Apoptosis in Mammalian Cells

7.16 2-10-05

Apoptosis is an important factor in many human diseases

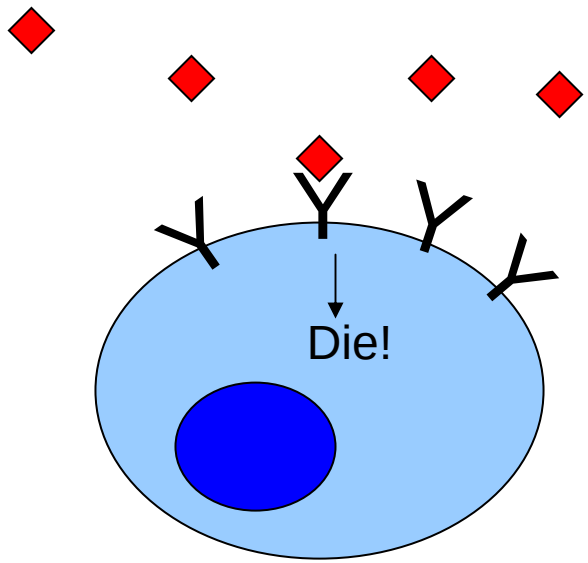
- **Cancer** – malignant cells evade death by suppressing apoptosis (too little apoptosis)

- **Stroke** – damaged neurons commit apoptosis, leading to more extensive damage (too much apoptosis)

- **Autoimmune disorders** – immune cells survive for too long, leading to over-reactive immune response (too little apoptosis)

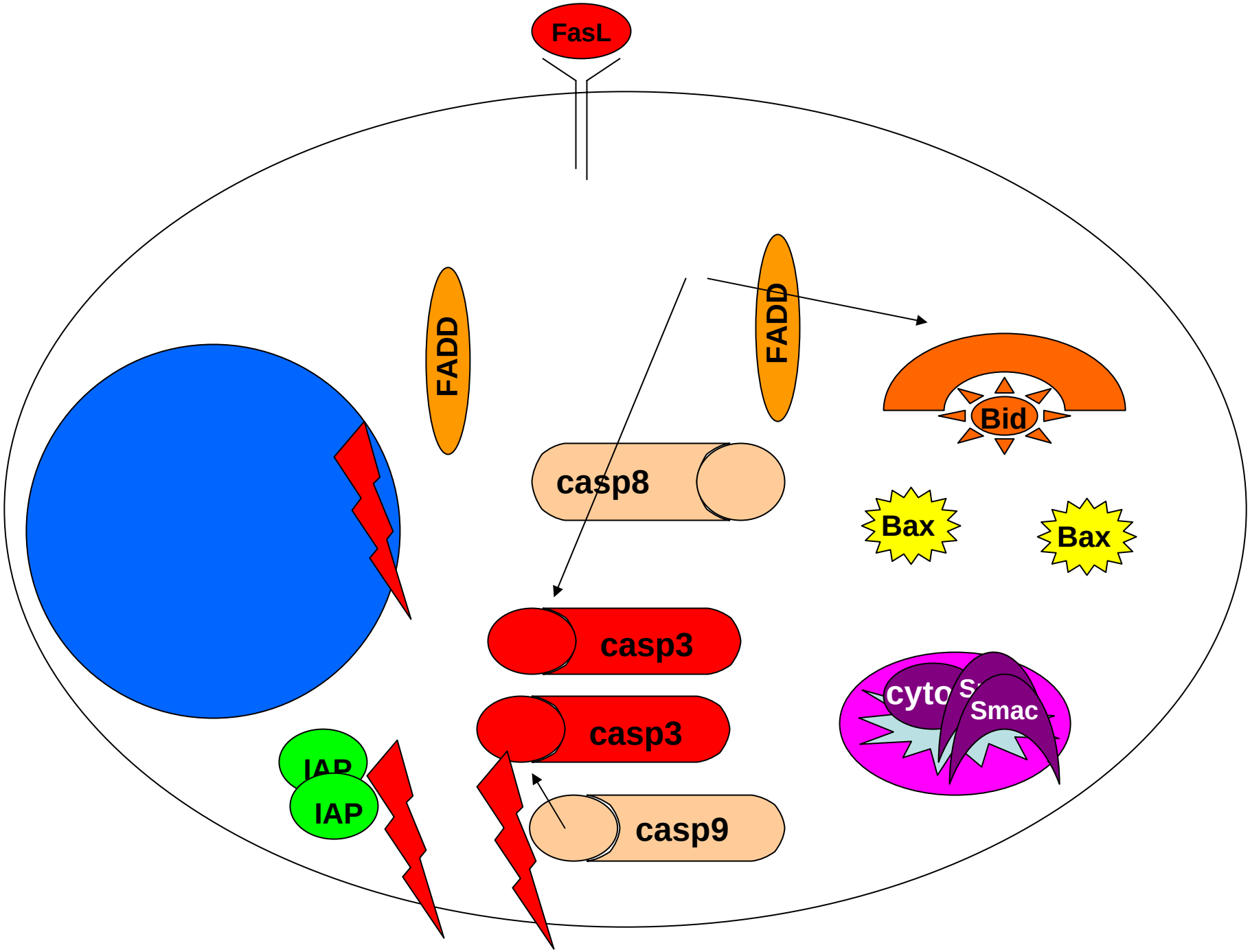
The death receptor pathway

- In mammals, there is a family of death ligands that bind to receptors on the cell surface, sending a pro-apoptotic signal to the cell



Death ligand family members

- **FasL** – very important in the immune system
- **TNF (Tumor necrosis factor)** – controls inflammatory responses
- **TRAIL** –selectively kills tumor cells; currently in clinical trials as an anti-cancer agent


