Game of Apoptosis

You Win or You Die

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Apoptosis - Background

- It is a process of ‘Programmed Cell Death’
- Regulated via Biochemical mechanisms
- Vital processes:
  - Maintaining cell population in tissues
  - Removing the cells that are no longer needed
  - Removing dysfunctional cells
- A faulty apoptosis mechanism:
  - neurodegenerative diseases
  - ischemic damage
  - autoimmune disorders, cancer, etc.

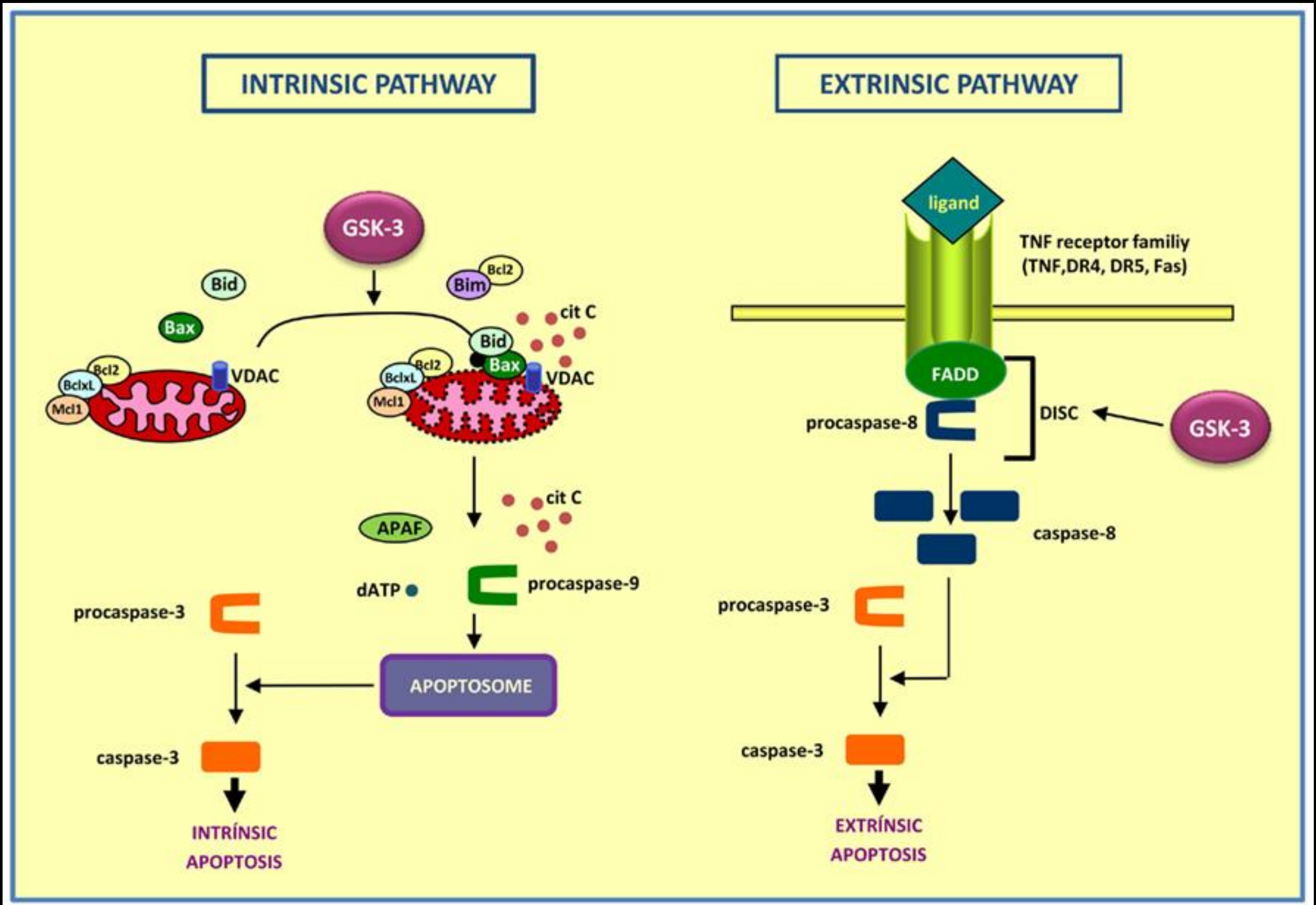
Two Pathways

- Mitochondrial Regulation - *Intrinsic*
  - Apoptotic proteins
    ➔ Mitochondrial swelling/membrane pores
    ➔ Apoptotic effectors leak out
  - Cytochrome C from mitochondria

