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Gravitropism mechanisms in single-celled organs and multicellular organs of plants

Porovnání mechanismů gravitropismu u jednobuněčných a mnohobuněčných orgánů rostlin

Bachelor's thesis

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval/a samostatně a že jsem uvedl/a všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 4.5.2022

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Abstract:

Plants react to various environmental stimuli by oriented growth. The growth responses are called tropisms. Gravitropism is a directed growth concerning the gravity vector. Plant shoots grow up, negatively gravitropically, to catch the light. Roots are positively gravitropic; they grow down to anchor the plant in the substrate and seek water and minerals. The process of gravitropism consists of three stages: signal perception, signal transmission, and growth response. These stages can all occur in a single cell or separately in different parts of a multicellular organ. Single-cell gravitropic systems are represented by algal rhizoids or moss protonemata. They need minimal signal transmission because gravity vector perception and growth response happen in the same cell. The multicellular systems, represented here by angiosperm roots, have a more robust signal transmission phase. This thesis compares mechanisms of plant gravitropism based on the two categories – single-cell vs. multicellular. Despite their different cellular arrangements, single-cell and multicellular gravitropism share several characteristics, such as statolith sedimentation, Ca^{2+} fluxes, pH changes, and altered vesicular trafficking. Still, the lack of knowledge about the single-cell systems and high inner variability within the categories prevents us from making reliable conclusions.

Keywords: gravitropism, rhizoids, roots, protonemata, statoliths, calcium, actin cytoskeleton

Abstrakt:

Rostliny reagují na různé stimuly z prostředí orientovaným růstem. Růstové odpovědi nazýváme tropismy. Gravitropismus je růst orientovaný ve vztahu ke směru gravitace. Rostlinný prýt je negativně gravitropní, roste nahoru, ke zdroji světla. Kořeny jsou pozitivně gravitropní, rostou dolů, aby zakotvily rostlinu v substrátu a našly vodu a minerály. Celý proces gravitropismu se skládá ze tří fází: zachycení signálu, přenos signálu a růstová odpověď. Tyto fáze mohou buď proběhnout v rámci jedné buňky, nebo odděleně každá v jiné části mnohobuněčného orgánu. Reprezentanti jednobuněčných gravitropních systémů jsou rhizoidy řas nebo protonemata mechů. Tyto si vystačí s minimální transmisní fází, protože k růstové odpovědi na gravitační signál dochází ve stejné buňce, v níž signál vznikl. Mnohobuněčné systémy, tady zastupované kořeny krytosemenných rostlin, mají transmisní fázi více vyvinutou. Tato práce porovnává mechanismy gravitropismu mezi těmito dvěma kategoriemi (jednobuněčný vs. mnohobuněčný). Přestože jsou z hlediska uspořádání buněk tolik rozdílné, spojuje je několik společných znaků, jako je sedimentace statolitů, toky Ca^{2+}, změny pH a změny v transportu váčků. Nedostatek informací o jednobuněčných systémech a velká vnitřní variabilita ve vymezených kategoriích ovšem neumožňují vyvodit spolehlivé závěry.

Klíčová slova: gravitropismus, rhizoidy, kořeny, protonemata, statolity, vápník, aktinový cytoskelet

Contents

1. Introduction

Gravity is a unique environmental factor, given its omnipresence and permanency. The life on Earth is influenced by gravity since it emerged. The gravity vector and force provide a constant, unchanging signal, reliable even when other environmental conditions are currently absent or rapidly changing. Therefore, many organisms have developed means to sense gravity and use the gravity vector for their orientation in space. Plants react to various stimuli by oriented growth, so called tropisms. Growth oriented on the basis of gravity vector is called gravitropism. Shoots grow negatively gravitropically to catch the light for photosynthesis, while roots grow positively gravitropically to the substrate to anchor the plant and seek water and nutrients.

Even though gravity acts on all equally, more than one way to sense it and response to it has developed. One apparent difference is in the arrangement of the gravitropic apparatus. Some plants perceive gravity and respond to it within a single cell, usually growing by tip growth. In contrast, other plants divide sensing and response between two different tissues. This implies that they must utilize some additional mechanisms of intercellular signal transduction and coordinated response to the gravitropic stimulus. On the other hand, dividing the process into more cells could allow better specialization of every single of them for a specific function.

In this thesis, I aim to explore this phenomenon by focusing on the Streptophyte lineage of green plants. In the first part, I describe the mechanisms of single-cell gravitropism. I have chosen three representatives, one alga and two mosses, which are the most examined in this regard. In the second part, I point out the crucial moments of far more intensively studied multicellular gravitropism, focusing only on the roots of euphyllophytes (*i.e.* vascular plants that have true leaves). The last part is dedicated to the comparison of these two gravitropic systems. I will track their structural and mechanistic similarities.

2. Single-cell gravitropism

2.1 General mechanisms of tip growth

In this chapter, the most important phenomena facilitating and characterizing tip growth will be introduced. In rhizoids of algae and mosses, this phenomenon is not so well examined. More research has been done on pollen tubes and root hairs of higher plants. Nevertheless, based on the information yet known about tip growth in rhizoids and their similarity to root hairs and pollen tubes, we can assume that the basic principles will be shared for all the model systems (Bibeau *et al.*, 2021). Still, it is important to keep in mind that there are some differences. For instance, pollen tubes and root hairs are not gravitropic, and pollen tubes are also under evolutionary pressure on the growth speed (Williams, 2012).

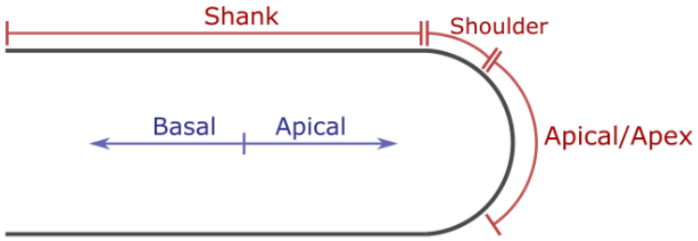

Figure 1 - Schematic representation of the termes related to the tip-growing cell

Tip growth is a very specialized type of cellular growth. The tip-growing cell is basically elongating in one extremely specific direction. The elongation by diffusion growth is done by loosening cellulose filaments deposited in the cell wall and then enlarging the cell's volume with the help of turgor (Braidwood, Breuer and Sugimoto, 2014). The tip-growth, in contrast, is based on a constant deposition of the new cell wall components into one region of the plasma membrane. The result of this type of elongation is a relatively thin and long, highly polarized cell of a typical tubular shape (Derksen et al., 1995; Guo and Yang, 2020).

The tip-growing cells have a unique inner organization. The large organelles, such as the nucleus and vacuole, are located in the middle- or basal part (Fig.1) of the cell. As tip growth is based on targeted delivery of cell wall material, the apical and subapical parts of the cell consist primarily of functionally related structures: ER, Golgi bodies, endosomes, multiple vesicles containing components of the cell wall, and cytoskeletal elements – actin filaments and microtubules (Lancelle and Hepler, 1992). Actin filaments guide the secretory vesicles to the tip and endocytosed vesicles away from the tip. They are oriented mainly in the longitudinal direction and are present in both the cortical and inner regions of the cytoplasm (Geitmann and Emons, 2000). Vesicles come to the tip along the cortical zone and leave

the apical region through the inner cytoplasm, by which they create a "reverse fountain" streaming (Derksen *et al.*, 1995). It is important to orchestrate the equilibrium between exocytosis and endocytosis to keep the right speed and direction of growth (Guo and Yang, 2020). In the pollen tube, the place of the most frequent exocytosis occurs at the very tip region and was found to correlate with the highest concentration of active small GTPase ROP1 (Rho-like GTPase of plants 1; (Luo *et al.*, 2017). This region is restricted by REN1 and REN4 (ROP1 *enhancer* 4) via deactivation and internalization of ROP1, respectively (Luo *et al.*, 2017; Li *et al.*, 2018).

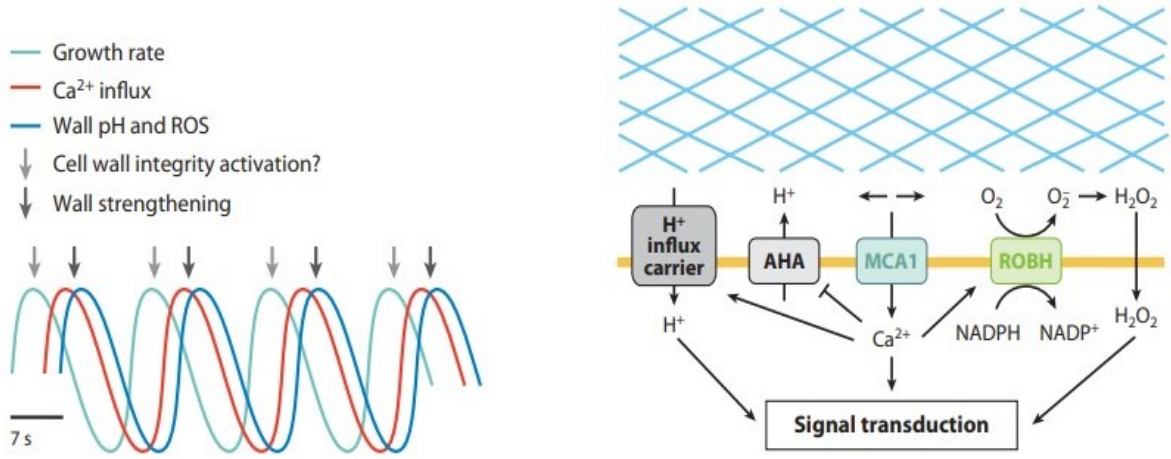

Figure 2 - Schematic representation of tip-growth regulation. The feedback loops create the phase shift and following oscillations of Ca^{2+} concentration, pH, ROS concentration, and growth rate, adapted from (Wolf, Hématy, Höfte 2012).

The centerpiece in regulating the growth rate is a Ca^{2+} gradient. The Ca^{2+} channels in the apical plasma membrane create a locally higher concentration of calcium ions in the tip. The concentration of the Ca^{2+} ions oscillates (Fig.2) and was indicated to be involved in the negative feedback regulation of ROP1 (Yan, Xu and Yang, 2009). Together they regulate the growth rate, which is also oscillatory (Li *et al.*, 2018). Changes in the Ca^{2+} concentration correlate with pH changes in the cell wall and regulate the production of reactive oxygen species (ROS). Both these factors play a role in the structure and strength of the cell wall (Wolf, Hématy and Höfte, 2012).

Apoplastic Ca^{2+} ions themselves are also involved in the cell wall assembly. In pollen tubes, pectins represent the main component of the cell wall. Pectins are deposited to the nascent apical cell wall via exocytosis in a methyl-esterified form, and in this form, they constitute a pliable region of the cell wall. In the shoulder region (Fig.1), pectins are demethyl-esterified by pectin methyl-esterases, allowing mutual cross-linking via Ca^{2+} ions and thus

toughening the cell wall. In the shank region (Fig.1), cellulose, hemicelluloses and callose are deposited to contribute to strengthening the wall, being more rigid than in the apical region (Chebli *et al.*, 2012).

The above-mentioned mechanisms of tip growth regulation were described mostly in pollen tubes, but the information about tip growth in algae and mosses is still sparse. The organelle localization is similar for all the organisms concerned in this thesis, and the organization of the cytoskeleton (although there are some deviances) is as well. The tip Ca^{2+} gradient was observed in both algae and mosses, but its role in tip growth is not described in such detail as it is in pollen tubes (reviewed by Braun and Limbach, 2006; Bibeau et al., 2021). The cell wall composition differs from pollen tubes. Cell walls of moss protonemata contain far fewer pectins and more cellulose (Berry *et al.*, 2016). Since pectins are important fast regulators of cell wall rigidity, it is possible that cells with walls containing less pectins developed another mechanism of cell wall strengthening.

2.2 Model organisms of single-cell gravitropism

Chara rhizoids and protonemata are often used models for studying single-cell gravitropism because they are large, transparent, and contain many statoliths, so it is easy to observe the inner processes (Braun, 2002). *Chara* is a genus of freshwater algae from the family *Characeae* in the Streptophyte branch of the green lineage. The genome of *Chara braunii* was published recently (Nishiyama *et al.*, 2018). Although the *Characeae* are closely related to land plants, they do not represent the sister clade (the Zygnematophyceae are now thought to hold this position; Fig.3; Turmel, Otis and Lemieux, 2006; Wodniok et al., 2011; Wickett et al., 2014). The gametophyte is multicellular and has an advanced structure consisting of the main axis and branches. While *Chara* is a multicellular organism, it has two types of tip-growing cells – rhizoids and protonemata. Both of these cells originate from nodes and grow only in the dark (Hodick, 1994). Rhizoids are positively gravitropic and anchor the alga in the substrate. Protonemata are negatively gravitropic, and they only develop when the thallus is buried in the substrate. Protonemata grow upwards through the sand, and when they reach light again, the tip-growth stops, the protonema cell starts to divide, and a new thallus is formed (Braun and Wasteneys, 1998).

