Early GI Cancer Detection

Human Health Team

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Impact

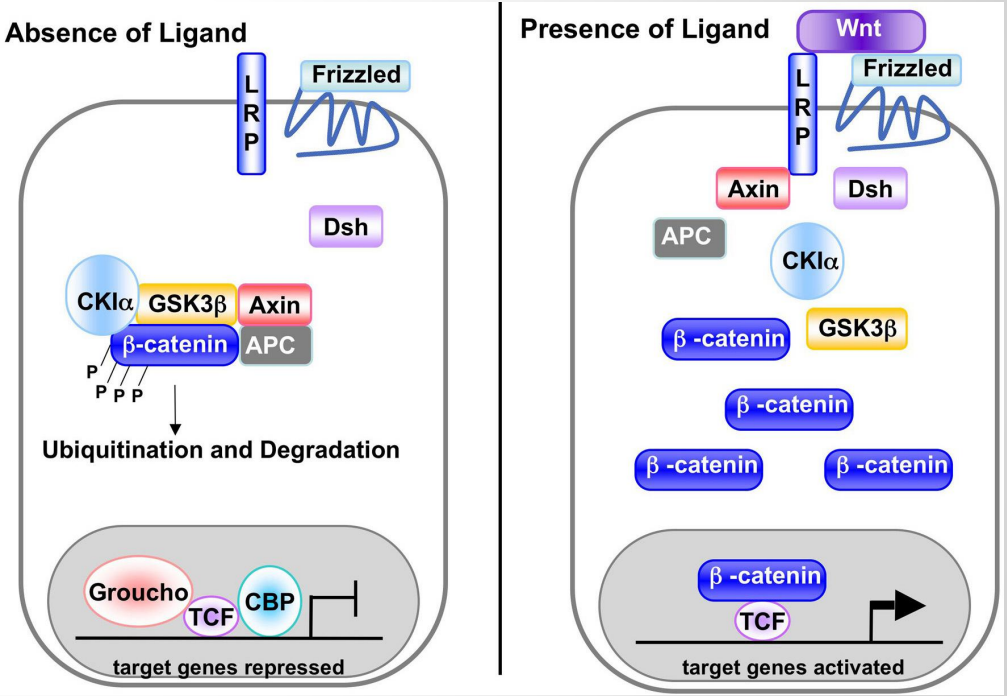
Early detection of cancerous cells, especially for patients in remission, will allow for more effective treatment

- Focusing on GI cancers because of relatively easy access

Target

Wnt Signaling Pathway

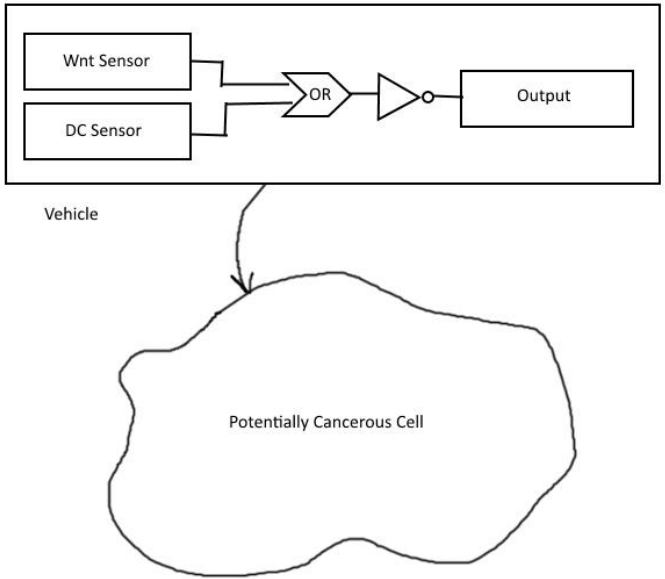
- Loss of function in APC gene is most common mutation in GI cancers
  - ASSUMPTION: Lack of APC function is a reliable marker for cancer



