Rhiannon Kearns
Science Research

Introduction

Cells communicate by transferring molecules from one to another. When a molecule is transferred it creates a reaction which then causes an effect. Within multicellular organisms there is cell-to-cell communication. Direct communication between cells can occur in two ways. One way is cell-cell recognition via interaction between surface proteins. When there is contact between cell surfaces, the proteins on the surface of the cell interact and create a signal. The other way is through cell junctions between adjacent cells. In animals this is called gap junction, in plants it is called plasmodesmata. Plasmodesmata are microchannels that act as intercellular cytoplasmic bridges that create an interconnected commune within adjacent cells and enable transport of materials between plant cells. The plasmodesmata connect the symplastic space in plants. They are specialized channels that permit intercellular movement of water, various nutrients, and other molecules (including signaling molecules) (Epel, 1994). Myosin VIII, a plant specific unconventional myosin has been localized within the plasmodesmata(Reichelt et al, 1997). The role of this protein within the plasmodesmata has yet to be determined.

Background

Eduard Tangl in 1897 noticed the presence of the plasmodesmata(PD) within the symplasm. In 1907, Strasburger termed these microchannels PD. The introduction of the electron microscope allowed the plasmodesmata to be studied more closely; in the 1980's scientists began to study the movement of molecules through the PD using fluorescent probes (Oparka, 2001).

Plant cells are surrounded by cell wall which separates neighboring cells. Plasmodesmata(PD) are located in areas in cell walls called primary pit fields. There is up to one million per square millimeter making up one percent of the entire area of the cell wall (Salisbury and Ross, 1992). There are two forms of PD, primary and secondary. Primary originates during cell division and remains in growing cells. Secondary PD are formed in mature plant cells. PD vary in formation as well. They can be simple, twinned, or branched. Simple PD is a single linear channel. Twinned occurs when two simple PD join together, commonly in Y-, V-, X-, or H-shaped. Branched PD are complex and have a common central cavity that connects the branched out channels. Branched PD are more commonly secondary PD, however simple secondary can occur (Roberts and Oparka, 2003).

Structure

The plasma membrane is continuous between cells, the inner leaflet contiguous with the plasmodesmal pore and the outer leaflet contiguous with the cell wall (Overall and Blackman, 1996; Oparka, 1993). A modified strand of endoplasmic reticulum(ER) termed the desmotubule runs the length of the PD(Hepler, 1982).Thus, the desmotubule is essentially a tube within a larger tube surrounded by the plasma membrane which is bordered by the cell wall. Tilney, et al. (1991) studied the structure of the desmotubule and how it relates to the overall structure of PD by using plasmolysis. The fern (Onoclea

sensibilis) gametophytes were plasmolyzed. The PD was found to remain intact as long as the desmotubule stayed in its regular, fixed position as the cells detached from the cell walls.Thus suggesting that the desmotubule provides a rigid stability to PD(Tilney, et al. ,1991) However, since the desmotubule is linked to the ER in adjacent cells a dynamic endomembrane continuum is formed. The ER can serve as a pathway for transporting various lipids between plant cells, including lipid signaling molecules (Grabski, et al., 1993).

The space between the plasma membrane and the desmotubule is called the cytoplasmic sleeve or cytoplasmic annulus. Plasmodesmal transport has been thought to occur both through the lipid portions of the desmotubule and the cytoplasmic sleeve. The neck region at each end of the plasmodesmal channel is where the plasma membrane closely associates with the central desmotubule. The neck region is proposed to contain proteins that regulate the passage of materials through the PD, much like gap junctions in animal cells(Roberts and Oparka, 2003). The accumulation of polysaccharide callose around the neck region of PD forms a collar, reducing the diameter and thereby controlling permeability of substances in the cytoplasmic sleeve(Robards, 1976).

