Molecular and Cell Biological Study on the *Arabidopsis thaliana* Actin Isoforms

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Table of contents

Abstract

Actin is one of the most important eukaryotic proteins, playing critical roles in a variety of cellular functions. Higher plants express multiple actin isoforms in a manner dependent on tissues and organs, and also on the stage of development. Genetic analyses suggested that individual actin isoforms have specific roles in cells. Moreover, it was demonstrated that different *Arabidopsis* actin isoforms possess distinct biochemical properties. These results suggest that plants developed a mechanism to diversify actin functions by expressing multiple functionally non-equivalent actin isoforms. However, it is unclear if and how multiple actin isoforms perform specific functions in plant cells.

In this study, I constructed plasmids that express major *Arabidopsis* vegetative actin isoforms (AtACT2 and AtACT7) directly fused to a fluorescent protein. By optimizing the linker sequence between actin and the fluorescent protein, I succeeded in transiently expressing and observing filaments containing expressed actin isoforms directly fused with the fluorescent protein in *Arabidopsis* protoplasts.

Different coloristic fluorescent proteins fused with AtACT2 and AtACT7 co-expressed in *Arabidopsis* protoplasts showed filaments containing both of the vegetative actin isoforms and those containing either one of the two vegetative actin isoforms. In leaf mesophyll cells of *Nicotiana benthamiana,* AtACT2 and AtACT7 were co-polymerized in a segregated manner along the filaments. In epidermal cells, actin filaments with different thicknesses were observed. I speculate that thick filaments are bundles, on the other hand, thin filaments are individual filaments or bundles of a small number of filaments. Surprisingly, AtACT2 and AtACT7 tended to polymerize into different thicknesses of filaments. AtACT2 was incorporated into thinner filaments than AtACT7, whereas AtACT7 was incorporated into thick bundles. I concluded that different actin isoforms are capable of constructing unique filament arrays, depending on tissues. These observations will help to understand the function of individual actin isoforms in plants.

Interestingly, staining patterns by two generic actin filament probes, Lifeact and

mTalin1, were different between filaments containing AtACT2 and those containing AtACT7 in leaf epidermal cells. Especially, Lifeact only partially labeled actin filaments containing AtACT2 *in vivo*. The binding analyses of Lifeact *in vitro* revealed that Lifeact was capable of binding to purified AtACT2 filaments, suggesting the competition of Lifeact with endogenous actin binding proteins along AtACT2 filaments *in vivo*. Because the two probes consist of the actin binding domains of different actin binding proteins, it was further suggested that filaments containing different actin isoforms bind specific actin binding proteins *in vivo*.

Finally, I attempted to establish plant lines expressing AtACT7 fused with GFP. However, expression of GFP-AtACT7 caused growth defects. The negative effect caused by expressing actin fused with a fluorescent protein has been reported in various organisms. Nevertheless, the direct actin observation method developed in this study is a powerful tool for imaging of not only actin isoforms but also mutant actins, and to broaden the range of research about actin cytoskeleton in plants. Hopefully, this problem can be alleviated by reducing the expression level of actin fused with a fluorescent protein within the detection range of the camera of the microscope, when establishing a plant line expressing actin fused with a fluorescent protein.

Abbreviations

F-actin: Filamentous actin

G-actin: Globular actin

ABP: Actin binding protein

ABD: Actin binding domain

CAP: Cyclase-associated protein

ADF: Actin depolymerization factor

PPI: Polyphosphoinositide

PCR: Polymerase chain reaction

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

Tris: Tris(hydroxymethyl)aminomethane

PIPES: Piperazine-1,4-bis(2-ethanesulfonic acid)

EGTA: *O,O*'-Bis(2-aminoethyl)ethyleneglycol-*N,N,N′,N′*-tetraacetic acid

EDTA: Ethylenediamine-*N,N,N′,N′*-tetraacetic acid, tetrasodium salt, tetrahydrate

DTT: Dithiothreitol

His-tag: Poly-histidine tag

Ni-NTA: Nickel-nitrilotriacetic acid

BSA: Bovine serum albumin

SDS: Sodium dodecyl sulfate

MES: 1-Morpholinoethanesulfonic acid monohydrate

ATP: Adenosine tri-phosphate

Pi: Inorganic phosphate

ADP: Adenosine di-phosphate

MBS: *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester

PAGE: Polyacrylamide gel electrophoresis

GFP: Green fluorescent protein

RFP: Red fluorescent protein

CaMV: Cauliflower mosaic virus

UTR: Untranslated region

cDNA: Complementary DNA

ER: Endoplasmic reticulum

General Introduction

Actin

Actin was first discovered in 1942 by Straub from rabbit skeletal muscle[1]. Each actin molecule binds nucleotide: ADP, ADP + Pi or ATP, complexed with a divalent ion (Mg^{2+} or Ca^{2+}). Monomeric or globular actin (G-actin) reversibly assembles into filamentous actin (F-actin). The actin filament is a double helical chain and has a distinct polarity[2]. One end is called the barbed end and the other is called the pointed end. The elongation rates from these two ends are different, and the barbed end grows faster than the pointed end[3]. As the polymerization progresses, the concentration of G-actin in solution is reduced until an equilibrium is reached. In this steady state, association and dissociation of G-actin continue, and in the presence of ATP, an asymmetric polymerization/depolymerization process called treadmilling occurs[4,5]. During treadmilling, G-actin bound with ATP associates with actin filaments preferentially at the barbed end, and the ATP bound to G-actin is hydrolyzed to ADP and phosphate. Actin bound with ADP dissociates from the pointed end after phosphate is released[6].

