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REVIEW ARTICLE

Isovariant Dynamics Expand and Buffer the Responses of Complex Systems: The Diverse Plant Actin Gene Family

Richard B. Meagher,[1] Elizabeth C. McKinney, and M. K. Kandasamy

Genetics Department, Life Sciences Building, University of Georgia, Athens, Georgia 30602-7223

INTRODUCTION

Most plant and animal genes are members of gene families that are differentially expressed and may encode diverse protein isovariants. With the recent explosion of information in plant genomics, researchers have become acutely aware that the gene families in plants are at least as diverse as their animal counterparts (McGrath et al., 1993; Newman et al., 1994; Henikoff et al., 1997; McKinney and Meagher, 1998). Among plant cytoskeletal gene families in Arabidopsis, there are at least 10 actins, nine α-tubulins, six β-tubulins, six profilins, and dozens of myosins. These and a few other examples of families of plant isoenzymes, signal transduction proteins, and regulatory proteins are listed in Table 1. The general view is that such family members are both selected out and preserved in evolution because they express varying levels of proteins in different temporal and spatial patterns (Meagher, 1995; Meagher et al., 1999). However, the expression of many of these gene family members overlaps considerably. This coexpression of protein isovariants in the same cells is expected to result in more dynamic behavior of these proteins, a process that we refer to as isovariant dynamics.

We define isovariant dynamics as the temporal and biochemical expansion of a biological system's responses as a result of the simultaneous expression and interaction of multiple isovariants of a protein. For isovariant dynamics to be operational in a cell, the coexpressed isovariants must be functionally distinct in at least one activity (e.g., binding a substrate or cofactor and/or interactions with other proteins). Because two or more members of each of the families of plant isovariants listed in Table 1 appear to be coexpressed in some cells, each has the potential to participate in protein–protein interactions that lead to isovariant dynamics.

It has been suggested that more highly networked biochemical systems are inherently more robust (Barkai and Leibler, 1997). If isovariant dynamics do indeed lead to more robust and highly buffered responses of cells, then they should be beneficial to the parent organisms and thus an important factor in the selection and/or preservation of the gene families encoding isovariants. By using a case study of the plant actin family to illustrate our points, we propose that the coexpression of multiple actin isovariants in the same cell results in isovariant dynamics that allow for more complex cytoskeletal responses.

Because dynamic processes are those in which energy, force, or motion are continuously changing, the cytoskeleton is an excellent system for studying isovariant dynamics. The cytoskeleton is constantly controlling cell structure and intercellular movement at the expense of chemical bond energy. However, enzymes and regulatory proteins (Table 1) also convert chemical bond energy in dynamic biochemical processes that can be expanded by the expression of multiple isovariants. By illustrating the properties of isovariant dynamics for actin, we hope to stimulate discussion and research on this complex problem for other families of isovariants.

FUNCTIONS AND CHARACTERISTICS OF PLANT ACTINS

Roles for Actin in Subcellular Processes

The plant actin cytoskeleton is central to many different subcellular processes that could be affected by interactions of actin isovariants. Figure 1 shows the complex network of actin filaments and bundles that reach into nearly every part of the cytoplasm of an Arabidopsis cell grown in suspension culture. The nucleus is positioned in the cell within a basket of actin filaments that is connected to the cortical cytoskeleton by strands of actin filaments and bundles.

The different cellular processes in which actin plays demonstrated or proposed roles include establishing cell polarity, division plane determination (by positioning the preprophase band), preprogramming of development and cell wall deposition, cell elongation, tip growth (e.g., of pollen tubes, root

[1] To whom correspondence should be addressed. E-mail meagher@arches.uga.edu; fax 706-542-3910.