There is an actin-myosin interaction network within the PD (White, et al.,1994) Overall and Blackman (1996) suggest the actin and myosin wrap around the central desmotubule and help stabilize the desmotubule. They treated plant tissue with cytochalasin (which interferes with actin structures) and found PD to be swollen. Actin has been shown to bind to viral movement proteins, which transport themselves through the plant via PD (Zambryski, 1995). Thus, various macromolecules require assistance to traffic through the PD. Actin has been thought to help alter the size exclusion limit of the neck region to allow for the transport of larger molecules (Overall and Blackman, 1996). In addition, myosin may also act as a cytoskeletal motor in plasmodesmal transport. Myosin has been found in several species of plants(Avisar, et al. 2008; Radford, et al. 1998). It is an ATP-dependent protein that generates directional movement by converting chemical energy into kinetic energy through hydrolysis. High ATPase activity has been demonstrated in PD(Fleurat-Lessard, et al. 1995). Alluding to the presence of myosin activity in the PD.

Function

PD are crucial for the transport of molecules(Lucas and Lee, 2004). Transport can occur in two forms: passive and active. Passive transport does not require energy and is simply the movement of molecules down the concentration gradient or from a high concentration to a low concentration(diffusion). Any molecule that is soluble can transport this way. If a molecule is soluble in lipids it is thought to diffuse within the desmotubule(Roberts and Oparka, 2003). Active Transport requires cellular energy. It uses ATP to pump molecules against the concentration gradient or from a low concentration to high concentration. Amino acids and other molecules can not diffuse or diffuse too slowly to survive and must be transported actively. There are two types of active transport: primary and secondary. In primary active transport, a group of ion channels exist and specialized trans-membrane proteins recognize the presence of a substance that needs to be transported, which then activate the channels to open and close causing change in concentration gradient. These pumps carry the desired

molecules across with the assistance of ATP(Physiology). In secondary active transport,proteins force the molecules across using an electromagnetic gradient. Often, this energy is gained by simultaneously moving another substance down the concentration gradient(Cooper, 2009). Molecules that can not simply diffuse are shuttled through plasmodesmata via unidentified active transport mechanisms(Oparka, 2003).

As mentioned earlier, a class of unconventional myosin is localized within the PD. Since myosin VIII is found only in plants it could perform plant specific functions. It is possible that myosin VIII along with actin regulates the architecture of PD via formation of radial spoke-like linkages between the central desmotubules and the plasma membrane (reviewed by Overall and Blackman,1996; Balusûka F et al. ,2001). Myosin VIII contains the RDALAK motif in its head domain which was proposed to be conserved for myosin I (Knight and Kendrick-Jones, 1993). Meaning myosin VIII contains a similar section of amino acid protein sequences that are like those of myosin I, whose function is to cargo molecules. Myosin VIII contain a unique C terminus which has several predicted phosphorylation sites for protein kinases A and C. Protein kinase A is associated with signal molecule regulation and when a protein kinase C reaction occurs it triggers the function of other proteins, this reaction is activated by an influx of Ca++ (Balusûka F et al. ,2001). Also the presence of four calmodulin-binding IQ motifs implies the regulation with both calcium and calmodulin, both of which are needed in myosin kinetic movement. These characteristics are key in other myosin motor proteins(Reichelt and Kendrick-Jones, 2000). With this knowledge, it is possible the myosin acts as a cargo-protein within the PD.

Myosin

Myosin is an mechanochemical enzyme or a type of enzyme that converts chemical energy(in this case ATP) into mechanical energy.Myosin is also an ATPase that traffics along the actin filaments by converting ATP via hydrolysis into conformational changes. Myosin is the motor; actin filaments are the tracks to which the myosin moves; ATP is the fuel that powers the interaction.Calcium too plays a key role in this interaction(Cheney and Mooseker, 1992).

Myosin are composed of three main domains. The head domain: contains actin and ATP-binding sites and is responsible for generating the force. The head domain among different classes of myosin generally are conserved. Next is the neck region, which regulates the activity of the head domain. Finally the tail domain contains binding sites that determine the function of that given myosin(Oliver et al, 1999). That being said, the tail domain's structure is a main way of characterizing a myosin(Oliver et al. 1999).

The first step of myosin ATPase activity is ATP binds to the myosin head which results in the myosin head releasing from the actin. Next ATP is converted into ADP+ a phosphate via hydrolysis. This "cocks" the myosin protein into a high energy conformation. In the high energy state the phosphate is released, which causes the myosin to push onto the actin. Finally the ADP is released which locks the myosin head in place and the cycle begins again(Tyska and Warshaw, 2002).