FasL
FADD
FADD
Bid
casp8
Bax
Bax
casp3
casp3
cyto
Smac
IAP
IAP
casp9

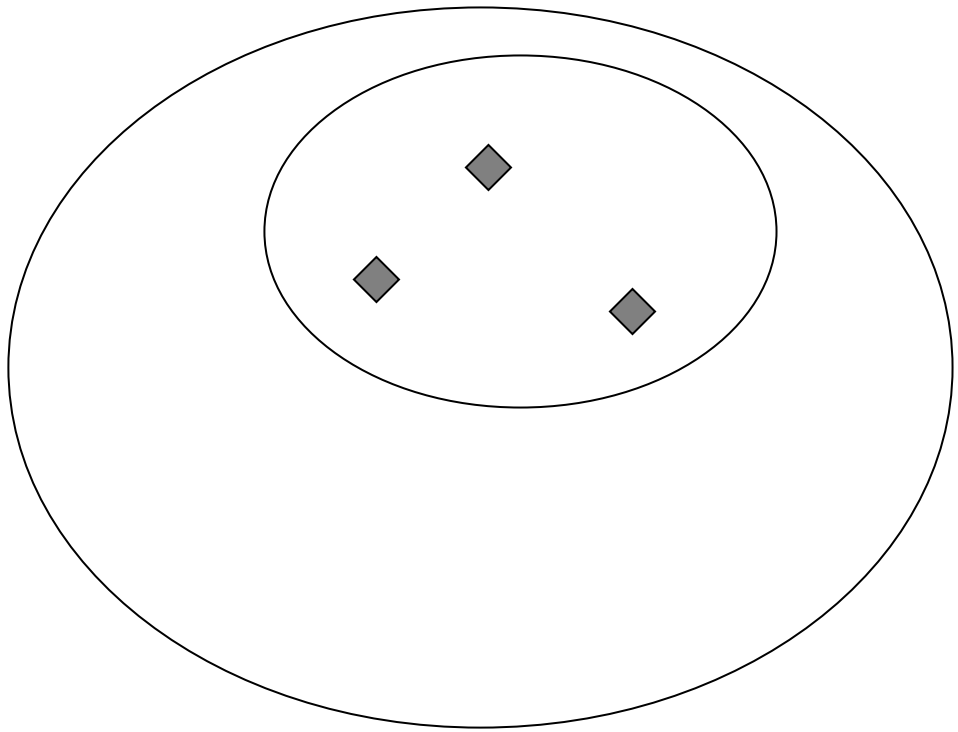
Tools for observing apoptosis

- Immunofluorescence mircroscopy
- Flow cytometry
- Live cell microscopy

Principles of Fluorescence

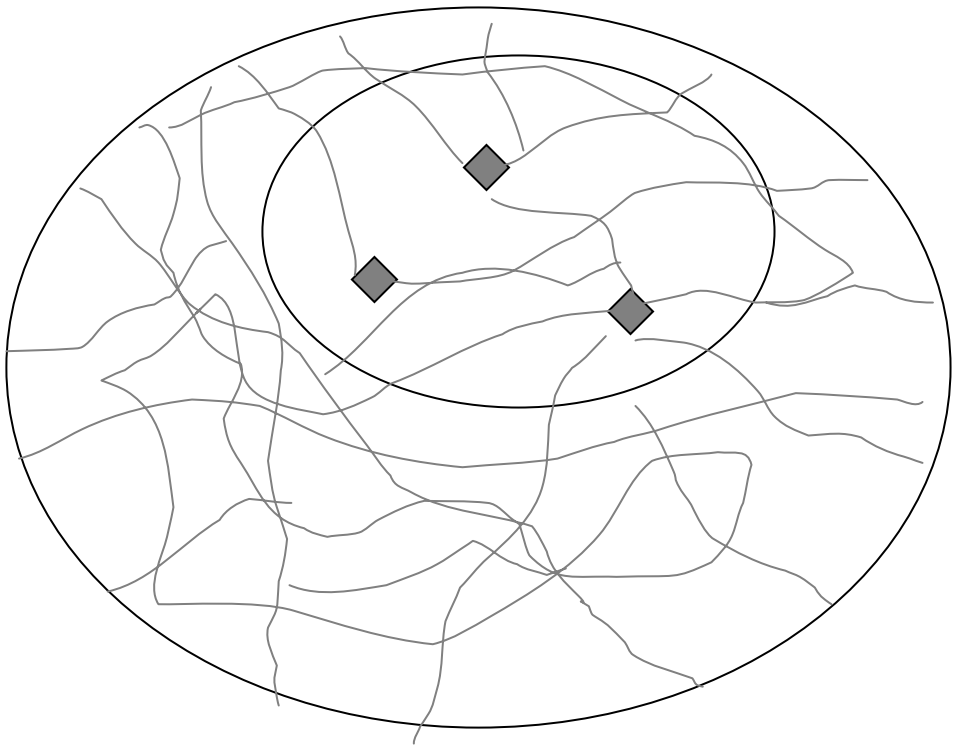
Two diagrams removed for copyright reasons.

Immunofluorescence can detect specific proteins in cells



Immunofluorescence can detect specific proteins in cells

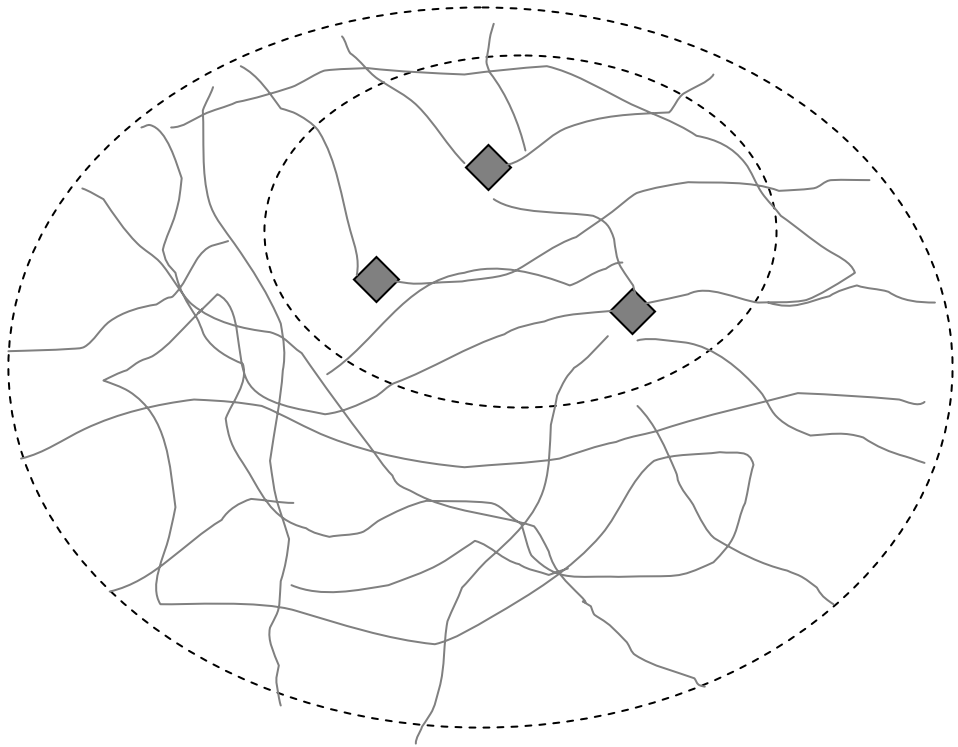
Fix with formaldehyde



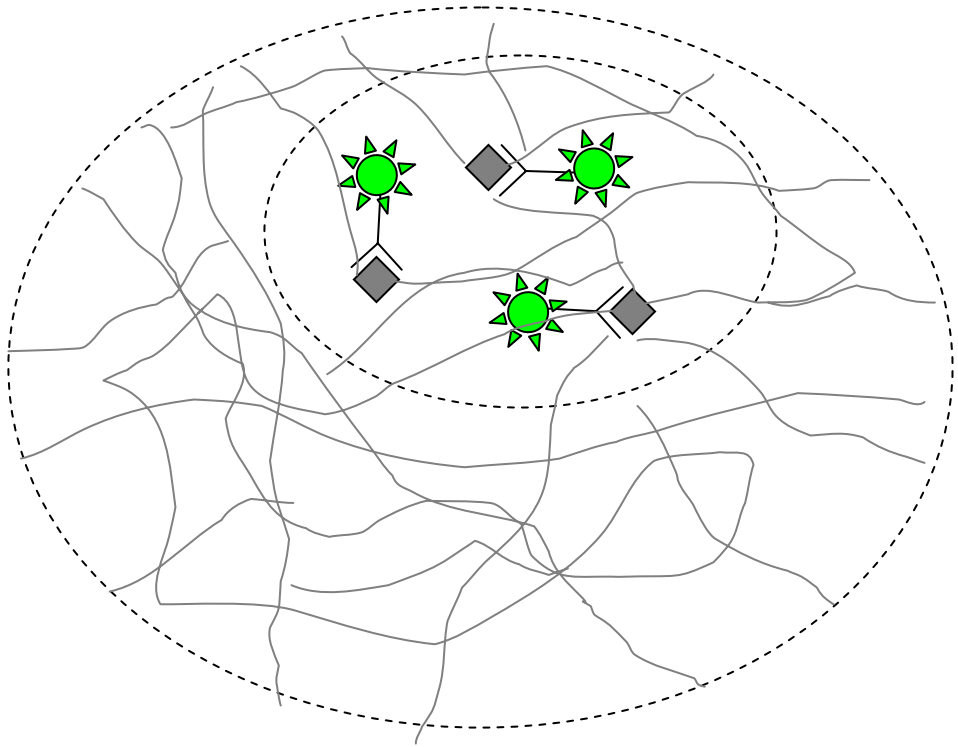
Immunofluorescence can detect specific proteins in cells

Fix with formaldehyde

Permeablize with detergent



Immunofluorescence can detect specific proteins in cells



Antibodies bind to specific proteins.

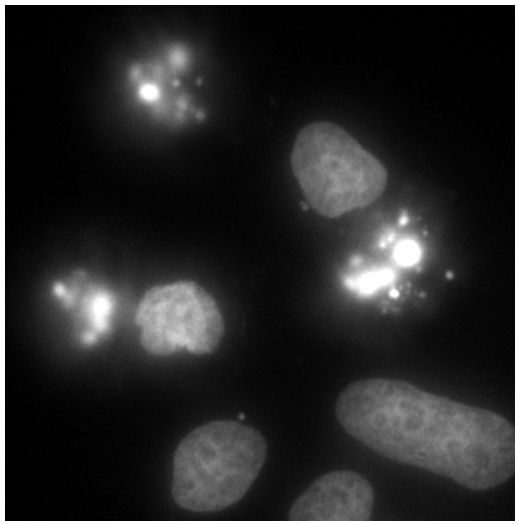
We can generate antibodies to nearly any protein we're interested in.

We can label antibodies with fluorescent molecules.

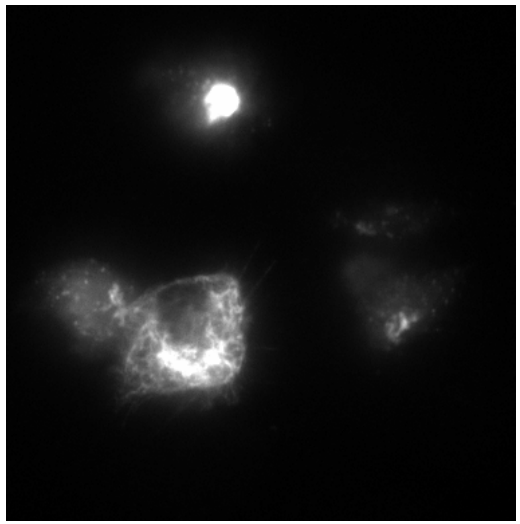
Fluorescently labeled antibodies can reveal the location of specific proteins in the cell.

