Mechanoactivation of Wnt/β-catenin pathways in health and disease

Dr Christina M. Warboys

Department of Bioengineering, Imperial College London

Contact details for correspondence:

Address: Dr C Warboys, 4.35 Royal School of Mines, Imperial College, London, SW7 2BP.

E-mail: c.warboys@ic.ac.uk

Telephone: +44 (0)207 594 6377

Abstract

Mechanical forces play an important role in regulating tissue development and homeostasis in multiple cell types including bone, joint, epithelial and vascular cells and are also implicated in the development of diseases e.g. osteoporosis, cardiovascular disease and osteoarthritis. Defining the mechanisms by which cells sense and respond to mechanical forces therefore has important implications for our understanding of tissue function in health and disease and may lead to the identification of targets for therapeutic intervention. Mechanoactivation of the Wnt signalling pathway was first identified in osteoblasts with a key role for β-catenin in loading-induced osteogenesis. Since then, mechanoregulation of the Wnt pathway has also been observed in stem cells, epithelium, chondrocytes and vascular and lymphatic endothelium. Wnt can signal through both canonical and non-canonical pathways and evidence suggests that both can mediate responses to mechanical strain, stretch and shear stress. This review will discuss our current understanding of the activation of the Wnt pathway in response to mechanical forces.

Introduction

Cells within the body are continuously exposed to a range of mechanical forces, e.g. cyclic stretch (1), strain (2,3) and shear stress (4) that are known to exert powerful effects on cell function in health and disease, thus pathways that transmit mechanical forces into biochemical signals are of significant interest. The Wnt pathway has long been recognised as an important mechanosignalling pathway in bone and evidence of its role in other mechanosensitive cells and tissues has become apparent in recent years. This review will provide a brief overview of canonical and non-canonical Wnt pathways and will summarise our current understanding of the mechanisms by which mechanosignalling through Wnt pathways regulates the function of bone, joint, epithelial and endothelial cells that are all continuously exposed to mechanical forces. Interestingly, there is little evidence of Wnt pathways being involved in mechanotransduction in skeletal and vascular smooth muscle cells, despite their regulation by mechanical forces (1) and this remains an interesting and important area for future research.

The Wnt pathway is an ancient and highly conserved signalling pathway that regulates a diverse set of cellular functions including proliferation, survival and differentiation and plays an important physiological role in embryonic development, stem cell differentiation, wound healing and angiogenesis (5). Wnt signalling is also implicated in several pathologies including cardiovascular disease (6), diabetes (7), osteoporosis (8), osteoarthritis (9), glaucoma (10) and cancer (11). The Wnt signalling pathway has been reviewed extensively elsewhere (5,12) and will be summarised here (see also Figure 1). The Wnt pathway is highly complex due to the presence of multiple glycoprotein Wnt ligands, Frizzled (Fzd) G-protein coupled receptors, lipoprotein receptor-related protein (Lrp) co-receptors and endogenous regulators that can combine in multiple combinations to regulate Wnt signalling (13).

Wnt signalling pathways

The canonical pathway is generally considered to act via stabilisation of cytosolic β-catenin. Under 'resting' conditions β-catenin is rapidly degraded due to its interaction with a destruction complex comprised of adenomatosis polyposis coli (APC), axin, casein kinase-1 (CK1) and glycogen synthase kinase-3β (GSK3β) that phosphorylate β-catenin, targeting it for ubiquitin-mediated proteasomal degradation (see Figure 1a). The binding of a Wnt ligand to a Fzd receptor results in the formation of a complex with an Lrp5/6 co-receptor that causes the recruitment of Dishevelled (Dvl). Phosphorylation of Lrp5/6 by Dvl leads to interaction with Axin thus disrupting and inhibiting the destruction complex (see Figure 1b). This results in β-catenin becoming activated (dephosphorylated) allowing it to translocate to the nucleus where it interacts with transcription factors e.g. T-cell factor (TCF)/lymphoid enhancer factor (LEF) to regulate gene expression (5,12). Several non-canonical Wnt pathways have also been identified that require the interaction of Wnt with Fzd receptors but function independently of Lrp5/6 and β-catenin (see Figure 1c). The best described are the Wnt/planar cell polarity (PCP) pathway that activates RhoA, Rac and JNK, and the Wnt/Ca^{2+} pathway that activate phospholipase C (14). Fzd receptors can also be activated by non-Wnt ligands e.g. Norrin and R-spondin-1 and several endogenous inhibitors also regulate Wnt signalling. Dickkopf-related protein-1 (DKK-1) and sclerostin (Sost) block the interaction between Fzd and Lrp5/6 thus inhibiting canonical Wnt signalling. Wnt inhibitory factors (WIFs) and secreted Fzd-related proteins (sFRP) also act to prevent Wnts from binding to Fzd on the cell surface. Recent studies have also highlighted an emerging role for regulation by microRNAs which adds further complexity (15).

Mechanical regulation of the Wnt pathway in bone

The mechanical environment plays a critical role in bone development and homeostasis (16). Mechanical forces are also implicated in the pathogenesis of osteoporosis, a disease that is characterised by a loss of bone strength and increased risk of fracture. Bone mass increases in response to increased loading and decreases in response to reduced loading (17) thus the mechanisms by which bone cells sense and respond to mechanical forces are of significant interest. The importance of Wnt signalling in regulating bone metabolism is apparent from the number of polymorphisms identified in Wnt pathway components that are associated with defects in bone

mineral density (18). Numerous experimental studies have confirmed that Wnt pathways play a key role in mechanosignalling in bone. Wnt signalling and nuclear localisation/activation of β-catenin is increased in osteoblasts in response to mechanical loading *in vivo* (19,20) and *in vitro* following application of cyclic strain (21,22) or shear stress (23–26) and is associated with the increased expression of osteogenic genes. Evidence also suggests that loss of bone strength in aged mice is associated with a failure to sustain Wnt activity in response to repeated mechanical loading (27). Furthermore, osteocyte-specific deletion of β-catenin results in severe bone loss and an osteoporotic phenotype (28) whilst targeted deletion of a single β-catenin allele in osteocytes abolishes the osteogenic response to mechanical loading positioning β-catenin as a key regulator of mechanosignalling in bone (29). Interestingly, the osteogenic effects of β-catenin in response to mechanical loading are attenuated when oestrogen receptor-α is absent or inhibited which may account for the increased prevalence of osteoporosis in post-menopausal women (20).

Although the importance of β-catenin activation in mediating responses to mechanical loading is clear, the precise mechanosignalling events are still uncertain with several different signalling pathways proposed. Loading of the mouse tibia using a 4-point bending method increased the expression of Wnt10B, sFRP1, sFRP2, DKK-1 and Fzd2 (19) although it is not clear whether other Wnt pathway components were mechanically regulated since an *ad hoc* PCR approach was used rather than microarray or RNAseq analysis. Several genome-wide association studies in humans have identified genetic variations within the *Wnt16* locus that are associated with defects in bone mineral density and osteoporosis (30–33) whilst transgenic mouse models have demonstrated that loss of Wnt16 reduces the formation of periosteal bone in response to mechanical loading (34) and increases the risk of osteoporosis (33). Wnt16 expression was also shown to be increased in cultured osteoblastic cells in response to shear stress for 30 min and *in vivo* in response to 4-point bending and axial compression of the mouse tibia (34) suggesting an important role for Wnt16 in mechanosignalling in bone.

Additionally, a critical role for Lrp5 in bone mechanosignalling has also been identified, further supporting the hypothesis that a canonical Wnt pathway mediates responses to mechanical loading in bone. $Lrp5^{-/-}$ mice have severely impaired osteogenic responses to axial loading of the ulna (35) whilst loss of function mutations in the *Lrp5* locus are associated with osteoporosis in humans (36). Interestingly, acute responses to shear stress (e.g. MAPK activation) remain intact in $Lrp5^{-/-}$ mice suggesting that loss of Lrp5 does not cause global defects in mechanosignalling but specifically alters osteogenic responses driven by the Wnt pathway. The osteogenic response to mechanical loading also requires the down-regulation of Sclerostin, an endogenous inhibitor of Lrp5/6 (37), confirming the importance of the canonical Wnt pathway. Transgenic mice that constitutively over-express Sclerostin in osteocytes exhibited significantly reduced periosteal bone formation in response to axial loading which was associated with a failure to up-regulate Wnt target genes suggesting that down-regulation of Sclerostin is a key step in Wnt-mediated mechanosignalling (37).

There is some evidence that Wnt-independent pathways may be also be activated in response to acute exposure to mechanical forces. Exposures of osteoblasts to laminar shear stress for 1 h causes β-catenin to dissociate from N-cadherin whereby it can translocate to the nucleus (24). The same study also reported increased phosphorylation (inhibition) of GSK3β in response to acute shear stress which may also promote the nuclear translocation of β-catenin (24). The authors did not assess the mechanisms of GSK3β inhibition but reported activation of Akt over the same time course

and speculate that this may inhibit GSK3β, however Wnt signalling cannot be ruled out. In another study, exposure of osteoblasts to laminar shear stress for 30 min resulted in mechanoactivation of β-catenin that was dependent on the activation of eNOS suggesting a role for eNOS/NO in early mechanosignalling (38). The authors propose that NO may promote stabilisation of β-catenin independently of Wnt and Fzd signalling in the initial response to mechanical forces, as has been shown in static endothelial cells where NO, via cGMP, activates cGMP-dependent protein kinase (PKG) that phosphorylates and inhibits GSK3β (39). These data raise the possibility that rapid mechanoactivation of β-catenin may be mediated by Wnt independent pathways whereas canonical Wnt signalling is important for sustained responses to mechanical force (38). Mechanosensitive Wnt/β-catenin pathways in bone are summarised in Figure 2 and Table 1.

