"Never Walk Alone: Clathrin-Coated Vesicle (CCV) Components in Plant Immunity"

Abstract

The plasma's protein makeup at the host-pathogen interaction membrane (PM) has significant effects on a plant cell's perception of and reacts to microbiological infections that invade. The capacity of a plant to adjust Its PM composition is essential for controlling the intensity, length, and immunological response integration. One method by which plant cells reconfigure the vesicular trafficking on their cell surface, encompassing secretion and endocytosis. During these trafficking procedures, cargo proteins (such as transporters, receptors for pattern recognition, and other proteins with immunological activities) by tiny, membrane-bound vesicles to or from the PM. Vesicles covered with clathrin (CCVs) that develop at the PM and trans-Golgi membranes Early endosomes and networks have become the most common vesicle form in the control of plant defense mechanisms.

Keywords:adaptor, CME, dynamin, endocytosis, FLS2, PRR

Introduction

In order to defend against possible infections caused by invasive pathogens, plants employ a two-layered immune system that consists of effector-triggered immunity (ETI) and pattern-triggered immunity (PTI). Pattern-recognition receptors (PRRs) located in the plasma membrane (PM) in PTI identify structurally varied microbe- or pathogen-associated molecular patterns (PAMPs) that are obtained from infections that are encroaching. Many immunological responses that lead to PTI are triggered by PAMP perception. In order to prevent the plant from mounting appropriate PTI responses, pathogens concurrently inject a variety of effector proteins into the host's cytoplasm and extracellular space (apoplast), partly by focusing on the host's essential cellular activities. Conversely, plants have developed resistance proteins that enable them to identify pathogen effectors either directly or indirectly (Ricotta *et al.,* 2012).

CLATHRIN CORE COMPONENTS

Evolutionarily conserved clathrin, a hexameric protein complex made up of three CLATHRIN HEAVY CHAINs (CHCs) and three CLATHRIN LIGHT CHAINs (CLCs) that combine to create a three-legged clathrin triskelion, is the central component of CCV and the source of its name. One CHC that attaches to the clathrin triskelion's C-terminal region makes up each leg. Through the C termini of CHCs, three CHC-CLC dimers join to create the clathrin triskelion, in addition to one CLC. The distinctive soccer ball- or basket cage-like structure of CCVs can then be formed by multiple clathrintriskelions self-assembling into a lattice of pentagons and hexagons.

