Modeling cell life and cell death in cancer

Andrei Zinovyev

"Computational Systems Biology of Cancer"

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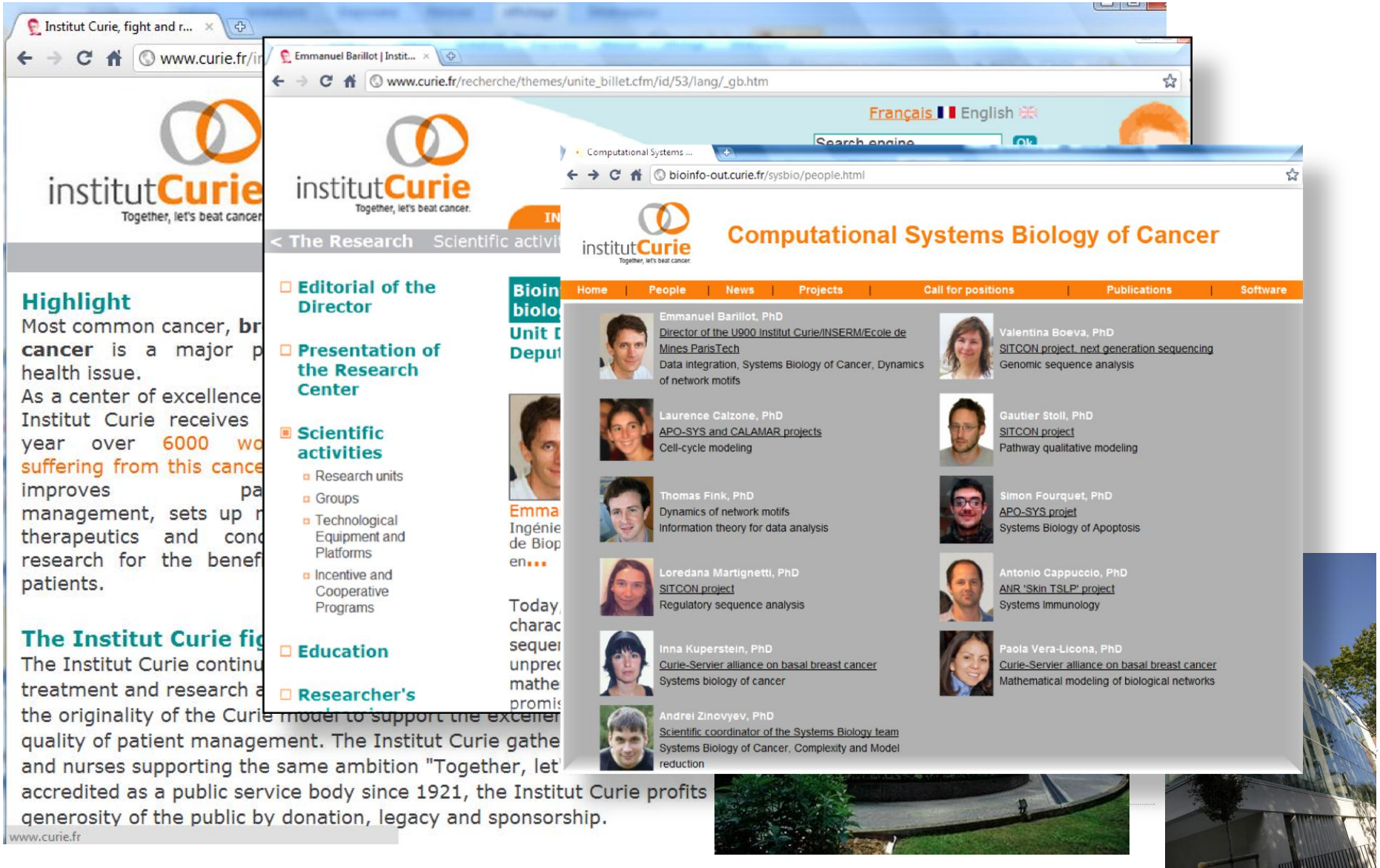
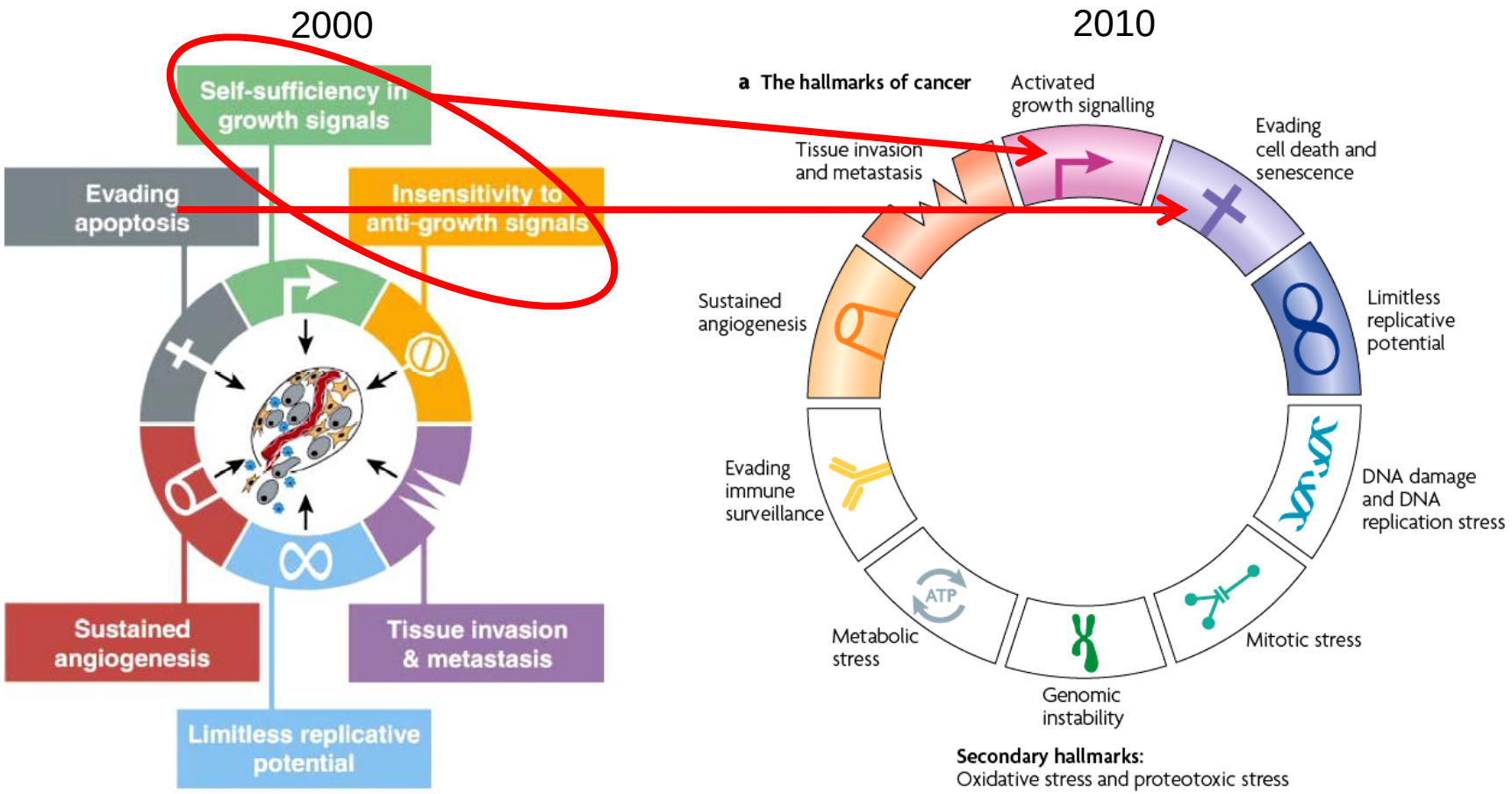
100 years of fighting with cancer



Hallmarks of cancer



Hanahan and Weinberg, 2000, Cell

Negrini et al, 2010, Nat Rev Mol Cell Bio

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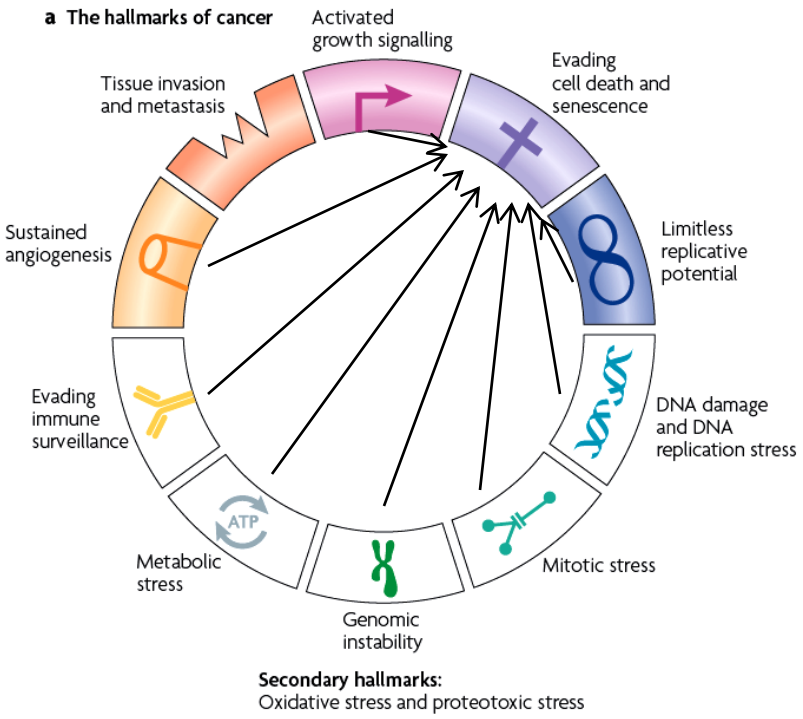
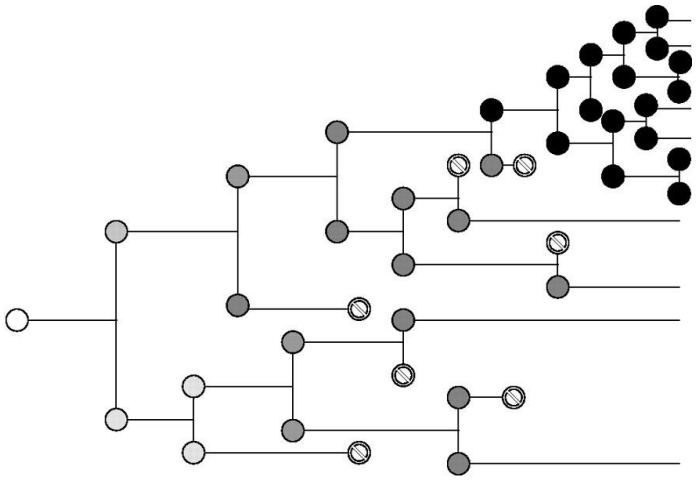
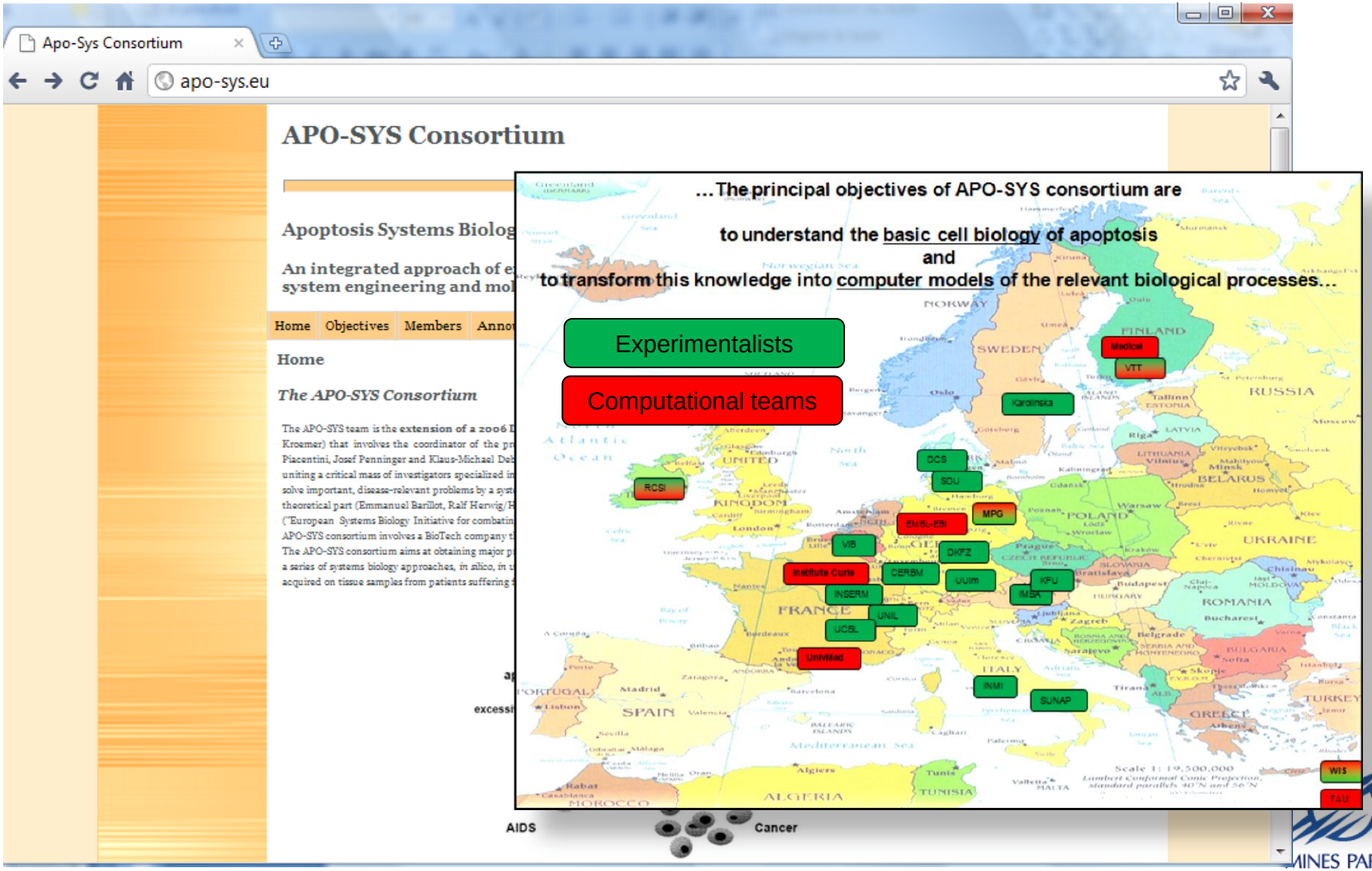
Cell life/death decisions in cancer

