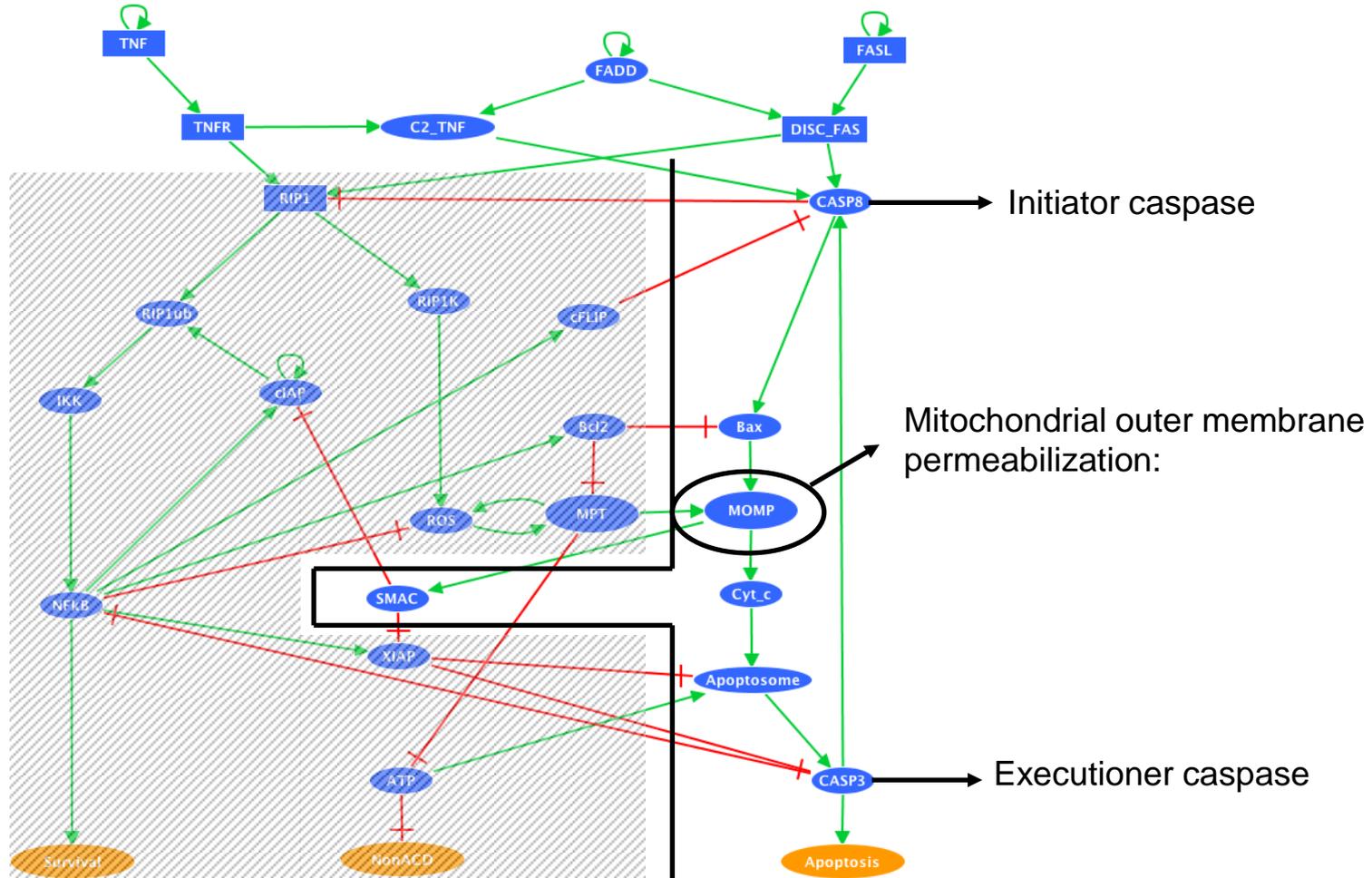
PLoS COMPUTATIONAL BIOLOGY

Mathematical Modelling of Cell-Fate Decision in Response to Death Receptor Engagement

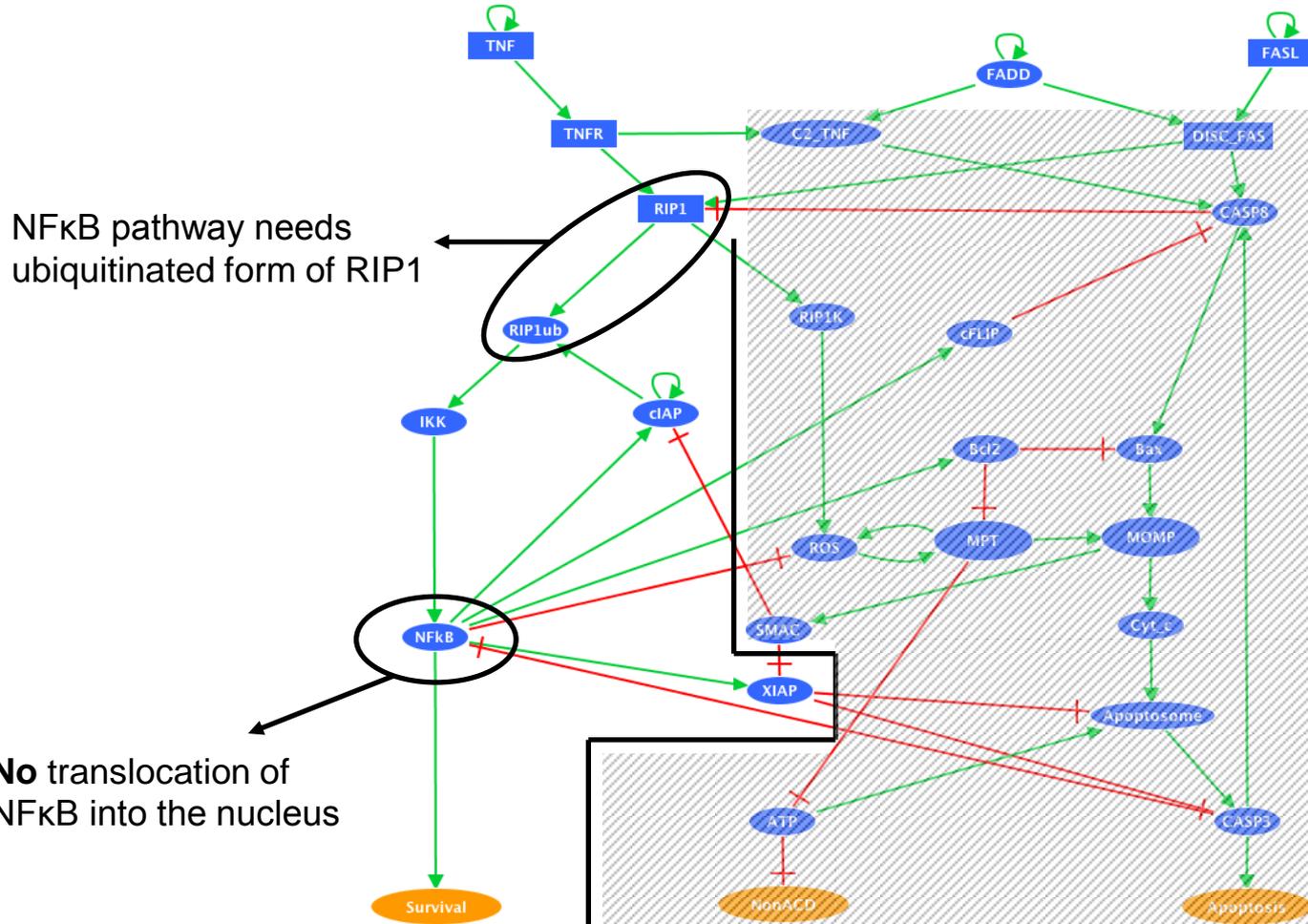
Laurence Calzone^{1,2,3*}, Laurent Tournier^{1,2,3}, Simon Fourquet^{1,2,3}, Denis Thieffry^{4,5}, Boris Zhivotovskiy⁶, Emmanuel Barillot^{1,2,3†}, Andrei Zinovyev^{1,2,3†}

