ROLE OF RNA –BINDING PROTEINS IN CONTROLLING CELL TO CELL COMMUNICATION and MOLECULAR TRAFFICKING IN PLANTS

ABSTRACT

Cell to Cell communication is the most important process in all living organisms. This communication network is essential for growth, differentiation, development and metabolic functions. As plant cell has a rigid cell wall so there are some special ways for using communication network through a special structure called Plasmodesmata. The messages from the DNA of dividing cells pass signals or messages to other cells through RNA molecules. But RNA molecules cannot pass independently to other cells , so some proteins are needed that are bound to RNA (RNA-binding Proteins). These proteins actually carry signals from one cell to another. In plants plasmodesmata is very small that can carry only water and micronutrients. But it has been noted that the size of plasmodesmata is not constant but is very dynamic meaning thereby that it their sizes can change according to their need. The role of plasmodesmata in communication and the role of RNA-binding proteins in communication as well as in molecular trafficking have been discussed.

Introduction

In any organism, cell does not remain in isolation but there is a cell to cell communication network for growth, differentiation, development and other metabolic functions. This cellular communication is also necessary for co-ordination of cellular functions particularly in multicellular organisms. Generally dividing cells pass signals or messages from one cell to another to co-ordinate growth and differentiation to the development of different organs. The messages from the DNA of dividing cells pass or communicate to other cells through RNA molecules. But RNA molecules cannot pass independently to other cells so some proteins are needed that are bound to RNA. This is known as RNA-binding proteins that carry signals from one cell to other. Animal cell has no problem as there is a thin cell membrane without any cell wall. But plant cell has a thick cell wall besides cell membrane. So the signalling process is different here. The signal molecules (RNA-binding) proteins pass through tiny pores of the cell wall called Plasmodesmata. Normally plasmodesmata is very small that only water and nutrients can pass through it. The size of plasmodesmata is not constant but it is dynamic meaning thereby that their size can change according to their need. This dynamism of plasmodesmata plays an important role in protecting plants from disease. When a plant cell is infected by a pathogen, the plasmodesmata can shrink so that it cannot enter the cell. Thus there are different processes or pathways or some networks for communication either through electrical or chemical signals or mass transfer through physical structures called plasmodesmata.

i) Plasmodesmata in communication

Cell to cell communication is very important in multicellular organisms for growth and development. Generally this is done through receptor-ligand reactions or through some carriers. The special structure present in plant cell for communication is plasmodesmata. It is the membrane bound structure, called channels that pass through cell wall to keep connection with the adjacent cell for the exchange of essential molecules from one cell to another. Plasmodesmata are nanochannels embedded in the cell wall. They mediate transport of various signals from cell to cell including proteins, RNA, hormones, ions and nutrients (Jackson 2022).The structure of plasmodesmata is different and this can be classified into three types likei) Simple; ii) Branched (Y-shaped) ; iii) and X-shaped on the basis of plant developmental stage and tissue specificity (Sun et al 2019). The transmission electron microscopic (TEM) studies found a cylindrically compressed endoplasmic reticulum (ER) which is called Desmotubule that formed a gap between the desmotubule and the plasma membrane. This formation of a gap functions as a channel for the transmission of some molecules to other cell. Some spoke like elements within the plasmodesmata made a connection between the desmotubule and the inner layer of plasmodesmata. These spoke like elements may be composed of actin and myosin like structures of the cytoskeleton. Plants use RNA as a way to relay messages from cell to cell through plasmodesmata. It has been clearly shown in experiment using fluorochromes in *Arabidiopsis thaliana* that RNA messages (stained orange) jump around inside a cell. When messages found tiny channels called plasmodesmata (stained blue) that allow them to pass leading to the transport of substances from cell to cell (Jackson and Kitagawa 2022). They also identified escort protein called AtRRP44a in *A.thaliana*. It has also been found that lowering the amount of AtRRP44a slowed the movement of RNA messages leading to hamper the development of plants (Jackson and Kitagawa 2022).