Table 1. Examples of Plant Gene Families Encoding Coexpressed Protein Isovariants

Type of Protein	References
Cytoskeletal proteins	
Actins	McDowell et al. (1996b)
Myosins	Kinkema et al. (1994)
Profilins	Staiger et al. (1993); Christensen et al. (1996); Huang et al. (1996b)
α-Tubulins	Carpenter et al. (1992, 1993); Kopczak et al. (1992)
β-Tubulins	Snustad et al. (1992)
Enzymes	
Aminocyclopropane-1-carboxylic acid synthases	Rottmann et al. (1991); Zarembinski and Theologis (1997)
Acetyl-CoA carboxylases	Yanai et al. (1995)
Anthranilate synthases	Niyogi and Fink (1992)
Aspartate aminotransferases	Schultz et al. (1998)
Chalcone synthases	Koes et al. (1989)
Fatty acid desaturases	Fukuchi-Mizutani et al. (1998)
Flavanone isomerases	van Tunen et al. (1988)
Glucanases, chitinases	Glazebrook et al. (1997)
Glutamine synthetases (cytosolic)	Peterman and Goodman (1991); Stanford et al. (1993)
Laccases	LaFayette and Dean (1997)
Plasma membrane H^+-ATPases	Sussman (1994)
S RNAses	Green (1994)
Tryptophane synthases	Last et al. (1991)
Xyloglucan endotransglucosylases	Xu et al. (1996)
Regulatory and signal transduction proteins	
Calmodulin-related proteins	Braam (1992)
Disease resistance (*R*) genes with leucine-rich repeats	Chasan (1994); Staskawicz et al. (1995); Reignault et al. (1996)
Ethylene receptors	Chang and Meyerowitz (1995)
MADS-box proteins	Davies and Schwarz-Sommer (1994)
Myb homologs	Li and Parish (1995); Abe et al. (1997)
Myc homologs (*R* genes)	Purugganan and Wessler (1994); Hu et al. (1996)
Phytochromes	Clack et al. (1994)
Poly(A) binding proteins	Belostotsky and Meagher (1993)

hairs, and moss protonema), transmembrane transport and positioning of receptors, mRNA transport within the cell, cytoplasmic streaming, and orientation of chloroplasts in response to light with appropriate repositioning of the nucleus. These and other functions for plant and algal actins have been reviewed elsewhere (Emons et al., 1991; Staiger and Lloyd, 1991; Meagher and Williamson, 1994; Bouget et al., 1996; Staehelin and Hepler, 1996). Because plant cells do not migrate, establishment of polarity, division plane determination, cell elongation, and directing cell wall deposition are processes critical to nearly all aspects of plant development and morphology. Moreover, combinations of various actin-mediated processes are thought to be necessary for the rapid response of plant cells to internal or external signals.

The Actin Cytoskeleton Is Dynamic

The dynamic activity of the actin network is a major factor contributing to the viscoelastic properties of cells (Ingber, 1993; Ingber et al., 1994). That is, cells spring back into shape when deformed suddenly, although they can be deformed by force applied over an extended period of time. Viscoelastic properties are partially explained by the fact that whereas most actin filaments have half-lives of $\sim$1 min (Theriot and Mitchison, 1991), most cross-links between filaments last $<$1 sec (Wachsstock et al., 1994).

How information about the intensity or quality of mechanical stress is conveyed to signaling pathways is not understood, but most likely it starts by the interaction of the actin or tubulin cytoskeleton with signal molecules. It seems reasonable to propose that the property of viscoelasticity contributes to the dynamic response of plant cells to different physical stresses in their environment (Braam and Davis, 1990). After the cytoskeleton is deformed from external mechanical stress, cues about the quality and quantity of force must be transmitted to cell information pathway(s), as proposed in Figure 2. It is likely that the cytoskeleton plays direct roles in mechanosensation processes (Wang et al., 1993; Bargmann, 1994), such as the touch response of leaves (Xu et al., 1996), the grasping of support by gyrating tendrils (Engelberth et al., 1995), or the avoidance of hard objects in the soil by roots (Okada and Shimura, 1990).

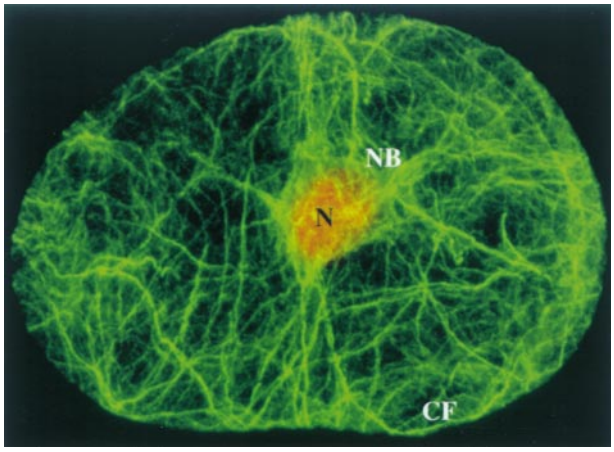

Figure 1. Actin Forms Complex Arrays of Filaments and Bundles Interacting with Nearly Every Part of the Cell.