¹Institut Curie, Paris, France, ²Ecole des Mines ParisTech, Paris, France, ³INSERM U900, Paris, France, ⁴TAGC – INSERM U928 & Université de la Méditerranée, Marseille, France, ⁵CONTRAINTE Project, INRA Paris-Rocquencourt, France, ⁶Karolinska Institutet, Stockholm, Sweden

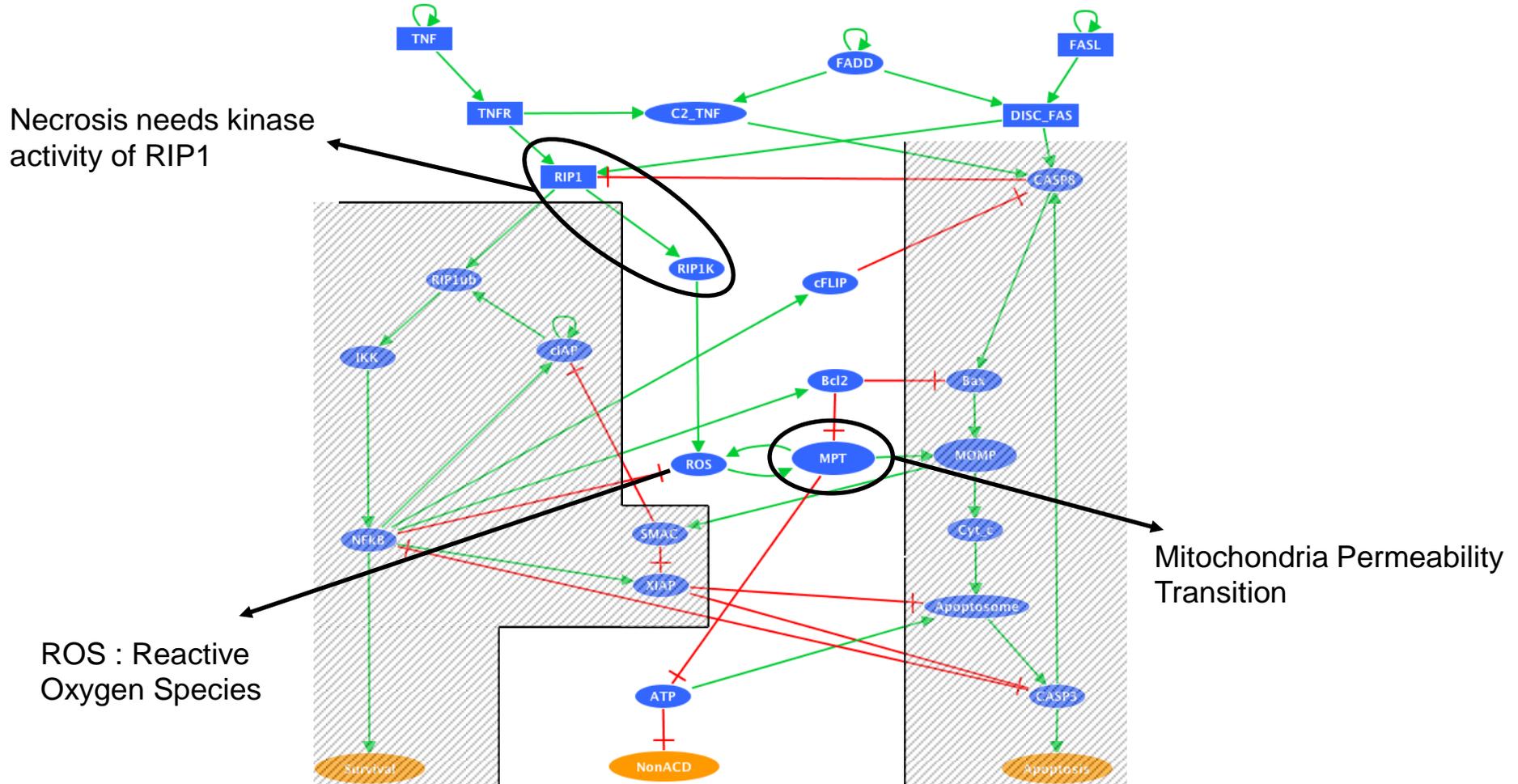
APOPTOSIS



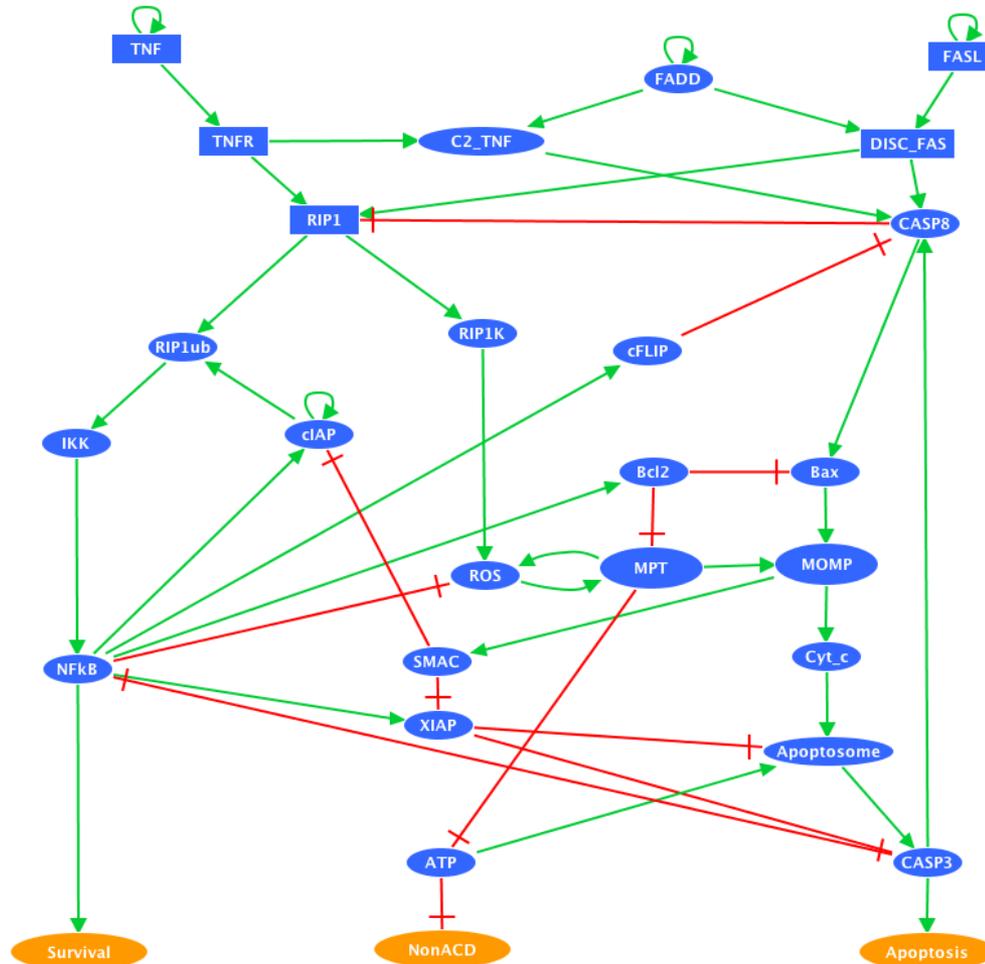
NFκB pathway



NECROSIS



ASSEMBLED MECHANISM OF THREE CELL FATE DECISION



Boolean modeling

Assign logic to nodes

Example of CASP8

CASP8 = 0 when

DISC-Fas=0 and DISC-TNF=0 and CASP3=0
(equivalent to no external signals from death receptors
and no intracellular problems)

cFLIP=1

(equivalent to inhibition by the NFkB pathway)

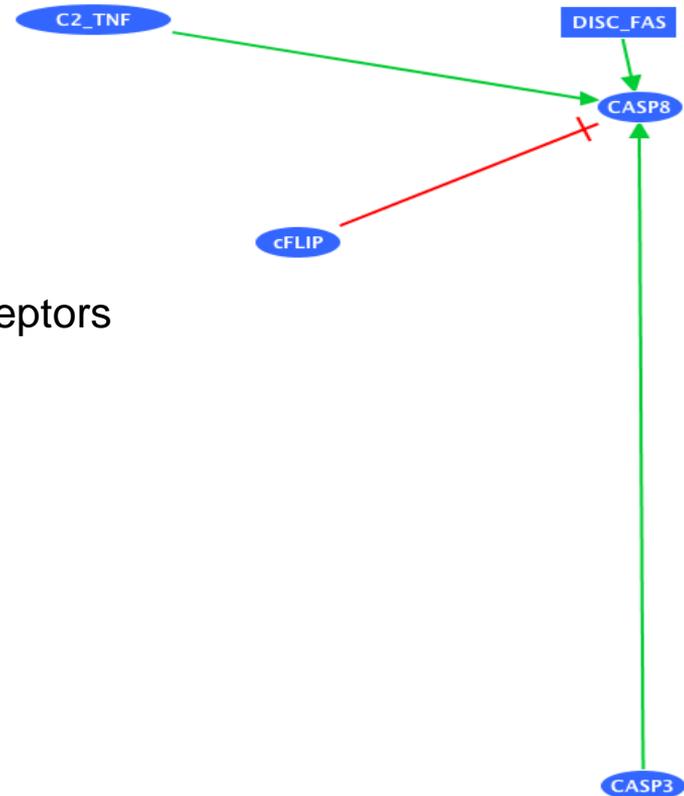
CASP8 = 1 when

DISC-Fas=1 or/and DISC-TNF=1
(equivalent to signal from death receptors)

CASP3=1

(amplification signal, feedback activation)

