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Effects of microgravity mechanotransduction in bone tissue and cells: systematic review on primary cilium-dependent mechanisms

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Bone density loss is a major concern for astronauts in space, largely due to altered mechanical stimuli in microgravity. These changes are thought to impact bone cells by directly affecting musculoskeletal cell physiology and disrupting mechanosensing and mechanotransduction pathways. This review focuses on the role of the primary cilium, a small, non-motile cellular structure, involved in these processes. Previously underestimated, the primary cilium is now known to act as a mechano- and chemo-sensor on the surface of most vertebrate cells, transmitting signals via multiple intracellular pathways. The primary cilium senses the extracellular fluid flow and its dynamic changes in physiological and pathological conditions, which may include the exposure to microgravity, connecting its inactivation to bone density loss. This systematic review will compile and analyze current data on how weightlessness affects the mechano-sensing functions of the primary cilium and its role in bone homeostasis disruption.

Keywords: microgravity, primary cilium, mechanosensing, mechanotransduction, bone, space, calcium channels, fluid flow, osteocyte

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1 INTRODUCTION

Gravity, as a universal force, has played a pivotal role throughout evolution, profoundly influencing life's development. Dynamic tissue homeostasis is strongly subjected to the effects that gravity exerts on cells, and in turn on the intracellular mechanisms through which cells adapt to the extracellular environment. Cells indeed exhibit noticeable alterations when exposed to microgravity (μG), or weightless conditions. Bone, being a highly complex tissue undergoing remodeling throughout postnatal life to adjust to mechanical stressors, is among the tissues that are most directly affected by μG [1]. The continuous bone remodeling is crucial for maintaining structure, strength, and functionality in response to daily loading variations that, under physiological conditions, ensure homeostasis and structural integrity through the coordinated activity of osteoblasts and osteoclasts [2]. By nullifying the direction of gravitational vector and reducing the load, μG significantly impacts bone acting directly on the remodeling process. The resulting adaptive changes in cellular homeostasis increase bone resorption, inhibiting bone formation [3–5].

Mechanobiology studies the mechanical properties and adaptation of cells to their mechanical environment. This emerging field merges engineering, biology, physics and chemistry to understand all the small facets of cell homeostasis signaling pathways.

By mechanically interacting with the extracellular environment through highly complex mechanisms and structures, all cells can sense and respond to cues from the local extracellular matrix (ECM), influencing their own physiology to regulate tissue viability [6].

This sequence of events is the consequence of the recruitment of specific mechano-molecular players capable of activating, interconnecting, and reorganizing themselves, following mechanical stimuli from the surrounding microenvironment (mechanosensing) to transduce these stimuli into intracellular biochemical signals (mechanotransduction) [7,8].

The maintenance of cell functions heavily relies on the physiological mechanical stimulation, and disruption in tissue mechanics and cellular mechano-signaling can lead to severe alteration of homeostasis, triggering tissue injury in diverse pathological conditions, such as fibrosis, cancer, cardiovascular diseases and aging [9].

Physiologically, mechanical homeostasis is sustained through negative feedback mechanisms sensing extracellular environmental changes and restore them to their baseline levels. Altered mechanosensing leads to loss of this negative feedback regulation that in turn causes upregulation of signaling cascades leading to pathological conditions [10].

Plasma membrane can sense diverse physical cues, including hydrostatic pressure (HP), tensile force (TF), fluid shear stress (FSS), ECM stiffness, tissue elasticity, and extracellular fluid (ECF) viscosity, through discrete structural domains involved in cell-ECM and cell-cell interactions and cross-talking [9].

On this regard, the primary cilium (PC), which has recently emerged as a key cell surface compartment, has been found to be involved in both chemosensing and mechanosensing. Specifically, PC-mediated sensing transduces intracellular signaling pathways [11], including Hedgehog [12], Wingless-Type (WNT) [12], Hippo [12], Yes-associated protein 1/Tafazzin (Yap/Taz) [13] cascades, thus playing a pivotal role in regulating cell proliferation, survival and differentiation.

Recent data proposed PC as a gravity sensor, as changes in earth's gravitational field (like tides) could affect gene

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expression through changes in extracellular FSS sensed by the PC [14]. Consistently, under μG conditions, the decrease in osteoblastic differentiation was restricted to cells bearing primary cilia, further underscoring their central role as mediators of the gravitational response [15].

Because of the load-related activity of osteoblasts and osteocytes, strictly associated with the peculiar nature of the calcified ECM that surrounds them, the PC is emerging as an essential player in bone tissue physiopathology. Nonetheless, its implication in the altered mechanobiology induced by μG is yet poorly explored and clarified, with new pieces of evidence been provided in the most recent scientific literature.

Bone tissue supports body weight through a specialized mineralized ECM that provides structural integrity, mechanical signals, and nutrients to resident bone cells. Depending on the kind of support role the bone has, it exists in two macroscopic forms: cortical bone, a dense outer layer that provides strength and protection **[16]**, and cancellous (or spongy) bone, a porous structure composed of trabeculae arranged to absorb stress and resist forces from multiple directions. The ECM is composed of about 60% inorganic minerals, mainly hydroxyapatite (Ca)10(PO4)6(OH)2, and 30% organic components **[17]**, such as type I collagen, forming a rigid scaffold that supports and regulates bone cell behavior.

Osteocytes are the most abundant and long-lived cells in bone, making up over 90% of the cellular population **[18]**. They originate from mesenchymal stromal cells that differentiate into mature osteoblasts, which, once embedded in the mineralized matrix during bone formation, become osteocytes. As they mature into osteocytes, they reduce in size and extend dendritic processes that pass through tiny channels called canaliculi, forming an interconnected network known as the Lacuno-Canalicular System (LCS) **[19]**. This system allows interstitial fluid flow and cellular communication, enabling osteocytes to function as mechanosensors. In contrast, osteoclasts are the least abundant bone cells, comprising about 1%, and play a crucial role in bone resorption. Derived from the monocyte lineage, they secrete acidic vesicles and enzymes that degrade hydroxyapatite and the organic matrix, contributing to bone turnover **[17]**.

Bone is a dynamic tissue that continuously remodels in response to environmental cues, with mechanical stimulation being one of the most potent drivers **[20]**. This remodeling process is activated by both mechanical and biochemical signals, which are sensed by osteocytes and transduced through pathways involving WNT, integrins, estrogen, and calcium **[21,22]**.

According to Roux's "use it or lose it" principle and Wolff's law, bones adapt structurally to the loads they experience **[23,24]** (**Fig. 1**). Everyday activities generate varying degrees of strain, promoting new bone formation when load increases. In this context, Frost introduced the concept of "windows of mechanical usage," where low strain (Disuse Window) leads to resorption, optimal strain (Overuse Window) promotes bone formation, and excessive strain (above 3500 με – Harmful Window) can result in bone damage **[25–28]** (**Fig. 1**).

The efficiency of mechanical signal transmission is crucial for bone health. Disruptions in load transfer—caused by changes in bone stiffness or extracellular fluid flow—can impair mechanotransduction and lead to bone loss **[16,29]**. Stiffness, dependent on bone elasticity, is essential for translating mechanical signals into cellular responses **[16]**.

With aging, skeletal properties like material strength and stiffness decline, prompting compensatory changes in bone architecture **[16]**. However, this often results in increased bone resorption, especially in weight-bearing bones. Similar effects are seen in astronauts, where μG impairs remodeling, particularly in load-dependent bones **[30]**. Cortical bone, known for its load-bearing capacity and mechanical anisotropy, also shows age-related changes, further impacting its mechanical function **[30]**.

Cells can sense mechanical signals and transduce them through their membrane to the intracellular compartment; this sequence of events define the mechanism called mechanosensing [31,32].

A four-step model has been proposed to explain mechanosensing: i. mechanopresentation: the mechanical cue is presented to the cell; ii. mechanoreception: the cell catches the input; iii. mechanotransmission: the signal is transferred from the outside of cell to the inside through a transmitter; iv. mechanotransduction: the mechanical force is transduced into a biochemical signal [8].

Eukaryotic cells orchestrate these processes through specialized surface domains and structures enabling membrane/cytoskeleton physical interactions, called mechanosensors. Among these mechanosensors, focal adhesions (FA) and glycocalyx transduce mechanical cues through molecules anchored to neighboring cells or stimuli arising from the surrounding ECM [33]. The PC, a mechano-sensory organelle that protrudes like an antenna from the cell surface, is able to sense both mechanical and chemical stimuli provided in the extracellular environment and transduce them into intracellular molecular cascades [34].

The first observations of the PC were made by the anatomist Karl Wilhelm Zimmerman in 1898, who described it as a vestigial organelle in renal tubule cells without any clear role for cellular functions [35]. The occurrence of this organelle in several other tissues, including bone, was demonstrated only 70 years later, though its function remained unclear for a long time [36].

Currently, we know that the PC is a single, non-motile microtubule-based organelle that projects from the surface of most non-proliferating mammalian cells [37]. The ciliary structure comprises different portions and components, namely: basal body, axoneme, transition zone, ciliary membrane, cilioplasm and ciliary pocket (**Fig. 2**).

The basal body is an apical microtubule organizing centre, with the classical centriole structure made of nine triplets of microtubules organized in circle and localized at the base of the cilium. This structure is connected to the cell membrane by fibrous proteins called transition fibers or alar sheets [38] that anchor it in place and create a filter zone that separates the cellular cytoplasm from the ciliary cytoplasm (cilioplasm) (**Fig. 2**).

Close to these alar sheets there are other conical structures that connect the basal body to the cytoskeletal microtubules, called basal feet [39]. Whereas in a motile beating cilium, there is always one basal foot oriented in the direction of the cilia beating [38], the PC can have from 1 to 5 basal feet projecting in all directions.