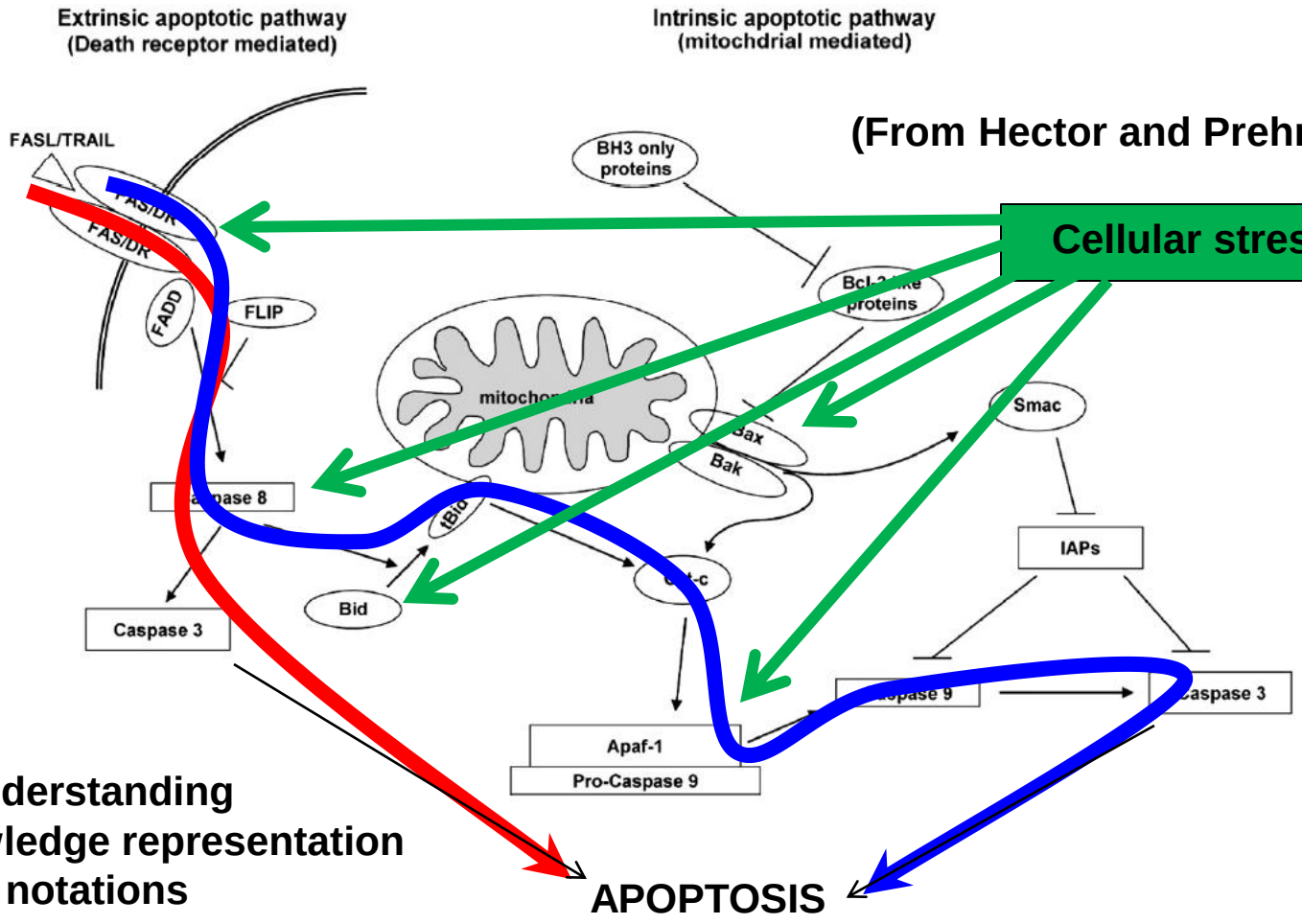




APO-SYS: First EU FP7 large-scale project on systems biology of cancer



A textbook view on apoptosis



(From Hector and Prehn, 2009)

Illusion of understanding
Art-like knowledge representation
Ambiguity in notations

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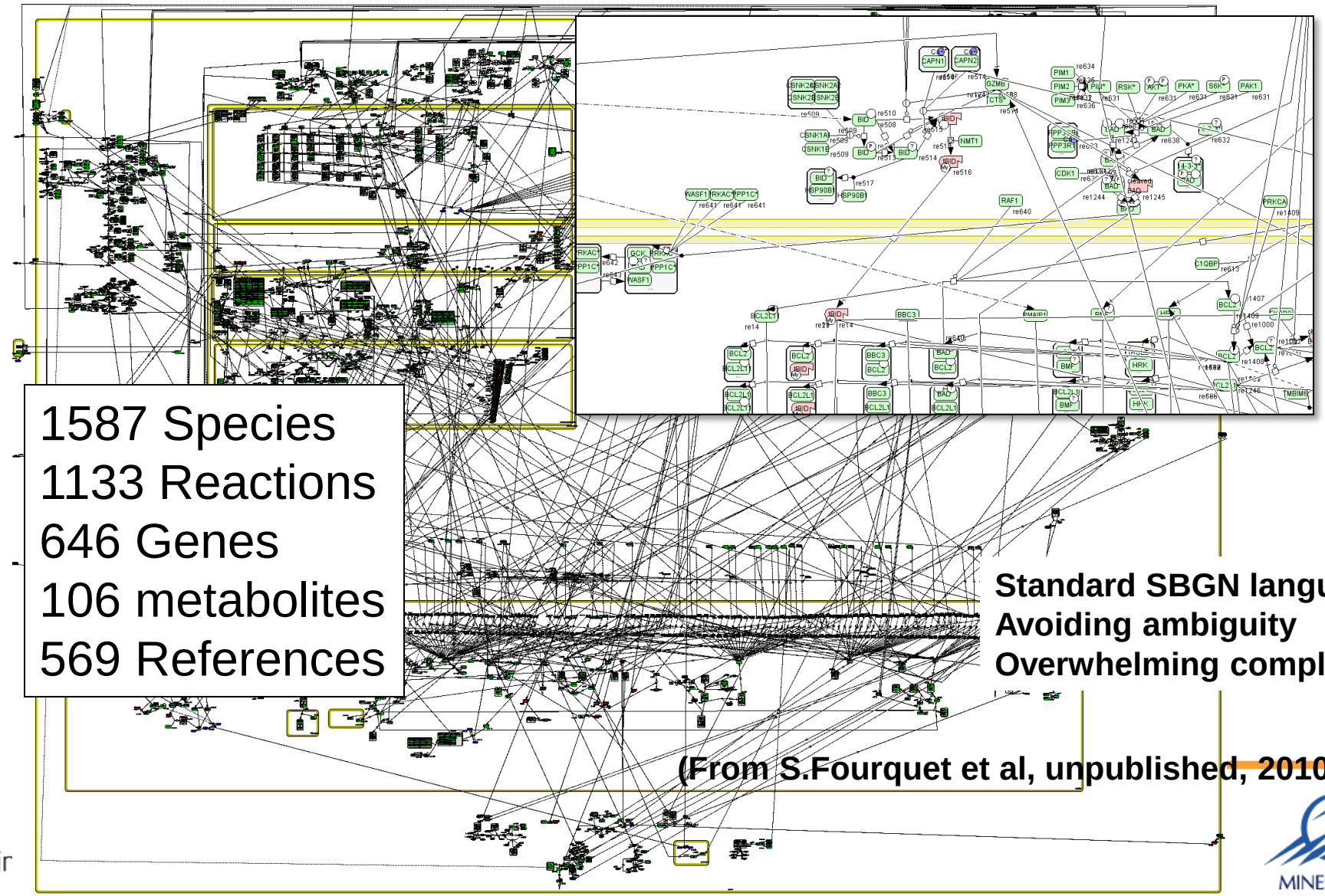
A systems biologist's view on apoptosis



1587 Species
1133 Reactions
646 Genes
106 metabolites
569 References

Standard SBGN language
Avoiding ambiguity
Overwhelming complexity

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

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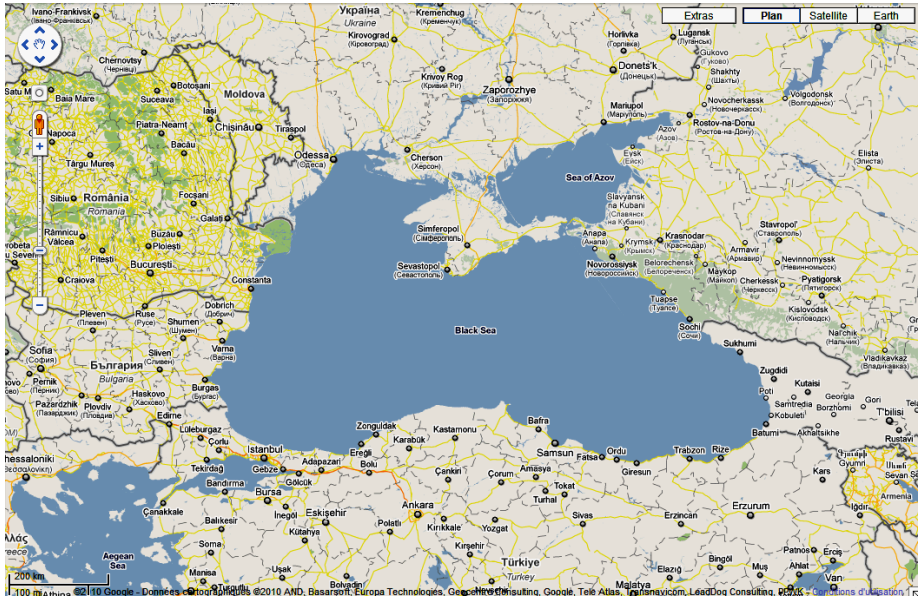
Map and Map

1559



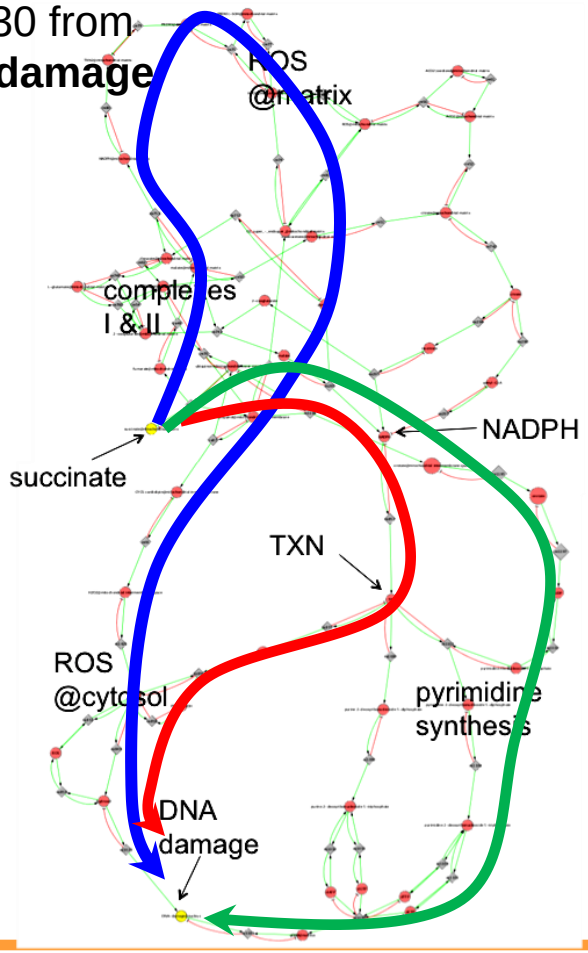
2000

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

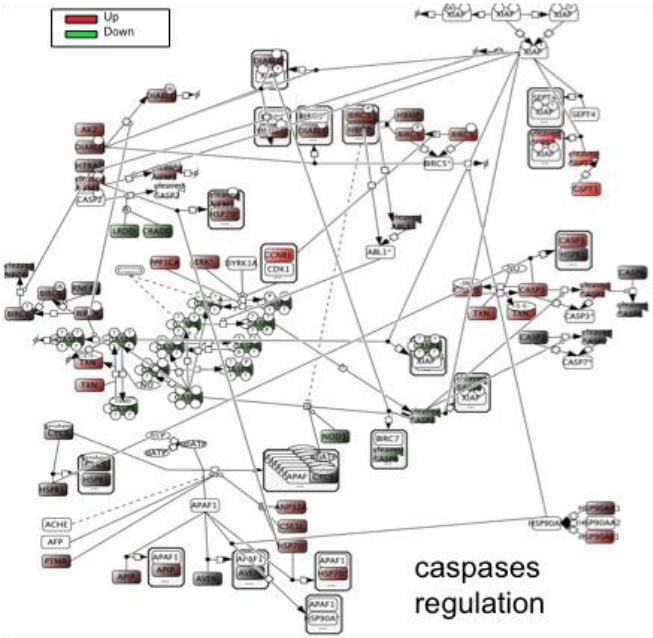
Through transfer of the reductive equivalents of succinate to NADPH and thioredoxin, then ROS detoxification or RNR activity and DNA repair

