SEASON 2

Understanding Cancer

Lecture 13

Types of signalling pathway: normal and dysregulated Wnt

DR HAFSA WASEELA ABBAS

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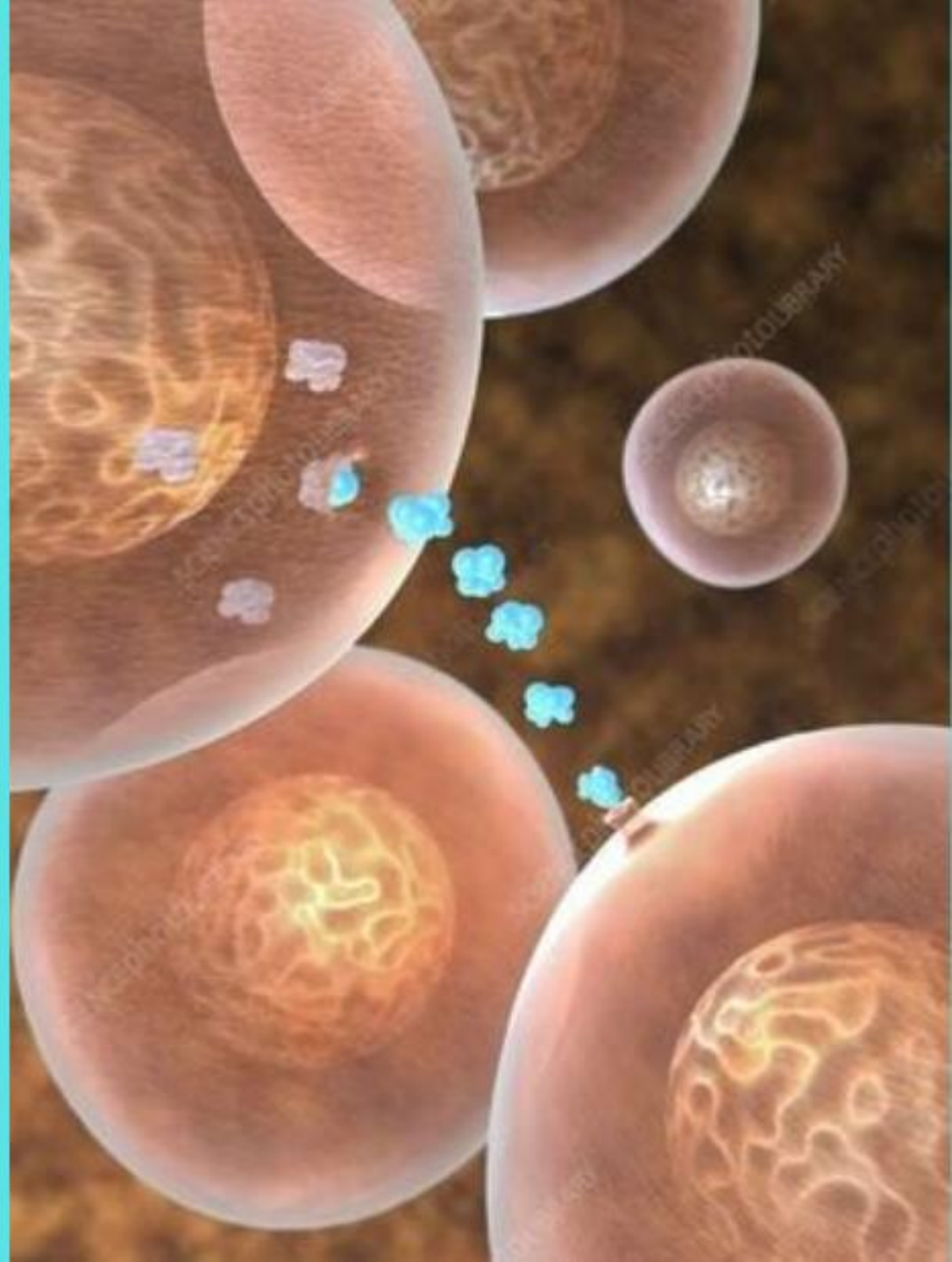

RECAP:

What you hopefully should understand so far from Lecture 12

- **Notch are transmembrane protein receptors.**
- **Four receptors found in mammals: Notch1, Notch2, Notch3 and Notch4.**
- **The ligand-receptor complex can be cleaved by three enzymes: Furin-like protease, ADAM family and E3 ligases.**
- **The intracellular domain of the activated receptor is released and can bind to CSL to mediate transcription in the nucleus. CSL can also bind without the intracellular domain to induce transcription.**
- **Notch can promote tumour progression and can also suppress.**

What will we learn today?

- ***The structure of the Wnt family***
- ***Canonical Wnt signaling (Receptor activation)***
- ***Canonical Wnt signaling (Signal transduction)***
- ***Canonical Wnt signaling (Cellular response)***
- ***Planar Cell Polarity pathway (PCP)***
- ***β-catenin Independent (non-canonical)***
- ***Key cellular functions***
- ***Dysregulated Wnt pathways***

GENTLE REMINDER
An ideal way of learning:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

Mini-lectures.

Approximate total time: 1 hour

Divide over 7 days at your **own pace**.

Challenge yourself with a quiz!

HN

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RECAP: How to support your learning?

- **Key facts with diagrams by HN designs presented in a simplified way.**
- **Glossary to help understand what key words mean.**
- **Summary doodle revision posters by HN designs.**
- **Quizzes to test your knowledge and reflect.**
- **Reference list for further reading.**

Acknowledgements: Special thanks to my parents, family, friends and colleagues for their support and the respected teachers and health professions who taught me and installed the passion of cancer/oncology.

The structure of Wnt family

The structure of Wnt family

They are a **family of ligands** that **bind to their respective receptors.**

The **ligands are glycoproteins** that are enriched with **cysteine amino acid residues.**