The axoneme is the core longitudinal shaft of the cilium, composed of nine peripheral microtubule doublets, extending from the nine triplets of the basal body and projecting towards the tip of the PC (**Fig. 2**). The doublets are asymmetric, having one microtubule made of thirteen protofilaments and one made of ten protofilaments [40].

The PC axoneme differs from that of motile cilia in the lack of the central pair of singlet microtubules. This "9+0" conformation makes the PC devoid of active motility while being more flexible and resilient to cell surface shear stresses induced by fluid flow. Nevertheless, recent studies have shown that the 9+0 circle architecture is consistent only at the base of the cilium, then it gradually becomes disorganized when approaching the ciliary tip. Microtubules transition to singlets and shift toward the center of the axoneme, forming a 7+2 configuration; as the PC tapers toward its distal end, its diameter decreases, leading to a further reduction in microtubule number, reaching a 3+0 arrangement [41,42] (**Fig. 2**). Collectively, these irregularities increase the bending proprieties of the organelle and partially explain the variable flexibility throughout the PC length [43,44].

The transition zone is a specialized region of the PC located between the basal body and the axoneme. It regulates the entry

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and exit of molecules in the cilium through protein structures called Y-links, thereby regulating its signaling and function (**Fig. 2**) [45].

The ciliary membrane differs from the rest of the plasma membrane [46]in its lipid composition, featuring a higher presence of sterols and sphingolipids [47]. This composition allows for increased fluidity balance underlying higher resistance to flow shear stress, in particular at the base of the cilium where they are more abundant [48].

The amount of cytoplasm surrounding the axoneme represents another specific PC sub-compartment, referred to as the cilioplasm. This is segregated from the cytoplasm by the transition zone and basal body. The cilioplasm has indeed a higher concentration of calcium compared to the cytoplasm, due to the selective membrane properties of the transition zone and the continuous influx of calcium through mechanosensitive ion channels. This calcium concentration difference is crucial for activating mechanotransduction [49].

Finally, the ciliary pocket (CiPo) is another noteworthy PC domain (**Fig. 2**). The CiPo is an introflexion of the membrane on the extracellular side, supported by the actin filament network [50]. Although its function remains largely unclear, the clustering of clathrin-coated pits (CCPs) at this level suggests a role in vesicle trafficking, which may be important in the early phases of cilium assembly [50].

The anterograde and retrograde transport known as intraflagellar transport (IFT) is the mechanism that enables the transport of multisubunit protein complexes, called IFT particles, along axonemal microtubules (MTs) beneath the ciliary membrane exploiting the microtubule-associated motor proteins kineins and dyneins. IFT is therefore crucial for ciliary structural assembly and maintenance, but also for ciliary signaling (see next paragraph) [51].

2 **RESULTS**

2.1 Primary cilium signaling in mechanotransduction

The PC is currently recognized as a vital signaling hub that translates external signals into internal responses playing a crucial role in maintaining the proper balance and growth of most cells, ensuring their overall stability. It is estimated that at least 1200 genes are expressed within the PC and involved in both its structure and function [52].

The PC can function as both a chemosensor and a mechanosensor, which in both cases entail an intense signaling acting in the ciliary and subsequently transmitted to the rest of the cell.". For the purpose of this review, only key molecular signaling involved in PC mechanotransduction will be explored.

PC-mediated mechanosensing signaling begins in every cell when an external mechanical input, as fluid flow, bends the cell membrane. This deformation activates multiple mechanisms that transduce the mechanical stimuli into specific biochemical responses. Among these mechanisms, the PC activates various signaling pathways crucial to mechanotransduction.

SACs are ion channels that respond to membrane deformation caused by mechanical inputs from inside and outside the cells. They play pivotal roles in sensing vibrations, pressure (osmotic, fluidic and atmospheric) and touch [53], being localized in areas subjected to high strains of fluid flow, like FA and PC itself.

SACs belong to many different protein families with specific functions and ion selectivity, as summarized in **Table 1**. The largest and most important among them is the transient receptor (TRP) channel family, that comprises 9 subfamilies, whit the most studied being subfamilies C, P and V.

They are vital for PC function and have been found to be highly correlated with bone osteogenesis and formation, hence it has been seen that various ciliopathies are associated with loss-of-function or other mutations in these channels, like polycystic disease or craniosynostosis (see **Table 1**).

SACs cluster on the ciliary membrane likely due to the cilium's elastic properties through which energy is transmitted to open these channels and facilitate specific calcium ion accumulation to act as second messengers in PC-mediated transduction [48,54,55]

The cilium can be indeed considered a significant storage site for intracellular calcium reservoir due to its high calcium concentration ([Ca^{2+}] of 500 nM, compared with 100-200 nM found in mitochondria) [31,56].

Lastly, the cell cortex, mostly composed of cytoskeletal actin microfilaments located in close proximity to the plasma membrane, has a role in mechanotransduction. Through specific protein domains, it enables crosstalk between actin and SACs, such as Potassium channel subfamily K member 2 (KCNK2, or TREK1) [57] and Transient receptor potential Vanilloid Cation Channel Subfamily V Member 6 (TRPV6) [32] creating a bridge from mechanical stimuli to the cytoskeleton and sometimes even to the nucleus, inducing epigenetic and transcriptional changes [58].

Also, intermediate filaments are other cytoskeletal components involved in mechanotransduction. Owing to their flexibility and resilience to tensile and compressive forces they mediate interactions among cells (in adherent junctions) and with the ECM (in desmosomes). They are also directly implicated in nuclear mechanotransduction as they establish indirect contact with the nuclear lamina through the LINC complex [59]. Due to their high sensitivity to shear stresses, they can modify their own stiffness as well as that of nearby cells [60,61].

The PC is implicated in the Hippo signaling pathway (**Fig 3A**; for a comprehensive review of this pathway see [62]). One of the core components, the mammalian sterile 20-like kinase 1/2 (MST1/2), is localized at the basal body of PC where it interacts with the nephronophthisis (NPHP) complex. This complex binds MST1/2 and the large tumor suppressor kinase 1/2 (LATS1/2), inhibiting their phosphorylation activity. This prevents the inactivation of the transcriptional co-regulators Yes-associated protein and Tafazzin family protein (YAP/TAZ) complex, allowing it to activate target gene transcription [63].

The PC is also essential for the development and fate determination of neuroepithelial cells through the activation of Notch signaling (**Fig 3B**; for a comprehensive review of this signaling pathway see [64,65]), which leads to the accumulation of Sonic hedgehog (SHH) in the cilium and determines ventral cell fate [66]. Presenilin, a key component of the Notch pathway, is also localized at the basal body of the PC. When a Notch receptor on the PC membrane binds its ligand -jagged or delta on a neighbouring cell-, Presenilin is activated, enabling the Notch intracellular domain (NICD) to translocate to the nucleus and activate downstream target genes [67].

Unlike other pathways, Hedgehog pathway relies on the PC's chemosensory function and will not be delved into. The movement of Hedgehog and Hippo signaling key factors can be helped by the IFT proteins, and when IFT proteins are mutated or the IFT mechanism is impaired these signaling pathways are compromised [68,69]. Although major evidence of the exact mechanism by which this happens is still lacking, PC role in modulating WNT pathway by non-canonical WNT/Ca^{2+} activation (**Fig. 3C**) as specialized calcium signaling compartment is widely accepted [12,70].

Supporting evidence for the connection between the WNT/Ca^{2+} pathway and the PC includes findings in rats and mice models (Choi and Robling reviewed the role of Wnt pathway in bone mechanotransduction [71]). Zhou and colleagues showed that the exposure of PC-expressing osteoblasts to a sinusoidal electromagnetic field (SEMF) led to increased expression of WNT10b, thereby activating downstream osteogenic signaling pathways, including Disheveled, Collagen-1α1, and β-catenin [72]. Moreover, WNT9b has been shown to interact specifically with the

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TRPP1/TRPP2 complex, acting as a ligand that activates the channel and enhances intracellular Ca^{2+} transport through a β-catenin-independent pathway [73].

Additionally, He and colleagues observed that a pulsatile electromagnetic field (PEMF) primarily activated iNOS and eNOS—the main producers of nitric oxide (NO)—within the PC (for a review of the NO/cGMP signaling pathway see [74]). They further demonstrated a strong correlation between the PC and the NO/cGMP signaling pathway, as inhibition of either one abolished the effects of PEMF [75]. It has also been suggested that this signaling pathway was regulated by the TRPP1/TRPP2 complex (referred also as PC1/PC2) [75].

A significant mechanosensing mechanism also localized to the PC of bone cells is cAMP/PKA signaling pathway [76] (reviewed by Sassone-Corsi, 2012 [77]), mediated by adenylyl cyclase isoform 6 (AC6) [78]. RNA interference against Polaris- an important component of IFT- have shown that the removal of PC prevented the fluid flow-dependent cAMP level reduction and osteocytes lacking AC6 exhibited similar behavior to those depleted of the PC, demonstrating the cAMP compartmentalization's importance as a second messenger for intracellular responses such as cell proliferation, differentiation, and apoptosis [79]. cAMP negatively regulates Hedgehog signaling by phosphorylating Gli2, blocking its activation, while SHH reduces cAMP and PKA activity, affecting Ca^{2+} dependent signal transduction [80].