It is well known that signalling molecules like transcription factors (TFs) and small non-coding RNAs (sRNAs) are transported from one cell to another in plants through plasmodesmata. The movement of first signal of plant was

noted as the homeodomain (HD) TF KNOTTED 1 in maize. KN1 gene is expressed in the L2 layers of the Shoot Apical Meristems (SAM) of maize and then the signal protein KN1 moves into the layer L1 for growth and development of shoot. During normal development KNAT 1 moves from the cortex to epidermal cells in stems and the movement of signals through plasmodesmata is required for epidermal differentiation and for the formation of plant architecture. Another mobile signal KN1 homologue is found in *Arabidiopsis*(Kitagawa and Jackson 2017). It is known that SAM in plants produce all shoot tissues like leaves, stems, flowers and reproductive tissues (germ line). It has also been noted that there are some receptor-like-kinases and receptor like proteins in plasmodesmata that also help in the movement of molecules through plasmodesmata. These receptors were identified in *Arabidopsis* and in rice cell wall proteome through Proteome analysis of plasma membrane of plasmodesmata (Kitagawa and Jackson 2017).

The dynamic character of plasmodesmata is clearly observed in the movement of substances in the phloem. Asymmetric plasmodesmata is found between companion cells and sieve elements to form a plasmodesmata-pore unit while at the sieve plate plasmodesmata is enlarged through callose-mediated cell wall restructuring to form expanded cytoplasmic pores through which the mass flow of phloem sap occur.Again funnel shaped plasmodesmata is found in the cell wall junctions between sieve elements and the phloem pole in pericycle cells using tools like electron tomography and 3D scanning electron microscopy in the phloem of root (Sager and Lee 2018). Several genes that control the transport of molecules through plasmodesmata have been identified in *Arabidiopsis*through mutant screening methods. Thus plasmodesmata is a dynamic structure forming a trans-wall channels connecting adjacent cells to transport water, nutrients, signalling molecules and other macromolecules. Generally the size of molecules that pass through plasmodesmata is less than 800-1000 Da but that can be modulated by environmental and developmental signals.

In addition to the signal KN1, there are other macromolecular signals like TFs (Transcription Factors) and some sRNAs that are transferred to target cells through plasmodesmata for morphogenesis and development. In *Arabidiosis*

some homologues of KN1 have been noted such as SHOOT MERSISTEMLESS (STM) and ARABIDIOPSIS KNOTTED-LIKE (KNAT1/BREVIPEDICELLUS (BP) yjayact as mobile proteins. These proteins move L1 layers of SAM to the inner layers for the development of other parts of the stem (Kitagawa and Jackson 2017). Again there are some small non-coding RNAs (sRNAs) and Micro RNAs (mi RNAs) that are also acting as signals in plants. Some receptor proteins like Kinases and other receptor like proteins arehelping in the plasmodesmata mediated movement.It has been noted that development of the plant organs depend on the symplastic movement of TFs through plasmodesmata and it can also give plant defense or immunity against any viral infection. In plants callose accumulation may also take part in the control of cell to cell communication by its deposition at the neck of plasmodesmata leading to regulate the mass symplastic flow by making constriction at the channel of plasmodesmata (Maule et al 2012).When the pathogen causes local infection to plants, the infected cells send signals to other uninfected cells making the cells resistant to pathogen to control the spread of infection by forming Salicyclic acid, Azelaic acid, Glycerol-3-phosphate etc.

Thus cell to cell communication is most important for all multicellular organisms including plants for growth, development and responses to environment and pathogens. The main component for communication is the signal molecules released from one cell and then these are transferred to other cells through receptor proteins. In addition to proteins plants also use RNA molecules for sending signals to adjacent cell with the help of plasmodesmata. But it has been noted that proteins select the suitable RNA for transport and bind with that RNA to form RNA-binding proteins which can help for faster movement.

Role of RNA-Binding Proteins in Communication and Molecular Trafficking in Plants

The process of Molecular trafficking is the way by which proteins and other molecules are transferred to different cells through some carriers (RNA-binding proteins).The molecular trafficking in plants have been well studied in mediating trafficking of RNAi (RNA interference). It is known that RNAi has

played an important role in growth and development, gene regulation andresponses to pathogen attack, biotic and abiotic stresses in plants. All these processes of molecular trafficking are carried out through RNA-binding proteins.

