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Molecular mechanisms of gravity perception and signal transduction in plants

Yaroslav S. Kolesnikov¹ · Serhiy V. Kretynin¹ · Igor D. Volotovskiy² · Elizabeth L. Kordyum³ · Eric Ruelland^{4,5} · Volodymyr S. Kravets¹

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Abstract Gravity is one of the environmental cues that direct plant growth and development. Recent investigations of different gravity signalling pathways have added complexity to how we think gravity is perceived. Particular cells within specific organs or tissues perceive gravity stimulus. Many downstream signalling events transmit the perceived information into subcellular, biochemical, and genomic responses. They are rapid, non-genomic, regulatory, and cell-specific. The chain of events may pass by signalling lipids, the cytoskeleton, intracellular calcium levels, protein phosphorylation-dependent pathways, proteome

changes, membrane transport, vacuolar biogenesis mechanisms, or nuclear events. These events culminate in changes in gene expression and auxin lateral redistribution in gravity response sites. The possible integration of these signalling events with amyloplast movements or with other perception mechanisms is discussed. Further investigation is needed to understand how plants coordinate mechanisms and signals to sense this important physical factor.

Keywords Gravity · Signalling · Phospholipase · Calcium · Cytoskeleton · Phosphorylation

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Abbreviations

ADK1	Adenosine kinase 1
PI-PLC	Phosphoinositide-specific phospholipase C
IP ₃	Inositol-1,4,5-trisphosphate
PIP5K	Phosphatidylinositol monophosphate 5-kinase
PI-4,5-P ₂	Phosphatidylinositol-4,5-bisphosphate
AtInsP5tase	<i>Arabidopsis thaliana</i> inositol polyphosphate 5-phosphatase
PI	Phosphatidylinositol
TOC	Translocon of outer envelope
PA	Phosphatidic acid
PLD	Phospholipase D
PP	Protein phosphatase
PDK1	Phosphoinositide-dependent protein kinase
ROS	Reactive oxygen species
SGR	Shoot gravitropic response

Introduction

The spatial orientation of immobile higher plants in the gravitational field is mainly determined by gravitropism and

phototropism. This orientation mobilizes physiological activity that results in differential growth. There are three main phases in the gravitropic response: perception of a gravitational stimulus, transduction of a signal, and the resulting differential growth that causes bending. Roots show positive gravitropism, growing in the direction of a gravitational vector, and stems show negative gravitropism, growing in the direction opposite to the gravitational vector.

To begin this reflection on gravitropism, it is useful to get an anatomical and cellular overview of the whole response. Gravitropism starts with gravity susception. Amyloplasts appear to act as gravity sensors in statocytes, the specialized graviperceptive cells, to fulfill a statolithic function by sedimenting in the direction of the gravitational vector to the lower part of a cell. The gravitropically responsive apical parts of alga *Chara globularis* rhizoids contain statoliths, compartments filled with crystallites of barium sulphate (Sievers et al. 1991). In mosses, gravity perception and gravitropic bending are both carried out in the same cell, an apical cell of the chloronema. Amylochloroplasts containing large starch grains in this cell are believed to play the role of statoliths (Demkiv et al. 1998; Walker and Sack 1990). In higher plants, the sites of gravity perception and reaction are spatially separated. In roots, statocytes are cells of the central zone of the root cap columella. In stems, statocytes are localized in the endodermis, particularly in hypocotyls, epicotyls, inflorescences, flower stalks, peduncles, gynophores, and in leaf petioles in dicots. In pistil, they are located in cells along the inner cortex layers below the epidermis. In monocot stems, they are also observed in bundle sheath of internodal pulvini, coleoptiles, and in stem leaf sheath (Blancaflor and Masson 2003; Kitazawa et al. 2005; Li et al. 2013; Mano et al. 2006; Philosoph-Hadas et al. 2015; Shimizu et al. 2005). In organs

with secondary growth, cambium may be suggested to be a gravisensing site (Coutand et al. 2014). Stamens, plumules, fruits, and leaves are also sensitive to gravity, but their statocytes are less studied (Philosoph-Hadas et al. 2015). Statocytes thus transform a physical signal, gravity, into biochemical and physiological ones. The physiological signal is a change in auxin concentration that is first initiated in root cap cells by the rapid downward movement of the PIN3/PIN7 auxin transporter. The differences in auxin level are further transmitted by other auxin transporters (PIN2) to the responsive elongation zones (Feraru et al. 2015; Friml et al. 2002; Kleine-Vehn et al. 2010; Rahman et al. 2010) (Fig. 1). This leads to differential growth on opposite sides of the root that causes its bending.

Gravity perception and signal transduction have been extensively studied for a long time. Different models of gravity signal perception have been proposed and tested: the starch statolith, the ligand-receptor, and the hydrostatic pressure models. Possible roles of the cytoskeleton, the plasmalemma control center, calcium ions and other messengers, and the related gene activity were considered from the outset (Sack 1997; Sievers et al. 1991). The field has seen many breakthroughs and developments in recent years. The specific roles of signalling lipids and phospholipases, cytoskeleton, calcium, protein phosphorylation, and transcription factors in gravity action and signalling can now be discussed.

Models of gravity perception

There are many hypotheses to explain the mechanisms of gravity perception by higher plants (Fig. 2). The conventional concept of gravity perception with statoliths, the starch-

Fig. 1 Sites of gravity perception and response in different organs: **a** gravity perception in roots; **b** gravity perception and response in stems