Interestingly, Polycystin-1 (TRPP1) and Polycystin-2 (TRPP2), key proteins implicated in ciliopathies such as autosomal dominant polycystic kidney disease (ADPKD), localize to the PC. Within this structure, they form a heterodimeric polycystin complex, which plays a crucial role in mechanotransduction processes, particularly in renal and hepatic tissues [81–83]. Although skeletal abnormalities remain a relatively underexplored aspect of ciliopathies, emerging evidence suggest that mutations in *PKD1/2-encoded polycystin (TRPP) proteins* also affect PC expression in bone cells. Indeed, its abnormal expression alters TRPP1/TRPP2-related ciliary mechanosensing in these cells, contributing to the skeletal phenotype observed in ADPKD, independently of renal dysfunction [84–86].

In this regard, Xiao et al., demonstrated that mouse models with mutations in *Pkd1*, displayed reduced bone mineral density, trabecular bone volume, and cortical thickness [87]. These mice also exhibited decreased expression of osteogenic markers such as Runx2-II, Osterix, and Osteocalcin, along with a reduced receptor activator of nuclear factor kappa-B ligand/osteoprotegerin (OPG/RANKL) ratio [87]. Notably, these defects appear in a gene-dose-dependent manner, underscoring a direct role for TRPP1 in osteoblast-mediated bone formation, likely via downregulation of key osteogenic pathways.

2.2 Microgravity effects on bone cells and mechanotransduction

In latest years, we have been witnessing an increased interest and demand for longer space travels. Yet, the space environment is inherently inhospitable to human life without proper protective gear, and even with the correct equipment, there are numerous threats that can severely disrupt the body's homeostasis.

The most problematic space condition affecting the human body is weightlessness, commonly referred to as μG, which occurs when individuals or objects experience an extremely weak gravitational field (10^{-6} times earth's gravity), making them appear to be weightless. A prominent example is the experience of astronauts aboard the International Space Station (ISS), where, despite being subjected to a gravity vector that is 90% of that on Earth, they float due to the free fall movement of the space station orbiting the planet. Prolonged exposure to weightlessness during spaceflight induces notable changes in the human body. Initially, the redistribution of internal fluids triggers motion sickness with symptoms such as headaches and nausea, resulting from increased blood pressure on the skull [88]. By only one month, the immune system and the musculoskeletal system start suffering the effects of μG too, which lead to immunosuppression [89], muscle atrophy, and loss of bone density [90].

Astronauts experiencing μG or weightlessness undergo a proportional reduction in bone and muscle density that correlates linearly with the duration of their space journey [91]. It may be reasonably hypothesized that the reduced mechanical load on bones leads to a gradual deactivation of the bone remodeling unit hence disrupts the delicate balance between bone resorption and formation.

To support the hypothesis that μG has a specific impact on weight bearing bones - because they are constantly subjected to mechanical loading [92], while having minimal-to-no effects on non-weight bearing bones [93]- bone density data from 148 astronauts has been evaluated pre- and post- spaceflight. This analysis showed a greater bone density loss on lumbar spine, lower limbs and pelvis, meanwhile the skull presented an increase in bone density because of the increase in intracranial pressure [21]. These results were also strengthened by an increase in biochemical bone resorption markers, confirming that μG exerts region-specific effects on the skeletal system [30]. Despite astronauts engaging in rigorous physical activity (2.5 hours of daily exercise) to mitigate bone and muscle density loss, the magnitude of loss remains substantial [94].

Another hypothesis regarding how μG affects bone homeostasis stems from physics. Water cohesion and gravity work in concert to maintain water persistence on the earth surface. In the absence of a consistent gravitational vector, smaller forces, such as surface tension and cohesion, influence the behavior of fluids [95]. This alteration can impact fluids within the human body, altering ECF dynamics, particularly near the cell membrane. This abnormal flow behavior, characterized by local vortexes, changes in diffusivity, etc., may affect fluid-structure interaction and subsequently influences the proper bending of PC and mechanotransduction. Bone represents one of the most remarkable examples of organ adaptation to Earth's gravity, and consequently, bone cells are presumed to have evolved in response to this gravitational force.

The main effect of μG on bone is a reduction of bone mass, suggesting a direct influence on osteoclastogenesis [96] and osteoblastogenesis [97]. While spaceflight provides the most physiologically relevant model for studying μG (μG)-induced bone loss, it is limited by infrequent missions, small sample sizes, and high costs. Due to logistical constraints, many studies now rely on simulated μG models on Earth using cellular and animal models, which have significantly advanced our understanding of the molecular mechanisms underlying bone loss in μG. Small animal models like rodents and fish help mitigate these issues, enabling larger cohorts and revealing bone loss patterns comparable to those seen in astronauts.

Different studies, briefly discussed in the following paragraphs, have demonstrated that bone loss due to μG exposure can be explained by alterations in the survival and differentiation of osteoblasts and osteocytes, along with increased osteoclastic activity [98].

2.3 Osteoblasts/Osteocytes impairment and reduced osteoblastogenesis due to microgravity

Microgravity appears to exert negative effects on osteoblasts, leading to the downregulation of key osteogenic markers and impairing their biological function. As previously described, μG diminishes the expression of *Tumor necrosis factor (TNF) receptor superfamily member 11b* (*TNFRSF11B*) [4] ,which codes for osteoprotegerin (OPG) that plays a vital role in

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inhibiting osteoclastogenesis by sequestering RANKL and is typically produced by osteoprogenitors and osteoblasts during excessive bone resorption [99]. Another gene affected by μG in osteoblasts is *Alkaline Phosphatase, Biomineralization Associated* (*ALPL*) – encoding for a hydrolytic enzyme playing a key role in matrix remodeling- representing a crucial marker for osteoblast differentiation and bone formation [100]. The expression of *ALPL* is reduced in osteoblasts subjected to μG, compared with those in standard conditions [4].

Osteoblast differentiation is further hindered through the downregulation of focal adhesion kinase (FAK) [101], mammalian target of rapamycin complex 1 (mTORC1), adenosine monophosphate-activated protein kinase (AMPK), and WNT/β-catenin. Specifically, the downregulation of the WNT/β-catenin pathway affects the expression of *Bone Morphogenetic Protein 2* (*BMP2*) and *collagen type I alpha 1 chain COL1A1*—both vital markers for osteoblast differentiation [101]. These effects are believed to be the consequence of the reduced expression of the *Runt-related transcription factor 2* (*RUNX2*) [102], a critical regulator of osteogenic differentiation [103]. In addition, when accompanied by the μG-induced increase in *Mitogen-activated protein kinase (*p38 MAPK) and *Peroxisome proliferator-activated receptor γ2* (PPARγ2) expression, human mesenchymal stromal cells (hMSCs) are induced to differentiate into adipocytes, reducing the osteoprogenitors pool [104].

Moreover, researchers have also explored if simulated μG influences and enhances cell apoptosis as a potential contributor to bone loss. Intriguingly, *in vitro* studies on bone cells have shown that μG upregulates both antiapoptotic genes— including *cyclin-dependent kinase inhibitor 1A* (*CDKN1A*), *B-cell lymphoma 2* (*BCL2*), and *p21*—as well as apoptotic genes—*p53* and *BCL2 Associated X* (*BAX*) [105]. All these effects result in a reduction in osteoblast differentiation and activity.

These findings were observed in short-duration spaceflight, suggesting that apoptosis is temporarily inhibited during brief space missions in *in vitro* osteoblasts culture. However, during extended spaceflights, cells become more predisposed to programmed cell death [106].

Once osteoblasts remain trapped within the osteoid, they terminally differentiate into osteocytes, which cease the matrix deposition and take on their mechanosensory role within bone lamellae [71]. When exposed to a μG environment, also osteocytes have been observed to undergo apoptosis. Histological analysis of postflight samples has revealed a doubling of empty bone lacunae, indicating osteocyte death [107]. Moreover, spaceflight induces alterations in lacunar shape and volume, causing osteocytes to gradually assume a more spherical and smaller shape [107]. A computational analysis of the 3D structure of osteocyte lacunae, aimed at explaining the activation of apoptosis, revealed that in μG, the velocity of solute transport is approximately 600 times slower [29]. This implies that osteocytes receive fewer nutrients and experience compromised waste removal, ultimately driving them towards programmed cell death [29].

To substantiate the hypothesis that osteocytes undergo apoptosis in μG, osteocyte cultures were subjected to μG and concurrently treated with Irisin, a myokine known for its antiapoptotic function on bone cells [108]. As expected, Irisin increased the expression of *Activating Transcription Factor 4 (Atf4)* mRNA, thereby mitigating bone loss in μG [108].

Unfortunately, osteocytes remain the least studied bone cell type in μG, primarily due to their inaccessibility within bone lacunae and the difficulty of replicating their native 3D matrix environment in experimental settings and function.

2.4 Osteoclastogenesis increase and osteoclasts' functional activation due to microgravity

Mechanosensitive osteoclasts contribute to space-related bone loss by disrupting normal bone homeostasis. In response to μG exposure, osteoclasts increase their resorptive activity, when compared to control cells exposed to gravity on Earth [4]. This evidence has been further supported by the effects of μG on the molecular regulation of pro-osteoclastogenic genes. The expression of the *Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL)* increased in osteoclast precursors subjected to μG [109], increasing their differentiation into mature form [110]. The upregulation of TRAIL indeed activated the TNF receptor-associated factor (TRAF6) signaling pathway, leading to increased osteoclast formation [111]. Furthermore, TRAIL can weakly bind to OPG, a protein that inhibits osteoclast differentiation, thereby reducing its availability and elevating the RANKL/OPG ratio [4].

Interestingly, the promotion of osteoclastogenesis through the increase in expression of sclerostin and RANKL has a negative effect also on osteocytes [112]. The increased expression of sclerostin can impair osteoblastic-osteoid deposition and osteocyte differentiation by negatively regulating the BMP/WNT signaling pathway [113] while RANKL promotes directly osteoclastogenesis and osteoid resorption.