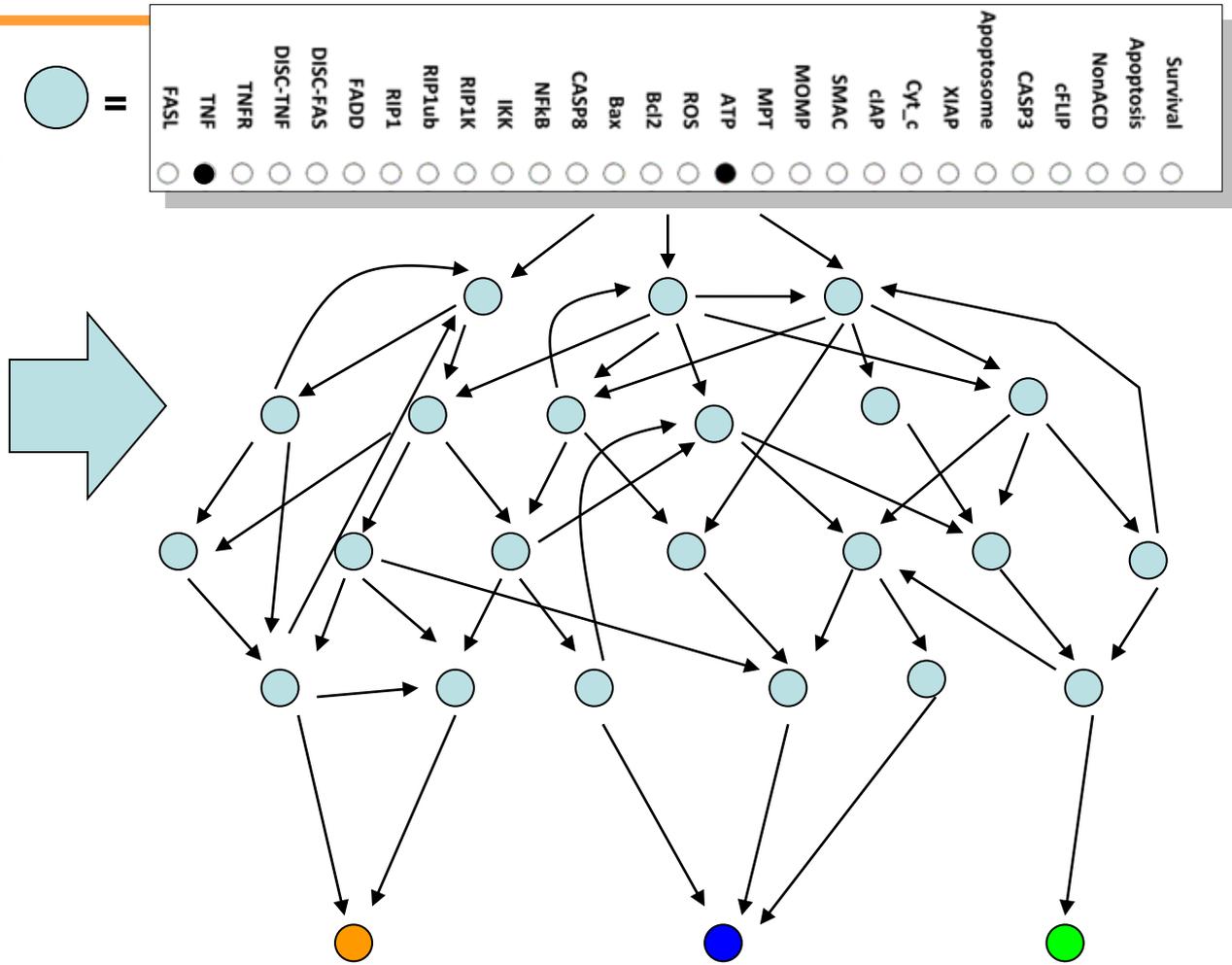
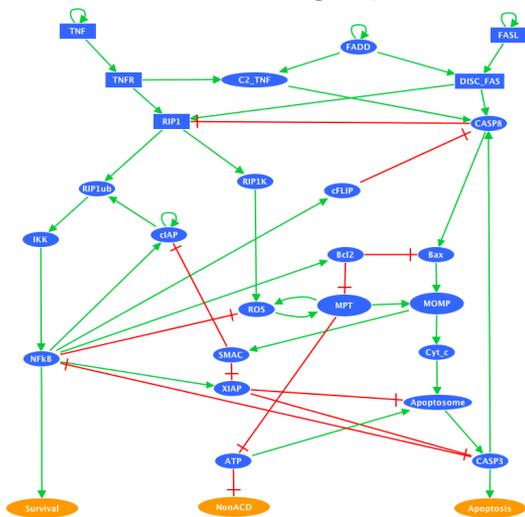
AND no cFLIP



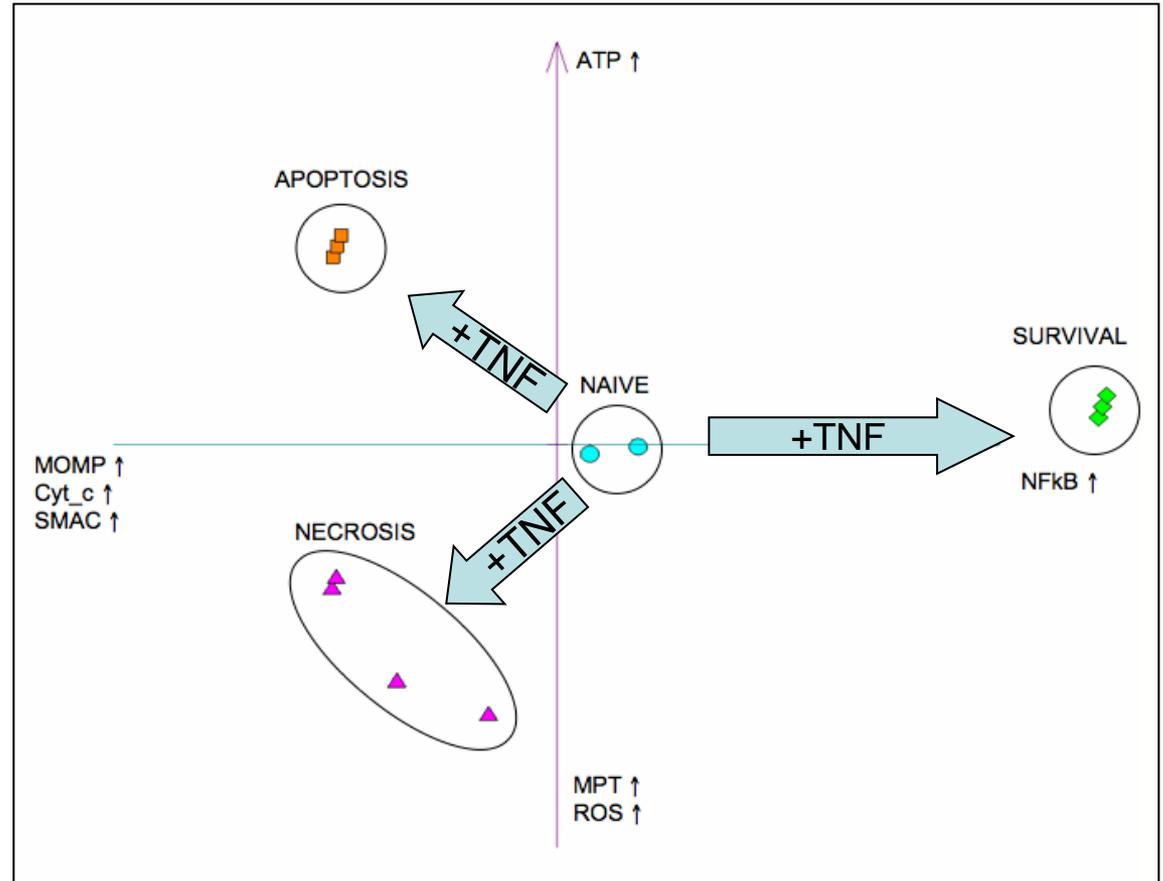
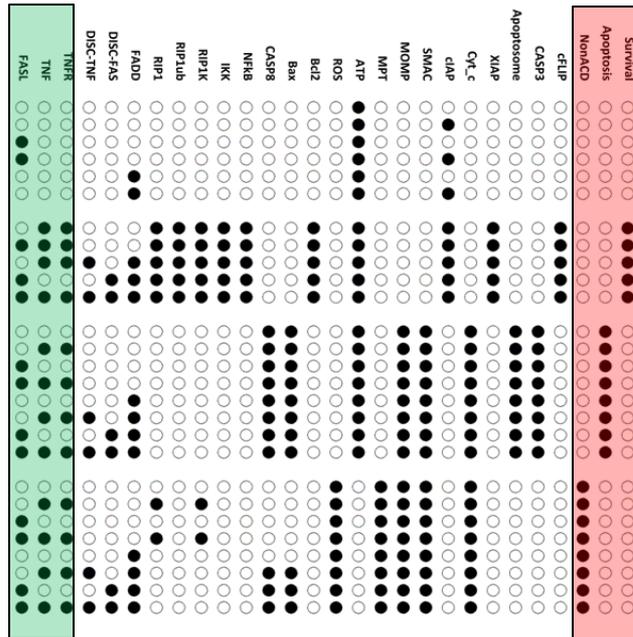
One node = one species