Actin filament arrays (green) in an Arabidopsis interphase suspension culture cell (Keith et al., 1991) are stained with a general plant actin monoclonal antibody, MAbGPa, that reacts with all isovariants of plant actin (Kandasamy et al., 1999). The nucleus, stained with 4′,6-diamidino-2-phenylindole, is shown in red. CF, cortical filaments; N, nucleus; NB, nuclear basket.

That the actin cytoskeleton responds dynamically to information from signal transduction pathways is better documented (Figure 2; Zigmond, 1996; Yamada and Geiger, 1997). For example, external stimuli activate different members of the Rho GTPase family (e.g., Rho, Rac, and Cdc42) that in turn signal distinct changes in the cytoskeleton (Tapon and Hall, 1997). In animal cells, these changes translate into defined changes in cell morphology and movement, such as the expression of membrane receptors and focal adhesion complexes linked to the cytoskeleton and stress fibers, the assembly of actin filaments just below the cell membrane that produce lamellipodia and ruffles, and the protrusion of filapodia (Hall, 1998). To produce these three different morphologies, each GTPase activity results in the expression of distinct adhesion complexes linking the cytoplasm to the cell membrane.

Similarly, in pollen tubes, localization of a Rho GTPase dubbed Rop1Ps suggests that this "molecular switch" might be involved in generative cell movement and tip growth (Lin et al., 1996). Moreover, microinjected anti-Rop1Ps antibodies inhibit pollen tube elongation but not cytoplasmic streaming (Lin and Yang, 1997). In accordance with the model elaborated in animal systems, this experiment helps to associate one plant signal transduction pathway with at least one cellular function.

Other plant-specific signal transduction pathways with links to the actin cytoskeleton are now being elucidated. For example, the phytohormone abscisic acid acts near the onset of a complex pathway that is triggered by physical environmental stresses, including temperature, humidity, and osmotic shock. Abscisic acid stimulates marked changes in the cell architecture and subsequent development (Pennisi, 1997; Wu et al., 1997). Thus, the actin cytoskeleton might have roles to play in signaling stress at the beginning of cell communication pathways (i.e., through mechanosensation) and/or in elaborating a developmental response at the end of these pathways. The breadth of possible activities and responses of the plant cytoskeleton is increased by the coexpression of multiple actin isovariants.

Extreme Variability among Plant Actin Isovariants

Plant actins comprise 376 to 377 amino acid residues. They share most of these residues with actins in other kingdoms, typically showing 83 to 88% identity with actins from green algae and most other protists, fungi, and animals. This high degree of conservation is thought to be a direct result of the fact that nearly every surface of actin is involved in protein–protein interactions (Sheterline and Sparrow, 1994; Furukawa and Fechheimer, 1997; Puius et al., 1998). Higher plants and animals contain relatively ancient families of actin proteins, the phylogenies of which can be traced to the origin of vascular plants and vertebrate animals, respectively (Meagher and Williamson, 1994; Meagher, 1995). There is ~94 to 95% amino acid sequence identity within each of these ancient families.

The actin gene family in Arabidopsis represents an excellent model system for understanding actin functions in plants. There are only 10 actin genes in Arabidopsis, all of which have been cloned, sequenced, and characterized in detail (McDowell et al., 1996b). The actin genes are all relatively small (i.e., $<$3 kb) and are individually dispersed in the genome (McKinney and Meagher, 1998). At least eight of the actin genes appear to be functional and are strongly expressed at some time and place during plant development (An et al., 1996a, 1996b; Huang et al., 1996a, 1997; McDowell et al., 1996a). Among the eight encoded functional actin proteins, there are a relatively large number of nonconservative amino acid substitutions (Meagher, 1991; McDowell et al., 1996b). For example, there are seven charged residue interchanges (e.g., His43→Thr43) and two changes between an α amino acid and proline (e.g., Lys272→Pro272), the latter of which are likely to alter the peptide backbone. All of these changes map to the surface of the eight Arabidopsis actin proteins. In addition, there are several nonconservative interchanges (e.g., Met201→Ser201) affecting amino acids with hydrophobic side chains (Hightower and Meagher, 1986; McDowell et al., 1996b).