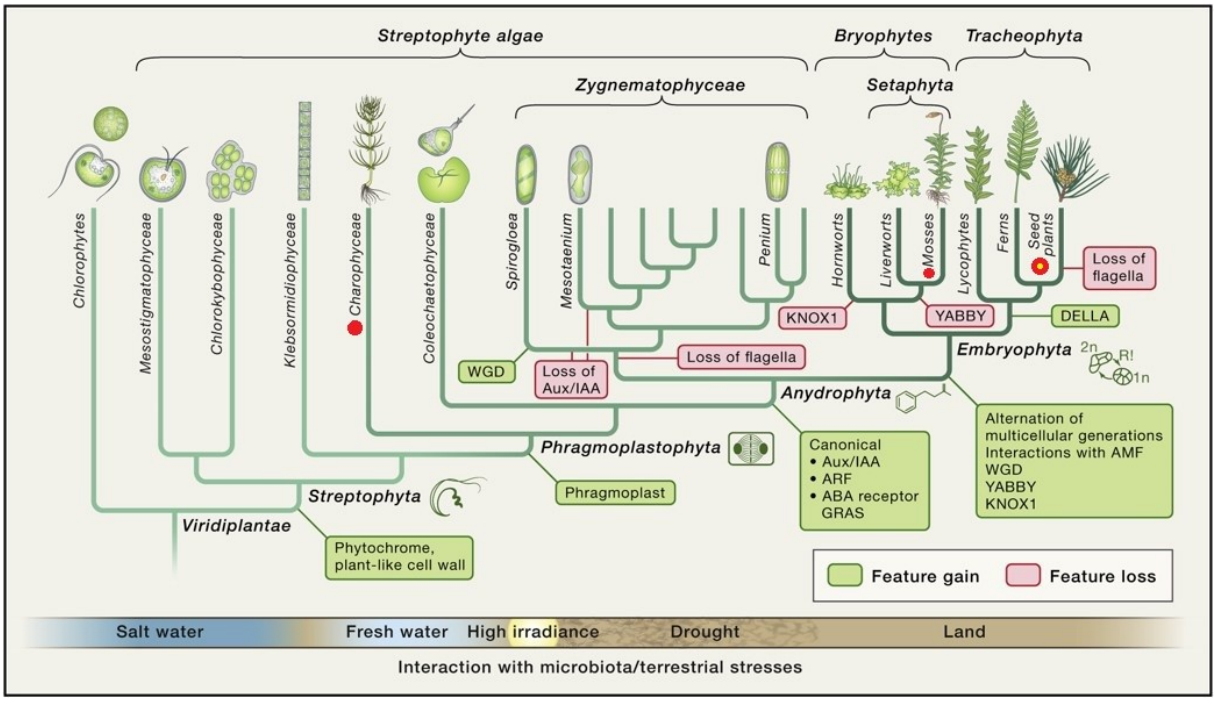

Figure 3 - Dendrogram illustrating the variability of Streptophytes and showing phylogenetic relationships of the examined organisms. The single-cell model systems are marked with red dot, the multicellular by red-yellow dot. Adapted from (Rensing 2020), modified.

The moss *Physcomitrium patens* (former name *Physcomitrella patens*) belongs to the *Fumariaceae* and represents mosses as a model organism in general. Two decades ago, it was discovered that it is easy to integrate transgenes into its genome by homologous recombination (Schaefer and Zrÿd, 1997). Moreover, single protoplasts can be regenerated into a whole new plant (Rensing *et al.*, 2020). In 2008, the nuclear genome was published (Rensing *et al.*, 2008).

Protonemata of *Physcomitrium patens* consist of two cell types – chloronemata and caulonemata. Chloronemal cells contain many chloroplasts, and their primary function is photosynthesis. In contrast, caulonemal cells contain less chlorophyll and are more involved in substrate colonization and seeking nutrition. Another difference is that chloronemal cells have perpendicular cross walls while the cell walls between caulonemal cells are oblique (Rensing *et al.*, 2020). In the absence of light, only caulonemata develop and grow negatively gravitropically (Cove *et al.*, 1978). Gametophores do not develop in the dark, but when already developed gametophores are put into darkness, they etiolate and show a negative gravitropic response. This means that *P. patens* allows us to study multicellular and single-cell gravitropic systems within the same organism. It was observed that some mutants with altered caulonemal gravitropism do not show changes in gametophore gravity response, so there must be at least

some gene products participating in gravity response that are not the same in single-cell and multicellular systems (Jenkins, Courtice and Cove, 1986).

Ceratodon is a moss from the Ditrichaceae family, a genus widespread all over the world. The species *Ceratodon purpureus* is also used as a model organism in investigating single-cell gravitropism. It can be cultivated under similar conditions as *P. patens* (Finiuk *et al.*, 2014) and it is capable of heterotrophic growth (it can use glucose as a source of carbon), which allows cultivation in the dark to avoid the effect of light to oriented growth (Thornton *et al.*, 2005). Protonemata perform negative gravitropism only in the dark; under directional illumination, they are positively phototropic. All wavelengths of visible light inhibit the gravitropic response. Red and far-red light induce phototropic growth. The effect of gravity can be studied only in infrared light since no measurable impact of these wavelengths on the gravitropic response was observed (Young and Sack, 1992). However, *Ceratodon* is not so commonly used model now. *P. patens* is preferred because it can be transformed with higher efficiency (Finiuk *et al.*, 2014).

2.3 Mechanisms of gravity sensing and response in single-cell organs

2.3.1 Inner organization of the gravity-sensing cell

Gravitropic cells have many specific adaptations to apical growth and to gravity sensing. As mentioned above, the common characteristic of apically growing cells is a tubular shape and position of the big organelles, such as the nucleus and vacuole in the middle or basal part of the cell, to leave the tip free for maintaining the growth. A characteristic common for most gravity sensing cells is the presence of the statoliths (*i.e.,* heavy sedimenting particles; (Schröter, Läuchli and Sievers, 1975; Sievers and Volkmann, 1977; Walker and Sack, 1990; Schwuchow, Kim and Sack, 1995; Braun and Sievers, 2000). Since gravitropic cell has two non-trivial functions, gravity sensing, and polarized growth, it is crucial for it to stay organized. However, the exact arrangement of the organelles differs among the studied species.
In gravitropic cells in general, approximately five zones can be distinguished (Fig.4):

- *Zone 1* is a zone of non-sedimenting organelles in the apical part of the cell
- *Zone 2* is a zone with very few to no statoliths that appears as an empty space on micrographs. Statoliths do not stay here for long; they just occasionally pass through in both directions. However, this zone is not an empty space but contains ER, Golgi, and other organelles important for cell growth (Furt *et al.*, 2012).

- *Zone 3* is the zone where statoliths show lateral sedimentation, the zone where the gravity signal originates
- *Zone 4* is the zone between the sedimentation zone (*Zone 3*) and the nucleus; it contains non-sedimenting organelles
- *Zone 5* is the most distal zone; it contains the nucleus and everything in a basal direction from it – vacuole and other organelles

(Schwuchow *et al.*, 2002)

Different species can have a different order of these zones, or some zone does not have to be present at all. In *Chara* rhizoids, *Zone 1* basically does not exist. The very apical part of the cell, where the plasma membrane is curved, is the apical dome. The apical dome of *Chara* rhizoids and protonemata contains the so-called Spitzenkörper, which is a growth organizing center. It consists of ER cisternae, many diverse secretory vesicles with the cell wall components, and actin filaments, which organize the vesicular transport (Limbach *et al.*, 2008). We can classify the extreme apex (less than 10 μm from the tip) as *Zone 2*, the "no statoliths zone". In *Zone 3*, there are 3-100 statoliths that can sediment in lateral directions, but in the axial direction, they are much more restricted. An equilibrium between the gravity force and a cytoskeleton-driven basipetal movement of the statoliths keeps them at a very exact distance (10-30 μm) from the apex (Hejnowicz and Sievers, 1981; Braun, 1997). In the basal part (more than 30 μm from the tip) of the cell, there is a nucleus, a large vacuole, and some plastids, which do not sediment (Kiss and Staehelin, 1993). In this part of the cell, there is also a quite strong cytoplasmic streaming that is not present in the apical part of the cell. This region could probably be divided into *Zone 4* and *Zone 5*, but the older literature about *Chara* uses a broader division to only three zones.

The *Chara* protonemata show the same zonation as rhizoids, except *Zone 3,* which can be a bit broader (Braun, 1997). The site of gravity perception is at the very tip, so after reorientation, statoliths move to *Zone 2* to trigger the gravity response (Fig.4C; Braun 2002).

The protonemata of the moss *Ceratodon purpureus* show a slightly different zonation. Up to 10 μm from the tip, there is *Zone 1*, which contains 2-10 plastids that act like statoliths in another zone, but do not sediment here. Then there is *Zone 2*, the statolith-free zone. 20-40 μm from the tip starts *Zone 3*, which contains the sedimenting statoliths, and continues up to 80-100 μm from the tip, where Zone 4 starts with the nucleus and other non-sedimenting organelles. (Young and Sack, 1992) The region basal to the nucleus we can call *Zone 5* even though older literature talks only about four zones (Kern *et al.*, 2001).

Apically growing protonemata of the moss *Physcomitrium patens* lack *Zone 1*, same as in *Chara*. The statolith-free *Zone 2* is located up to 20-40 µm from the tip, followed by *Zone 3*, which lasts up to 80-100 µm, where, same as in the *Ceratodon* protonemata, starts *Zone 4* and behind nucleus *Zone 5.* (Schwuchow *et al.*, 2002)

The position of organelles is maintained by cytoskeleton – actin microfilaments and microtubules, their organization is described in the following chapter.

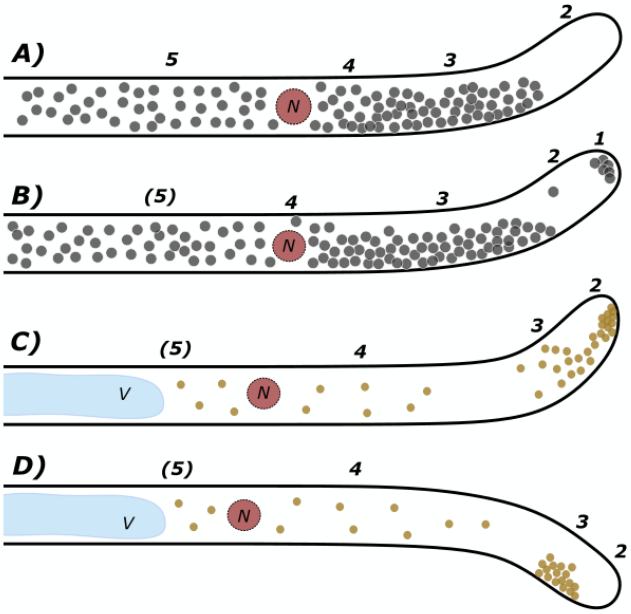

Figure 4 - Schematic comparison of the cytoplasmic zonation of different model systems. The drawings are not to scale for easier comparison of the zones. This figure represents the process of gravitropic response to accent the regions of statolith sedimentation. A) caulonema of *Physcomitrium patens,* B) protonema of *Ceratodon purpureus*, C) protonema of *Chara*, D) positively gravitropic rhizoid of *Chara*. Amyloplasts are represented by grey dots, yellow dots represent vacuoles with BaSO4, N – nucleus, V – vacuole. Gravity vector is to the bottom of the figure.

2.3.2 Organization and role of the cytoskeleton

The cytoskeleton of algal and moss gravitropic rhizoids is adjusted for maintaining the cell's polarity, tip growth, and gravity sensing. The alga *Chara* has two types of gravitropically growing cells – positively gravitropic rhizoids and negatively gravitropic protonemata. Interestingly, despite their opposite gravitropic reactions, *Chara* rhizoids and protonemata seem very similar in structure even though they show opposite gravitropic responses, so they will be described together in this text.

In the basal region of the gravitropic cell of *Chara*, there are parallelly organized thick actin bundles that generate the rotational cytoplasmic streaming. In the apical part, however, actin forms a three-dimensional (3D) web of predominantly axially oriented thin filaments that fill the volume of the cell and surround statoliths. There is a strong fluorescent signal of labeled

actin on the microphotographs correlating with the position of Spitzenkörper, which suggests that microfilaments participate in its structure (Braun and Wasteneys, 1998). As mentioned above, statoliths are nonrandomly distributed in the gravitropic cells; they show a specific longitudinal zonation. Hejnowicz and Sievers (1981) noticed that statoliths stay at the same distance from the tip and do not sediment as one could expect. An experimental treatment with cytochalasin B, the actin-depolymerizing drug, caused the sedimentation of statoliths to the tip. Based on this, they concluded that actin helps keep the dynamically stable position of statoliths in longitudinal directions so that statoliths stay in the region adapted to sense their lateral sedimentation. Later, myosin-like proteins were discovered on the statolith membrane, which supported the theory that statoliths are kept in place by the equilibrium between gravitational force and active actomyosin transport in the opposite direction (Braun, 1996).

Microtubules are not present at all in the apical region of the *Chara* rhizoid. In the subapical region, they form a complex 3D meshwork and in the basal zone are predominantly cortical and axially oriented microtubules (Braun and Wasteneys, 1998). However, microtubules seem to be essential for keeping the cell's polarity. When the microtubular cytoskeleton was experimentally disrupted, the organization of organelles was randomized as all parts of the cytoplasm got involved in cytoplasmic streaming ((Braun and Sievers, 1994), article not found, cit. from Braun 1997). Direct involvement of microtubules in gravitropism is though unlikely because the gravitropic response was not affected by microtubule-depolymerizing drugs until all organelles, including statoliths and Spitzenkörper, were involved in cytoplasmic streaming, the whole organization of the cell was broken, and growth stopped (Braun, 1997).