Myosin ATPase activity will not occur unless it is associated with actin. Therefore conformational changes of the actin results in the regulation of myosin movement. Two

proteins that assist with regulation of myosin are tropomyosin and troponin. Tropomyosin coils around the actin protein and blocks myosin binding sites on the actin. Troponin attaches the tropomyosin to the actin. In its unmodified conformation these two proteins prevent myosin from moving. The only way to reactivate the myosin activity is to have a conformational change of the tropomyosin and troponin. Calcium ions bind to the troponin which then changes the configuration of the tropomyosin, revealing the myosin binding sites. When there is a high concentration of calcium the myosin is able to move. When there is a low concentration the tropomyosin and troponin go back to their blocking states(Robertson et al, 2010).

Myosin Within Plasmodesmata

Following is a hypothetical scenario of the role of myosin. It is a combination of Karl J. Oparka's potential pathway model with other known regulatory mechanisms within the PD(Oparka, 2003). Oparka's model borrows some recent developments in yeast and animal biology (Wu et al. 2003). In this scenario, I will guide you on the potential way a molecule is transported via a myosin VIII cargo protein.

A signal molecule interacts with a cytoplasmic chaperone which then traffics the molecule to a proper motor protein(Gaestel, 2006),in this case it is a specific myosin motor. When the chaperone-assisted molecule reaches the myosin it bind to the tail of the myosin. This interaction then determines the transport specificity to travel to the PD(Oparka, 2003). When the molecule reaches the neck or threshold of the PD it binds to a putative docking protein. A myosin-specific kinase occurs which adjusts the size exclusion limit of the PD(Balusûka et al. 2001). The molecule is then free to travel via the myosin motor domain along the actin filaments found closely associated with the desmotubule(which is the main storage of calcium)(Koch, 1990). Calcium ions are needed in a actin-myosin interaction(Robertson et al, 2010). Calcium ion gates on the desmotubule release Ca^{2+} and the concentration of calcium allows the binding sites on the actin to reveal.When it reaches the other neck region of the PD another kinase occurs which results in the release of the chaperone-assisted molecule from the motor domain(Oparka, 2003). When it reaches its destination, it is released from the chaperone protein(Gaestal,2006).

Future Directions

This all being said, in order to see if the myosin truly is acting as a cargo protein within the PD, there must be experimental research specifically related to finding the function of myosin VIII. This can be done using numerous methods. Variable changes such as calcium concentration in correlation with molecular trafficking(Oparka, 2003). Since calcium is needed in myosin activity perhaps if the change in calcium affects how much is being trafficked that then alludes to the function of myosin. Also GFP tracking and gold immunofluorescence with the use of electron microscopes are ways of documenting visual evidence of myosin(Lee et al. 2011). In any case the worlds knowledge of the PD are far from complete. There are still many questions unanswered and many studies to be performed. Perhaps one day the function of myosin in the PD will be determined.

Acknowledgements
I thank Dr. Patricia Zambryski, Dr. Robert Turgeon, and Jeff Mann for assisting me in my research thus far.

References

1. AW Robards (1976) Plasmodesmata in higher plants. In: Intercellular communications in plants: studies on plasmodesmata. Edited by BES Gunning and AW Robards Springer-Verlag Berlin pps 15-57.

2. Avisar D, Prokhnevsky AI, Dolja VV (2008) Class VIII myosins are required for plasmodesmatal localization of a closterovirus Hsp70 homolog. J Virol 82: 2836–2843

3. Balusûka F, Cvrcûkova´ F, Kendrick-Jones J, Volkmann D. 2001a. Sink plasmodesmata as gateways for phloem unloading. Myosin VIII and calreticulin as molecular determinants of sink strength? Plant Physiology 126, 39–46.