As a result of this sequence diversity, five to six distinct actin isovariants can be resolved from many plant species by using two-dimensional electrophoretic separations of polypeptides (McLean et al., 1990). The isoelectric points of the plant actins vary over a relatively wide range of 0.7 pH units (Meagher and McLean, 1990).

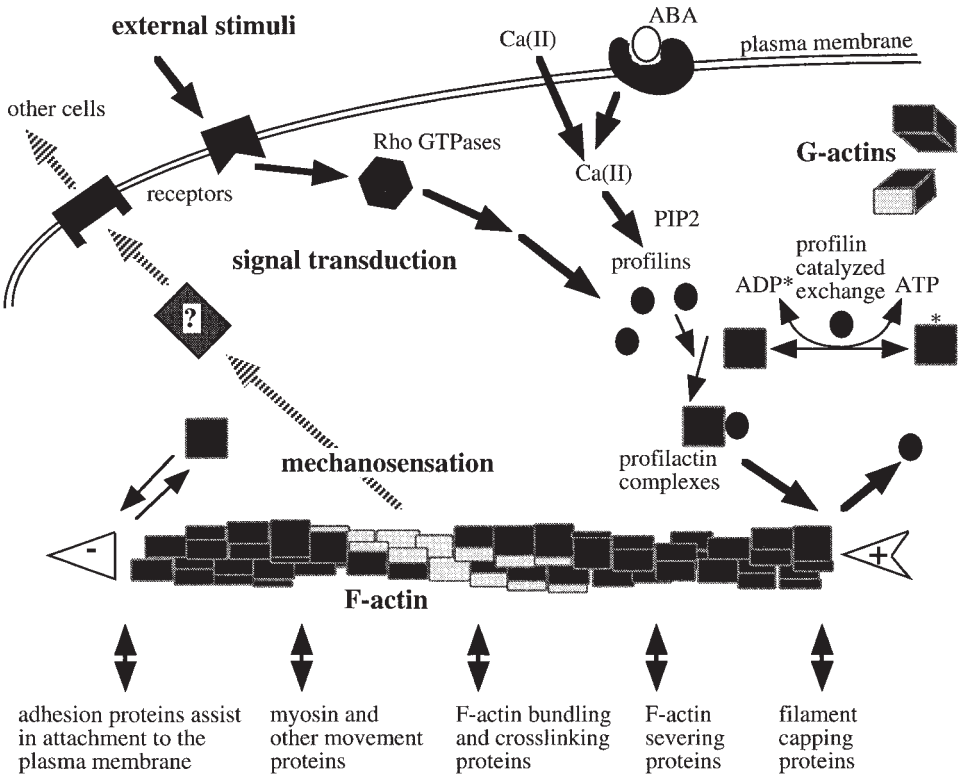

Figure 2. G-Actin Monomers and F-Actin Filaments Interact with Profilin and Many Other Actin Binding Proteins.

Proteins from multiple signal transduction cascades direct change in cytoarchitecture by acting directly on actin. The actin cytoskeleton may itself be involved in signaling mechanical stress to the rest of the organism through the same or other proteins. Numerous actin binding proteins interact with actin monomers, filaments, and bundles to effect these changes. The dynamics of these interactions should be greatly expanded by the expression of multiple actin isovariants in most plant cells. Pointed (−) and barbed (+) ends of an F-actin filament are indicated. ABA, abscisic acid; Ca (II), calcium ions; PIP2, phosphatidylinositol diphosphate; ?, unknown pathway; *, ADP and ADP-bound actin monomers.