In contrast to *Chara*, microtubules fill the whole gravitropic cell in mosses, including the apical region. In *Ceratodon* protonemata, microtubules have primarily axial orientation. The distribution of the microtubule cytoskeleton seems to be influenced by gravitropism. In most protonemata, an area with a higher concentration of microtubules forms after reorientation near the lower flank (the one more expanding to produce curvature) of the amyloplast-free zone (proximal to sedimented amyloplasts; Schwuchow, Sack, Hartmann 1990). So microtubules are likely to be involved in the gravitropic response of *Ceratodon* protonemata. In addition, disruption of microtubules with oryzalin and APM (amiprophos methyl) disturbed the plastid zonation, so it seems that microtubules are connected to plastids and help them keep their position. On the other hand, microtubules seem not to be directly necessary for plastid sedimentation and gravity perception (Schwuchow, Sack and Hartmann, 1990).

Microfilaments in the *Ceratodon* protonemata fill the apical cell with a web of fine, primarily axially oriented filaments and mostly peripherally located thick actin bundles. Actin filaments extend even into the extreme apex. Peripheral microfilaments are more abundant than internal ones. In contrast to *Chara*, the dense actin array in the apical region of *Ceratodon* protonemata was not detected (Walker and Sack, 1995).

In caulonemata of *Physcomitrella patens,* microtubules also fill the whole apical cell. They form bundles that undulate throughout the cytoplasm in a mainly axial direction, surround the nucleus and other organelles, and form a three-dimensional network by crossing each other. In the apical part, the web is denser and composed of thinner filaments than in the basal part of the cell. Microtubules also fill the apical dome, where their ends converge (Doonan, Cove and Lloyd, 1985; Hiwatashi, Sato and Doonan, 2014). In contrast to the previously described organisms, there is evidence that microtubules are directly involved in the gravity response. A recently discovered minus-end kinesin KCHb, when mutated, reverses the direction of gravitropic bending. The exact mechanism is yet unknown, but it appears that activity of the kinesin results in upward shifting of a growth-organizing apical actin cluster (Li *et al.*, 2021).

Actin filaments are also present in the whole caulonemal cell, and in addition, they accumulate on a small limited area in the apical dome. It is not a static structure; a very rapid remodeling of the actin filaments was observed within the area (Vidali *et al.*, 2009). Wu and Bezanilla (2018) investigated the position of this apical actin cluster during the growth of a caulonemal cell, and they suggested that it predicts the direction of cell expansion. They also suggest that actin, together with myosin VIII, mediates the convergence of microtubule ends and that microtubules, in return, direct localization of formins, which are actin nucleation factors. In other words, actin and microtubules cooperate closely and together maintain the polarized growth (Wu and Bezanilla, 2018).

2.3.3 Statolith sedimentation

As statoliths, we term particles whose density is higher than the surrounding cytoplasm and even higher than other organelles (DARWIN, 1903). It is thought that the role of statoliths is to sediment to the current bottom of the cell and determine this way the orientation of the cell in space (the exact mechanisms will be minutely discussed below). In vascular plant models (in the protonema of the fern *Ceratopteris*) and in the moss protonemata described here, statoliths are represented by amyloplasts – plastids containing a high-density starch (Walker and Sack, 1990; Schwuchow, Kim and Sack, 1995; Driss-Ecole, Lefranc and Perbal, 2003). However, in *Chara,* small vacuoles with barium sulfate ($BaSO_4$) crystals act like statoliths. (Schröter, Läuchli and Sievers, 1975; Braun and Sievers, 2000)

The gravitropic structures of mosses are protonemata that creep on the ground and give rise to upright, leafy gametophores after some time. As protonemata do not naturally grow in the dark, but on the surface, they contain chloroplasts that allow them to photosynthesize. In the apical and subapical zones of the gravisensing cells of *Ceratodon purpureus* protonemata, both amyloplasts and plastids showing autofluorescence of chlorophyll were observed (Walker and Sack, 1995). However, it was not examined whether the same plastid can contribute to both the gravitropic sedimentation and photosynthesis or whether they are different organelles, each specialized for one function. The words "plastid" and "amyloplast" are used as synonyms in the literature. The role of plastid sedimentation in gravitropism was experimentally proven by (Kuznetsov *et al.*, 1999), who displaced plastids with magnetophoresis, leading to negative curvature of protonema concerning the direction of the magnetophoretic force.

In *P. patens* caulonemata, the sedimentation of plastids is so poorly pronounced that it was not even observed at first (Jenkins, Courtice and Cove, 1986). On a closer look, amyloplasts were found to sediment after all. Still, the amyloplasts were larger than in *Ceratodon* and so abundant that they almost filled the tip and their sedimentation was hardly possible to detect (Schwuchow, Kim and Sack, 1995). No more research has been done on the nature and sedimentation of *P. patens* statoliths, but it is tempting to speculate whether such weak sedimentation is sufficient for gravitropism or if *P. patens* uses some additional way to sense gravity.

According to *Chara* internodal cells, it seems unnecessary for gravity perception to contain statoliths at all. *Chara* has large cells with robust cytoplasmic streaming, which is generated by the movement of myosin motors along with actin cables. This streaming has a gravity-dependent polarity. The downward stream is ca. 10 % faster than the upward one in vertically oriented cells, whereas, in horizontally positioned cells, both streams have equal

velocities. Moreover, when the surrounding medium is denser than the cell, the cytoplasmic streaming is reversed, *i.e.*, the upward stream is faster than the downward one. Based on these observations, a protoplast-pressure model was suggested. According to this model, a cell can sense gravity by perceiving different tensions and pressures between the plasma membrane and cell wall on different areas of the cell's surface. In the medium of a high density, the cell is "floating" within its own cell wall (reviewed by Staves, 1997).

Amyloplasts seem to be the most common statoliths, maybe because starch-filled plastids are formed in plants anyway, as storage organelles. However, the *Chara* model shows that it is possible to have statoliths of a different nature or not to have statoliths at all. This raises the question of whether the starch-statolith and protoplast-pressure ways of gravity sensing can cooperate in fine-tuning the response or whether there are other structures that could act like statoliths. For example, the nucleus is dense enough to carry out this function, and it was observed to sediment along with amyloplasts in *Equisetum* (Ridge and Sack, 1992).

2.3.4 Gravity perception

As described above, statoliths keep a specific distance from the growing tip in a vertically oriented rhizoid or protonema. When the cell is rotated 90°, statoliths sediment to the lower flank because actin does not control the lateral movement of statoliths as much as it does in the longitudinal direction (Braun, 2002). It was assumed that sedimenting statoliths exert pressure on the plasma membrane or stretch actin filaments, and the signal is perceived mechanically. However, Braun (2002) noticed that when the cell is rotated at angles different from 90°, statoliths do not sediment straight down, but they are transported along actin filaments to a specific belt-like region 10-35 μm away from the tip in rhizoids, or directly into the tip in protonemata. It is necessary for the gravitropic response that statoliths touch the plasma membrane in the specific region. When they were forced (by optical laser tweezers) to sediment to a different place, the gravitropic response was not initiated (Braun 2002). At the same time, intensifying the force which statoliths exert on the plasma membrane (by centrifugation) does not enhance the gravitropic response. On the contrary, when weightless statoliths touched the responding PM region in microgravity, *i.e.*, exerting very little or no force, the gravitropic response was not weaker. This proves that contact of statoliths with the specific region of the plasma membrane and their contact with yet unknown receptors is more likely to act in gravity sensing than the pressure or tension exerted on some cytoskeleton components (Limbach *et al.*, 2005). Unfortunately, the molecular details as to what kind of receptors could

interact with statoliths and what kind of pathway they would activate, remain unclear. A hypothesis of how could the sedimented statoliths induce gravitropic curvature will be presented in the next chapter.

In contrast to *Chara* rhizoids and protonemata, the rate of curvature of *Ceratodon* protonemata is likely to depend on the rate of the sedimentation force. Kuznetsov et al. (1999) suggested it based on the observation that protonemata with more abundant and larger statoliths responded stronger to a magnetophoretic displacement of statoliths. Interestingly, a mutation of some undetermined gene or genes (mutant generated by UV radiation) is able to reverse the gravity response of the moss protonemata (Wagner, Cove and Sack, 1997). It is also worth reminding that amyloplasts in *Ceratodon* sediment in a region ca 30 μm from the tip, which may be too far for statoliths to directly alter tip growth as hypothesized for *Chara*. So is there any other mechanism of the signal transduction? Sack et al. (2001) suggested one, and it will be closer described in the next chapter.

Although *Physcomitrium patens* is among nonvascular plants a popular model organism, almost no information about its gravity perception is available.

2.3.5 Gravity response

Although *Chara* rhizoids and protonemata show so many similarities regarding their cytoplasmic zonation and organization of the cytoskeleton, they perform two remarkably different ways of gravity response.

In *Chara* rhizoids, statoliths sediment on the plasma membrane in the belt-like region 10-35 μm from the tip, which results in differential elongation of the upper flank and curve blandly by so-called "bending by bowing" (Fig.5). The position of the Spitzenkörper in the apex is not shifted, nor is an apical Ca^{2+} gradient (Braun and Richter, 1999). It was hypothesized that statoliths trigger some unknown receptors in the perception area or that the sedimentation of statoliths sterically blocks secretory vesicles from fusing with the plasma membrane, which would result in differential deposition of the new cell wall only to the upper flank of the cell (Sievers, Heinemann and Rodriguez-Garcia, 1979; cited from Braun and Limbach, 2006).

In *Chara* protonemata, the sedimenting statoliths are guided by actin filaments to the extreme apex, which is likely to disturb the structure of the growing region. It was observed that the calcium gradient relocalizes to a slightly asymmetric position in an upward direction, and so does also the Spitzenkörper. As a result, a bulge is formed in the new direction of growth,

and the protonema grows straight following this new direction. The bending is much more acute in comparison to rhizoids and is called "bending by bulging" (Braun and Richter, 1999).

Not enough research has been done on the mechanisms of a gravity signal transduction

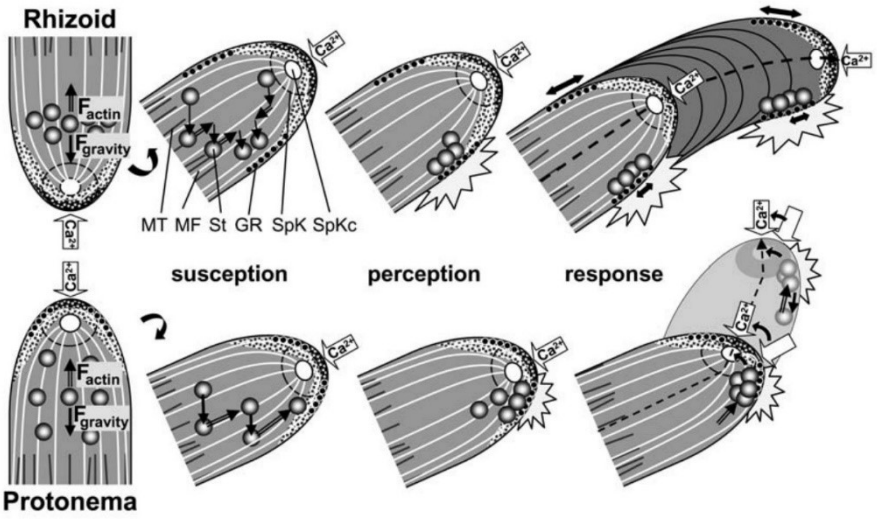

Figure 5 - Schematic representation of the difference between "bending by bowing" and "bending by bulging" of *Chara* rhizoid and protonema, respectively, adapted from (Braun, Limbach 2006)

and response to it in the model of *Ceratodon* protonemata. Basically, there are two theories of how the gravity response could function. The first hypothesis is that the mechanism is similar to *Chara* rhizoids or protonemata, *i.e.*, the statoliths sterically block exocytosis, or they mechanically shift the growth organizing center. This explanation is unlikely due to the long distance of the sedimenting-statolith region from the tip. Based on this assumption, Sack et al. (2001) came up with another hypothesis. They suggested that an ion (most likely calcium) current could flow inward in the apical region, and the sedimentation region would be the place of outflow. Sedimented statoliths would block the outflow on a specific flank, and this disturbance of symmetric ion flux would provide spatial information for the tip to change growth direction. However, this theory seems not to be supported by any experimental evidence.

Although the molecular mechanisms of gravitropism in *Ceratodon* have not been revealed, another interesting fact was spotted. Shortly after 90° reorientation, before the statoliths are fully sedimented, the protonema starts to curve downwards (the wrong way) at first, and it takes a several minutes before the correct negatively gravitropic response is established (the upward curvature is clearly noticeable 30-45 min after reorientation; Young and Sack 1992). The initial slight wrong way curvature was hard to detect on some of

the protonemata but was always present. Moreover, the positively gravitropically growing *wwr* (wrong way response) mutant mirrored the gravitropic response of the wild type, *i.e.*, it started to grow upwards at first and then performed the full positive gravitropism (Wagner, Cove and Sack, 1997). This surprising detail could be a valuable piece to the puzzle of understanding gravity responses. Unfortunately, no one has taken a deeper look at the molecular basis of this phenomenon yet.