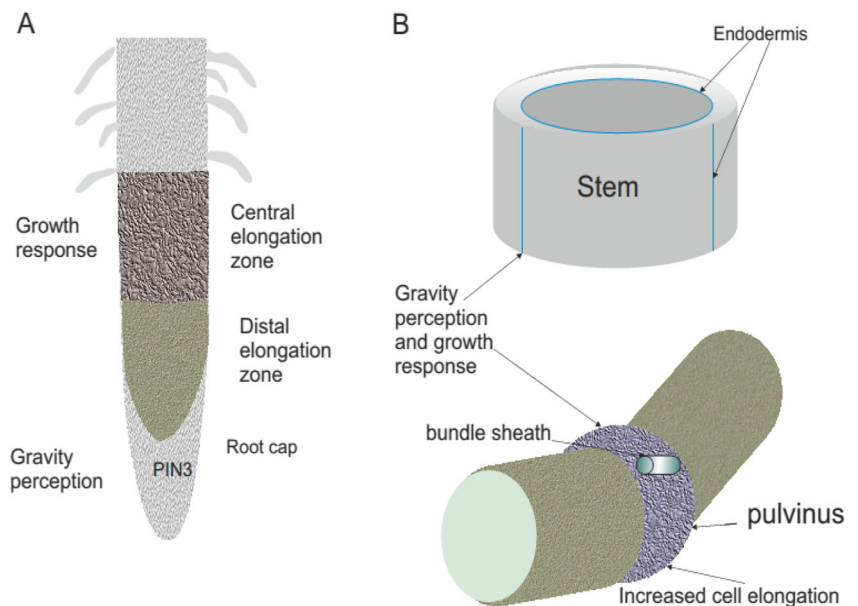
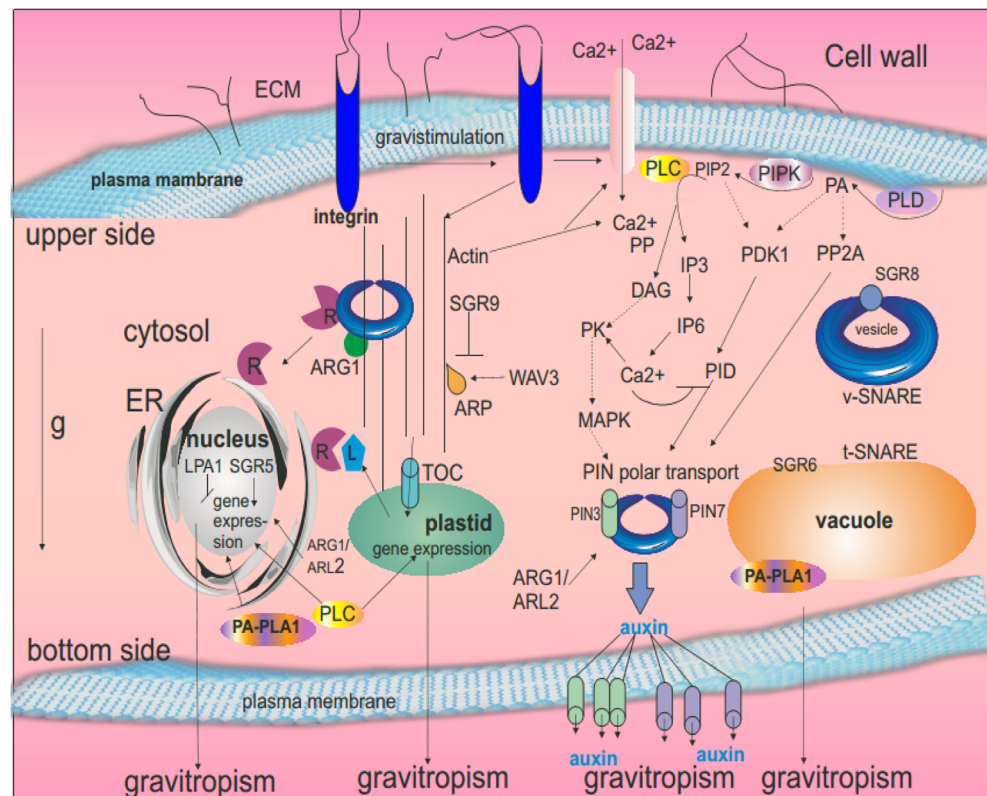


Fig. 2 Integrated model of known gravity signalling pathways in plant cells. *DAG* diacylglycerol, *IP3* inositol trisphosphate, *IP6* inositol hexakisphosphate, *L* ligand, *ECM* extracellular matrix, *PA* phosphatidic acid, *PA-PLA1* phosphatidic acid-specific phospholipase A₁, *PIP2* phosphatidylinositol bisphosphate, *PIPK* phosphatidylinositol phosphate kinase, *PK* protein kinase, *PLC* phospholipase C, *PLD* phospholipase D, *PP* protein phosphatase, *PDK1* phosphoinositide-dependent protein kinase, *PID* ser/thr protein kinase PINOID, *R* receptor, *TOC* translocator of outer envelope of plastids, *ARP* actin-regulatory proteins



statolith theory of Nemeč and Haberlandt, still stands (Haberlandt 1900; Nemeč 1900). This theory explains how special cells, the statocytes, in plant axial organs are stimulated by gravity. The stimulus causes amyloplasts, 1.5–3 μm in diameter, to move to the distal end of the cell to transiently interact with specific cellular structures. The amyloplasts are thus thought to act as the sensors of the gravitational force, so are called “statoliths.” The starch-statolith model is the most generally accepted model supported by a range of data (Band et al. 2012; Leitz et al. 2009; Wolverton et al. 2011). (1) There is a correlation between statolith localization and the site of gravity sensing. (2) Significant redistribution (sedimentation) of amyloplasts occurs in statocytes under gravistimulation in comparison with the displacement of other cell organelles. (3) Roots, shoots, pulvini, and gynophores are less able to react to gravity when there is no starch in the amyloplasts, starchless *Arabidopsis thaliana* (e.g., *pgm1*), tobacco, or rice (*Oryza sativa*, *agpl1*) mutants are weakly gravitropic (Chang et al. 2001; Fitzelle and Kiss 2001; Fujihira et al. 2000; Kiss and Sack 1990; Kordyum 2014; Kuznetsov and Hasenstein 1996; Moctezuma and Feldman 1999; Okamura et al. 2015; Rioux et al. 2015; Vitha et al. 2007; Weise and Kiss 1999). Normal moss protonemata gravitropism in surrounding media with high-density also suggests the role of sedimenting amyloplasts as statoliths in gravitropism (Schwuchow et al. 2002). This is also supported by the fact that in mangrove roots with

different growth direction, gravity nevertheless drives downward location of amyloplasts to different cell walls of statocytes (Purnobasuki and Suzuki 2004). Starch in amyloplasts is not required for gravity perception, but is necessary for full gravisensitivity. Sedimentation of plastids in stem endodermis possibly acts as an accelerator but is not required to elicit a gravitropic response (Kiss et al. 1989; Kiss 2000; Weise and Kiss 1999). In contrast to roots, where gravity signal is perceived in cap columella cells and transduced to elongation zone, sedimentation of plastids and the site of gravity perception appears to be diffuse throughout the inflorescence stem endodermis and the responsive signal resulted from gravity perception is spatially restricted (Weise and Kiss 1999; Weise et al. 2000).

Alternative mechanisms of gravity sensing may also exist in plants. A hypothesis of gravity perception without statoliths was first proposed by Pollard in 1965 (Pollard 1965). The so-called “model of hydrostatic pressure” was proposed to explain several observations. Starchless mutants have some residual gravitropism, and starch-deficient mutants have a normal graviresponse to hypergravity (Toyota et al. 2013b; Wolverton et al. 2011). Cells in the root distal elongation zone of higher plants and characean algae internodal cells contain no sedimentable amyloplasts, and lateral root gravitropism is initiated before their amyloplasts emerge. So, there must be another

amyloplast-independent gravity perception mechanism in these plant organs (Bai and Wolverton 2011; Guyomarc'h et al. 2012; Wolverton et al. 2002). Decreased gravitropism of rice aquatic roots grown in high-density external medium without changes in amyloplast sedimentation also supports this idea (Staves et al. 1997). The perception of protoplast pressure on the cell wall could be one of the gravity sensing mechanisms. A change in orientation according to the gravity field will result in changes in tension and compression in the different sites of plasma membrane attachment to the cell walls on opposite sides of the cell. Changes in tension and compression exerted by constituents of extracellular matrix junctions may be perceived by specific receptors, possibly cell wall-associated receptor-like kinases and integrins as cell wall status sensors. In the latter case, this may result in stretch-dependent activation of ion channels at the site of tension, calcium flux, and further signalling events (Staves et al. 1995; Wayne and Staves 1996; Wayne et al. 1992) (Fig. 2). Immunolocalization studies indicate that integrin-like proteins are enriched in plasma membranes of root cap cells of *Arabidopsis* and rhizoid tips of *Chara*. They are also found in *Arabidopsis* shoots and *Chara* intermodal cells (Katembe et al. 1997). Integrin colocalization with matrix proteins vitronectin and fibronectin in plant cell plasma membrane may suggest their role in perception and transduction in mechanical signal during graviperception (Gens et al. 1996). In the “ligand-receptor model,” unidentified ligands associated with plastids interact with receptors located on gravisensitive membranes. Graviperception in characean rhizoids requires direct contact of some component on the surface of sedimented statoliths with membrane-bound receptor molecules rather than pressure or tension exerted by the weight of statoliths (Limbach et al. 2005). In higher plants, components of plastid preprotein recognition (e.g., TOC132) may facilitate insertion of an unidentified ligand protein into the outer amyloplast membrane. When the amyloplast sediments, this ligand could interact with a membrane-associated receptor modulating its activity. The TOC75 isoform of the central pore complex may mediate this insertion (Stanga et al. 2009; Strohm et al. 2014). The integrin receptor-based “model of hydrostatic pressure” may actually be tightly linked to the “ligand-receptor model.”