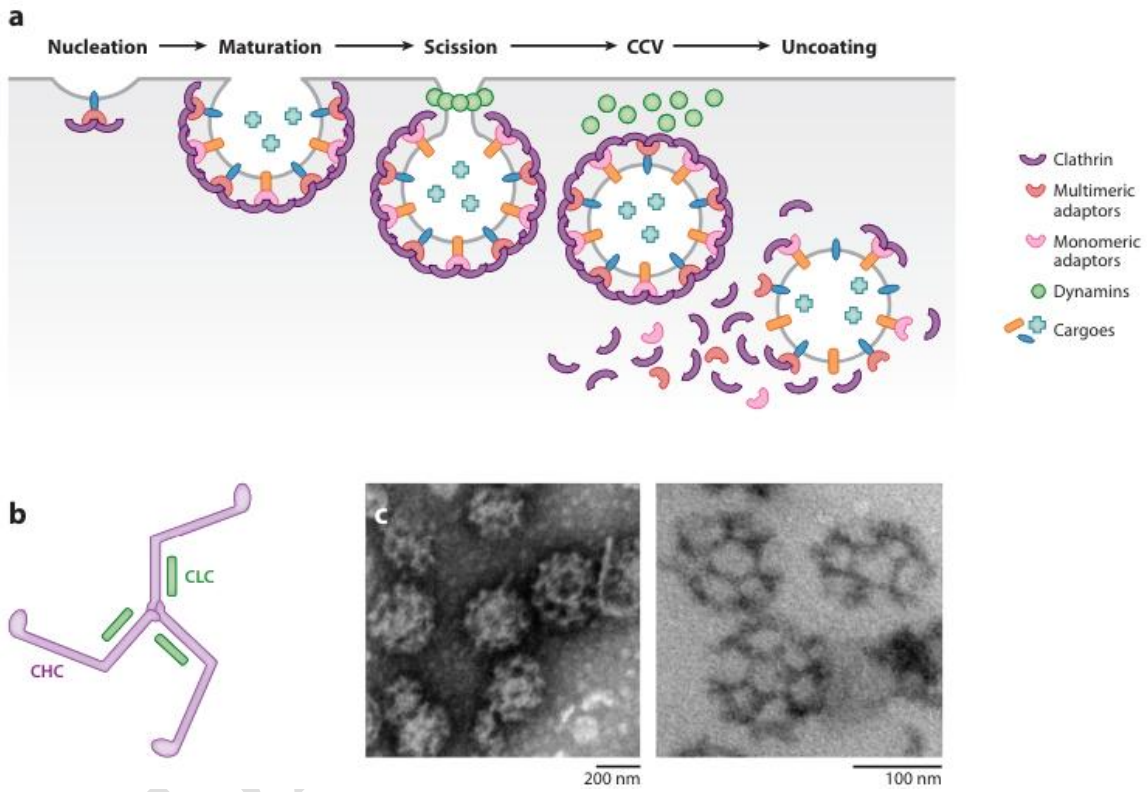

Fig 1(a) Proposed model of CCV assembly and delivery in plants. (b) Diagram of a clathrin triskelion composed of three CHC monomers and three CLC monomers. (c) Transmission electron microscopy of enriched CCVs from Arabidopsis: (left) negative-stained CCVs and (right) conventional transmission electron microscopy of CCVs. Images provided by Dr. Sebastian Bednarek, University of Wisconsin–Madison. Abbreviations: CCV, clathrin-coated vesicle; CHC, clathrin heavy chain; CLC, clathrin light chain **(**Kirchhausen., 2009).

CLATHRIN-COATED VESICLE ADAPTORS

CCV adaptors are essential for cargo sorting because they can identify "sorting motifs" in

cargo proteins. Short peptide sequences, such the di-Leu [DE]XXXL[LI] motif or the Tyr-based YXXφ motif (where φ denotes a bulky hydrophobic amino acid), can be used as sorting motifs. which particular adaptor protein (AP) complex components recognize Furthermore, APs with ubiquitin-interaction motifs are able to detect ubiquitination, and posttranslational changes like phosphorylation and ubiquitination can function as endocytic sorting signals. At many stages of vesicular trafficking in plants, such as endocytic internalization, endosomal sorting, and vacuolar targeting of ubiquitinated plant cargo proteins, mono- and polyubiquitination of cargo proteins have emerged as sorting signals. Additionally necessary for ligand-induced breakdown is cargo ubiquitination **(**Praefcke., 2014).

Monomeric Clathrin-Coated Vesicle Adaptors

Monomeric CCV adaptors integrate each of the distinct roles into a single polypeptide. monomeric adaptors feature a modular domain architecture made up of many domains and peptide motifs that are involved in cargo recognition in each subunit of multimeric adaptors as well as attaching to clathrin, lipids, multimeric adaptor subunits, and other vesicular trafficking proteins (78). At the PM or TGN, distinct monomeric adaptors take part in the synthesis of CCV frequently cooperating with multimeric adaptors (78). Nonetheless, far less is known about the cellular functions of monomeric adaptors in plants than that of multimeric adaptors, Members of the ENTH/ANTH/VHS families are monomeric adaptors that contain an ANTH, VHS (Vps27, Hrs, and STAM), or ENTH (EPSIN N-terminal homology) domain at the N termini where they are engaged (Takei and Haucke., 2011).