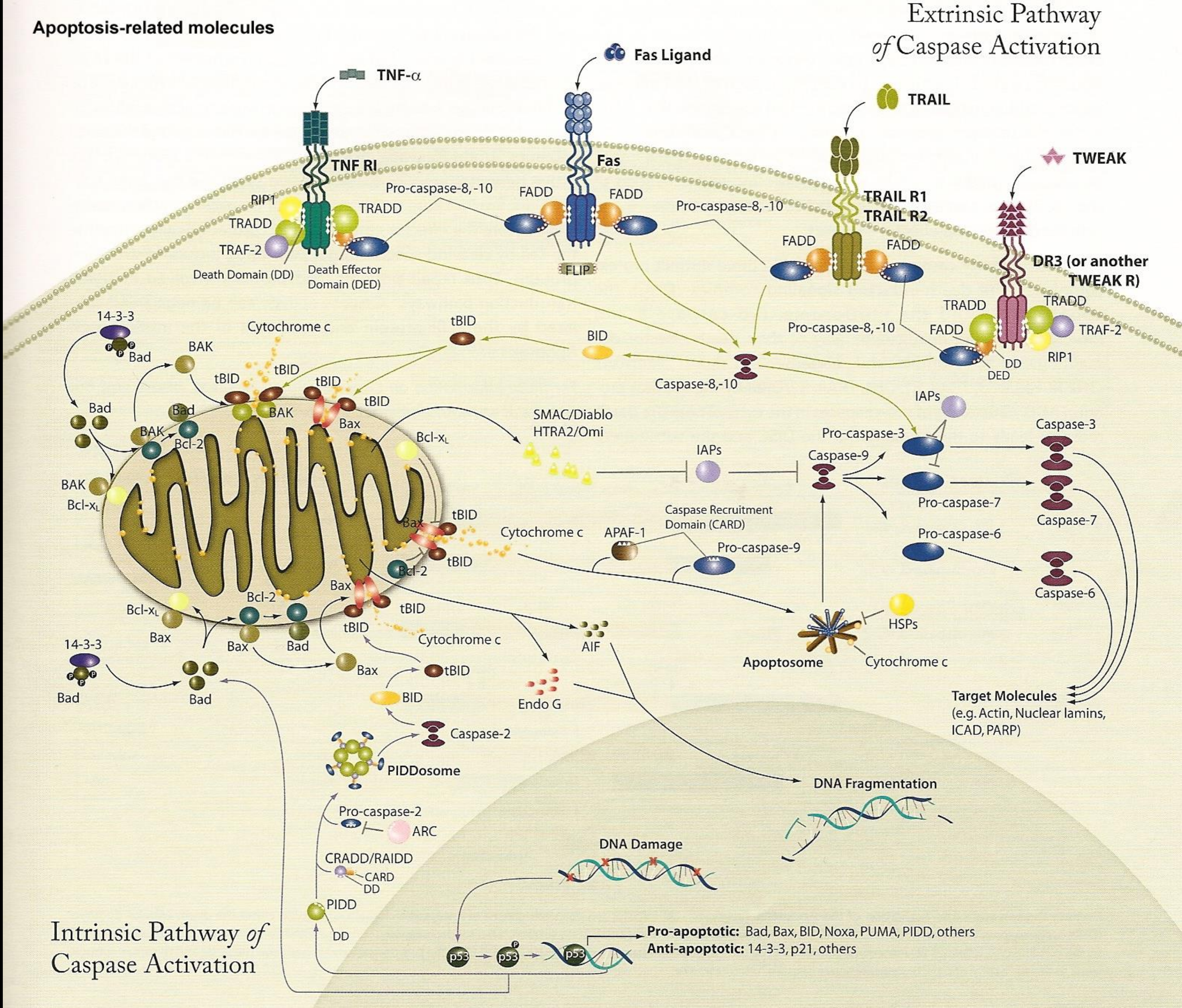
- Direct Signal Transduction - *Extrinsic*
  - TNF Induced
  - Fas-Fas ligand-mediated model

Cell Degradation

- Organized degradation of cellular organelles by activated proteolytic caspases
- Breakdown of the proteinaceous cytoskeleton by caspases
- Pyknosis - condensation of chromatin
- Karyorrhexis – DNA fragmentation


INTRINSIC PATHWAY
EXTRINSIC PATHWAY
GSK-3
Bid
Bim
Bcl2
Bax
cit C
VDAC
BclxL
Mcl1
APAF
dATP
procaspase-9
procaspase-3
APOPTOSOME
caspase-3
INTRÍNSIC
APOPTOSIS
ligand
TNF receptor familiy
(TNF,DR4, DR5, Fas)
FADD
procaspase-8
DISC
caspase-8
EXTRÍNSIC
APOPTOSIS

Apoptosis-related molecules



Modelling the Pathway

- Signal ➔ Surviving outcome OR Apoptosis
- Stability of the network [definite trigger]
- Irreversibility of the survival to apoptotic transition

  [Once started cannot be stopped, attributed to non-linear dynamics associated with feedback loops]
- ODE vs. Boolean Analysis
  - Complex dynamics
  - Not every system property is known
  - Difficult to explore the initial condition states
  - Building a complete big picture

Apoptosis - Boolean Model

- 40 Nodes (including two inputs: TNF, GF and one output: DNA Damage Event)
- Both Anti Apoptosis and Pro Apoptosis pathways considered for a better picture
- Both Intrinsic and Extrinsic pathways considered for a complete picture*
- Identify key network components

*Model based on extensive literature review as well as expert-curated databases, especially *reactome*.