In all eukaryotes including fungi, animals and plants, actin is involved in a large variety of cellular functions. In plant cells, actin participates in activities such as cell division and morphogenesis, tip growth, movement and repositioning of organelles, cytoplasmic streaming, fertilization, hormone transport and responses to external signals[7–11]. In this way, actin is one of the most important proteins to fulfill various cellular functions. *Arabidopsis* actin consists of 376 or 377 amino acid residues. It is well known that the amino acid sequence of actin is highly conserved among most of the eukaryotic actins. For example, the sequence homology between rabbit skeletal muscle actin and *Arabidopsis* AtACT2 actin is 86%.

Actin binding proteins

Actin achieves above-mentioned diverse and complex functions by interaction with various actin-binding proteins (ABPs). ABPs regulate actin structures and dynamics by modifying the rates of polymerization, depolymerization and nucleation, and by inducing branching, bundling, unbundling, severing, capping, *etc.* (Fig. 1). Myosin moves various cargos along actin filaments or use them as scaffolds to change the cell shape and cell motility[12]. Profilin is one of the most important ABPs[13,14]. Profilin binds to G-actin and forms a heterodimeric complex. Actin bound with profilin is unable to spontaneously nucleate polymerization of actin filaments and to associate with the pointed ends of filaments. Therefore, profilin acts as a sequestering protein, which maintains high levels of G-actin pool when the barbed ends are capped[15,16]. Cyclase-associated protein (CAP) is also a G-actin binding protein. CAP increases the rate of nucleotide exchange on actin in plants[17]. Formin is another group of important ABPs and promotes incorporation of G-actin bound with profilin to the barbed ends of filaments[18]. Actin depolymerization factor (ADF)/cofilin is a major group of actin severing factors. ADF is probably more abundant in plant cells than in other eukaryotes, since ADF is present at a 1:3 molar ratio with actin (ADF: actin) in *Arabidopsis* leaf cells[19] whereas in platelets, the molar ratio of cofilin to actin is 1:10[20]. ADF/cofilin binds to both G-actin and actin filaments to promote actin dynamics. Severing actin filaments results in generation of new filament barbed ends for polymerization[21]. Polymerization is usually terminated by capping of the barbed end with a capping protein[22]. Thus, polymerization of actin *in vivo* is strictly regulated in a manner different from that *in vitro*. In addition, a cell contains many more ABPs including villin[23], fimbrin[24] and Arp2/3 complex[25], each of which confers specific functions on actin filaments by modifying network structures of the filaments such as bundling or branching. However, it is poorly understood how each ABP selectively binds to appropriate actin molecules for performing specific functions in a cell, although several mechanisms have been suggested, as described below.

Regulation of actin binding proteins

The regulation mechanisms for binding of ABPs to actin molecules are broadly separated into following two mechanisms. One mechanism is the biochemical regulation of ABPs including phosphorylation, and binding of a small molecule such as Ca^{2+} and nucleotide[7]. For example, biochemical study revealed that the activities of gelsolin and villin are regulated by Ca^{2+}[26,27] and inhibited by PPI, polyphosphoinositide, which is the second messenger involved in cellular signaling[28]. Activity of cofilin is suppressed by phosphorylation at the N-terminal Ser-3[29]. Another mechanism is that actin itself affects the binding of ABPs. Actin is subject to various posttranslational modifications which affect the interaction with ABPs[30]. For example, the N-terminal acetylation of actin molecules facilitates actomyosin interactions[31]. Moreover, the affinity of certain ABPs, such as cofilin and profilin, varies with the nucleotide state of actin molecules[32,33]. The conformational flexibility of actin filaments is also important to regulate the affinity for or activity of ABPs. Restriction of conformational changes of actin filaments inhibits the motility of myosin[34]. Moreover, it is suggested that conformational changes of actin filaments induced by binding of ABPs such as myosin and cofilin affect the affinity for these ABPs[35]. In this study, I focus on actin isoforms as the component in the latter mechanism.

Actin isoforms

Here, isoform is defined as a set of highly similar proteins that have a certain number of amino acid substitutions, but excluding splicing isovariants. Higher plants and animals have actin isoforms in this sense. The model plant, *Arabidopsis thaliana*, has eight actin isoforms that are grouped into two classes, vegetative (AtACT2, 7 and 8) and reproductive (AtACT1, 3, 4, 11 and 12) actins, according to their expression patterns (Fig. 2)[36,37]. Interestingly, the *Arabidopsis* actin isoforms have more non-conservative amino acid substitutions than do mammalian actin isoforms, and these amino acid substitutions in *Arabidopsis* actin isoforms are frequently located on the surface of the molecule[36]. For example, there are 28 amino acid substitutions between AtACT2 and AtACT7 which are scattered throughout the molecule (Fig. 3)[36]. This is in sharp contrast to the difference between human cytoplasmic β and γ actin isoforms, the two major actin isoforms in non-muscle cells, that have only four conservative changes at the N-terminus (Fig. 3)[38].