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

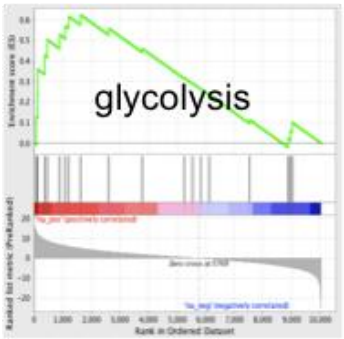
(see Khutornenko AA et al., PNAS, 2010,107,12828)

Using the cell death map: map high-throughput data

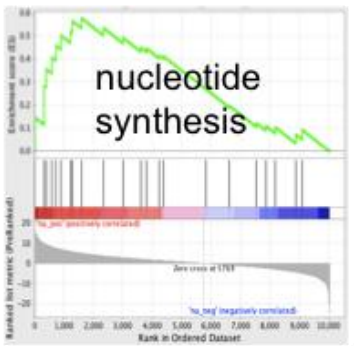
Basal breast cancer gene expression compared to healthy adypocytes



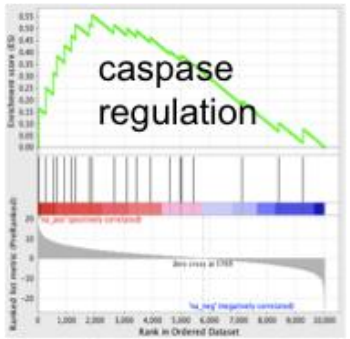
Map high-throughput data and infer “differentially deregulated subnetworks”



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	0.04495614
FWER p-Value	0.06



Enrichment Score (ES)	0.5580466
Normalized Enrichment Score (NES)	1.6066436
Nominal p-value	0.010324484
FDR q-value	0.055582277
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**

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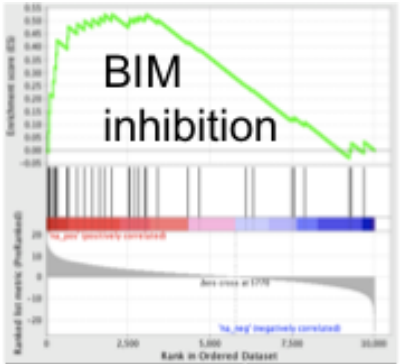
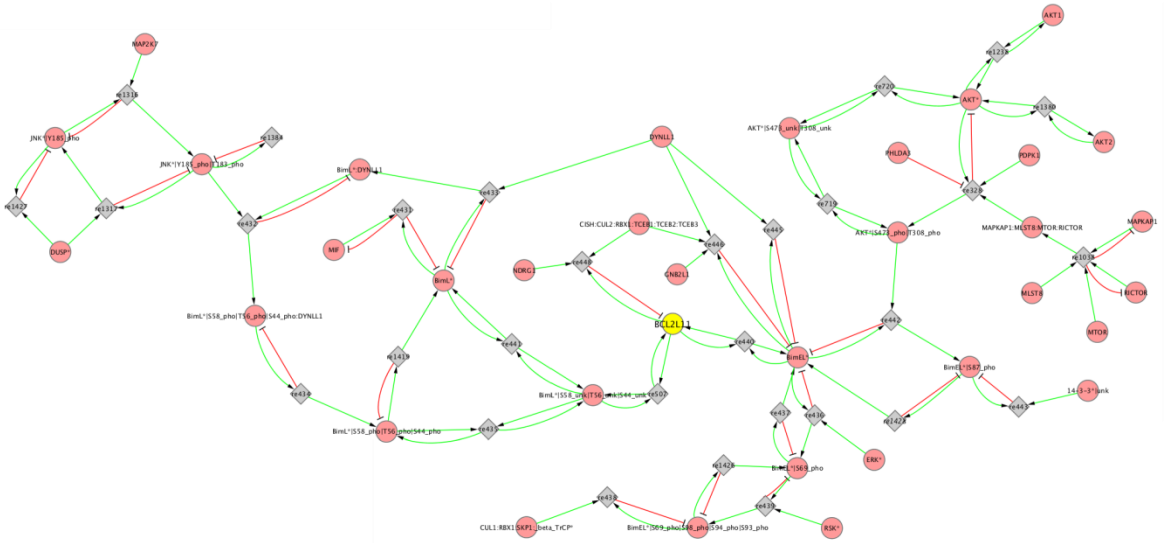
Using the cell death map: detecting hot spots of activity



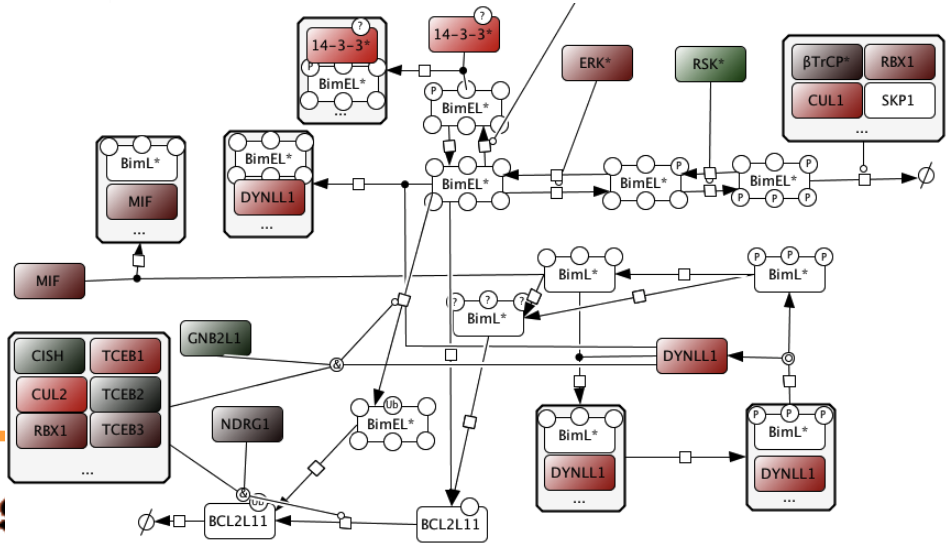
Enrichment Score (ES)	0.5258896
Normalized Enrichment Score (NES)	1.6449205
Nominal p-value	0.009749304
FDR q-value	0.009749304
FWER p-Value	0.0070



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



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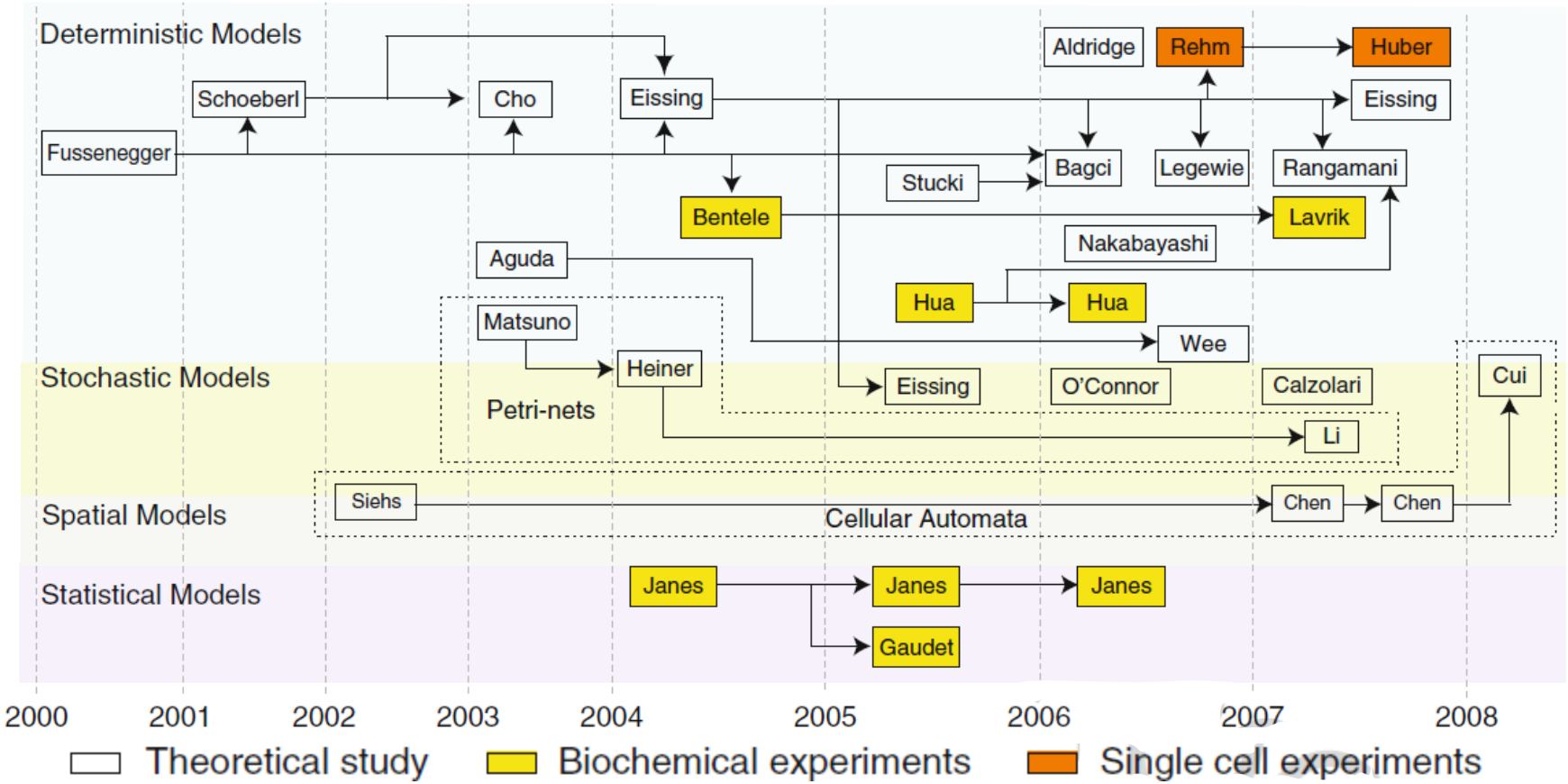
Systems Biology of Apoptosis



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

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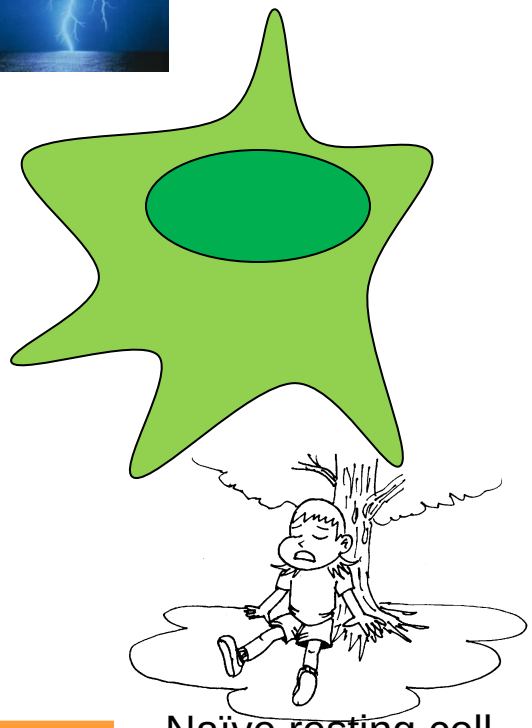
“Passive” vs “active” survival