Gravity signal transduction in plant cells

Gravity perceived in cells must be transduced by specific signalling cascades in order to establish the formation of auxin gradients in specific response zones. Among them, a lipid signalling is emerging (Smith et al. 2013). A well-known outcome of gravity signalling is rapid PIN3 translocation to the side of the root columella cell that now

becomes the bottom of the cell after root reorientation/bending. This subsequently leads to changes in gravity-specific auxin lateral redistribution and gene expression (Friml et al. 2002; Kimbrough et al. 2004; Salinas-Mondragon et al. 2010). The rapid biochemical gravity responses that are initially produced by gravistimulation are listed in Table 1 and Fig. 2. The nature, similarities, and differences between gravity signalling in sensitive organs and different stages of gravity action in cells are summarized in Tables 2, 3, and 4.

Lipid signalling

Phosphoinositide-specific phospholipase C

Phosphoinositide-specific phospholipase C (PI-PLC) enzymes hydrolyze phosphatidylinositol-4,5-bisphosphate (PI-4,5-P₂) into inositol-1,4,5-trisphosphate (IP₃) and diacylglycerol (Pokotylo et al. 2014). IP₃ plays a well-defined role in calcium release from intracellular stores in cells (Zhang et al. 2015). Different investigations indicate a primary role for PI-PLC and its product IP₃ in gravity signalling. Biphasic changes in IP₃ levels were detected in response to gravity. During the first phase, IP₃ levels increase initially during the first 10–15 s of gravity action in the lower halves of maize (*Zea mays*) and in the upper and lower halves of oat (*Avena sativa*) pulvini. Then, they fluctuate between the upper and lower halves with a period of 90 s over the first 30 min of gravistimulation. After 30 min of gravistimulation, the second phase of dynamics was detected, with a stable increase to a high IP₃ level in the elongating lower halves of pulvini (Perera et al. 1999, 2001; Yun et al. 2006). A two-phase rise in IP₃ levels was also observed in the total *Arabidopsis* inflorescence stems in response to short-term gravity. This second IP₃ peak is similar to the pattern seen with the lower sides of the pulvini. This indicates the universality of the IP₃ generation in gravity signalling (Perera et al. 2006). Duration of the second IP₃ phase in different plants correlates in timing with the initiation of their bending response (Perera et al. 1999, 2001, 2006). The PLC-dependent long-term sustained increase in IP₃ could possibly induce biochemical asymmetry that precedes differential cell growth of each pulvinus half (Perera et al. 2001).

It is not really known how gravity activates PI-PLC. The increase in IP₃ in response to gravity is independent on auxin transport (Perera et al. 2001; Yun et al. 2006). On the other hand, okadaic acid, an inhibitor of protein phosphatases 1 and 2A, and lanthanum ions, inhibitors of calcium channels, inhibit IP₃ accumulation and the response to gravistimulation (Yun et al. 2006). When starch levels are reduced in the lower halves of shoot pulvinus statoliths, the IP₃ increase is delayed. Some internode tissues that do not contain amyloplasts showed no changes in the IP₃ levels upon gravistimulation (Perera et al. 1999, 2001). This

Table 1 Onset of early biochemical events in response to gravity action

Time of detectable changes	Signalling event	Notes	Amyloplast dependency	Reference
2–3 s	NO	Rapid transient burst in root transition zone	Unknown	Mugnai et al. (2014)
10–15 s	IP ₃	Rapid transient, long sustained	+	Perera et al. (1999), Perera et al. (2001)
20–35 s	Ca ²⁺	Transient	Independent	Toyota et al. (2008a, b)
		Flux reorientation	Unknown	Salmi et al. (2011)
1 min	ROS accumulation	NADPH-oxidase-independent	Localized under amyloplasts	Clore et al. (2008)
1–2 min	pH changes	Transient, in columella cytosol	+	Hou et al. (2004)
			(not in distal elongation zone)	Fasano et al. (2001)
2 min	PIN3 relocalization	Detected in roots	+	Friml et al. (2002)
	Proteome changes	GSTF9 and HSP81 are involved in gravitropism	Unknown	Schenck et al. (2013)
	Gene expression	Transient, in root apex	Unknown	Kimbrough et al. (2004)
	Ethylene emission	Transient, in seedlings and hypocotyls	Unknown	Harrison and Pickard (1984)
3 min	Protein phosphorylation	32, 35 kDa	Unknown	Friedmann and Poovaiah (1991)
Within 5 min	Auxin gradient formation	Transient, asymmetric, basally redistributed	+	Band et al. (2012)
	Calcium increase in cytoplasm	Transient, detected in stem endodermis	Unknown	Zhang et al. (2011c)
5 min	Protein phosphorylation	38, 50 kDa (pulvini), <i>Zm</i> MAPK5 (roots, H ₂ O ₂ -dependent)	Unknown	Chang and Kaufman (2000), Joo et al. (2001), Clore et al. (2003)
	Gene expression	Detected in root apex	Unknown	Salinas-Mondragon et al. (2010)

suggests that amyloplast sedimentation and calcium and protein dephosphorylation can influence PI-PLC activation during gravity signalling (Fig. 2). Short-term IP₃ changes were shown to be unaffected by PI-PLC inhibitor, in contrast to long-term changes, suggesting the involvement of different pools of PI-4,5-P₂ and associated PI-PLC in different phases of IP₃ generation that may not be equally sensitive to the inhibitor (Perera et al. 2001). These results may suggest that different mechanisms of PI-PLC regulation and possibly distinct PI-PLC isoforms are involved in gravity signalling.

Biochemical and genetic studies provide stronger evidence that PI-PLC and IP₃ have major roles in gravity signalling. Inhibition of PI-PLC activity and gravity-induced IP₃ accumulation leads to a delay in the formation of auxin gradients in the root apex and in pulvini (Perera et al. 2006). Root and pulvinus gravitropism and the accompanying gravity-evoked changes in gene expression are also delayed and decreased by PI-PLC inhibitors (Andreeva et al. 2010; Salinas-Mondragon et al. 2010; Yun et al. 2006). The ectopic expression of human inositol polyphosphate phosphatase in *Arabidopsis* interferes with the IP₃ levels in the plant tissues. It dramatically reduces the gravitropism of roots, hypocotyls, and

inflorescence stems (Perera et al. 2006). The usually rapid gravity-dependent gene expression is also reduced (Perera et al. 2006; Salinas-Mondragon et al. 2010). Defective root gravitropism in *mips1* (*myo*-inositol-1-phosphate synthase, MIPS1) mutant is due to dysfunction of PIN2 trafficking and root columella cells organization (Chen and Xiong 2010). On the contrary, plants lacking the inositol polyphosphate 5-phosphatase 13 (*AtInsP5tase13*), *AtInsP5tase6*, or inositol polyphosphate 1-phosphatase (*SUPO1*) genes show an enhanced gravitropism, with elevated cellular IP₃ levels and accelerated recycling of PIN1 and PIN2 auxin transporters to the plasma membrane (Wang et al. 2009; Zhang et al. 2011a). Elevation of the IP₃ levels in the mutant stabilizes PIN2 protein levels via mechanism that is likely transcription-independent and possibly calcium-mediated. As result an increased lateral redistribution of auxin can be observed (Zhang et al. 2011a). Results of pharmacological studies show that IP₃ action in gravity response is calcium-mediated and the second phase of calcium increase is dependent on PI-PLC production of IP₃ (Perera et al. 2001; Plieth and Trewavas 2002; Toyota et al. 2013a). Therefore, PI-PLC and IP₃ are regulators of gravity-dependent auxin transport.