Previous genetic studies showed distinct expression patterns of actin isoforms and their possible specific functions[39]. Loss of function of the reproductive AtACT11 caused delayed pollen germination and enhanced pollen tube growth, accompanied by an increase in the rate of actin turnover[40]. The AtACT7-, but not AtACT2-, knock down plants were slow to produce callus tissues from roots or leaf tissues in response to hormones to induce callus[41]. A defect in root hair growth caused by the AtACT2 knock-out was not complemented by over-expression of AtACT7[42]. Ectopic expression of a reproductive actin isoform in vegetative tissues caused abnormal growth by accumulating aberrant bundled filaments[43]. These phenotypes, which resulted following the ectopic expression of a reproductive actin, were suppressed by the ectopic co-expression of corresponding reproductive ABPs, profilin or ADF/cofilin[44,45]. These reports imply that individual actin isoforms interact with specific ABPs to fulfill a specific cellular function.

Moreover, different *Arabidopsis* actin isoforms, more specifically, major vegetative actin isoforms, AtACT2 and AtACT7, and major reproductive actin isoforms, AtACT1 and AtACT11, have significantly different biochemical properties as mentioned

below (Table. 1)[46]. Phalloidin bound normally to the filaments of the two reproductive actins as well as to the filaments of skeletal muscle actin. However, phalloidin bound only weakly to AtACT7 filaments and hardly at all to AtACT2 filaments, despite the conserved sequence of the phalloidin-binding site. Polymerization and phosphate release rates among these four actin isoforms were also significantly different. Moreover, interactions with profilin were also different among the four *Arabidopsis* actin isoforms. Two profilin isoforms, PRF1 and PRF2, inhibited the polymerization of ACT1, ACT11 and ACT7, while ACT2 was only weakly affected.

These genetic and biochemical studies suggested that individual actin isoforms fulfill specific roles in plant cells. In other words, actin isoform is one important factor to consider for understanding the actin regulation mechanism. However, little is known about how differently actin isoforms work in plant cells. In this study, I attempted to reveal the subcellular distribution of different actin isoforms and between actin isoforms and ABPs. In Chapter I, new actin probes which are fusion proteins of actin and a fluorescent protein were developed for direct imaging of individual actin isoforms in plant cells. The expression of actin fused with a fluorescent protein revealed the distributions of different vegetative actin isoforms in plant leaf cells. In Chapter II, I analyzed the binding of actin binding domain derived from ABPs to actin filaments of each vegetative actin isoform by imaging and biochemical experiments, in order to understand the relationship between ABPs and actin isoforms. This observation suggested the presence of ABPs that prefer actin filaments containing particular actin isoforms. In Chapter III, I applied the actin fused with a fluorescent protein to investigate the nature of a mutant actin in plants, with a combination of a biochemical approach. Moreover, I attempted to establish a transgenic plant line expressing actin fused with a fluorescent protein, with the aim of observing the localization of individual actin isoforms or mutant actin in various tissues under various conditions in plants.

General Discussion

In Chapter I, I compared the intracellular distribution between the two vegetative actin isoforms, AtACT2 and AtACT7, in *N. benthamiana* leaf cells. This study revealed that different actin isoforms form unique filament arrays in leaf epidermal and spongy mesophyll cells. It is speculated that another vegetative actin isoform, AtACT8, behaves the same way as AtACT2, because the difference of amino acid sequences between AtACT2 and AtACT8 is only one residue (Glu-3 to Asp) and previous genetic study demonstrated functional equivalent between AtACT2 and AtACT8[42]. Gunning *et al*. proposed that there are two different types of relationships between function and isoform of actin; a one-filament-one-function system and a universal force provider system[67]. Many bacteria have major three types of actin-like proteins, MreB, FtsA and ParM, whose amino acid sequences are dramatically different among these actin-like proteins (*e.g.*, only~20% identity between ParM and MreB)[118]. MreB is involved in maintenance of the cell shape and cell wall synthesis[119]. FtsA participates in cell division together with a tubulin homolog, FtsZ, for forming Z-rings[120]. ParM (also called StbA) is responsible for plasmid segregation and stability[121]. In this way, individual actin-like proteins, which have completely different roles, are referred to as "a one-filament-one-function system". In contrast, in eukaryotes such as fungi and animals, actin is referred to as "a universal force provider"[67]. In these organisms, actin fulfills various complex functions through interactions with a variety of ABPs. Gunning *et al.* emphasized that diversified tropomyosins increase the actin functions[67]. In addition to the regulation by ABPs, the importance of the regulation by actin itself such as flexibility of actin filaments has also attracted attention[122,123]. For example, it is suggested that cooperative conformational changes within actin filaments alter affinities for different ABPs[35]. In other eukaryotes, plants, Gunning *et al.* focused on the diversification in actin isoforms[67]. However, the relationship between actin isoforms and these actin regulation systems in plant cells has been unclear. I propose the following hypothesis through this study. In leaf spongy mesophyll cells, AtACT2 and AtACT7 co-polymerized in a segregated manner and appear to function cooperatively. Thus, the regulation system of vegetative actin in mesophyll cells seems to belong to the universal force provider system. In contrast, AtACT7 and AtACT2 were incorporated into obviously different types of filaments in epidermal cells. In leaf epidermal cells, AtACT2 and AtACT7 might have taken an evolutionary route toward partially isoform-specific functions, although not quite to the state of one-filament-one function system as seen in bacteria. Since genetic study showed

both functional redundancy and specificity of *Arabidopsis* actin isoforms[42], it is reasonable to suppose that plant actins employee both actin functionalization systems of bacteria and fungi/animal actin. Our observations about the localization of different *Arabidopsis* actin isoforms should provide platforms to understand functions of actin isoforms in plant cells.