Additionally, μG has been demonstrated to enhance autophagy processes in pre-osteoclast cells, leading to increased expression of autophagy-related proteins such as Autophagy Related 5 (ATG5), light chain protein 3 (LC3), and autophagy-related protein 16L (Atg16L) [114]. Autophagy, as a recycling process for nutrients and energy, gives a boost to pre-osteoclast to differentiate and activate their bone resorption activity [114]. This enhancement is also facilitated by a higher presence of pro-inflammatory cytokines [115], including Chemokine Tumor Necrosis Factor Ligand Superfamily Member 10 (Tnfsf10). Sambandam et al, demonstrated that the administration of autophagy inhibitors, like 3'-metyladenine, greatly decreased Cathepsin K expression (marker for bone resorption [116]), suggesting a possible target to modulate osteoclastogenesis in μG [114].

Collectively, these data suggest the potential for abnormal osteoclast differentiation and overgrowth, which—if sustained over time—could lead to the significant bone resorption observed in astronauts. Notably, this surge in osteoclast numbers is primarily observed during the initial 15 days of exposure to μG [98], as well as during immobilization during bed-rest experiments [106]. In this early stage, bone loss occurs at a rate of 12-24% per month [98]. Subsequently, the rate decreases to the well-documented 1-2% loss per month [4].

2.5 PC in bone cells' response to microgravity

PC are significantly reduced in osteoblasts exposed to μG [97] (**Fig 4**). Shi and collaborators demonstrated that PC are significantly reduced in osteoblasts exposed to μG by 60.5±10.4% in PC-presenting cells (reaching a maximum reduction of 85±1.9% after 24 hours) as soon as after 6 hours of culture in simulated μG [97]. Additionally, they observed an average reduction in PC length from 5.11±0.89 μm to 0.79±0.18 μm [97]. On this regard, Shi and colleagues demonstrated also that PC abrogation may impair osteoblastogenesis [97]. The Authors treated cultured osteoblasts with Cytochalasin D, an inhibitor of actin polymerization that blocks PC elongation, and then subjected them to simulated μG. As a result, osteoblasts' number and differentiation capabilities were not affected anymore by μG in the absence of PC [97]. This was accompanied by restored expression of osteogenic marker, an increase of PC presenting cells and acetylated-α tubulin (a microtubules post translational modification that is highly present in PC axoneme) levels, which was also downregulated in μG [97].

By contrast, osteoclasts and osteocytes, do not rely primarily on PC presence to be active and receptive to mechanical stimuli (**Fig 4**).

Indeed, to fully differentiate from monocyte precursors, osteoclasts must undergo the PC disassembly. In fact, macrophages treated with fenoldopam mesylate - a dopamine D1-like receptor agonist used to increase PC length and

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stability-, the expression of key regulators of osteoclast differentiation including c-Fos, CTSK and acp5, was downregulated [117].

Nonetheless, μG was also shown to increase osteoclasts functionality by increasing resorption pit [118] (**Fig 4**). Because these effects were shown to increase the osteolytic processes [118] and the effect of PC reduction was seen in other cell type [97], we could infer that μG affects pre-osteoclasts maturation to fully functional osteoclasts by disrupting primary cilium incidence on cells.

The contribution of the PC in bone cell signaling yet represents a topic of debate, partially due to the limited space gap between osteocyte and pericellular matrix (PCM) inside the bone lacunae, often considered too small for the PC to bend

The osteocyte-lacunar wall gap was measured to be from 0.1 μm to 2 μm [119] with a PCM that does not touch cell body that is 0.5-1 μm thick [54]. While the PC length was measured to be 4-9 μm in vitro [120]and 1-4 μm in vivo, with an average of 1.62 μm [34,82,121,122]. In the first case, if the PC was long as the in vitro condition, inside the lacunae it will be curled or it will be touching and connected to the PCM; gaining a different behavior, more alike to the PC in the chondrocytes, hence being more prone to stretching than bending [123].

The second case can be split into two: the short-length scenario and the long-length scenario. If we consider the PC to be below 1 μm, its functionality will be completely abolished or highly compromised [124,125]. In such cases, other mechanotransducers—such as the tethering elements within the osteocytes processes of the LCS system—may compensate for the loss of PC function in osteocytes [82]. This scenario may be plausible given the high fluid drag within the canaliculi; however, it may not be sufficient to transmit mechanotransduction signals as effectively as the PC.

Alternatively, a length of 1.62 μm may allow the PC to maintain its role as a mechanosensor by permitting sufficient bending and mobility in response to ECF flow [34,82].

All these different measurements demonstrated how particular and unique the osteocyte lacuna is, and a 2D culture will never be totally perfect for studying certain pathway and especially for mechanosensors.

Given that, as mentioned earlier, shorter PC are less stiff and more prone to bending compared to longer ones [43], it might be speculated that the PC senses the reduction of fluid flow due to μG and try to compensate it by depolymerizing the axoneme and the microtubules' network to reduce its length and flexural rigidity.

2.6 Microgravity effects on bone cell tensegrity and ciliary mechanosensing

The PC represents a notable paradigm of cellular adaptation to gravity, responsible not only for establishing planar cell polarity but also for cell mechanosensing. The functionality of the PC has been correlated with cyclical changes in Earth's gravitational field, as evidenced in zebrafish embryos, where gene expression activation was dependent on the presence of the cilium during low and high tides, representing lower and higher Earth gravity, respectively [14].

Over the past two decades, researchers have intensified their investigations into the PC, especially focusing on its role in μG conditions, sparked by the reduction in the number of osteoblasts observed in μG and the understanding that μG alters mechanical stimuli.

Microgravity also significantly impacts cell shape and overall cell architecture, from FA to cell morphology, all of which undergo changes during weightlessness. Typically, osteoblasts exhibit a cuboidal cell morphology, but simulated μG in a random positioning machine (RPM) causes cells to adopt a more spindle-shaped or rounder form [126,127]. These changes in cellular shape are primarily a consequence of cytoskeletal reorganization. Stress fibers (actin and myosin) and FAs (Paxillin) are drastically reduced, meanwhile microtubules undergo a gradual depolymerization in cells exposed to μG [15] compared to control cells grown in normal gravity [101]. This results in the disruption of PC and in decreased interconnectivity between cells and the ECM, which plays a key role in mechanotransduction [127], connecting external stimuli to the cytoskeleton. Therefore, the reduction in Paxillin expression could alter the mechanosensitivity of osteoblasts.

Microgravity causes also an alteration of cell tensive and compressive forces, compromising cell tensegrity [128] and directly inhibiting differentiation, as well as reducing ciliary length. Osteoblasts were seen larger because of F-actin and internal stress-fiber thinning and reorganization, along with changes in nuclear morphology. This compromises IFT, which represents a critical component for mechanosensitivity and mechanotransduction [129].

Although these effects were not directly correlated with the PC, previous in vitro studies on mice subjected to μG have shown a reduction in FA maturation and stabilization, which is associated with a reorganization of acetylated microtubules, which leads to increased microtubule restructuring. Acetylated microtubules are highly abundant in the PC axoneme, and during μG, they have been observed to become shorter and curlier, preventing them from extending long distances from the perinuclear area. This, in turn, alters osteoblast tensegrity [118].

Cell swelling leads to increased activation of phospholipase A2 (PLA2), which promotes the metabolism of arachidonic acid, thereby raising levels of epoxyeicosatrienoic acid (EET)[130]. These EETs can directly bind a site on TRPV4, a SAC highly localized in the PC, (notably 5′,6′-EET), thereby gating the channel in response to osmotic/mechanical stress [131]. This binding causes increased channel opening and activity, which can result in Ca^{2+} overload and mitochondrial dysfunction [125,131].

Notably, when cells are pretreated with either chloral hydrate - a sedative-hypnotic drug known to induce ciliary loss – or siRNA against *IFT88* - blocking IFT trafficking and ciliogenesis - the depolymerization of microtubules induced by μG conditions is attenuated [15]. Consistently, a recent work shown that microgravity alters ciliary dynamics by increasing ratio of anterograde/retrograde IFT, leading to primary cilium shortening [132], suggesting that simulated microgravity-induce depolymerization of microtubules requires the presence of PC. Additionally, cells treated with docetaxel trihydrate (DOC) - a microtubule stabilizer - under μG conditions, exhibit reduced levels of PC expression, from 60.8% to 26.7%. However, the effects of μG on ALP activity as well as on the expression of RUNX2 and Osterix (OSX) osteospecific genes are rescued by DOC treatment, leading to enhanced osteogenesis and bone mineralization through stabilization of the microtubule network [15]. These results suggest the pivotal role of the PC in signaling the μG condition to the cell and reducing osteoblastic differentiation. It appears that microtubule depolymerization starts from the primary cilium tip and then is sent to the ciliary transition zone, where the PC's microtubules are interconnected with the entire microtubule network system and then hindering the cytoskeleton structure and transducing the μG effects inside the cell body [15].

2.7 Targeting the primary cilium to counteract microgravity effects

Given its key role as bone cells' mechanosensor and its importance in maintaining cellular homeostasis under μG, the PC can be considered a promising target for mitigating the adverse effects of spaceflights.

A few studies have already tested various effectors to protect the PC expression and function, primarily by mimicking normal physiological conditions, thereby providing new insights for future research.

As already introduced before, an interesting mechanical induction method used to increase PC presence or activity is the usage of Electromagnetic field (EMF), such SEMF or PEMF [133]. These physical stimuli have been already recognized as safe and effective treatments for osteoporosis

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also in space environment, due to their ability to increase bone density and promote osteogenic differentiation [134,135]. Notably, the efficacy of this mechanical stimulation required the presence of PC on bone cells to activate bone deposition. EMF exposure has been shown to directly promote elongation of the PC axoneme and enhance the activity of its associated signaling pathways, thereby facilitating bone formation [75].