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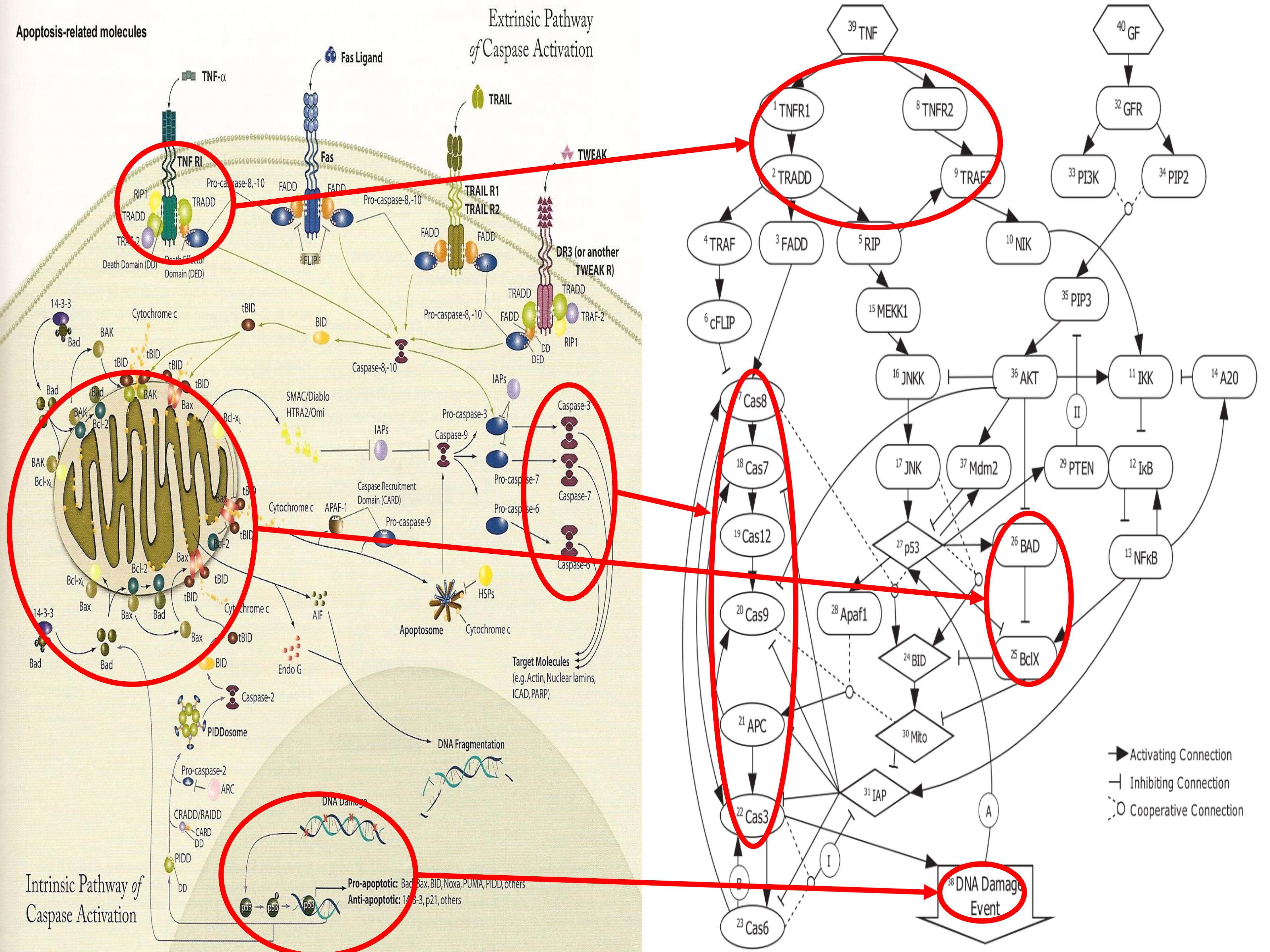
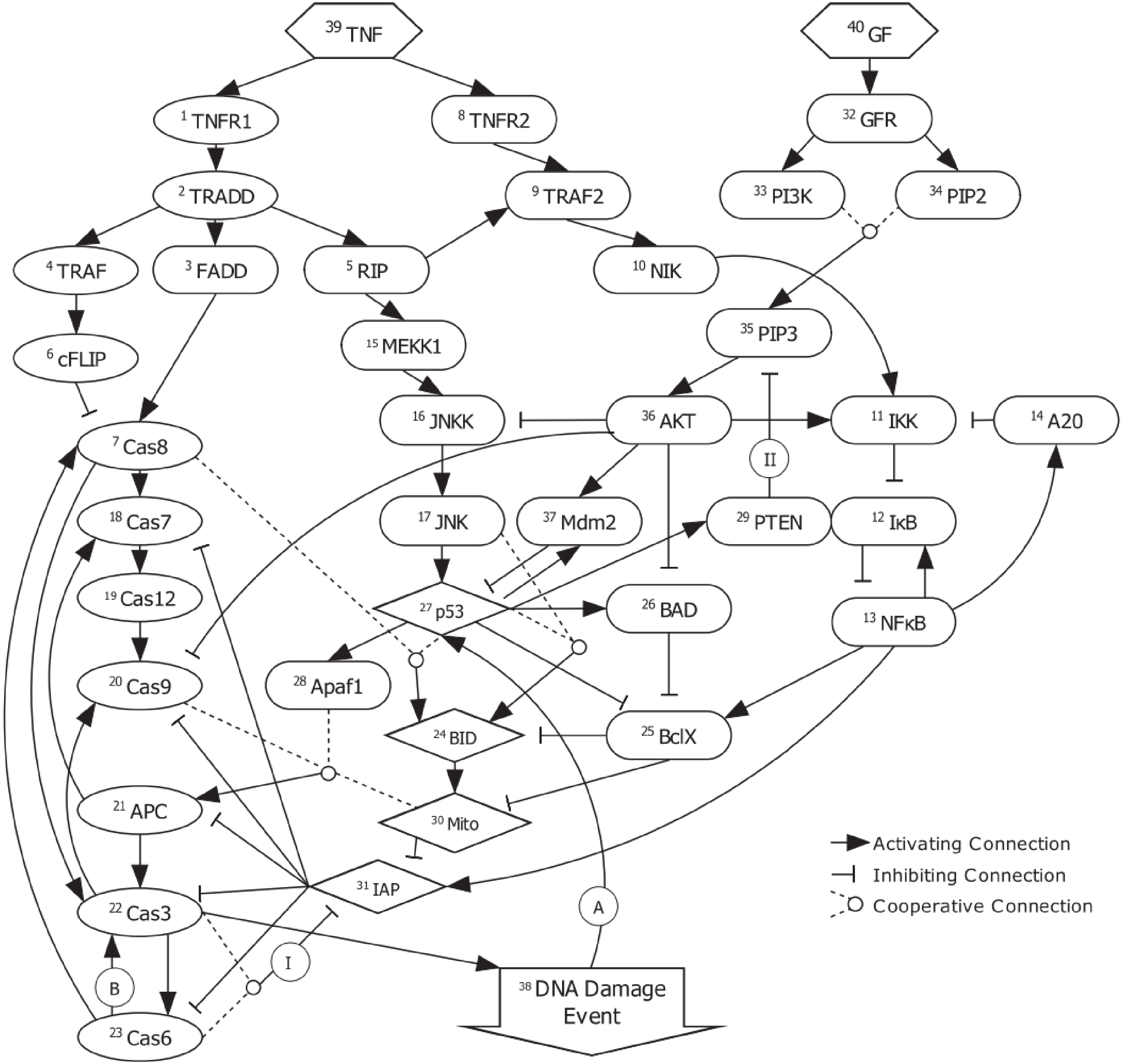