Detecting apoptosis with immunofluorescence microscopy

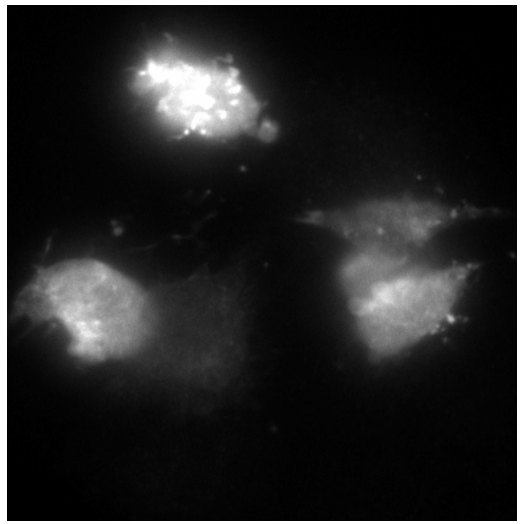
DNA

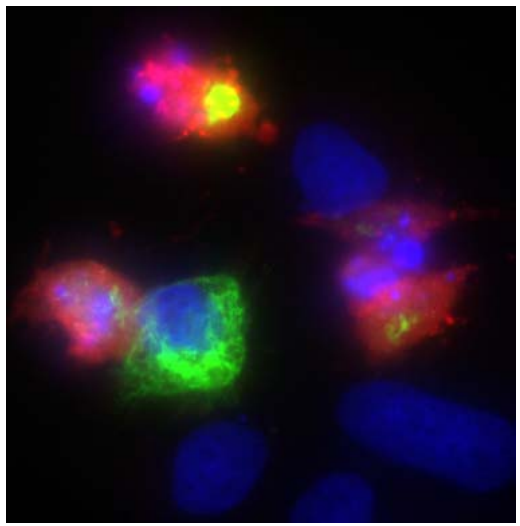


Cleaved cytokeratin



Cleaved caspase-3

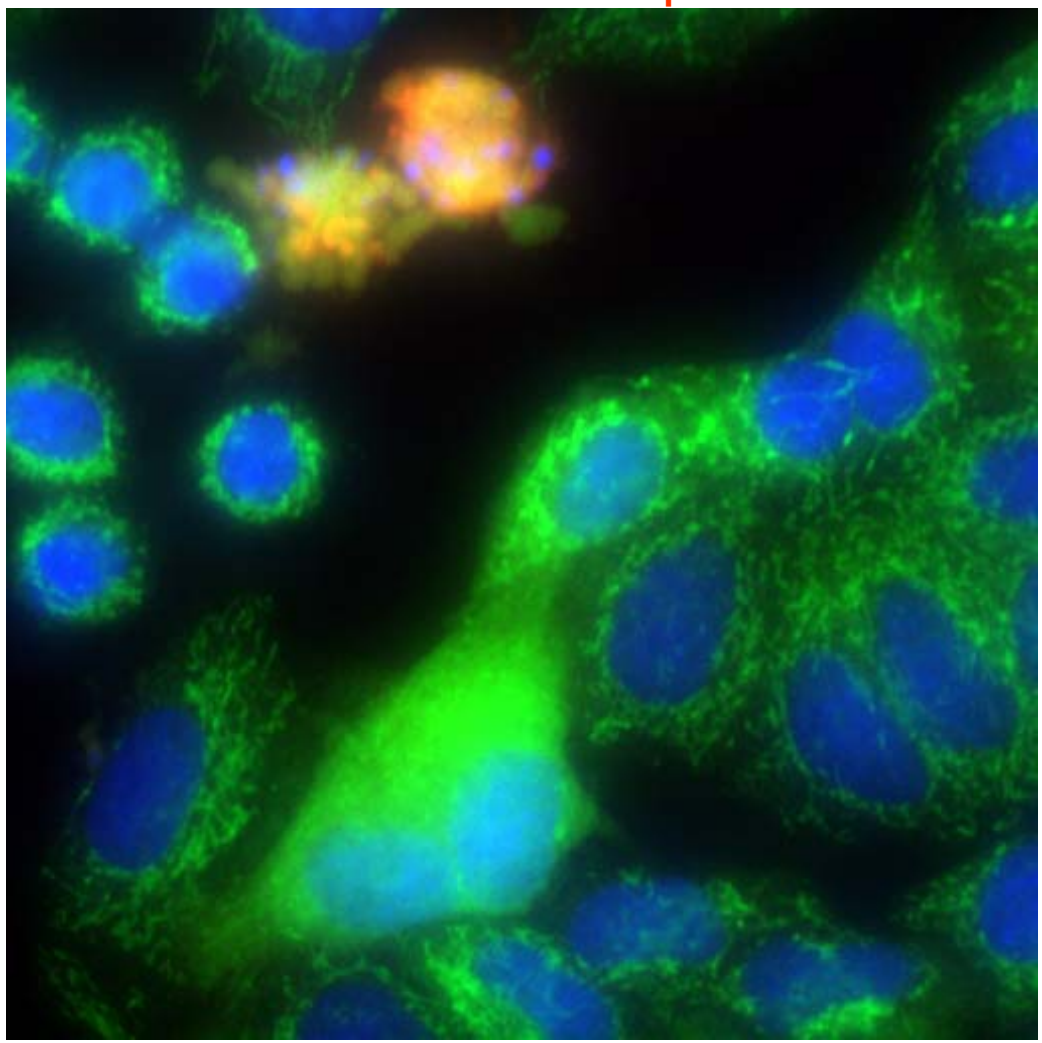




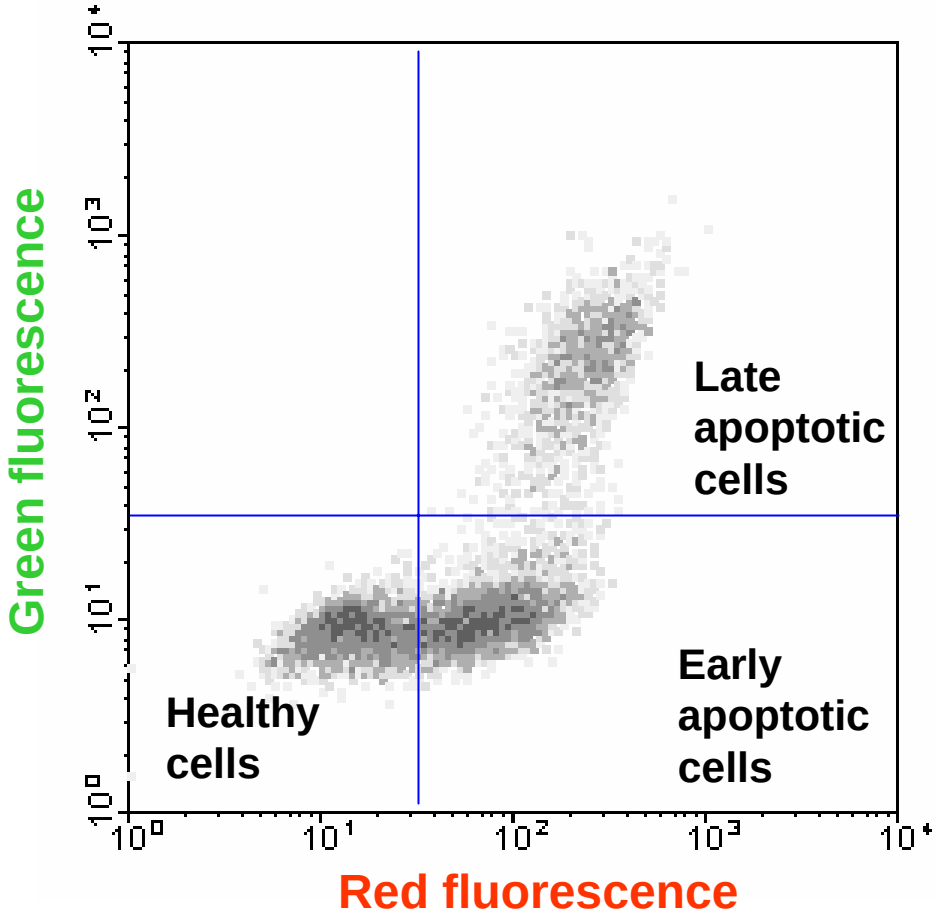
Blue: DNA
Green: cleaved cytok
Red: cleaved casp3

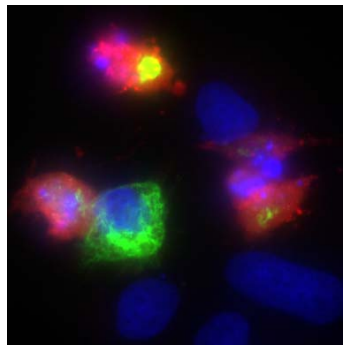
Determining the order of apoptotic events