ACCESSORY COMPONENTS INVOLVED IN CLATHRIN-COATED VESICLE SCISSION, UNCOATING, AND OTHER FUNCTIONS

Dynamin-Related Proteins

Dynamins and dynamin-related proteins (DRPs) mediate the mechanochemical release of CCVs into the cytoplasm subsequent to vesicle maturation, cargo selection, and clathrin recruitment. Membrane fission is catalyzed in a GTP-dependent manner by these high molecular weight GTPases, which assemble into contractile helical polymers around the neck of the budding CCV. Six DRP families are encoded by plants, and members of the DRP1 and DRP2 families are thought to be involved in the scission of CCVs DRP2A and DRP2B, members of the evolutionarily conserved DRP2 family, are regarded as authentic (or classical) dynamins due to their fivedomain design, which they share with animal

dynamins. Conversely, pleckstrin homology is absent from plant-specific DRP1s, which only have an N-terminal catalytic GTPase, a middle domain for self-assembly, and a GTPase effector domain.

DRP2s in plant immunity

DRP2A and DRP2B are functionally redundant for the gametophytic development of Arabidopsis, sharing 93% of their amino acid identity. DRP2A and DRP2B do not, however, play redundant roles in every cell response. Strong ligand-induced FLS2 endocytosis and flg22-induced ROS generation depend on AtDRP2B but not AtDRP2A Gene In*N. benthamiana*, silencing of NbDRP2 family members also hinders activated FLS2 endocytosis. According to research activated FLS2 is internalized via ligand-induced endocytosis, which downregulates signaling. Delaying the removal of active FLS2 from the PM is associated with early and amplified flg22 responses, including increased ROS generation and Ca2+ flux in *Arabidopsis*. Thus, early flg22 signaling is associated with FLS2 endocytosis. Interestingly, drp2b mutants have a noncanonical mix of immunological function (Perraisand Merrifield., 2015).

Uncoating Proteins

To reveal vesicular trafficking proteins that facilitate fusion with the target membrane, CCVs must lose their clathrin coat after scission from the donor membrane. *Arabidopsis* AUXILIN-LIKE PROTEIN 1 (AX1) and AX2 are implicated in CCV uncoating, same like in mammals and yeast, most likely with the assistance of SH3 domain-containing proteins such AtSH3P1 and HEAT SHOCK COGNATE 70. At the PM, AX1 and AX2 partially colocalize with CLC2 and coimmunoprecipitate with CLC1. AX1/2-ox hinders FM4-64 and CME-dependent endocytosis of numerous payloads, although *Arabidopsis* ax1/2 single and double mutants show no discernible abnormalities in development or endocytosis, therefore their significance in CME is only derived from overexpression experiments (Mashl and Bruinsma., 2018).

Auxilin-like proteins in plant immunity

Insights into AUXILIN-LIKE PROTEIN function in plant defensesignaling and immunity are also based on AUXILIN-LIKE PROTEIN overexpression. In Arabidopsis, AX2-ox impairs uptake of the fluorescently tagged DAMP AtPEP1 and MAPK phosphorylation after AtPEP1 elicitation. In rice, the AUXILIN-LIKE PROTEIN Xa21-BINDING PROTEIN 21 (XB21) interacts with Xa21, the immune receptor that confers resistance to the bacterial pathogen *Xanthomonas oryzae*pv. *oryzae* and may undergo

endocytosis. XB21-ox results in increased resistance against this pathogen and elevated expression of rice genes likely involved in cell death and vesicle-mediated transport.However, silencing of XB21 does not result in altered resistance phenotypes.