By contrast, there are no analogous nonconservative changes among the six vertebrate actin proteins, even though the muscle and cytoplasmic actins have not shared a common ancestor for an estimated 500 million years. Moreover, the isoelectric points within animal actin families vary over only 0.3 pH units, and as a result, no more than three isovariants can be resolved by standard isoelectric focusing (see Meagher and McLean, 1990). Even the few conservative charged residue interchanges (e.g., Glu2→Asp2) that do occur among the first few N-terminal residues of vertebrate actins can lead to different physical properties for the isovariants (Garrels and Gibson, 1976).

Despite their relative similarity, vertebrate nonmuscle actins bind profilin and thymosin in preference over muscle actins (Larsson and Lindberg, 1988; Oshima et al., 1989; Weber et al., 1992). In addition, vertebrate nonmuscle actins polymerize less readily but are ADP ribosylated far more efficiently by *Botulinum chlostridium* C2 toxin than are muscle actins, and smooth muscle actins are more readily heat denatured than are other muscle isoforms (Rubenstein, 1990). Thus, even minor changes in the amino acid sequence of an actin can alter protein function.

The six Drosophila actins also can be subdivided into cytoplasmic and muscle-specific classes (Fyrberg et al., 1981, 1983), but these classes are thought to have evolved independently of the analogous two classes in vertebrates. There is only one full charged-residue interchange (Gln360→Glu360) among all six Drosophila actins. Even so, ectopic expression of cytoplasmic actin instead of flight muscle actin in Drosophila muscle tissues leads to dominant flightless mutants. Moreover, substituting a modified gene with the seemingly harmless interchange from Ile76 (found in the four muscle actins) to Val76 (found in the two cytoplasmic actins) for the normal adult muscle actin gene *Act88F* created a flightless phenotype (Fyrberg et al., 1998). Whereas most single–amino acid changes from the normal adult flight muscle actin sequence produced no measurable deficiencies, changing multiple residues to those of another isovariant generally disrupted flight muscle function. It should be reiterated that these functional differences are observed even though there

is much less variability in the Drosophila actin family than in any plant actin family. These data taken together suggest that the highly variable plant actin isovariants must vary in their physical chemical parameters and must interact differentially with some actin binding proteins (ABPs).

Plant Actin Genes Show Distinct but Overlapping Expression Patterns

An analysis of steady state RNA levels and the expression of actin translational fusions to the β-glucuronidase reporter in transgenic plants has been performed on the eight functional Arabidopsis actin genes (An et al., 1996a, 1996b; Huang et al., 1996a, 1997; McDowell et al., 1996a). Based on their distinct temporal and spatial expression patterns, the eight functional Arabidopsis actin genes can be divided into vegetative and reproductive classes (McDowell et al., 1996b; Meagher et al., 1999). There are two vegetative subclasses. Subclass 1 includes *ACT2* and *ACT8*, the expression of which predominates in nearly all vegetative tissues. Subclass 2 comprises *ACT7*, the expression of which is strongest in young, rapidly growing vegetative tissues and is responsive to most phytohormones. There are three reproductive subclasses. Subclass 3 includes *ACT11*, the expression of which is concentrated in gynoecia and pollen. Subclass 4 includes *ACT1* and *ACT3*, which are predominantly expressed in young ovules, pollen, and organ primordia. Subclass 5 includes *ACT4* and *ACT12*, which are expressed during pollen development. Interestingly, the relationships among actin classes and subclasses defined by tissue-specific expression patterns corresponds precisely with those based on actin sequence comparisons (McDowell et al., 1996b).

If each subclass of actin were expressed in only one cell type, tissue, or organ exclusive of other actins, there would be no chance for isovariant dynamics to play a role in regulating the plant actin cytoskeleton. In fact, two or more actin subclasses are strongly expressed in most tissues and organs. For example, in a developing inflorescence, *ACT1*, *ACT2*, *ACT3*, *ACT7*, and *ACT11* are expressed in the floral meristem; *ACT2* and *ACT7* continue to be expressed in the developing nonreproductive parts of the flower, including sepals, petals, stigma, style, and filaments, whereas *ACT1*, *ACT3*, and *ACT11* continue to be expressed in young developing ovules. Five genes, *ACT1*, *ACT3*, *ACT4*, *ACT11*, and *ACT12*, are strongly expressed late in pollen development and during pollen tube growth. All eight of the actin genes appear to be expressed at reasonable levels in developing vascular tissues.