Mechanical regulation of Wnt signalling in mesenchymal stem cells

Following the discovery that mechanical forces play a critical role in the development of bone, attention has also been directed to understand the mechanical regulation of human mesenchymal stem cells (hMSC) that play a role in osteogenesis. Exposure of hMSCs to oscillatory shear stress causes the rapid activation of β-catenin along with increased expression of Wnt5A, receptor tyrosine kinase-like orphan receptor-2 (Ror2) and the osteogenic gene, runt-related transcription factor-2 (Runx2). Mechanical up-regulation of Runx2 was inhibited following knockdown of Wnt5a suggesting a direct link between Wnt5a and Runx2 although, interestingly, β-catenin activation occurred independently of Wnt5a induction. Instead, oscillatory shear stress resulted in the dissociation of β-catenin from N-cadherin (40). N-cadherin can inhibit β-catenin activity in un-loaded osteocytes forming a complex with Lrp5 and Axin2 that promotes β-catenin degradation (41). It is possible that mechanical forces induce a conformational change in N-cadherin that disrupts this complex leading to activation of β-catenin. It is unclear whether this is a general response to shear stress since only one flow condition was assessed and compared to static conditions.

As well as promoting osteogenesis, mechanical forces acting via β-catenin further direct the lineage specification of hMSCs by inhibiting adipogenesis (42). These effects appear to be mediated by inhibition of GSK3β but are independent of Wnt-Fzd signalling, although the mechanism by which mechanical loading inhibits GSK3β is unclear (43). Interestingly, one study found that β-catenin was inhibited in hMSCs following acute exposure to oscillatory shear stress although these analyses were performed on cells from a single donor and so may not be indicative of a typical response to shear stress (44). The functional outcome of Wnt signalling in osteogenic cells can also vary depending on the differentiation state of the stem cells being studied and endogenous Wnt activity/expression (45,46) and requires further consideration when describing mechanoactivation of Wnt pathways in these and other mechanoresponsive cells.

Recent evidence demonstrates that β-catenin can be directly activated by mechanical forces in hMSCs using functionalised magnetic nanoparticles (MNPs) coated with an anti-Fzd2 antibody (47). This was not associated with any alterations in Lrp5/6 phosphorylation and was not affected by co-treatment with DKK-1 suggesting a non-canonical mechanism. A subsequent study by the same group reveals that direct mechanical activation of Fzd2 leads to receptor clustering and can promote osteogenesis and bone formation (48). Mechanosensitive Wnt/β-catenin pathways in MSC are summarised in Figure 3 and Table 1.

Mechanical regulation of Wnt signalling in joints

Mechanical forces are also important in the development and maintenance of joints and are believed to be linked to the degeneration of the joint in osteoarthritis, a disorder of the joint associated with degeneration of articular cartilage and re-modelling of the surrounding bone (49,50). Canonical Wnt/β-catenin signalling is required for the formation of synovial joints during skeletogenesis in the developing mouse embryo (51) and in mouse models where muscle contraction is absent, the formation of joints is disrupted (52). This is associated with reduced transcriptional activity of β-catenin suggesting a mechanistic link (52). The potential importance of Wnt signalling in the mechanical regulation of joint development was underscored by a microarray analysis of humeri from *splotch* mutants that lack limb skeletal muscle and hence display abnormal joint development due to loss of mechanical forces. Gene ontology analysis of differentially expressed transcripts revealed significant enrichment of genes associated with Wnt signalling (53). Furthermore, recent studies in zebrafish demonstrate that mechanical activation of Wnt/β-catenin in response to high strain is important in the development of the lower jaw and that immobilisation of the joint leads to reduced β-catenin activity (54). In zebrafish lacking Wnt16 the migration and proliferation of chondrocytes in response to mechanical force is attenuated suggesting that Wnt16 and β-catenin play a key role in mechanosignalling in the developing joint (54). Mechanosensitive Wnt/β-catenin pathways in chondrocytes are summarised in Figure 4 and Table 1.

The mechanosignalling mechanisms that drive osteoarthritis are poorly defined although it is known that polymorphisms in the *FrzB* gene are associated with osteoarthritis of the hip (55) and the expression of both Wnt16 and β-catenin is increased in articular cartilage from osteoarthritis patients (56). Furthermore, inhibition of Wnt/β-catenin via intra-articular injection of a Wnt pathway inhibitor appears to reduce the severity of osteoarthritis in a mouse model of the disease (9). Although these data suggest that Wnt/β-catenin plays a role in the pathogenesis of osteoarthritis, their role in transducing mechanical signals associated with disease progression remains unclear since Wnt pathways have been shown to be increased in response to injury and inflammation (56).

Mechanical regulation of Wnt signalling in the vasculature

Evidence has also begun to emerge over the last few years to suggest that Wnt signalling may play an important role in mechanotransduction in the vasculature. Endothelial cells that line the blood vessels are continuously exposed to the mechanical drag exerted by the flowing blood (known as shear stress) and it is widely recognised that shear stress plays a crucial role in determining endothelial phenotype (57). Moreover, mechanical forces play a critical role in the development of atherosclerosis. Lesions develop at areas of high curvature, branching and bifurcation where blood flow is ‘disturbed’ whereas vessels exposed to uniform flow with high shear stress are spared (58–60). The mechanisms by which endothelial cells sense and respond to shear stress are therefore of great importance in understanding the development of cardiovascular disease.

The first evidence that β-catenin may play a role in endothelial mechanosensing came to light following the identification of the junctional mechanosensory complex comprised of platelet

endothelial cell adhesion molecule-1 (PECAM-1), vascular-endothelial (VE)-cadherin and vascular endothelial growth factor receptor-2 (VEGFR2) (61). The interaction between VEGFR2 and VE-cadherin is indirect and is facilitated by β-catenin, furthermore, deletion of β-catenin blocked the activation of integrins in response to acute application of shear stress or force. Although these data suggest a mechanosensory role for β-catenin in response to acute application of mechanical force to single cells, its role in mechanotransduction under conditions of sustained shear stress in confluent endothelial monolayers remains to be defined although several independent studies have shown that β-catenin activation (nuclear translocation) is increased in response to atherogenic flow patterns (62–64). Recently, a role for Wnt signalling in mechanosensing by primary cilia has also emerged (64). Dvl2 was shown to localise to primary cilia in endothelial cells exposed to very low wall shear stress (but not high shear stress) although the role of Wnt and β-catenin in cilia-mediated mechanosignalling was not studied (64).

Microarray analysis of atheroprone and protected regions of the pig aorta revealed that the expression of Fzd4 and Fzd5 is increased in endothelial cells exposed to 'disturbed' flow. These data suggest that the Wnt pathway may be regulated by mechanical forces, although they may also be increased secondary to other mechanically activated pathways. Interestingly, the expression of Lrp6 is increased in cells exposed to uniform flow (65) suggesting that different Wnt pathways may be active under different flow conditions. Nuclear accumulation of β-catenin has also been observed in an atheroprone region of the mouse aorta, in the absence of lesions, suggesting flow-dependent regulation. This was confirmed *in vitro* by exposing cultured endothelium to an atherogenic wave form (62). Subsequent analysis determined that β-catenin activation occurred as a consequence of PECAM-1 mediated inactivation of GSK3β suggesting that flow-dependent regulation of β-catenin may be independent of Wnt signalling, although this was not studied directly (62).

Increased nuclear translocation and transcriptional activity of β-catenin can also be induced following short-term exposure to oscillatory shear stress (63) which was associated with elevated expression of angiopoietin-2 (Ang-2). Laminar shear stress also increased the expression of Ang-2 although to a much smaller degree (63). The shear-dependent increased in Ang-2 was reduced in the presence of the Wnt inhibitor IWR-1. Parallel studies revealed that Wnt3-dependent increases in Ang2 expression were inhibited by DKK-1 suggesting activation via a canonical mechanism although these experiments were performed under static conditions, thus it is unclear which Wnt pathway(s) are activated in response to mechanical force (63). Oscillatory shear stress also promotes nuclear translocation of β-catenin in lymphatic endothelium and has been shown to be required for formation of lymphatic valves and for lymphatic vascular patterning in mice (66).

Non-canonical Wnts also play a role in flow-mediated vessel remodelling although it is not clear if these responses require β-catenin. Conditional inhibition of Wnt signalling using a tamoxifen-inducible Cre to knockout *Wls* (a chaperone protein required for Wnt secretion), resulted in significantly reduced vascular density compared to littermate controls with evidence of increased vascular regression (67). This was attributed to loss of non-canonical Wnt signalling since vessel regression was phenocopied in mice with an endothelial specific inactivation of Wnt5a and global knockout of Wnt11 (67). Analysis of cultured cells exposed to laminar shear stress for 4h suggests that loss of Wnt5a and Wnt11 increases the sensitivity of cells to shear stress leading to increased polarisation. Interestingly, typical transcriptional responses to shear stress (i.e. induction of Krüppel-like factor-2 and -4) were not altered following deletion of Wnt5a and Wnt11 suggesting that Wnts

act only to modulate the threshold for flow-induced polarisation (67). Mechanosensitive Wnt/β-catenin pathways in endothelial cells are summarised in Figure 5 and Table 1.