Eisenmann (2005) *Wormbook*

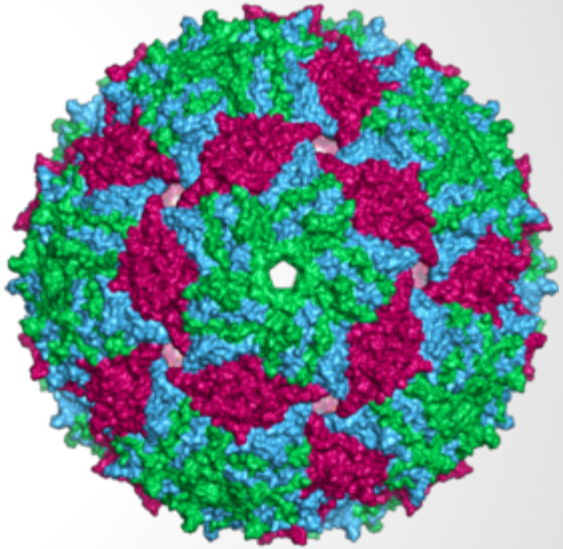
Devices

- Vehicle
- Wnt Sensor
- Destruction Complex Sensor
    - ASSUMPTION: Mutated APC cannot form DC and bind CTNNβ
- Logic Gate
- Output



Vehicle

- EBV/PAC Combo viral vector (Lufino, Edser, Wade-Martins, 2008)
- Pack a huge amount of DNA
- Low risk-- most people have already had EBV
    - Clinical trials showed little ill-effect, even in high dosage
- Has been used successfully on a wide variety of cells
- Well-understood, widely used



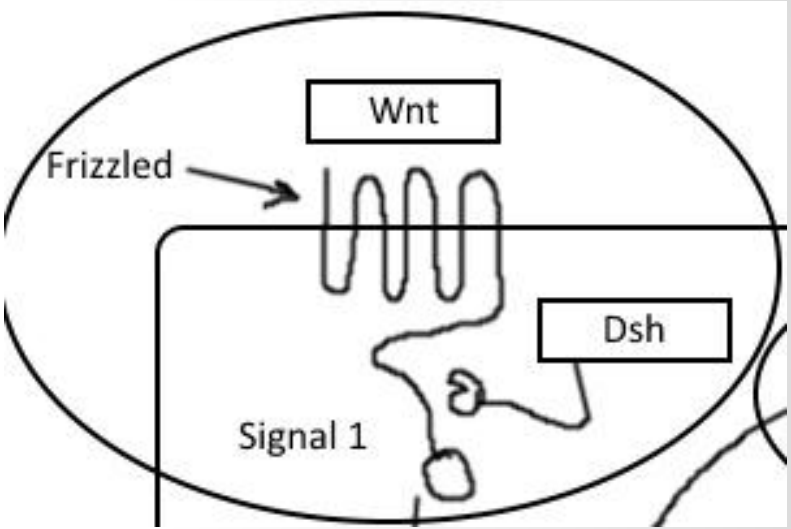
Wnt Sensor

Goal: Signal released when Wnt present

- Frizzled Protein (Wnt receptor) + S_1 production
- Disheveled Protein recruited + protease (P_1) to release S_1

P_1 - TEV protease

- Cleavage domain - Glu-X-X-Tyr-X-Gln/Ser



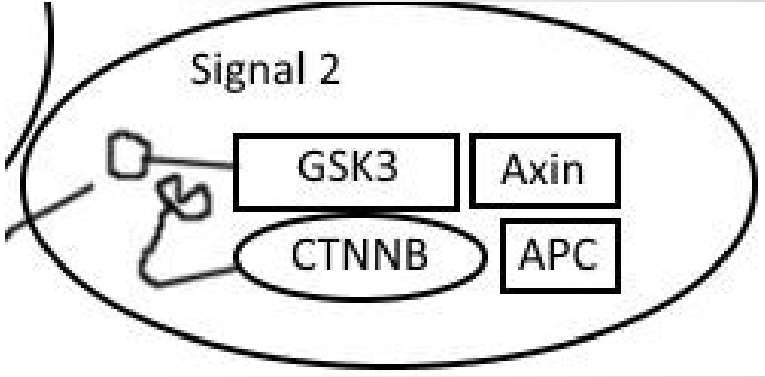
Destruction Complex Sensor

Goal: Detect DC formed and release signal

- GSK* (in complex) + S_2 produced
- β-catenin (surrounded by complex) + protease (P_2) to release S_2