Genetic studies suggested that, in addition to leaf epidermal cells discussed above, root hair also employs the one-filament-one-function like system[42]. To provide the basis for a one-filament-one-function like system in plants, it is considered that plants diverged actin isoforms to fulfill complex actin functions. Šlajcherová *et al*. described that the total number of actin family members partially reflects the complexity of the organisms; *e.g.*, algae contain only 2-3 actin isoforms while gymnosperms and angiosperms have 8 or more isoforms (Table 2)[39]. Perhaps those diversified actin isoforms, which presumably perform specific functions in specific tissues, may be the products of an evolutionarily acquired strategy for immobile plants to respond to various environmental stresses.

In Chapter II, I analyzed the binding between each actin isoform and ABD, mTalin1 and Lifeact, *in vivo* and *in vitro*, for understanding the relationship between actin isoforms and ABPs. The results suggested some ABPs bind specifically to specific actin isoforms. As mentioned in General Introduction, the amino acid substitutions among *Arabidopsis* actin isoforms are more frequently located on the surface of the molecule than human actin isoforms[36]. Actually, there are amino acid substitutions between AtACT2 and AtACT7 in the residues implicated in interaction with major ABPs (Fig. 26). In the previous study, I demonstrated that the extent of phalloidin binding is significantly different between purified AtACT2 and AtACT7 filaments, even though the amino acid sequences of phalloidin binding site are identical[46]. This result suggested that the amino acid substitutions between these vegetative actins allosterically affect the global structure of the actin molecules, resulting in different conformations at the phalloidin binding site. These different filamentous structures also would affect the affinities for the ABPs. Further research on the relationship between actin isoforms and ABPs would clarify the molecular regulation mechanism of individual actin isoforms. However, the interactions between actin isoforms and ABPs are probably complex because ABPs also have multiple isoforms (*e.g.*, *Arabidopsis* has 12 ADF, 5 profilin and 21 formin isoforms)[39,67].

The direct actin observation method in plant cells, which I developed in this

study, is useful to study not only isoforms but also mutant actin. Moreover, the method will allow observation of phenomena which an indirect observation method using ABD is unable to detect, such as treadmilling and direct actin dynamics including elongation, depolymerization, annealing, and severing. Although the expression of the GFP-actin fusion protein currently causes the dwarf phenotype in plants, this problem should be solved in the near future. I believe that this novel observation method of individual actin isoforms will be a powerful tool for future actin research in plants.

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Figures and Tables

Table 1. Biochemical properties of *Arabidopsis* actin isoforms in comparison with skeletal muscle actin.
The kinetics of polymerization and phosphate release rates were normalized by dividing by the rates of skeletal muscle actin. This table is modified from Kijima *et al.* (2016).

	Skeletal muscle actin	AtACT2 (veg)	AtACT7 (veg)	AtACT1 (rep)	AtACT11 (rep)
Binding of phalloidin	++	−	+	++	++
Relative polymerization rate	1	3.2	1.9	0.70	1.0
Relative phosphate release rate (During polymerization)	1	1.6	2.3	1.4	3.8
Relative phosphate release rate (During treadmilling)	1	3.3	4.4	7.2	0.78
Effect of AtPRF1	+	−	++	++	++
Effect of AtPRF2	+	+/−	++	++	++

Table 2. The number of actin genes in representative plant species.
The total number of actin gene family members partially reflects the complexity of the organisms. Modified from Slajcherová *et al*. (2012).

	Species	The number of actin genes
Algae	*Volvox carteri*	2
	Chlamydomonas reinhardtii	1-2
	Porphyra purpurea	2-3
Moss	*Physcomitrella patens*	10
Gymnosperms	*Pinus taeda*	10
Monocots	*Zea mays*	21
	Oryza sativa	8-10
Dicots	*Populus trichocarpa*	8-9
	Glycine max	17
	Arabidopsis thaliana	10