Blue: H33342
Green: cytochromec
Red: cleaved casp3

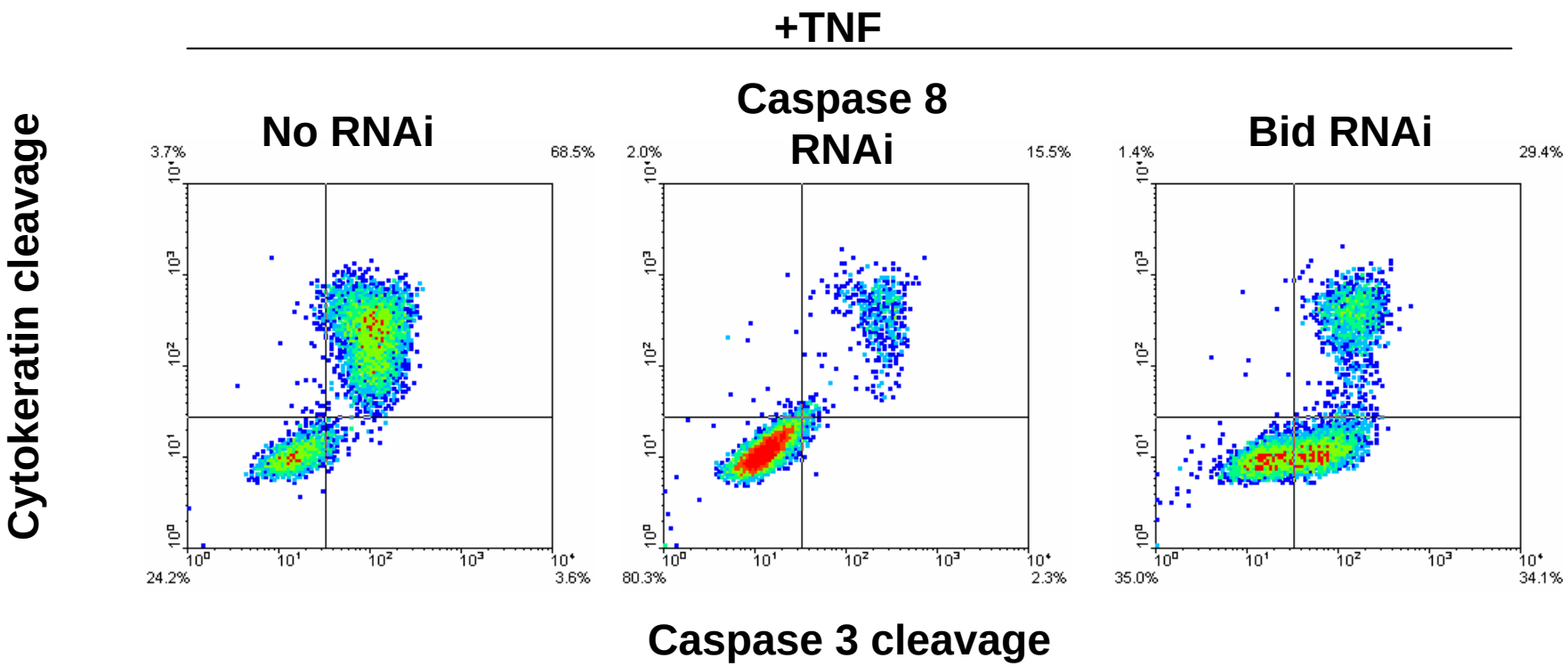


Quantitating apoptosis using flow cytometry





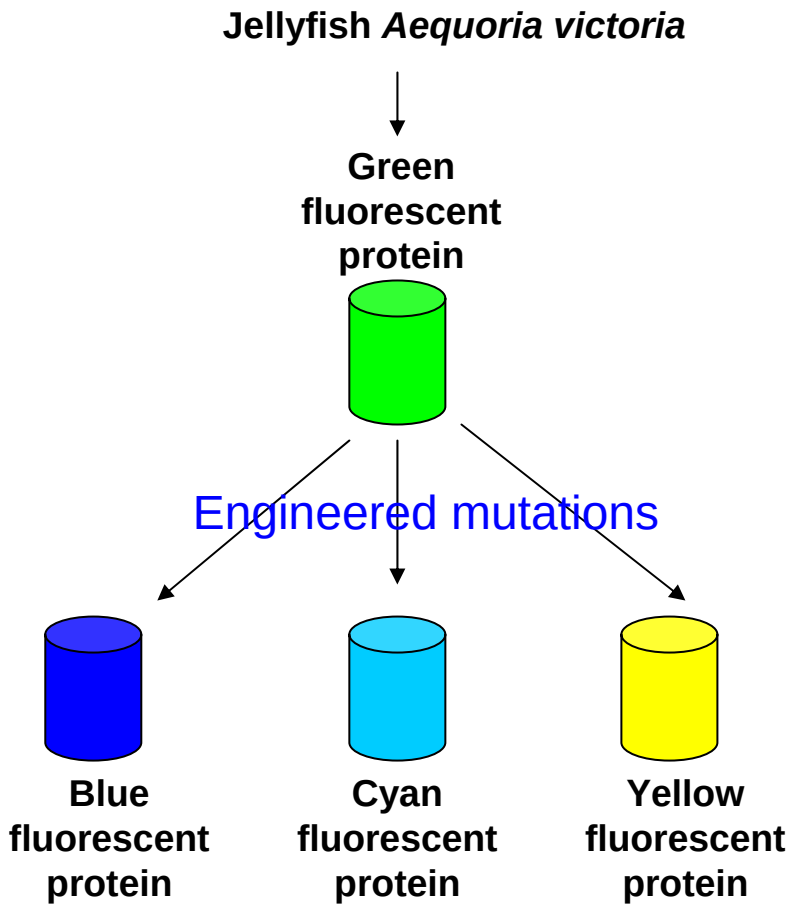
Combining flow cytometry with RNAi

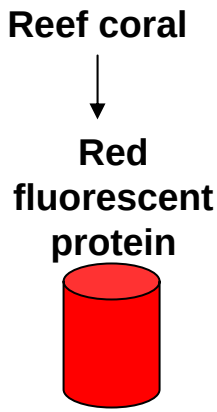


Green Fluorescent Protein – a genetically encoded fluorescent tag

Diagram removed for copyright reasons.

The fluorescent protein family





GFP has been engineered to make blue and yellow variants; red fluorescent proteins have been isolated from other sea creatures