Apoptosis-related molecules
Extrinsic Pathway of Caspase Activation
Intrinsic Pathway of Caspase Activation
TNF-α
Fas Ligand
TRAIL
TWEAK
TNF RI
Fas
TRAIL R1
TRAIL R2
DR3 (or another TWEAK R)
RIP1
TRADD
TRAF-2
FADD
Pro-caspase-8,-10
FLIP
Death Domain (DD)
Death Effector Domain (DED)
DD
DED
Caspase-8,-10
BID
tBID
Cytochrome c
14-3-3
Bad
BAK
Bax
Bcl-2
Bcl-xL
SMAC/Diablo
HTRA2/Omi
IAPs
Pro-caspase-3
Caspase-9
Caspase-3
Pro-caspase-7
Caspase-7
Pro-caspase-6
Caspase-6
Caspase Recruitment Domain (CARD)
APAF-1
Pro-caspase-9
HSPs
Apoptosome
AIF
Endo G
Target Molecules
(e.g. Actin, Nuclear lamins, ICAD, PARP)
Caspase-2
PIDDosome
Pro-caspase-2
ARC
CRADD/RAIDD
CARD
PIDD
DNA Fragmentation
DNA Damage
p53
Pro-apoptotic: Bad, Bax, BID, Noxa, PUMA, PIDD, others
Anti-apoptotic: 14-3-3, p21, others
39 TNF
40 GF
1 TNFR1
8 TNFR2
32 GFR
2 TRADD
9 TRAF2
33 PI3K
34 PIP2
4 TRAF
3 FADD
5 RIP
10 NIK
6 cFLIP
15 MEKK1
35 PIP3
7 Cas8
16 JNKK
36 AKT
11 IKK
14 A20
18 Cas7
17 JNK
37 Mdm2
29 PTEN
12 IκB
II
19 Cas12
27 p53
26 BAD
13 NFκB
20 Cas9
28 Apaf1
24 BID
25 BclX
21 APC
30 Mito
22 Cas3
31 IAP
A
I
B
23 Cas6
38 DNA Damage Event
Activating Connection
Inhibiting Connection
Cooperative Connection



39 TNF
40 GF
1 TNFR1
8 TNFR2
32 GFR
2 TRADD
9 TRAF2
33 PI3K
34 PIP2
4 TRAF
3 FADD
5 RIP
10 NIK
35 PIP3
15 MEKK1
6 cFLIP
16 JNKK
36 AKT
11 IKK
14 A20
7 Cas8
II
18 Cas7
17 JNK
37 Mdm2
29 PTEN
12 IκB
19 Cas12
27 p53
26 BAD
13 NFκB
20 Cas9
28 Apaf1
24 BID
25 BclX
21 APC
30 Mito
31 IAP
22 Cas3
A
I
B
23 Cas6
38 DNA Damage Event
Activating Connection
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Cooperative Connection