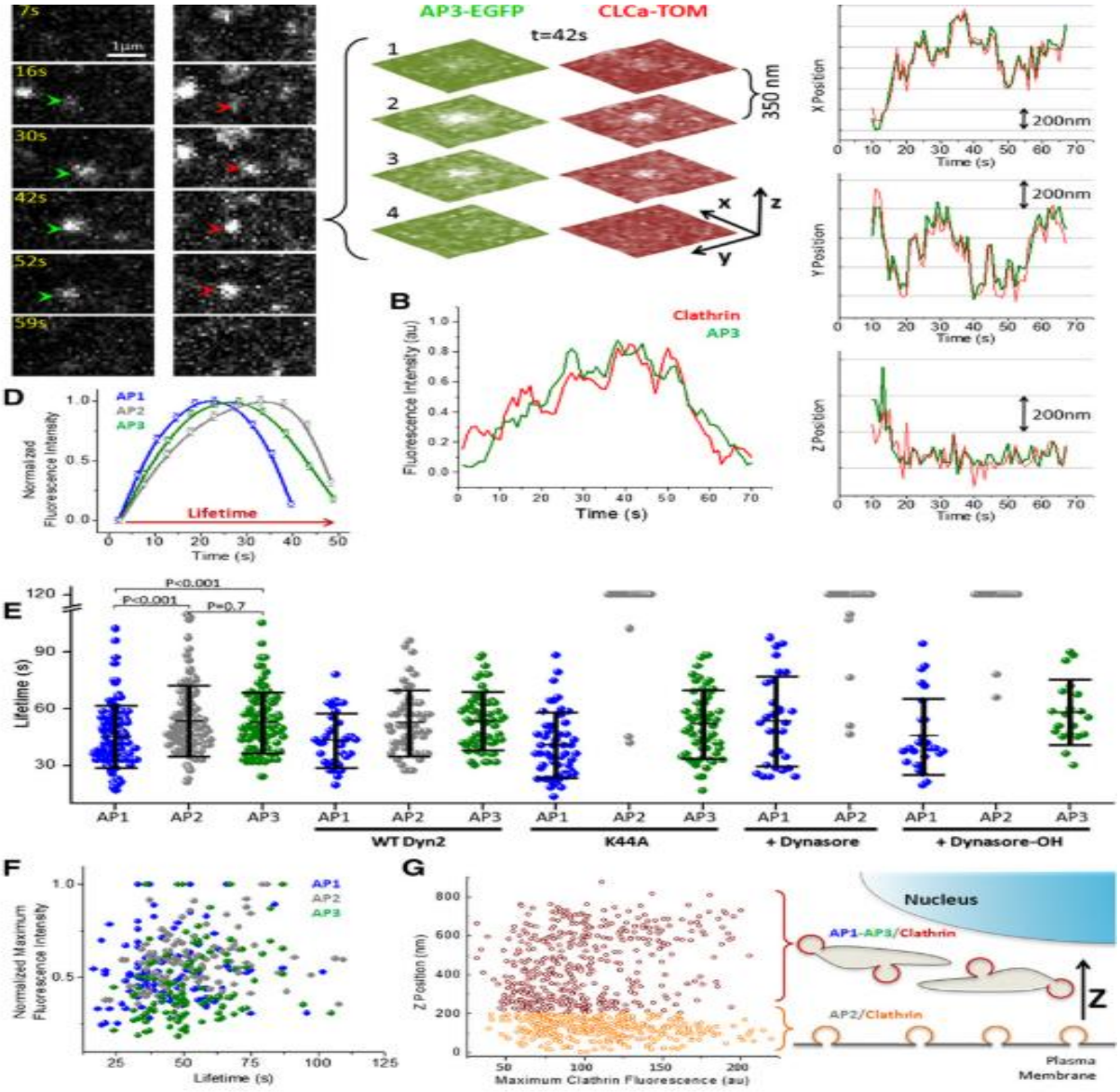

Figure 2- 3D Live Cell Imaging of Intracellular Clathrin Carriers in Living Cells (Heuser, and Kirchhausen., 1985)

DEFENSE HORMONE SALICYLIC ACID IN CLATHRIN-MEDIATED ENDOCYTOSIS

A growing body of research links SA to the regulation of vesicular trafficking pathways, which include the production of CCV at the PM and TGN/EE. Endoplasmic reticulum and

TGN/EE gene expression are both coordinatedly induced by SA before SA-dependent elevation of secreted defense genes occurs. To handle the increased demand on the secretory route during defense responses, resident proteins—including vesicular trafficking components—must be present. *Zea mays* also exhibits some variation in this process. The ZmCHC1 promoter contains SA responsive elements, as evidenced by the upregulation of ZmCHC1 (but not ZmCHC2) following SA treatment. Moreover, SA upregulates FLS2 and its coreceptor BAK1 in the Arabidopsis plant, presumably enhancing the plant's reactivity to PAMPs (Nossal., 2011).