Physcomitrium patens has the slowest gravitropic response among these models. The reorientation consists of two phases – in the first 12 h, protonemata reach the curvature of 15-30°; the second phase is much slower, and it can take more than 100 h until protonema reaches the 90°curvature (Jenkins, Courtice and Cove, 1986). In contrast, the *Ceratodon* protonemata reached the mean curvature angle of 84° in 24 h (Walker and Sack, 1990), and *Chara* protonemata reached the full 90° reorientation in about 3 hours (Braun, 1997). An interesting fact already mentioned above is that negative gravitropism of protonemata can be reversed to positive by a single-gene mutation of minus-end directed kinesin. When the kinesin is functional, the growth organizing center (formed by cross-linking of actin and microtubules) shifts upwards after reorientation. In the roots with the mutated kinesin, the growth organizing center shifts to the lower region of the cell. These observations imply that the transport of some yet unknown determines the negative gravitropic response of the protonema (Li *et al.*, 2021). Further details concerning the gravitropic responses of *Physcomitrium* still remain to be revealed.

2.4 Summary of the single-cell gravitropism

To sum up, although the research on this topic started decades ago, the knowledge is still fragmentary. The cytoplasmic zonation and cytoskeleton organization are thoroughly described and appear to be more or less similar in all mentioned models. Many discussions were held about the involvement of statoliths in gravitropism, and it was concluded that statoliths are necessary for gravity sensing or at least strongly contribute to it. However, the nature of statoliths and their distance from the apex differ among the model organisms. This might also be the case of the molecular mechanisms of gravitropism being still on a hypothetical level. Therefore, it is hard to make conclusions when the current understanding of the topic is far from complete. Nonetheless, maybe it is misleading to make any generalizations and conclusions about single-cell gravitropism because these models indicate a wide range of ways to respond to gravity.

3 Multicellular gravitropism

Plants have evolved root-like structures to anchor their bodies in the substrate and to take up water and nutrients. Nonvascular plants, such as algae, liverworts, and mosses, have filamentous rhizoids. Vascular plants have developed complex roots consisting of multiple layers and specialized cell types. Roots have evolved among vascular plants two times – in Lycophytes and in Euphyllophytes (*i.e.,* vascular plants with true leaves; Raven and Edwards 2001). This thesis will regard only the Euphyllophyte roots.

3.1 Anatomy of the root

Euphyllophyte roots are complex specialized structures consisting of 5 different radially organized cell files (Fig.6). On the surface of the root is the epidermis, the most exposed file, which is in direct contact with soil and provides mechanical and chemical protection. In the tip, the columella root cap and the lateral root cap cover the root tip and provide mechanical protection of the root. Under the epidermis, there is the cortex, which can consist of one (in *Arabidopsis*) or more layers of cells. The third layer is the endodermis, which provides additional protection. It is the place where we can find apoplastic barriers that force water and nutrients to undergo controlled transcellular transport (Robards and Robb, 1972). Under endodermis, there is the pericycle, a layer that gives rise to lateral roots. The most inner layer is the stele, which contains vasculature – elements of phloem and xylem – and cambium – populations of cells that maintain secondary thickening of the root (Dolan *et al.*, 1993).

The apical meristem of roots is characteristic for its two-directional activity. It produces the root "body" in one direction and the root cap in the opposite direction. Root cap function as mechanical protection when the root is pushing through the soil. Bábovka. On the interface between the "body" of the root and the root cap of *Arabidopsis*, there are four central cells. These cells do not divide; they function as a quiescent center (QC), a stable point in the middle of a dynamic area. QC is the organizing center maintaining the identity of surrounding stem cells, the initials of different tissues. The cells that abut the QC in a tipward direction are the columella initials (Dolan *et al.*, 1993). These are surrounded by initials shared by the lateral root cap and epidermis (Duckett *et al.*, 1994). The growth of the columella root cap and lateral

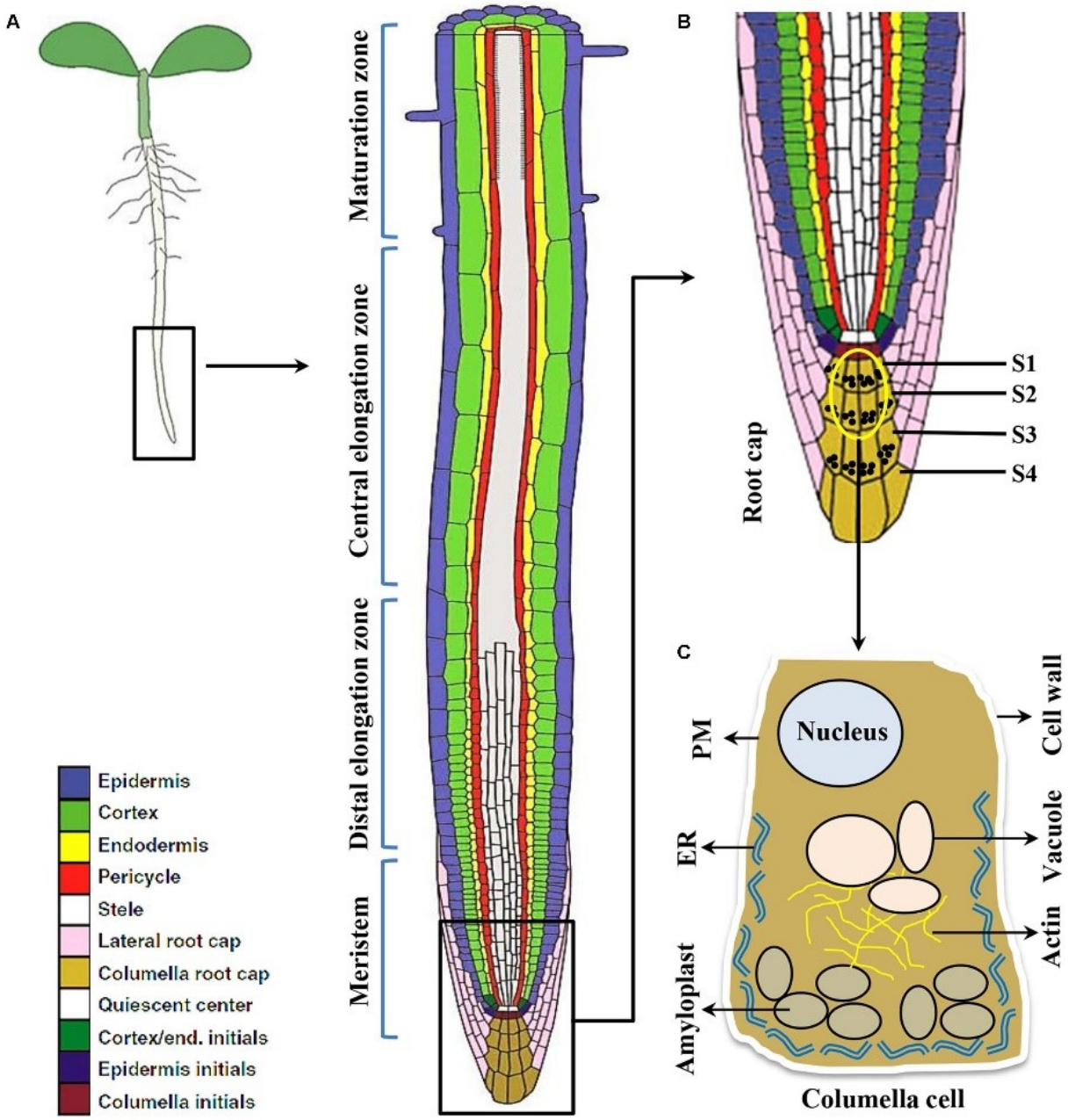

Figure 6 – Schematic representation of different cell files in the root and their arrangement, adapted from (Singh *et al.,* 2017).

root cap has to be synchronized even though they do not share the same ancestor. Shootward from the quiescent center, there is a layer of stele initials. In all lateral directions, the quiescent center is surrounded by initials common for cortex and endodermis (Dolan *et al.*, 1993). Stem cells divide asymmetrically – one of the daughter cells keeps the stem character, and the other one turns into a transit-amplifying RAM (root apical meristem) cell, which continues to divide rapidly (Heimsch and Seago, 2008).

In the axial direction, there are five zones of the root. The first one is the root cap (columella+lateral root cap), which functions as protection. The root cap cells are continually

rubbed down from the root tip, and new ones arise from the meristem. The columella cells from S1 and S2 tiers (Fig.6) function as statocytes in gravity perception (Leitz *et al.*, 2009). Then there is the meristematic zone, where cells divide dynamically, and all the tissues are established, followed by the transition zone, where the cells are preparing for fast elongation. The cells in this zone are also very sensitive to mechanical stimuli and to external auxin treatments (Verbelen *et al.*, 2006). The following zone is the elongation zone, which maintains the length gain. In the differentiation zone, the cells do not elongate anymore but run the specialized developmental programs to fully fulfill their fates. This is also the zone of the emergence of root hairs and even lateral roots (Duckett *et al.*, 1994).

Regarding gravitropism, the root cap zone is the place of gravity sensing, and the elongation zone maintains the gravitropic bending. The gravity signal originates in the S1 and S2 layers of columella cells and is transduced through the lateral root cap and epidermis to the epidermal cells of the elongation zone (Swarup *et al.*, 2005).

3.2 Model organisms of multicellular gravitropism

Arabidopsis thaliana, a small, inconspicuous dicot plant from the family *Brassicaceae*, is surely the most examined plant in the world. It was chosen as a model for molecular genetics research in the 1970s. In these times, researchers agreed that with a single model organism used by everyone, discoveries from various science fields could be gathered. *A. thaliana* is a useful model organism for its small size, short generation time, easy cultivation, and solid seed production through self-pollination (Koornneef and Meinke, 2010). Nowadays, *A. thaliana* has been the most popular model plant since 50 years ago, and its popularity is likely to rise along with the level of knowledge of it.

Oryza sativa and *Zea mays* (both *Poaceae*) are also often used as model plants. Rice was chosen as the second model organism at the end of the last century for many reasons. It is a staple food for more than half of the world's population, has a relatively small genome, can be transformed with high efficiency, and belongs to monocots, which complements the dicot *Arabidopsis* model (Izawa and Shimamoto, 1996; Wang and Han, 2022). *Zea is* also examined for being an important crop, very resistant to abiotic stress (*e.g.,* it has C4 photosynthesis; Yassitepe *et al.*, 2021).

Most experiments with gravity were realized on *A. thaliana* or rice. Some of them were, however, done also on maize, *Medicago truncatulata*, *Lepidium sativum,* and others. Still, in comparison with the single-cell gravitropic model systems, the model organisms of

multicellular gravitropism are evolutionarily very close to each other, so it is presumed that the mechanisms will be more or less shared for all of them.

3.3 Mechanisms of gravity sensing, transduction, and response in roots

Unlike in the single gravitropic cells, in the multicellular gravitropic systems, the gravity perception and growth response are spatially separated. The perception happens in specialized cells called the statocytes, located in the central part (tiers S1 and S2; Fig.6) of the columella root cap (Leitz *et al.*, 2009), while the gravitropic bending takes place in the elongation zone by elongating the cells on one root flank more than the cells on the opposite side of the root. This arrangement requires a middle step between signal perception and response –transduction of the signal for quite a long distance from the root cap to the elongation zone. The transduction is done through an asymmetric flow of the plant hormone auxin (Ottenschläger *et al.*, 2003). This topic has been studied and discussed a lot in the last decades; a lot is known about it, and more remains yet to be discovered. For the case of this thesis, the following chapters will focus on the topics comparable with single-cell gravitropism: the inner organization of the gravisensing cells, the role of the cytoskeleton, and the mechanism of sensing and transduction. The gravitropic bending of the root will be presented just briefly because it is not comparable with the single-cell systems.

3.3.1 Inner organization of statocytes

Root statocytes are specialized cells located in the tiers S1 and S2 in the central columella (Leitz *et al.*, 2009). The most typical structure in these cells are statoliths, represented by dense starch-containing plastids (same as in *Ceratodon*, but in contrast to *Chara*), which always sediment to the physical bottom of the cell (Sievers and Volkmann, 1977).

While in the non-differentiated meristematic cell, the nucleus is in the middle of the cell, and the other organelles are more or less randomly distributed around it; the statocytes are more polarized. The nucleus is located by the proximal wall (the one closer to the QC). In contrast, the nuclei of *Zea* fall down to the distal position, along with statoliths; unfortunately, it has not been revealed if the nuclei contribute to gravity perception (Baluška *et al.*, 1997). Statoliths fall onto multiple layers of ER, which are stacked near the distal (in relation to meristematic cells) cell wall (Sievers and Volkmann, 1977). The ER also rises along the lateral walls to form a cup-shaped structure at the distal end of the statocyte (Perbal and Driss-Ecole, 1989).

The polarized distribution of ER and nucleus was shown to be dependent on actin (Hensel, 1985), which in contrast to surrounding cells, forms a network of fine filaments rather

than thick bundles in the central part of statocytes. This organization is likely important for the free sedimentation of statoliths (Baluška *et al.*, 1997; Blancaflor, 2013). In addition, it was demonstrated that actin dynamically interacts with the statoliths when the basipetal movement of statoliths was observed in microgravity (Volkmann *et al.*, 1991). Myosin-like proteins were detected on the surface of statoliths of maize (Baluška and Hasenstein, 1997) and lentil (Driss-Ecole, Lefranc and Perbal, 2003). Moreover, Huang et al. (2018) visualized actin forming ring-like structures around statoliths. Additionally, they suggested that Rice Morphology Determinant (RMD) protein is linking statoliths with actin filaments and slowing down their sedimentation.