P_2- Enteropeptidase

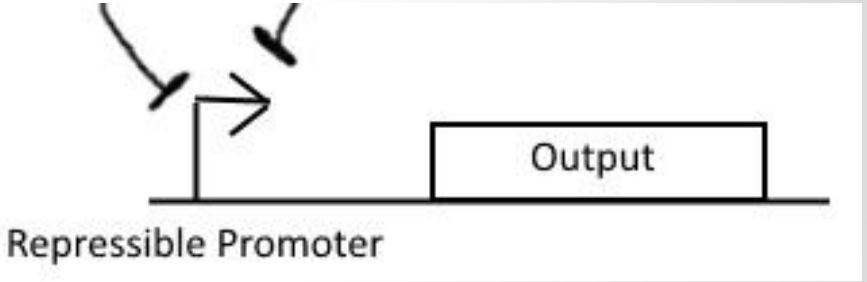
- cleaves high specificity at Asp-Asp-Asp-Asp-Lys-|-X



Logic Gate/Output

Goal: $!(S_1||S_2)$:

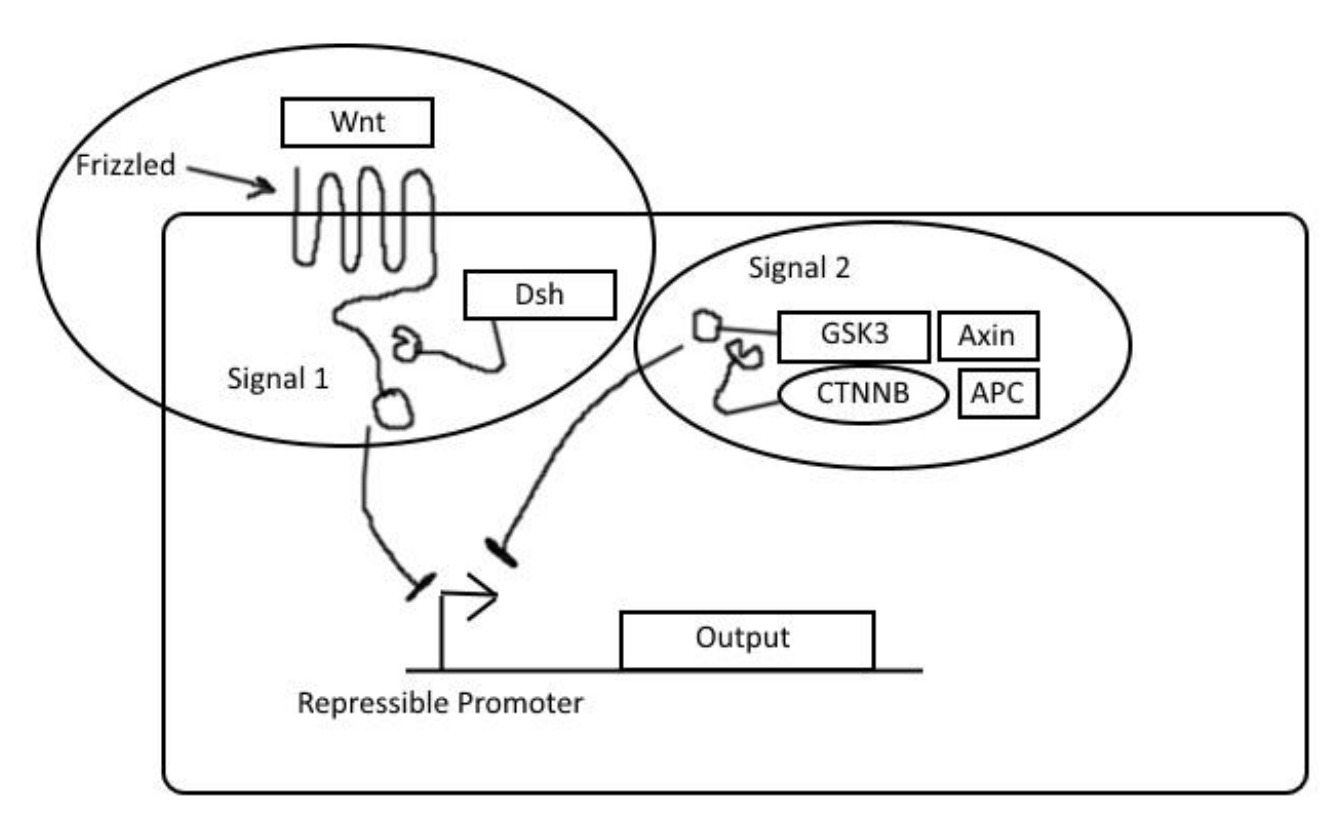
- Produce output if neither signal is present
- A repressible promoter regulated by both “signals” accomplishes this
- Make both “signal” molecules TetR and our promoter the TetR repressible promoter