However, SA suppresses the constitutive endocytosis and bulk membrane of certain PM proteins, such as PIN1 and PIN2, in contrast to its beneficial effects on the secretory pathway. More precisely, elevated SA inhibits AP-2, CLCs, and CHCs at the PM, but not TPC subunits, disrupting clathrin-mediated endocytosis. This suggests that there are SA-sensitive and SA-insensitive CCV trafficking routes. Fascinatingly, FLS2 endocytosis triggered by FLG22 is insensitive to SA, which is surprising given that a chc2 mutant has significantly reduced FLS2 endocytosis in response to flg22. It is unknown if SA influences constitutive endocytosis of FLS2 or other immunological payloads. It may disrupt CCV-dependent constitutive endocytosis of cargo proteins exclusively, but not ligand-induced endocytosis.

CHC2 in trafficking of immune cargo and immune signaling against bacterial pathogens

It is evident that CHC2 is necessary for pathogen defense, which begs the questions, What are the molecular mechanisms behind CHC2's contribution to immunity, and Which immunological components require CHC2-dependent trafficking to function in immunization against DC3000 PTO. Two recent studies have revealed immunological payloads that need CHC2 to be internalized from the cell surface, providing preliminary insights. The internalization of the bacterial PAMP flg22and the endogenous damage-associated molecular pattern (DAMP) AtPep1 in Arabidopsis roots and cotyledons, respectively, is impaired by loss of CHC2, indicating that CHC2 is involved in endocytosis of DAMPs/PAMPs in a variety of plant tissues. In both investigations, live-cell imaging was used to track the cellular uptake of physiologically active peptide ligands that were fluorescently tagged with 5-TAMRA (5-carboxytetramethylrhodamine).

Chc2 mutants also have altered levels of RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD), the PM-localized NADPH oxidase that causes apoplastic ROS

generation minutes after DAMP/PAMP elicitation. Quantitatively characterizing the localization and dynamics by single-molecule tracking and variable-angle total internal reflection fluorescence microscopy. Using fluorescently tagged RBOHD, researchers discovered that in the absence of any stimulus, loss of CHC2 causes an increase in the number and size of fluorescently tagged RBOHD foci in the PM in addition to a decrease in lateral mobility. It is interesting to note that flg22 elicitation increases RBOHD's diffusion and clustering within PM, however, it is unclear if these changes in PM mobility are dependent on CHC2 and are required to activate RBOHD. Given that chc2 mutations exhibit a comparatively (Ungewickell and Hinrichsen., 20107).

Auxilin-like proteins in plant immunity

AUXILIN-LIKE PROTEIN overexpression also provides insights into the role of AUXILIN-LIKE PROTEIN in plant defensesignaling and immunology. AX2-ox inhibits the absorption of the fluorescently tagged DAMP AtPEP1 in Arabidopsis and the phosphorylation of MAPK following AtPEP1 elicitation. The AUXILIN-LIKE PROTEIN in rice (Mousavi *et al*., 2014).

The immunological receptor Xa21, which provides resistance to the bacterial disease *Xanthomonas oryzae*pv. *oryzae*, interacts with Xa21-BINDING PROTEIN 21 (XB21) and may proceed through endocytosis. Higher expression of rice genes probably involved in cell death and vesicle-mediated transport, as well as enhanced resistance to this pathogen, are the outcomes of XB21-ox. Nevertheless, XB21 silencing does not change the resistance phenotypes.

Tyrphostin A23

Many animal and plant labs have used pharmacological interference with tyrphostin A23 (TyrA23) in the past to determine whether a certain PM protein and/or an immune response needs CME and AP2µ in particular. These investigations are predicated on TyrA23 obstructing AP2µ's interaction with cargo proteins containing YXXφ, which prevents CME. However, a recent work demonstrating that TyrA23 does not particularly target CME calls for a reevaluation of the meaning of these TyrA23 trials. Rather, it works by disabling mitochondria and causing protonophoric activity, which causes cytoplasmic acidification, which seems to be the main obstacle to CME; for a thorough review of probable side effects as well as the benefits (Loerke *et al.*, 2011).