Stress

Toxic stress

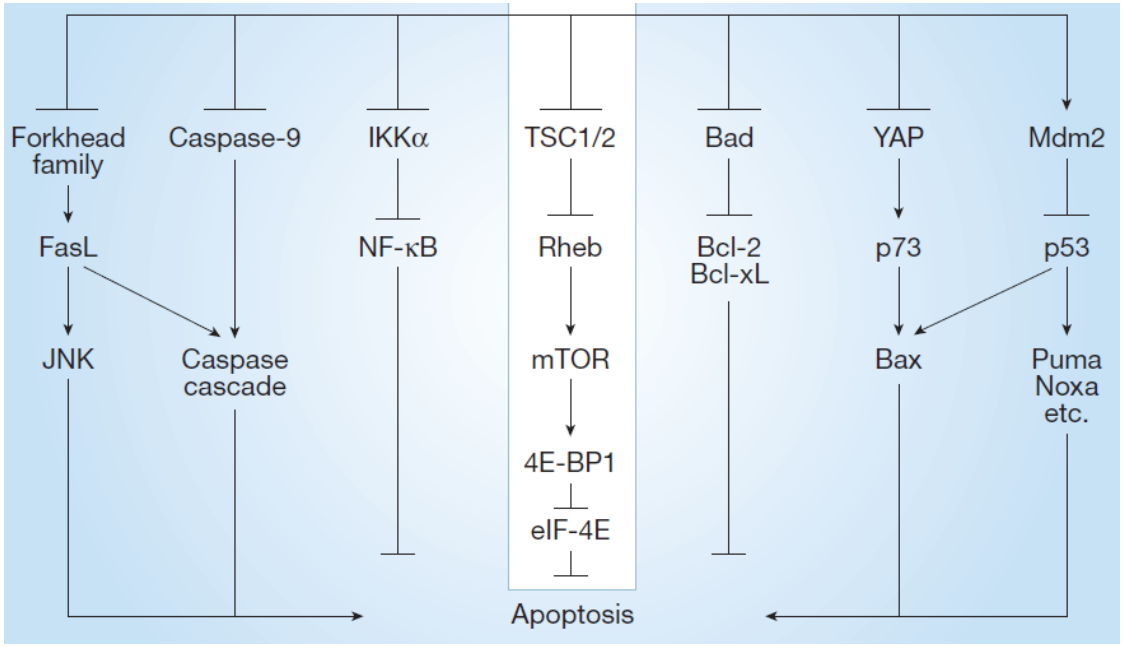
DNA damage

Nutrient deprivation



Naïve resting cell

AKT Survival signalling pathways

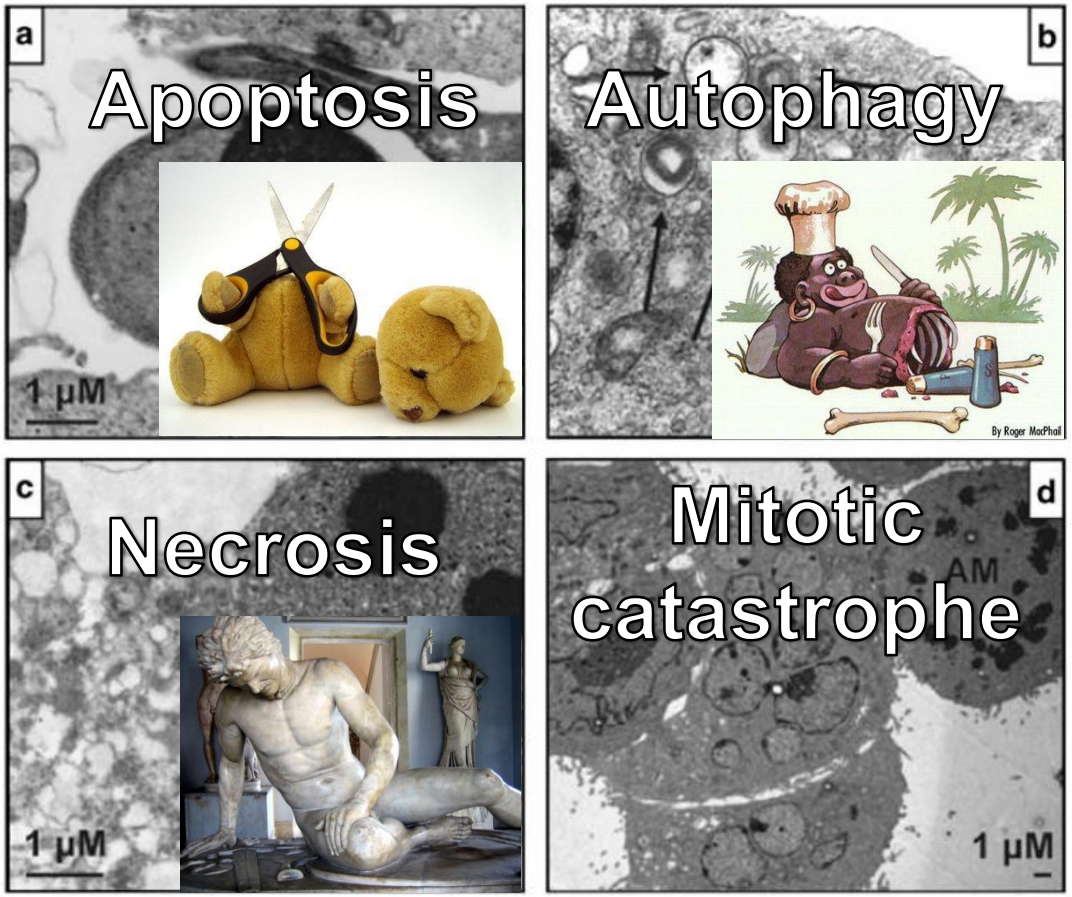


(From McCormick, Nature, 2004)

Four Faces of Cell Death

Cell Death Modalities

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



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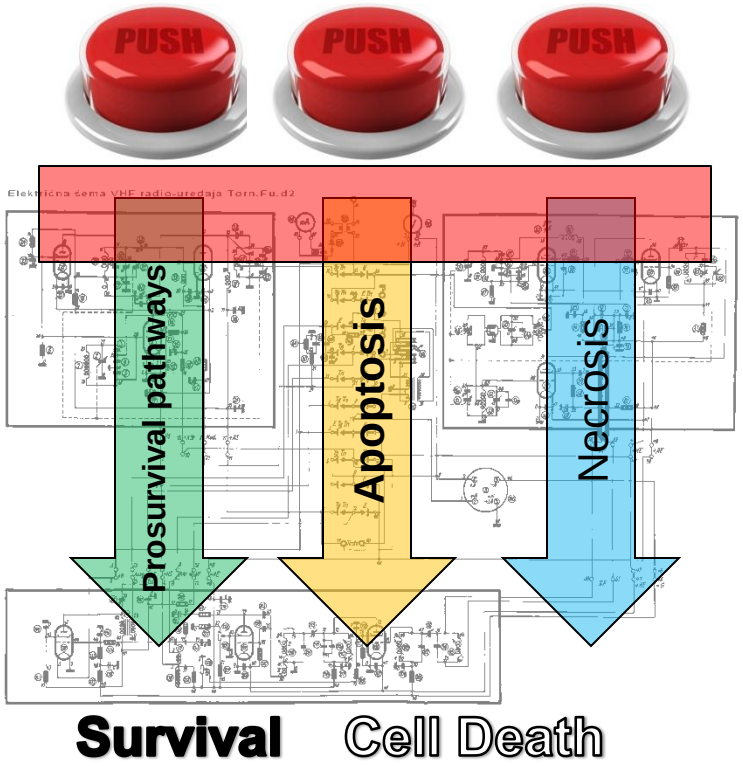
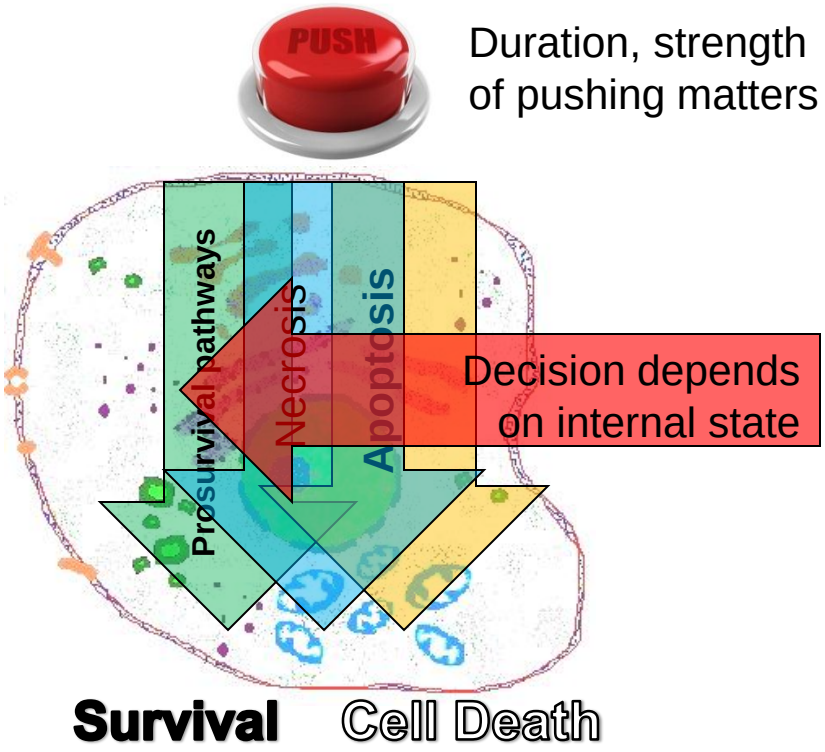
Engineering vs Biology

Engineering solution

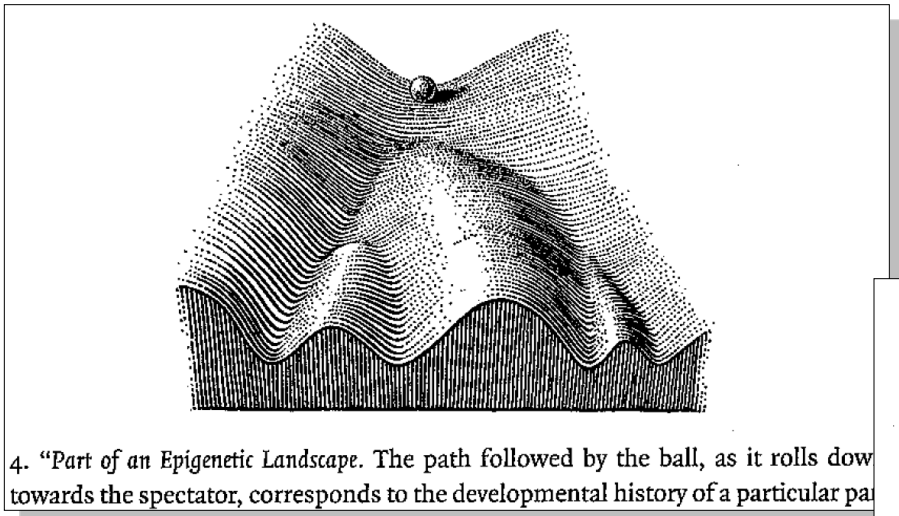


Biological solution



Cell fate decisions

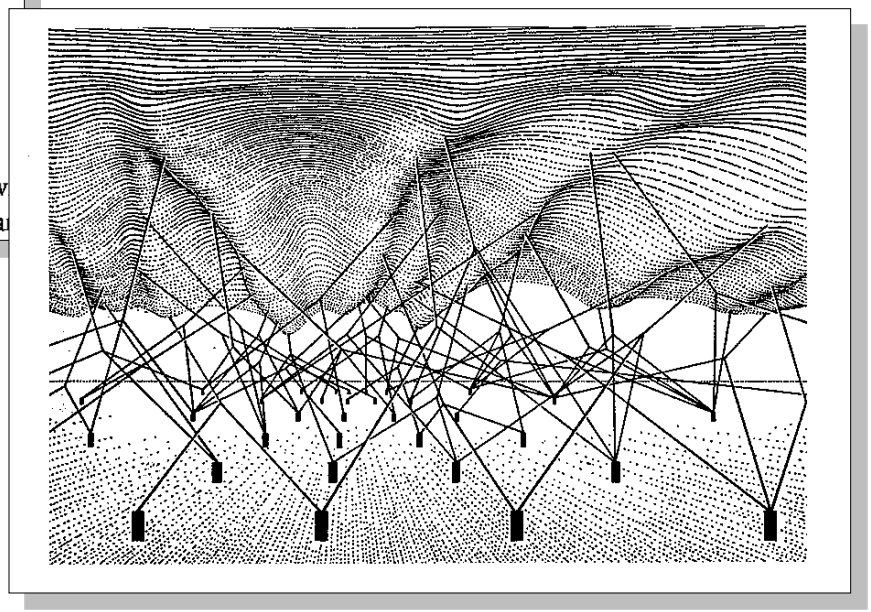
Conrad Hal Waddington, Professor of Animal Genetics at the University of Edinburgh, 1957.