Microtubules are not present deep inside the statocytes, only in the cortical regions. The cortical microtubules likely interact with sedimented amyloplasts (Baluška and Hasenstein, 1997), and they seem to be necessary for keeping the statocytes' polarity (Hensel, 1984).

3.3.2 Signal perception

A remaining mystery in both single-cell and multicellular systems is, what happens during and after the statolith sedimentation to the new bottom of the cell, and how is the mechanical signal of sedimenting particles transformed into a biochemical signal. On the electron microphotographs of the root cap of *Lepidium sativum* that were taken by Sievers and Volkmann, it was visible that in vertically oriented cells, the statoliths lie down on the distal ER. When the root is rotated 90°, statoliths leave their position on the distal ER cisternae and sediment to the new bottom of the cell. As the root is bending in response to gravity, the statoliths slowly return to the distal ER. Based on this observation, they came up with a theory that gravity is perceived through sensing the different forces exerted on different regions of ER (Sievers and Volkmann, 1977).

Later, myosin-like proteins were observed on the surface of the statoliths, implicating a putative interaction between these two structures. Another theory has arisen that sedimenting statoliths pull at actin filaments, which activate stretch-sensitive receptors on the ER- or plasma membrane (Baluška and Hasenstein, 1997).

A tensegrity-based model suggests that statoliths are not directly linked to actin filaments, but as they sediment, they disrupt the actin network, by which they activate stretch-sensitive receptors on the plasma membrane (Yoder *et al.*, 2001).

Experiments with the actin-depolymerizing drug Latrunculin B (100 nM) showed that roots of maize and *A. thaliana* with disturbed actin filaments have faster and stronger gravitropic responses than untreated roots. The Lat B stimulated roots even exceeded the reorientation angle, *i.e.,* the bending did not stop when the tip reached the vertical orientation again. Based on these observations, it was suggested that actin filaments are not necessary for the perception itself but most likely act as negative regulators of gravitropism that continuously reset the gravitropic-signaling pathway. (Hou *et al.*, 2004)

Leitz, Kang, and Schoenwaelder (2009) took microphotographs showing in detail the interaction between statoliths and ER. The statoliths sedimented onto the ER cisternae and deformed their membranes. After reorientation of the root, the equilibrium of forces shifted, ER membranes returned quickly to their original shape, and amyloplasts bounced away. Based on these observations, they support the theory that statoliths activate mechanosensitive Ca^{2+} channels by compression of the ER cisternae.

To sum up, the latest hypothesis explaining the perception of the gravity signal says that amyloplasts fall down to the membranes of ER, where they change their shape and open mechanosensitive Ca^{2+} channels (Leitz *et al.*, 2009). Statoliths yet are not sedimenting completely freely. Actin interacts with them, slowing down their sedimentation and facilitating timely termination of the gravitropic response, acting as a negative regulator of gravitropism (Baluška and Hasenstein, 1997; Huang *et al.*, 2018).

In contrast to all these studies, Hans Edelmann came up with the idea that statoliths are not necessary for gravitropism at all. He experimented with maize coleoptiles and roots, from which he surgically removed amyloplast-containing tissues (vascular bundle with its amyloplast-containing sheath from coleoptiles and root cap from roots). The no-amyloplast coleoptiles showed gravitropism comparable to an intact control. The decapped roots did not show a gravitropic response; however, when they were incubated in latrunculin for one hour before reorientation, they responded to gravity again, although in an opposite way. Based on these observations, he suggests that statolith sedimentation in the specific tissues is not necessary for the regulation of gravity perception (Edelmann, 2018). However, these quite drastic experiments stand against many others performed by various researchers on various models that support the role of statoliths in gravity sensing.

3.3.3 Signal transduction

It is well proven that the signal is transduced from the columella cells to the elongation zone by the asymmetrical flux of the plant hormone auxin (Band *et al.*, 2012), a regulator of many aspects of plant growth and development. Auxin distribution in plant tissues is controlled by polar localization of its membrane efflux carrier proteins PIN-FORMED (PINs; Vanneste, Friml 2009). In the columella cells, the main PIN creating the asymmetric auxin transport is PIN3, followed by PIN7. After gravistimulation, they are internalized from the plasma membrane to endosomes and subsequently transferred to the bottom of the cell (Kleine-Vehn *et al.*, 2010). However, the signaling pathway leading to PIN repolarization is still not completely understood.

As mentioned before, the sedimentation of statoliths causes Ca^{2+} influx hypothetically by opening mechanosensitive Ca^{2+} channels on ER membrane (Leitz *et al.*, 2009). An additional middle step between ER and Ca^{2+}, such as inositol-1,4,5-triphosphate (ISP3), is also possible (Tatsumi *et al.*, 2014). Ca^{2+} is a ubiquitous signaling molecule; therefore, it could contribute to gravitropism in many ways. If the Ca^{2+} ions were released from ER in reaction to sedimented statocytes, they would create a localized Ca^{2+} signal (Leitz *et al.*, 2009), which could contribute to subsequent polarization of the cell. For example, Ca^{2+} can modulate the auxin transport on the lower side of the cell through the regulation of PINOID activity (Robert and Offringa, 2008). Calmodulins were also found to be highly expressed in columella cells, indicating that the cells are ready to perceive the Ca^{2+} signal (Stinemetz *et al.*, 1987). Last but not least, the increase of cytoplasmic calcium levels coincides with alkalinization of the cytoplasm and following acidification of apoplast, which both can trigger a different enzymatic activity (Scott and Allen, 1999). Although the involvement of Ca^{2+} in gravitropic signaling is certain, these are still hypothetical options of how it could participate. The exact molecular mechanisms have not yet been revealed (Su, Keith and Masson, 2020).

A family of genes named LAZY by their mutant phenotype discovered in rice over 20 years ago, has been under an intense investigation in the last years in several model organisms because its members appear to be a crucial part of the pathway leading to the asymmetric auxin flow (reviewed by (Nakamura, Nishimura and Morita, 2019a). In *lazy3 (i.e., dro1)* mutants, a lesser or no asymmetric flow of auxin was observed after reorientation (Waite, Collum and Dardick, 2020), although starch synthesis or amyloplast sedimentation did not appear to be affected (Kawamoto *et al.*, 2020). These results implicate that LAZY genes belong somewhere between these two events on the pathway. Among the LAZY family genes, five evolutionarily conserved regions were discovered, and the region 5, the C terminus (CCL; conserved C

terminus in LAZY1) domain, was proven to have a role in gravity sensing and be the most conserved at the same time (Taniguchi *et al.*, 2017).

In relation to gravitropism, the most is probably known about the LAZY3 gene (also referred to as DRO1). This one was observed to localize both in the nucleus and on the plasma membrane in *A. thaliana* root tips (Waite, Collum and Dardick, 2020). Its' role is still not completely clear. In l*azy3* mutant, the roots react very slowly to reorientation, and lateral roots keep a greater inclination from the vertical axis than the wild type. Mutation in LAZY3 affects the expression of many other genes, among which several are related to gravitropism (Waite, Collum and Dardick, 2020). In *lazy234* triple mutant, roots grow upwards, hinting towards a cumulative function of these proteins (Taniguchi *et al.*, 2017). Later it was proven that *lazy234* triple mutants show regular negative gravitropism caused by a reversed auxin transport. It appears that LAZY genes control the polarization of auxin transporter PIN3 to the bottom of the cell (Ge and Chen, 2019). However, what determines the reversed lateral auxin flow in the absence of LAZY proteins, is still unknown. After reorientation o the root, the CCL domain of LAZY3 interacts with the BRX (Brevis radix) domain of RLD1 (Regulator of Chromosome Condensation 1-like domain) protein and recruits it to the plasma membrane, where they polarize to the bottom of the cell (Furutani *et al.*, 2020). This polarized RLD1 could possibly direct the incorporation of PINs back to the PM, as they modulate exocytosis by a guanine nucleotide exchange factor (GEF) activity (Jensen *et al.*, 2001; Furutani *et al.*, 2020).

3.3.4 Bending of the root

As mentioned above, asymmetrical auxin flux is the carrier of the information about reorientation from the columella cells to the elongation zone (Band *et al.*, 2012). Auxin is transported from the columella mainly through the lateral root cap and then through the epidermis. The auxin flow is facilitated mainly by PINs (PIN3 and PIN7 in the root cap and PIN2 in the epidermis), which are auxin efflux carriers and have a polarized localization. AUX1, an auxin influx carrier, does not contribute to the directionality but increases the speed of the auxin transport (Swarup *et al.*, 2005). On the lower flank of the elongation zone, increased cytosolic levels of auxin lead to an influx of Ca^{2+} ions and trigger H^{+} influx into cells. This results in apoplast alkalinization, cell wall stiffening, and inhibition of elongation (Monshausen *et al.*, 2011). Debates have been held about how cells respond to auxin. Besides the transcriptional TIR1/AFB-Aux/IAA pathway (where auxin acts like an inhibitor of transcriptional repressors; Tan *et al.*, 2007) it appears that there is another, faster nontranscriptional pathway. Its mechanisms, though, remain unknown (Fendrych *et al.*, 2018).

On the upper flank, there is less auxin present in the elongation zone cells, which supports the activity of H^+ pumps, leading to acidification of the cell walls, which results in breakage of intermolecular cross-links and allows cells to elongate (Monshausen *et al.*, 2011). Besides Ca^{2+} and pH, NO and ROS (reactive oxygen species) are also thought to be involved in the gravity response (Terrile *et al.*, 2012; Krieger *et al.*, 2016).

3.4 Summary of the multicellular gravitropism

The roots of the Euphyllophytes are complex organs containing several cell types adjusted to their specific function (Dolan *et al.*, 1993). Gravity perception is localized to the central columella (Leitz *et al.*, 2009). There are statocytes, cells with specifically polarized organization and with high-density starch-containing plastids. These plastids, statoliths, can move more or less freely in the cell (Sievers and Volkmann, 1977). They always sediment to the current bottom of the cell, where they are supposed to deform ER membrane and release Ca^{2+} ions (Leitz *et al.*, 2009). Actin interacts with statoliths and serves here most likely as the negative regulator of gravitropism (Baluška and Hasenstein, 1997; Hou *et al.*, 2004; Huang *et al.*, 2018).

The following steps of the gravitropic pathway are not yet well examined, but it is certain that a concerted action of Ca^{2+} ions, pH changes, and activity of LAZY proteins (Nakamura, Nishimura and Morita, 2019b) leads to the relocalization (Friml *et al.*, 2002) or differential activation (Barbosa et al., 2014) of PINs and to asymmetrical auxin transport. Auxin is transported through the lateral root cap and epidermis to the elongation zone, where its high concentration on the lower flank of the root inhibits cell elongation (Band *et al.*, 2012). The unequal elongation rates on the upper and lower flank lead to the bending of the root (Monshausen *et al.*, 2011).

Although the multicellular gravitropic systems are much more complex than the single-cell ones, it is still possible to find some comparable principles.

4 Comparison of the two gravitropic systems

4.1 Statoliths

Even though an opinion appeared questioning the necessity of statoliths for gravity perception (Edelmann, 2018), still many studies from the last more than 150 years indicate that they are somehow involved in sensing the gravity vector.

All of the model systems described in this thesis (except the internodal cells of *Chara;* Staves, 1997) have statoliths (Sievers and Volkmann, 1977; Walker and Sack, 1990; Schwuchow, Kim and Sack, 1995; Braun and Sievers, 2000), and even animals use statoliths for orientation in the gravity field. I think the reason is that when an organism "needs" to sense gravity for orientation, the easiest way to materialize the gravity force is to utilize the basic physical principles and have some heavy particles that can undergo a free fall.

The statolith-independent gravity sensing in *Chara* internodal cells is not examined enough to compare it with the statolith-dependent pathway. It is still intriguing to know that it is possible that statolith sedimentation is not the only option. Actually, the possibility of an additional gravity signal from outside of the root cap (*e.g.,* elongation zone) in vascular plants as an additional mechanism was already discussed (Wolverton *et al.*, 2002).

Considering the structure and composition of statoliths in mosses and vascular plants, they are represented by amyloplasts – plastids full of high-density starch granules. *Chara* rhizoids and protonemata do not contain sedimenting plastids but vesicles filled with $BaSO_4$ crystals that are heavy enough to serve as statoliths. From this point of view, it looks like the phenomenon of statoliths emerged at least twice in the evolution because plastids and crystal-filled vesicles seem unrelated structures. Interestingly, *Chara* is not the only one to produce $BaSO_4$ crystals. Another one to be endowed with this feature is, for example, the desmid genus *Closterium*, a single-cell alga that is not known to sense gravity (Brook *et al.*, 1980).

The location of the statoliths is another aspect in which plants differ. The single-cell gravitropic systems just contain statoliths in the one gravitropic cell, but the variability among the multicellular organisms is interesting. (Zhang *et al.*, 2019) noticed that Lycophytes have amyloplasts, but only in the tissues above the root apex, never within it. Ferns contain amyloplasts both within and above the root apex, and seed plants concentrate the gravity perception only to the root apex. (Zhang *et al.*, 2019) explain the absence of amyloplasts in Lycophyte root apices by the hypotheses that roots as a structure evolved multiple times and that it is an example of convergent evolution between Lycophytes and Euphyllophytes

(Hetherington and Dolan, 2018). During the evolution of the Euphyllophytes, amyloplasts were possibly focused from more scattered distribution present in the fern bodies to the more limited area of the root apex only.