Mediation of Clathrin-Dependent Trafficking during Cytokinesis and Cell Expansion

Stable transformation of HUB-ox in BY-2 tobacco suspension cells prevents constitutive clathrin-coated pit formation from occurring, but not from forming cryptogein-induced clathrin-coated pits (10 min post-elicitation). HUB-ox had no effect on the formation of ROS early but lessens PR-10 mRNA induction (4 hours post-elicitation) and extracellular alkalization (a few minutes post-elicitation). Following exposure to EIX, the 22-kDa fungal elicitor protein from *Trichoderma viride*, transient HUB-ox also impairs immunological responses. The study found that *Nicotiana tabacum* L. cv. *Xanthi* cell culture and leaf epidermal cells showed a reduction in EIX-induced cell death (2 hours post-elicitation) and electrolyte leakage (24 hours post-elicitation), respectively. It is unknown if HUB-ox obstructs ligand-induced endocytosis of other plant PRRs and the EIX receptor LeEix2.

Distinct Clathrin-Coated Pits Sort Different GProtein-Coupled Receptor Cargo

Normally three methods are used to investigate the crucial question of whether arrestins differentially regulated the receptors. First, while P2Y1 receptor internalization was not considerably impacted, overexpression of a DNM variant of arrestin-2 (arrestin-DNM) preferentially reduced agonist-induced P2Y12 receptor internalization (Figure 3A). Both arrestin-2 and arrestin-3 compete with this arrestin-DNM for clathrin binding. Secondly, we looked at the redistribution of arrestins tagged with green fluorescent protein (GFP) since the activation of several GPCRs causes arrestins to be recruited to the plasma membrane quickly (Figures 3B).

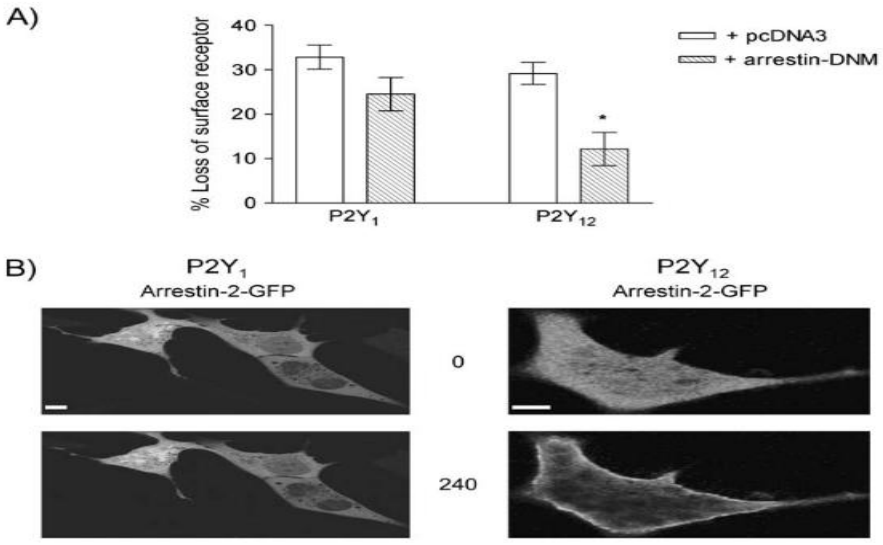

Figure 3-A. P2Y12but not P2Y1receptor internalization is mediated by arrestin in a manner dependent upon GRK2 and GRK6.A)P2Y1- or P2Y12-expressing cells were transiently transfected with 5mg of DNM forms of arrestin-2 (arrestin-DNM) or vector(pcDNA3) alone. Cells were subsequently challenged with ADP (10mM; 30 min) and surface receptor loss assessed by ELISA. The datarepresent means SEM of five independent experiments. *p<0.05 compared with respective pcDNA3 vector-transfected controls

B) Cells grown on poly-L-lysine coverslips were transiently transfected with 0.5mg of peGFP-N1-arrestin-2-GFP.Prior to stimulation and viewing, coverslips were mounted in an imaging chamber at 378C. The initial diffuse cytoplasmic distribution ofarrestin-2-GFP is shown prior to agonist stimulation (0 second). ADP (10mM) was added and the redistribution of arrestin-2 was monitoredin real time. The images shown were collected before agonist addition (0) or 240 seconds after agonist addition. The scale bar represents10mm. Data shown are representative of three independent experiments (Gaudarov*et al.*, 1999).