RNA-binding proteins with more than 2000 proteins regulate genes at the post transcriptional level by controlling splicing,polyadenylation, RNA trafficking etc (Huh and Paek 2013, Corley et al 2020). The transport of macromolecules in plants takes place through the phloem through wide range of signalling pathways with the help of RNA binding proteins (assisting translocation). It has been noted that RNA binding proteins contain some sequence motifs like RBA recognition motif (RBM) and K homology domain (KH) that can be combined with each other or with other protein domains (Pallas and Gomez 2013).In addition to these motifs, there are other motifs also found in plants like Glycine rich motif, double stranded RNA binding motif, Zinc finger etc. These motifs are plant specific (Pallas and Gomez 2013) and are found in the transport system of phloem. The mRNAs, small RNAs(Si RNA) and other macromolecules like sugars, amino acids, mineral nutrients, hormones , proteins etc are transported through phloem. It has been noted that very limited RNA degradation is taking place in the sieve tubes as no RNase activity is found there (Lin et al 2009).

Molecular Trafficking in Plants

It has been found that small RNAs (siRNAs) have played an important role in trafficking macromolecules from cell to cell through plasmodesmata, particularly in mediating long distance delivery in phloem tissues.In studying the molecular mechanism behind the transport of small RNAs has been identified theprotein that mediates the cell tocell communication of siRNA. This protein is found to be PHLOEM SMALL RNA BINDING PROTEIN1 (Cm PSRP1) isolated from the phloem of Pumpkin (*Cucurbita maxima)*. This protein binds to si RNA in the form of Ribonucleoprotein complex. The stability of this complex is strengthened by the phosphorylation of protein CmPSRP1 leading to the systemic trafficking of the bound siRNA signalling agents (Yan et al 2020).

During studies of molecular trafficking in plants, it has been noted that the composition of cell wall is not same always but is different among cell types. It is also modified in response to environmental conditions (Ebine and Ueda 2015). Many proteins are required for the modification of cell wall and endo-membrane through transport of macro-molecules like proteins, lipids, polysaccharides within the organelles. The mechanism of membrane trafficking is generally done by three steps like i) through budding of vesicle from organelles sometimes with coat protein complexes (Fig.1); ii) attaching the vesicle to the membrane with the help of Rabproteins and Rab effectors. Rab protein is a member of the Ras superfamily of G proteins which has played an important role in regulating many steps of molecular trafficking; iii) fusion of vesicle membrane with the target membrane of organelle is done through a special protein known as SNARE. It is called as SNAP receptor. This receptor is also known as soluble NSF(N-ethylmaleimide sensitive Factor) attachment protein. Once the vesicle is attached to the target membrane, SNARE protein is activated to fuse the membrane of vesicle and target membrane(Rognilien and Woodbury2003). Most of the proteins are synthesized in the endoplasmic reticulum (ER) and polysaccharides in the Golgi apparatus are transported to the organelle or the plasmamembrane/extracellular space and finally to the vacuoles of plant cells through Golgi independent trafficking pathway via trans-Golgi network (Ebine and Ueda 2015). The SNARE protein helps in the fusion of transport vesicles and target membrane.Thus the transport of macromolecules like proteins, lipids and polysaccharidesin plant cells is done through vesicular carriers which are formed by the coat protein complex in the donor organelles or cells with the help of GTPases and protein receptors SNARE (Cevher-Keskin 2020).

In the secretory pathway of plants, the lytic vacuoles of plants transport the soluble proteins to the lumen of endoplasmic reticulum. If the protein is not retained in the endoplasmic reticulum then it is transported to the Golgi apparatus for further processing. Proteins with vacuolar sorting signals are also known as cargo proteins that are finally sorted into the lytic vacuoles. It has been noted that the transport of storage proteins 2S albumin and 11S globulin of pumpkin seeds, globulin of rice endosperm are sometimes done

directly in the protein storage vesicles from the endoplasmic reticulum without going through Golgi apparatus (Zhu et al 2019). If there is no vacuolar sorting signal in the protein then it is transported through the trans-Golgi network pathway. Endocytosis is also another pathway of plants that plays an important role in cell to cell communication of membrane proteins, lipids and extra-cellular materials (Zhu et al 2019). Different methods using organelle markers are used to identify the protein subcellular localization and also for the analysis of trafficking process. These methods used are the Immunofluorescence technique, Organelle Fractionation, Immuno Electron Microscopy and others (Zhu et al 2019).