4.2 The relationship between actin and statoliths

In both single-cell and multicellular systems, the actin network plays a significant role in mediating gravity sensing (Hensel, 1985; Limbach *et al.*, 2005). This is not surprising, as actin is an inherent component of all cells, where it discharges many roles crucial for diverse functions essential to life. It is natural that cells simply "use what is available". However, the ways how did different gravity sensing systems made use of actin filaments are remarkably diverse.

The actin cytoskeleton, in general, maintains the inner organization of cells – controls the position of organelles, guides diverse vesicles, and provides additional mechanical support. In tip growing cells, its function is even more pronounced as it controls the extreme polarization of the cytoplasm and maintains vesicular trafficking to all parts of the untypically shaped cell (Braun and Wasteneys, 1998). In single-cell gravitropic systems, actin filaments are known to actively keep the position of the statoliths along the longitudinal axis. Lateral sedimentation is much less restrained, but the longitudinal position of statoliths is still controlled even during this process (Hejnowicz and Sievers, 1981). In *Chara,* an active longitudinal transport was observed during lateral sedimentation of statoliths, which guided them to sensitive areas of PM. This effect is more pronounced in *Chara* protonemata, where sedimenting statoliths are directed to the very apex, just next to the Spitzenkörper (Braun, 2002). Actin filaments are also abundant inside the Spitzenkörper (Braun and Wasteneys, 1998), where they most likely organize the ER cisternae and secretory vesicles. In *P. patens*, actin cooperates with microtubules, and together they maintain the direction of growth (Wu and Bezanilla, 2018).

The cells involved in multicellular gravitropism do not stand on their own but are a part of a complex structure, the root (Dolan *et al.*, 1993). They also have a more cube-like shape that is controlled mainly by cellulose fibrils in the cell wall, which were deposited on the basis of the organization of cortical microtubules. However, as the statocytes (in the columella) are highly specialized to sense gravity, even the organization of the actin cytoskeleton is highly conformed to this function. Actin filaments keep the organelles in their places, as was described above (3.3.1 Inner organization of statocytes). It was observed that statocytes interact with actin filaments, similarly to these in the tip growing cells, but with less intensity (Perbal *et al.*, 1997). Experiments with Latrunculin B, an actin-depolymerizing drug, revealed that actin probably

serves as a negative regulator of gravitropism. Sedimentation of statoliths is slowed by interactions with actin (Hou *et al.*, 2004).

In conclusion, the organization of actin in the single-celled gravitropic systems seems to be adjusted mainly for tip growth with the extra ability to facilitate the statolith sedimentation in gravity sensing. On the contrary, actin and also the whole subcellular organization are specified for gravity sensing in the statocytes (*i.e.,* multicellular gravitropism). The interactions between statoliths and actin filaments are present in both systems but more intensive in the single-cell gravitropic systems. Moreover, the effect of the actin network on gravitropism is opposite in the two described systems. It enhances the gravitropism in single cells by guiding the statoliths to the sites of perception, and it inhibits gravitropism in multicellular systems by slowing down the sedimenting statoliths. In both cases, actin serves as a tool, as one component of a complex mechanism of gravity sensing, which evolved at least twice independently and in pursuance of that evolved also two different ways to involve actin in the process of gravitropism.

4.3 Perception

A common characteristic of both single-cell and multicellular gravitropism is the enigma around gravity perception. An answer to the question 'what exactly happens right after statolith sedimentation?' is, after decades of research, still not available (Su, Keith and Masson, 2020).

In *Chara* rhizoids, it was observed that the altered weight of statoliths does not affect the gravitropic response. Therefore, it was suggested that for conversion of the signal from physical to biochemical, *Chara* uses a ligand-receptor system rather than a mechanoreceptor sensitive to pressure (Limbach *et al.*, 2005). However, it is not clear how would the ligand-receptor system fit in the hypotheses of gravity response (*i.e.*, sterically blocking exocytosis in rhizoids and mechanical displacement of Spitzenkörper in protonemata) what would be their function in the chain. And especially in the *Chara* protonemata, weightless statoliths (in microgravity) could have problems pushing the Spitzenkörper to the side.

In protonemata of the mosses, some mechanoreceptor is likely, because a larger mass of amyloplasts resulted in a stronger bending (Kuznetsov *et al.*, 1999). A similar mechanism was also proposed for the roots of higher plants (Leitz *et al.*, 2009).

Maybe inspired by the single-cell systems, where statoliths are closely associated with actin, the theory of restrained gravitropism came up. It suggests that statoliths are firmly

connected to the actin filaments, which are associated with stretch-activated channels on PM. However, depolymerization of actin enhanced the gravity response instead of inhibiting it (Hou *et al.*, 2004). This observation was inconsistent with the current hypothesis and inspired scientists to search for new theories.

The most recent hypothesis turned back to the idea of mechanoreceptors. The curvature of distal ER by sedimented statoliths could open mechanosensitive Ca^{2+} channels (Leitz *et al.*, 2009). Moreover, the existence of some additional ligand-receptor system that would contribute to the higher sensitivity of the statocytes to smaller inclinations cannot be ruled out (Wolverton *et al.*, 2002).

Intriguingly, a genome sequence-based mapping revealed that *Physcomitrium patens* has some homologs of LAZY family genes. It was identified that the homologs contain the C-terminal domain, which is the most conserved one (Dardick *et al.*, 2013). This domain is also the one that plays an important role in euphyllophyte gravitropism. The gene sequence is, for now, everything we know about these proteins in *P. patens,* but it would be interesting to find out if *P. patens* utilizes the gene and what function it possibly has.

The mechanisms of this step of the gravitropic chain still remain on a hypothetical level, so it is hard to look for some evolutionary analogies. Interestingly, the hypotheses for both single-cell and multicellular systems "evolve" together. The researchers from both fields are taking into account the discoveries from the other field and contemplating whether some of the mechanisms could apply to their model system.

4.4 The role of calcium in gravitropism

Both examined gravitropic systems use calcium ions for gravity-related signaling. In tip-growing cells, there is a constant Ca^{2+} gradient in the apex, contributing to holding the restriction of the growing area. In *Chara* protonemata, a shift of this Ca^{2+} maximum was observed after reorientation, together with a change of location of the growth-organizing center. In *Chara* rhizoids, no change in calcium distribution was observed (Braun and Richter, 1999). In mosses, Ca^{2+} ions were hypothesized to play a crucial role in gravity signal transduction by providing spatial information about the position of the statoliths to the growing tip (Sack *et al.*, 2001), although there was probably no experimental evidence supporting it at the time.

The localization of Ca^{2+} ions to a specific part of the cytoplasm could play a role even in flowering plant roots. The Ca^{2+} ions release is most likely triggered by the statolith

sedimentation at the bottom of the cell, which could provide spatial information for relocalization and activity of PIN proteins (Plieth and Trewavas, 2002; Leitz *et al.*, 2009).

Shared mechanisms of calcium function can also be found in cell walls; alkalinization together with the Ca^{2+} ions cross-links the components of the cell wall, making it more rigid and not allowing further growth.

However, in single-cell gravitropic systems, the fluxes of Ca^{2+} come from apoplast, while in multicellular systems, calcium is speculated to be released from ER. In single-cell gravitropic systems, the local Ca^{2+} maximum is permanent (though it can oscillate), maintained by continually opened channels (Braun and Richter, 1999), while in multicellular systems, the increase of calcium level follows statolith sedimentation(Plieth and Trewavas, 2002; Tatsumi *et al.*, 2014). These clues indicate that it is a kind of different calcium signal in both systems.

Calcium is a ubiquitous second messenger among plants and animals. It contributes to a wide range of signaling pathways. Single-cell and multicellular gravitropic systems appear to use the calcium signal in different ways, so it is likely that they both just utilized a common signaling molecule in the gravity signaling pathway, the same as many others.

4.5 Gravity response or signal transduction?

In multicellular gravitropic systems, the gravity response (the bending of the root) occurs in a different part of the root than where the perception occurs. Therefore, the multicellular gravitropic systems need differential auxin flux to transport the signal between different tissues. Contrarily, in the single-celled systems, almost no transduction of the signal is necessary, as the perception and response happen within the same cell.

Taken together, the bending of single-cell systems corresponds more with the transduction mechanisms of multicellular systems than with their bending. From this point of view, the two gravitropic systems show some similarities.

The first step of single-cell gravity response is probably, at least in some of the model organisms, calcium fluxes and local changes in Ca^{2+} concentrations (Braun and Richter, 1999). The response is then manifested by a differential growth resulting from altered vesicular trafficking. The exocytosis of cell-wall components is most likely guided by the spatial information provided by calcium (probably through reorganization of actin filaments; Braun, Limbach 2006). In addition, pH changes in both cytosol and apoplast contribute to cellular signaling and modification of cell wall structure, respectively (Wolf, Hématy and Höfte, 2012).

In the statocytes of multicellular gravitropic systems, the first reaction to reorientation is a rise in cytosolic Ca^{2+} concentration (Scott, Allen 1999). The response is here manifested by an asymmetrical auxin transport, which results from the translocation of PINs to the bottom side of the cell (Kleine-Vehn *et al.*, 2010), which, in other words, can be called altered vesicular trafficking. Moreover, rapid cytosolic and apoplastic pH changes occur (Scott, Allen 1999). The apoplastic acidification facilitates auxin transport into cells (Boonsirichai *et al.*, 2003), contributing to better control over the auxin flux.

To sum up, both single-cell and multicellular systems respond to gravity by Ca^{2+} concentration and pH changes in apoplast and cytosol, followed by changes in vesicular trafficking, which executes the response. In single-cell systems, the response is a differential growth, while in multicellular systems, the statocyte responds "only" by transduction of the signal from the cellular- to tissue level.

5 Conclusions

In this thesis, I divided the gravitropic organs of plants in the Streptophyte lineage into two groups according to the number of cells involved in gravitropism – single-cell systems and multicellular systems. I was looking deeper into the mechanisms used by these two systems to sense and respond to the gravity vector and thinking about their similarities and differences, revealing the relation between these two systems. However, during this process, I came across two main hindrances. The first one is the level of knowledge. The molecular mechanisms of gravitropism are not fully unraveled even in higher plant models, such as *Arabidopsis* or rice; even less is known about the lower plants, which get much less attention. The second obstacle was the internal variability of the groups. Some members of the same gravitropic system showed up a similar amount of variability among themselves as in comparison with the members of the other group.

It appears that these two groups, single-cell vs. multicellular, are artificial, and their comparison does not provide much information about the evolution of gravitropic mechanisms.

To embrace the variability while not getting lost in it, I suggest relying on phylogenetic links rather than morphological similarities. A deeper look into the Lycophyte roots compared to those from Euphyllophytes could provide intriguing discoveries related to root development, growth, and gravitropism (and soon, it may become a reality: (Fang *et al.*, 2021). And then it is possible to proceed deeper into the evolution, closer to the roots of the phylogenetic tree. Did the common ancestors of *Characean* algae and land plants even have some root-like structures? Were they able to sense gravity?

References

Baluška, F. *et al.* (1997) "Central root cap cells are depleted of endoplasmic microtubules and actin microfilament bundles: Implications for their role as gravity-sensing statocytes," *Protoplasma*, 196(3–4), pp. 212–223. doi:10.1007/bf01279569.

Baluška, F. and Hasenstein, K.H. (1997) "Root cytoskeleton: its role in perception of and response to gravity," *Planta*, 203(S1), pp. S69–S78. doi:10.1007/PL00008117.

Band, L.R. *et al.* (2012) "Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism," *Proceedings of the National Academy of Sciences*, 109(12), pp. 4668–4673. doi:10.1073/pnas.1201498109.

Barbosa, I.C.R. *et al.* (2014) "D6 PROTEIN KINASE Activates Auxin Transport-Dependent Growth and PIN-FORMED Phosphorylation at the Plasma Membrane," *Developmental Cell*, 29(6), pp. 674–685. doi:10.1016/j.devcel.2014.05.006.

Berry, E.A. *et al.* (2016) "Immuno and Affinity Cytochemical Analysis of Cell Wall Composition in the Moss Physcomitrella patens," *Frontiers in Plant Science*, 7(MAR2016), p. 248. doi:10.3389/fpls.2016.00248.

Bibeau, J.P. *et al.* (2021) "Quantitative cell biology of tip growth in moss," *Plant Molecular Biology 2021 107:4*, 107(4), pp. 227–244. doi:10.1007/S11103-021-01147-7.

Blancaflor, E.B. (2013) "Regulation of plant gravity sensing and signaling by the actin cytoskeleton," *American Journal of Botany*, 100(1), pp. 143–152. doi:10.3732/ajb.1200283.

Boonsirichai, K. *et al.* (2003) "ALTERED RESPONSE TO GRAVITY Is a Peripheral Membrane Protein That Modulates Gravity-Induced Cytoplasmic Alkalinization and Lateral Auxin Transport in Plant Statocytes," *The Plant Cell*, 15(11), pp. 2612–2625. doi:10.1105/TPC.015560.

Braidwood, L., Breuer, C. and Sugimoto, K. (2014) "My body is a cage: mechanisms and modulation of plant cell growth," *New Phytologist*, 201(2), pp. 388–402. doi:10.1111/nph.12473.

Braun, M. (1996) "Immunolocalization of myosin in rhizoids ofChara globularis Thuill," *Protoplasma*, 191(1–2), pp. 1–8. doi:10.1007/BF01280820.