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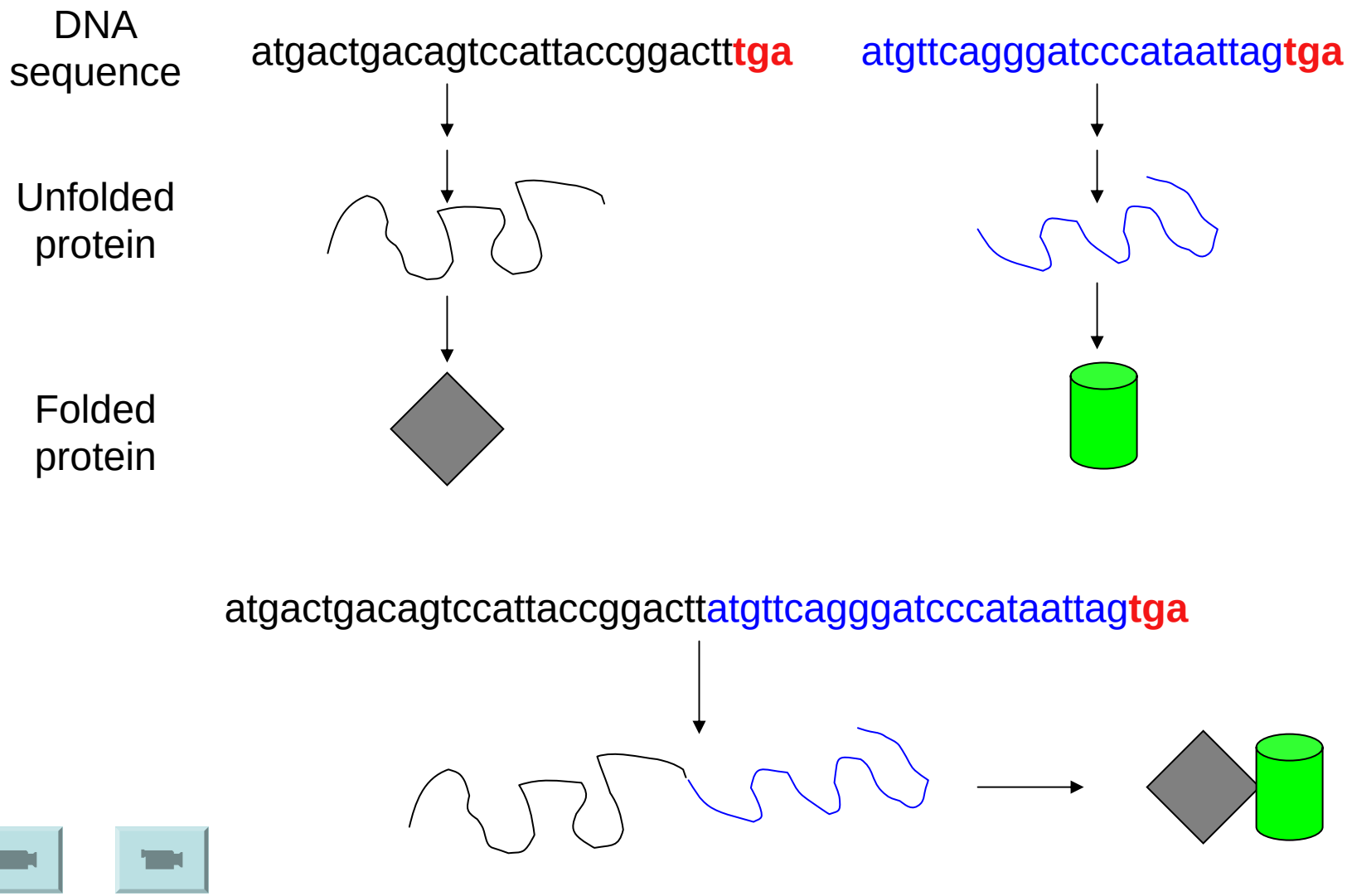
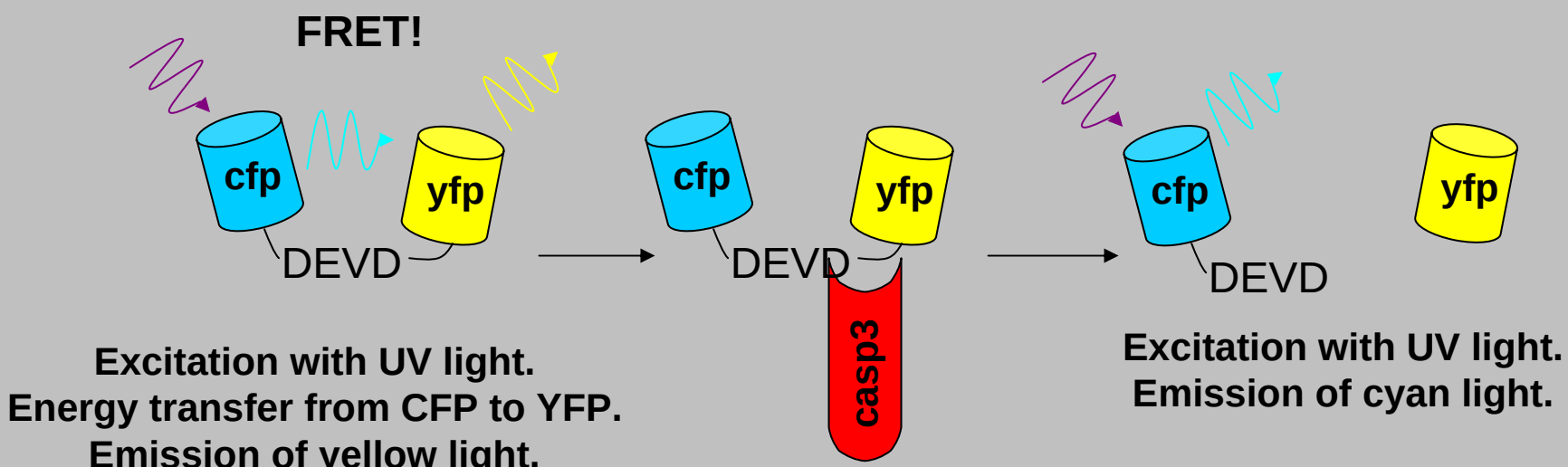
GFP can be spliced into any gene



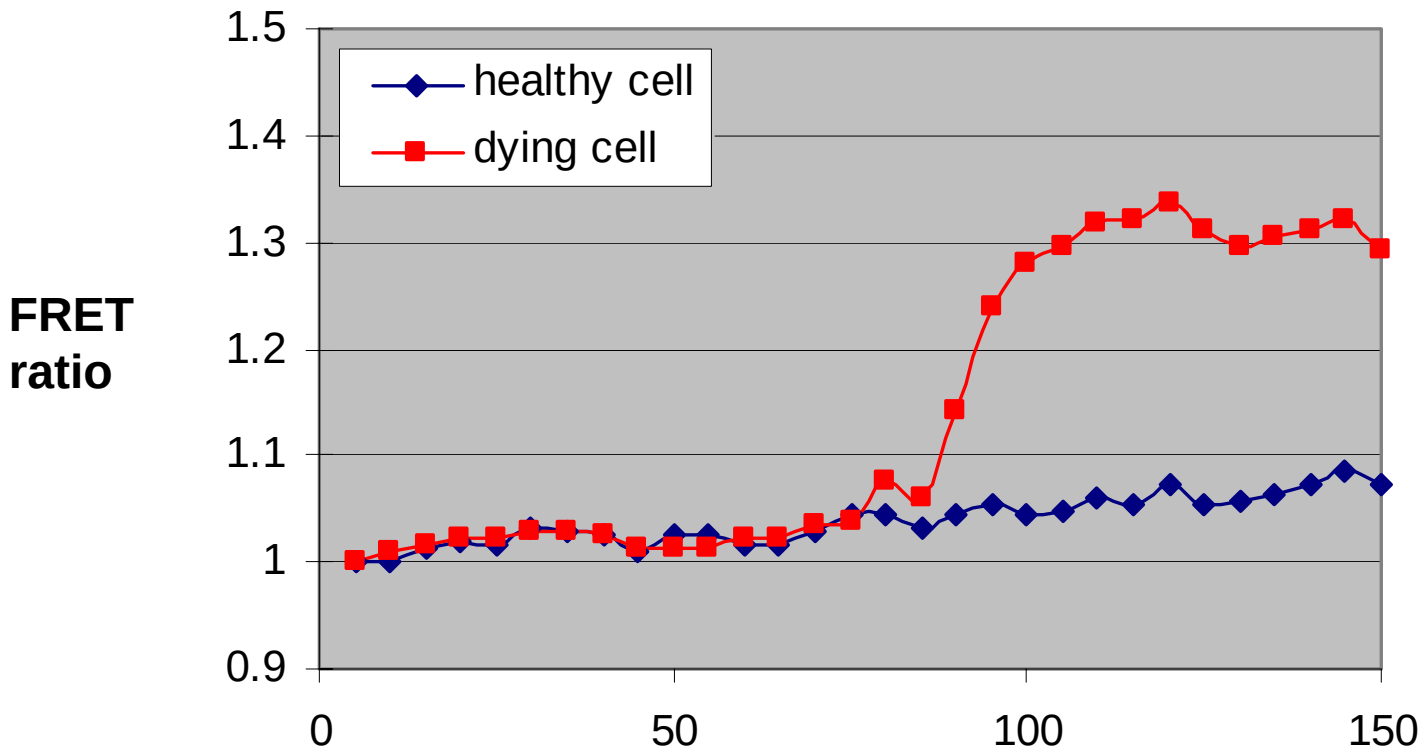
FRET – a phenomenon that occurs when 2 fluorescent molecules are very close