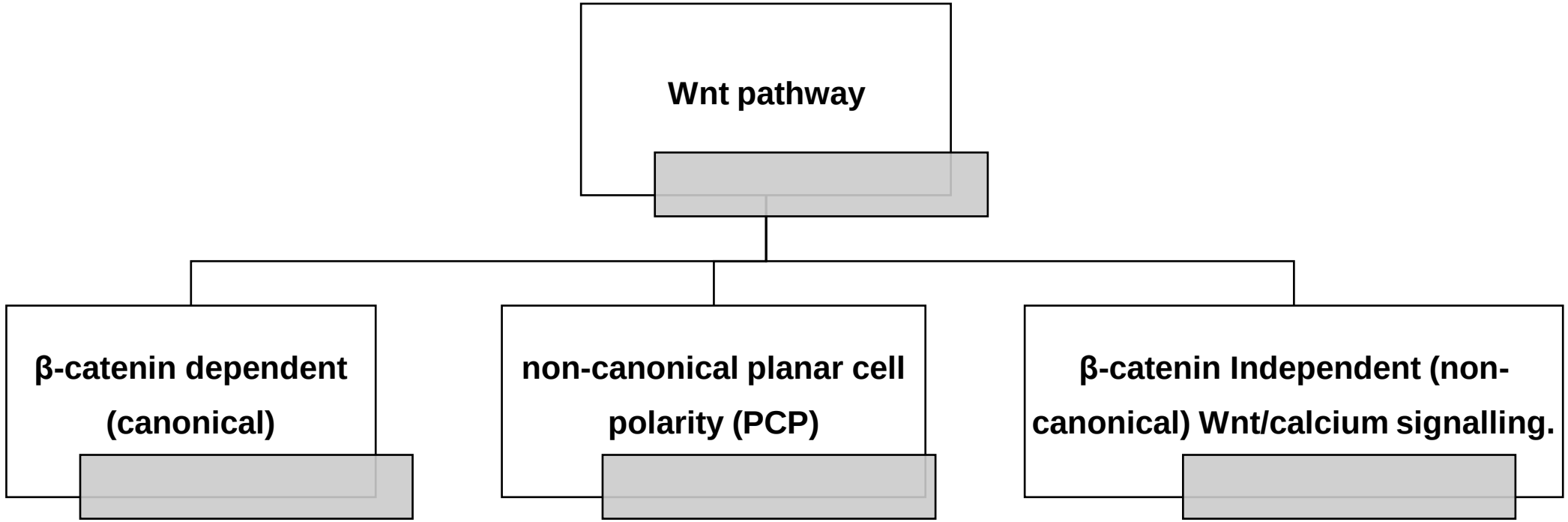
The total number of ligands present in the **Wnt family are 19 for more than 15 receptors.**

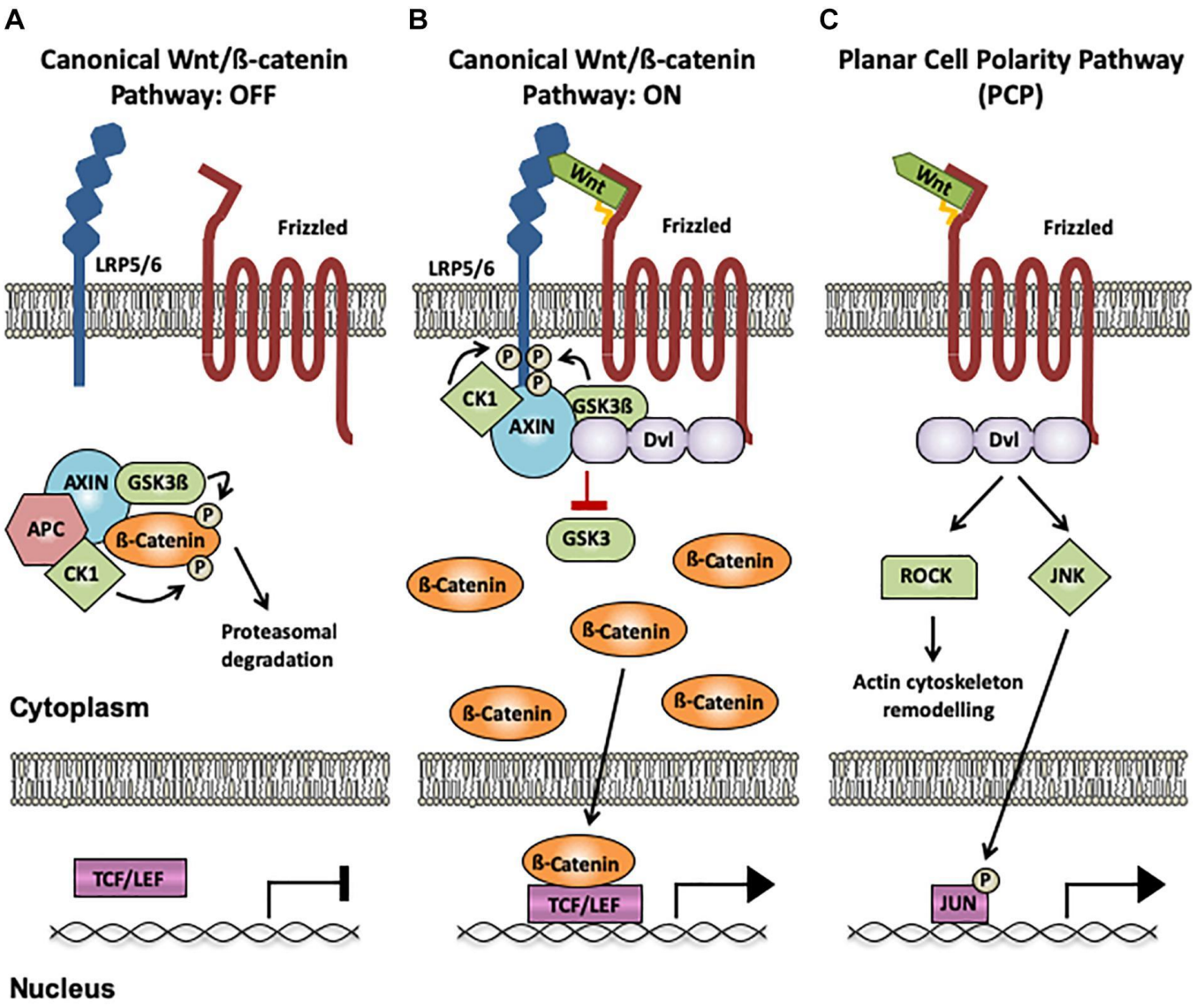
Examples of receptors:

- Frizzled
- Receptor Like Tyrosine Kinase (RYK)
- Retinoic acid receptor-related orphan receptor (ROR)

(Duchartre, Kim and Kahn, 2016).

Types of Wnt signalling pathways





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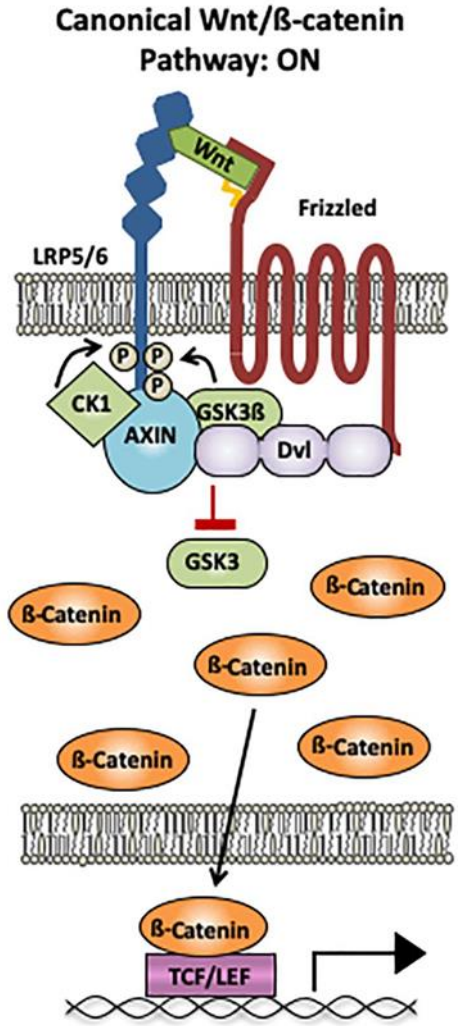
Canonical Wnt signaling (Receptor activation)