Asynchronous state transition graph

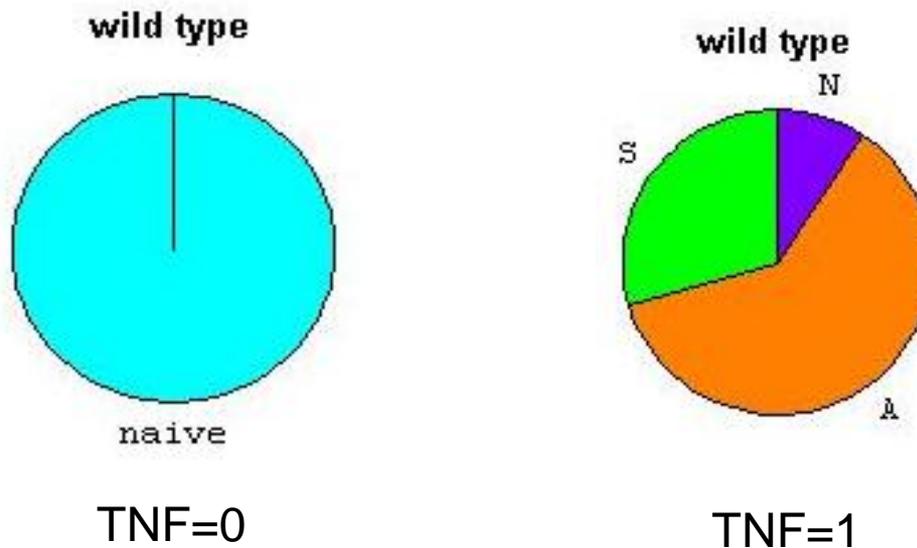
Influence graph



Structure of attractors: distribution of logical stable states



« Probabilities » of reaching phenotypes from physiological initial conditions:



Confront the model to existing data: verify the structure of the network by comparing the simulations to published data