It is known that the localization of mRNAs is the first step to drive protein targeting both in prokaryotes and eukaryotes for the accumulation of locally translated proteins to specific areas of the cell for establishing cell polarity, patterning, fate determination and protein sorting (Tian et al 2020). During mRNA localization, cis- acting elements (RNA Zipcodes) are bounded by RNA binding proteinsto form mRNA-nucleoprotein complex that are transported to the cytoplasm. These proteins are finally transferred to the target site after some modificationsthat was observed by Tian and others during transport βGlutelin mRNAs in the endosperm of Rice (Tianet al 2020).

It has also been found that the Zipcode binding protein (a member of highly conserved family of RNA-binding protein) is important for the transportation of specific protein to the target (Doyle and Kiebler 2012). Of the RNA binding proteins (RBP), Zipcode binding protein (Highly conserved family of RBPs) plays an important role in the localization of target RNA sequence. Although this Zipcode binding protein has not yet been identified in plants but its role has been detected in targeting β-actin mRNA of neurons (Doyle and Kiebler 2012). However, it has been noted in Rice plants that the localization and transport of mRNAs encoding glutelin and prolamine of storage proteins to the Endoplasmic reticulum has been done through cis-acting elements (RNA zipcodes) bound by RNA-binding proteins to form a primary mRNA-nucleoprotein complex. Then this complex undergoes remodelling with attachment of new factors for enabling cytoskeleton-transport to the target site (Tian et al 2020).

The transport of macromolecules like proteins, lipids and polysaccharides from compartment to compartment in plants is being done through vesicular carriers that are formed by the assembly of coat protein complexes (COPs) with donor organelles (Cehver-Keskin2020) which is mediated by ARF/SAR1 (ADP-ribosylation factor/ secretion- associated RAS superfamily) GTP-ases(and coat proteins in many cases) .Finally, the fusion of the transport vesicle to the target membrane has been done by the Soluble N-ethylmalemide-sensitive factor Attachment protein receptors (SNARE) followed by recycling transport machinery components to the donor membrane (Cehver-Keskin 2020). This fusion process is regulated by tethering proteins with the help of RAB GTPase (RAS associatedproteins) [Fig.1]. The term tether is used in cells which form bonds with a substrate to connect the desired area or object to the main body of the cell.

In plants VacuolarTransport systemis the most important for trafficking macromolecules that are essential for all types of plant development with the help of some important components like Rab GTPases. This enzyme falls under the family of small GTPases . It has been noted 57 members of RabGTPasrs in the genome of *Arabidiopsis thaliana* that are important in Vacolar trafficking pathways of plants. This vacuolar trafficking pathway is the moat important

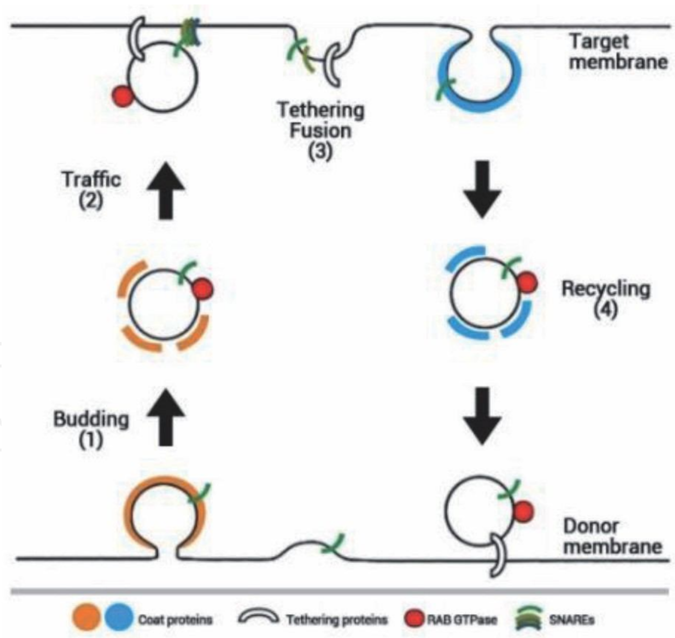

Fig.1. Showing Membrane Trafficking pathway (Taken from Cehver-Keskin 2020).

pathways for molecular trafficking in plants . The actual mechanism is not clearly known.