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

Epigenetic landscape, canalization

Complex system of genes, underlying the landscape



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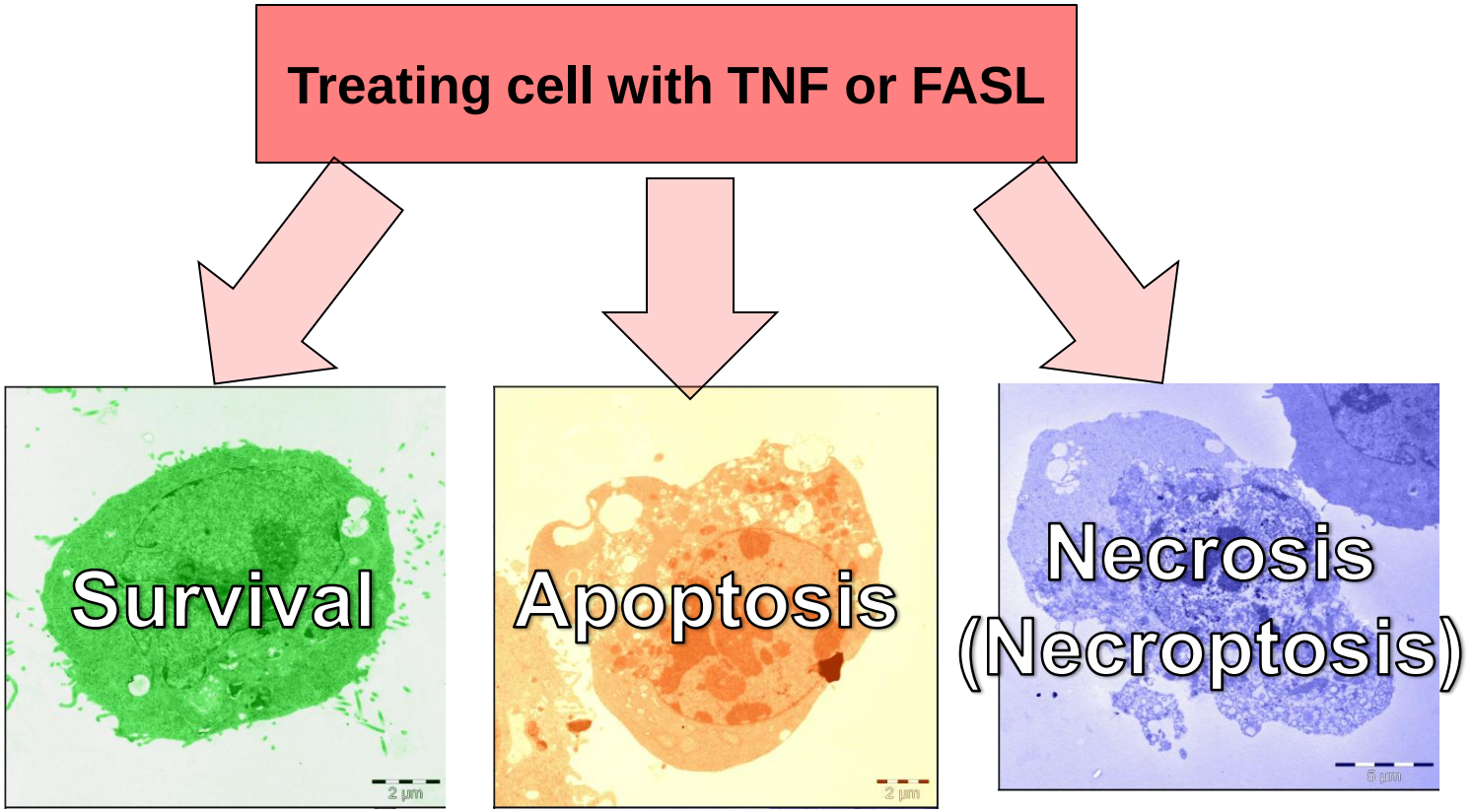
Apoptosis vs Necrosis vs Survival



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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 Zhivotovsky[6], Emmanuel Barillot[1,2,3¶], Andrei Zinovyev[1,2,3¶]

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

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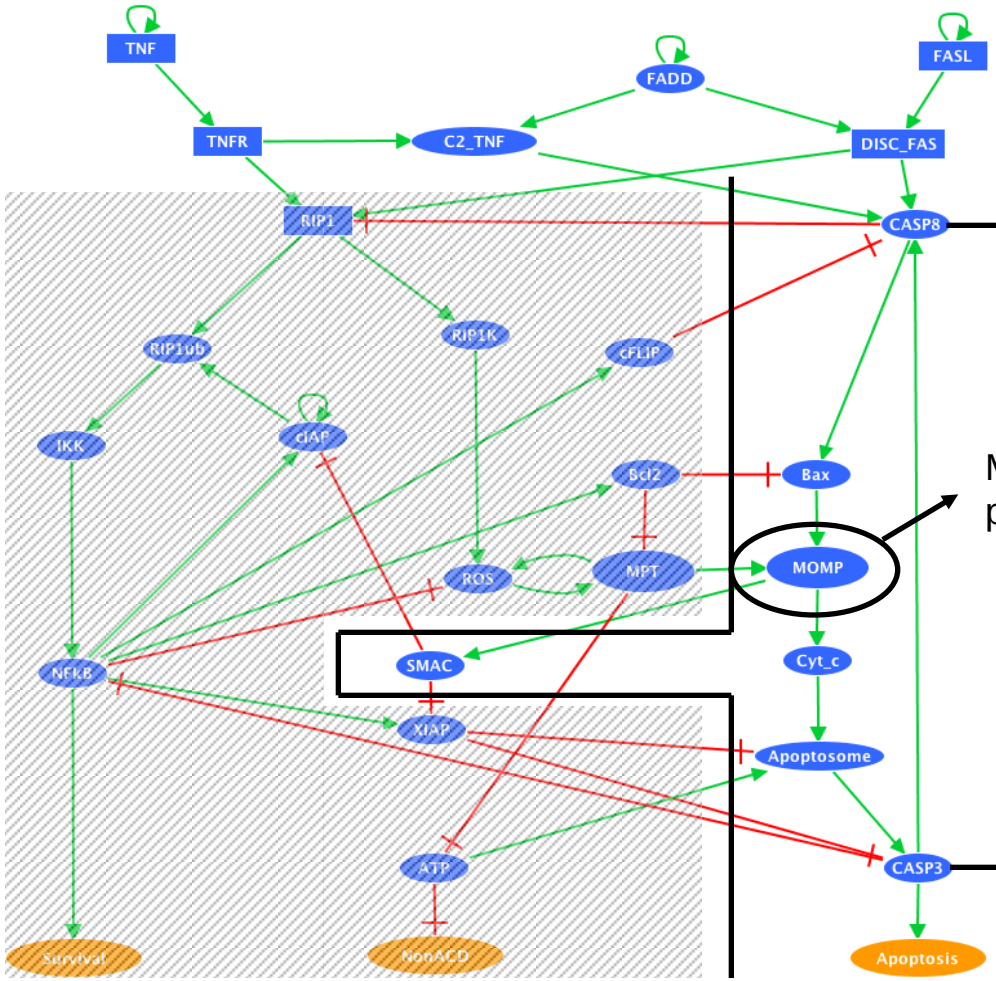
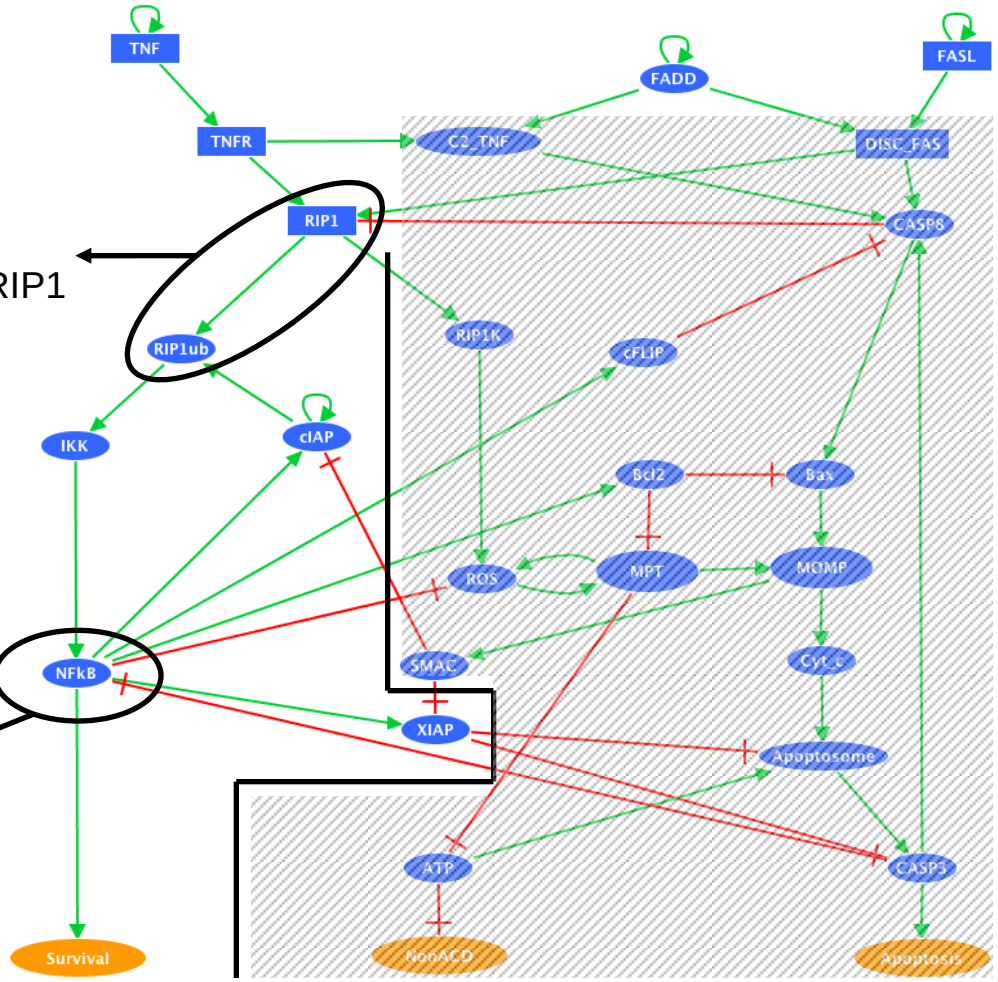
APOPTOSIS


TNF
FADD
FASL
TNFR
C2_TNF
DISC_FAS
RIP1
CASP8
Initiator caspase
RIP1ub
RIP1K
cFLIP
IKK
cIAP
Bcl2
Bax
Mitochondrial outer membrane permeabilization:
ROS
MPT
MOMP
SMAC
Cyt_c
NFkB
XIAP
Apoptosome
ATP
CASP3
Executioner caspase
Survival
NonACD
Apoptosis

NFκB pathway

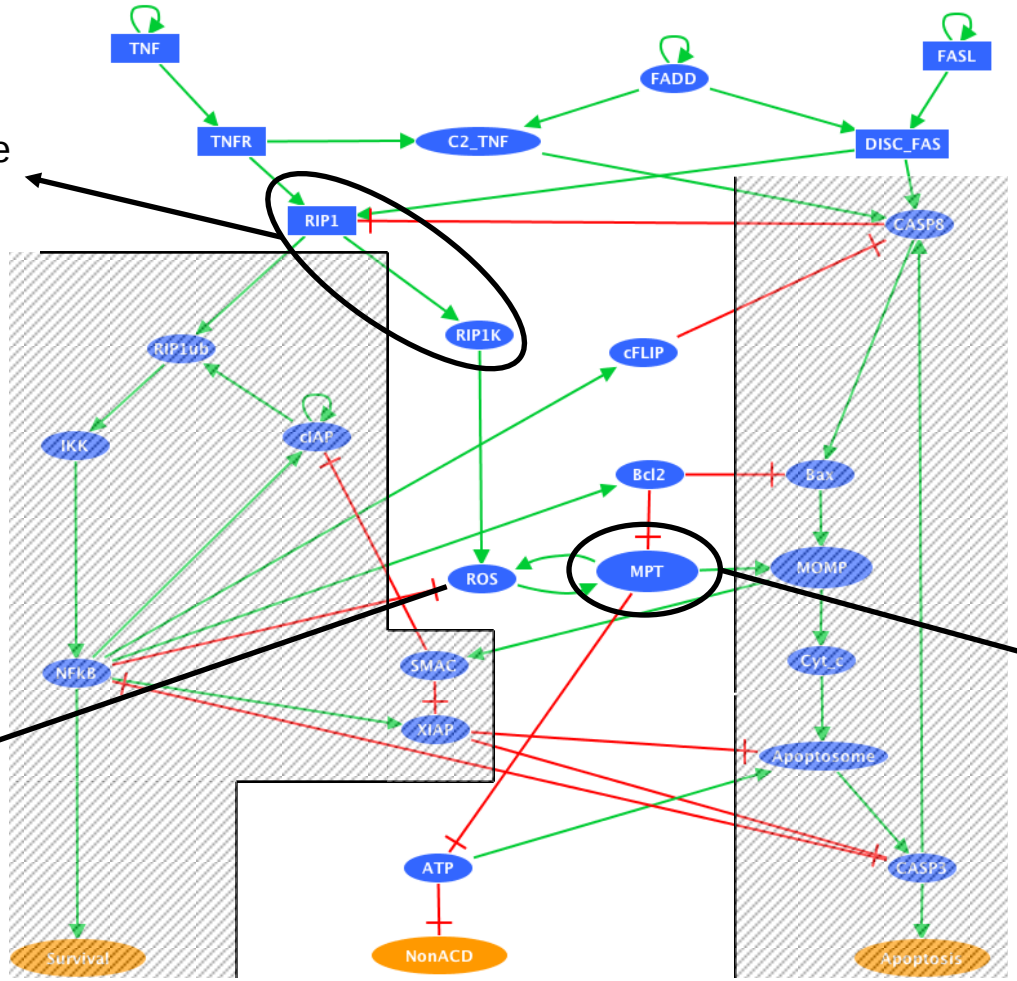
NFκB pathway needs ubiquitinated form of RIP1