⇒ Simulations of mutants or drug treatments

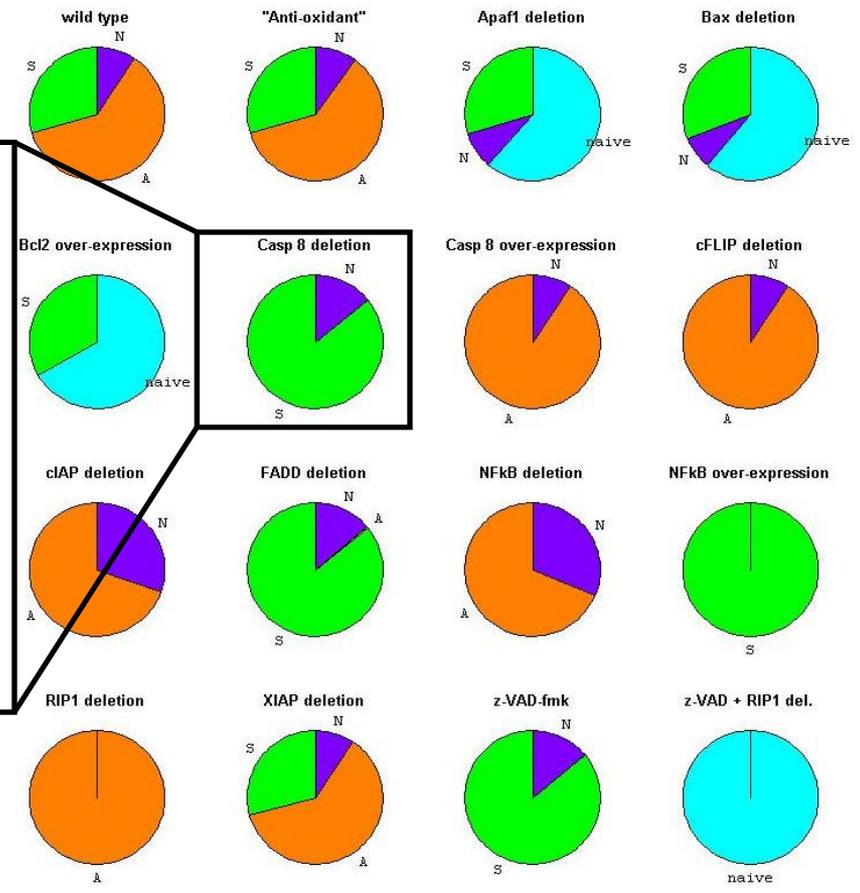
Name	Modified rules	Expected phenotypes	Qualitative results
Anti-oxidant	$ROS' = (RIP1 \text{ OR } MPT)$		Suppression of $NF\kappa B$ anti-oxidant effect leads to no change in the decision process (the computed probabilities are noticeably close to the wild type).
APAF1 deletion	$C3' = 0$	APAF1 ^{-/-} mouse thymocytes are not impaired in Fas-mediated apoptosis (Yoshida <i>et al</i> , 1998)	Apoptosis disappears. Necrosis and survival are close to the wild type case. Lacking apoptosis is mainly replaced by the 'naïve' state
BAX deletion	$MOMP' = MPT$	BAX deletion blocks Fas or TNF+CHX - induced apoptosis in some cell lines, such as HCT 116 (LeBlanc <i>et al</i> , 2002)	BAX deletion prevents apoptosis.
BCL2 over-expression	$MOMP' = MPT$ $MPT' = 0$	FAS induces the activation of $NF\kappa B$ pathway (Kreuz <i>et al</i> , 2001)	As expected, $NF\kappa B$ pathway is a reachable attractor. The second reachable attractor is the 'naïve' state, which means that both death pathways are inhibited.
C8 deletion	$C8' = 0$	Caspase 8 deficient MEFs (Varfolomeev <i>et al</i> , 1998) or Jurkat cells (Kawahara <i>et al</i> , 1998) are resistant to Fas-mediated apoptotic cell death.	As expected, apoptosis is no longer reachable. Compared to the wild type, a slight increase of necrosis is observed, while $NF\kappa B$ survival becomes the main cell fate.
constitutively activated CASP8	$C8' = 1$		Over-expression of caspase 8 leads to an increased disappearance of $NF\kappa B$ activation.
cFLIP deletion	$C8' = TNF \text{ OR } FAS \text{ OR } C3$	cFLIP ^{-/-} MEFs are highly sensitive to FasL and TNF α (Yeh <i>et al</i> , 2000)	The increase of apoptosis is effectively observed in the cFLIP mutant; however we also observe that $NF\kappa B$ pathway can no longer be sustained.
cIAP deletion	$cIAP' = 0$	$NF\kappa B$ activation in response to TNF is blocked (Varfolomeev <i>et al</i> , 2008)	$NF\kappa B$ activation is impaired, and only the apoptotic or necrotic attractors are reached.