Mechanical regulation of Wnt signalling in epithelial cells.

Mechanotransduction in epithelial cells has gained traction recently following the discovery that tumour cells and tumour-adjacent cells are exposed to a range of mechanical insults that can increase malignant transformation promoting tumour growth and metastasis (68). Several studies have suggested a role for β-catenin in mechanosignalling in quiescent epithelium and within the tumour microenvironment, for example increased tension or matrix stiffness has been shown to increase β-catenin stabilisation in epithelial cells in a Rho-associated protein kinase (ROCK)-dependent manner via activation of focal adhesion kinase, Akt and GSK3β (69,70). Similarly, mechanical strain activates β-catenin in quiescent epithelium causing cell cycle re-entry via an E-cadherin dependent mechanism (71). This may be arise as a consequence of conformational changes within the interacting site of β-catenin and E-cadherin leading to release of β-catenin (72). In tumour-adjacent cells, increased mechanical strain leads to the activation of β-catenin and increased expression of β-catenin target genes that drive proliferation, resulting in increased tumour mass (73). Interestingly, in cells where β-catenin is constitutively activated i.e. SW480 colon cancer cells, laminar shear stress has been shown to reduce β-catenin activation through an α6β4-integrin dependent mechanism (74). The functional relevance of this finding in response to tumour progression where other mechanical forces may dominate is unclear although it may indicate that mechanosignalling may differ in cells with mutations in the Wnt/β-catenin pathway. There is currently no evidence of mechanoactivation of canonical Wnt signalling pathways in epithelial cells and warrants further study considering its important role in mechanosignalling in other cell types. Mechanosensitive Wnt/β-catenin pathways in epithelial cells are summarised in Figure 6 and Table 1.

Summary

- Wnt/β-catenin signalling pathways are activated in a number of mechanoresponsive cells (summarised in Table 1), playing an important role in regulating cell phenotype. They are also implicated in several mechanically-driven diseases.

- Mechanoactivation of the canonical Wnt pathway and stabilisation of β-catenin appears to play an important role in signalling in all mechanosensitive cells suggesting the presence of a common mechanosignalling mechanism.

- Non-canonical Wnt pathways are also regulated by mechanical forces and may play a role in determining cell function/fate alongside canonical pathways. Similarly, β-catenin can be activated independently of Wnt signalling via its interaction with cadherins which may further dictate cellular responses to mechanical force.

- Wnt/β-catenin pathways are highly complex and context-dependent. The precise signalling mechanisms involved in the mechanoactivation of canonical and/or non-canonical pathways appears to depend on the type and duration of mechanical force, the cell type and underlying cellular factors.

- Future studies into the mechanoactivation of Wnt/β-catenin pathways should address temporal effects (acute *vs.* sustained force) and whether pathways are differentially activated by different magnitudes or types of force (e.g. low *vs*. high shear stress) instead of comparing responses to unphysiological static conditions.

- Better understanding of the precise signalling mechanisms involved in the mechanoactivation of Wnt/β-catenin pathways may provide valuable targets for future treatment or prevention of mechanically-driven diseases.

References

1. Haga JH, Li Y-SJ, Chien S. Molecular basis of the effects of mechanical stretch on vascular smooth muscle cells. *J Biomech*. 2007;**40**(5):947–60. Available from: doi:10.1016/J.JBIOMECH.2006.04.011

2. Rubin CT, Lanyon LE. Regulation of bone mass by mechanical strain magnitude. *Calcif Tissue Int*. 1985;**37**(4):411–7.

3. Freeman PM, Natarajan RN, Kimura JH, Andriacchi TP. Chondrocyte cells respond mechanically to compressive loads. *J Orthop Res*. 1994;**12**(3):311–20. Available from: doi:10.1002/jor.1100120303

4. Baeyens N, Bandyopadhyay C, Coon BG, Yun S, Schwartz MA. Endothelial fluid shear stress sensing in vascular health and disease. *J Clin Invest*. 2016;**126**(3):821–8. Available from: doi:10.1172/JCI83083

5. Clevers H, Nusse R. Wnt/β-Catenin Signaling and Disease. *Cell*. 2012;**149**(6):1192–205. Available from: doi:10.1016/J.CELL.2012.05.012

6. Mill C, George SJ. Wnt signalling in smooth muscle cells and its role in cardiovascular disorders. *Cardiovasc Res*. 2012;**95**(2):233–40. Available from: doi:10.1093/cvr/cvs141

7. Bretón-Romero R, Feng B, Holbrook M, Farb MG, Fetterman JL, Linder EA, et al. Endothelial Dysfunction in Human Diabetes Is Mediated by Wnt5a–JNK Signaling. *Arterioscler Thromb Vasc Biol*. 2016;**36**(3):561.

8. Lerner UH, Ohlsson C. The WNT system: background and its role in bone. *J Intern Med*. 2015;**277**(6):630–49. Available from: doi:10.1111/joim.12368

9. Lietman C, Wu B, Lechner S, Shinar A, Sehgal M, Rossomacha E, et al. Inhibition of Wnt/β-catenin signaling ameliorates osteoarthritis in a murine model of experimental osteoarthritis. *JCI Insight*. 2018;**3**(3). Available from: doi:10.1172/JCI.INSIGHT.96308

10. Wang X, Huai G, Wang H, Liu Y, Qi P, Shi W, et al. Mutual regulation of the Hippo/Wnt/LPA/TGF‑β signaling pathways and their roles in glaucoma (Review). *Int J Mol*

Med. 2017;**41**(3):1201–12. Available from: doi:10.3892/ijmm.2017.3352

11. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene*. 2017;**36**(11):1461–73. Available from: doi:10.1038/onc.2016.304

12. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. *Dev Cell*. 2009;**17**(1):9–26. Available from: doi:10.1016/j.devcel.2009.06.016

13. Dijksterhuis JP, Petersen J, Schulte G. WNT/Frizzled signalling: receptor-ligand selectivity with focus on FZD-G protein signalling and its physiological relevance: IUPHAR Review 3. *Br J Pharmacol*. 2014;**171**(5):1195–209. Available from: doi:10.1111/bph.12364

14. Mill C, George SJ. Wnt signalling in smooth muscle cells and its role in cardiovascular disorders. *Cardiovasc Res*. 2012;**95**(2):233–40. Available from: doi:10.1093/cvr/cvs141

15. Song JL, Nigam P, Tektas SS, Selva E. microRNA regulation of Wnt signaling pathways in development and disease. *Cell Signal*. 2015;**27**(7):1380–91. Available from: doi:10.1016/J.CELLSIG.2015.03.018

16. Rubin J, Rubin C, Jacobs CR. Molecular pathways mediating mechanical signaling in bone. *Gene*. 2006;**367**:1–16. Available from: doi:10.1016/j.gene.2005.10.028

17. Mullender MG, Huiskes R. Proposal for the Regulatory Mechanism of Wolff's Law [Internet]. Vol. 13, The Journal of Bone and Joint Surgery. 1995.

18. Hsu Y-H, Kiel DP. Genome-Wide Association Studies of Skeletal Phenotypes: What We Have Learned and Where We Are Headed. *J Clin Endocrinol Metab*. 2012;**97**(10):E1958–77. Available from: doi:10.1210/jc.2012-1890

19. Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C, et al. Wnt/β-Catenin signaling is a normal physiological response to mechanical loading in bone. *J Biol Chem*. 2006;**281**(42):31720–8. Available from: doi:10.1074/jbc.M602308200

20. Armstrong VJ, Muzylak M, Sunters A, Zaman G, Saxon LK, Price JS, et al. Wnt/β-catenin signaling is a component of osteoblastic bone cell early responses to load-bearing and requires estrogen receptor alpha. *J Biol Chem*. 2007;**282**(28):20715–27. Available from: doi:10.1074/jbc.M703224200

21. Case N, Ma M, Sen B, Xie Z, Gross TS, Rubin J. β-catenin levels influence rapid mechanical responses in osteoblasts. *J Biol Chem*. 2008;**283**(43):29196–205. Available from: doi:10.1074/jbc.M801907200

22. Hens JR, Wilson KM, Dann P, Chen X, Horowitz MC, Wysolmerski JJ. TOPGAL Mice Show That the Canonical Wnt Signaling Pathway Is Active During Bone Development and Growth and Is Activated by Mechanical Loading In Vitro. *J Bone Miner Res*. 2005;**20**(7):1103–13. Available from: doi:10.1359/JBMR.050210

23. Kamel MA, Picconi JL, Lara-Castillo N, Johnson ML. Activation of β-catenin signaling in MLO-Y4 osteocytic cells versus 2T3 osteoblastic cells by fluid flow shear stress and PGE2: Implications for the study of mechanosensation in bone. *Bone*. 2010;**47**(5):872–81. Available from: doi:http://dx.doi.org/10.1016/j.bone.2010.08.007