Table 2 Localization of regulatory and signalling components in gravity responses of different plant organs

Signalling component	Organ							
	Root	Shoot	Hypocotyl	Pulvinus	Spike	Coleoptile	Protonema	Rhizoid
Ca ²⁺	+	+					+	
PI-PLC	+	+	+	+				
Peripheral membrane proteins	+	+	+					
v-SNARE		+						
PA-PLA ₁		+						
ADK1	+							
Ubiquitin ligase		+						
Transcriptional factor		+		+				
Actin	+	+	+		+		+	+
Tubulin						+	+	
NO	+							
ROS	+			+				
pH	+			+				

It should be noted that interfering with PI-PLC activity or IP₃ levels in inhibitor and genetic studies does not fully abolish gravitropism. Rather the time course of the response is delayed and the magnitude of the response is decreased (Andreeva et al. 2010; Perera et al. 2001, 2006; Salinas-Mondragon et al. 2010; Yun et al. 2006). Taken together, these results indicate that PLC may act as one of the primary components in gravity signal transduction in graviperceptive cells and as a regulator of polar auxin transport in gravitropic response zones.

Phosphatidylinositol phosphate kinases

Polyphosphoinositides are the phosphorylated forms of phosphatidylinositol (PI) that may also contribute to gravity-induced biochemical asymmetry that induces auxin redistribution. PI-4-P is formed through the phosphorylation of membrane phospholipid PI by phosphatidylinositol 4-kinase, whereas PI-4,5-P₂, a substrate of PI-PLC, is generated by subsequent phosphorylation of PIP by phosphatidylinositol monophosphate 5-kinase (PIP5K) (Delage et al. 2013). An

Table 3 Role of different regulatory and signalling components in gravitropism

Signalling component	Organ					
	Root		Shoot		Pulvinus	
	Upper side	Lower side	Upper side	Lower side	Upper side	Lower side
PI-PLC	+		+		+	
Ca ²⁺ channel					+	
MAPK	-	+				
Protein kinases/phosphatases					+	
H ₂ O ₂	-	+			+	No
PA	+					
pH increase ^a	+				+	
Actin	-		-			
Actin, myosin			+			

+ Stimulation, - inhibition

No no effect

^a pH increase in statocyte cytosol

Table 4 Participation of regulatory and signalling components in different phases of gravity responses

Component	Susception	Signal transduction	Response
PGM1	+		
SGR2-6	+	p	
SGR9	+		
LPA1	+		
ACTIN2	+	p	+
FtsZ3	+		
Ca ²⁺	p	+	+
PI-PLC		+	
PIPK1/2		p	+
FAB1A/B			+
PPLA-I-1			+
ARG1/ARL2	p	+	
ADK1		+	
MAP kinase		+	+
LA1			+
PLD ζ 2			+

p Possible role

increase in PIP5K activity in membranes of elongating lower halves of pulvini occurs at the same time as the second phase of IP₃ increase (Perera et al. 1999). Transgenic *Arabidopsis pip5k2* plants had a delayed root gravitropic response that could not be bypassed by auxin application. In these mutants, there was less auxin in roots, and PIN2- and PIN3-containing vesicle cycling in non-stimulated cells as well as PIN2 redistribution in gravistimulated cells were suppressed. The resulting limited PIN2 redistribution and basipetal auxin transport could be the cause of the delayed gravitropic response. PI-4,5-P₂ treatment of wild type and *pip5k2* plants indicates that the PIP5K2-generated product in wild type positively regulates vesicle transport. As PI(4,5)P₂ binds to all endocytic clathrin adapters, it was also proposed that PIP5K2 may regulate PIN transport by stimulating the assembly of clathrin-coated vesicles (Mei et al. 2012). During root and hypocotyl gravitropism, PIP5K1 and PIP5K2 both positively regulate membrane association of PIN1/PIN2 transporters and basipetal auxin transport by modulating clathrin-mediated endocytosis (Ischebeck et al. 2013). Therefore, the role of these enzymes may be connected with auxin transport and/or action. However, it is possible that PIP5K may have an overall effect on constitutive vesicle transport in cells and cell growth regulation (Sousa et al. 2008). So, the observed gravitropism effects seen in the *pip5k* mutant may be indirect. More evidence is needed then to prove that the PIP5K connection to PI-PLC has a direct role in primary gravity signalling responses in gravisensitive cells.

A role of 3'-phosphorylated phosphoinositides in gravitropism is also possible. PI-3-P is formed in result of PI

phosphorylation by phosphatidylinositol 3-kinase, whereas PI-3,5-P₂ is a product of phosphatidylinositol-3-phosphate 5-kinase activity (Gao and Zhang 2012). Genetic studies with phosphatidylinositol-3-phosphate 5-kinase gene FAB1A/B and inhibition of phosphatidylinositol 3-kinases by wortmannin also indicate that these enzymes have a positive role in gravitropism, auxin action, and sorting of PIN2 to the vacuole in gravity response zones (Hirano et al. 2011; Kleine-Vehn et al. 2008, 2010). PI3P is a well-known negative regulator of vacuolar fusion and wortmannin treatment restored gravitropic response of v-SNARE protein VTI11 mutant hypocotyls (Zheng et al. 2014). As this protein participates in membrane fusion and its mutation impaired sedimentation of amyloplasts due to increased vacuolar fragmentation, phosphatidylinositol 3-kinases and their product may participate in regulation of amyloplast movements during gravity perception.

Phospholipase D

Phospholipase D (PLD) hydrolyses structural membrane phospholipids generating phosphatidic acid (PA), a well-known second messenger of signalling pathways (Liu et al. 2013). PLD isoenzymes in plants constitute a heterogeneous family. They differ in biochemical properties and roles in plant cell physiology (Kolesnikov et al. 2012; Kravets et al. 2010). The expression of PLD ζ 2 gene in the root elongation zone of *A. thaliana* coincides with sites of auxin accumulation and response. Root gravitropism, auxin sensitivity, cycling of PIN2-enriched vesicles, and vesicular secretion of auxin were blocked in *pld ζ 2* knockout plants. However, all these phenotypes were increased by PLD ζ 2 over-expression or PA treatment of wild-type plants (Li and Xue 2007; Mancuso et al. 2007). The effects of *n*-butanol, a PLD substrate that produces phosphatidylbutanol to the detriment of PA (Rainteau et al. 2012), on root gravitropism are stronger than the effects of the *pld ζ 2* mutation, suggesting that other PLD isoenzymes may also be involved (Li and Xue 2007). However, many regulatory aspects of this enzyme class in stem gravitropism and gravity signalling remains to be investigated.