FADD deletion	$C8' = C3 \text{ AND NOT } NF\kappa B$ $RIP1' = NOT \ C8 \text{ AND } TNF$	FADD ^{-/-} mouse thymocytes are resistant to Fas mediated apoptosis (Zhang <i>et al</i> , 1998). FADD ^{-/-} MEFs are resistant to FasL and TNF α (Yeh <i>et al</i> , 1998). In Jurkat cells treated with TNF α +CHX, FADD deletion turns apoptosis into necrotic cell death (Harper <i>et al</i> , 2003)	In response to FasL, signalling is blocked, thus the 'naïve' attractor is the only reachable one. In response to TNF, apoptosis disappears.
$NF\kappa B$ deletion	$NF\kappa B' = 0$	TNF α induces both apoptosis and necrosis in $NF\kappa B$ p65 ^{-/-} cells (Sakon <i>et al</i> , 2003) or in IKK β ^{-/-} fibroblasts (Kamata <i>et al</i> , 2005)	This mutant shows a strong increase of necrosis (to be related with concomitant apoptosis/necrosis)
constitutively active $NF\kappa B$	$NF\kappa B' = 1$		Both death pathways are shut down in this mutant.
RIP1 deletion	$RIP1' = 0$	RIPK1 ^{-/-} MEFs are hypersensitivity to TNF α , no TNF α -induced $NF\kappa B$ activation, (Kelliber <i>et al</i> , 1998)	Both $NF\kappa B$ and necrosis become unreachable. The effect of RIP1 silencing leads to a complete loss of the decision process (apoptosis becoming the only outcome).
XIAP deletion	$C3' = ATP \text{ AND } MOMP$	No effect on TNF α -induced toxicity in XIAP ^{-/-} MEFs (Harlin <i>et al</i> , 2001)	S

TNF=1

Example : Caspase 8 deletion

≈ 85% survival (NFκB)
 ≈ 15% necrosis
 No apoptosis

Qualitatively consistent with the literature
 “TNF-induced apoptosis is blocked though not necrosis”
 [Kawahara, Ohsawa *et al.*, *J Cell Biol* 1998]
 (Jurkat cells, C8-/-)



“Ligand dosage” experiments

What if the signal was removed...
at which point would the cell commit to one
or the other phenotype?

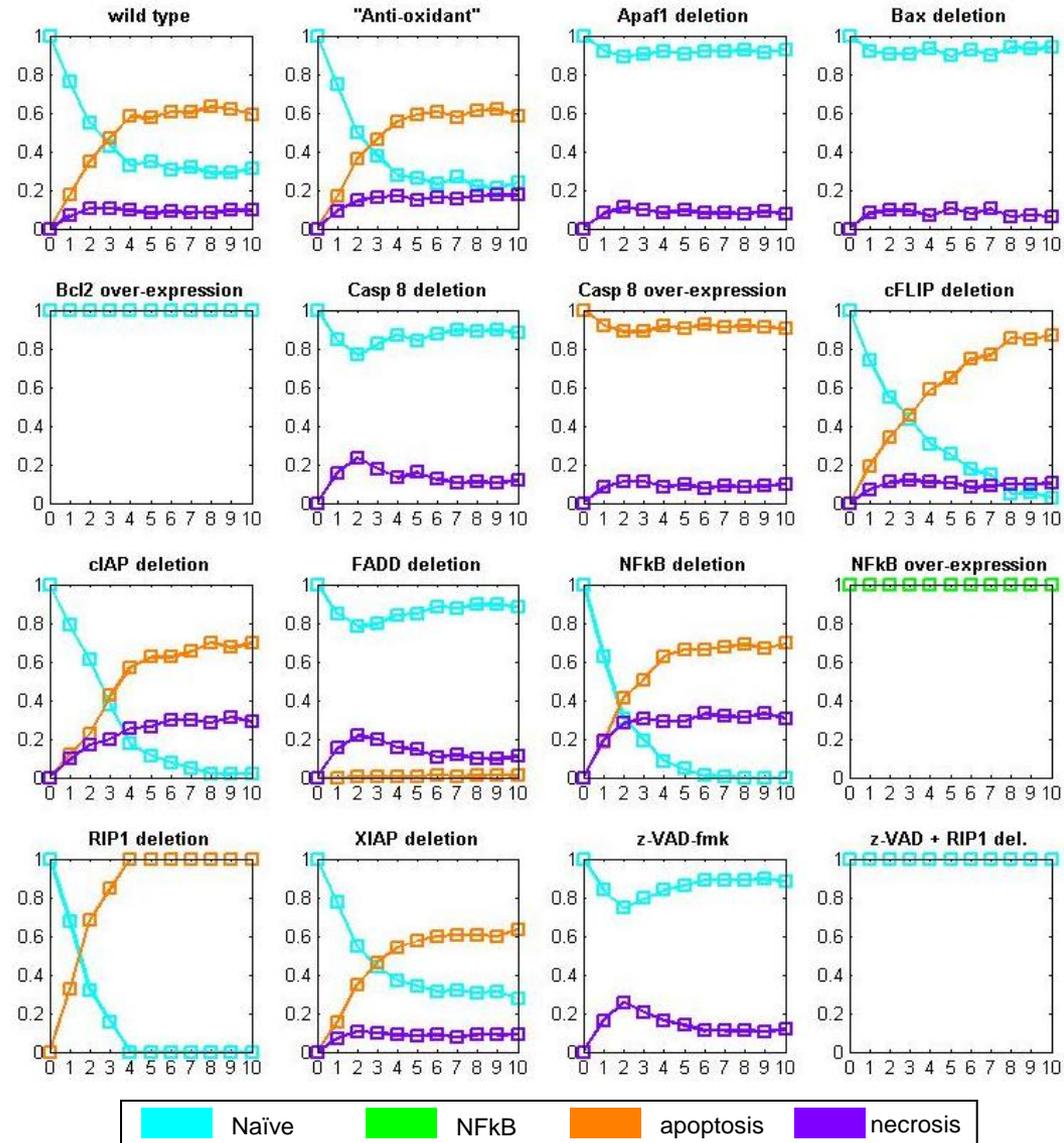
Introduction of “pulse” of TNF instead of
constant induction