During μG the whole cytoskeleton is subjected to a different load causing MT depolymerization and impaired axoneme assembly [118]. To counteract these effects and stabilize the axoneme, two pharmacological strategies have been explored: Cytochalasin D, an inhibitor of actin polymerization that indirectly favors microtubule polymerization, and Docetaxel, a well-known microtubule stabilizer [15,97]. Although only Cytochalasin D increased PC presence and PC length on bone cells, both treatments were able to decrease bone resorption, while increasing bone formation [15,97].

Lastly, μG is known to enhance free radical and oxidative stress formation that leads to bone density loss [125]. Recent findings suggest that PC abrogation may be one of the underlying causes. On this regard, the use of antioxidants to protect the PC has been associated with increased osteogenesis and a higher prevalence of ciliated cell [125]. Specifically, the proposed mechanism behind the increase in oxidative stress involves dysregulated TRPV4 activity. Following the loss of the PC, TRPV4 expression significantly increases and translocates to the cytoplasm, where it elevates intracellular calcium levels. This, in turn, leads to mitochondrial Ca^{2+} overload and a consequent rise in reactive oxygen species (ROS) production [125].

To understand and evaluate if TRPV4 could be a possible therapeutic target to attenuate μG-driven oxidative stress, a TRPV4 antagonist called HC-067047 has been evaluated [125]. Miao and colleagues demonstrated that when administered to osteoblasts cultures this compound decreased Ca^{2+} influx and reduced ROS formation, increasing osteogenesis, thus confirming the importance of PC in maintaining low and localized TRPV4 expression *in vitro* [125]. Also, plant-derived molecules and extracts were screened based on their known antioxidant activity. Among these, moslosooflavone (MFL), a flavonoid isolated from natural compounds with antioxidant and anti-inflammatory properties, was identified as a promising candidate. Cell treatment with MFL increased PC length, reduced TRPV4 expression and restored osteogenic potential of bone cells during μG [125]. Interestingly other studies using plant-derived molecules with antioxidant properties had similar effects on preventing bone cell loss during μG [136–139].

3 DISCUSSIONS

"Life as we know it" has evolved under Earth's gravitational field, as reflected in the way cells have developed the ability to sense mechanical stimuli and transduce them into intracellular signals. Mechanotransduction is crucial for maintaining tissue homeostasis, and any alteration in mechanical input, such as in space and μG, increases the likelihood of health issues. The wide and heterogeneous pieces of scientific evidence collected and analyzed in this review focus on the modification or perturbation of bone tissue homeostasis in μG conditions due to its crucial role in sustaining other organs and to its direct correlation implication in mechanical sensing throughout the body.

Microgravity affects the movement and transport of fluids, directly impacting bone cells by reducing nutrient transport and increasing apoptosis in osteocytes, increases osteoclasts growth rate and differentiation speed, meanwhile also affecting osteoblasts differentiation and reducing the mesenchymal stromal cells pool, affecting astronauts bone healing speed process when coming back on earth. Moreover, it indirectly alters the mechanical input sensed by PC or other cellular organelles, disrupting downstream signaling pathways. The PC undergoes various changes due to μG, including its disappearance from most cells and shortening in the remaining ones, μG completely alters cell shape and cytoskeletal architecture, compromising IFT within the PC and the whole cell.

In conclusion, μG detrimentally affects every aspect of bone homeostasis, from microfluidic alterations to subcellular and protein changes, ultimately accelerating bone density loss compared to the natural course of an osteoporotic patient on Earth, in all of these effects the primary cilium related pathways are in the majority of the cases to get affected. Considering the significance of deep space exploration and the colonization of new planets for humanity, it is imperative to investigate these mechanisms fundamental to cell viability. An increasing number of emerging studies are uncovering novel mechanisms by which the PC contributes to altered mechanotransduction and bone loss under μG conditions. As a result, the PC is now considered a promising therapeutic target for mitigating the adverse effects of μG on bone cells. This has initiated the screening of molecules aimed to reduce space effects on bone tissue that are mainly directed to exert mechanical induction, enhancing PC functionality or counteracting the oxidative stress induced by spaceflight. Among these treatment strategies, modulation of TRPV4 overexpression stands out as particularly promising, as it addresses one of the primary drivers of ROS production and mitochondrial dysfunction.

An improved knowledge in this field may lead to the development of novel potential strategies to prevent and restore correct cell homeostasis in astronauts. Moreover, understanding key concepts in extreme environments like space can propel a better understanding of bone and PC-related diseases, such as osteoporosis.

4 METHODS

4.1 Objective of the review

This review has been conceived to delve into a deeper understanding of the μG effects that appear to act via the PC in bone tissue. We will analyze the details of PC-mediated mechanobiology to give a comprehensive picture on its impact on human bone cells physiology and homeostasis and deduce the implication in space biology. Collecting and critically analyzing existing scientific evidence that connects the PC to the mechanosensing and mechanotransduction governing bone homeostasis, would provide new insights towards clarifying the mechanisms involved in tissue adaptation to μG. Focusing on the PC might be particularly relevant for future human space explorations, being a discrete spot on cell surface, hence a putative druggable target. This will possibly provide novel hints paving the way to the design of innovative therapeutic interventions targeting mechanotransduction-related bone diseases, such as osteoporosis.

4.2 Study design and search strategy

The present study is a systematic review reported according to the Preferred Reporting Items for Systematic review and Meta-Analyses (PRISMA) guidelines [140] PubMed, ScienceDirect & Scopus database were searched using the keywords: "Bone", "microgravity", "space", "weightlessness", "primary cilium/a", "osteoblast", "osteoclast", "osteocyte", "mechanotransduction", "mechanosensitive", "cytoskeleton", "microtubules", "actin", "calcium", "ion channel" and "fluid dynamics", and their MeSH terms in any possible combination using the logical operators "AND" and "OR".

4.3 Inclusion and exclusion criteria

All the reviews, original articles, and book chapters, which were with free open access or were available as full text

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versions in scientific publication, databases and open access resources (PubMed, ScienceDirect, Scopus), describing the effects of space or microgravity on bone cells/primary cilium and/or regarding the change of mechanotransduction/fluid dynamics in microgravity/primary cilium, were considered eligible. No time limits were established regarding the publication date of the articles. The studies to be included had to be written in English only.

4.4 Data extraction and analysis

The Authors independently searched and collected data from all the studies found by the inclusion/exclusion criteria (**Fig. 5**).

The exclusion process of not relevant, duplicates or out of focus articles was performed with the website "Rayyan" [141].

DECLARATION

Data availability: All data generated or analysed during this study are included in this published article.

Author Contributions: Conceptualization, D.D.T and W.L.; writing—original draft preparation, D.D.T., F.T., L.P.; writing review and editing, W.L., F.T., A.A., A.M., and L.D.P; funding acquisition, A.M., W.L. and O.P. All authors have read and agreed to the published version of the manuscript.

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Figures legend and Tables

Figure 1. **Historical theories for bone adaptation and remodeling:** Schematic overview of the most relevant theories for bone adaptation and remodeling by Roux, Wolff and Frost theories [23-27]. Created with BioRender.com

Figure 2. Primary Cilium (PC) structure: Schematic representation of the PC structure: PC microtubules arrangement changes from the bottom, where they are arranged in doublets and in a ring conformation, to the top, where they are disorganized and in a singlet conformation [42]. The basal feet are connections from the basal body to the cytoskeletal microtubules [38]. Cytoskeletal structures are reduced in number and in length for simplicity purposes. Image not to scale. Created with BioRender.com

Figure 3. Primary-cilium related signaling pathways: Schematic representation of primary cilium-related signaling pathways: Hippo pathway in the OFF state (left) does not present PC, thus leading to phosphorylation of MST1/2 by Rassf and subsequently degradation of YAP/TAZ, and in the ON state (right) where the presence of PC blocks the phosphorylation of MST1/2 complex permitting the localization of YAP/TAZ protein in the nuclear compartment [62] (**a**), the NOTCH pathway is regulated the Presenlin, present in the basal body of the PC, cleaving the NCID domain from the NOTCH membrane protein, leading it to migrate to the nucleus and activating target genes expression [64-65] (**b**); the non-canonical WNT/Ca2+ pathway is activated by fluid flow that by bending primary cilium permit the entrance of Ca2+ (green circles) from TRPP1/2, this then increase the expression of Inversin, present on the primary cilium base. Inversin then migrates to the cytoskeleton and blocks β-catenin translocation to the nucleus. Meanwhile calcium is stored inside the cilioplasm [71] (**c**). Created with BioRender.com

Figure 4. Microgravity effects on bone cells: Synthetic representation of microgravity effects on osteoclasts (**a**), osteoblasts (**b**) and on bone cell tensegrity [127] (**c**). Created with BioRender.com

Figure 5. Studies identification via databases: The flow diagram represents the flow of information through the different phases of the systematic, with paper identification number, inclusion and exclusion criteria, following the "Prisma 2020 flow diagram chart" [139]. Created with BioRender.com

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Table 1. List of most important families of stretch activated ion channel (SAC)