No translocation of NFκB into the nucleus



NECROSIS

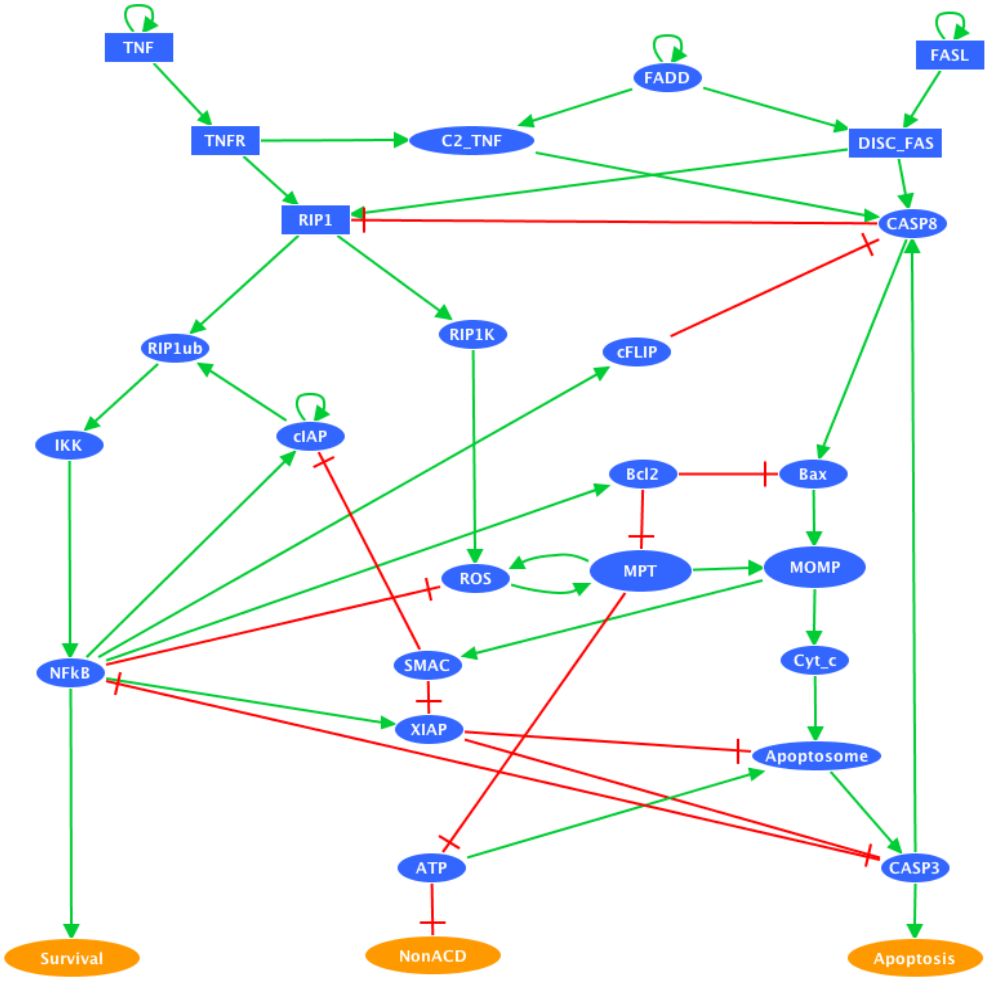
Necrosis needs kinase activity of RIP1



Mitochondria Permeability Transition

ROS : Reactive Oxygen Species

ASSEMBLED MECHANISM OF THREE CELL FATE DECISION



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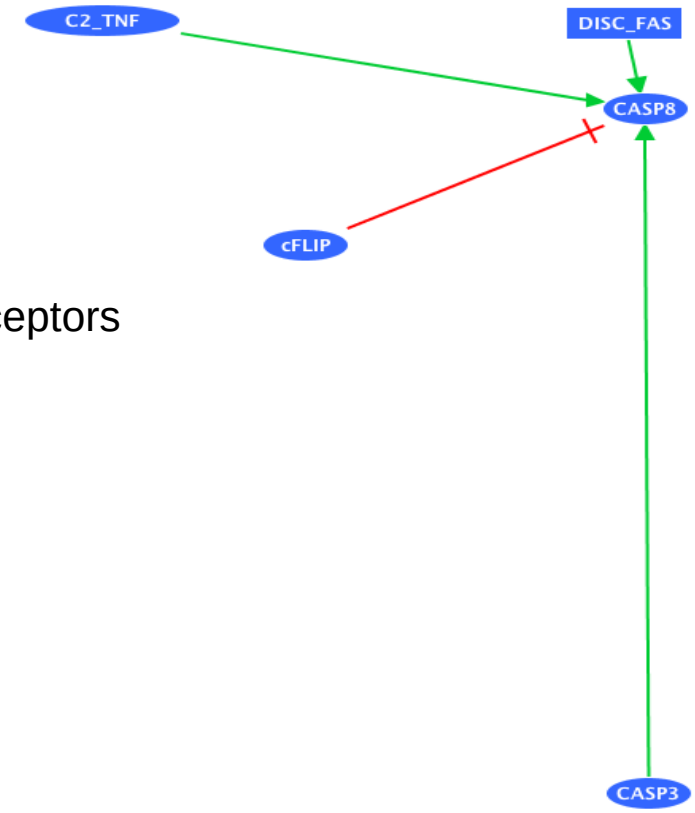
Boolean modeling

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)

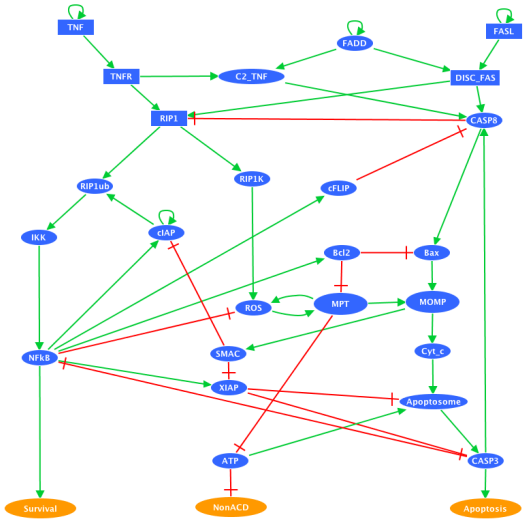
CASP8 = 1 when
DISC-Fas=1 or/and DISC-TNF=1
(equivalent to signal from death receptors)
CASP3=1
(amplification signal, feedback activation)
AND no cFLIP



One node = one species

Asynchronous state transition graph

Influence graph



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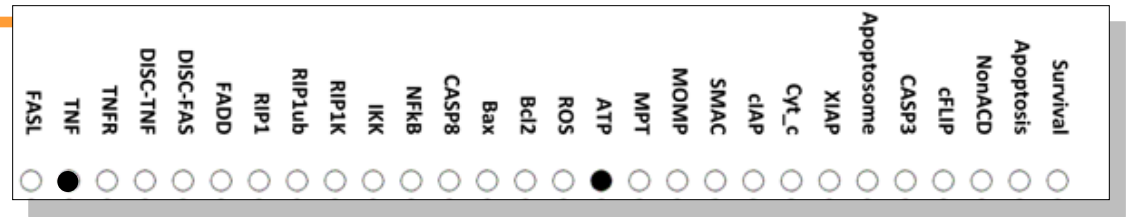

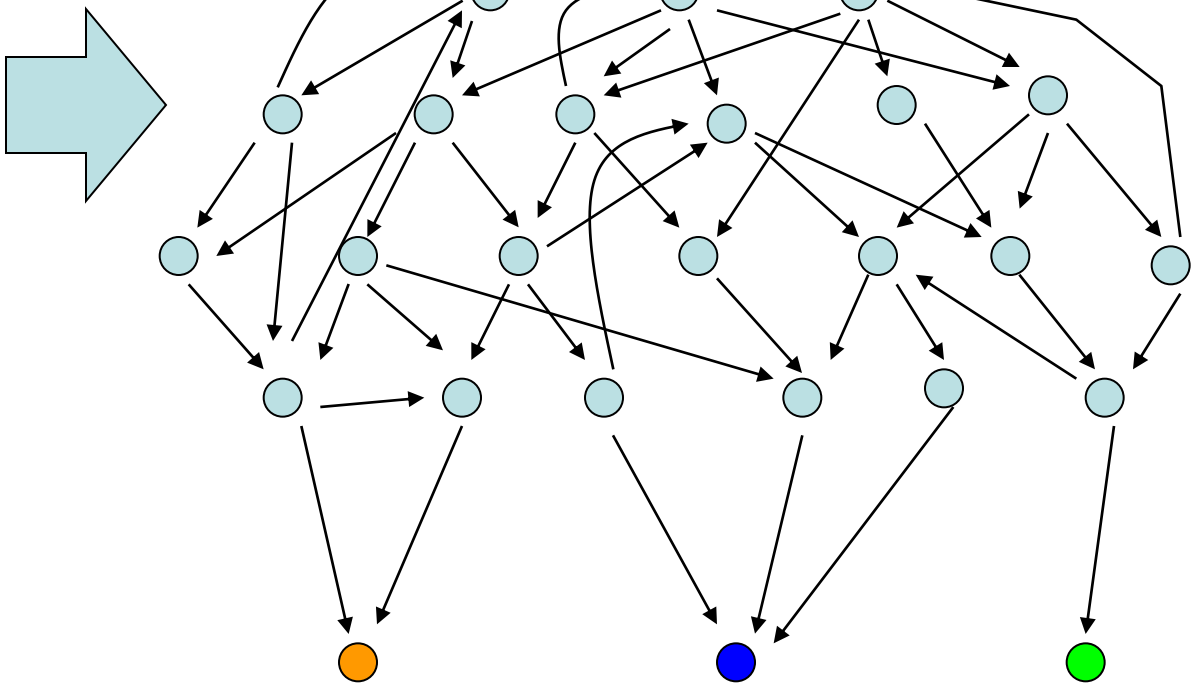

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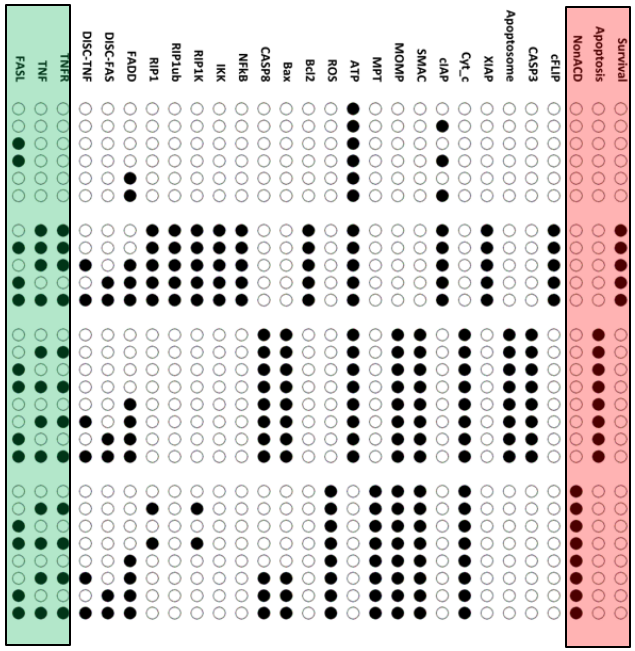
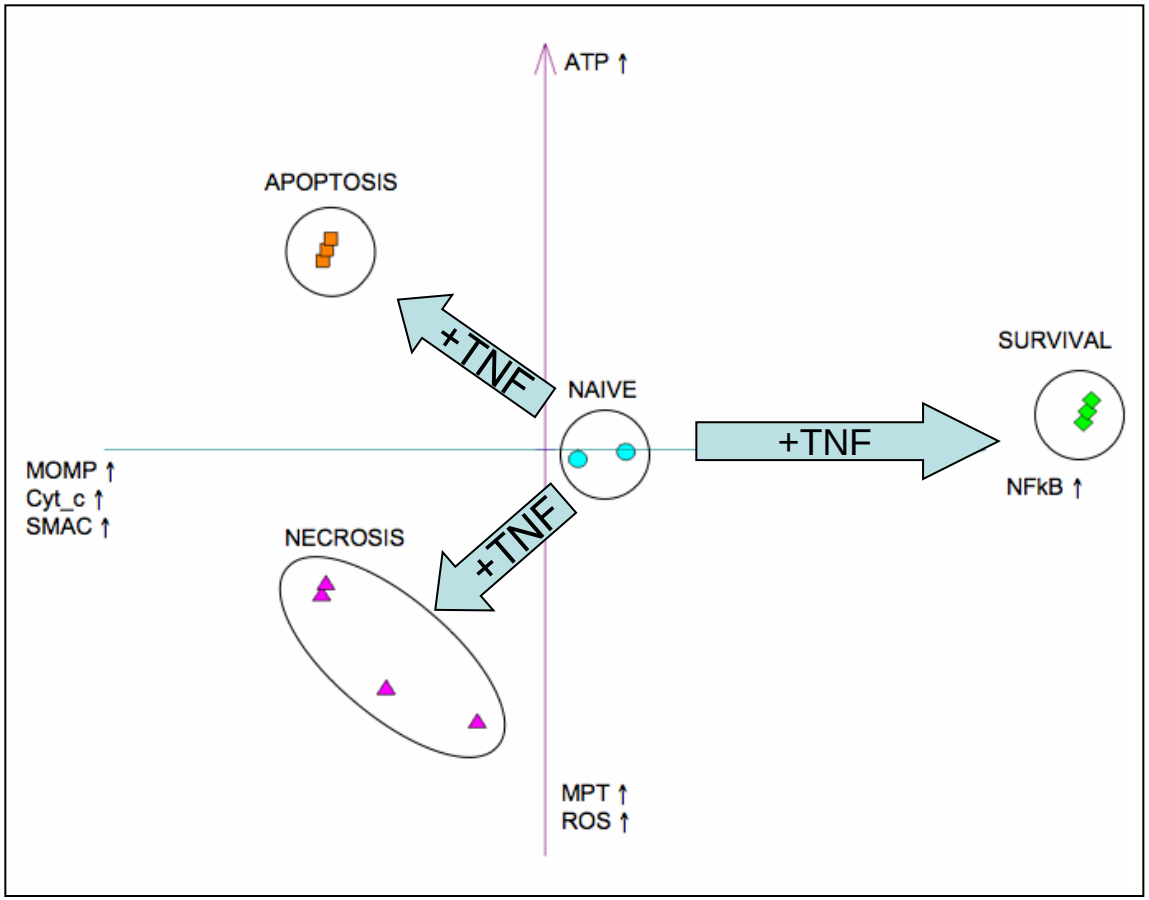
Structure of attractors: distribution of logical stable states