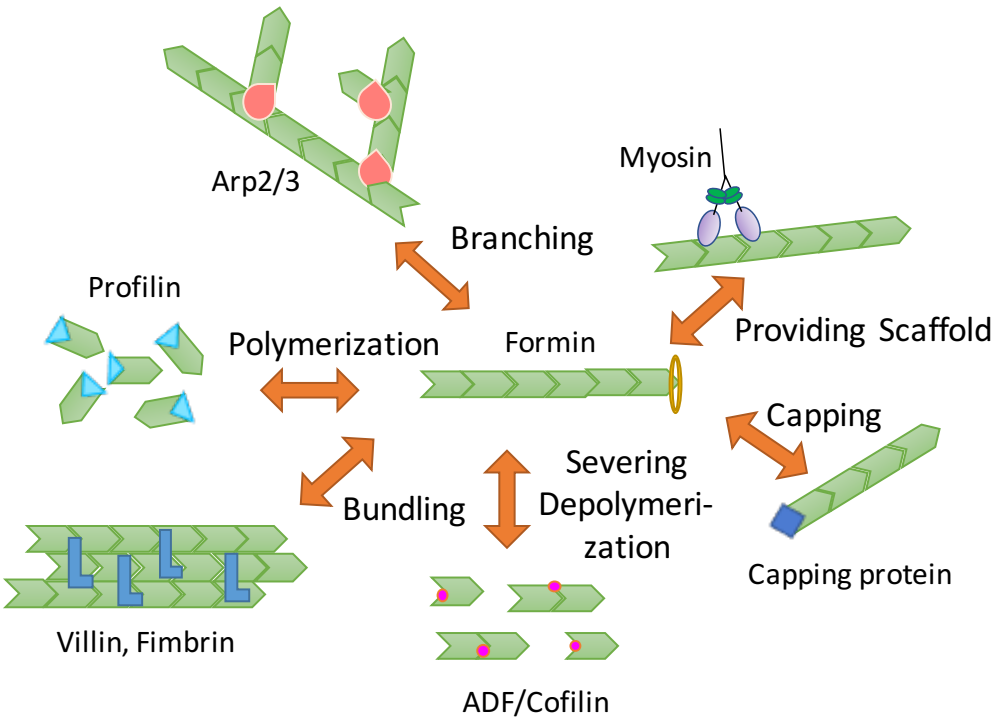


Figure 1. The functions of actin binding proteins.
Intracellular actin functions are regulated by various actin-binding proteins. Actin-binding proteins regulate actin structures and dynamics by modifying the rates of polymerization, depolymerization and nucleation, and by inducing branching, bundling, unbundling, severing, capping, *etc.*. Myosin moves various cargos along actin filaments or use them as scaffolds to change the cell shape and cell motility.

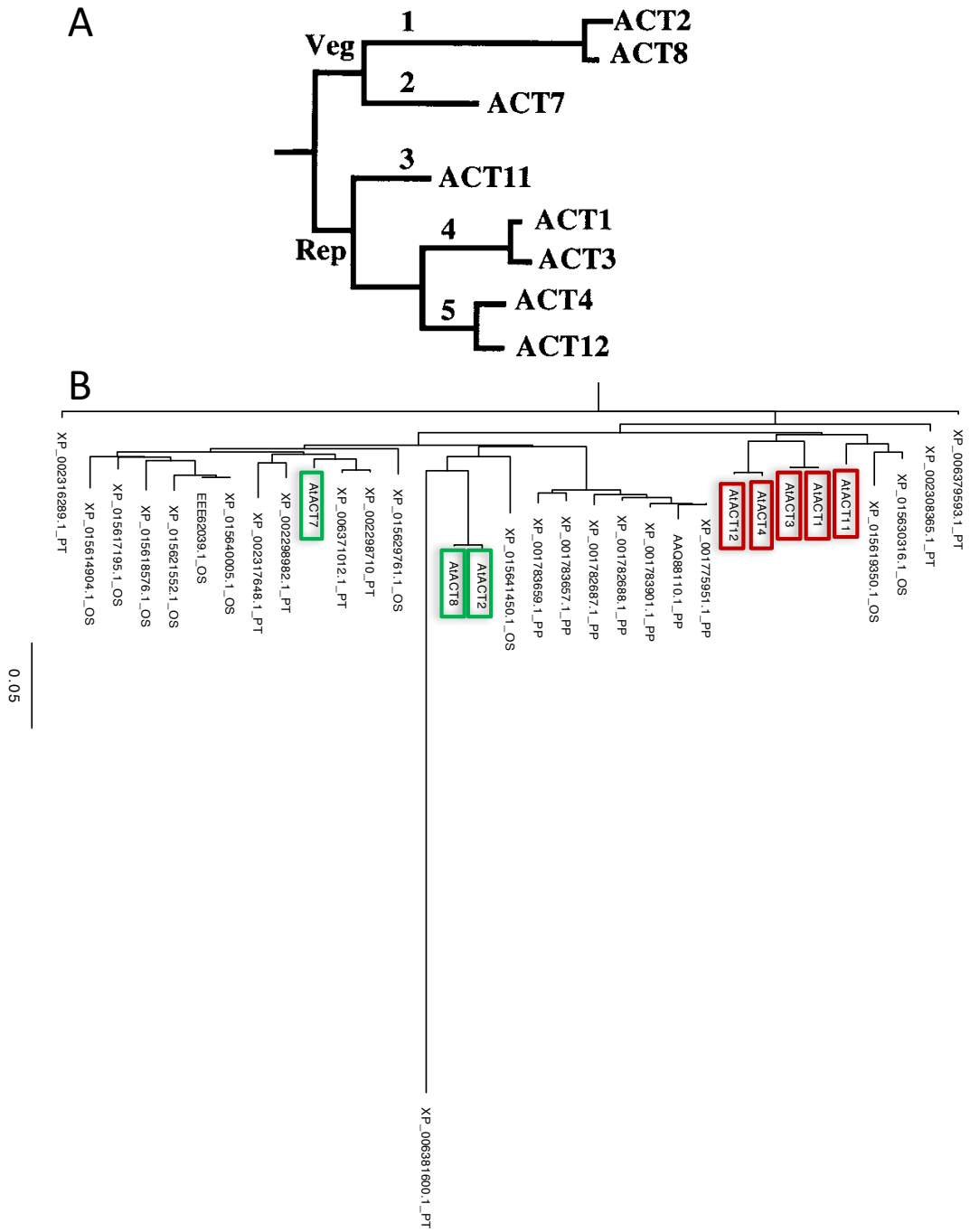


Figure 2. Actin isoforms of plants.