24. Norvell SM, Alvarez M, Bidwell JP, Pavalko FM. Fluid shear stress induces β-catenin signaling in osteoblasts. *Calcif Tissue Int*. 2004;**75**(5):396–404. Available from: doi:10.1007/s00223-004-0213-y

25. Santos A, Bakker AD, Zandieh-Doulabi B, de Blieck-Hogervorst JMA, Klein-Nulend J. Early activation of the β-catenin pathway in osteocytes is mediated by nitric oxide, phosphatidyl

inositol-3 kinase/Akt, and focal adhesion kinase. *Biochem Biophys Res Commun*. 2010;**391**(1):364–9. Available from: doi:10.1016/J.BBRC.2009.11.064

26. Santos A, Bakker AD, Zandieh-Doulabi B, Semeins CM, Klein-Nulend J. Pulsating fluid flow modulates gene expression of proteins involved in Wnt signaling pathways in osteocytes. *J Orthop Res*. 2009;**27**(10):1280–7. Available from: doi:10.1002/jor.20888

27. Holguin N, Brodt MD, Silva MJ. Activation of Wnt Signaling by Mechanical Loading Is Impaired in the Bone of Old Mice. *J Bone Miner Res*. 2016;**31**(12):2215–26. Available from: doi:10.1002/jbmr.2900

28. Kramer I, Halleux C, Keller H, Pegurri M, Gooi JH, Weber PB, et al. Osteocyte Wnt/β-catenin signaling is required for normal bone homeostasis. *Mol Cell Biol*. 2010;**30**(12):3071–85. Available from: doi:10.1128/MCB.01428-09

29. Javaheri B, Stern AR, Lara N, Dallas M, Zhao H, Liu Y, et al. Deletion of a Single β-Catenin Allele in Osteocytes Abolishes the Bone Anabolic Response to Loading. *J Bone Miner Res*. 2014;**29**(3):705–15. Available from: doi:10.1002/jbmr.2064

30. Zhang L, Choi HJ, Estrada K, Leo PJ, Li J, Pei Y-F, et al. Multistage genome-wide association meta-analyses identified two new loci for bone mineral density. *Hum Mol Genet*. 2014;**23**(7):1923–33. Available from: doi:10.1093/hmg/ddt575

31. García-Ibarbia C, Pérez-Núñez MI, Olmos JM, Valero C, Pérez-Aguilar MD, Hernández JL, et al. Missense polymorphisms of the WNT16 gene are associated with bone mass, hip geometry and fractures. *Osteoporos Int*. 2013;**24**(9):2449–54. Available from: doi:10.1007/s00198-013-2302-0

32. Medina-Gomez C, Kemp JP, Estrada K, Eriksson J, Liu J, Reppe S, et al. Meta-Analysis of Genome-Wide Scans for Total Body BMD in Children and Adults Reveals Allelic Heterogeneity and Age-Specific Effects at the WNT16 Locus. Gibson G, editor. *PLoS Genet*. 2012;**8**(7):e1002718. Available from: doi:10.1371/journal.pgen.1002718

33. Zheng H-F, Tobias JH, Duncan E, Evans DM, Eriksson J, Paternoster L, et al. WNT16 influences bone mineral density, cortical bone thickness, bone strength, and osteoporotic fracture risk. *PLoS Genet*. 2012;**8**(7):e1002745. Available from: doi:10.1371/journal.pgen.1002745

34. Wergedal JE, Kesavan C, Brommage R, Das S, Mohan S. Role of WNT16 in the regulation of periosteal bone formation in female mice. *Endocrinology*. 2015;**156**(3):1023–32. Available from: doi:10.1210/en.2014-1702

35. Sawakami K, Robling AG, Ai M, Pitner ND, Liu D, Warden SJ, et al. The Wnt Co-receptor LRP5 Is Essential for Skeletal Mechanotransduction but Not for the Anabolic Bone Response to Parathyroid Hormone Treatment. *J Biol Chem*. 2006;**281**(33):23698–711. Available from: doi:10.1074/jbc.M601000200

36. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, et al. LDL Receptor-Related Protein 5 (LRP5) Affects Bone Accrual and Eye Development. *Cell*. 2001;**107**(4):513–23. Available from: doi:10.1016/S0092-8674(01)00571-2

37. Tu X, Rhee Y, Condon KW, Bivi N, Allen MR, Dwyer D, et al. Sost downregulation and local Wnt signaling are required for the osteogenic response to mechanical loading. *Bone*. 2012;**50**(1):209–17. Available from: doi:10.1016/J.BONE.2011.10.025

38. Santos A, Bakker AD, Zandieh-Doulabi B, de Blieck-Hogervorst JMA, Klein-Nulend J. Early activation of the β-catenin pathway in osteocytes is mediated by nitric oxide, phosphatidyl inositol-3 kinase/Akt, and focal adhesion kinase. *Biochem Biophys Res Commun*.

2010;**391**(1):364–9. Available from: doi:http://dx.doi.org/10.1016/j.bbrc.2009.11.064

39. Warboy CM, Chen N, Zhang Q, Shaifta Y, Vanderslott G, Passacquale G, et al. Bidirectional cross-regulation between the endothelial nitric oxide synthase and β-catenin signalling pathways. *Cardiovasc Res*. 2014;**104**(1):116–26.

40. Arnsdorf EJ, Tummala P, Jacobs CR. Non-Canonical Wnt Signaling and N-Cadherin Related β-Catenin Signaling Play a Role in Mechanically Induced Osteogenic Cell Fate. Bergmann A, editor. *PLoS One*. 2009;**4**(4):e5388. Available from: doi:10.1371/journal.pone.0005388

41. Haÿ E, Laplantine E, Geoffroy V, Frain M, Kohler T, Müller R, et al. N-cadherin interacts with axin and LRP5 to negatively regulate Wnt/β-catenin signaling, osteoblast function, and bone formation. *Mol Cell Biol*. 2009;**29**(4):953–64. Available from: doi:10.1128/MCB.00349-08

42. Sen B, Xie Z, Case N, Ma M, Rubin C, Rubin J. Mechanical strain inhibits adipogenesis in mesenchymal stem cells by stimulating a durable β-catenin signal. *Endocrinology*. 2008;**149**(12):6065–75. Available from: doi:10.1210/en.2008-0687

43. Sen B, Styner M, Xie Z, Case N, Rubin CT, Rubin J. Mechanical loading regulates NFATc1 and β-catenin signaling through a GSK3β control node. *J Biol Chem*. 2009;**284**(50):34607–17. Available from: doi:10.1074/jbc.M109.039453

44. Kuo YC, Chang TH, Hsu WT, Zhou J, Lee HH, Hui-Chun Ho J, et al. Oscillatory shear stress mediates directional reorganization of actin cytoskeleton and alters differentiation propensity of mesenchymal stem cells. *Stem Cells*. 2015;**33**(2):429–42. Available from: doi:10.1002/stem.1860

45. de Boer J, Wang HJ, van Blitterswijk C. Effects of Wnt Signaling on Proliferation and Differentiation of Human Mesenchymal Stem Cells. *Tissue Eng*. 2004;**10**(3–4):393–401. Available from: doi:10.1089/107632704323061753

46. Quarto N, Behr B, Longaker MT. Opposite Spectrum of Activity of Canonical Wnt Signaling in the Osteogenic Context of Undifferentiated and Differentiated Mesenchymal Cells: Implications for Tissue Engineering. *Tissue Eng Part A*. 2010;**16**(10):3185–97. Available from: doi:10.1089/ten.tea.2010.0133

47. Rotherham M, El Haj AJ. Remote Activation of the Wnt/β-Catenin Signalling Pathway Using Functionalised Magnetic Particles. Tang S-J, editor. *PLoS One*. 2015;**10**(3):e0121761. Available from: doi:10.1371/journal.pone.0121761

48. Rotherham M, Henstock JR, Qutachi O, El Haj AJ. Remote regulation of magnetic particle targeted Wnt signaling for bone tissue engineering. *Nanomedicine Nanotechnology, Biol Med*. 2018;**14**(1):173–84. Available from: doi:10.1016/J.NANO.2017.09.008

49. Carter DR, Beaupré GS, Wong M, Smith RL, Andriacchi TP, Schurman DJ. The mechanobiology of articular cartilage development and degeneration. *Clin Orthop Relat Res*. 2004;(427 Suppl):S69-77.