Step 1: Canonical Wnt signaling (Receptor activation)

The **Wnt ligand** specifically binds to its **specific receptor Frizzled.**

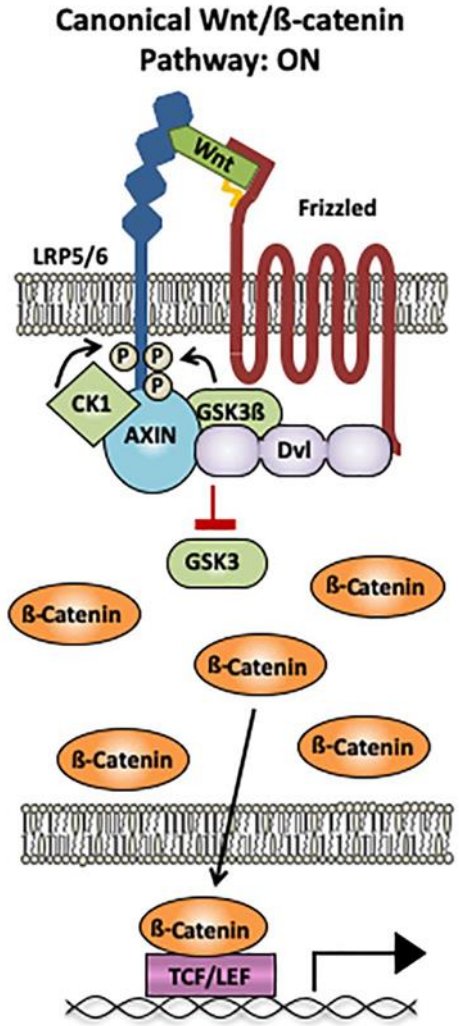
Frizzled is a **transmembrane receptor** that consists of **7 heterodimeric proteins**.



(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023a).

Step 2: Canonical Wnt signaling (Receptor activation)

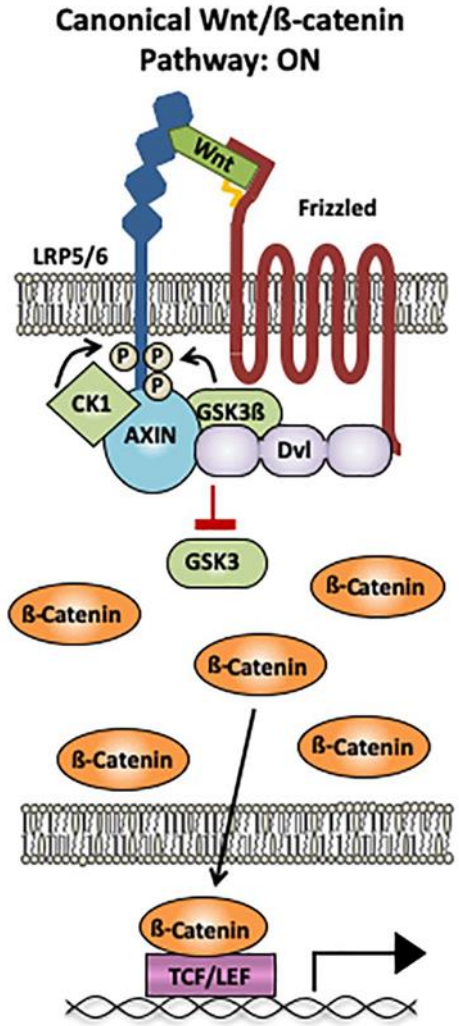
The **ligand** binds to the **low-density lipoprotein receptor-related protein (LRP).**



(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023a).

Step 3: Canonical Wnt signaling (Receptor activation)

The **LRP protein** particular 5 or 6 undergoes **phosphorylation** (addition of a phosphate group).

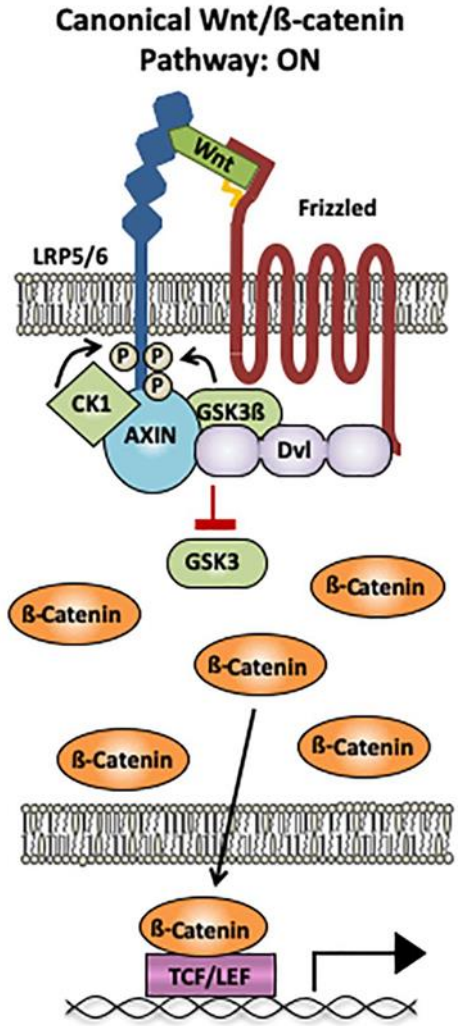


(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023a).

Step 4: Canonical Wnt signaling (Receptor activation)

The **phosphorylated LRP phosphorylates proteins called Dishevelled**.

Dishevelled (Dvl) is a **negative regulator** of the **destruction complex**. They are **recruited to the plasma membrane** where they **undergo polymerization.**



(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023a).

Canonical Wnt signaling (Signal transduction)

Step 5: Canonical Wnt signaling (Signal transduction)

The **activated Dishevelled polymer suppresses and deactivates the destruction complex.**

The destruction complex consists of the following:

- AXIN – scaffold protein.
- APC (adenomatous polyposis coli) – negatively regulates Wnt.
- Two kinases CK1α (casein kinase 1α) and Glycogen Synthase Kinase 3β (GSK3β)

GSK3β is a protein kinase enzyme that regulates growth, proliferation, differentiation, adhesion and survival. It deactivated β-catenin by phosphorylating serine and threonine residues.