As the targets of the PA generated by PLD in response to gravity are unknown, it is reasonable to look at other studies in which the lipid may modulate protein kinase/phosphatase activity regulating rapid PIN transport and hence growth regulation (Fig. 2). PA accumulation induces membrane targeting and activation of protein phosphatase 2A (PP2A). PP2A induces dephosphorylation of PIN proteins and modulates their polar localization (Gao et al. 2013). PA also activates phosphoinositide-dependent protein kinase (PDK1), a phosphoinositide-dependent protein kinase that modulates PINOID (PID) Ser/Thr protein kinase activity, subsequent regulation of PIN phosphorylation and polar targeting (Anthony et al. 2004; Sukumar et al. 2009; Zegzouti et al. 2006). In addition, PA may stimulate endocytosis of auxin transporters

regulating tropism (Galvan-Ampudia et al. 2013). Also, PA may regulate gene expression in response to gravity as it is known that some MYB transcription factors modulate growth in response to this lipid (Yao et al. 2013).

Guanosine-nucleotide exchange factors are important regulators of PLD in animal cells (Kolesnikov et al. 2012). Genetic and pharmacological studies indicate that gravity induces the recruitment of PIN3 to the polar targeting pathway governed by GNOM, a guanosine-nucleotide exchange factor of ADP-ribosylation factors. These factors generate asymmetric PIN3 relocalization in graviperceptive cells (Kleine-Vehn et al. 2010; Rakusová et al. 2011). Nucleoside-diphosphate kinase, an enzyme that may generate GTP for these factors, is the only protein that was not found to be properly targeted to plastids in roots of *toc132*, the gravitropic mutant of receptor component of chloroplast membrane translocon (Strohm et al. 2014). Overall, these factors are likely to act early in gravity signalling in plant statocytes.

Phospholipase A

Phospholipase A enzymes hydrolyze phospholipids at the *sn-1* or *sn-2* positions generating free fatty acids and lyso-phospholipids. These enzymes are divided into the phospholipase A₁ and phospholipase A₂ classes. The phospholipase A₁ class is divided into five groups based on the structure and substrate specificity of the enzymes. One of the groups is PA-hydrolyzing phospholipase A₁. The phospholipase A₂ class includes secretory (two groups) and patatin-related (three groups) enzymes (Chen et al. 2011). Genetic studies of the secretory phospholipase A₂β in relation to the auxin sensitivity of its gene expression indicated that the enzyme is needed for stem and hypocotyl gravitropism and auxin action during gravity response (Lee et al. 2003). A knockdown mutant of patatin-related phospholipase A-I (*ppla-I-1*) was shown to have delayed gravitropic responses in roots, hypocotyls, and flower stalks (Scherer and Pietrzyk 2014). A possible reason for this is that the enzyme mediates auxin-induced gene expression (Effendi et al. 2014).

Abnormal amyloplast sedimentation in graviperceptive endodermal cells and dramatic reduction in the gravitropism of inflorescence stems, hypocotyls, and petioles were observed in *shoot gravitropism (sgr) 2* plants (Kato et al. 2002; Mano et al. 2006; Morita et al. 2002; Toyota et al. 2013b). The protein sequence of SGR2 is homologous to PA-hydrolyzing phospholipase A₁. It contains a transmembrane domain and is localized to vacuolar membranes (Kato et al. 2002; Morita et al. 2002). According to electron microscopy studies of *sgr2* mutants, SGR2 modulates rigidity and elasticity of vacuolar membranes in the stem endodermis indicating that hydrolysis of PA may regulate vesicular trafficking during gravity sensing (Morita et al. 2002; Saito et al. 2011). Early induction of auxin response genes in response to gravistimulation in

inflorescence stems was also shown to be positively regulated by SGR2 (Taniguchi et al. 2014). This strong genetic evidence for phospholipase A action in early gravity signalling events needs more confirmation at the biochemical level.

Cytoskeleton

The cytoskeleton is known to participate in cytoplasmic streaming, organelle motion, mitosis, cytokinesis, endocytosis, exocytosis, and intracellular transport, all activities that are potentially gravisensitive (Capriano 1993). The cytoskeleton and extracellular matrix are indispensable for cellular and developmental processes that are directly or indirectly linked through the intermediary of the cell membrane, with the macromolecular organization of these three compartments making them sensitive to gravity.

In root statocytes, there is a very thin actin network where single filaments are arranged in webs or fine bundles. Amyloplasts are enmeshed in this actin network, with their envelopes being in contact with actin filaments (Driss-Ecole et al. 2003). Amyloplasts are located in actin hollows, which may form the cages confining these organelles (Zheng et al. 2015). This arrangement suggests that the actin network could be easily destroyed during amyloplast sedimentation. Artificial disruption of actin filaments results in a more rapid and free diffusive sedimentation of amyloplasts after an orientation change and increased gravisensitivity in roots, hypocotyls, and inflorescence stems (Hou et al. 2003, 2004; Nakamura et al. 2011; Saito et al. 2005; Yamamoto and Kiss 2002; Yoder et al. 2001; Zheng et al. 2015). Similarly, the *act2-5* mutation in the *ACTIN2* gene induces hypersensitivity to gravitropic stimuli and mutation in *ACT8*-facilitated amyloplast sedimentation (Lanza et al. 2012; Nakamura et al. 2011). Actin-disrupting drugs also lead to the persistence of gravitropic bending, prolonged root cap statocyte alkalisation, and lateral auxin gradient formation. It also reduces the time necessary for plants to become committed to bending (Hou et al. 2003, 2004). Artificial actin destabilization also increases gravitropism of slowly responding decapped roots, in contrast to intact roots (Mancuso et al. 2006). This demonstrates the existence of another site of gravity perception outside the root cap that depends on a high rate of actin turnover. In contrast, drug-induced stimulation of actin polymerization was shown to compromise root bending (Mancuso et al. 2006). Therefore, the actin cytoskeleton may play a negative role in gravity perception, or a positive role in stopping the graviperception or response. These results are consistent with the “tensegrity model” of amyloplast-cytoskeleton interaction during gravity signal transduction. In this model, collective pressure exerted on the actin cytoskeleton by settling amyloplasts may transduce a signal to membrane-localized stretch receptors or ion channels (Perbal et al. 2004).

It is possible that actin may regulate the timing of gravitropic bending when columella cells attain a specific angle to the gravity vector (Hou et al. 2004). It was also suggested that the actin cytoskeleton may be required to control the duration and amplitude of the initial stages of gravity signalling in roots to ensure a correct root graviresponse (Hou et al. 2003). Given that during seed germination, gravity perception does not require a fully developed actin cytoskeleton, actin may also participate in resetting the gravitropic signalling by impeding amyloplast sedimentation in reoriented roots, relying on saltatory (non-stimulated) amyloplast movements, but not by direct gravity perception in roots and stems (Hou et al. 2004; Ma and Hasenstein 2006; Nakamura et al. 2011; Saito et al. 2005). Curvature and partial fragmentation of actin microfilaments detected in *Arabidopsis* root elongation zone in response to 15–60 min of gravistimulation were explained by their involvement in the mechanism of extensive cell growth during gravibending (Pozhvanov et al. 2013).