The transport of macromolecules in plants takes place from the plasma membrane to the cytoplasm through another important pathway called the Endocytic pathway. In the life cycle of plant, endosomes have played an important role in developmental processes like lateral organ differentiation, signal transduction, root hair formation, embryo patterning etc (Cehver-Keskin 2020; Reyes et al 2011).In all these cases, RBA-binding proteins (RBPs) have a distinct role which has been discussed in some important functions.

Role of RNA-binding proteins in signalling

RNA-binding proteins (RBPs) are mediating many important functions of plants by binding RNA through single or multiple RNA-binding domains. One of their important roles are in plant signalling during environmental stress conditions. The function of these proteins have been reported not only in higher plants but also in unicellular alga (*Chlamydomonas reinhardtii)*, several bacterial species like *Shigella sonnei , Enterococcus faecalis, Bacillus subtilis* (Muleya and Marondedze 2020).Some of the RBPs have an important role in unicellular organisms in the cellular localization of RNAs. These proteins are functionally conserved in *Arabidiopsis thaliana* and *Oryza sativa* during cold stress adaptation. It has also been noted that RBPs have played an important role in signalling of stress in plants. In addition to this these proteins are important in carbohydrate metabolic pathways like Glycolysis and tri-carboxylic cycle (Muleya andMarondedze 2020).

During stress responses, RBPs are playing a regulatory role in producing stress granules through the interaction of RBPs with each other along with the association of proteins with mRNAs. These stress granules are also found to produce during heat shock stress. Under normal condition the translation process is going on in plants. But when stress signal is detected, the normal translation process is stopped leading to the formation of stress granules followed by the transcription and translation process of stress responsive genes to overcome the stress conditions (Muleya and Marondedze 2020). Thus RBPs have played an important role in signalling various environmental stresses.

Adaptation to various stress conditions like drought, heat and salinity leads to reprogramming of cellular events by signalling network to form stress granules for overcoming the stress conditions. These activities have been done with the help of RBPs. Thus various studies have indicated that RBPs have played a crucial role in stress adaptation and in controlling metabolic processes, RNA splicing and other processes (Marondedze2020).

Researches are going on to identify the KNOTTED 1 Gene in *Arabidiopsis thaliana* which keeps plant stem cells in undifferentiated state and is very important for plant growth and development. This gene (KNOTTED 1) receives signal to communicate the message for initiation of growth tothe target cells via RNA signal molecule. It has been identified RBPs to escort the signal molecule from KNOTTED 1 gene through the plasmodesmata so that it can move between cells (Jackson 2022). Jackson and his team used a screening technique to search for genes that control the transport of KNOTTED 1 RNA signal molecule and also identified the gene that encodes RNA-binding protein. Jackson also demonstrated the movement of the KNOTTED 1 RNA signal molecule between cells using a fluorescent tag or marker using a super resolution Confocal microscope. They also showed that RNA-binding proteins have played the most important role in plant communication by binding to specific RNA molecule that may be either due to the presence of a specific code in the RNA molecule or due to some chemical modifications in the RNA molecule that help to identify the binding proteins.

Thus the importance of RNA-binding protein in plant cell communication has been established.