Arrestin-2-GFP had a diffuse cytoplasmic distribution before agonist treatment (Figure 5B). In P2Y12-expressing cells, but not P2Y1-expressing cells, there was a rapid translocation of arrestin-2-GFP from cytosol to membrane upon the injection of 10mMADP. Similar results were obtained with arrestin-3-GFP, which likewise demonstrated colocalization at focal locations of the plasma membrane.

Stochastic Model of Clathrin-Coated Pit Assembly

We believe that reversible monomer binding at the pit's edge causes a pit to expand. The kinetic design for pit assembly. With site-dependent rate constants, the technique transfers the dynamics of CCPs onto a one-dimensional nearest-neighbor continuous-time random walk.

We assume that a pit is pinched off when it reaches vesicle size N, but we don't consider the specifics of the kinetics involved in dynamin and other protein-based vesicle scission. A CCV normally contains between 60 and 140 clathrin molecules, as was previously mentioned. In light of this, we maintain the vesicle size constant in our model at N ¼ 100. Small modifications in the value have no substantial impact on our results (Liu *et al.*, 2009).

The growth process is characterized by the forward rate constants an, n ¼ 1,., N – 1, which are assumed to be a ¼ kbmfðn\ ¼ gfðnÞ. In this case, fðn\ ¼ fðn; c\, m is the free monomer concentration, and g is a constant. Additionally, kb is the bimolecular rate constant of a monomer binding to an accessible site on the pit. When the pit is near a hemisphere, the value of f(n) and, consequently, that of an, reaches its maximum. The scission of a vesicle from the membrane is characterized by a rate constant, aN. We will assume for convenience that aN ¼ N.The backward rate constants, bnþ1, can be found using the condition of detailed balance after the forward rate constants, an, are known. The connection bnþ1 ¼ mfðn\exp½Eðn þ 1\ Eðn\, n follows from this.

Using kinetic Monte Carlo simulations, we apply the above scheme to determine the fate and lives of pits. In our models, a pit can either develop until it achieves vesicle size N (productive pits) or finally decrease in size and fall below a detection threshold (abortive pits). The values of the forward and backward rate constants, a and bn, are the inputs needed to execute the simulations. The energy function E(n) and the unknown values of g and m determine the rate constants (Traub., 2019).

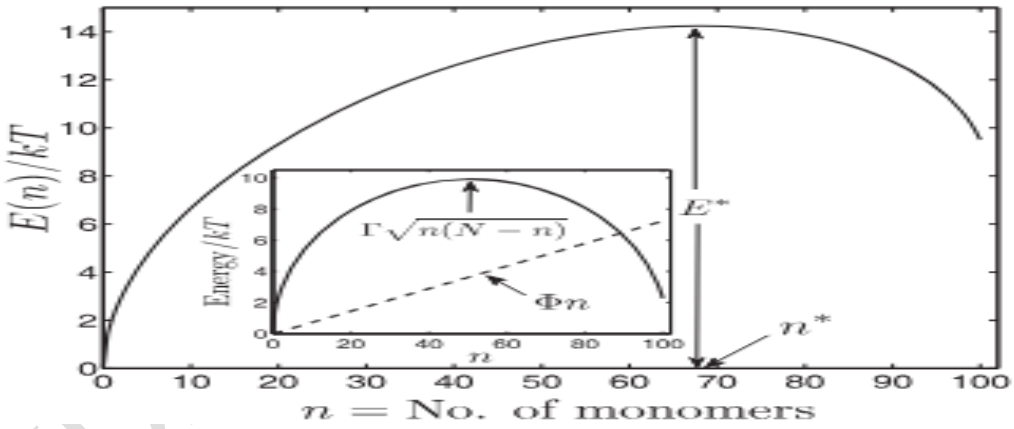

Figure 4-Dependence of pit energy on pit size, n (Eqs. 4–6). (Inset) The two terms contributing to E(n), Eq. 5. The parameter values are km ¼ 20kT, kp ¼ 200kT, cp ¼ 1/45 nm1 , b ¼ 5.5kT, and s ¼ 1kT. For these parameter values, F ¼ 0.07kT and G ¼ 0.2kT (Edeling *et al*., 2006).