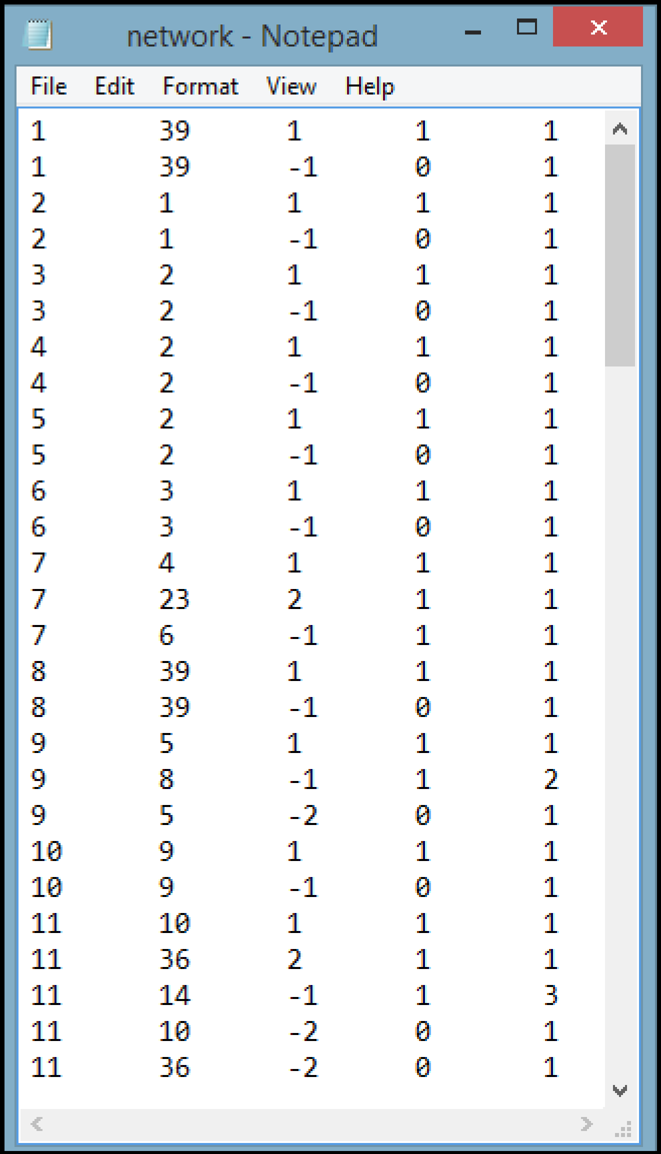
Rules of the *Game*

- Dynamics:

$$S_i(t+1) = \begin{cases} \text{OFF} & \text{if } A_i(t) < H_i(t), \\ \text{ON} & \text{if } A_i(t) > H_i(t), \\ S_i(t) & \text{if } A_i(t) = H_i(t). \end{cases}$$

  - DNA Damage is a special case
- Ending criteria and ending states:
  - DNA damage event has remained continuously on for a predefined number of steps (APOP_THRESH)➔ Apoptosis
  - Maximum number of steps have been reached (NUM_STEPS) ➔ Survival

#	Node	Type	Condition	Contribution
1	TNFR1	activating	TNF ON	1
		inhibiting	TNF OFF	1
2	TRADD	activating	TNFR1 ON	1
		inhibiting	TNFR1 OFF	1
3	TRAF	activating	TRADD ON	1
		inhibiting	TRADD OFF	1
4	FADD	activating	TRADD ON	1
		inhibiting	TRADD OFF	1
5	RIP	activating	TRADD ON	1
		inhibiting	TRADD OFF	1
6	cIAP	activating	TRAF ON	1
		inhibiting	TRAF OFF	1
7	Cas8	activating	FADD ON	1
		activating	Cas6 ON	1
		inhibiting	cIAP ON	1
8	TNFR2	activating	TNF ON	1
		inhibiting	TNF OFF	1
9	TRAF2	activating	RIP ON	1
		inhibiting	TNFR2 ON	2
			RIP OFF	1
10	NIK	activating	TRAF2 ON	1
		inhibiting	TRAF2 OFF	1
11	IKK	activating	NIK ON	1
			Akt ON	1
		inhibiting	A20 ON	3
			NIK OFF and Akt OFF	1



1	39	1	1	1
1	39	-1	0	1
2	1	1	1	1
2	1	-1	0	1
3	2	1	1	1
3	2	-1	0	1
4	2	1	1	1
4	2	-1	0	1
5	2	1	1	1
5	2	-1	0	1
6	3	1	1	1
6	3	-1	0	1
7	4	1	1	1
7	23	2	1	1
7	6	-1	1	1
8	39	1	1	1
8	39	-1	0	1
9	5	1	1	1
9	8	-1	1	2
9	5	-2	0	1
10	9	1	1	1
10	9	-1	0	1
11	10	1	1	1
11	36	2	1	1
11	14	-1	1	3
11	10	-2	0	1
11	36	-2	0	1

Initialising the *Game*

- Initial States
  - 2^{37}? Random initial states; probability of 0.5
  - Simulated using many initial states to get good statistics
    - How much is good enough?

- Input Signals

• No input	TNF OFF	GF OFF	00
• Only TNF	TNF ON		01
• Only GF		GF ON	10
• Both TNF & GF	TNF ON	GF ON	11

Metrics for Network Evaluation – Part 1

- Lethal States:
  - Initial states that, independent of input, always end up in apoptosis. Natural?
- Apoptosis Ratios:
  - For each of the 4 signal combinations, evaluate the "probability" of state ending in apoptosis.

$$\text{Apop\%} = \frac{\text{Number of apoptotic initial states}}{\text{Total number of initial states}} \times 100\%$$

- p(ON/lethal_state)
- Knockout experiments: removal of edges

Results and Comparisons - 1

- Apoptotic Ratio:

	Apop%				
Comments	No signal	GF only	TNF only	Double Input	# lethal states
IS=1000	53.6	53.3	70.7	66.4	
	0.64	0.21	34.05	24.84	533
IS=5000	55.98	55.76	69.94	66.28	
	0.99	0.49	32.39	24.16	2777
IS=10000	55.19	55.07	70.59	66.78	
	0.93	0.66	34.98	26.55	5477
	48	47	97	64	
IS=20000	55.575	55.415	71.1	67.225	
	1.00	0.65	35.60	26.96	11025