Stretch-activated ion channel (SAC)	Location	Function	Ion selectivity
Transient Receptor Potential (TRP) channels, subfamily C			
Transient Receptor Potential Canonical 1 cation channel (TRPC1)	Most tissues, localize in PC	It mainly functions as an auxiliary subunit, working as a heteromeric channel with other TRP subtypes [20,142–144]. Its expression is positively regulated by mechanical stimulation, like positive or negative pressure [145].	Non-selective Ca^{2+} permeable ion channel
Transient Receptor Potential Canonical 3 & Transient Receptor Potential Canonical 6 cation channels (TRPC3/TRPC6)	Mainly brain, lungs, heart and, in less degree, bone cells, localize in PC	They have redundant function and can work as hetero-oligomers. When they are mutated or silenced, neurons decrease mechanosensitive-ion channel activation and in bone enhance osteoclast formation [146,147]	Non-selective Ca^{2+} permeable ion channel
Transient Receptor Potential (TRP) channels, subfamily P			
Transient Receptor Potential Polycystic 2 cation channel (TRPP2, also called PC2)	Mainly kidneys [148], bone [84, 145] and bile ducts [149], localize in PC	It is responsible for sensing the fluid flow and increasing intracellular Ca2+. Its localization is in the Endoplasmic Reticulum (ER) and PC [150]. TRPP2 interact with TRPP1 (that has only a supporting role) to sense the mechanical input of the shear stress.	Non-selective Ca^{2+} permeable ion channel
Transient Receptor Potential Polycystic 3 cation channel (TRPP3, also called PC2-L1)	Mainly kidneys, localize in PC	It has the same function and has a high homology to TRPP2 [151] and can interact with TRPP1 to sense mechanical signal [152].	Non-selective Ca^{2+} permeable ion channel
Transient Receptor Potential (TRP) channels, Subfamily V			
Transient Receptor Potential Vanilloid 2 cation channel (TRPV2)	Motor neurons	It has a role in connecting strain-sensing response and FA and cytoskeleton [153].	Non-selective Ca^{2+} permeable ion channel
Transient Receptor Potential Vanilloid 4 cation channel (TRPV4)	Ubiquitous expression, strongly present in kidneys, localize in PC	It is an osmo-sensitive and mechanosensitive receptor that increases the intracellular concentration of Ca^{2+}. Its mechanotransducive activity is associated with the PC [154], and there is evidence that it can cooperate with both TRPC1 and TRPP2, when interacts with TRPC1 it can prolong the calcium influx [56] and with TRPP2 forms a complex that acts as a thermosensitive sensor in the PC [154]. TRPV4 mutations or inactivation have been found to both diminish MSC osteogenesis and impair regulation and function of mitochondria [155,156] and also associated to ciliopathies [157].	Non-selective Ca^{2+} permeable ion channel
Piezo family			
Piezo Type Mechanosensitive Ion Channel Component 1 & 2 (Piezo1/Piezo2)	Piezo 1: Mainly bone and cartilage localize in PC Piezo2: mainly sensory neurons	Although is still poorly understood their mechanosensitivity [34] there are some studies that correlates the membrane tension to their activation [158,159]. Piezo1/2 mediate the influx of Ca2+ in bone marrow stromal cells, promoting osteoblasts differentiation [160].	Non-selective Ca^{2+} ion channels
Other important families			
Epithelial Sodium channel superfamily (ENaC superfamily)	No specific tissue, localize in PC	Their function has been linked to mechanotransduction, but the mechanism is yet to be fully understood [161].	Selective Na^{+} ion channels

Historical theories for bone adaptation and remodeling

Wilhelm Roux (1883)

↓

Use it or lose it principle

↓

When bone is subjected to mechanical stress it grows stronger, if not it goes through degradation

Julius Wolff (1892)

↓

Wolff's law of bone remodelling

↓

The greatest the load on the bone, the greater it adapts to it and remodel its structure to withstand the new stress

Harold Frost (1987)

↓

Mechanostat theory

↓

Bone remodeling is regulated by "windows of mechanical usage"

Burr et al. & Fritton et al. quantified the intensity:

- Disuse Window- when strain <1000 με (increased bone resorption)
- Overuse Window- when strain 1000<x<3000 με (increased bone formation)

Later confirmed also a "Harmful Window", with strain >3000 με that causes bone damage

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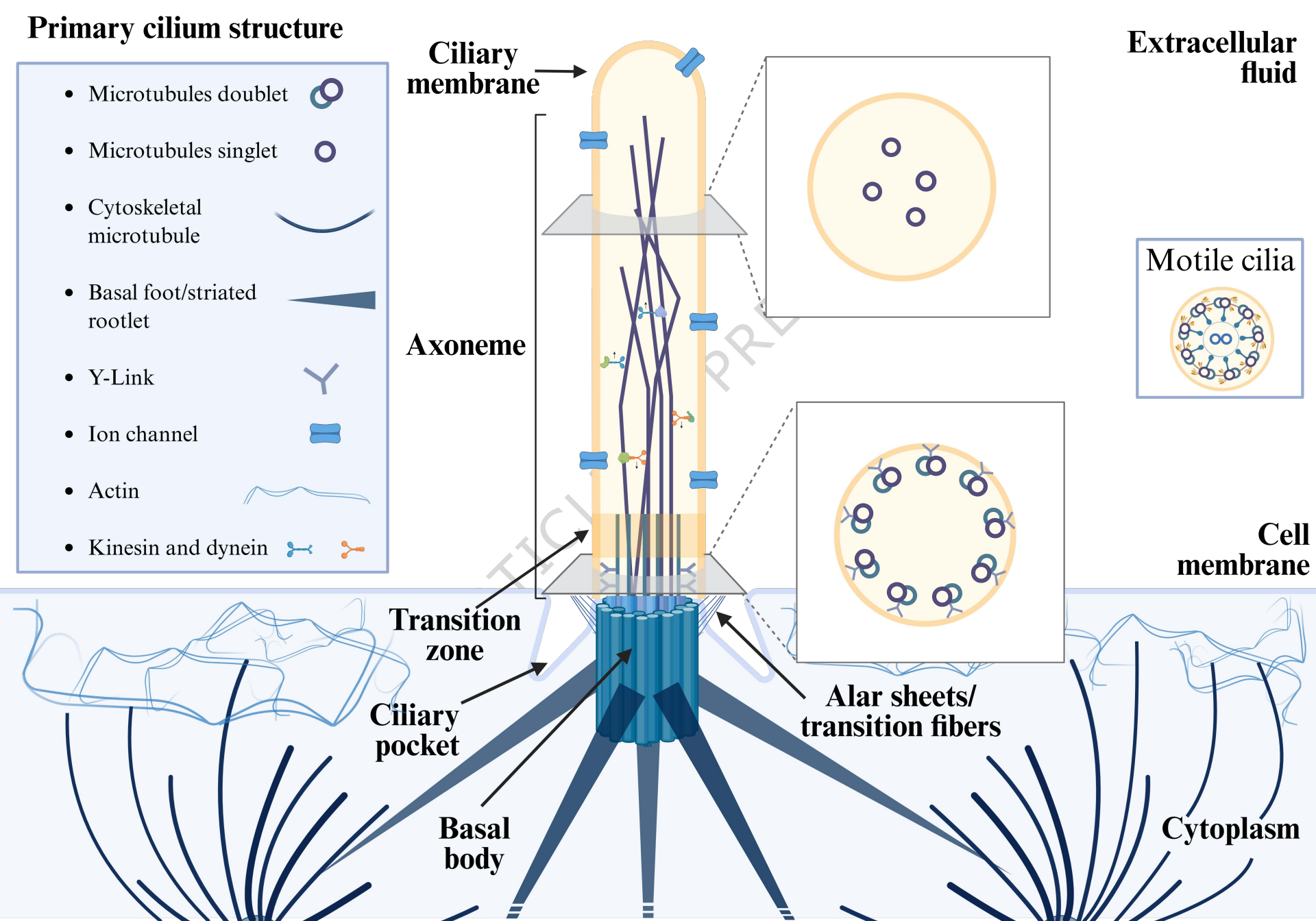
Primary cilium structure
Microtubules doublet
Microtubules singlet
Cytoskeletal microtubule
Basal foot/striated rootlet
Y-Link
Ion channel
Actin
Kinesin and dynein
Ciliary membrane
Extracellular fluid
Axoneme
Motile cilia
Cell membrane
Transition zone
Ciliary pocket
Basal body
Alar sheets/ transition fibers
Cytoplasm