(A) *Arabidopsis thaliana* has eight actin isoforms. These isoforms are divided into two major classes, reproductive (Rep) and vegetative (Veg), and five subclasses that are numbered 1-5. This actin family tree is modified from Kandasamy *et al.* (2002). (B) Phylogenetic relationships among *A. thaliana, Populus trichocarpa, Oryza sativa* and *Physcomitrella patens* actin isoforms. Red and green boxes indicate reproductive and vegetative *A. thaliana* actin isoforms, respectively. The last "_PT", "_OS" and "_PP" of individual accession numbers indicate *Populus trichocarpa, Oryza sativa* and *Physcomitrella patens* actin isoforms, respectively. This phylogenetic tree was constructed using Clustal W website (http://www.genome.jp/tools-bin/clustalw) and visualized by Fig Tree 1.4.3. The accession numbers and amino acid sequences of all actins were acquired from the NCBI Protein database (https://www.ncbi.nlm.nih.gov/protein/).

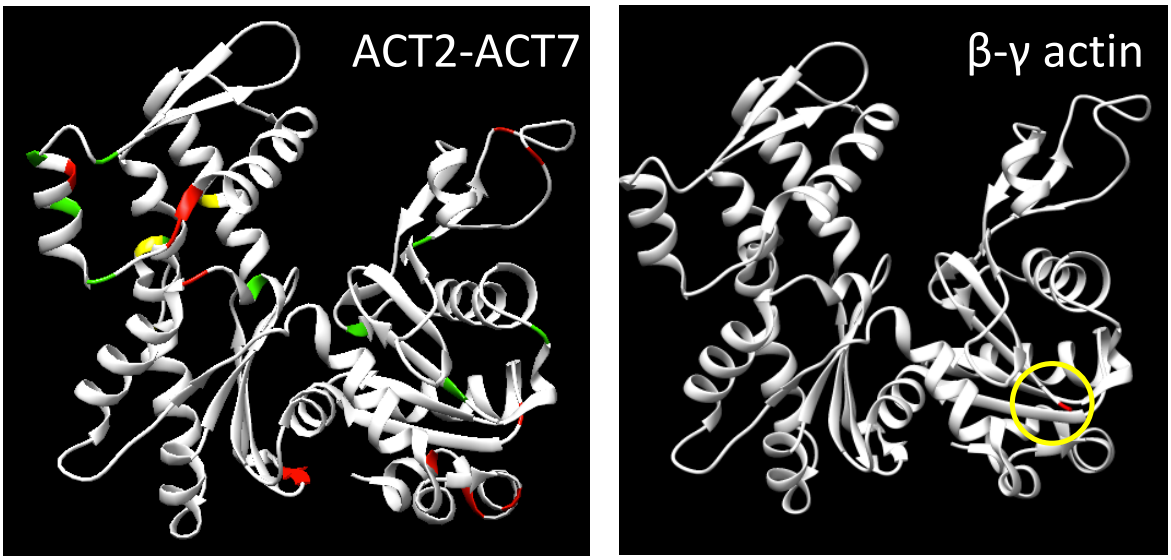
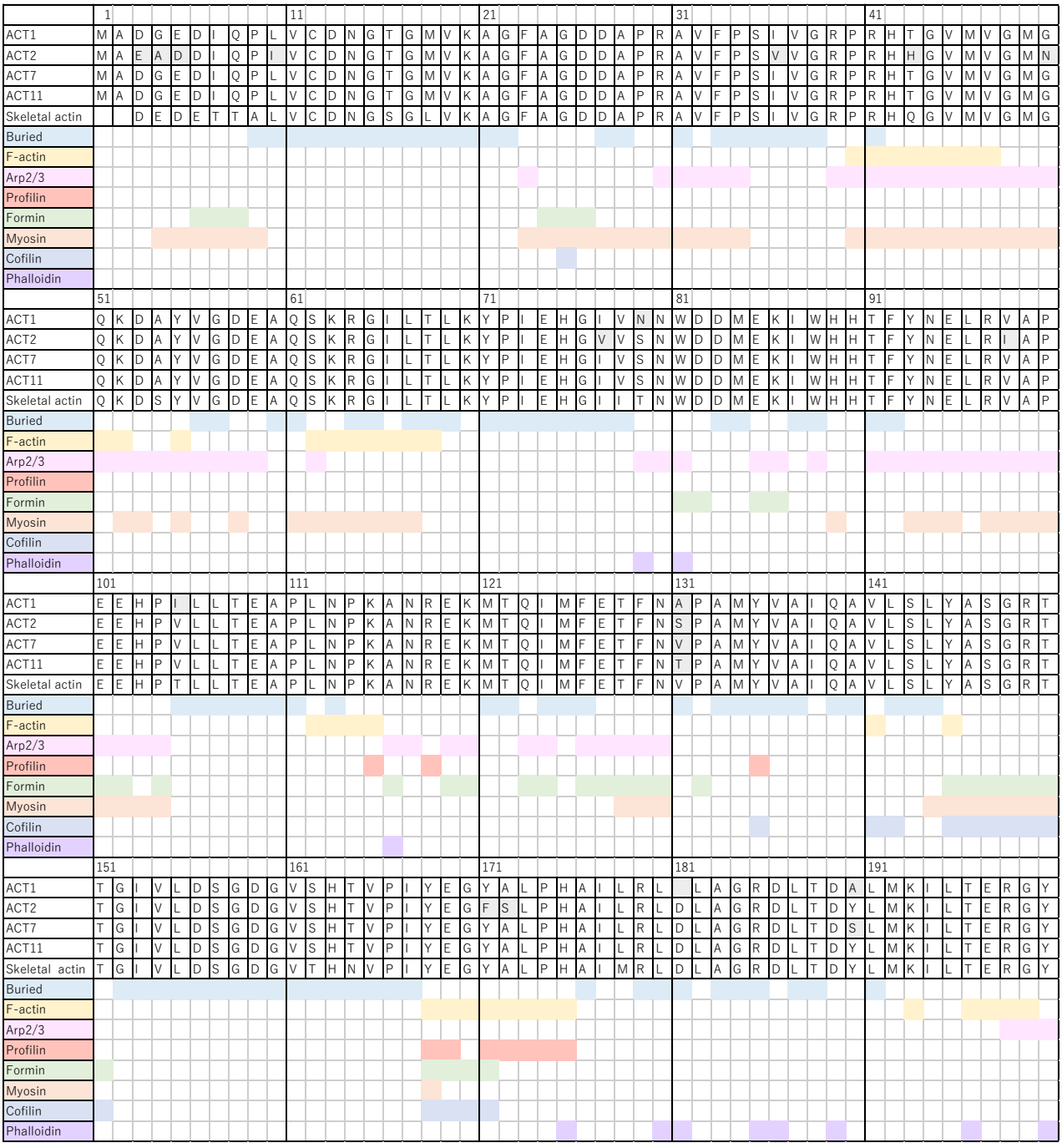