50. Vincent KR, Conrad BP, Fregly BJ, Vincent HK. The pathophysiology of osteoarthritis: a mechanical perspective on the knee joint. *PM R*. 2012;**4**(5 Suppl):S3-9. Available from: doi:10.1016/j.pmrj.2012.01.020

51. Guo X, Day TF, Jiang X, Garrett-Beal L, Topol L, Yang Y. Wnt/β-catenin signaling is sufficient and necessary for synovial joint formation. *Genes Dev*. 2004;**18**(19):2404–17. Available from: doi:10.1101/gad.1230704

52. Kahn J, Shwartz Y, Blitz E, Krief S, Sharir A, Breitel DA, et al. Muscle Contraction Is Necessary

to Maintain Joint Progenitor Cell Fate. *Dev Cell*. 2009;**16**(5):734–43. Available from: doi:10.1016/J.DEVCEL.2009.04.013

53. Rolfe RA, Nowlan NC, Kenny EM, Cormican P, Morris DW, Prendergast PJ, et al. Identification of mechanosensitive genes during skeletal development: alteration of genes associated with cytoskeletal rearrangement and cell signalling pathways. *BMC Genomics*. 2014;**15**:48. Available from: doi:10.1186/1471-2164-15-48

54. Brunt LH, Begg K, Kague E, Cross S, Hammond CL. Wnt signalling controls the response to mechanical loading during zebrafish joint development. *Development*. 2017;**144**(15):2798–809. Available from: doi:10.1242/dev.153528

55. Loughlin J, Dowling B, Chapman K, Marcelline L, Mustafa Z, Southam L, et al. Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. *Proc Natl Acad Sci*. 2004;**101**(26):9757–62. Available from: doi:10.1073/pnas.0403456101

56. Dell'Accio F, De Bari C, Eltawil NM, Vanhummelen P, Pitzalis C. Identification of the molecular response of articular cartilage to injury, by microarray screening: Wnt-16 expression and signaling after injury and in osteoarthritis. *Arthritis Rheum*. 2008;**58**(5):1410–21. Available from: doi:10.1002/art.23444

57. Chiu J-J, Chien S. Effects of Disturbed Flow on Vascular Endothelium: Pathophysiological Basis and Clinical Perspectives. *Physiol Rev*. 2011;**91**(1):10.1152/physrev.00047.2009. Available from: doi:10.1152/physrev.00047.2009

58. Mohamied Y, Rowland E, Bailey E, Sherwin S, Schwartz M, Weinberg P. Change of Direction in the Biomechanics of Atherosclerosis. *Ann Biomed Eng*. 2015;**43**(1):16–25. Available from: doi:10.1007/s10439-014-1095-4

59. Peiffer V, Sherwin SJ, Weinberg PD. Computation in the rabbit aorta of a new metric – the transverse wall shear stress – to quantify the multidirectional character of disturbed blood flow. *J Biomech*. 2013;**46**(15):2651–8. Available from: doi:10.1016/J.JBIOMECH.2013.08.003

60. Peiffer V, Sherwin SJ, Weinberg PD. Does low and oscillatory wall shear stress correlate spatially with early atherosclerosis? A systematic review. *Cardiovasc Res*. 2013;**99**(2):242–50. Available from: doi:10.1093/cvr/cvt044

61. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, et al. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature*. 2005;**437**(7057):426–31. Available from: doi:10.1038/nature03952

62. Gelfand BD, Meller J, Pryor AW, Kahn M, Bortz PDS, Wamhoff BR, et al. Hemodynamic Activation of β-Catenin and T-Cell-Specific Transcription Factor Signaling in Vascular Endothelium Regulates Fibronectin Expression. *Arterioscler Thromb Vasc Biol*. 2011;**31**(7):1625–33. Available from: doi:10.1161/atvbaha.111.227827

63. Li R, Beebe T, Jen N, Yu F, Takabe W, Harrison M, et al. Shear Stress–Activated Wnt-Angiopoietin-2 Signaling Recapitulates Vascular Repair in Zebrafish Embryo. *Arterioscler Thromb Vasc Biol*. 2014;**34**(10):2268.

64. Sheng X, Sheng Y, Liu Y, Li X, Shu B, Li D. Effects of FSS on the expression and localization of the core proteins in two Wnt signaling pathways, and their association with ciliogenesis. *Int J Mol Med*. 2018; Available from: doi:10.3892/ijmm.2018.3758

65. Serbanovic-Canic J, de Luca A, Warboys C, Ferreira PF, Luong LA, Hsiao S, et al. Zebrafish Model for Functional Screening of Flow-Responsive Genes. *Arterioscler Thromb Vasc Biol*.

2017;**37**(1):130–43. Available from: doi:10.1161/ATVBAHA.116.308502

66. Cha B, Geng X, Mahamud MR, Fu J, Mukherjee A, Kim Y, et al. Mechanotransduction activates canonical Wnt/β-catenin signaling to promote lymphatic vascular patterning and the development of lymphatic and lymphovenous valves. *Genes Dev*. 2016;**30**(12):1454–69. Available from: doi:10.1101/gad.282400.116

67. Franco CA, Jones ML, Bernabeu MO, Vion AC, Barbacena P, Fan J, et al. Non-canonical wnt signalling modulates the endothelial shear stress flow sensor in vascular remodelling. *Elife*. 2016;**5**(FEBRUARY2016).

68. Ou G, Weaver VM. Tumor-induced solid stress activates β-catenin signaling to drive malignant behavior in normal, tumor-adjacent cells. *Bioessays*. 2015;**37**(12):1293–7. Available from: doi:10.1002/bies.201500090

69. Samuel MS, Lopez JI, McGhee EJ, Croft DR, Strachan D, Timpson P, et al. Actomyosin-mediated cellular tension drives increased tissue stiffness and β-catenin activation to induce epidermal hyperplasia and tumor growth. *Cancer Cell*. 2011;**19**(6):776–91. Available from: doi:10.1016/j.ccr.2011.05.008

70. Mouw JK, Yui Y, Damiano L, Bainer RO, Lakins JN, Acerbi I, et al. Tissue mechanics modulate microRNA-dependent PTEN expression to regulate malignant progression. *Nat Med*. 2014;**20**(4):360–7. Available from: doi:10.1038/nm.3497

71. Benham-Pyle BW, Pruitt BL, Nelson WJ. Cell adhesion. Mechanical strain induces E-cadherin-dependent Yap1 and β-catenin activation to drive cell cycle entry. *Science*. 2015;**348**(6238):1024–7. Available from: doi:10.1126/science.aaa4559

72. Röper J-C, Mitrossilis D, Stirnemann G, Waharte F, Brito I, Fernandez-Sanchez M-E, et al. The major β-catenin/E-cadherin junctional binding site is a primary molecular mechano-transductor of differentiation in vivo. *Elife*. 2018;**7**. Available from: doi:10.7554/eLife.33381

73. Fernández-Sánchez ME, Barbier S, Whitehead J, Béalle G, Michel A, Latorre-Ossa H, et al. Mechanical induction of the tumorigenic β-catenin pathway by tumour growth pressure. *Nature*. 2015;**523**(7558):92–5. Available from: doi:10.1038/nature14329

74. Avvisato CL, Yang X, Shah S, Hoxter B, Li W, Gaynor R, et al. Mechanical force modulates global gene expression and β-catenin signaling in colon cancer cells. *J Cell Sci*. 2007;**120**(Pt 15):2672–82. Available from: doi:10.1242/jcs.03476

Figure Legends

Figure 1. Canonical and non-canonical Wnt signalling pathways

(A) In the 'resting' or 'off' state, β-catenin is phosphorylated by a destruction complex consisting of APC, CK1, axin and GSK3β that targets it for ubiquitin-mediated proteasomal degradation. Activation of the Wnt pathway is inhibited by endogenous mediators including WIF and sFRPs that reduce Wnt-Frizzled binding, and DKK-1 and sclerostin that prevent binding of Lrp co-receptors to Frizzled. **(B)** Binding of a Wnt ligand to a Frizzled receptor triggers the recruitment of Lrp5/6(1) leading to recruitment of dishevelled (Dvl)(2) which phosphorylates and activates Lrp(3). Once activated, Lrp6 binds to axin(4) triggering the dismantling of the destruction complex. Dephosphorylated (active) β-catenin then accumulates and translocates to the nucleus where it binds to other transcription factors to regulate gene expression. **(C)** Non-canonical pathways also require the interaction of Wnt with Frizzled receptors but are independent of β-catenin activity. The Wnt/Ca^{2+} pathway requires activation of PLC to raise intracellular Ca^{2+} levels following Wnt-Frizzled interaction but does not require Lrp, dishevelled or other Wnt pathway components. In contrast, the Wnt/JNK pathway requires recruitment of dishevelled in response to Wnt-Frizzled binding that activates Rac and RhoA which regulate JNK activation.

Figure 2. Wnt/β-catenin signalling in osteocytes

(A) Osteocytes and osteoblasts respond to acute and chronic application of mechanical forces such as shear stress, stretch and increased mechanical loading by activating canonical Wnt signalling. Several Wnts have been shown to activate the pathway with strong evidence that Wnt16 plays an important role. The resulting increase in the nuclear accumulation of β-catenin promotes the expression of osteogenic genes. A reduction in sclerostin levels is also necessary for functional activation of the Wnt pathway in response to chronic mechanical loading. **(B)** Osteocytes also rapidly activate β-catenin in response to acute shear stress via Wnt independent pathways. Acute exposure to pulsatile shear stress triggers the release of β-catenin from N-cadherin which can then translocate to the nucleus. Acute exposure to shear stress also inhibits GSK3β, via activation of FAK, Akt and eNOS leading to the phosphorylation and inhibition of GSK3β, resulting in the stabilisation of β-catenin.

Figure 3. Wnt/β-catenin signalling in mesenchymal stem cells

(A) Acute exposure of human mesenchymal stem cells to oscillatory shear stress leads to the release of β-catenin from N-cadherin alongside elevation of Wnt5a that also increases β-catenin via activation of Ror2 and RhoA. Increased stabilisation of β-catenin leads to increased expression of osteogenic genes. **(B)** Frizzled-2 responds directly to the application of force applied through magnetic beads leading to activation of β-catenin and increased expression of osteogenic genes.