ACTIN ISOVARIANT DYNAMICS

The expression of multiple actin isovariants in the same cells facilitates extraordinary flexibility in the dynamic behavior of the cytoskeleton. The majority of this flexibility is likely to be achieved via distinct types of actin isovariant dynamics that come into play during, for example, the polymerization of various actin monomers into F-actin polymers and during the interaction of actin with numerous ABPs (Figure 2). These two categories of isovariant dynamics and their possible effects on the cytoskeleton are discussed below.

Formation of Actin Homopolymers versus Heteropolymers

When two actin isovariants, A_i and A_j, are expressed in the same cell, there is the potential to form separate homopolymers $[(A_i)_n, (A_j)_n]$ or heteropolymers $(A_iA_j)_n$ with various mixtures of the two isovariants. We consider the addition of actin monomers to just one end of an actin filament (i.e., the plus or "barbed" end), which already ends with an A_i or an A_j isovariant. In this instance, there are four association constants to consider—$K_{di}^{\,i}$, $K_{dj}^{\,j}$, $K_{di}^{\,j}$, and $K_{dj}^{\,i}$, where, for example, $K_{di}^{\,j} = [A_i][A_j]/[A_iA_j]$ and represents the addition of A_j to an A_i end. If $K_{di}^{\,i} < K_i^{\,j} < K_j^{\,i} < K_{dj}^{\,j}$, then A_i-rich polymers will be formed even when A_i monomer concentrations are relatively low, so long as $A_i \geqslant A_j$. A_j-rich polymers are formed only when $A_j >> A_i$. The degree of heteropolymer formation depends on monomer concentrations and the specific K_d values. However, because $K_i^{\,j} < K_j^{\,i}$, A_i-rich heteropolymers would be favored. If, for example, A_i-rich polymers have a stronger tendency to bundle, then this property of isovariant dynamics would have a significant impact on cell morphology and response.

Dynamic instability is a related concept that was first used to describe the effect of coordinated amino acid substitutions among ancient classes of coexpressed animal tubulin isovariants (Burns and Surridge, 1990; Caplow and Shanks, 1998). In this specific type of isovariant dynamics, microtubules assembled in vitro from highly mixed populations of isovariants are less stable than those assembled from pure or less mixed populations of isovariants due to differences in the rates of assembly-dependent GTP hydrolysis among isovariants. The greater protein sequence variability among plant tubulin isovariants appears to result in significantly higher rates of microtubule shortening for plant tubulin heteropolymers than for the animal heteropolymers (Moore et al., 1997).

Impact of Isovariant Structure on Interactions with ABPs

Monomeric G-actin and filamentous F-actin bind to myriad other proteins affecting changes in cell architecture (Figure 2; Kreis and Vale, 1993; Puius et al., 1998). We consider the impact of isovariant dynamics on the sequestration of actin monomers by profilin into profilactin complexes. Profilin is a major APB that is found in high concentrations in most cells (i.e., >50 μM in most animal cells). Profilin is involved in the

sequestration of actin into profilactin complexes, ADP/ATP nucleotide exchange on G-actin, addition of actin monomers from profilactin complexes to the barbed ends of actin filaments, and binding to PIP2 that inhibits profilin binding to actin. We focus on just the first of these activities, the binding of actin into profilactin complexes.

During late pollen development in Arabidopsis, at least three actin subclasses are coexpressed (Christensen et al., 1996; Huang et al., 1996b). The equilibrium expression can be written as $K_{di}^{k} = [A_i][P_k]/[A_iP_k]$, where K_{di}^{k} is the dissociation constant of the profilactin complex (A_iP_k) into monomeric actin isovariant A_i and profilin isovariant P_k. The interaction of only one actin isovariant, ACT1, with one profilin, PRF4 (Huang et al., 1996b), is relatively simple. There would be a distinct dissociation constant, K_{d1}^{4}, and various rate constants (not discussed) for the formation and dissociation of the A_1P_4 complex.