REFERENCES

1. Sun, Yanbiao, Dingquan Huang and Xu Chen. 2019. Dynamic regulation of plasmodesmatal permeability and its application to horticultural research. Hort. Res. 6: 47. Doi.org/10.1038/s41438-019-019-0129-3.
2. Kitagawa, Munenoriand David Jackson. 2017.Plasmodesmata-mediated Cell-to-Cell Communication in the Shoot Apical Meristem : How Stem Cells talk. Plants 6 ;12-26. . doi:10.3390/plants 60010012.
3. Sager, Ross E and Jung-Youn Lee. 2018. Plasmodesmata at a glance. Journal of Cell Science 131,jcs 209346.
4. Maule, Andy, Christine Faulkner and Voselin Benitez-Alfonso. 2012. Plasmodesmata “ incommunicado”. Frontiers in Plant Science 3. Article 30. 1-4. doi:10.3389/fpls.2012.00030.
5. Sang Un Huh and Kyung-HeePaek 2013. Plant RNA Binding proteins forcontrol of virus infection. Frontiers in Physiology.Vol.4 (Article 397) :1-5.
6. Corley, Meredith, Margaret C. Burns and Gene W. Yeo.2020. Molecular Cell 78 (April 2): 9- 29.

7. Pallas, Vicente and Gustavo Gomez. 2013. Phloem RNA Binding proteins as potential components of the long distance RNA transport system. Frontiers in Plant Science. Vol.4 (Article 30) : 1-6.
8. Lin, MK, Lee,Y-J, Lough, TJ, Phinney, BS and Lucas, WJ. 2009. Analysis of the pompkin phloem proteome provides insights into Angiosperm sieve tube function. Mol. Cell Proteomics 8: 343-356.
9. Jackson, David and M. kitagawa. 2022. Plants: RNA notes to self. Cold Spring HarborLaboratory . 13 January . (written by Luis Sandoval., Communications specialist sandova@cshl.edu)
10. Yan Yan, Byung-Kook Ham, Yee Hang Chong, Shyi- Dong Yeh and William J. Lucas.2020. Molecular Plant 13:331-335.
11. Ebine, Kazuo and Takashi Ueda. 2015. Roles of membrane trafficking in plant cell wall dunamics. Frontiers in Plant Science. Vol.6, Article 878.
12. Rognilien, KT and DJ Woodbury.2003. Planar Lipid Bilaters (BLMS) and their Applications. Chapter 16. In Plant Lipid Bilayers (BLMS) and their Application. eds. HT, Tien and A. Ottova-Leitmannova. Elsevier science. B.V.
13. Cevher-Keskin, Birsen. 2020. Endomembrane Trafficking in Plants. Chapter In the Book Electrodialysis . DOI. http;//dx.doi.org./10.5772/intechopen.91642.
14. Zhu, Dongmei, Mengdi Zhang, caiji Gao and Jinbo Shen. 2019. Protein Trafficking in Plant Cells. Science China Life Sciences. 62: 1-21. https ://doi.org./10.1007/s 11427-019-9598-3.
15. Tian, Li, Kelly A. Doroshenk, Laining Zhang, Masako Fukuda et al. 2020. Zipcode RNA binding proteins and Membrane Trafficking Proteins Cooperate to transport Glutelin mRNAs in Rice endosperm. The Plant Cell, Vol.32: 2566-2581.
16. Doyle, Michael and Michael A. Kiebler .2012. A zipcode unzipped. Gene and Development. 26: 110-113.
17. BirsenCevher-Keskin. 2020, Endomembrane Trafficking in Plants . In Electrodialysis (Ed.TanerYonar) Published by Intech Open. DOI: http:// dx.doi.org./10.5772/intechopen.91642 chapter pp.1-22.
18. Reyes, FC, B. Rafael, M.S Otegui .2011. Plant endosomal trafficking pathways . Current Opinion in Plant Biology. 14(6): 666-673.

19. Muleya , Victor and Claudius Marondedze. 2020. Functional Roles of RNA-binding proteins in Plant signalling. Life 10: 288; doi: 10.3390/life 10110288.
20. Marondedze, C. 2020.,The increasing diversity and complexity of the RNA-binding protein repertoire in plants. Proc. Roy. Soc. B. 287: 20201397. http:// dx,.doi.org./10. 1098/rspb.2020.1397.
21. Jackson, Dave. 2022. Can we decipher the mechanisms that allow plant cells to communicate? Newsletter Cold Spring Harbor Laboratory (CSHL), NewYork, USA.