Figure 4. Wnt/β-catenin signalling in chondrocytes

In chondrocytes, increased expression of Wnt4, Wnt9a, Wnt14 and Wnt16 in response to muscle contraction (mechanical force) leads to stabilisation of β-catenin which plays a critical role in the development of the joint.

Figure 5. Wnt/β-catenin signalling in endothelial cells

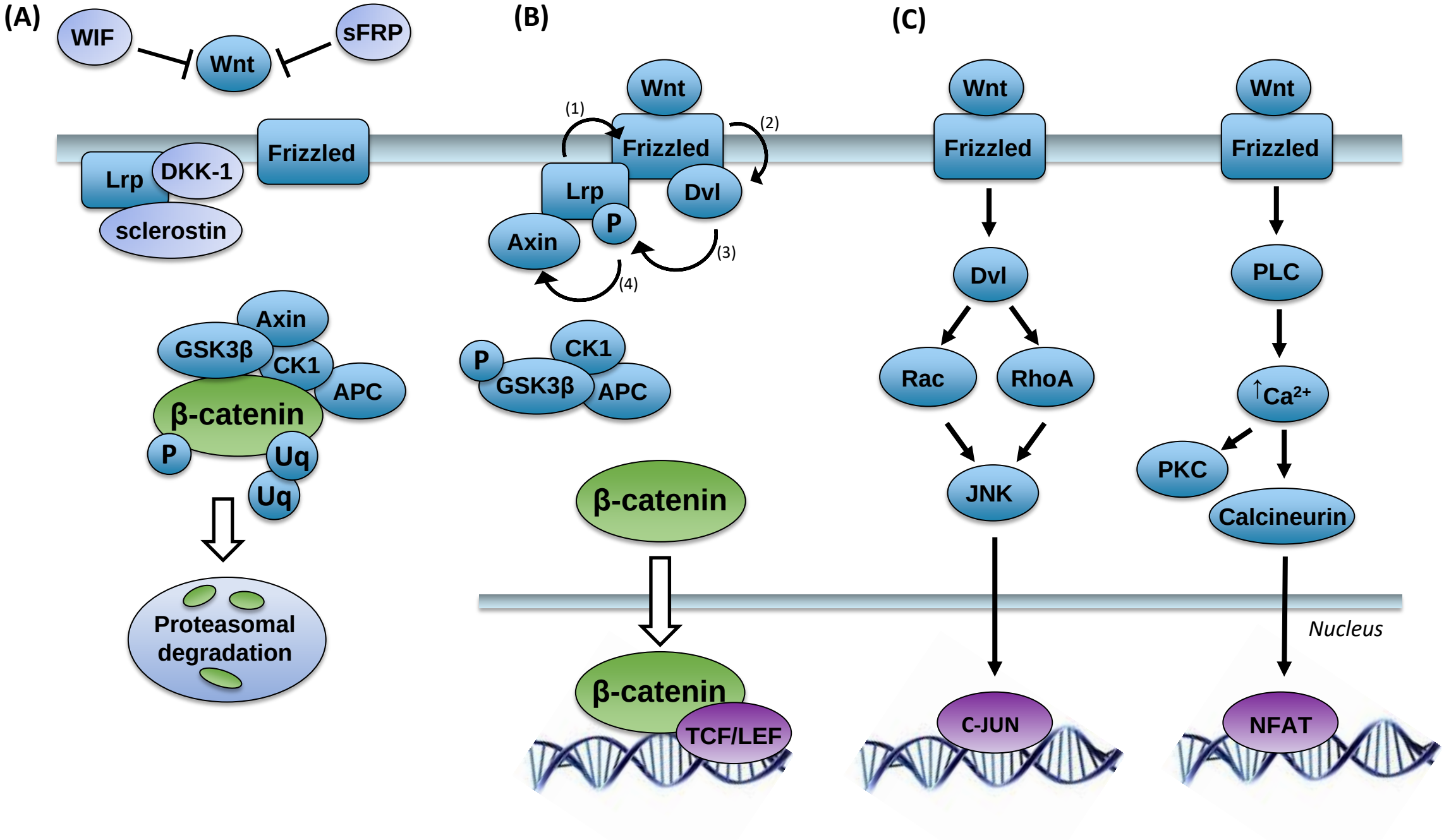
(A) Acute exposure of endothelial cells to laminar shear stress leads to the rapid activation of a mechanosensory complex comprised of VE-cadherin, PECAM-1 and VEGFR2 that requires the presence of β-catenin to activate integrins. **(B)** Exposure to laminar shear stress for 4h leads to increases in Wnt5a and Wnt11 that control flow-dependent polarisation. **(C)** Wnts can also be increased following application of oscillatory shear stress leading to stabilisation of β-catenin via the canonical Wnt signalling pathway resulting in increased expression of angiopoietin-2 that can promote vascular repair. **(D)** Atheroprone flow acts on PECAM-1 to inhibit GSK3β leading to stabilisation of β-catenin with a consequent increase in the expression of fibronectin and IL-8.

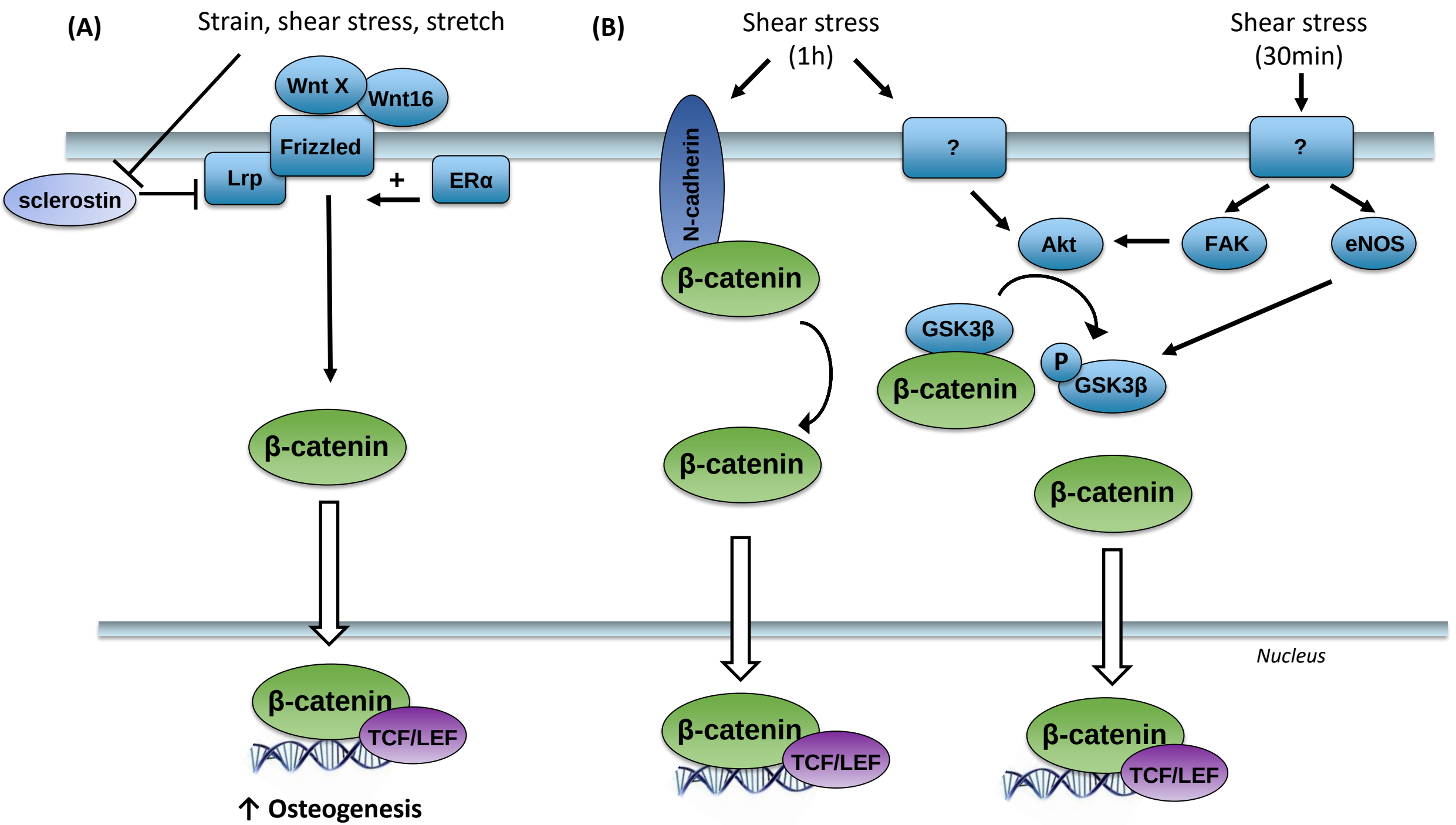
Figure 6. Wnt/β-catenin signalling in epithelial cells