Actin may contribute to gravitropism via its involvement with membrane transport and regulation. In contradiction with data mentioned above, the disruption of actin in hypocotyls retarded amyloplast movement, in parallel with a decrease or increase in the gravitropic response (Friedman et al. 2003). In the latter example, an increased contact of amyloplasts with the tonoplast may increase the interaction with a hypothetical gravity receptor (Palmieri and Kiss 2005). Dramatic reductions in inflorescence stem and hypocotyl gravitropism were observed in a series of loss-of-function mutants in PA-hydrolyzing phospholipase A₁, t-SNARE protein VAM3, v-SNARE protein VTI11, DnaJ-domain-containing peripheral membrane protein GRV2, and HEAT repeat-containing protein SGR6 (Hashiguchi et al. 2014; Kato et al. 2002; Mano et al. 2006; Saito et al. 2005, 2011; Silady et al. 2004, 2008; Yano et al. 2003). These gene products are involved in membrane trafficking from the Golgi apparatus to vacuoles and protein sorting. In the knockout mutants, loss of vacuolar membrane dynamics and flexibility interferes with the configuration of cytoplasmic spaces which impedes and delays amyloplast sedimentation. Although root gravitropism was not affected in these mutants, data from chemical genomics indicate a possible role of vacuolar morphology also in root gravitropism (Surpin et al. 2005). Therefore, the mutant phenotypes could be mediated by disruption of vacuolar membrane interactions with F-actin. However, the real molecular link between actin and vacuolar membranes in gravity signalling needs to be identified.

A role of actin in gravity signalling in connection with membrane transport was advanced in other studies. ARG1 and ARL2 are specific DnaJ-like actin-binding proteins with outer membrane localization which are associated with components of vesicle transport pathways (Boonsirichai et al. 2003; Guan et al. 2003; Harrison and Masson 2008a; Sedbrook et al. 1999). They play positive roles in gravitropism

of *Arabidopsis* roots and stems by mediating gravity-induced cytoplasm alkalinisation, rapid PIN3 translocation to the lower side of statocytes, and auxin gradient formation in root cap columella cells (Fukaki et al. 1997; Harrison and Masson 2008b; Kumar et al. 2008; Luesse et al. 2010). In roots, ARG1 and ARL2 act in a pathway other than amyloplast sedimentation (Guan et al. 2003; Harrison and Masson 2008b). It is possible that these proteins modulate vesicle (e.g., PIN3-containing) transport by interaction with actin cytoskeleton. Direct interaction of ARG1 and ARL2 with HSP70 suggests they could be molecular adapters or co-chaperones that modulate folding, sorting, and protein complex formation of putative gravity signalling components (Harrison and Masson 2008a). TOC75 and TOC132, enhancers of *arg1* and *pgm1* phenotype, are components of an outer pore for protein import into chloroplasts that may play a role with ARG1 and ARL2 in gravity signalling according to a “ligand-receptor” model as proposed for gravity perception of *Chara* rhizoids (Limbach et al. 2005). ARG1 and ARL2 could modulate the location and activity of a putative receptor at the sensitive membranes that interact with possible plastid-localized and TOC75- and TOC132-transported ligands or non-ligand molecules, activating a signal transduction (Stanga et al. 2009; Strohm et al. 2014) (Fig. 2).

Another set of experiments has been done on gravitropism of snapdragon (*Antirrhinum majus* L.) spikes. Inhibitors of actin organization and myosin ATPases retard amyloplast sedimentation, auxin basipetal transport, and snapdragon spike gravitropism (Zhang et al. 2011b). Myosin ATPase inhibitor reduces gravitropic curvature and amyloplast movement only in hypocotyls of reoriented seedlings (Palmieri et al. 2007). It is suggested that during gravistimulation, amyloplasts remain connected with actin with the help of myosin as a motor protein. In this case, the actin filaments and myosin have an active role in facilitating amyloplast movement in response to gravistimulation. In this respect, spike gravitropism may resemble that of *Chara* rhizoids and moss protonemata, where active actomyosin-mediated movement of statoliths is required to direct them to graviperception sites of plasma membrane (Braun et al. 2002). In contrast, inhibition of myosin ATPase stimulated the bending of decapped maize roots but not of intact roots (Mancuso et al. 2006). A secondary site of root graviperception may function when myosin motor activity is weakened.

In stem statocytes of *A. thaliana*, the dynamic equilibrium of amyloplasts between sedimentation and saltatory movements is provided by their physical interaction with actin via SGR9 (SHOOT GRAVITROPIC RESPONSE 9). It is a plastid-localized C3H2C3-type ring finger protein with ubiquitin E3 ligase activity. During gravity perception, SGR9 facilitates amyloplast dissociation from actin allowing them to sediment. No changes in actin filament organization were observed in response to gravity, but in this model, actin serves as

a negative regulator of gravitropism. SGR9 also acts in root and hypocotyl gravitropism (Nakamura et al. 2011). WAV3 encodes another ring-finger E3 ligase. Roots of the *Arabidopsis wav3* mutant displayed enhanced gravitropism, a phenotype similar to roots treated with an inhibitor of actin polymerization (Sakai et al. 2012). However, the molecular WAV3 connection with actin needs to be shown. Ubiquitin ligases like WAV3 may target actin-regulatory proteins for degradation, thus modulating amyloplast movement and gravitropism. Plants mutated in components of the ARP2/3 actin-related protein nucleating complex, a complex which binds to existing filaments to initiate their branching, exhibit defects in gravitropism (Reboulet et al. 2010; Zheng et al. 2015), and inhibited cooperative cage escape movement of root amyloplasts. This indicates their stronger actin-dependent cage confinement in the mutant (Zheng et al. 2015). Different proteins that regulate amyloplast interaction with actin or modulate actin-binding protein activity (ubiquitin ligases) implicate actin in different stages of gravity perception, whether in active directional movement of amyloplasts or their sedimentation.

Microtubules may also participate in gravitropism. Changes in microtubule orientation during 15–30 min in upper flank of coleoptiles and shoots are a primary response to gravity, not a secondary effect of cell growth, although they were proposed as a result of auxin action (Himmelspach and Nick 2001; Zhang et al. 2008). Artificial microtubule depolymerisation increases gravity-mediated amyloplast sedimentation in root caps (Baluška et al. 1997). However, artificial disruption of microtubules perturbed rice coleoptile gravitropism after 15 min. This is in striking contrast to the much later inhibition of gravitropism by auxin transport inhibitors (Gutjahr and Nick 2006). Short-time gravistimulation of moss protonemata induced localization of higher density of microtubules near the lower flank compared to the upper flank proximal to sedimented plastids. They were localized near the more elongated part of the tip and their artificial disruption that inhibited gravitropism did not prevent plastid sedimentation. This suggests microtubules participation in further gravity-induced growth responses (Schwuchow et al. 1990). It is not clear whether the tubulin cytoskeleton is needed for the relocalization of auxin transporters in gravity response sites or it may be primary involved in graviresponse. Disappearance of moss gravitropism in an FtsZ3 knockout points to the importance of a plastid-cytoskeleton interaction in gravity perception. This is because FtsZ3 is a GTPase related to eukaryotic tubulin that regulates chloroplast division (Martin et al. 2009). In *Euglena gracilis*, flagellar microtubules activity that is regulated by c-AMP-activated protein kinase A may be the target of gravitactic signal transduction (Daiker et al. 2011).

Investigation of rapid changes in cytoskeletal structure and dynamics in graviperceptive cells and their connection with

amyloplast movements and downstream signalling events will confirm whether the cytoskeleton is involved in gravity perception. Statocytes may induce physical pressure on the cortical cytoskeleton, stretching the cytoskeletal network when they sediment (Perbal et al. 2004). This may evoke calcium uptake into the protoplast through stretch-activated ion channels. Therefore, although the role of cytoskeleton in gravity perception is yet to be clearly established and the different data obtained are controversial, these cellular components certainly play a regulatory role in statocyte gravity signalling.