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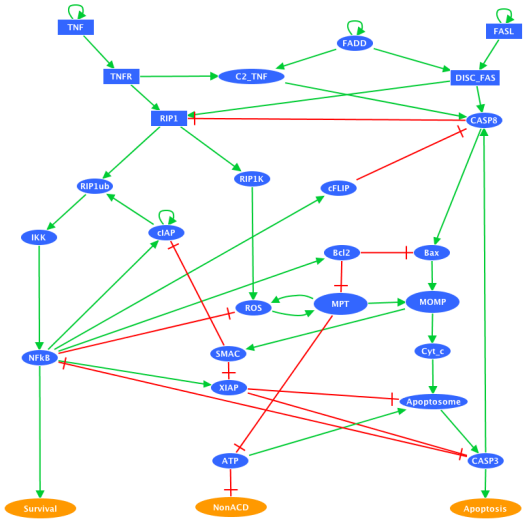
Asynchronous state transition graph

Influence graph



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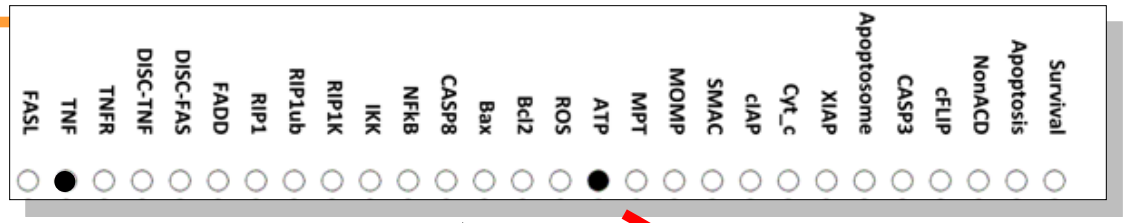

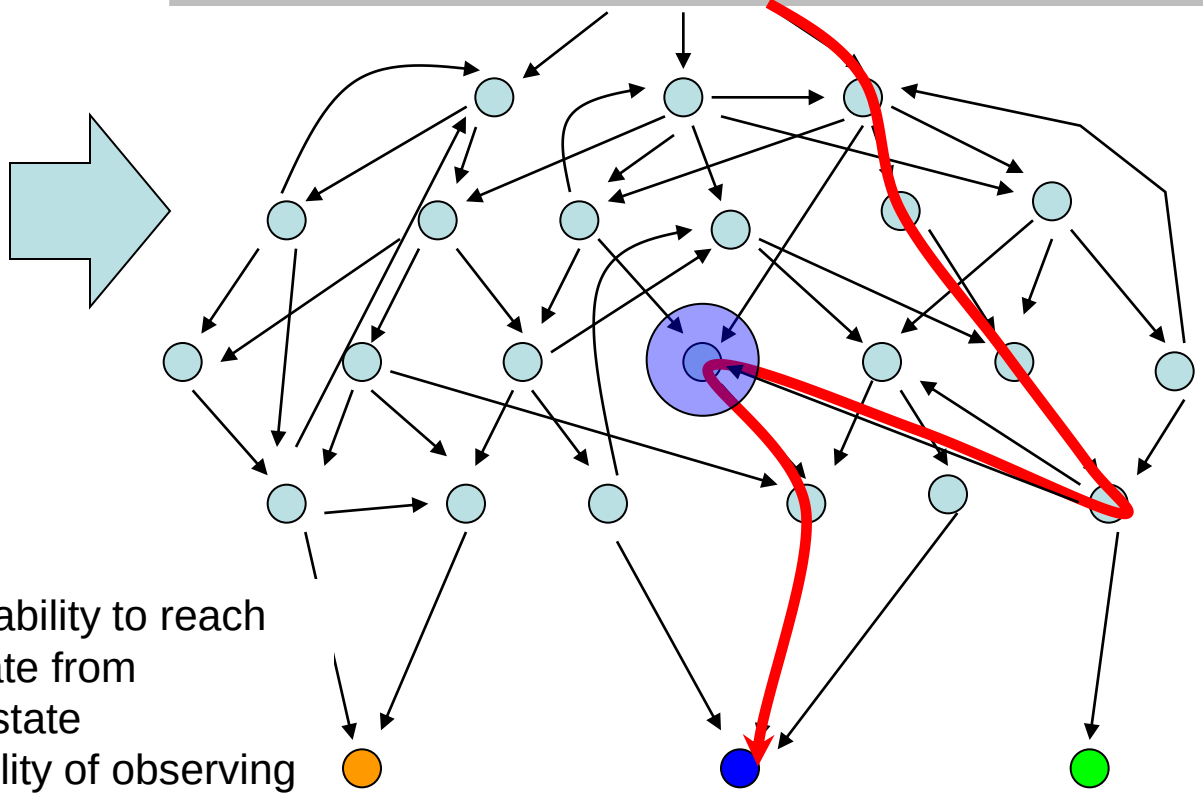

The probability to reach
a final state from
an initial state
= probability of observing
a phenotype in
experiment

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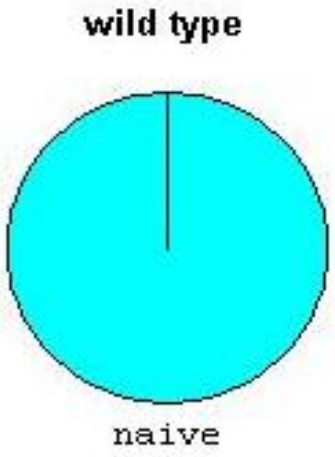
« Probabilities » of reaching phenotypes from physiological initial conditions:



TNF=0

TNF=1

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

$\Rightarrow$ Simulations of mutants or drug treatments

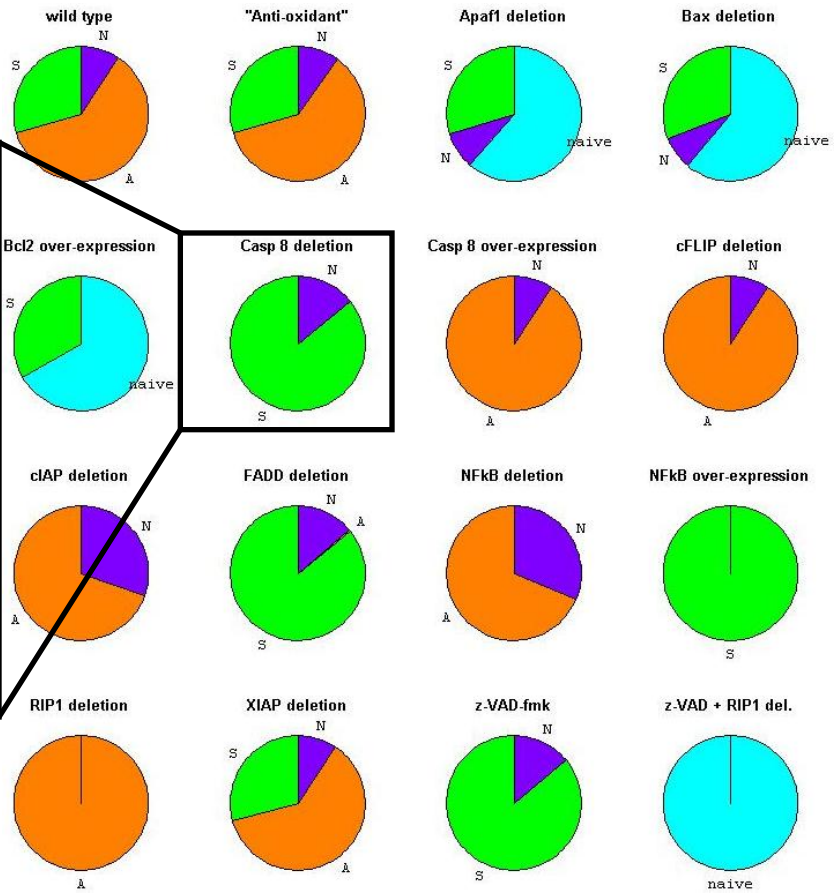
Name	Modified rules	Expected phenotypes	Qualitative results
Anti-oxidant	ROS′=(RIP1 OR MPT)		Suppression of NFκB anti-oxidant effect leads to no change in the decision process (the computed probabilities are noticeably close to the wild type).
APAF1 deletion	C3′=0	APAF1$^{-/-}$ mouse thymocytes are not impaired in Fas-mediated apoptosis (Yoshida *et al*, 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 HCT116 (LeBlanc *et al*, 2002)	BAX deletion prevents apoptosis.
BCL2 over-expression	MOMP′=MPT MPT′=0	FAS induces the activation of NFκB pathway (Kreuz *et al*, 2001)	As expected, NFκB 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 (Varfolom eev *et al*, 1998) or Jurkat cells (Kawahara *et al*, 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 NFκB survival becomes the main cell fate.
constitutively activated CASP8	C8′=1		Over-expression of caspase 8 leads to an increased disappearance of NFκB activation.
cFLIP deletion	C8′=TNF OR FAS OR C3	cFLIP-/- MEFs are highly sensitive to FasL and TNFα (Yeh *et al*, 2000)	The increase of apoptosis is effectively observed in the cFLIP mutant; however we also observe that NFκB pathway can no longer be sustained.
cIAP deletion	cIAP′=0	NFκB activation in response to TNF is blocked (Varfolo meev *et al*, 2008)	NFκB activation is impaired, and only the apoptotic or necrotic attractors are reached.
FADD deletion	C8′=C3AND NOT NFκB RIP1′=NOT C8 AND TNF	FADD-/- mouse thymocytes are resistant to Fas mediated apoptosis (Zhang *et al*, 1998). FADD-/- MEFs are resistant to FasL and TNFα (Yeh *et al*, 1998) In Jurkat cells treated with TNFα+CHX, FADD deletion turns apoptosis into necrotic cell death (Harper *et al*, 2003)	In response to FasL, signalling is blocked, thus the 'naïve' attractor is the only reachable one. In response to TNF, apoptosis disappears.
NFκB deletion	NFkB′=0	TNFα induces both apoptosis and necrosis in NF-κB p65$^{-/-}$ cells (Sakon *et al*, 2003) or in IKKβ$^{-/-}$ fibroblasts (Kamata *et al*, 2005)	This mutant shows a strong increase of necrosis (to be related with concomitant apoptosis/necrosis)
constitutively active NFκB	NFkB′=1		Both death pathways are shut down in this mutant.
RIP1 deletion	RIP1′=0	RIPK1$^{-/-}$ MEFs are hypersensitivity to TNFα, no TNFα-induced NFκB activation, (Kelliher *et al*, 1998)	Both NFκB 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 AND MOMP	No effect on TNFα-induced toxicity in XIAP$^{-/-}$ MEFs (Harlin *et al*, 2001)	S

TNF=1

Example : **Caspase 8 deletion**

≈ 85% survival (NFkB)
≈ 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-/-)



Naïve survival | NFkB survival | apoptosis | necrosis

“Ligand dosage” experiments

What if the signal was removed…

at which point would the cell commit to one or the other phenotype?