Conclusion

A growing body of research indicates that components of clathrin-coated vesicles (CCVs) support efficient defense against a variety of plant diseases, including as bacteria, oomycetes, fungi, and viruses.CCVs are thought to regulate the quantity of immune cargo at the cell surface for the efficient initiation and/or attenuation of defensive responses. They can originate at the plasma membrane as well as the trans-Golgi network/early

endosome.Up until now, research on plant immunity has concentrated on three essential CCV elements: DYNAMIN-RELATED PROTEIN 2, ADAPTOR PROTEIN 2μ, and CLATHRIN HEAVY CHAIN 2 (CHC2). There is still much to learn about the underlying molecular mechanisms and identities of other CCV adaptors and accessory components required for CCV function in plant defense. Highlighting their significance in plant immunity, CCV components such DRP2 and CHC2 function as potential targets.

REFERENCES

Edeling, M. A., C. Smith, and D. Owen. 2006. Life of a clathrin coat: insights from clathrin and AP structures. Nat. Rev. Mol. Cell Biol. 7:32–44.

Gaidarov, I., F. Santini, ., J. H. Keen. 1999. Spatial control of coatedpit dynamics in living cells. Nat. Cell Biol. 1:1–7.

Heuser, J., and T. Kirchhausen. 1985. Deep-etch views of clathrin assemblies. J. Ultrastruct. Res. 92:1–27.

Kirchhausen, T. 2009. Imaging endocytic clathrin structures in living cells. Trends Cell Biol. 19:596–605.

Liu, J., Y. Sun, ., G. F. Oster. 2009. The mechanochemistry of endocytosis. PLoS Biol.

Loerke, D., M. Mettlen, ., G. Danuser. 2011. Measuring the hierarchy of molecular events during clathrin-mediated endocytosis. Traffic. 12:815–825.

Mashl, R. J., and R. F. Bruinsma. 2018. Spontaneous-curvature theory of clathrin-coated membranes. Biophys. J. 74:2862–2875.

Mousavi, S. A., L. Malerød, ., R. Kjeken. 2014. Clathrin-dependent endocytosis. Biochem. J. 377:1–16.

Nossal, R. 2011. Energetics of clathrin basket assembly. Traffic. 2: 138–147. 23. Zandi, R., P. van der Schoot, ., H. Reiss. 2006. Classical nucleation theory of virus capsids. Biophys. J. 90:1939–1948.

Perrais, D., and C. J. Merrifield. 2015. Dynamics of endocytic vesicle creation. Dev. Cell. 9:581–592.

Praefcke, G. J. K., M. G. J. Ford, ., H. T. McMahon. 2014. Evolving nature of the AP2 alpha-appendage hub during clathrin-coated vesicle endocytosis. EMBO J. 23:4371–4383.

Ricotta, D., S. D. Conner, ., S. Honing. 2012. Phosphorylation of the AP2 mu subunit by AAK1 mediates high affinity binding to membrane protein sorting signals. J. Cell Biol. 156:791–795

Takei, K., and V. Haucke. 2011. Clathrin-mediated endocytosis: membrane factors pull the trigger. Trends Cell Biol. 11:385–391.

Traub, L. M. 2019. Clathrin couture: fashioning distinctive membrane coats at the cell surface. PLoS Biol. 7:e1000192.

Ungewickell, E. J., and L. Hinrichsen. 2017. Endocytosis: clathrinmediated membrane budding. Curr. Opin. Cell Biol. 19:417–425.