Braun, M. (1997) "Gravitropism in tip-growing cells," *Planta*, 203(S1), pp. S11–S19. doi:10.1007/PL00008098.

Braun, M. (2002) "Gravity perception requires statoliths settled on specific plasma membrane areas in characean rhizoids and protonemata," *Protoplasma*, 219(3–4), pp. 150–159. doi:10.1007/s007090200016.

Braun, M. and Limbach, C. (2006) "Rhizoids and protonemata of characean algae: model cells for research on polarized growth and plant gravity sensing," *Protoplasma*, 229(2–4), pp. 133–142. doi:10.1007/s00709-006-0208-9.

Braun, M. and Richter, P. (1999) "Relocalization of the calcium gradient and a dihydropyridine receptor is involved in upward bending by bulging of Chara protonemata, but not in downward bending by bowing of Chara rhizoids," *Planta*, 209(4), pp. 414–423. doi:10.1007/s004250050744.

Braun, M. and Sievers, A. (2000) "Plant cells on earth and in space," *Korean Journal of Biological Sciences*, 4(3), pp. 201–214. doi:10.1080/12265071.2000.9647546.

Braun, M. and Wasteneys, G.O. (1998) "Distribution and dynamics of the cytoskeleton in graviresponding protonemata and rhizoids of characean algae: exclusion of microtubules and a

convergence of actin filaments in the apex suggest an actin-mediated gravitropism," *Planta*, 205(1), pp. 39–50. doi:10.1007/s004250050294.

Brook, A.J. *et al.* (1980) "Barium accumulation by desmids of the genus Closterium (Zygnemaphyceae)," *British Phycological Journal*, 15(3), pp. 261–264. doi:10.1080/00071618000650251.

Chebli, Y. *et al.* (2012) "The Cell Wall of the Arabidopsis Pollen Tube—Spatial Distribution, Recycling, and Network Formation of Polysaccharides," *Plant Physiology*, 160(4), pp. 1940–1955. doi:10.1104/pp.112.199729.

Cove, D.J. *et al.* (1978) "Genetic and physiological studies of the effect of light on the development of the moss, Physcomitrella patens," *Photochemistry and Photobiology*, 27(2), pp. 249–254. doi:10.1111/j.1751-1097.1978.tb07596.x.

Dardick, C. *et al.* (2013) "PpeTAC1 promotes the horizontal growth of branches in peach trees and is a member of a functionally conserved gene family found in diverse plants species," *The Plant Journal*, 75(4), pp. 618–630. doi:10.1111/tpj.12234.

DARWIN, F. (1903) "The Statolith Theory of Geotropism 1," *Nature*, 67(1746), pp. 571–572. doi:10.1038/067571a0.

Derksen, J. *et al.* (1995) "Quantitative analysis of the distribution of organelles in tobacco pollen tubes: implications for exocytosis and endocytosis," *Protoplasma*, 188(3–4), pp. 267–276. doi:10.1007/BF01280379.

Dolan, L. *et al.* (1993) "Cellular organisation of the Arabidopsis thaliana root," *Development*, 119(1), pp. 71–84. doi:10.1242/dev.119.1.71.

Doonan, J.H., Cove, D.J. and Lloyd, C.W. (1985) "Immunofluorescence microscopy of microtubules in intact cell lineages of the moss, Physcomitrella patens. I. Normal and CIPC-treated tip cells," *Journal of Cell Science*, 75(1), pp. 131–147. doi:10.1242/jcs.75.1.131.

Driss-Ecole, D., Lefranc, A. and Perbal, G. (2003) "A polarized cell: the root statocyte," *Physiologia Plantarum*, 118(3), pp. 305–312. doi:10.1034/j.1399-3054.2003.00121.x.

Duckett, C.M. *et al.* (1994) "Clonal relationships and cell patterning in the root epidermis of Arabidopsis," *Development*, 120(9), pp. 2465–2474. doi:10.1242/dev.120.9.2465.

Edelmann, H.G. (2018) "Graviperception in maize plants: is amyloplast sedimentation a red herring?," *Protoplasma*, 255(6), pp. 1877–1881. doi:10.1007/s00709-018-1272-7.

Fang, T. *et al.* (2021) "Early 'Rootprints' of Plant Terrestrialization: Selaginella Root Development Sheds Light on Root Evolution in Vascular Plants," *Frontiers in Plant Science*, 12. doi:10.3389/FPLS.2021.735514.

Fendrych, M. *et al.* (2018) "Rapid and reversible root growth inhibition by TIR1 auxin signalling," *Nature Plants*, 4(7), pp. 453–459. doi:10.1038/s41477-018-0190-1.

Finiuk, N.S. *et al.* (2014) "Genetic transformation of moss Ceratodon purpureus by means of polycationic carriers of DNA," *Cytology and Genetics*, 48(6), pp. 345–351. doi:10.3103/S0095452714060048.

Friml, J. *et al.* (2002) "Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis," *Nature*, 415(6873), pp. 806–809. doi:10.1038/415806a.

Furt, F. *et al.* (2012) "Quantitative analysis of organelle distribution and dynamics in Physcomitrella patens protonemal cells," *BMC Plant Biology*, 12(1), pp. 1–15. doi:10.1186/1471-2229-12-70/FIGURES/6.

Furutani, M. *et al.* (2020) "Polar recruitment of RLD by LAZY1-like protein during gravity signaling in root branch angle control," *Nature Communications*, 11(1), p. 76. doi:10.1038/s41467-019-13729-7.

Ge, L. and Chen, R. (2019) "Negative gravitropic response of roots directs auxin flow to control root gravitropism," *Plant, Cell & Environment*, 42(8), pp. 2372–2383. doi:10.1111/pce.13559.

Geitmann, A. and Emons, A.M.C. (2000) "The cytoskeleton in plant and fungal cell tip growth," *Journal of Microscopy*, 198(3), pp. 218–245. doi:10.1046/j.1365-2818.2000.00702.x.

Guo, J. and Yang, Z. (2020) "Exocytosis and endocytosis: coordinating and fine-tuning the polar tip growth domain in pollen tubes," *Journal of Experimental Botany*. Edited by K. Vissenberg, 71(8), pp. 2428–2438. doi:10.1093/jxb/eraa134.

Heimsch, C. and Seago, J.L. (2008) "Organization of the root apical meristem in angiosperms," *American Journal of Botany*, 95(1), pp. 1–21. doi:10.3732/ajb.95.1.1.

Hejnowicz, Z. and Sievers, A. (1981) "Regulation of the position of statoliths inChara rhizoids," *Protoplasma*, 108(1–2), pp. 117–137. doi:10.1007/BF01276887.

Hensel, W. (1984) "A role of microtubules in the polarity of statocytes from roots of Lepidium sativum L.," *Planta*, 162(5), pp. 404–414. doi:10.1007/BF00393452.

Hensel, W. (1985) "Cytochalasin B affects the structural polarity of statocytes from cress roots (Lepidium sativum L.)," *Protoplasma*, 129(2–3), pp. 178–187. doi:10.1007/BF01279915.

Hetherington, A.J. and Dolan, L. (2018) "Stepwise and independent origins of roots among land plants," *Nature*, 561(7722), pp. 235–238. doi:10.1038/s41586-018-0445-z.

Hiwatashi, Y., Sato, Y. and Doonan, J.H. (2014) "Kinesins have a dual function in organizing microtubules during both tip growth and cytokinesis in physcomitrella patens," *Plant Cell*, 26(3), pp. 1256–1266. doi:10.1105/TPC.113.121723.

Hodick, D. (1994) "Negative gravitropism in Chara protonemata: A model integrating the opposite gravitropic responses of protonemata and rhizoids," *Planta*, 195(1), pp. 43–49. doi:10.1007/BF00206290.

Hou, G. *et al.* (2004) "The promotion of gravitropism in Arabidopsis roots upon actin disruption is coupled with the extended alkalinization of the columella cytoplasm and a persistent lateral auxin gradient," *The Plant Journal*, 39(1), pp. 113–125. doi:10.1111/j.1365-313X.2004.02114.x.

Huang, G. *et al.* (2018) "Rice actin binding protein RMD controls crown root angle in response to external phosphate," *Nature communications*, 9(1). doi:10.1038/S41467-018-04710-X.

Izawa, T. and Shimamoto, K. (1996) "Becoming a model plant: The importance of rice to plant science," *Trends in Plant Science*, 1(3), pp. 95–99. doi:10.1016/S1360-1385(96)80041-0.

Jenkins, G.I., Courtice, G.R.M. and Cove, D.J. (1986) "Gravitropic responses of wild-type and mutant strains of the moss Physcomitrella patens," *Plant, Cell and Environment*, 9(8), pp. 637–644. doi:10.1111/j.1365-3040.1986.tb01621.x.

Jensen, R.B. *et al.* (2001) "FYVE zinc-finger proteins in the plant model Arabidopsis thaliana: identification of PtdIns3P-binding residues by comparison of classic and variant FYVE domains," *Biochemical Journal*, 359(1), p. 165. doi:10.1042/0264-6021:3590165.

Kawamoto, N. *et al.* (2020) "Gravity-Sensing Tissues for Gravitropism Are Required for 'Anti-Gravitropic' Phenotypes of Lzy Multiple Mutants in Arabidopsis," *Plants (Basel, Switzerland)*, 9(5). doi:10.3390/PLANTS9050615.

Kern, V.D. *et al.* (2001) "Amyloplasts That Sediment in Protonemata of the Moss Ceratodon purpureus Are Nonrandomly Distributed in Microgravity," *Plant Physiology*, 125(4), pp. 2085–2094. doi:10.1104/pp.125.4.2085.

Kiss, J.Z. and Staehelin, L.A. (1993) "Structural Polarity in the Chara rhizoid: A Reevaluation," *American Journal of Botany*, 80(3), pp. 273–282. doi:10.2307/2445350.

Kleine-Vehn, J. *et al.* (2010) "Gravity-induced PIN transcytosis for polarization of auxin fluxes in gravity-sensing root cells," *Proceedings of the National Academy of Sciences of the United States of America*, 107(51), pp. 22344–22349. doi:10.1073/pnas.1013145107.

Koornneef, M. and Meinke, D. (2010) "The development of Arabidopsis as a model plant," *The Plant Journal*, 61(6), pp. 909–921. doi:10.1111/J.1365-313X.2009.04086.X.

Krieger, G. *et al.* (2016) "Reactive Oxygen Species Tune Root Tropic Responses," *Plant Physiology*, 172(2), pp. 1209–1220. doi:10.1104/PP.16.00660.

Kuznetsov, O.A. *et al.* (1999) "Curvature Induced by Amyloplast Magnetophoresis in Protonemata of the Moss Ceratodon purpureus 1," *Plant Physiology*, 119(2), pp. 645–650. doi:10.1104/pp.119.2.645.

Lancelle, S.A. and Hepler, P.K. (1992) "Ultrastructure of freeze-substituted pollen tubes of Lilium longiflorum," *Protoplasma*, 167(3–4), pp. 215–230. doi:10.1007/BF01403385.

Leitz, G. *et al.* (2009) "Statolith Sedimentation Kinetics and Force Transduction to the Cortical Endoplasmic Reticulum in Gravity-Sensing Arabidopsis Columella Cells," *The Plant Cell*, 21(3), pp. 843–860. doi:10.1105/tpc.108.065052.

Li, H. *et al.* (2018) "The REN4 rheostat dynamically coordinates the apical and lateral domains of Arabidopsis pollen tubes," *Nature Communications*, 9(1), p. 2573. doi:10.1038/s41467-018-04838-w.

Li, Y. *et al.* (2021) "A minus-end directed kinesin motor directs gravitropism in Physcomitrella patens," *Nature Communications*, 12(1), p. 4470. doi:10.1038/s41467-021-24546-2.

Limbach, C. *et al.* (2005) "How to Activate a Plant Gravireceptor. Early Mechanisms of Gravity Sensing Studied in Characean Rhizoids during Parabolic Flights," *Plant Physiology*, 139(2), pp. 1030–1040. doi:10.1104/pp.105.068106.

Limbach, C. *et al.* (2008) "Electron tomographic characterization of a vacuolar reticulum and of six vesicle types that occupy different cytoplasmic domains in the apex of tip-growing Chara rhizoids," *Planta*, 227(5), pp. 1101–1114. doi:10.1007/s00425-007-0684-y.

Luo, N. *et al.* (2017) "Exocytosis-coordinated mechanisms for tip growth underlie pollen tube growth guidance," *Nature Communications*, 8(1), p. 1687. doi:10.1038/s41467-017-01452-0.

Monshausen, G.B. *et al.* (2011) "Dynamics of auxin-dependent Ca2+ and pH signaling in root growth revealed by integrating high-resolution imaging with automated computer vision-based analysis," *The Plant Journal*, 65(2), pp. 309–318. doi:10.1111/j.1365-313X.2010.04423.x.

Nakamura, M., Nishimura, T. and Morita, M.T. (2019a) "Bridging the gap between amyloplasts and directional auxin transport in plant gravitropism," *Current Opinion in Plant Biology*, 52, pp. 54–60. doi:10.1016/j.pbi.2019.07.005.

Nakamura, M., Nishimura, T. and Morita, M.T. (2019b) "Gravity sensing and signal conversion in plant gravitropism," *Journal of Experimental Botany*, 70(14), pp. 3495–3506. doi:10.1093/jxb/erz158.

Nishiyama, T. *et al.* (2018) “The Chara Genome: Secondary Complexity and Implications for Plant Terrestrialization,” *Cell*, 174(2), pp. 448-464.e24. doi:10.1016/j.cell.2018.06.033.