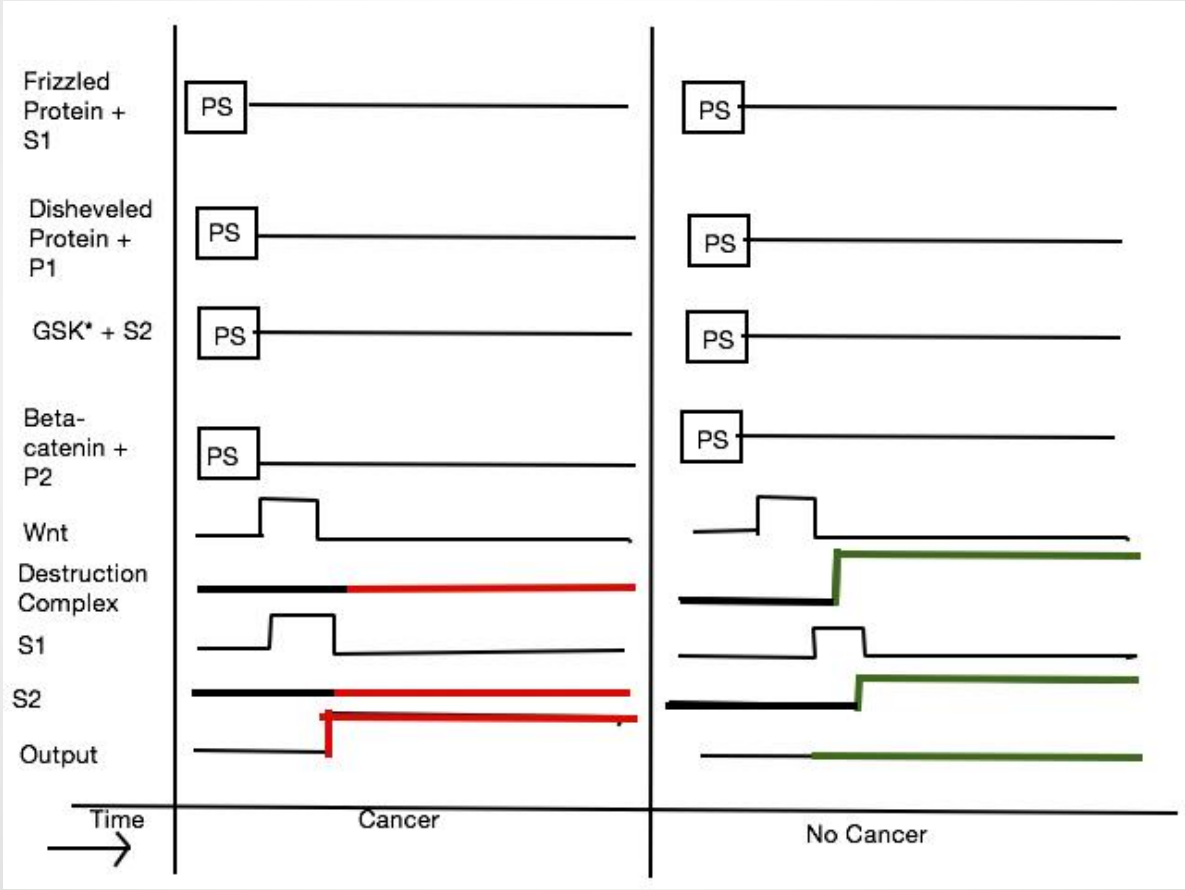
Logic Gate/Output

Cancer	Wnt	S_1	Dest. Comp.	S_2	Output (Luci.)
0	1	1	0	0	0
0	0	0	1	1	0
1	1	1	0	0	0
1	**0**	**0**	**0**	**0**	**1**

Putting it all Together



Timing Diagram



Testing and Debugging

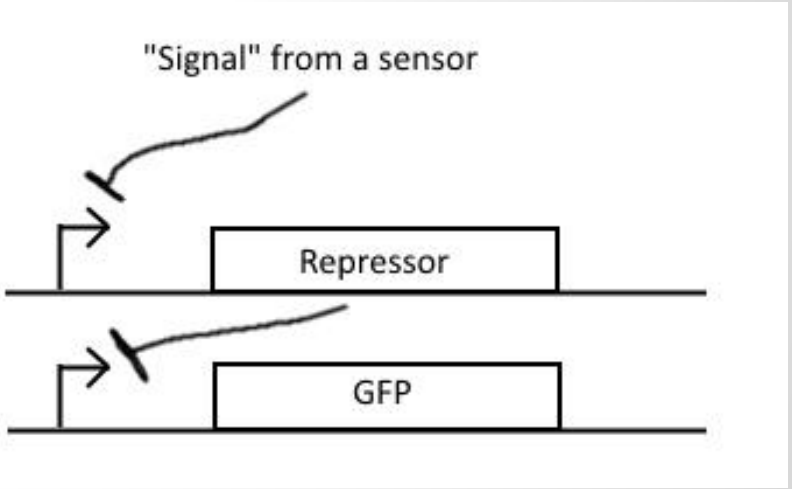
We'll want to test each device independent of the entire system

- Attempt to conditionally produce GFP with Wnt Sensor
- Attempt to conditionally produce GFP with DC sensor
- Attempt to constitutively express output protein in target cells
- Attempt to transfect GFP using our vector and compare to better characterized system

Conditional GFP Production

GFP could be conditionally expressed with this circuit

- Check basic function by growing cells with “signal”
- Check Wnt Sensor by growing with/without Wnt
- Check DC Sensor by operating circuit in normal cells and cells with mutated APC



Open Issues

- Ethical Issue: Intentionally infecting humans with viruses
- How could the introduction of our circuit interfere with normal cell function?
- Is lack of DC too specific of an indicator/will it be reliable?

Go or No Go?

Despite the uncertainties, through experimentation we believe we can work out all substantial issues, and the idea is viable.

Questions?