FRET: Forster Resonance Energy Transfer

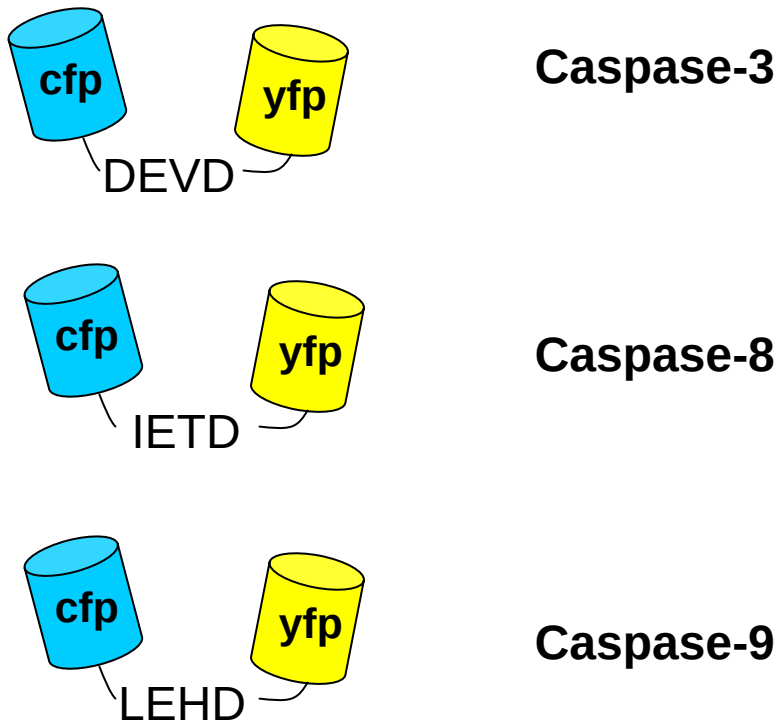
A FRET-based caspase activity reporter



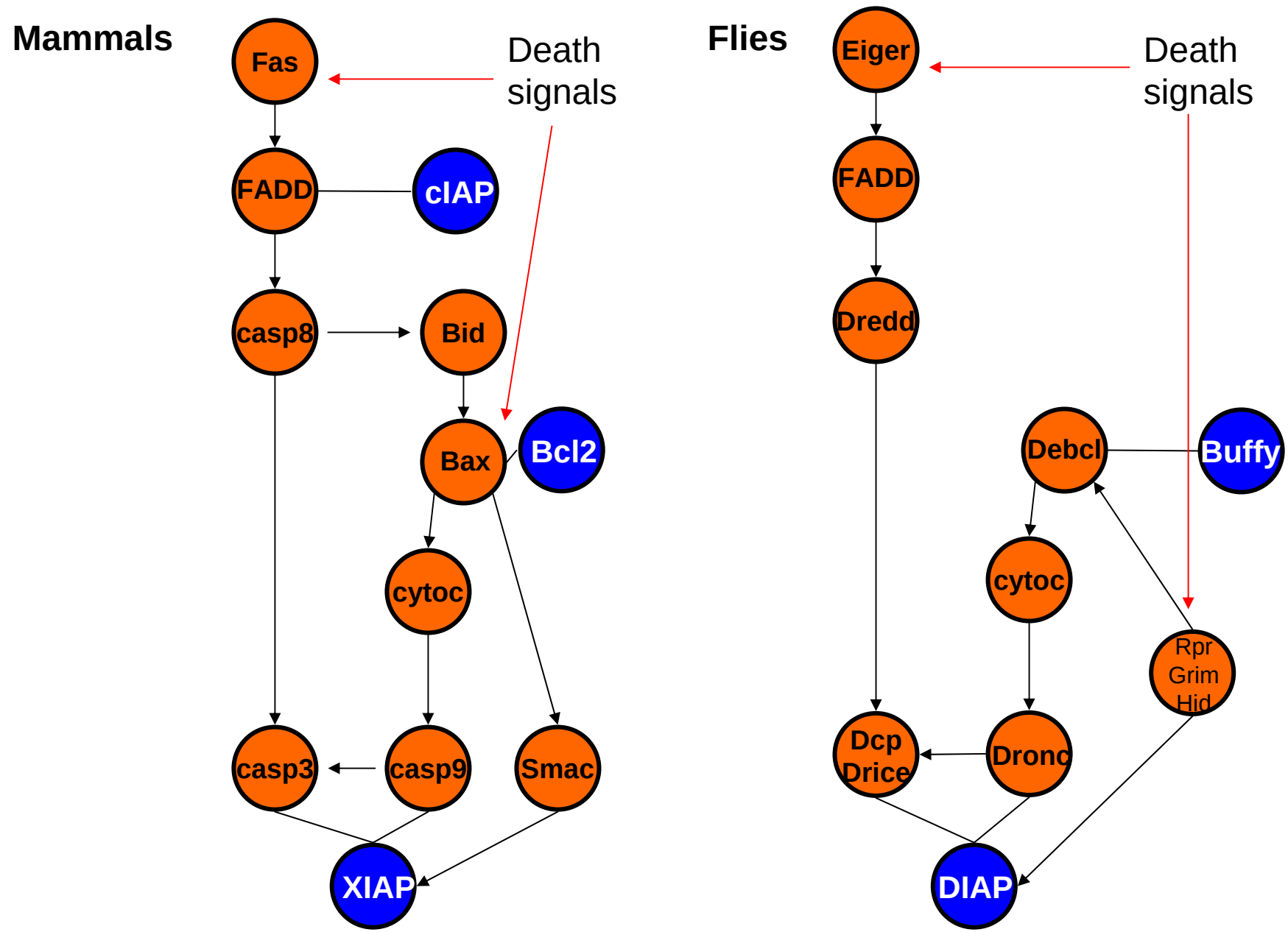
Monitoring caspase activity over time in living cells



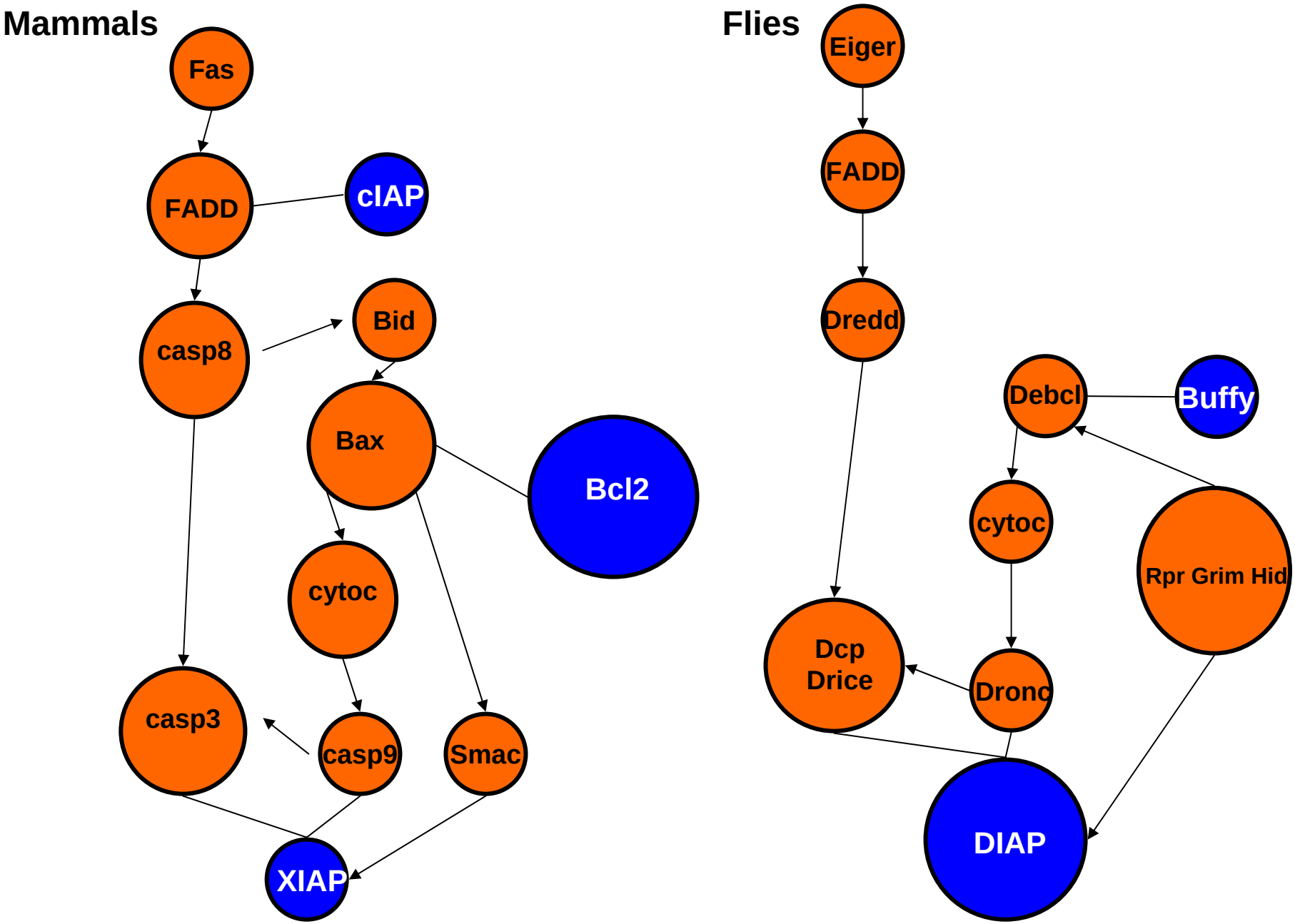
FRET reporters can be modified to detect different caspase activities



Much of the apoptotic pathway is conserved



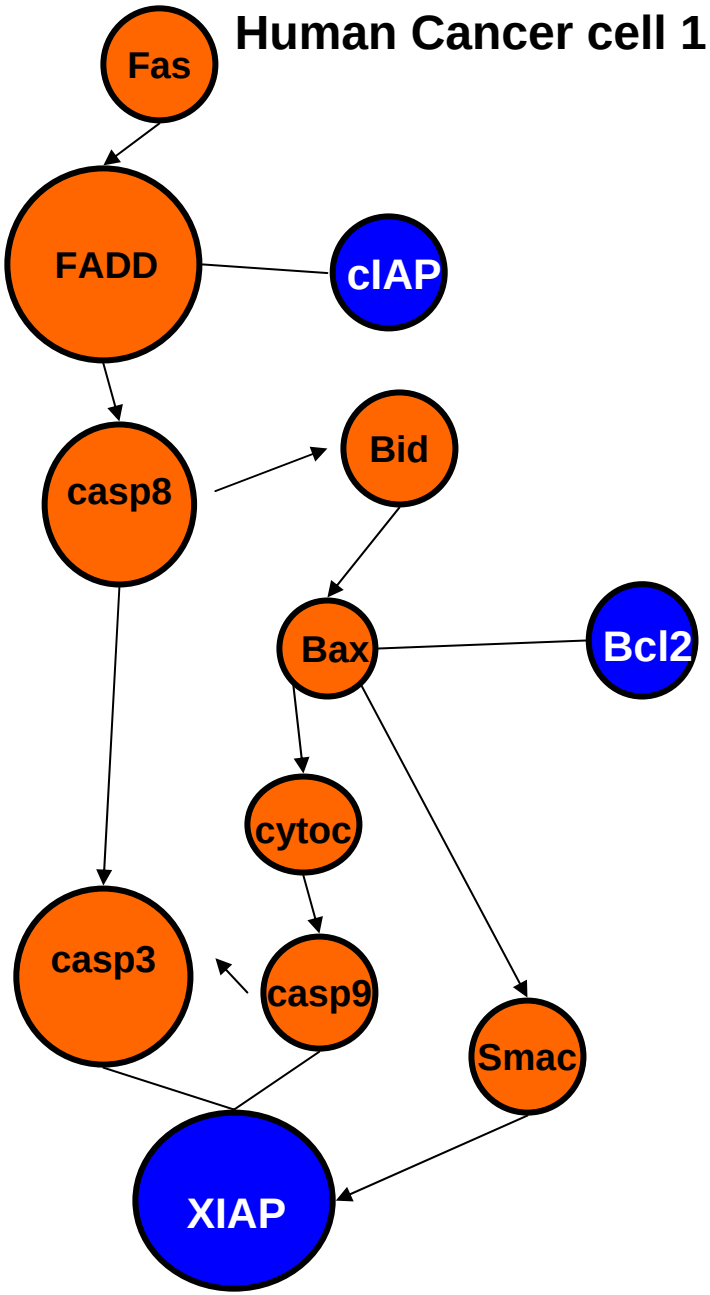
But the importance of certain pathways changes