Ottenschläger, I. *et al.* (2003) “Gravity-regulated differential auxin transport from columella to lateral root cap cells,” *Proceedings of the National Academy of Sciences of the United States of America*, 100(5), pp. 2987–2991. doi:10.1073/PNAS.0437936100.

Perbal, G. *et al.* (1997) “Statocyte polarity and gravisensitivity in seedling roots grown in microgravity,” *Planta*, 203(S1), pp. S57–S62. doi:10.1007/PL00008115.

Perbal, G. and Driss-Ecole, D. (1989) “Polarity of statocytes in lentil seedling roots grown in space (Spacelab D1 Mission),” *Physiologia Plantarum*, 75(4), pp. 518–524. doi:10.1111/j.1399-3054.1989.tb05618.x.

Plieth, C. and Trewavas, A.J. (2002) “Reorientation of Seedlings in the Earth’s Gravitational Field Induces Cytosolic Calcium Transients,” *Plant Physiology*, 129(2), pp. 786–796. doi:10.1104/pp.011007.

Raven, J.A. and Edwards, D. (2001) “Roots: evolutionary origins and biogeochemical significance,” *Journal of Experimental Botany*, 52(suppl_1), pp. 381–401. doi:10.1093/jxb/52.suppl_1.381.

Rensing, S.A. *et al.* (2008) “The Physcomitrella Genome Reveals Evolutionary Insights into the Conquest of Land by Plants,” *Science*, 319(5859), pp. 64–69. doi:10.1126/science.1150646.

Rensing, S.A. *et al.* (2020) “The Moss Physcomitrium (Physcomitrella) patens : A Model Organism for Non-Seed Plants,” *The Plant Cell*, 32(5), pp. 1361–1376. doi:10.1105/tpc.19.00828.

Ridge, R.W. and Sack, F.D. (1992) “Cortical and Cap Sedimentation in Gravitropic Equisetum Roots,” *American Journal of Botany*, 79(3), p. 328. doi:10.2307/2445023.

Robards, A.W. and Robb, M.E. (1972) “Uptake and Binding of Uranyl Ions by Barley Roots,” *Science*, 178(4064), pp. 980–982. doi:10.1126/science.178.4064.980.

Robert, H.S. and Offringa, R. (2008) “Regulation of auxin transport polarity by AGC kinases,” *Current Opinion in Plant Biology*, 11(5), pp. 495–502. doi:10.1016/J.PBI.2008.06.004.

Sack, F.D. *et al.* (2001) “Gravity sensing in moss protonemata,” *Advances in Space Research*, 27(5), pp. 871–876. doi:10.1016/S0273-1177(01)00151-X.

Schaefer, D.G. and Zrÿd, J.P. (1997) “Efficient gene targeting in the moss Physcomitrella patens,” *Plant Journal*, 11(6), pp. 1195–1206. doi:10.1046/j.1365-313X.1997.11061195.x.

Schwuchow, J., Sack, F.D. and Hartmann, E. (1990) “Microtubule distribution in gravitropic protonemata of the mossCeratodon,” *Protoplasma*, 159(1), pp. 60–69. doi:10.1007/BF01326635.

Schwuchow, J.M. *et al.* (2002) “Conservation of the Plastid Sedimentation Zone in All Moss Genera with Known Gravitropic Protonemata,” *Journal of Plant Growth Regulation*, 21(2), pp. 146–155. doi:10.1007/s003440010048.

Schwuchow, J.M., Kim, D. and Sack, F.D. (1995) “Caulonemal gravitropism and amyloplast sedimentation in the moss Funaria,” *Canadian Journal of Botany*, 73(7), pp. 1029–1035. doi:10.1139/b95-112.

Scott, A.C. and Allen, Nina Strömgren (1999) “Changes in cytosolic pH within Arabidopsis root columella cells play a key role in the early signaling pathway for root gravitropism,” *Plant Physiology*, 121(4), pp. 1291–1298. doi:10.1104/PP.121.4.1291.

Scott, A.C. and Allen, Nina Strömgren (1999) “Changes in Cytosolic pH within Arabidopsis Root Columella Cells Play a Key Role in the Early Signaling Pathway for Root Gravitropism,” *Plant Physiology*, 121(4), pp. 1291–1298. doi:10.1104/pp.121.4.1291.

Sievers, A. and Volkmann, D. (1977) “Ultrastructure of gravity-perceiving cells in plant roots,” *Proceedings of the Royal Society of London. Series B. Biological Sciences*, 199(1137), pp. 525–536. doi:10.1098/rspb.1977.0160.

Staves, M.P. (1997) “Cytoplasmic streaming and gravity sensing in Chara internodal cells,” *Planta*, 203(S1), pp. S79–S84. doi:10.1007/PL00008119.

Stinemetz, C.L. *et al.* (1987) “Correlation between Calmodulin Activity and Gravitropic Sensitivity in Primary Roots of Maize,” *Plant Physiology*, 84(4), pp. 1337–1342. doi:10.1104/PP.84.4.1337.

Su, S.H., Keith, M.A. and Masson, P.H. (2020) “Gravity signaling in flowering plant roots,” *Plants*. Multidisciplinary Digital Publishing Institute, pp. 1–23. doi:10.3390/plants9101290.

Swarup, R. *et al.* (2005) “Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal,” *Nature Cell Biology*, 7(11), pp. 1057–1065. doi:10.1038/ncb1316.

Tan, X. *et al.* (2007) “Mechanism of auxin perception by the TIR1 ubiquitin ligase,” *Nature*, 446(7136), pp. 640–645. doi:10.1038/NATURE05731.

Taniguchi, M. *et al.* (2017) “The Arabidopsis LAZY1 Family Plays a Key Role in Gravity Signaling within Statocytes and in Branch Angle Control of Roots and Shoots,” *The Plant Cell*, 29(8), pp. 1984–1999. doi:10.1105/tpc.16.00575.

Tatsumi, H. *et al.* (2014) “Calcium mobilizations in response to changes in the gravity vector in Arabidopsis seedlings: possible cellular mechanisms,” *Plant signaling & behavior*, 9(8). doi:10.4161/PSB.29099.

Terrile, M.C. *et al.* (2012) “Nitric oxide influences auxin signaling through S-nitrosylation of the Arabidopsis TRANSPORT INHIBITOR RESPONSE 1 auxin receptor,” *Plant Journal*, 70(3), pp. 492–500. doi:10.1111/j.1365-313X.2011.04885.x.

Thornton, L.E. *et al.* (2005) “Physcomitrella patens and Ceratodon purpureus, mosses as model organisms in photosynthesis studies,” *Photosynthesis Research*, 83(1), pp. 87–96. doi:10.1007/s11120-004-5577-3.

Turmel, M., Otis, C. and Lemieux, C. (2006) “The Chloroplast Genome Sequence of Chara vulgaris Sheds New Light into the Closest Green Algal Relatives of Land Plants,” *Molecular Biology and Evolution*, 23(6), pp. 1324–1338. doi:10.1093/MOLBEV/MSK018.

Vanneste, S. and Friml, J. (2009) “Auxin: A Trigger for Change in Plant Development,” *Cell*, 136(6), pp. 1005–1016. doi:10.1016/J.CELL.2009.03.001.

Verbelen, J.P. *et al.* (2006) “The root apex of arabidopsis thaliana consists of four distinct zones of growth activities: Meristematic zone, transition zone, fast elongation zone and growth terminating zone,” *Plant Signaling and Behavior*, 1(6), pp. 296–304. doi:10.4161/PSB.1.6.3511.

Vidali, L. *et al.* (2009) “Lifeact-mEGFP Reveals a Dynamic Apical F-Actin Network in Tip Growing Plant Cells,” *PLoS ONE*. Edited by I. Baxter, 4(5), p. e5744. doi:10.1371/journal.pone.0005744.

Volkmann, D. *et al.* (1991) “Oriented movement of statoliths studied in a reduced gravitational field during parabolic flights of rockets,” *Planta*, 185(2), pp. 153–161. doi:10.1007/BF00194056.

Wagner, T.A., Cove, D.J. and Sack, F.D. (1997) “A positively gravitropic mutant mirrors the wild-type protonemal response in the moss Ceratodon purpureus,” *Planta*, 202(2), pp. 149–154. doi:10.1007/s004250050113.

Waite, J.M., Collum, T.D. and Dardick, C. (2020) “AtDRO1 is nuclear localized in root tips under native conditions and impacts auxin localization,” *Plant Molecular Biology*, 103(1–2), pp. 197–210. doi:10.1007/s11103-020-00984-2.

Walker, L.M. and Sack, F.D. (1995) “Microfilament distribution in protonemata of the moss Ceratodon,” *Protoplasma*, 189(3–4), pp. 229–237. doi:10.1007/BF01280177.

Walker, LoniM. and Sack, FredD. (1990) “Amyloplasts as possible statoliths in gravitropic protonemata of the moss Ceratodon purpureus,” *Planta*, 181(1), pp. 71–77. doi:10.1007/BF00202326.

Wang, C. and Han, B. (2022) “Twenty years of rice genomics research: From sequencing and functional genomics to quantitative genomics,” *Molecular Plant*, 15(4), pp. 593–619. doi:10.1016/j.molp.2022.03.009.

Wickett, N.J. *et al.* (2014) “Phylotranscriptomic analysis of the origin and early diversification of land plants,” *Proceedings of the National Academy of Sciences*, 111(45), pp. E4859–E4868. doi:10.1073/pnas.1323926111.

Williams, J.H. (2012) “Pollen tube growth rates and the diversification of flowering plant reproductive cycles,” *International Journal of Plant Sciences*, 173(6), pp. 649–661. doi:10.1086/665822.

Wodniok, S. *et al.* (2011) “Origin of land plants: Do conjugating green algae hold the key?,” *BMC Evolutionary Biology*, 11(1). doi:10.1186/1471-2148-11-104.

Wolf, S., Hématy, K. and Höfte, H. (2012) “Growth Control and Cell Wall Signaling in Plants,” *Annual Review of Plant Biology*, 63(1), pp. 381–407. doi:10.1146/annurev-arplant-042811-105449.

Wolverton, C. *et al.* (2002) “Root gravitropism in response to a signal originating outside of the cap,” *Planta*, 215(1), pp. 153–157. doi:10.1007/s00425-001-0726-9.

Wu, S.-Z. and Bezanilla, M. (2018) “Actin and microtubule cross talk mediates persistent polarized growth,” *Journal of Cell Biology*, 217(10), pp. 3531–3544. doi:10.1083/jcb.201802039.

Yan, A., Xu, G. and Yang, Z.B. (2009) “Calcium participates in feedback regulation of the oscillating ROP1 Rho GTPase in pollen tubes,” *Proceedings of the National Academy of Sciences of the United States of America*, 106(51), pp. 22002–22007. doi:10.1073/pnas.0910811106.

Yassitepe, J.E. de C.T. *et al.* (2021) “Maize Transformation: From Plant Material to the Release of Genetically Modified and Edited Varieties,” *Frontiers in Plant Science*, 12. doi:10.3389/FPLS.2021.766702/FULL.

Yoder, T.L. *et al.* (2001) “Amyloplast Sedimentation Dynamics in Maize Columella Cells Support a New Model for the Gravity-Sensing Apparatus of Roots,” *Plant Physiology*, 125(2), pp. 1045–1060. doi:10.1104/pp.125.2.1045.

Young, J.C. and Sack, F.D. (1992) “Time-lapse analysis of gravitropism in Ceratodon protonemata,” *American Journal of Botany*, 79(12), pp. 1348–1358. doi:10.1002/j.1537-2197.1992.tb13744.x.

Zhang, Y. *et al.* (2019) “Evolution of fast root gravitropism in seed plants,” *Nature Communications*, 10(1), p. 3480. doi:10.1038/s41467-019-11471-8.

Secondary references

Braun, M. and Sievers, A. (1994) “Role of the microtubule cytoskeleton in gravisensing Chara rhizoids.,” *European Journal of Cell Biology*, 63(2), pp. 289–298. Available at: https://europepmc.org/article/med/8082653 (Accessed: May 4, 2022).

Cited from: Braun, M. (1997) “Gravitropism in tip-growing cells,” *Planta*, 203(S1), pp. S11–S19. doi:10.1007/PL00008098.

Schröter, K., Läuchli, A. and Sievers, A. (1975) “Mikroanalytische Identifikation von Bariumsulfat-Kristallen in den Statolithen der Rhizoide von Chara fragilis, Desv.,” *Planta*, 122(3), pp. 213–225. doi:10.1007/BF00385269.

Cited from: Braun, M. and Sievers, A. (2000) “Plant cells on earth and in space,” *Korean Journal of Biological Sciences*, 4(3), pp. 201–214. doi:10.1080/12265071.2000.9647546.

Sievers, A., Heinemann, B. and Rodriguez-Garcia, M.I. (1979) “Nachweis des subapikalen differentiellen Flankenwachstums im Chara-Rhizoid während der Graviresponse,” *Zeitschrift für Pflanzenphysiologie*, 91(5), pp. 435–442. doi:10.1016/S0044-328X(79)80257-3.

Cited from: Braun, M. and Limbach, C. (2006) “Rhizoids and protonemata of characean algae: model cells for research on polarized growth and plant gravity sensing,” *Protoplasma*, 229(2–4), pp. 133–142. doi:10.1007/s00709-006-0208-9.