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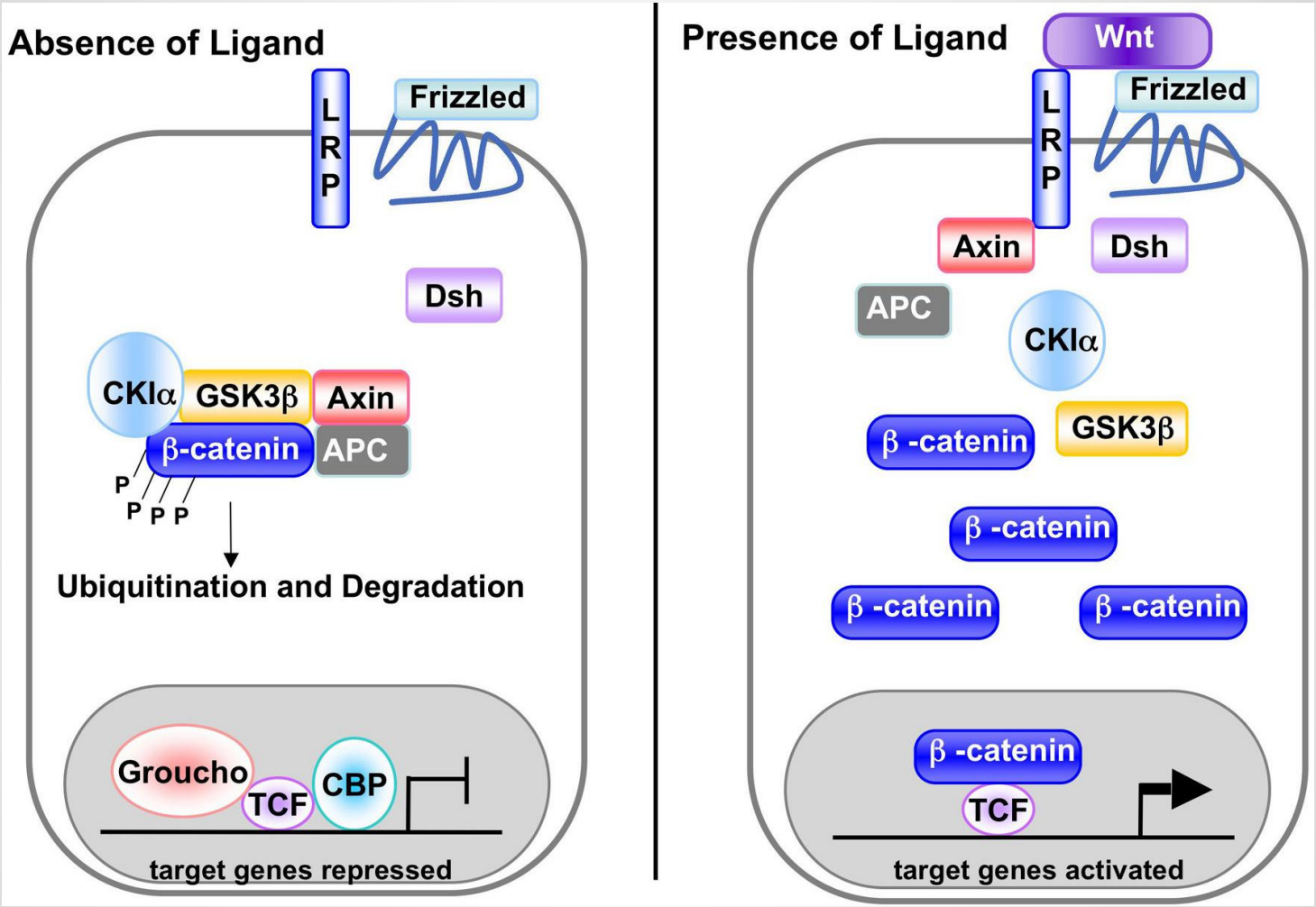
Absence of Ligand
LRP
Frizzled
Dsh
CKIα
GSK3β
Axin
β-catenin
APC
P
P P P
Ubiquitination and Degradation
Groucho
TCF
CBP
target genes repressed
Presence of Ligand
Wnt
LRP
Frizzled
Axin
Dsh
APC
CKIα
β -catenin
GSK3β
β -catenin
β -catenin
β -catenin
β -catenin
TCF
target genes activated