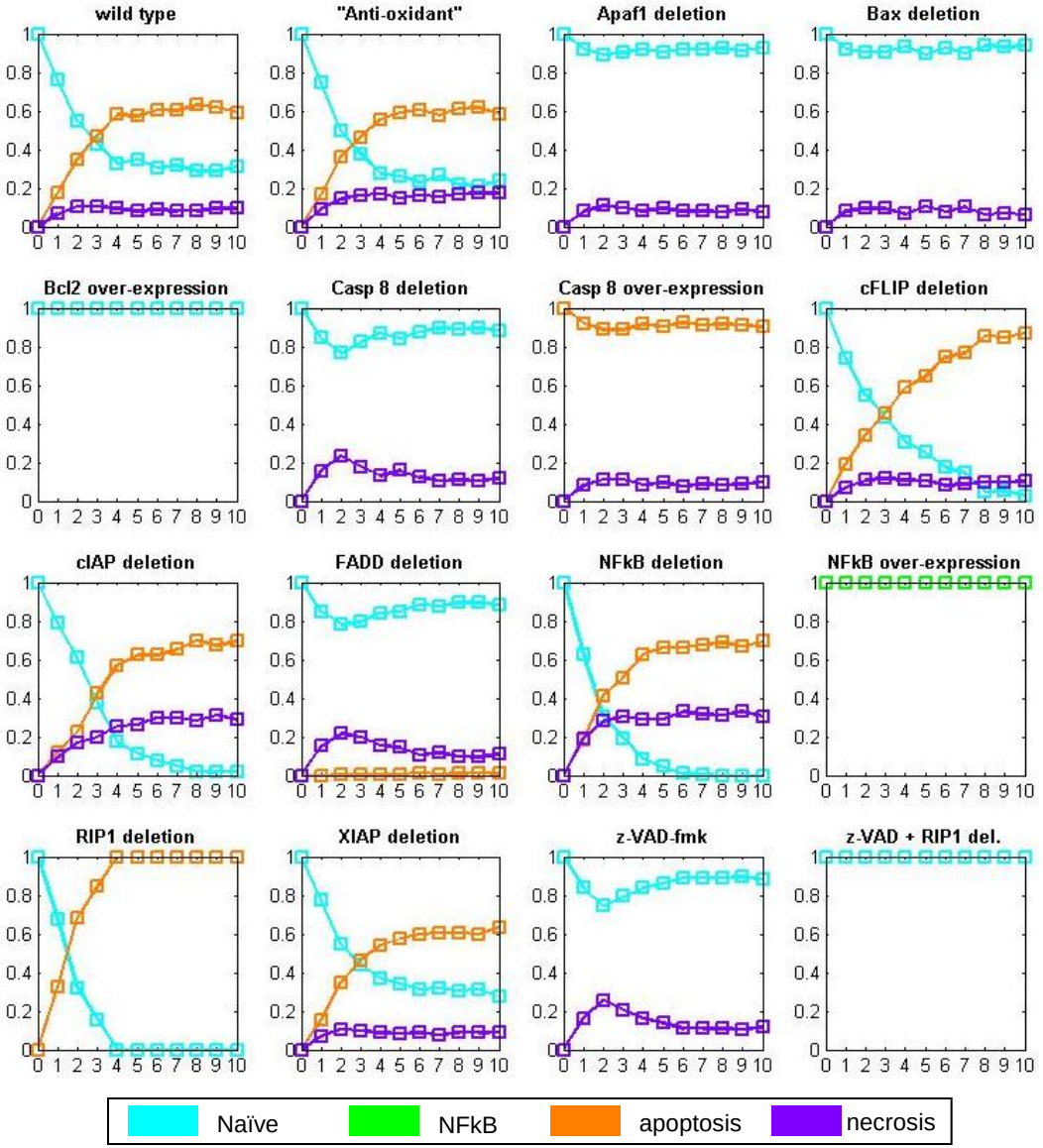
Introduction of “pulse” of TNF instead of constant induction

t : integer

During *t* steps, the system evolves with TNF=1

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

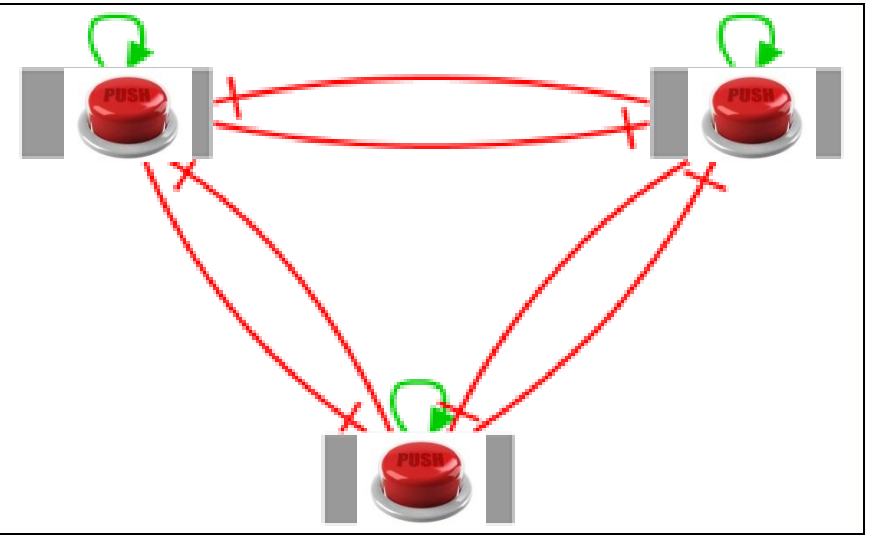
(x-axis → duration of TNF “pulse”)



Simplify to understand!

A conceptual 3-node model:

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



Feedback circuits

MPT => MPT

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

NFkB => NFkB

2) NFkB => cIAP => RIP1ub => IKK => NFkB (+)
3) NFkB => cFLIP -| CASP8 -| RIP1 => RIP1ub => IKK => NFkB (+)

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

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 => NFkB (-)

NFκB -| MPT

14) NFkB -| ROS => MPT (-)
15) NFkB => BCL2 -| MPT (-)
16) NFkB => cFLIP -| CASP8 -| RIP1 => RIP1K => ROS => MPT (+)

CASP3 -| MPT

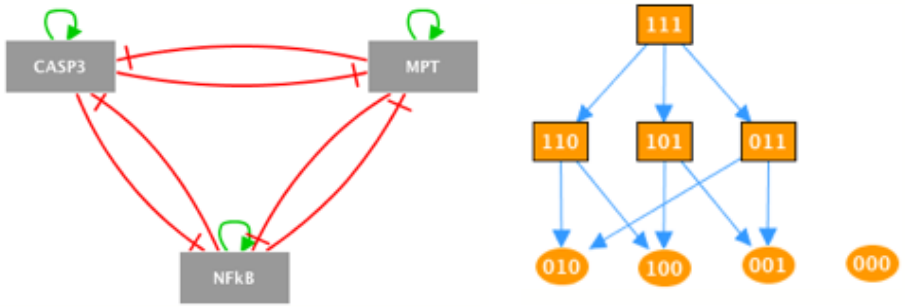
17) CASP3 => CASP8 -| RIP1 => RIP1K => ROS => MPT (-)

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

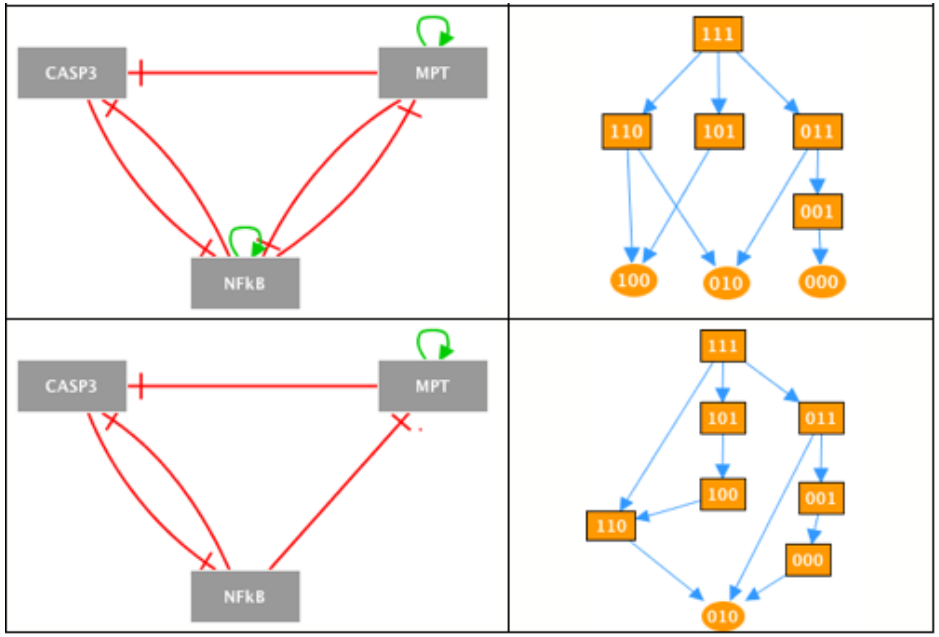
SIMULATE WILD TYPE



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!

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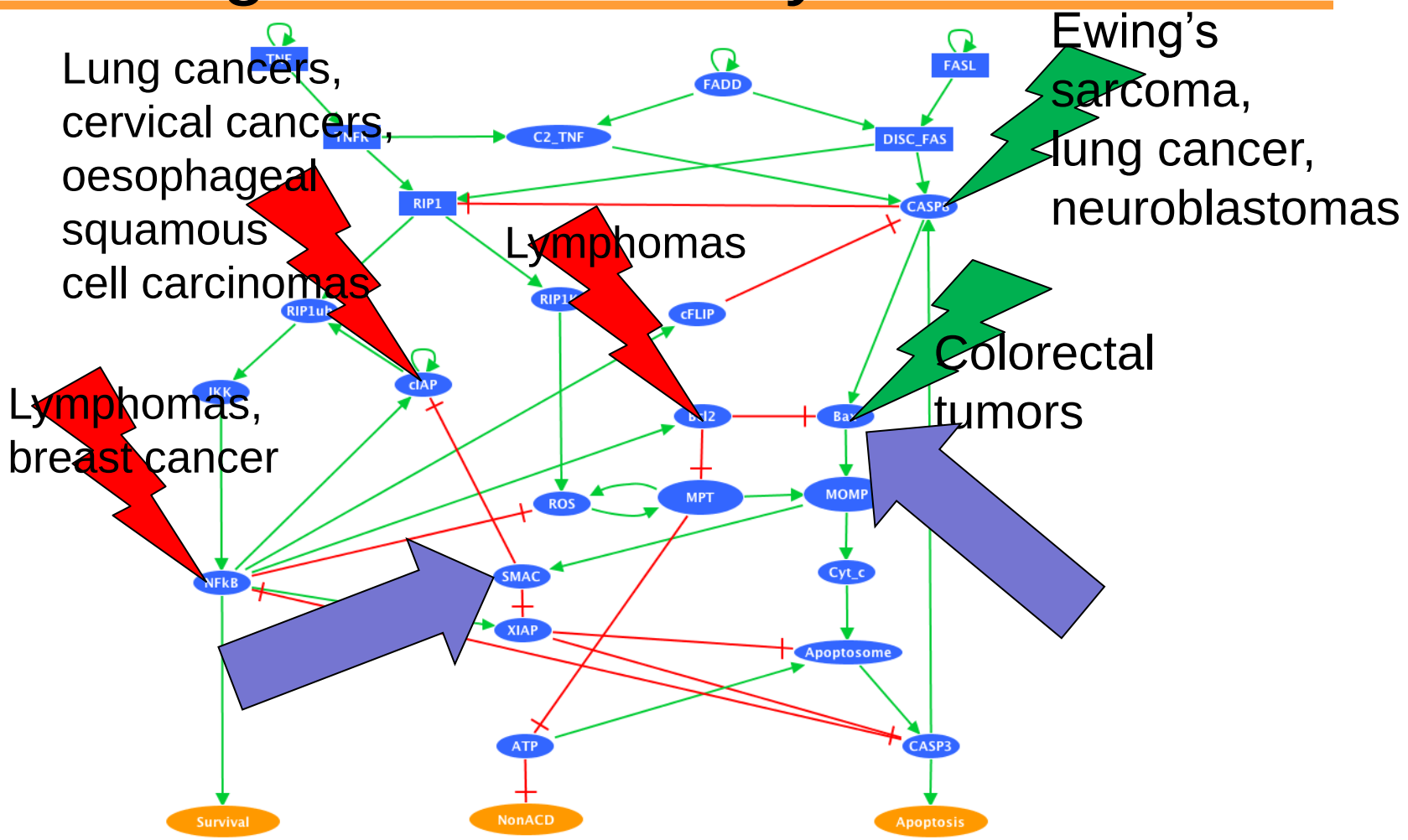
Cell fate decision mechanism fragilities utilized by cancers



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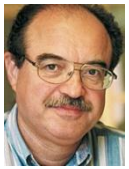

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