4. Cheney, Richard E.; Mooseker, Mark S. (1992). Unconventional myosins. Current opinion in cell biology 4 (1): 27–35.

5. Cooper, Geoffrey(2009) The Cell: A Molecular Approach. Washington DC: ASM PRESS. p 65

6. Epel, BL. (1994) Plasmodesmata: Composition, structure, and trafficking. Plant Mol Bio 26 (5):1343-1356.

7. Gaestel M. (2006)Molecular chaperones in signal transduction. Handb Exp Pharmacol.;(172):93-109.

8. Grabski, S, de Feijter, AW, and Schindler, M. (1993) Endoplasmic reticulum forms a dynamic continuum for lipid diffusion between contiguous soybean root cells. The Plant Cell 5:25-38.

9. Hepler, PK. (1982) Endoplasmic reticulum in the formation of the cell plate and plasmodesmata. Protoplasma 111:121-133.

10. Koch, GL. (1990) The endoplasmic reticulum and calcium storage. Bioessays.Nov; 12(11):527-31.

11.Knight AE, Kendrick-Jones J (1993) A myosin-like protein from a higher plant. J Mol Biol 231:143–154.

12. Lee JY, Wang X, Cui W, Sager R, Modla S, Czymmek K, Zybaliov B, van Wijk K, Zhang C, Lu H, Lakshmanan V. (2011)A plasmodesmata-localized protein mediates

crosstalk between cell-to-cell communication and innate immunity in Arabidopsis. Plant Cell. Sep;23(9):3353-73.

13. Lucas WJ, Lee JY (2004) Plasmodesmata as a supracellular control network in plants. Nat Rev Mol Cell Biol. Sep;5(9):712-26

14. Oliver, T. N.; Berg, J. S.; Cheney, R. E. (1999). Tails of unconventional myosins. Cellular and molecular life sciences 56 (3–4): 243–57.

15. Oparka, KJ. (1993) Signalling via plasmodesmata--the neglected pathway. Semin. Cell Bio. 4: 131-138.

16. Oparka , KJ. (2001) "Plasmodesmata. A Not So Open-and-Shut Case." Plant Physiol 125: 123-125.

17. Oparka, KJ.(2003) Getting the message across: how do plant cells exchange macromolecular complexes? Trends in Plant Science (9):-33-42.

18. Overall, R and Blackman, L. (1996) A model of the macromolecular structure of plasmodesmata. Trends in Plant Science 1 (9):307-311.

19. P. Fleurat-Lessard, S. Bouché-Pillon, C. Leloup. (1995) Absence of plasma membrane H+-ATPase in plasmodesmata located in pit-fields of the young reactive pulvinus ofMimosa pudica L. Protoplasma, Volume 188, 3-4, 180

20. Physiology at MCG 7/7ch05/7ch05p11

21. Radford, Janine E.; White, Rosemary G., 1998: Localization of a myosin-like protein to plasmodesmata. Plant Journal. 14(6): 743-750, E

22. Reichelt S, Kendrick-Jones J (2000) Myosins. in Actin: A Dynamic Framework for Multiple Plant Cell Functions. eds Staiger CJ, Baluška F, Volkmann D, Barlow PW (Kluwer Academic Publishers, Dordrecht, The Netherlands), pp 29–44.

23. Roberts, A. G. and Oparka, K. J. (2003), Plasmodesmata and the control of symplastic transport. Plant, Cell & Environment, 26: 103–124.

24. Robertson I, Sun YB, MX Li, BD Sykes (2010). A structural and functional perspective into the mechanism of Ca2+-sensitizers that target the cardiac troponin complex. Journal of Molecular and Cellular Cardiology 49: 1031–1041

25. S Reichelt, AE Knight, TP Hodge, F Baluska, J Samaj, D Volkmann and J Kendrick-Jones (1999) Characterization of the unconventional myosin VIII in plant cells and its localization at the post-cytokinetic cell wall. Plant Journal 19: 555–569

26. Tilney, LG, Cooke, TJ, Connelly, PS, and Tilney MS. (1991) The structure of plasmodesmata as revealed by plasmolysis, detergent extraction, and protease digestion. The Journal of Cell Biology 112 (4):739-747.

27. Tyska, Matthew J.; Warshaw, David M. (2002). The myosin power stroke. Cell Motility and the Cytoskeleton 51 (1): 1–15.
28. Wu, X. et al. (2003) Modes of intercellular transcription factor movement in the Arabidopsis apex. Development 130, 3735 – 3745