t : integer

During t steps, the system evolves with
TNF=1

At step $t+1$, TNF is switched to 0 (until the
end)

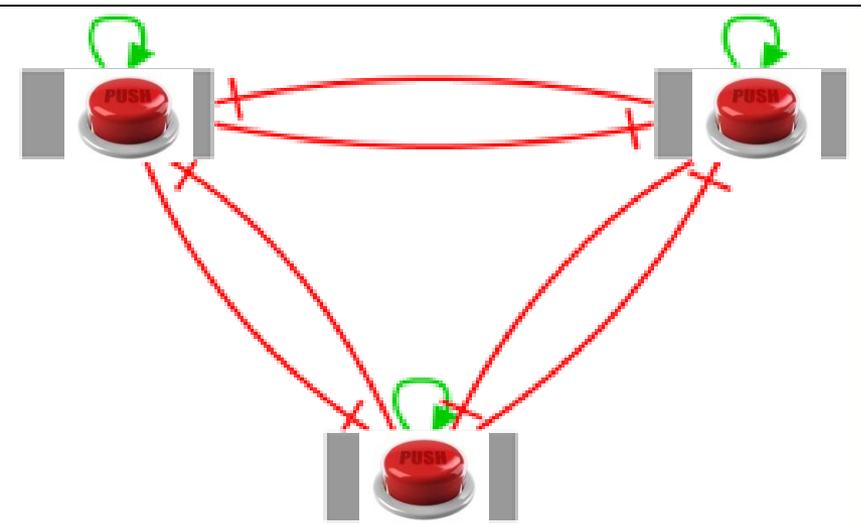
(x-axis → duration of TNF “pulse”)



Simplify to understand!

A conceptual 3-node model:

- 3 nodes to represent the 3 pathways (CASP3, NFκB, MPT)
- Each arrow summarizes one or several path(s) / cycle(s)



Feedback circuits

MPT => MPT

1) MPT => ROS => MPT (+)

NFκB => NFκB

2) NFκB => cIAP => RIP1ub => IKK => NFκB (+)

3) NFκB => cFLIP -| CASP8 -| RIP1 => RIP1ub => IKK => NFκB (+)

CASP3 => CASP3

4) CASP3 => CASP8 => BAX => MOMP => SMAC -| XIAP -| CASP3 (+)

5) CASP3 => CASP8 => BAX => MOMP => Cyt_c => apoptosome => CASP3 (+)

Other regulatory pathways

CASP3 -| NFκB

6) CASP3 => CASP8 -| RIP1 => RIP1ub => IKK => NFκB (-)

7) CASP3 => CASP8 => BAX => MOMP => SMAC -| cIAP => RIP1ub => IKK => NFκB (-)

8) CASP3 -| NFκB (-)

NFκB -| CASP3

9) NFκB => cFLIP -| CASP8 => BAX => MOMP => Cyt_c => apoptosome => CASP3 (-)

10) NFκB => XIAP -| CASP3 (-)

11) NFκB => XIAP -| Apoptosome => CASP3 (-)

12) NFκB => BCL2 -| BAX => MOMP => Cyt_c => apoptosome => CASP3 (-)

MPT -| NFκB

13) MPT => MOMP => SMAC -| cIAP => RIP1ub => IKK => NFκB (-)

NFκB -| MPT

14) NFκB -| ROS => MPT (-)

15) NFκB => BCL2 -| MPT (-)

16) NFκB => cFLIP -| CASP8 -| RIP1 => RIP1K => ROS => MPT (+)

CASP3 -| MPT

17) CASP3 => CASP8 -| RIP1 => RIP1K => ROS => MPT (-)

MPT -| CASP3

18) MPT => MOMP => Cyt_c => apoptosome => CASP3 (+)

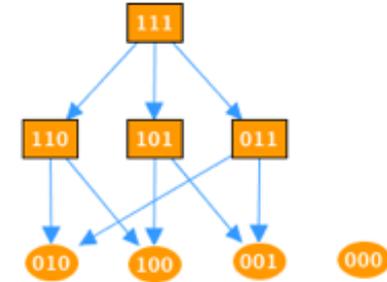
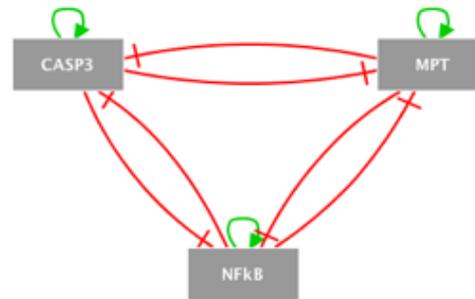
19) MPT => MOMP => SMAC -| XIAP -| CASP3 (+)