Figure 3. Amino acid substitutions between human cytoplasmic β and γ actins and *Arabidopsis* vegetative actins, AtACT2 and AtACT7.
The colored residues indicate actin amino acid substitutions. Since the structure of the N-terminus of actin is not determined, the neighborhood of the N-terminus is surrounded with a yellow circle. Amino acids are grouped into 4 groups depending on the natures of their side chains, which are hydrophobic side chains, polar uncharged side chains, positive charged side chains or negative charged side chains. Red residues indicate the amino acid substitutions between two different groups, such as Ala-Ser. Green residues indicate the amino acid substitutions within the same group, such as Val-Leu. Yellow residues indicate the amino acid substitutions that likely change the bulkiness within the same group, such as Tyr-Ser. The structure of actin monomer was obtained from the Protein Data Bank (PDB) (PDB ID: 1ATN).


1 11 21 31 41
ACT1 M A D G E D I Q P L V C D N G T G M V K A G F A G D D A P R A V F P S I V G R P R H T G V M V G M G
ACT2 M A E A D D I Q P I V C D N G T G M V K A G F A G D D A P R A V F P S V V G R P R H H G V M V G M N
ACT7 M A D G E D I Q P L V C D N G T G M V K A G F A G D D A P R A V F P S I V G R P R H T G V M V G M G
ACT11 M A D G E D I Q P L V C D N G T G M V K A G F A G D D A P R A V F P S I V G R P R H T G V M V G M G
Skeletal actin D E D E T T A L V C D N G S G L V K A G F A G D D A P R A V F P S I V G R P R H Q G V M V G M G
Buried
F-actin
Arp2/3
Profilin
Formin
Myosin
Cofilin
Phalloidin
51 61 71 81 91
ACT1 Q K D A Y V G D E A Q S K R G I L T L K Y P I E H G I V N N W D D M E K I W H H T F Y N E L R V A P
ACT2 Q K D A Y V G D E A Q S K R G I L T L K Y P I E H G V V S N W D D M E K I W H H T F Y N E L R I A P
ACT7 Q K D A Y V G D E A Q S K R G I L T L K Y P I E H G I V S N W D D M E K I W H H T F Y N E L R V A P
ACT11 Q K D A Y V G D E A Q S K R G I L T L K Y P I E H G I V S N W D D M E K I W H H T F Y N E L R V A P
Skeletal actin Q K D S Y V G D E A Q S K R G I L T L K Y P I E H G I I T N W D D M E K I W H H T F Y N E L R V A P
Buried
F-actin
Arp2/3
Profilin
Formin
Myosin
Cofilin
Phalloidin
101 111 121 131 141
ACT1 E E H P I L L T E A P L N P K A N R E K M T Q I M F E T F N A P A M Y V A I Q A V L S L Y A S G R T
ACT2 E E H P V L L T E A P L N P K A N R E K M T Q I M F E T F N S P A M Y V A I Q A V L S L Y A S G R T
ACT7 E E H P V L L T E A P L N P K A N R E K M T Q I M F E T F N V P A M Y V A I Q A V L S L Y A S G R T
ACT11 E E H P V L L T E A P L N P K A N R E K M T Q I M F E T F N T P A M Y V A I Q A V L S L Y A S G R T
Skeletal actin E E H P T L L T E A P L N P K A N R E K M T Q I M F E T F N V P A M Y V A I Q A V L S L Y A S G R T
Buried
F-actin
Arp2/3
Profilin
Formin
Myosin
Cofilin
Phalloidin
151 161 171 181 191
ACT1 T G I V L D S G D G V S H T V P I Y E G Y A L P H A I L R L L A G R D L T D A L M K I L T E R G Y
ACT2 T G I V L D S G D G V S H T V P I Y E G F S L P H A I L R L D L A G R D L T D Y L M K I L T E R G Y
ACT7 T G I V L D S G D G V S H T V P I Y E G Y A L P H A I L R L D L A G R D L T D S L M K I L T E R G Y
ACT11 T G I V L D S G D G V S H T V P I Y E G Y A L P H A I L R L D L A G R D L T D Y L M K I L T E R G Y
Skeletal actin T G I V L D S G D G V T H N V P I Y E G Y A L P H A I M R L D L A G R D L T D Y L M K I L T E R G Y
Buried
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Arp2/3
Profilin
Formin
Myosin
Cofilin
Phalloidin