The situation becomes considerably more complex with the coexpression of the ACT11 and ACT12 subclass isovariants, where each has a different dissociation constant, K_{d11}^{4} and K_{d12}^{4}, for the formation of the $A_{11}P_4$ and $A_{12}P_4$ profilactin complexes, respectively. All three actins compete in pollen for PRF4, and all three are in the actin monomer pool. If these three actin–profilin interactions each have different equilibrium dissociation constants and rate constants, then these kinetic relationships would be an important factor in the plant cytoskeleton. The ability of the actin cytoskeleton to respond to signals that affect nucleotide exchange, polymerization, and cycling and its ability to buffer or manipulate total actin monomer concentrations would be greatly expanded compared with the situation with a single actin isovariant. For example, if ACT1 underwent more efficient nucleotide exchange catalyzed through a preferential interaction with PRF4, then ACT1 filaments could also undergo more rapid treadmilling. The consequences of this could be rapid ATP-catalyzed growth of ACT1-rich filaments at the growing tip of the pollen tube (Wang, 1985) at the expense of other more static filaments.

The plant profilin family in Arabidopsis appears to be as large and diverse as the Arabidopsis actin family (Christensen et al., 1996; Huang et al., 1996b). Based on these studies and the data on maize pollen profilins (Staiger et al., 1993), it is extremely likely that there will be multiple profilin isovariants also expressed in Arabidopsis pollen. Moreover, recent results examining maize pollen profilins (Gibbon et al., 1998) and data on Arabidopsis profilins (C.J. Staiger and R.B. Meagher, unpublished data) demonstrate that different profilin isovariants vary over a two- to threefold range in their affinity (i.e., K_d) for actin and poly-L-proline. Thus, isovariant dynamics are a very real part of plant cytoskeletal systems. Furthermore, the coexpression of profilin isovariants adds another dimension to actin isovariant dynamics. Considering that all the other actin binding proteins for gelling, capping, bundling, severing, and depolymerizing, as well as motor proteins like myosin, may be encoded by gene families in plants, the potential for extreme isovariant dynamics in the plant actin cytoskeleton is staggering. Perhaps this diverse cytoskeletal system has evolved not only to direct plant cell developmental processes but also to respond rapidly to a wide variety of environmental changes.

Barkai and Leibler (1997) have presented a compatible model in which the highly networked structure of such a complex system increases its robustness. From this view, the simultaneous expression of actin and profilin isovariants would result in this cytoskeletal system being relatively insensitive to variations in biochemical parameters of the interacting components and in less demand for fine tuning of each protein. In other words, networked systems are better buffered against changes from either temporal differences in gene expression or genetic variation among individuals.

Impact of Isovariant Dynamics on the Actin Cytoskeleton

Physical changes in the actin network are brought about by a number of events. These include creating or breaking actin adhesions to the cell membrane, bundling or cross-linking filaments, interacting with myosin and other movement proteins, capping or decapping ends and sequestering monomers (i.e., profilin) to control cycling rates at filament ends, and dissolving and severing existing filaments (Figure 2). These processes are brought about by the interaction of distinct actin surfaces with diverse ABPs (Kreis and Vale, 1993; Puius et al., 1998).

Among dozens of possible examples of these processes, the spatial arrangement of actin filament links to the plasma membrane is particularly fundamental to programming cell architecture and development (Cowin and Burke, 1996; Yamada and Geiger, 1997). Another remarkable aspect of dynamic actin behavior is how actin filaments can elongate in one part of a cell by the addition of monomers, while they are static or being broken down in another part of the cell. This can even occur on the different ends of the same actin filament (Wang, 1985). The myosin motor–driven streaming of cytoplasm moves organelles along actin bundles and is thought to be essential to gas and nutrient exchange between layers of plant cells (Williamson, 1993). Each of these and many other actin-based processes may be enhanced by isovariant dynamics.

If isovariant dynamics are of any great significance to the function of the plant cytoskeleton, then it is conceivable that they might be subject to selective constraint over evolutionary time. By this hypothesis, natural selection operates beyond the level of single genes or proteins, acting as well at the level of isovariant dynamics in plants. The coexpression of isovariants may have resulted in the temporal and biochemical expansion and buffering of the properties of these biochemical systems, which in turn may have given a selective advantage to the plants expressing the isovariants.