(Racaud-Sultan and Vergnolle, 2021)

Step 6: Canonical Wnt signaling (Signal transduction)

Stopping the **complex helps stabilizes and increases the accumulation** of the protein called **β-catenin.**

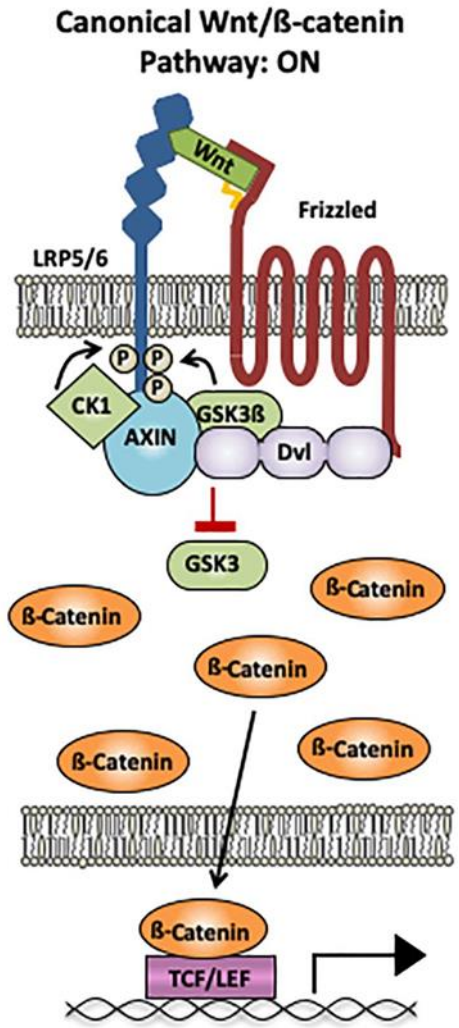
The **β-catenin protein is unstable** and has **no cytoplasm.**

The cytoplasm is where chemical reactions takes place.

(Duchartre, Kim and Kahn, 2016).

Step 7: Canonical Wnt signaling (Signal transduction)

β-catenin translocates to the nucleus.



(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023a).

Canonical Wnt signaling (Cellular response)

Step 8 Canonical Wnt signaling (Cellular response)

β-catenin forms a complex with **two types of transcription factors** inside the nucleus to increase transcription:

- **T-cell factor (TCF)**
- **Lymphoid enhancer factor (LEF)**

This is achieved by **removing the transducin-like enhancer of split (TLE) and Grorelated gene (Grg) proteins that repress transcription.**

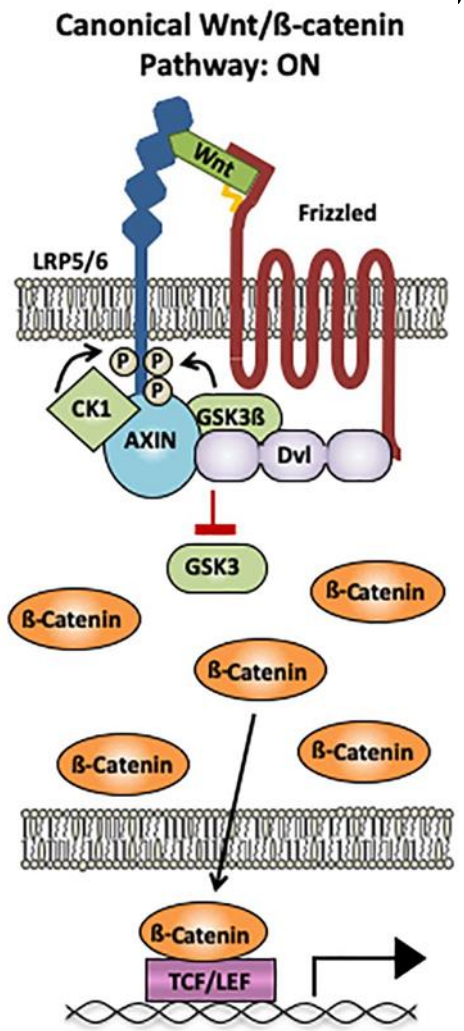
TLE/Grg are removed by histone deacetylases enzymes that allow proteins called histones to wrap around DNA more tightly.

(Jennings and Ish-Horowicz, 2008)

Step 9 Canonical Wnt signaling (Cellular response)

β-catenin can also recruit the **transcriptional activators that aim for the Wnt target genes**:

- **CBP/p300p**
- **CBP (CREB-binding protein) BRG1**
- **BCL9**
- **Pygo**



(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023a).

β-catenin Independent (non-canonical)

Activated β-catenin is targeted by:

Destruction complex

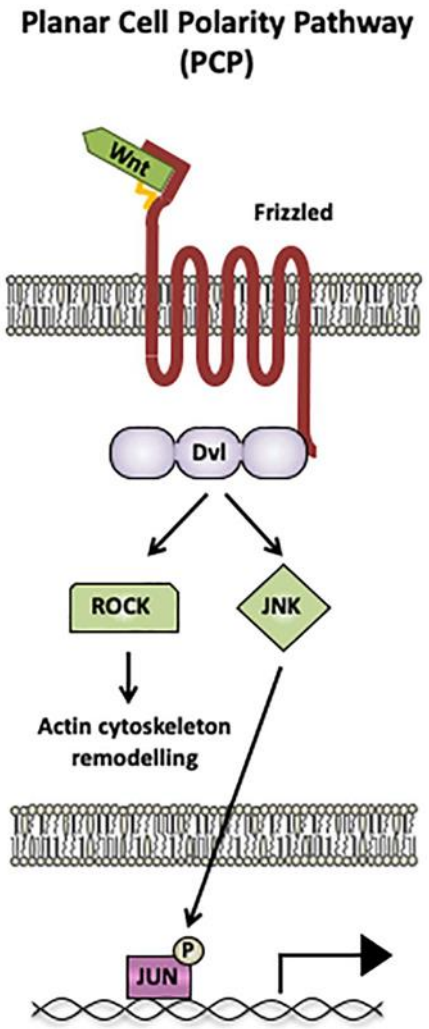
β-Transducin that causes poly-ubiquitination and degradation by the proteosome.

Planar Cell Polarity pathway (PCP)

Step 1 Planar Cell Polarity pathway (PCP)

The **Wnt ligands binds to the Frizzled receptors.**

This activates **Dishevelled (Dvl).**

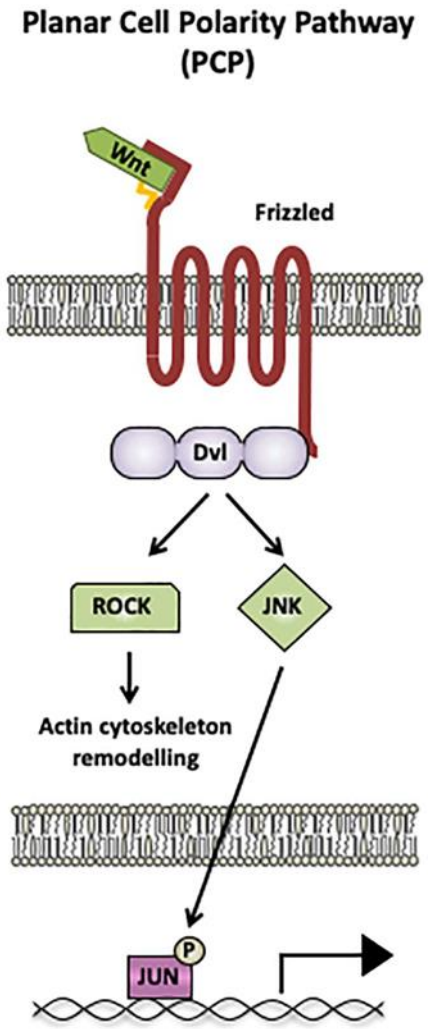


(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023a).