Results and Comparisons - 2

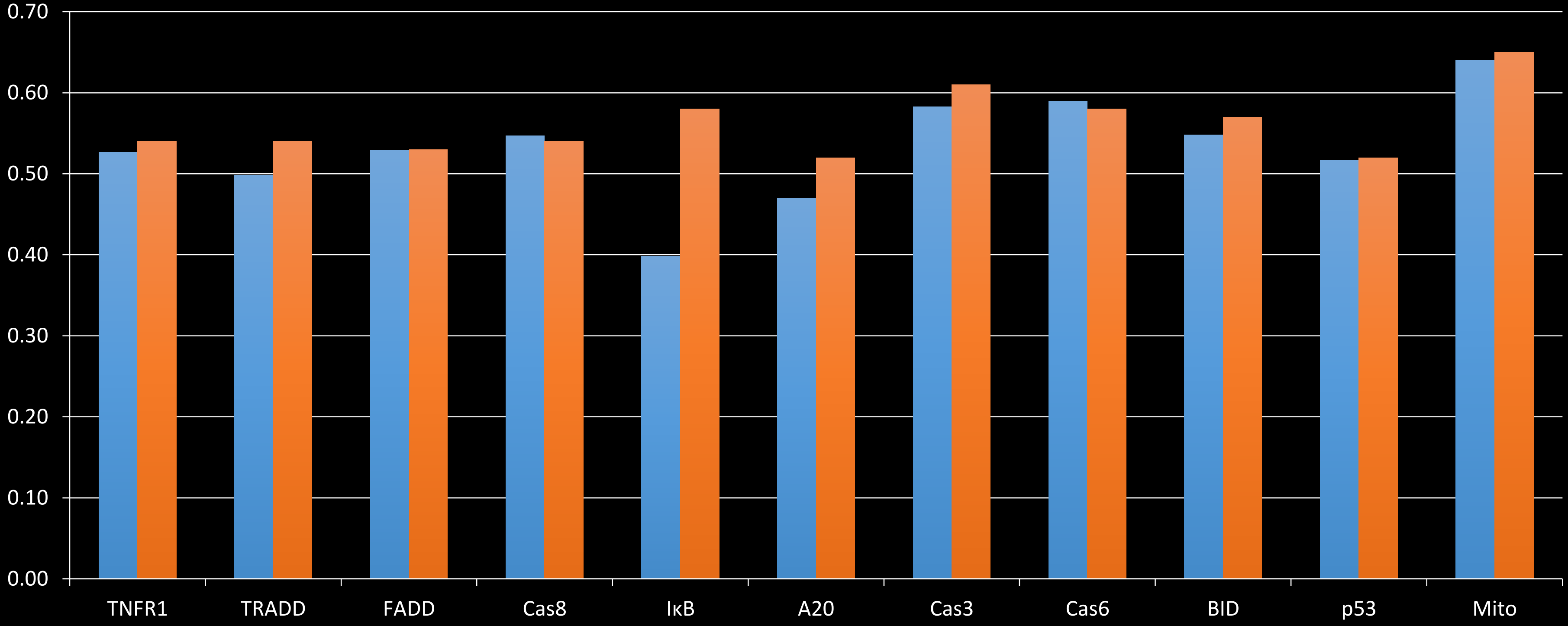
- Apoptotic Ratio

Comments	00	01	10	11
IS=10,000 / A	57.05	56.96	74.89	75.99
	0.88	0.67	42.05	44.59
	48	47	97	62
IS=10,000 / B	68.93	68.93	97.99	97.99
	0.00	0.00	93.53	93.53
	22	22	97	58
IS=10,000/ I	55.94	55.87	70.95	68.54
	0.97	0.81	34.70	29.29
	18	15	97	54
IS=10,000/ II	54.61	54.6	69.38	69.27
	0.90	0.87	33.14	32.90
	47	47	97	60

Results and Comparisons - 2

- p(ON/lethal_state) for important nodes: Pro-Apoptotic

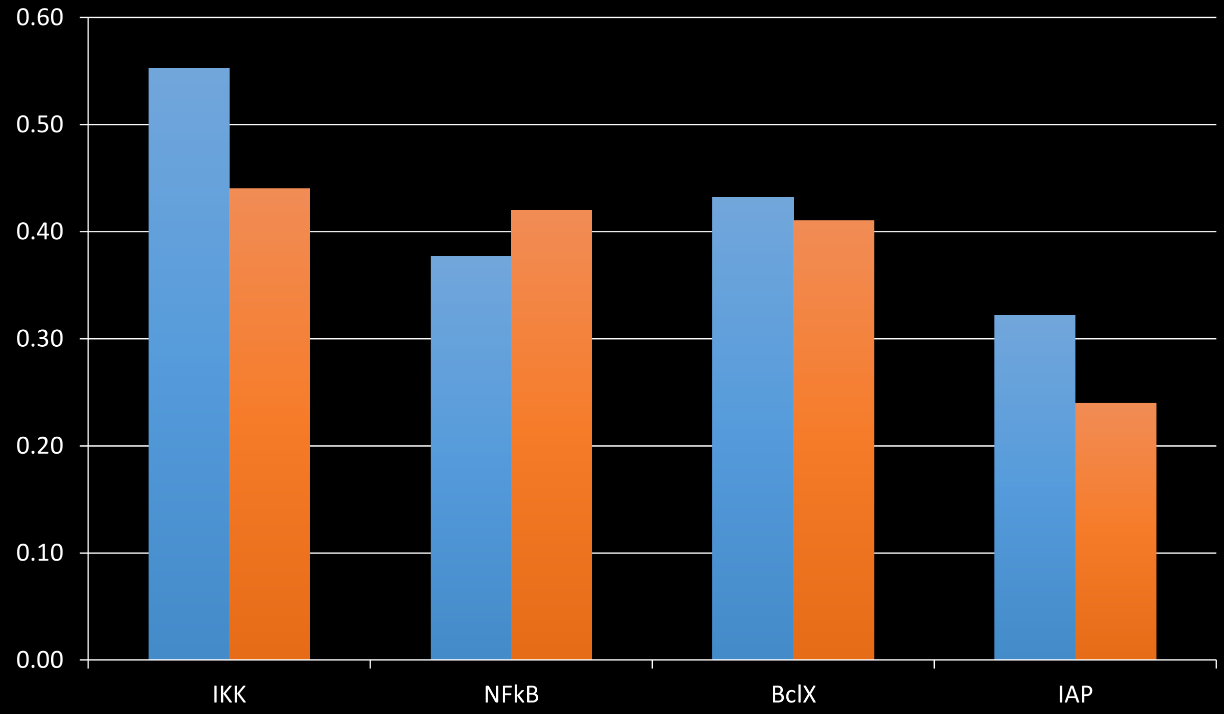
TNFR1	TRADD	FADD	Cas8	IκB	A20	Cas3	Cas6	BID	p53
0.53	0.50	0.53	0.55	0.40	0.47	0.58	0.59	0.55	0.52
0.54	0.54	0.53	0.54	0.58	0.52	0.61	0.58	0.57	0.52