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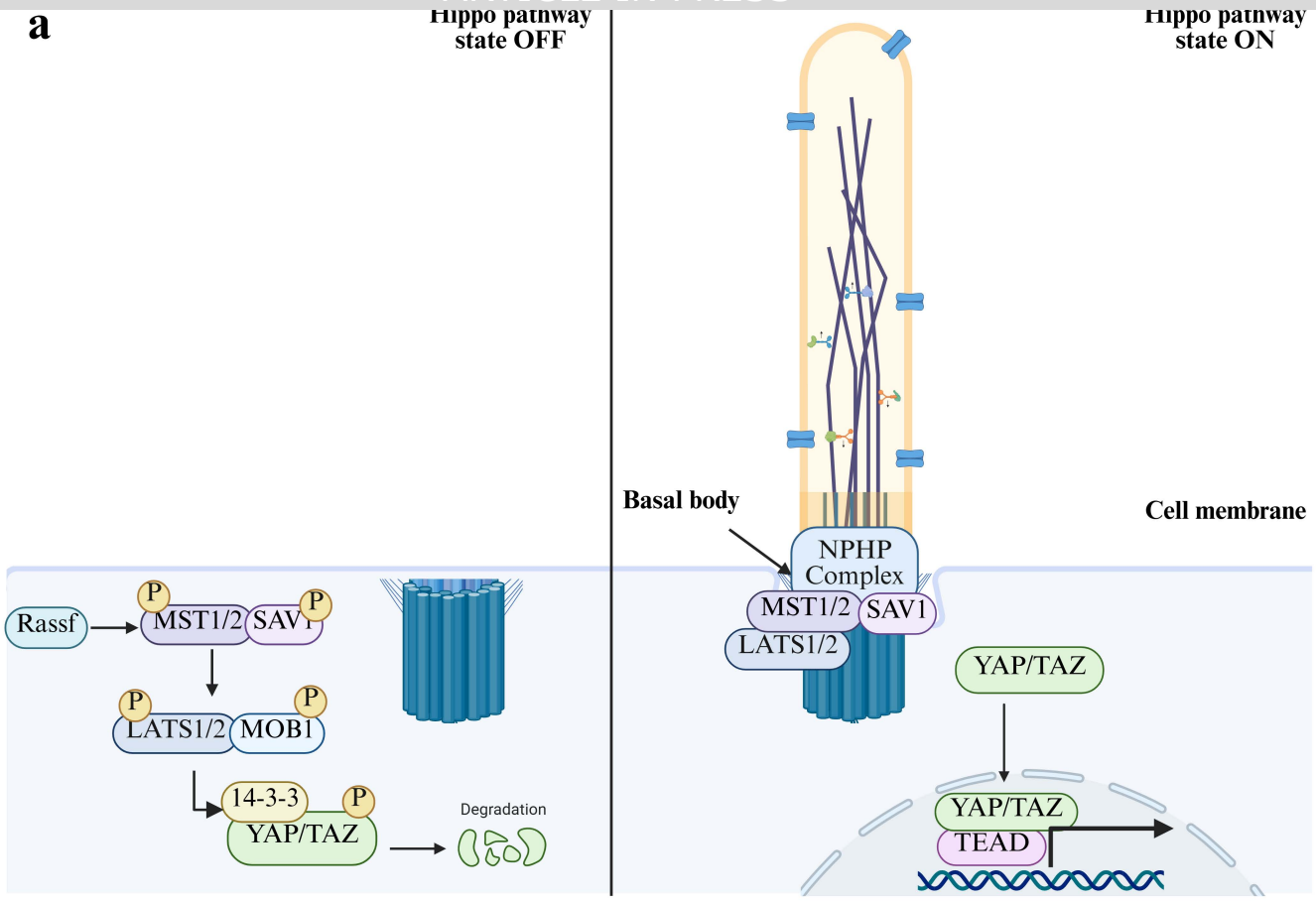
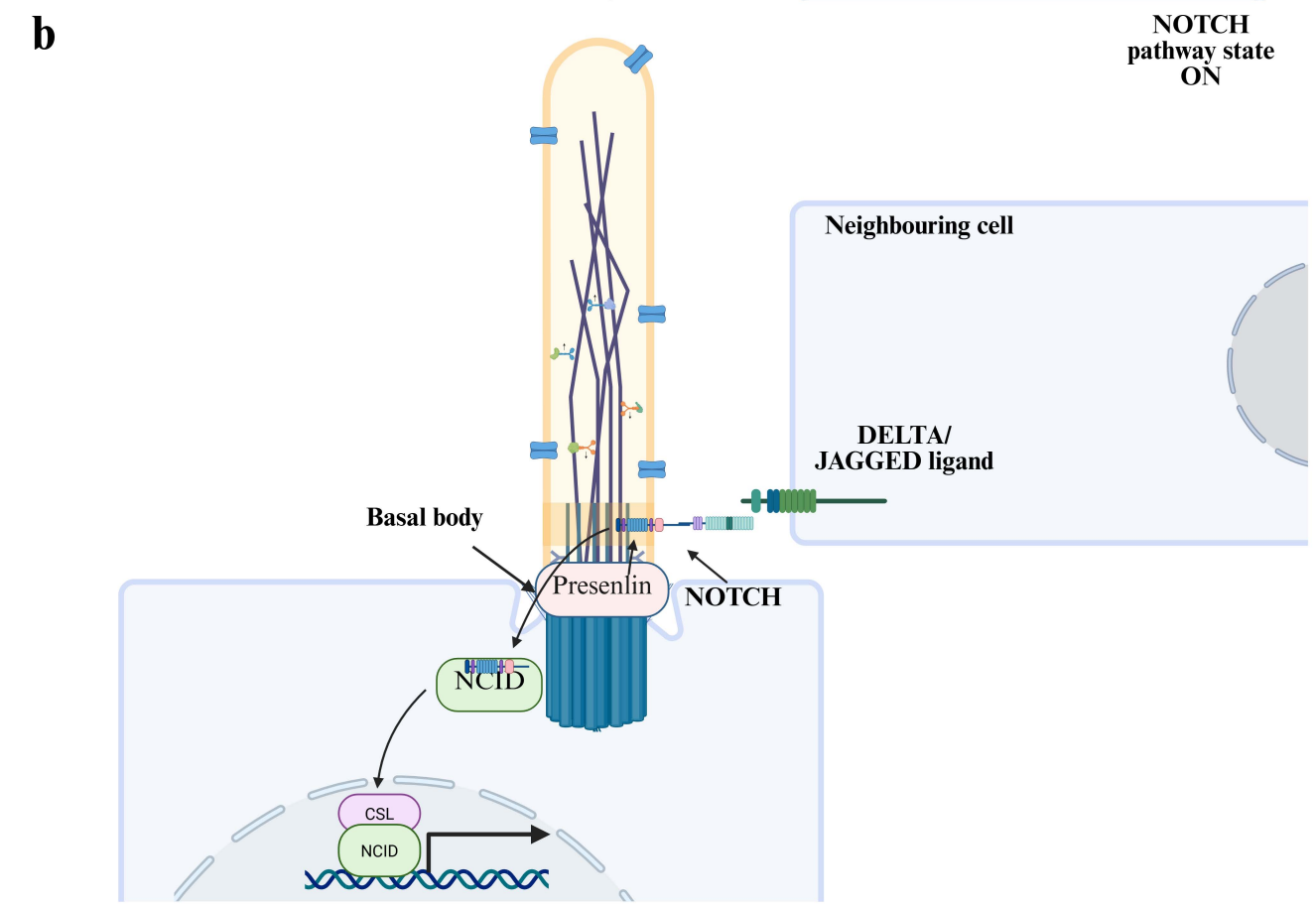
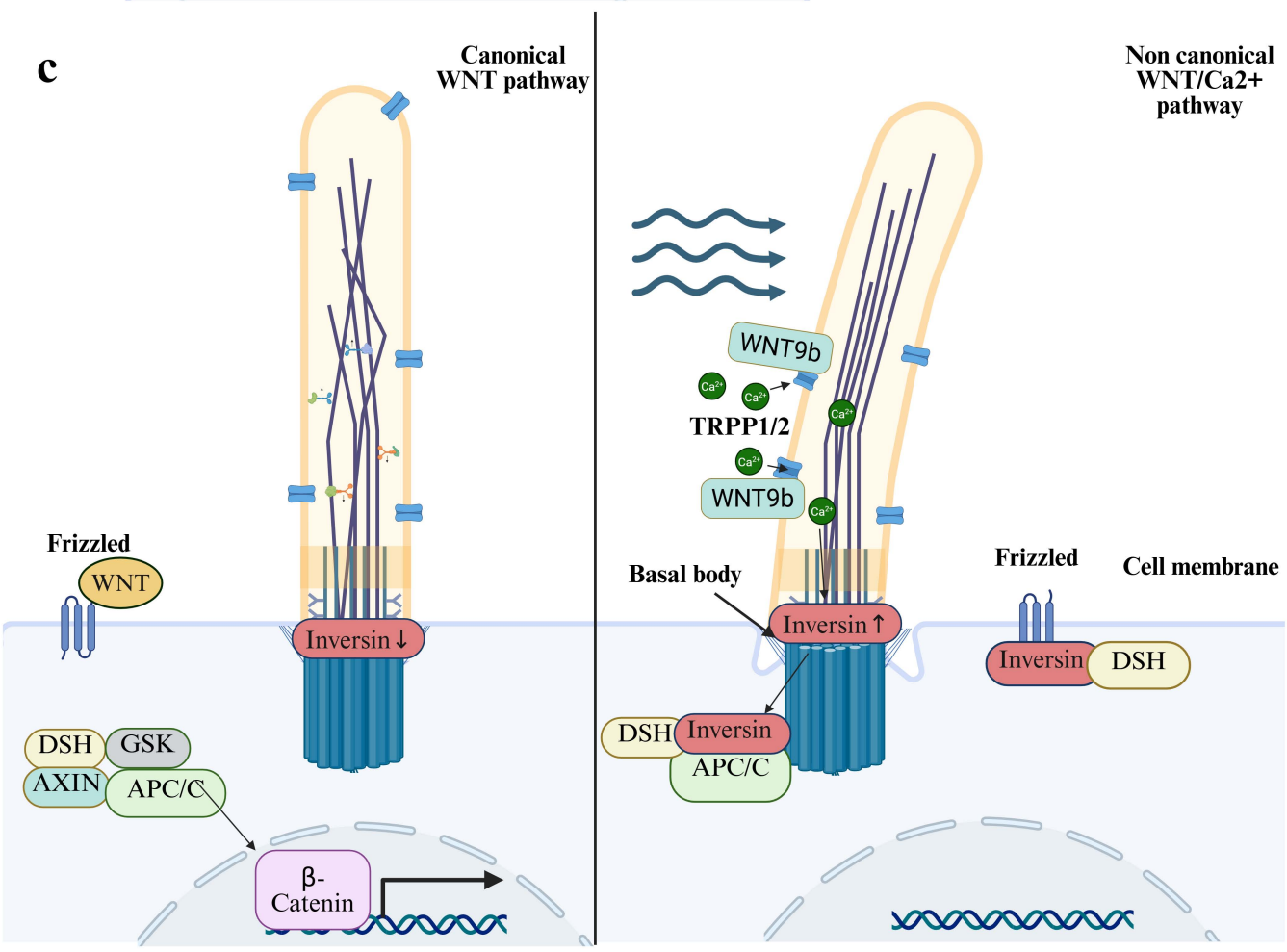
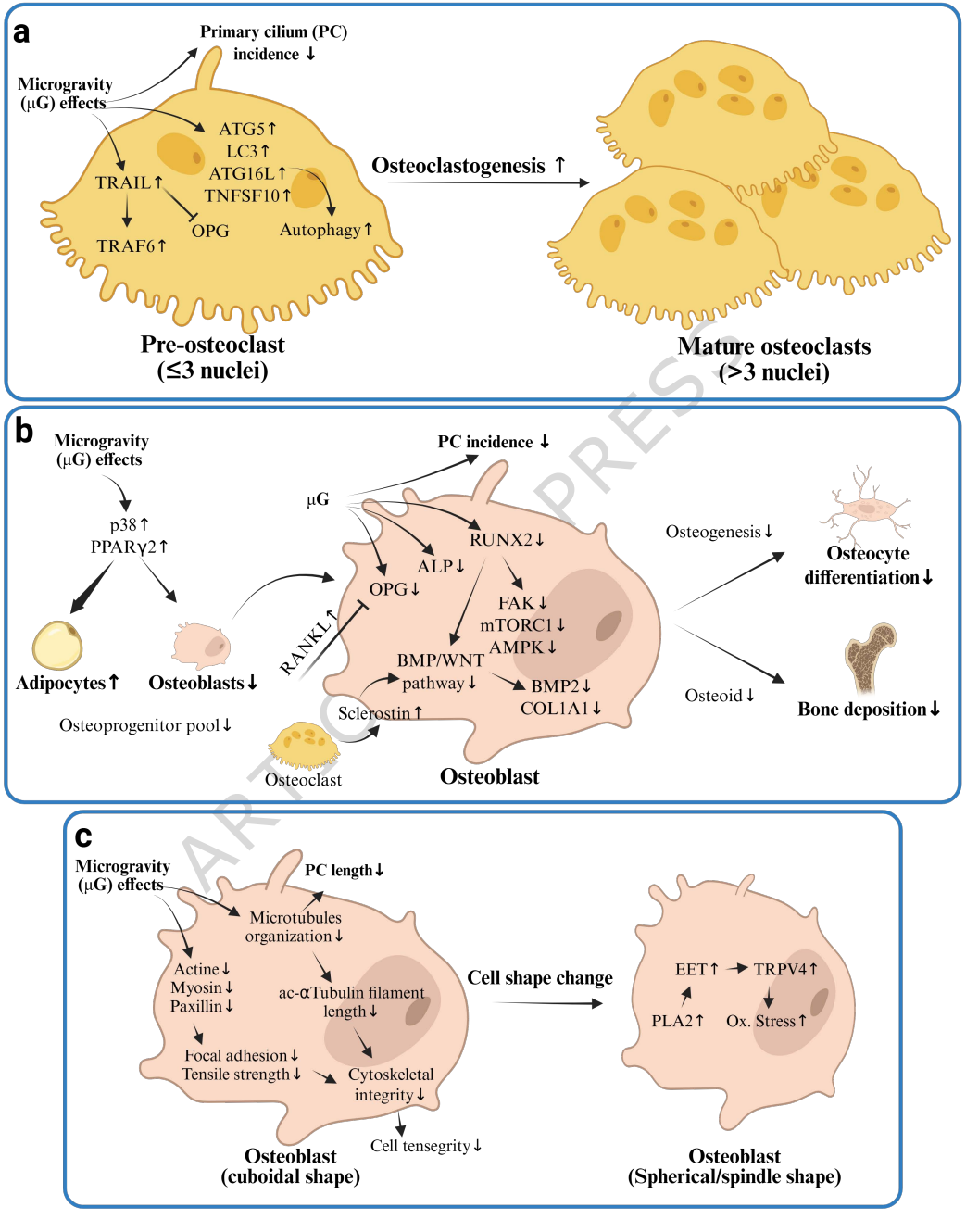
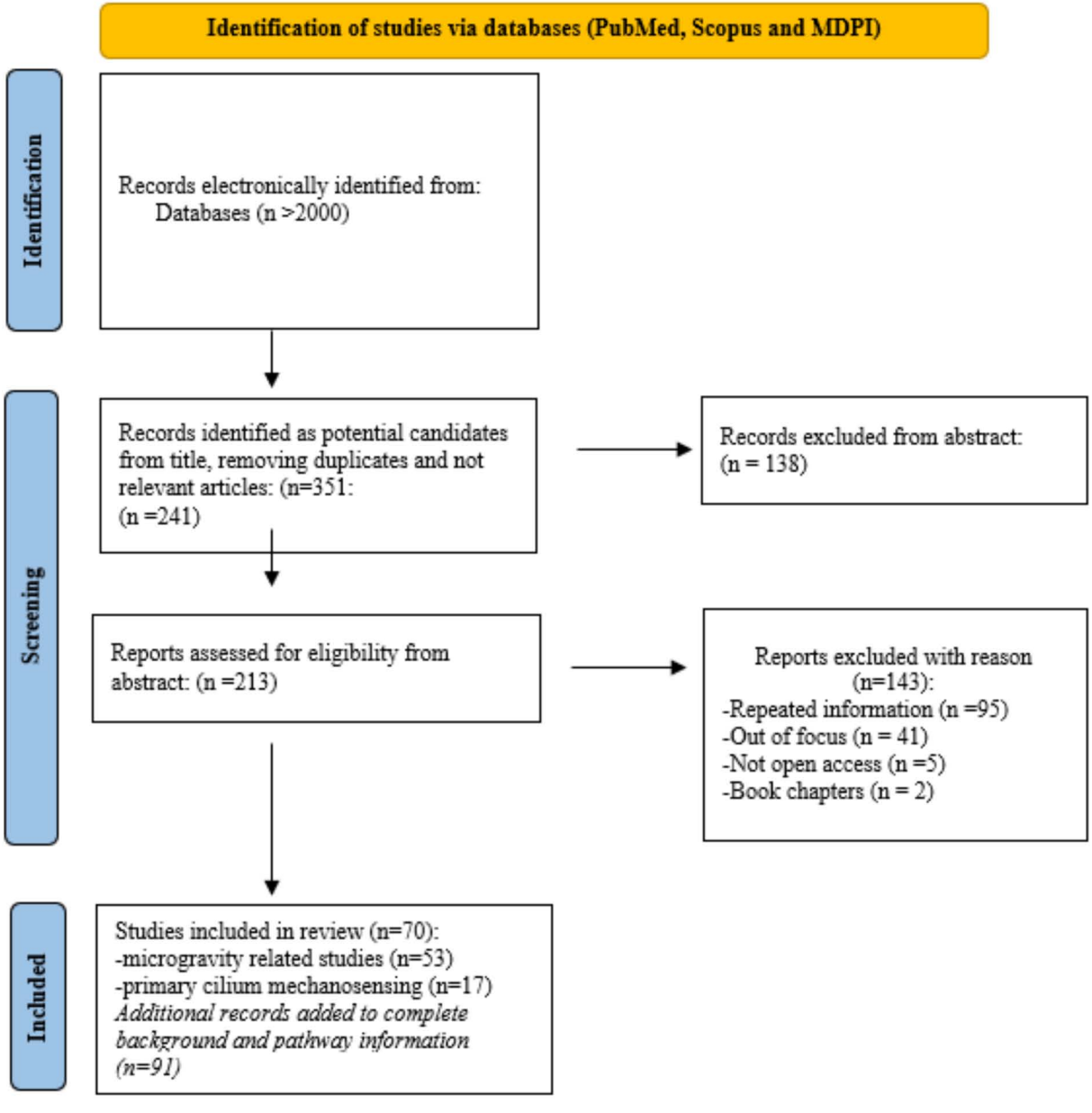