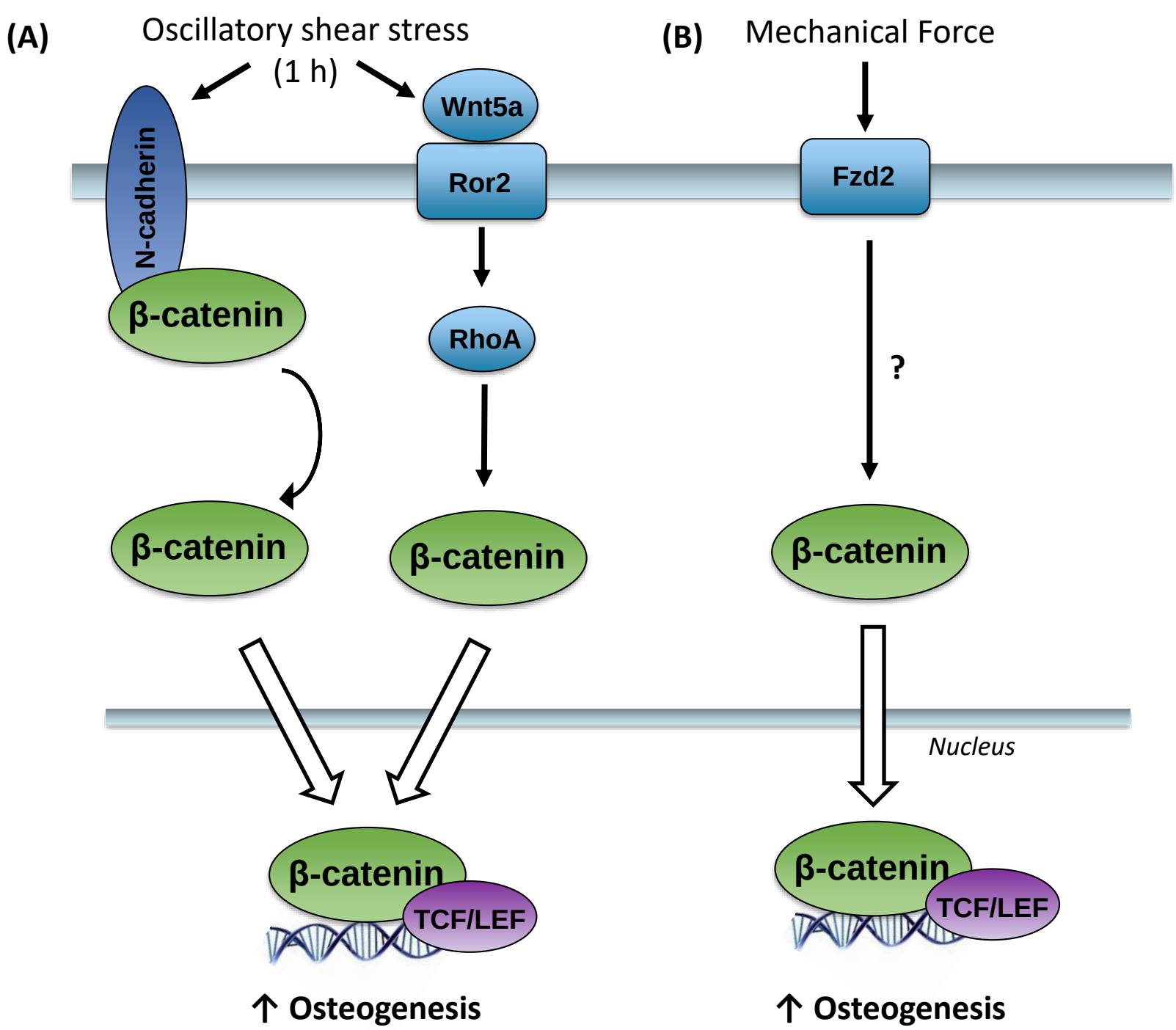
(A) In epithelial cells, exposure to mechanical strain triggers the release of β-catenin from E-cadherin leading to accumulation in the nucleus and increased expression of genes that promote proliferation. **(B)** Increased substrate stiffness or tension leads to the activation of FAK that inhibits GSK3β (via Akt activation) leading to the stabilisation of β-catenin and increased expression of proliferative genes.

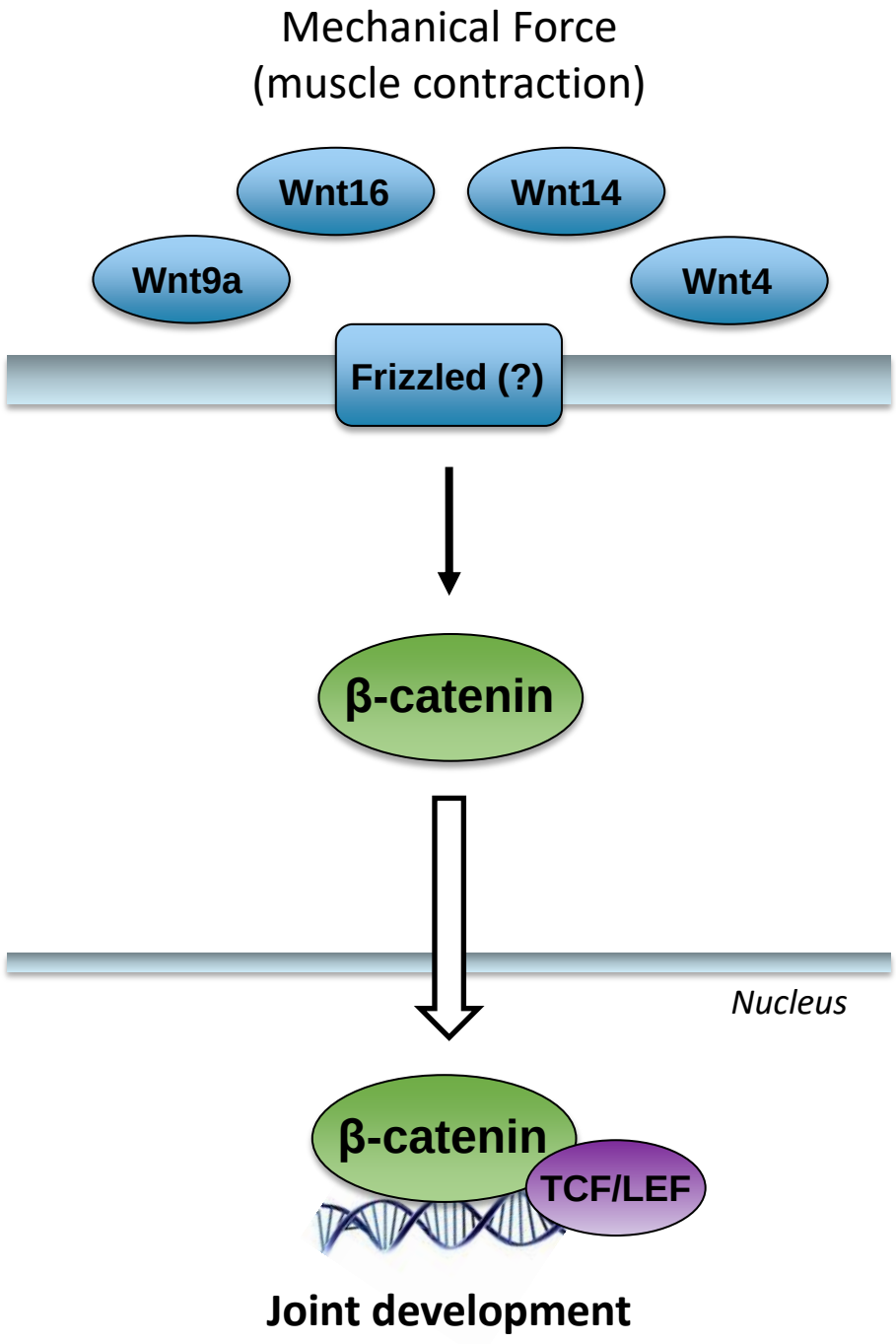
Table 1. Mechanoactivation of Wnt/β-catenin pathways. A summary of the mechanisms by which Wnt/β-catenin pathways are activated in the following mechanoresponsive cells; endothelial cells (EC), epithelial cells (EpC), osteocytes and/or osteoblasts (OST), chondrocytes (CND), mesenchymal stem cells (MSC).