20) MPT => MOMP => SMAC -| XIAP -| apoptosome => CASP3 (+)

21) MPT -| ATP => apoptosome => CASP3 (-)

The conceptual model as a predictive tool

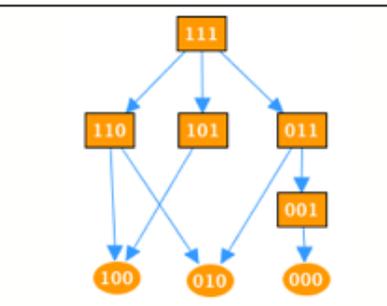
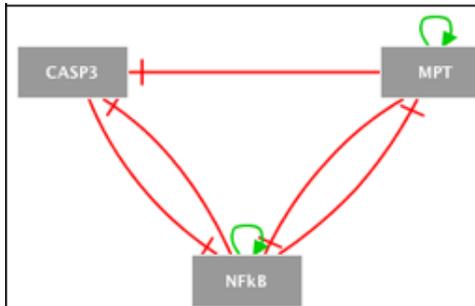
SIMULATE WILD TYPE



TEST MUTANTS

Casp8 deletion

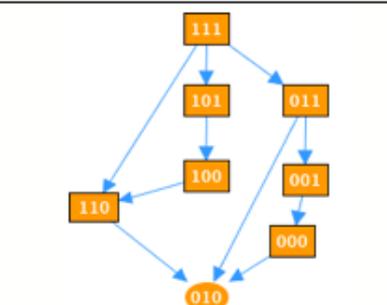
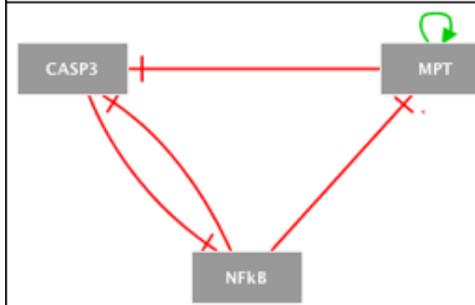
Apoptosis (CASP3 stable state) disappears



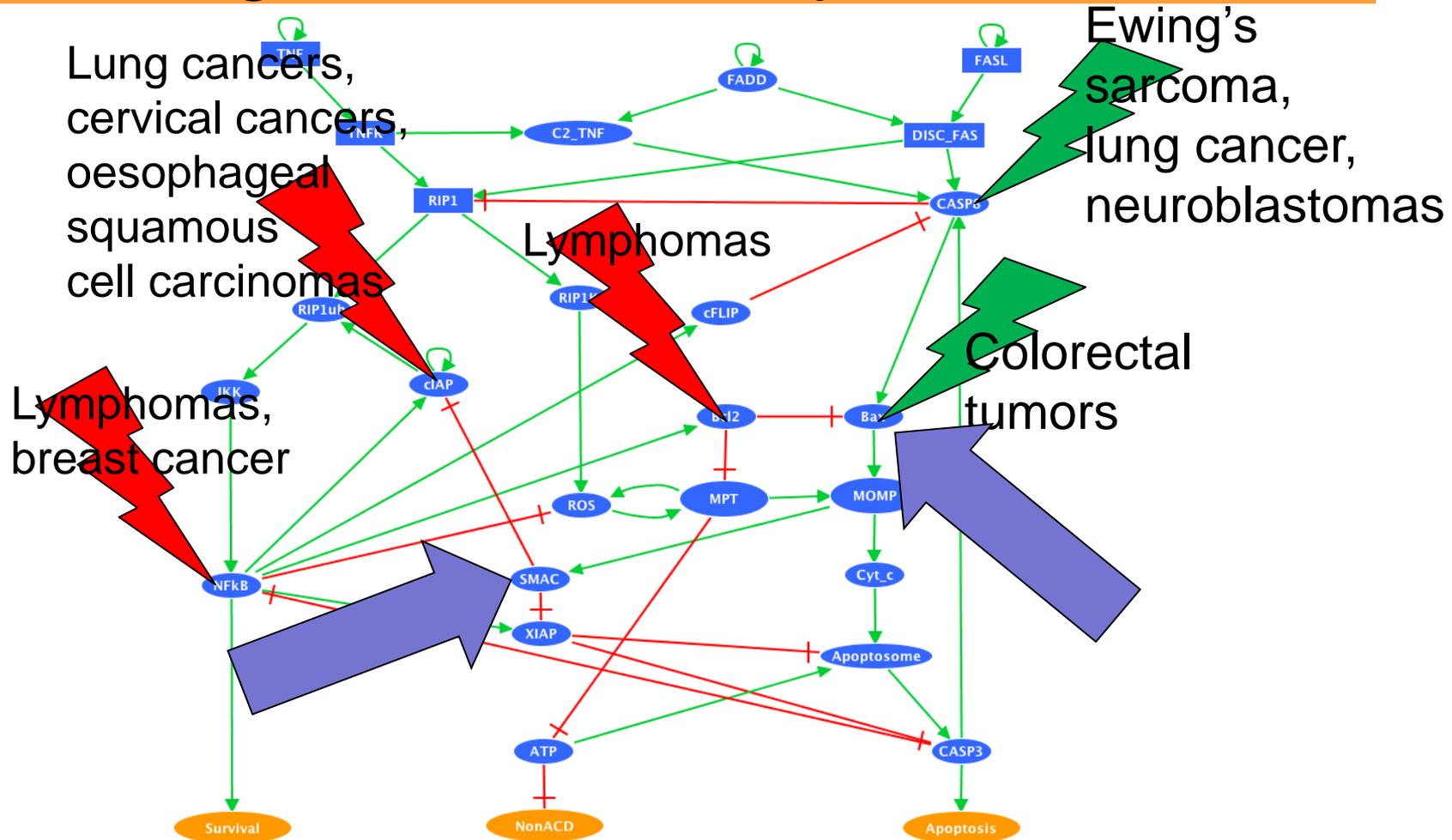
TEST VERSIONS OF THE MODEL

Casp8 deletion + no cIAP

Apoptosis and necrosis disappear
=> Confirms the necessity of cIAP!



Cell fate decision mechanism fragilities utilized by cancers



Acknowledgements

Laurence Calzone



Simon Fourquet



Denis Thieffry



Laurent Tournier



Boris Zhivotovsky



Emmanuel Barillot



Institut Curie

École normale supérieure

Karolinska Institutet



Inserm

Institut national
de la santé et de la recherche médicale