Step 2 Planar Cell Polarity pathway (PCP)

Active Dishevelled associates with small GTPase Rho e.g. Ras homolog gene family member A (RhoA).

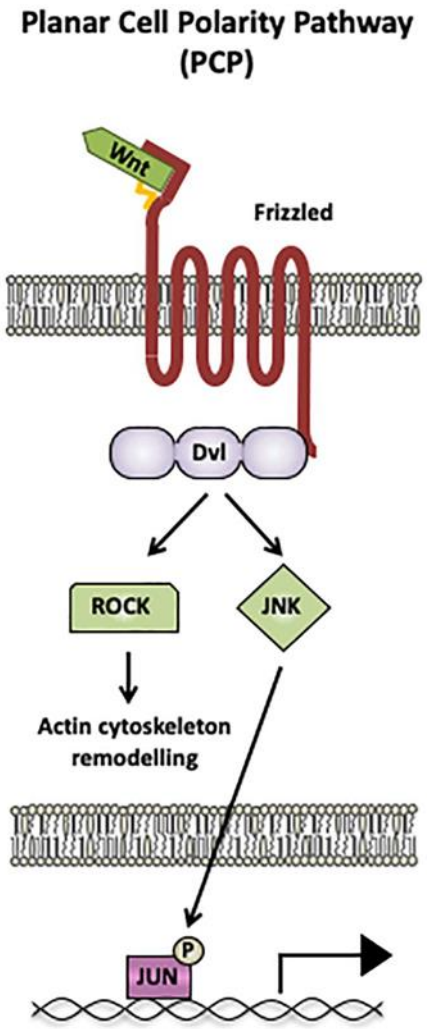
This is achieved by lowering the inhibitory control of the protein **Dvl associated activator of morphogenesis 1 (DAAM1)** in the cytoplasm.



(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023a).

Step 3 Planar Cell Polarity pathway (PCP)

Active Dishevelled also binds to the small GTPase Ras-related C3 botulinum toxin substrate (Rac1) and cell division control protein 42 (Cdc42).

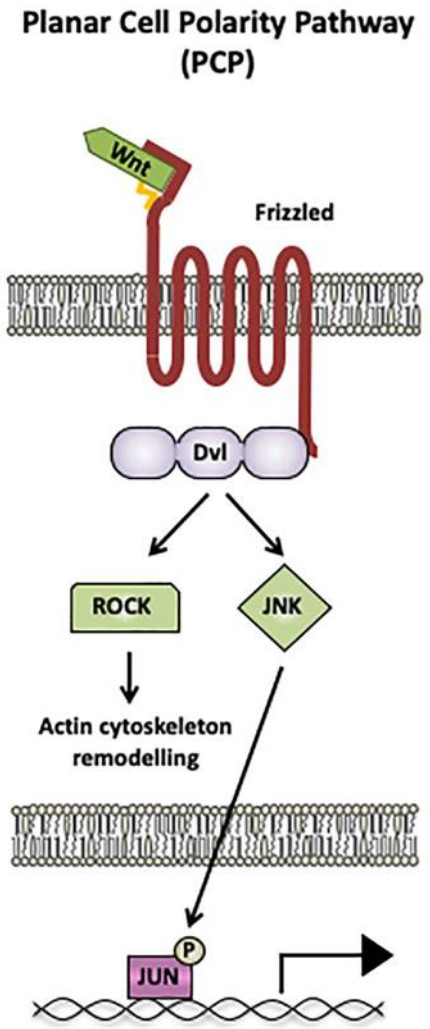


(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023a).

Step 4 Planar Cell Polarity pathway (PCP)

Rac and Rho stimulates:

- **Rho kinase (ROCK)**
- **c-Jun N-terminal kinases (JNK).**



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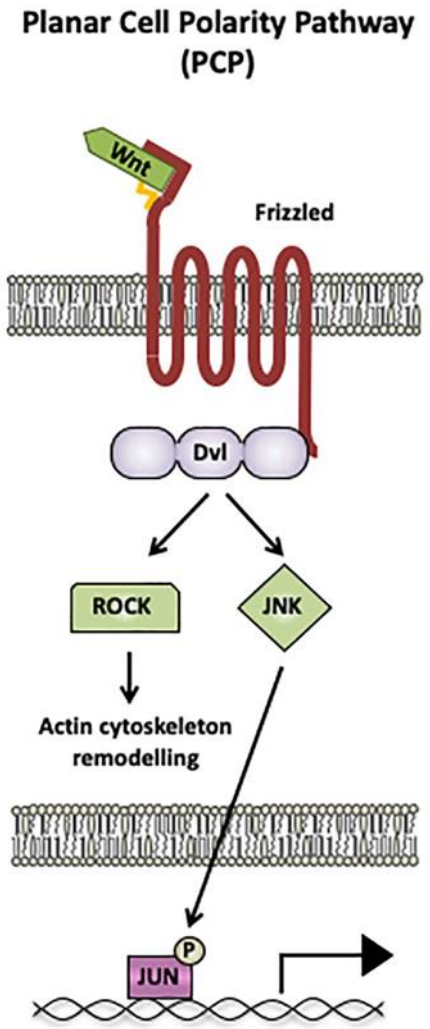
Step 5 Planar Cell Polarity pathway (PCP)

One of the proteins found in the cytoskeleton is **actin.**

It stimulates **ATF2 (activating transcription factor 2).**

Target genes involved in **cell adhesion and migration.**



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β-catenin Independent (non-canonical)