Results and Comparisons - 3

- p(ON/lethal_state) for important nodes: Pro-Survival

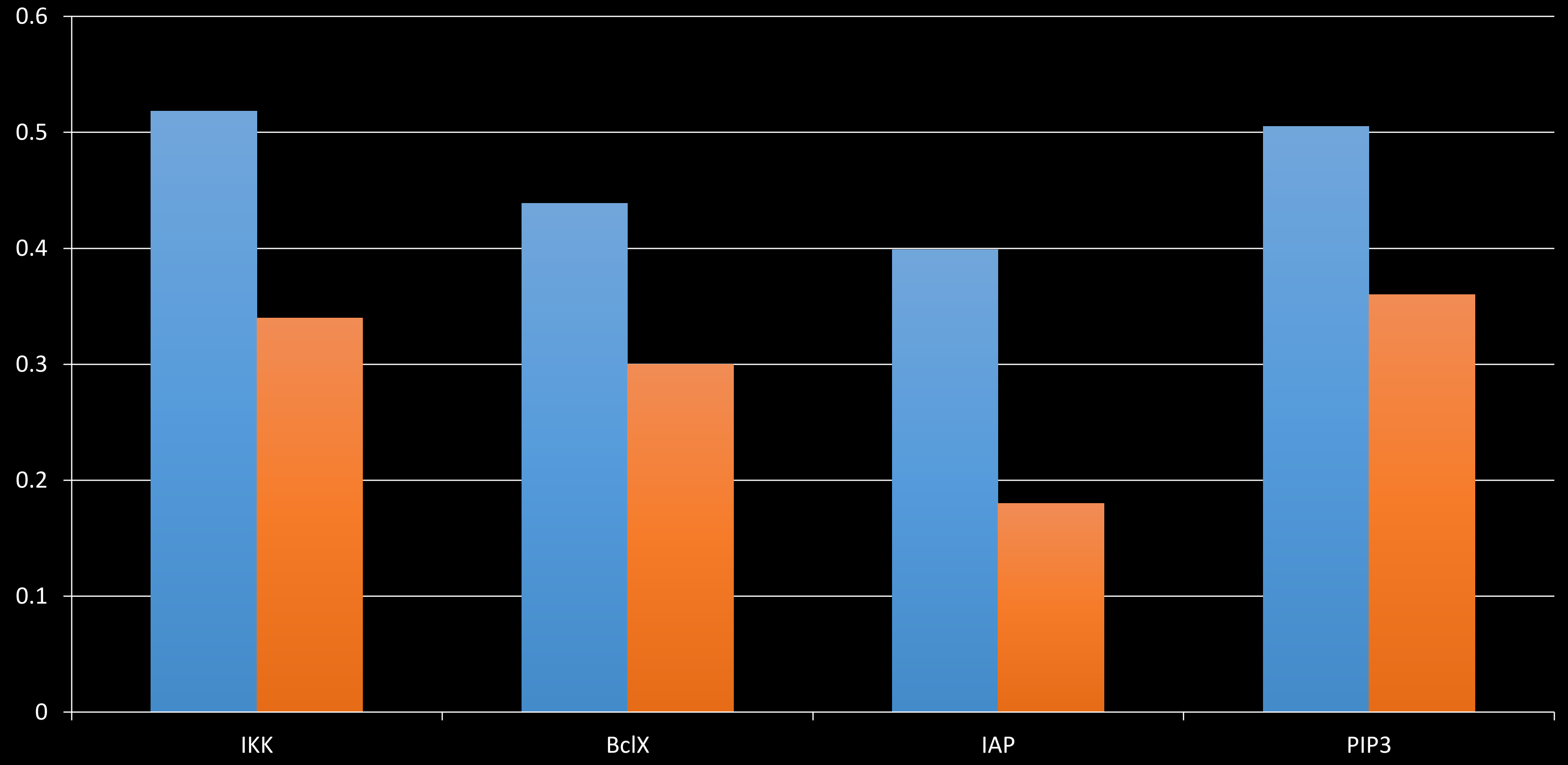
IKK	NFkB	BclX	IAP
0.55	0.38	0.43	0.32
0.44	0.42	0.41	0.24