a
Primary cilium (PC) incidence ↓
Microgravity (μG) effects
ATG5↑
LC3↑
ATG16L↑
TNFSF10↑
TRAIL↑
OPG
Autophagy↑
TRAF6↑
Pre-osteoclast (≤3 nuclei)
Osteoclastogenesis ↑
Mature osteoclasts (>3 nuclei)
b
Microgravity (μG) effects
p38↑
PPARγ2↑
Adipocytes↑
Osteoblasts↓
Osteoprogenitor pool↓
PC incidence ↓
μG
RUNX2↓
ALP↓
OPG↓
RANKL↑
FAK↓
mTORC1↓
AMPK↓
BMP/WNT pathway↓
BMP2↓
COL1A1↓
Sclerostin↑
Osteoclast
Osteoblast
Osteogenesis↓
Osteocyte differentiation↓
Osteoid↓
Bone deposition↓
c
Microgravity (μG) effects
PC length↓
Microtubules organization↓
Actine↓
Myosin↓
Paxillin↓
ac-αTubulin filament length↓
Focal adhesion↓
Tensile strength↓
Cytoskeletal integrity↓
Cell tensegrity↓
Osteoblast (cuboidal shape)
Cell shape change
EET↑
TRPV4↑
PLA2↑
Ox. Stress↑
Osteoblast (Spherical/spindle shape)


Identification of studies via databases (PubMed, Scopus and MDPI)
Identification
Records electronically identified from:
Databases (n >2000)
Screening
Records identified as potential candidates from title, removing duplicates and not relevant articles: (n=351:
(n =241)
Records excluded from abstract:
(n = 138)
Reports assessed for eligibility from abstract: (n =213)
Reports excluded with reason (n=143):
-Repeated information (n =95)
-Out of focus (n = 41)
-Not open access (n =5)
-Book chapters (n = 2)
Included
Studies included in review (n=70):
-microgravity related studies (n=53)
-primary cilium mechanosensing (n=17)
Additional records added to complete background and pathway information (n=91)