Step 1 Wnt signaling (β-catenin-independent) Wnt/calcium pathway

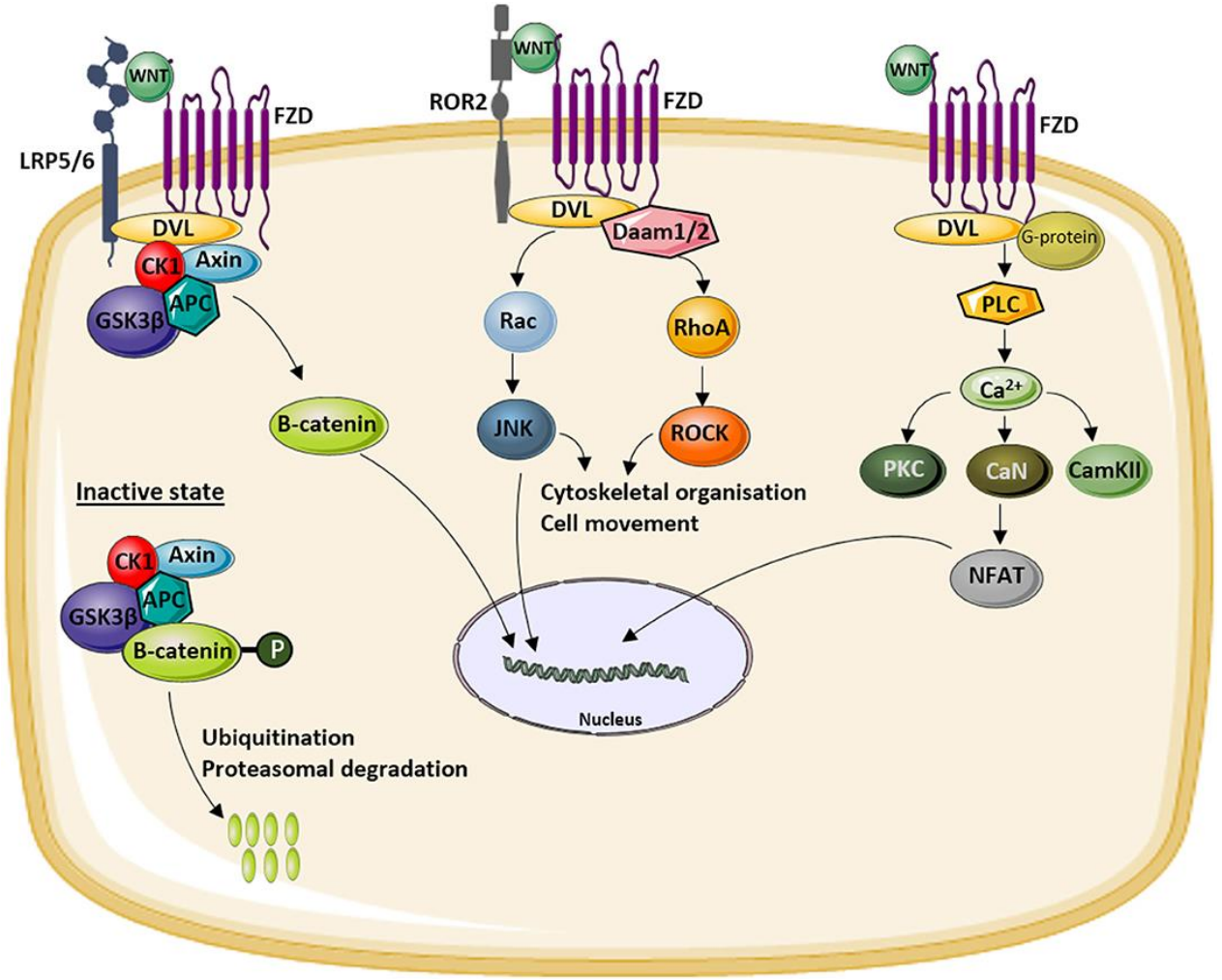
The Wnt ligands bind to **Frizzled receptors.**

This activates via two ways:

- **Dishevelled protein**
- **Trimeric G-proteins (Gα,β,γ).**

This activated complex stimulates:

Phospholipase C (PLC)



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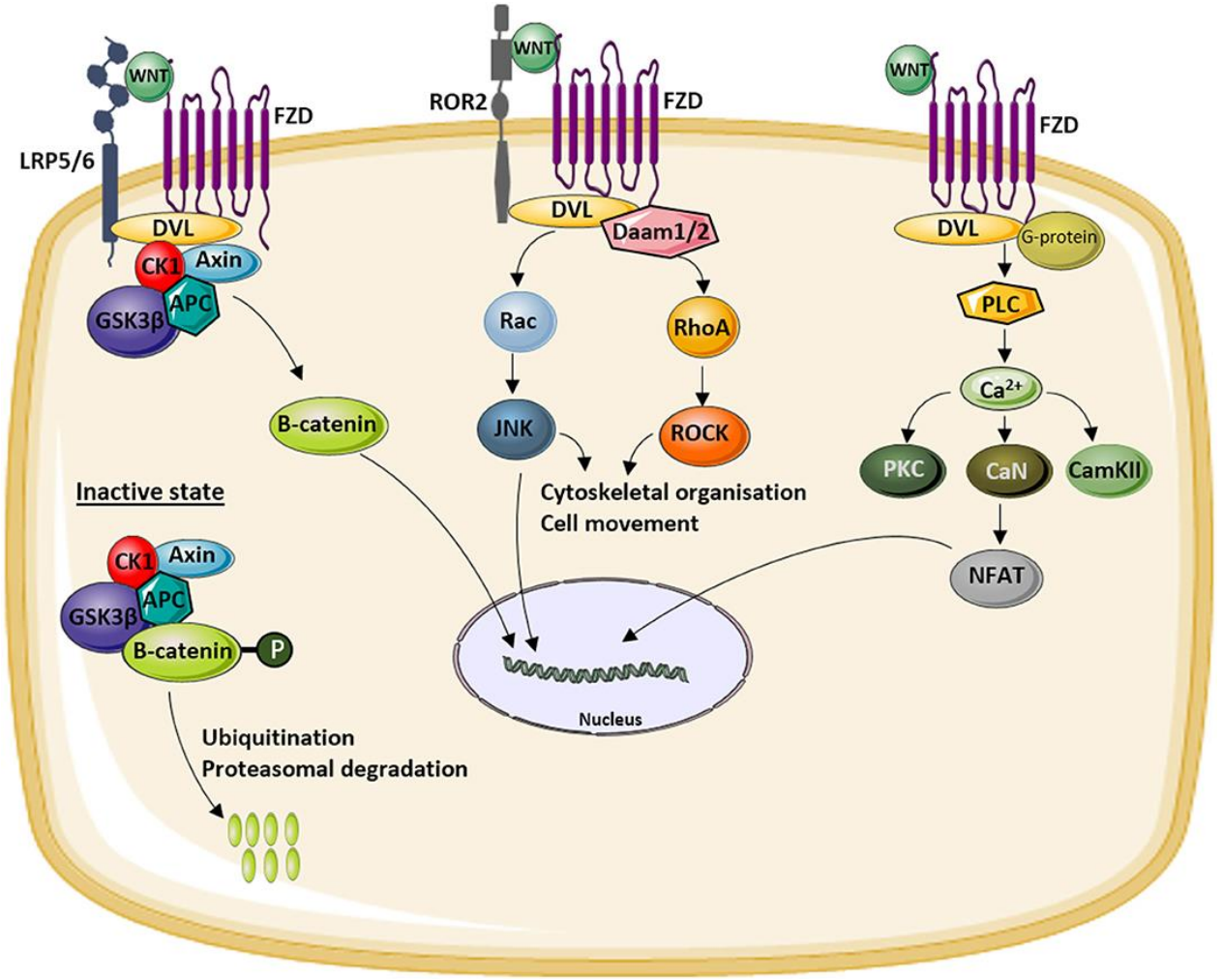
Step 2 Wnt signaling (β-catenin-independent) Wnt/calcium pathway

Phospholipase C leads to the activation

of the secondary messengers:

- **IP_3 (inositol 1,4,5-triphosphate)**
- **DAG_2 (diacylglycerol)**



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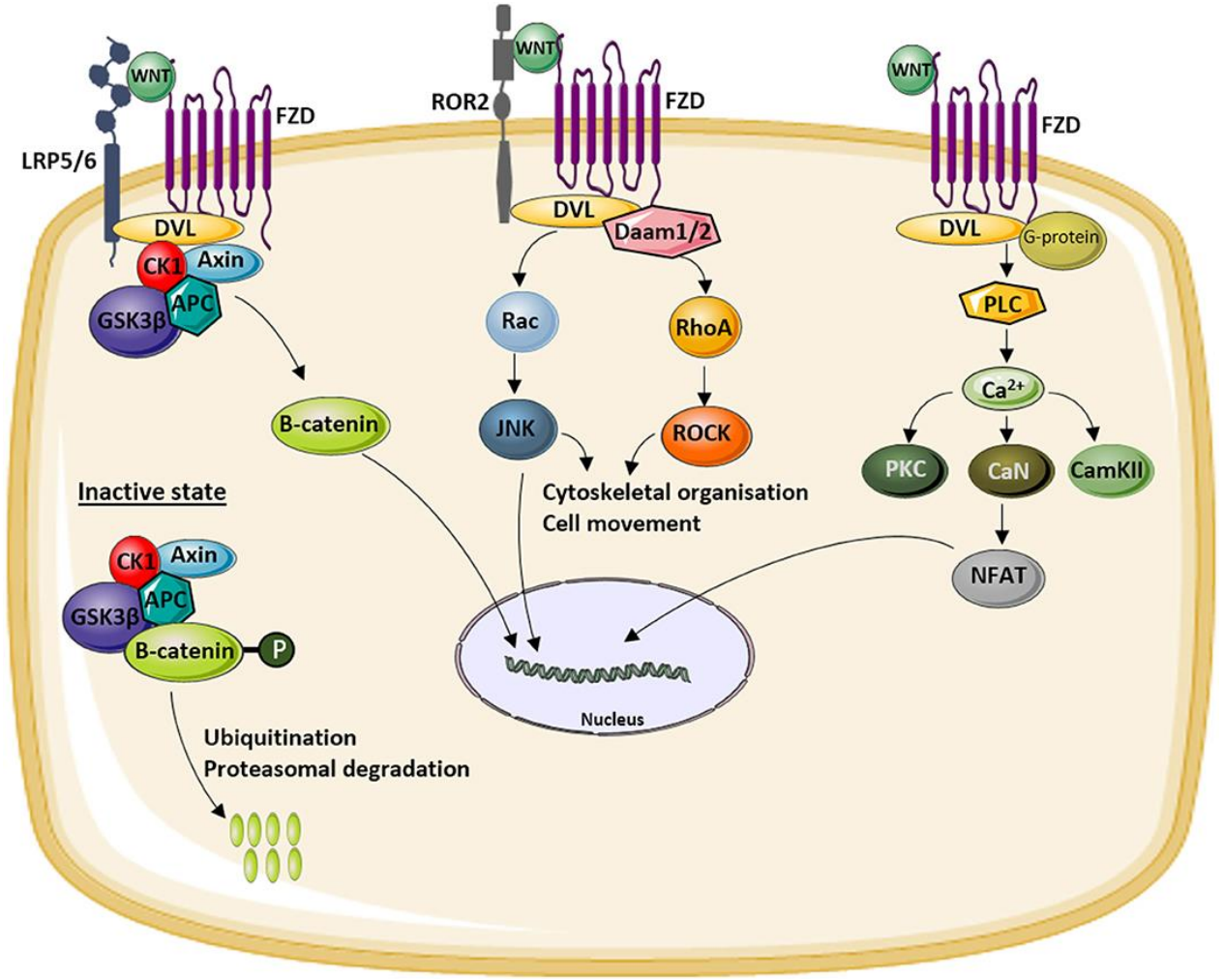
Step 3 Wnt signaling (β-catenin-independent) Wnt/calcium pathway

IP_3 release calcium ions from the endoplasmic reticulum to **the calcium-binding protein calmodulin.**



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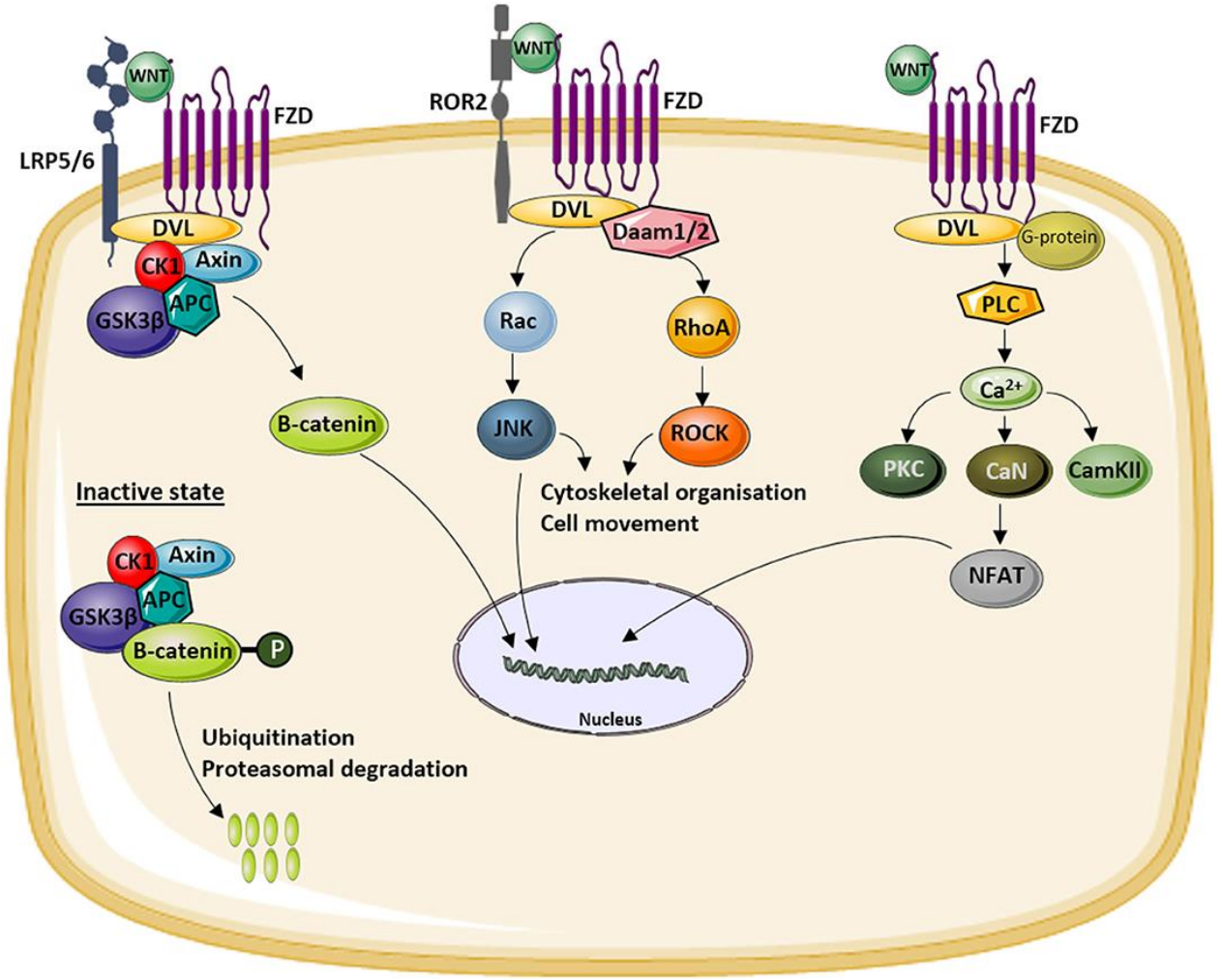
Step 4 Wnt signaling (β-catenin-independent) Wnt/calcium pathway

Calcium ions stimulates **calcium-dependent enzymes:**

- Protein kinase C
- Calcineurin.