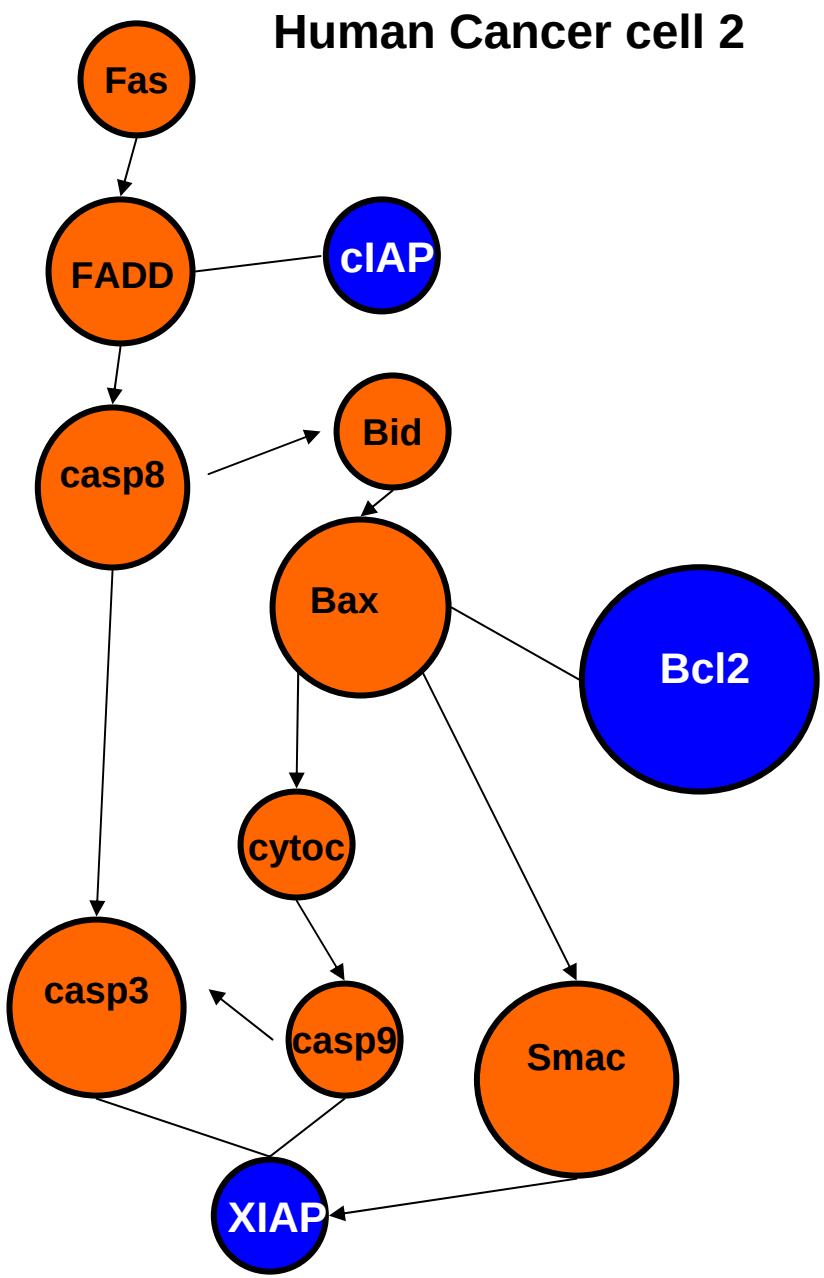


Recent development – XIAP is important in human cancer cells

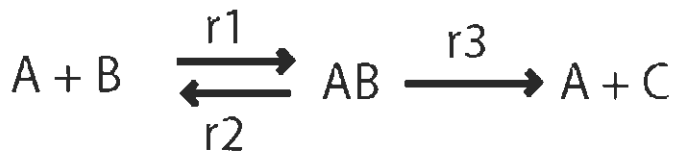
See Li, L. et al. “A Small Molecule Smac Mimic Potentiates TRAIL- and TNF-α Mediated Cell Death.” Science 305 no. 5689 (2004 Sep 3): 1411-3.

Even within a species, differences are observed





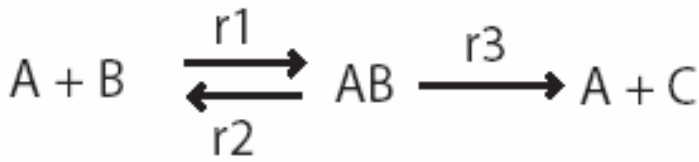
Modeling biochemical systems with differential equations



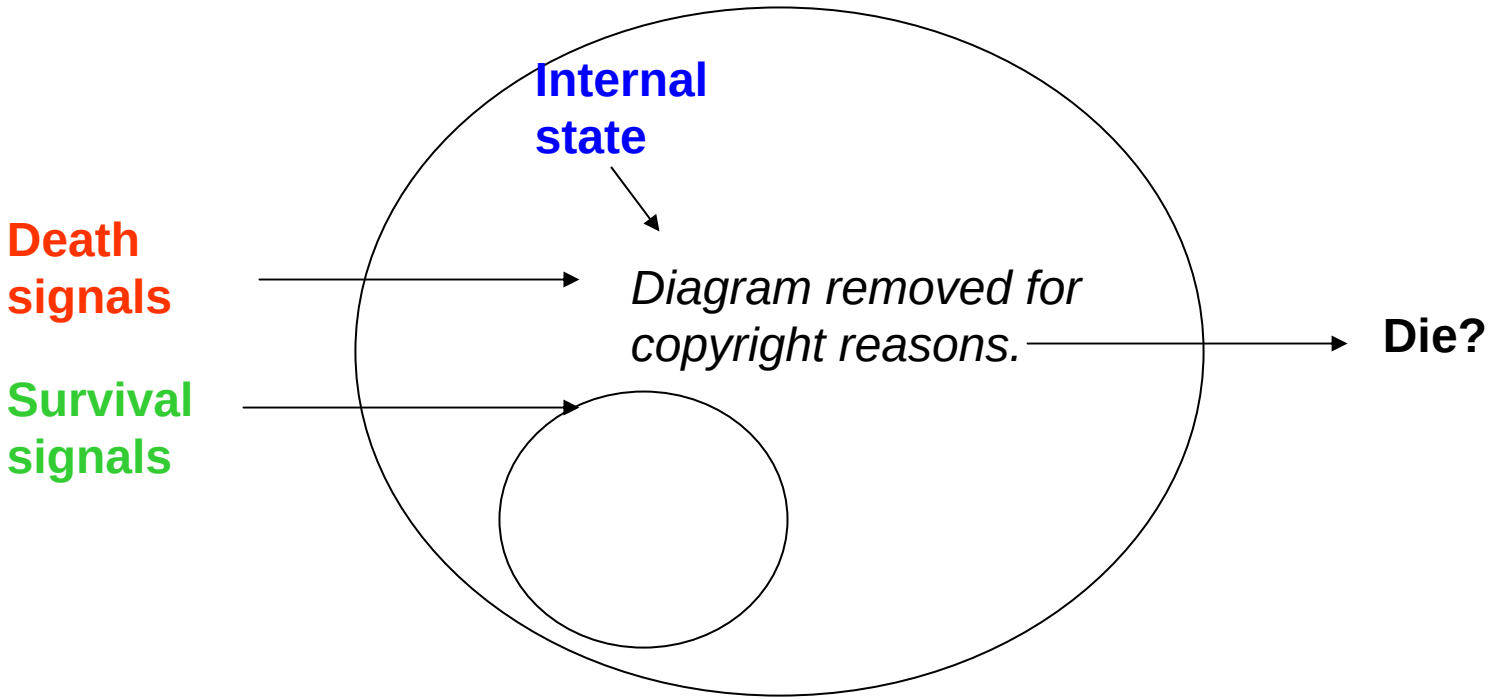
r1=k1[A][B]
r2=k2[AB]
r3=k3[AB]

d[A]/dt = -r1+r2+r3 = -k1[A][B]+k2[AB]+k3[AB]
d[B]/dt = -r1+r2
d[AB]/dt = r1-r2-r3
d[C] /dt = r3