Results and Comparisons - 4

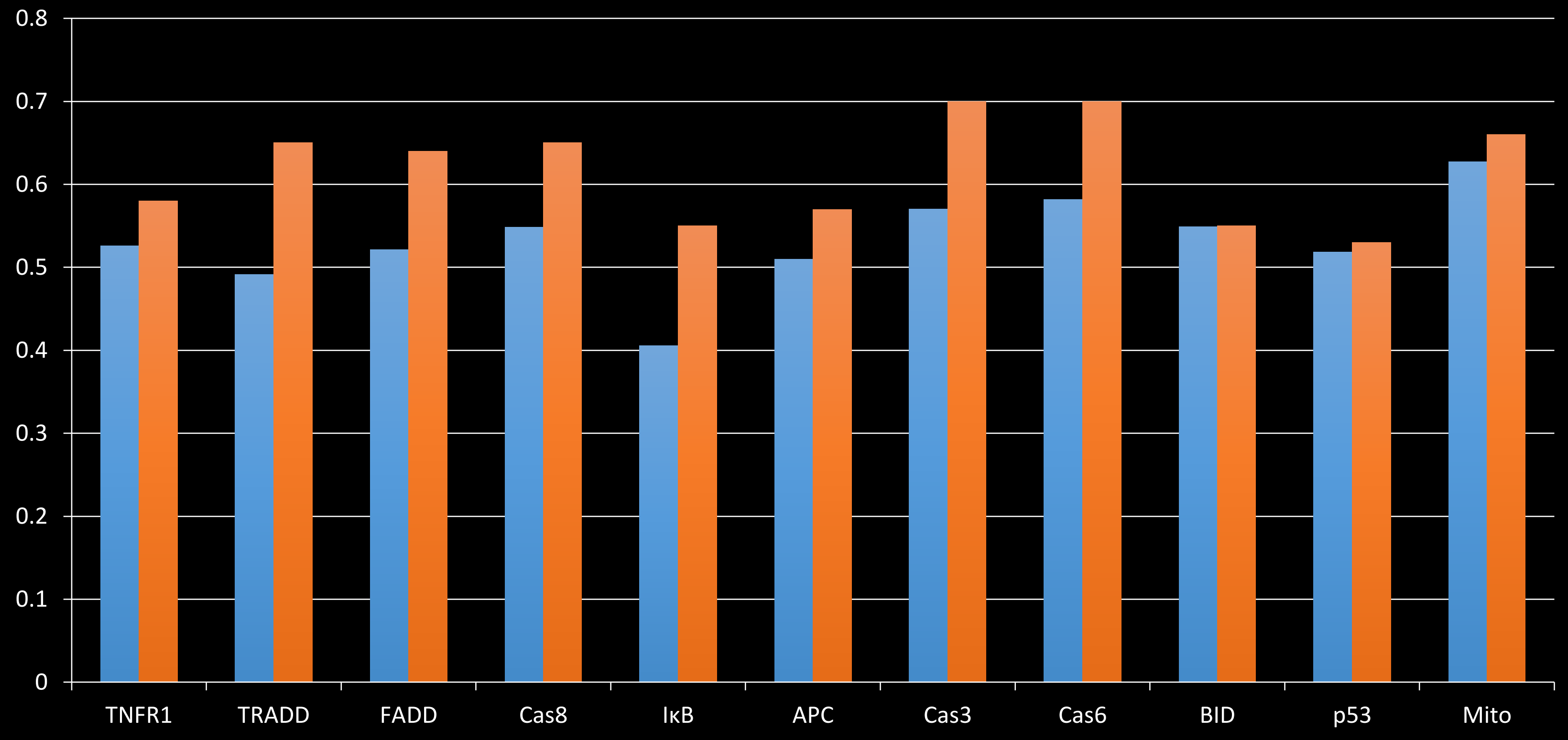
- p(ON/lethal_state) for important nodes: Pro-Survival – B loop elimination

IKK	BclX	IAP	PIP3
0.52	0.44	0.40	0.51
0.34	0.3	0.18	0.36



Results and Comparisons - 5

TNFR1	TRADD	FADD	Cas8	IκB	APC	Cas3	Cas6	BID	p53	Mito
0.53	0.49	0.52	0.54	0.40	0.51	0.57	0.58	0.55	0.52	0.63
0.58	0.65	0.64	0.65	0.55	0.57	0.7	0.7	0.55	0.53	0.66



Metrics for Network Evaluation – Part 2, an extension

- Lethal States:
  - Must be dealt with for better network evaluation.
  - Attach probabilities to initial states based on previously learned classification parameters of lethal and non-lethal states.
- Apoptosis Ratios:
  - A better picture would be to only consider non-lethal states in our sample space.
  - $Apop\% = \frac{Number\ of\ apoptotic\ and\ non{-}lethal\ initial\ states\ X100}{Total\ number\ of\ non{-}lethal\ initial\ states}$
- Studying irreversibility of apoptosis: the *dna_damage_run* switch

DNA_damage_run 'Switch'

Win or Die?

- We found out the expectation of the maximum DNA-damage-run in the states that end in survival, and not apoptosis.
- Turned out to be very small (see table below).
- Maximum of maximum of DNA-damage-run only 2.
- Switch 2->20 : Survival ->Apoptosis

Comments	No signal	GF only	TNF only	Double Input
Avg_DNA_Damage IS=10000	0.39267134	0.39521769	0.56568458	0.50979192
Avg_DNA_Damage IS=1000	0.42307692	0.42792793	0.60915493	0.55417957
Avg_DNA_Damage IS=100	0.36363636	0.36363636	0.46875	0.43243243

Failures

- Potential Weakness of network: Removal of nodes experiments?
- [No perfect matchings to the given paper.]
- Better treatment of lethal states.

Future Work

- Ranking nodes according to importance.
- Parametrisation of Lethal States.

Game Over

Questions?