This activates:

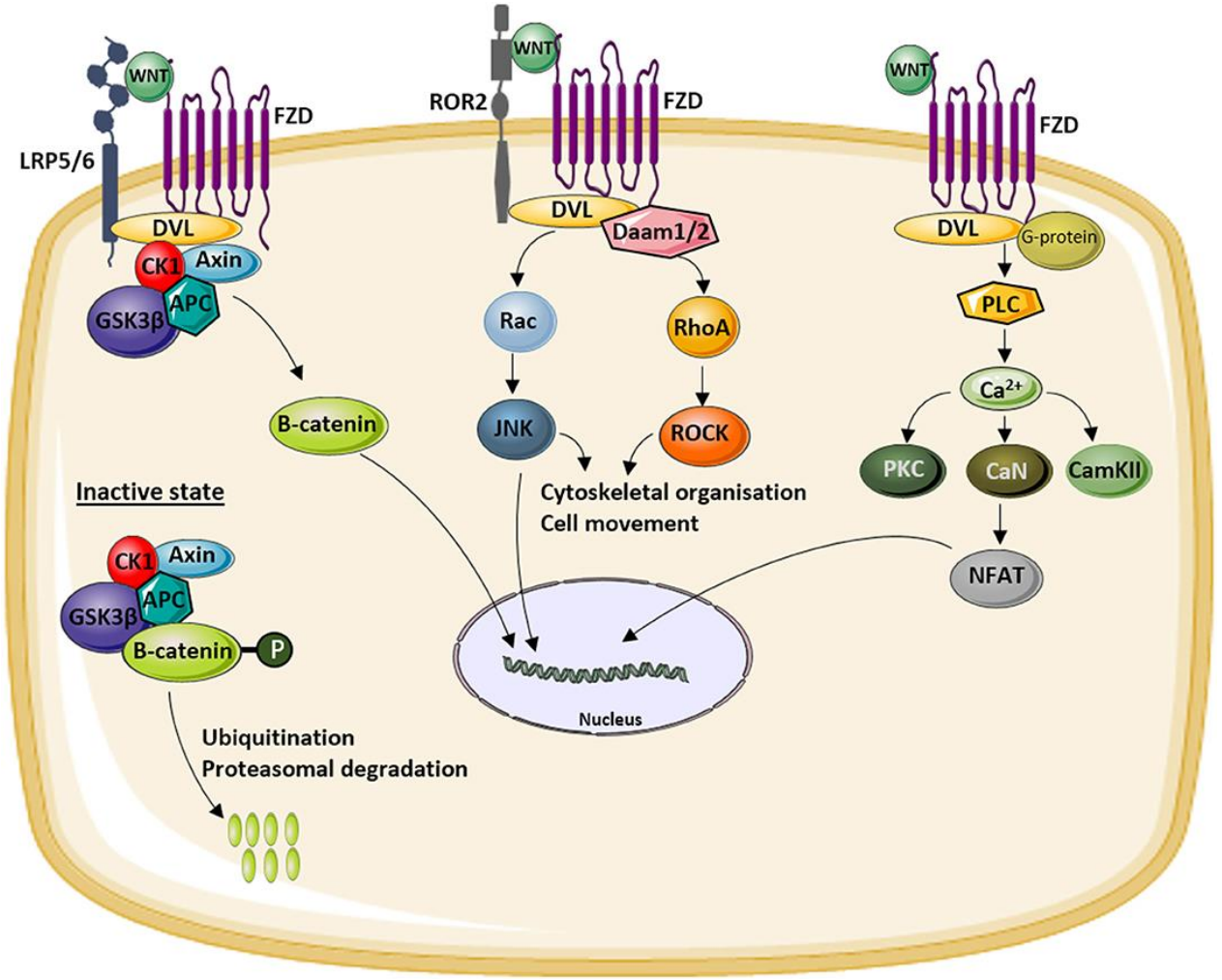
- CAMKII (calcium/calmodulin- dependent kinase II (CAMKII)
- TGF-β activated kinase 1 (TAK-1)



(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023b).

Step 5 Wnt signaling (β-catenin-independent) Wnt/calcium pathway

Nuclear Factor of Activated T-cells (NFAT) targets nucleus to **activate transcription of target genes.**



(Duchartre, Kim and Kahn, 2016; Cancer Research from Technology Networks, no date; Creative Commons, 2023b).

Key cellular functions

Key cellular functions

Cell proliferation

Cell migration

Tissue development

Differentiation

Cell survival

(Duchartre, Kim and Kahn, 2016).

Dysregulated Wnt pathway

Dysregulated Wnt pathway

Examples of disease

Metabolic	Degenerative	Cancer
• Type 2 Diabetes	• Parkinson's disease • Alzheimer's disease	• Liver • Gastrointestinal • Leukaemia • Breast • Melanoma (skin)

- **Increased Wnt activity**
- **GSK3β mutations**
- **Increased β-catenin accumulation**

(Cancer Research from Technology Networks, no date; Ahmad *et al*. 2021; Zhan, Rindtorff, and Boutros, 2016)

USP7 protein	• Protects β-catenin • APC gene is mutated, β-catenin accumulates and overactivates Wnt signalling pathway in colorectal tumour cells.
Regulators of epithelial-to-mesenchymal transition (EMT)	• Low expression of E-Cadherin protein triggers the translocation of β-catenin to the nucleus. Normally they E-Cadherin and β-catenin are tightly bound in the lining (epithelium) • Slug and Twist translocates β-catenin
Wnt5a/ROR2 activity	• Increases cell migration • Resistance to chemotherapy and radiotherapy.

(Cancer Research from Technology Networks, no date)

By the end of this lecture, you should understand

- **The Wnt ligands are cysteine-rich glycoproteins that bind to a specific receptor called Frizzled and a protein called LRP.**
- **There are three types of Wnt signalling pathways: β-catenin dependent, β-catenin Independent** And **planar cell polarity**
- **The Wnt ligands can also bind to RYK and ROR receptor families.**
- **The receptor-ligand complex leads to the activation and polymerization of Dishevelled protein.**
- **The activated Dishevelled polymer suppresses and deactivates the destruction complex.**
- **The destruction complex consists of the following: AXIN, APC and two kinases CK1α and GSK3β.**
- **Repressing the complex increases the accumulation of the β-catenin protein that lead to transcription of genes.**
- **Dysregulated Wnt signalling pathway is caused by: Increased Wnt activity, GSK3β mutations And increased β-catenin accumulation**

Reference list for further reading

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SEASON 2

Understanding Cancer

Lecture 14

Types of signalling pathway: normal and dysregulated Oestrogen

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