A computational model of caspase activation



Studying apoptosis to learn how cells make decisions



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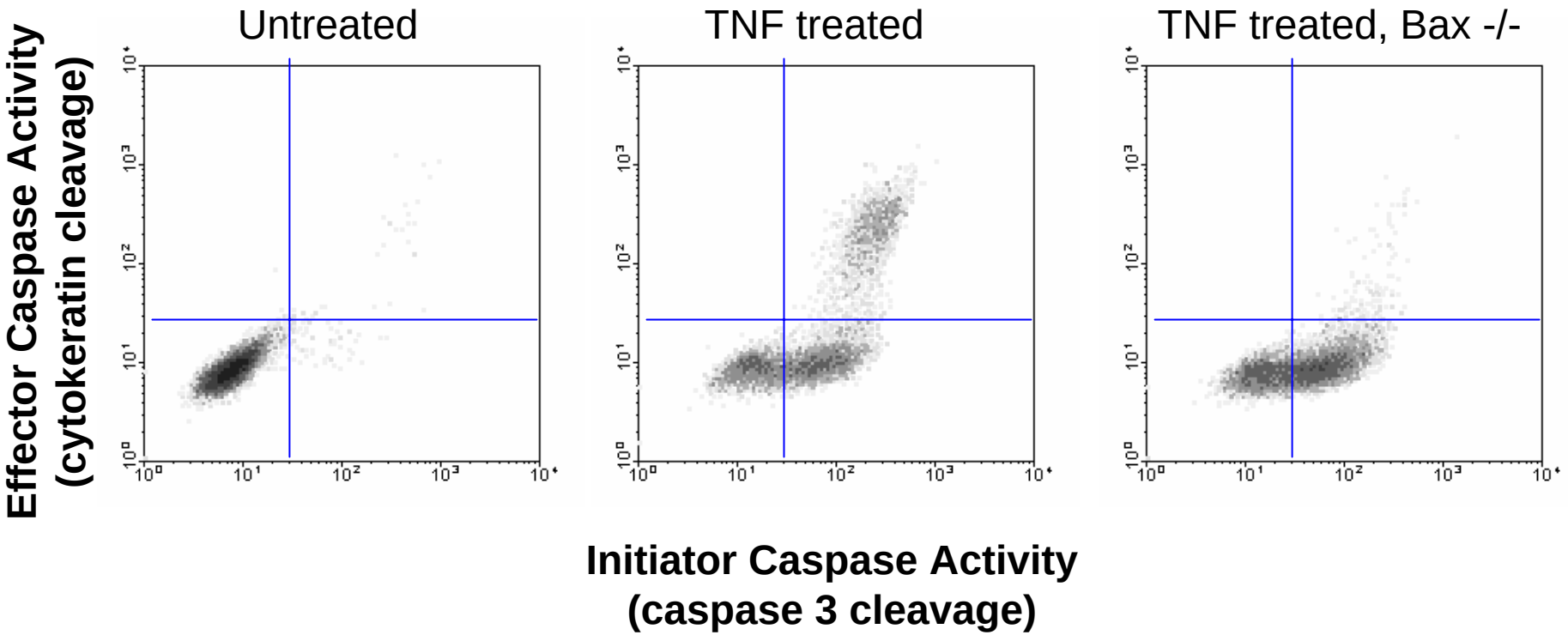
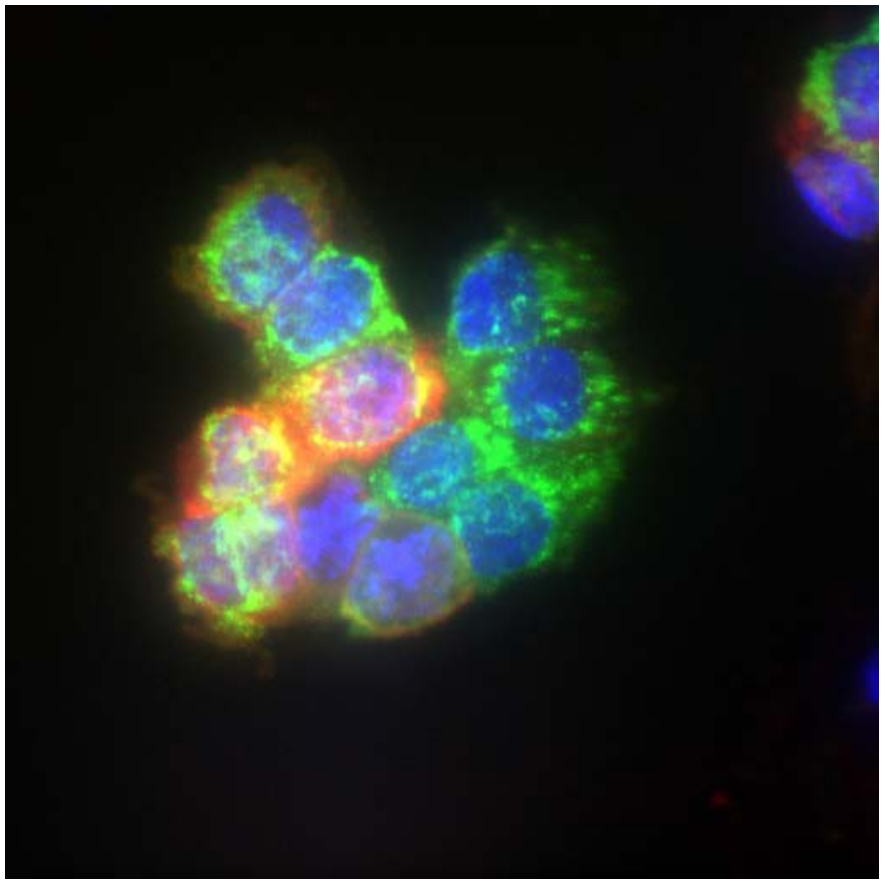
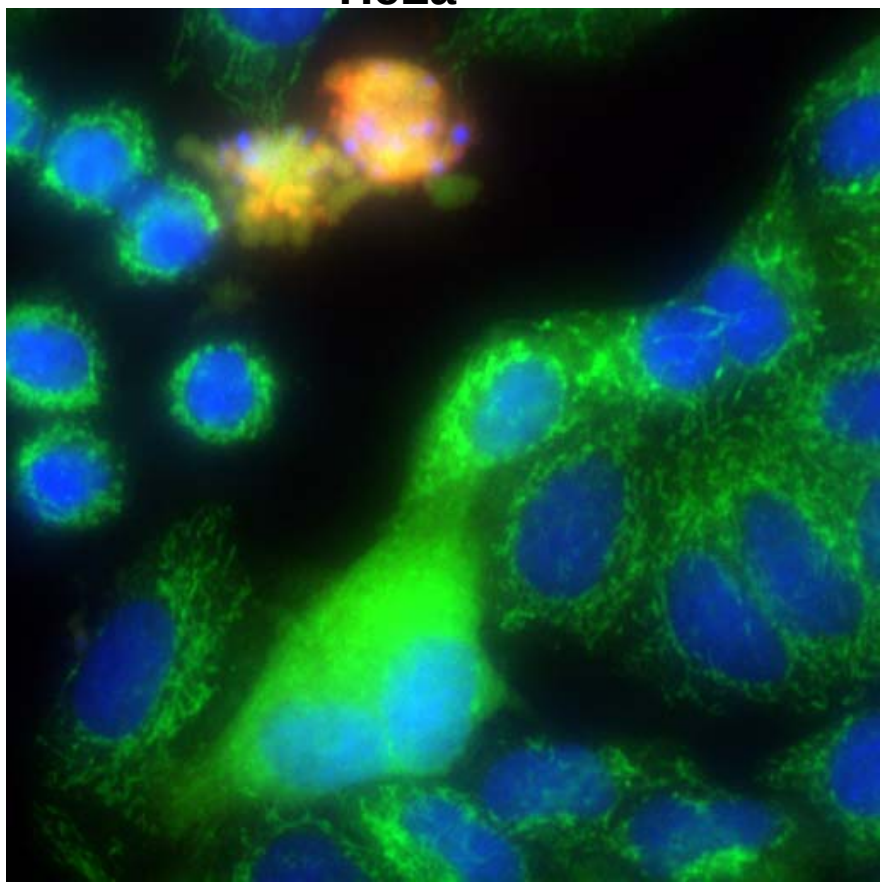


The order of apoptotic events varies between human cancer cells

Hct-116



HeLa



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