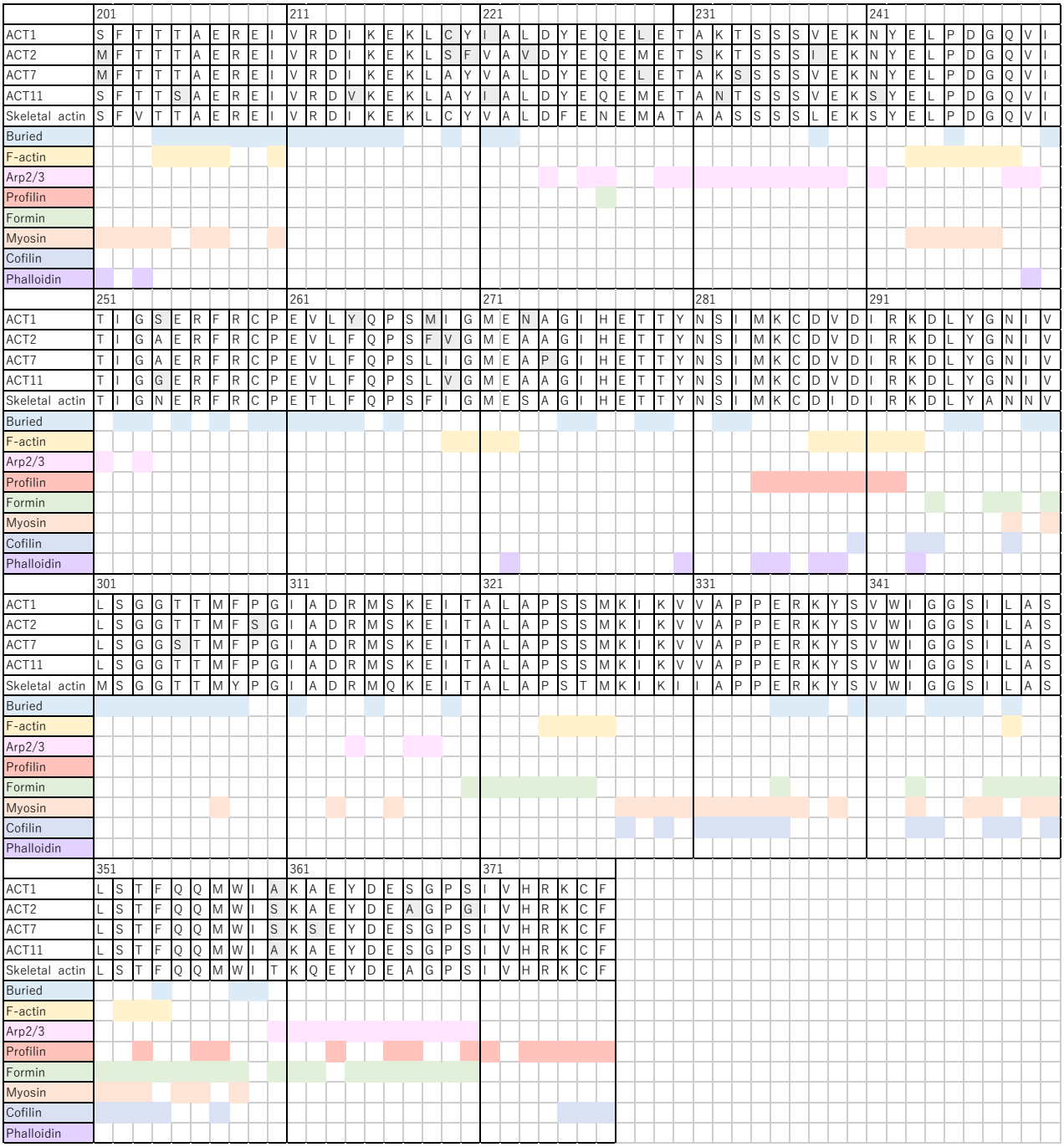

Figure 26. Alignment of amino acid residues among *Arabidopsis* actin isoforms.
The alignment shows the major *Arabidopsis* actin isoforms (ACT1, 2, 7 and 11) and human α skeletal muscle actin (Skeletal actin), with the interaction properties of each amino acid residue indicated below the alignment. The sequence of AtACT8 has only Glu to Asp substitution at residue 3 of that of AtACT2. “Buried” indicates buried residues in the G-actin structure, “F-actin” indicates residues interfacing neighboring actin molecules in the actin filaments, “Arp2/3”, “Profilin”, “Formin”, “Myosin”, “Cofilin” and “Phalloidin” indicate residues implicated in interaction with each protein or phalloidin peptide. These amino acid residues with the interaction properties were organized by Gunning *et al*. (2015). The phalloidin binding site in actin filaments was identified by Lorenz *et al.* (1993) and Oda *et al.* (2005).