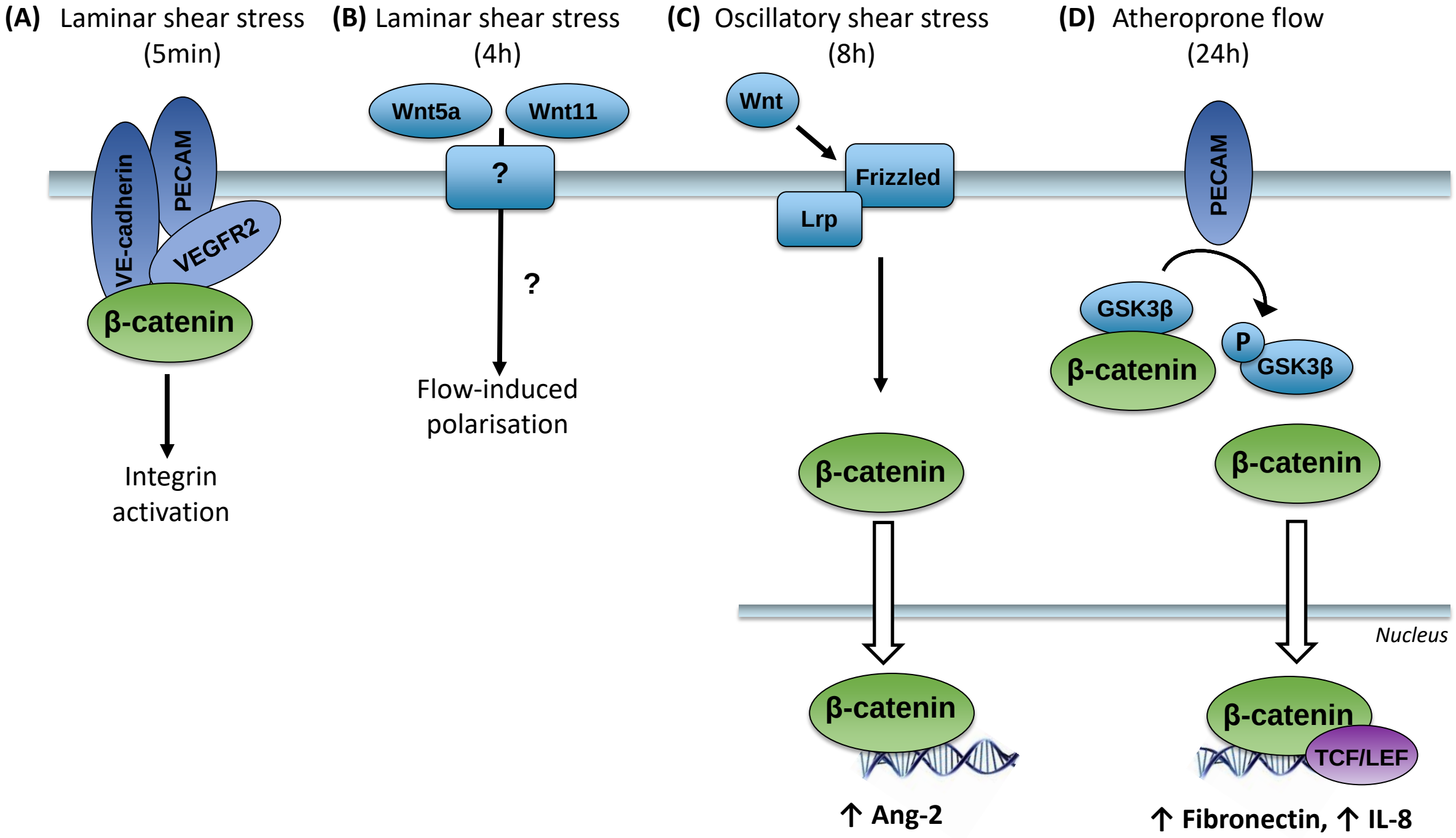
Mechanoactivation of Wnt/β-catenin in response to mechanical forces	**Loading** ***(strain, tension)***	**Shear stress** ***(laminar, oscillatory)***
Evidence of canonical Wnt signalling	OST, CND	OST, EC
Evidence of non-canonical Wnt signalling		MSC, EC
Evidence of cadherin-mediated signalling	EC, EpC	OST, EC
Evidence of Wnt and cadherin-independent signalling	OST, MSC, EpC	OST, MSC, EpC

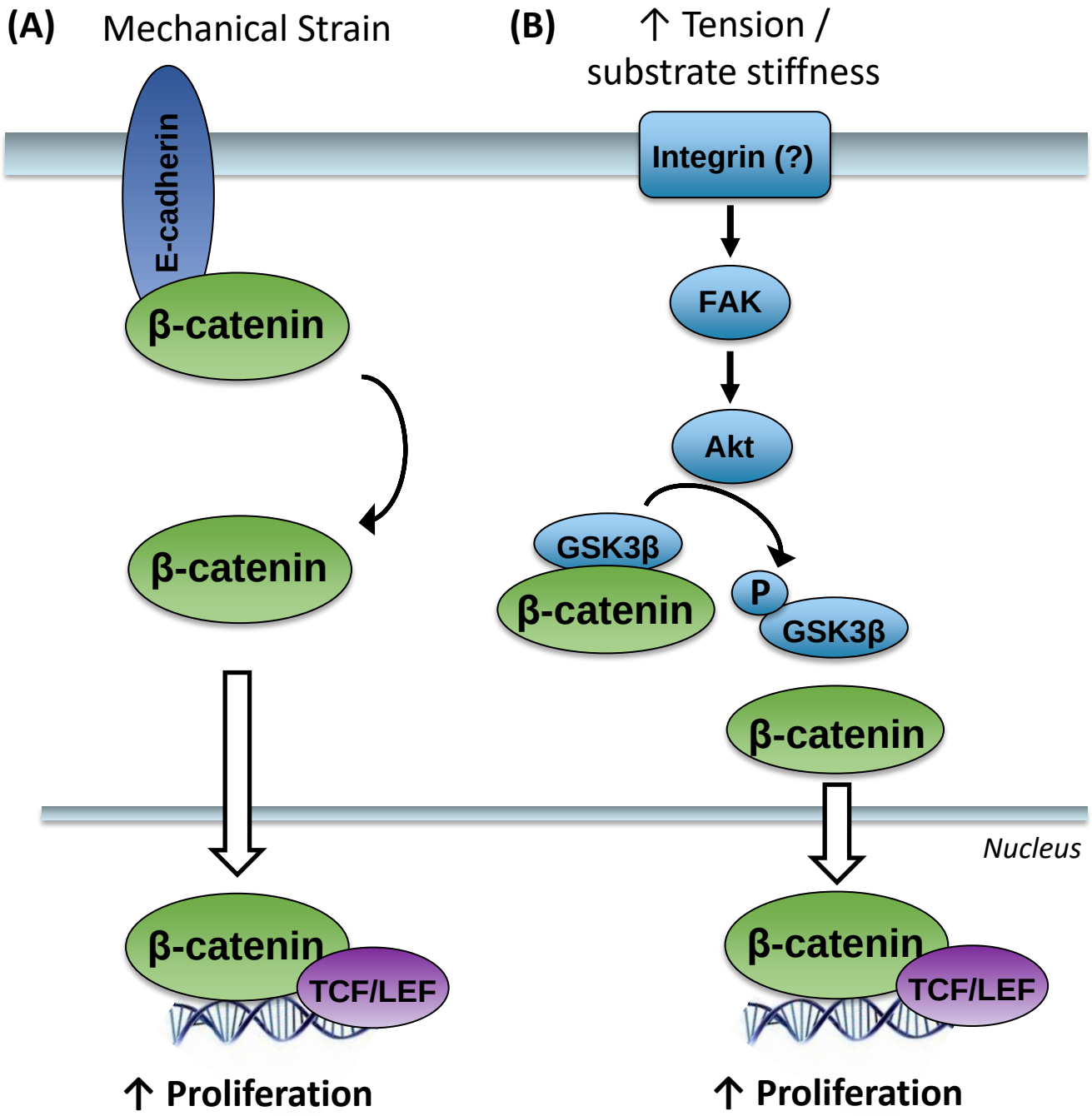

(A)
WIF
Wnt
sFRP
Frizzled
Lrp
DKK-1
sclerostin
Axin
GSK3β
CK1
APC
β-catenin
P
Uq
Uq
Proteasomal degradation
(B)
Wnt
(1)
Frizzled
(2)
Lrp
Dvl
P
Axin
(3)
(4)
P
CK1
GSK3β
APC
β-catenin
β-catenin
TCF/LEF
(C)
Wnt
Frizzled
Dvl
Rac
RhoA
JNK
C-JUN
Wnt
Frizzled
PLC
↑Ca2+
PKC
Calcineurin
NFAT
Nucleus


(A)
Strain, shear stress, stretch
Wnt X
Wnt16
Frizzled
Lrp
sclerostin
+
ERα
β-catenin
β-catenin
TCF/LEF
↑ Osteogenesis
(B)
Shear stress
(1h)
Shear stress
(30min)
N-cadherin
β-catenin
?
?
Akt
FAK
eNOS
GSK3β
β-catenin
P
GSK3β
β-catenin
β-catenin
TCF/LEF
β-catenin
TCF/LEF
Nucleus


(A)
Oscillatory shear stress
(1 h)
Wnt5a
Ror2
N-cadherin
β-catenin
RhoA
β-catenin
β-catenin
β-catenin
TCF/LEF
↑ Osteogenesis
(B)
Mechanical Force
Fzd2
?
β-catenin
Nucleus
β-catenin
TCF/LEF
↑ Osteogenesis


Mechanical Force
(muscle contraction)
Wnt16
Wnt14
Wnt9a
Wnt4
Frizzled (?)
β-catenin
Nucleus
β-catenin
TCF/LEF
Joint development


(A) Laminar shear stress (5min)
(B) Laminar shear stress (4h)
(C) Oscillatory shear stress (8h)
(D) Atheroprone flow (24h)
VE-cadherin
PECAM
VEGFR2
β-catenin
Integrin activation
Wnt5a
Wnt11
?
?
Flow-induced polarisation
Wnt
Frizzled
Lrp
β-catenin
↑ Ang-2
PECAM
GSK3β
β-catenin
P
GSK3β
β-catenin
Nucleus
β-catenin
TCF/LEF
↑ Fibronectin, ↑ IL-8


(A)
Mechanical Strain
E-cadherin
β-catenin
β-catenin
β-catenin
TCF/LEF
↑ Proliferation
(B)
↑ Tension /
substrate stiffness
Integrin (?)
FAK
Akt
GSK3β
β-catenin
P
GSK3β
β-catenin
Nucleus
β-catenin
TCF/LEF
↑ Proliferation