Calcium

Calcium is an important second messenger in many signal transduction pathways. Aequorin, a calcium-sensitive luminescent indicator, was used to show that a biphasic change in cytosolic calcium occurs in response to gravistimulation in *A. thaliana*. This effect was observed in hypocotyls and petioles, but not in cotyledons. The first peak of calcium was shown to be independent of the angle of orientation of the plant in the gravity field. However, the second peak is more robust increasing from 20 to 35 s and is dependent on changes in the seedling position relative to the gravitational vector (Plieth and Trewavas 2002; Toyota et al. 2008b, 2013a). The low level of fluorescence observed with this marker indicates that a limited number of graviresponsive cells are involved in the calcium changes or that these changes are localized in specific cellular compartments. In unicellular spores of the ceratodon *Ceratopteris richardii*, a transcellular calcium flux from the extracellular medium to the lower side of cells and from the upper side to the extracellular medium was detected by calcium-sensitive electrodes. This flux changes its orientation during 25 s of gravistimulation (Salmi et al. 2011). In cells of stem endodermis of the creeping *Chrysanthemum morifolium*, calcium deposition in cytoplasm was shown to be increased after 5 min of gravistimulation. Cell walls of the lower side of reoriented cells contained more calcium than that of the upper one (Zhang et al. 2011c).

The regulators of calcium transport during gravity signalling are far from understood. Exogenous calcium application enhances root and hypocotyl gravity responses, while inhibitors of mechanosensitive ion channels, calcium channels, and Ca^{2+} -ATPases interfere with gravitropism (Bushart et al. 2013, 2014; Perera et al. 2006; Salmi et al. 2011; Urbina et al. 2006; Zhang et al. 2011a). Specific inhibitors were also used to demonstrate that gravity induces stretch-activated channels to release ATP along the lower side of the spore cell. This ATP could further induce the opening of calcium channels by binding to purinoreceptors (Bushart et al. 2013). In *Arabidopsis*, gravity-evoked calcium changes are dependent on mechanosensitive ion channels and intracellular calcium stores, actin and PI-PLC (Braun and Richter 1999; Toyota et al. 2008b, 2013a). It is possible that calcium influx through

mechanosensitive ion channels may evoke PI-PLC activation that causes calcium efflux from intracellular stores. In contrast to *Chara protonemata*, the gravity-evoked calcium changes are independent on amyloplasts in *Arabidopsis*. This is known because the *eal1* mutant, which has no intact amyloplasts, retains the response to gravity-induced calcium changes (Braun and Richter 1999; Toyota et al. 2008b, 2013a). This fits the hydrostatic pressure model of gravity perception in plants where the weight of the cell protoplast is transmitted into changes in membrane tension resulting in channel activation in response to gravity. In *Chara* intermodal cells, calcium flux through dihydropyridine-sensitive and mechanosensitive channels is required for hydrostatic pressure and gravity-induced polarity of cytoplasmic streaming (Staves et al. 1992).

Pathways connecting calcium changes with mechanisms of auxin transport also remain to be investigated (Zhang et al. 2011c). The transient ion changes mentioned above may be converted and multiplied into long-lasting biochemical changes by calmodulins and calcium-dependent protein kinases/phosphatases, phospholipases, or calcium-responsive protein gene expression. One role of calcium in regulating gravitropism is through the modulation of PIN1 transport. Indeed, transgenic plants co-overexpressing artificial microRNA that collectively targeted nine genes encoding Ca^{2+} -ATPases had increased cytosolic calcium. These plants had defects in basal PIN1 localization in root tip epidermal cells disrupting gravitropic root growth. Protein kinase PID is an important regulator of auxin transport and is negatively regulated by calcium (Zhang et al. 2011a) (Fig. 2). However, the effect of calcium on rapid gravity-dependent PIN3 relocalization is unknown. Calcium may therefore be assumed to be upstream of auxin in gravity signalling, but pharmacological studies suggest that the first peak of the calcium response in stems and the plateau of the second response are both auxin-transport dependent (Toyota et al. 2008a). Calcium chelator-induced loss of root gravitropism was compromised in ACS7 (key ethylene biosynthesis enzyme) mutant, suggesting that ACS7 is involved in gravitropism downstream to calcium (Huang et al. 2013). Inhibitors of mechanosensitive ion channels reduce the sedimentation of starch-rich chloroplasts in gravisensitive stem cells (Friedman et al. 1998). So calcium could also be upstream of amyloplast sedimentation.

Protein phosphorylation/dephosphorylation

Results from pharmacological experiments suggest that MAP kinases play an important role in gravity response mechanisms acting at different stages of gravitropism in different organs (Clore et al. 2003). MAP kinases may also mediate the hydrogen peroxide effect on gravitropic bending (Liu et al. 2009). On the upper side of horizontally oriented maize roots, MAP kinases may play a negative role, whereas on the lower side, they positively regulate gravitropism (Clore et al.

2003; Liu et al. 2009). Gravity-dependent MAP kinase activity fluctuates between the upper and lower sides of pulvini (Clore et al. 2003). The relatively long time (75 min) needed for the MAP kinase activity to rise suggests that it only acts after the onset of gene expression or as the result of auxin transport/action. In maize roots, rapid reactive oxygen species (ROS)-dependent phosphorylation of ZmMAPK5 was shown to be evoked by gravistimulation (Joo et al. 2001). It is not known whether this effect is auxin-dependent though. Similarly, a 50 kDa protein with autokinase activity was shown to be phosphorylated during 5 min of gravistimulation in the lower halves of oat pulvini, whereas a 38 kDa protein was phosphorylated in the upper half (Chang and Kaufman 2000; Clore et al. 2003). The timing of this initial phosphorylation correlates with the minimal gravistimulation time needed to activate transduction pathways leading to the curvature response (5.2 min for oat pulvini). Although the phosphorylation of soluble 50 and 53 kDa proteins induced by gravistimulation is apparently rapid and regulated by calcium channel activity, it also depends on protein synthesis (Clore et al. 2003). Gravity during 3 min also induced calcium-dependent phosphorylation of 32 and 35 kDa proteins in corn roots, but translational dependency of this response is unknown (Friedmann and Poovaiah 1991). These results suggest calcium and ROS integration with protein phosphorylation during gravity signalling.

Inhibitor studies also indicate that different protein phosphatases (especially PP1 and PP2A) regulate the phosphorylation state of soluble 38 and 50 kDa proteins on different sides of oat pulvini in response to gravistimulation. This asymmetry was suggested to be important for gravibending (Chang et al. 2001; Chang and Kaufman 2000). However, the response to protein phosphatase inhibitor may be mediated through an inhibitor-promoted decrease in starch content (Chang et al. 2001). Accelerated gravitropic response of *rcn1* (a protein phosphatase 2A mutant) with affected auxin transport but not auxin response and ethylene signalling may suggest a negative role of this phosphatase in gravity signalling (Muday et al. 2006).

Other types of protein kinases/phosphatases with their respective regulatory mechanisms and targets could also be involved in gravity signalling. One example is ZmLA1. Loss-of-function of ZmLA1 is responsible for the *lazy* phenotype in hypocotyls and primary inflorescence stems. ZmLA1 was shown to interact with a putative protein kinase in the plasma membrane and stimulate lateral auxin transport (Dong et al. 2013; Yoshihara et al. 2013). Deep proteomic studies should further clarify the particular target proteins modified by phosphorylation during gravity signalling. For example, five serine-threonine phosphorylation sites in the hydrophilic loop of PIN3 were shown to be required for PIN3 plasma membrane targeting, auxin redistribution, and the action of PIN3 in root gravitropism (Ganguly et al. 2012). In *Arabidopsis*

hypocotyls endodermis cells, PINOID protein kinase negatively regulates gravity-induced PIN3 polarization and gravitropism (Rakusová et al. 2011).

Nuclear events

One of the possible gravity signalling components, adenosine kinase (ADK1), was shown to positively regulate rapid PIN3 relocalization and gene expression in response to gravity, suggesting a role for the *S*-adenosylmethionine methyl-donor pathway in gravity signalling. Gravistimulation for 12 min increased protein levels of ADK1, but the genome dependency of this event and pathways of its regulation have not been investigated (Yester et al. 2006; Young et al. 2006). Beside the genome-independent auxin redistribution, one of the possible outcomes of gravity signalling is the changes in gravity-responsive gene expression (Kimbrough et al. 2004; Yester et al. 2006). Specific nuclear-localized transcriptional regulators must exist for rapid transduction of gravity signal perception to gene expression. SGR5, a zinc-finger C2H2-type transcription factor, and LPA1, a transcriptional repressor, were shown to participate in the primary stages of gravity signal transduction in inflorescence stem and leaf pulvini statocytes, respectively (Morita et al. 2006; Wu et al. 2013). However, the mechanisms of activation and their roles in later gravity signalling events have not been investigated in depth. SGR5 positively regulates starch accumulation in amyloplasts, and LPA1 and SGR5 both contribute to the sedimentation rate of amyloplasts (Tanimoto et al. 2008; Wu et al. 2013). This suggests connection of nuclear events with amyloplast dynamics. Not only amyloplasts but also nucleus was shown to sediment in response to gravity in elongation zone of aquatic roots of *Limnobium* (Sack et al. 1994). However, the effect of nucleus as well as extracarp amyloplast sedimentation on gene expression is unknown. It is very important to state that, among the genes identified as sensitive to gravity and dependent on PI-PLC product, IP₃, there are not only nuclear genes but also genes encoded by chloroplast and mitochondrial genomes (Salinas-Mondragon et al. 2010). This makes the picture of gravity signalling even more complex, as we do not know how the regulators of organelle gene expression are connected to the other elements of the signalling network.

Concluding remarks

Gravitropism is a complex process starting with gravity perception and resulting into auxin relocalization and subsequent changes in cell growth. As studies of gravity signalling were mostly focused on model plants, other species may have evolved very different gravity sensing, signal transduction, and/or response strategies as well as pathways of evolution of gravity signalling mechanisms. Similarity and differences

between gravity signalling in different sensitive organs that are not only roots or stems (Tables 2 and 3) indicates existence of organ-specific similar or different signal transduction pathways and gravity sensing mechanisms. Therefore, the next question is how one signal (changes in orientation within the gravity field) is interpreted differently by plant organs or by the same organ at different developmental times or under distinct environmental conditions. Here, we considered different models that have been created to explain gravity perception and signal transduction mechanisms which have been added to and developed during the recent years (Fig. 2). However, these models contain many gaps and further research of them mostly increases new question delaying the drawing of an integrated view on gravity signalling. Taking the amyloplast statolith function as central, we proposed an integrated model of gravity signalling (Fig. 2) which pays special attention to roles of lipids and the cytoskeleton. As targets of PA generated by phospholipase D in response to gravity are unknown, it is possible that lipids may modulate protein kinase/phosphatase activity regulating rapid PIN transport. In addition, other possible signal transduction events need to be further studied: rapid NO burst in maize root apex, fast proteome changes in *Arabidopsis* inflorescence, and plastid-associated hydrogen peroxide accumulation and cytosol acidification in pulvini/root cap cytosol (Clare et al. 2008; Fasano et al. 2001; Johannes et al. 2001; Mugnai et al. 2014; Schenck et al. 2013).

According to recent investigation, the integrated model of gravity signalling mechanism may be wide and complex. However, lack of clear information about upstream signalling components, weakly studied relations between downstream different signalling pathways, their convergence that may be not only to auxin redistribution represent a challenge for further research. What structures in amyloplast bind a putative gravireceptor, what is the nature of this gravireceptor, how it initiates signalling pathway, and how this information from different settled plastids becomes to be integrated are also important questions. It may be that plastid detachment from unknown cellular structure, modification of the contacts or their interaction with nearby located specific structure after a short initial movement could be rapidly decoded by graviperceptive cell. In addition, study of plastid-mediated chloroplast-to-nucleus retrograde signalling in early gravity action should also be followed. As mechanisms of light-mediated chloroplast movement are well investigated, study of their nature and involvement in gravity-regulated plastid movement should also be conducted. Alternatively, apart from statoliths, possible role of integrins or possibly cell wall-associated receptor-like kinases in gravity perception should be also deeply studied. Close dissection of secondary gravity perception mechanisms and building of new or advanced models of amyloplast-dependent and independent pathways requires sensitive and specific tools to identify minor alterations in signalling in real time and non-invasive manner. It

is also possible that gravity vector reorientation may affect proper localization or dynamics of specific components of other signalling pathways in specific cellular compartments thus possibly inducing localized biochemical events. Changes in action or activity of different gravity signalling components in mutants (e.g., mutants of PI-PLC, PIPK, PLD) with altered gravitropic responses at the cellular and tissue level require more rapid and sensitive ways or methods to study (Table 4). Investigation of molecular targets of second messengers (e.g., PA, PIPs, DAG, IP₃, calcium) and their connection with protein phosphorylation pathways is also in a paramount importance. Identification of how cellular components, events, and generation of local signals are connected with amyloplast movement under gravity action in spatiotemporal manner may answer the question about how short-term signalling events are connected to long-term downstream tropic events. This could lead to understanding of how such molecular complexity underlies this rather simple growth behavior. How plants differentiate gravity signal from noise is another important question for future research. However, there are also well-known difficulties of studying only one gravitropism without contribution of other tropisms. Space flight experiments have contributed to our understanding of gravitropism, since mechanical stress and continuous unilateral gravity signal present on Earth interfere with tight analysis of gravity action components. Removal of the effective gravity vector in spaceflight and the administration of gravity in controlled periods of time by an on-board centrifuge have enabled investigations of different steps of gravitropism, for example, statolith sedimentation, actin interaction with amyloplasts, calcium currents polarity, and gravisensitivity thresholds. The absence of gravity in space affects plant growth and development, providing information about real functions of gravistimulus in plant biology on Earth (Correll and Kiss 2008; Correll et al. 2013; Paul et al. 2013).

In addition to auxin, other hormones like brassinosteroids are also well-known regulators of root and shoot gravitropism (Kim et al. 2007; Vandenbussche et al. 2013). Whether gravity induces rapid changes in brassinosteroid biosynthesis, transport, or action on genomic and non-genomic level remains to be studied. Early auxin-independent gravity-induced changes in transport and metabolism of jasmonic acid are known, but its regulation and detailed action mechanisms remain to be investigated (Gutjahr et al. 2005). Rapid and transient pulse of ethylene production in response to tomato gravistimulation and induction of gravicompetence of gravi-incompetent rye leaves suggest that ethylene formation and perception either constitutes a decisive part of graviperception mechanism or that it is a cardinal step within the transduction of the perceived gravity signal (Harrison and Pickard 1984; Kramer et al. 2003).

New tools derived from systems biology, including integrated genetic, genomic, proteomic, metabolomic, cell-biological, and biochemical approaches, should fill the gaps of known gravity signalling pathways, discover the others and find connection between them in the future. Attempts to integrate known gravity signalling mechanisms will help to create the overall picture of these primary cellular responses to gravity.

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