This hypothesis can be partially tested with the ectopic expression of isovariants in a limited number of cell types in mutant and wild-type plants, as discussed above for Dro-

sophila actin isovariants (Fyrberg et al., 1998). For the null hypothesis, we can argue for neutrality of the isovariant interactions. This argument would hold that the actin gene family members may have been selected for strong expression in particular tissues but that their coexpression in other tissues represents true redundancy resulting from the inability to regulate genes perfectly (Meagher, 1995).

Although the proof is not definitive, there are several reasons why this hypothesis seems unlikely to apply for the Arabidopsis actins. First, strong selective forces have acted to preserve the distinct protein sequences of each of the five subclasses of actin. Very clear evidence for this comes from the conservation of three pairs of actin protein sequences (i.e., ACT2 and ACT8; ACT1 and ACT3; and ACT4 and ACT12) since the divergence of each pair from three ancestral sequences (McDowell et al., 1996b). Second, mutants in three different Arabidopsis actin genes (e.g., *act2-1*, *act4-1*, and *act7-1*) act as deleterious alleles that are lost rapidly from plant populations (Gilliland et al., 1998).

One clear but uncommon exception to these two extreme possibilities (i.e., the positive selection of isovariant dynamics versus true redundancy) is represented by the plant gene family encoding the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). In this case, ancient and unlinked gene family members undergo frequent gene conversion events, and only organisms with protein sequence homogeneity among the protein family members survive (Meagher et al., 1989). Evidently, isovariant dynamics cannot be tolerated among Rubisco small subunit proteins, and we must assume that they are under strong negative selection.

Isovariant Dynamics in Other Plant Protein Families

Many other gene/protein families with coexpressed and divergent isovariants have been identified in plants, as shown by the examples given in Table 1. The number, size, and diversity of many of these gene families appear to be greater in plants than in animals and should lead to more complex behavior of plant systems (Moore et al., 1997). The effects of isovariant dynamics may be qualitatively slightly different for families of enzymes and regulatory proteins than they are for cytoskeletal proteins. The variations in structures among enzyme and regulatory protein isovariants could alter substrate affinity (K_d), substrate specificity, protein–protein interactions, turnover number, response to accessory factors, and positioning of the protein within the cell. Although the biological significance is not clear, heterooctamers formed from two isovariants of cytosolic glutamine synthetase have novel kinetic properties relative to either homooctamer (Robert and Wong, 1986; Bennett and Cullimore, 1989; Green and Wong, 1992; Carvalho et al., 1997). Isovariant dynamics in regulatory protein families could affect their binding to target nucleotide sequences or affinity for numerous other *trans*-acting or signaling factors. For example, heterodimers of the two MADS domain homeotic proteins, PISTILLATA1 and APETALA3, can bind the appropriate DNA sequence regulating Arabidopsis flower development, but each protein alone cannot (Riechmann et al., 1996). In one additional example of the potential for dynamic interactions among coexpressed protein family members, Arabidopsis calmodulin (CaM) isovariants bind and activate G/C box binding of nuclear proteins differentially. In particular, the CaM-6 isovariant enhances the DNA binding of TGA3 two to three times more efficiently than does either CaM-2 or CaM-4 (Szymanski et al., 1996).

Future Research on Isovariant Dynamics

Determining the contributions of isovariant dynamics to cellular and organismal functions is complex and will require the informed application of a wide variety of tools and approaches. In particular, it must be demonstrated that isovariants are not redundant and that differences in protein sequence and not differential gene regulation are responsible for the effect being examined. For example, quantitative molecular evolutionary studies can suggest whether genes encoding two isovariants belong to separate ancient gene subclasses that have been preserved among distant species (Huang et al., 1996b) or whether they are recently duplicated genes (Meagher et al., 1989). Such comparisons can be used to predict if the amino acid residue variability observed is likely to have an impact on protein function (Meagher and McLean, 1990; McDowell et al., 1996b). Genetic approaches can determine if isovariants are functionally redundant (Brookfield, 1997) by identifying potentially harmful mutant phenotypes (Oliver et al., 1992) or, failing this test, by demonstrating that negative selection acts on a mutant allele (Gilliland et al., 1998). The degree of suppression of a mutant allele obtained with a different isovariant allele should be one of the most definitive techniques to demonstrate the biological significance of an isovariant dynamics phenotype (Fyrberg et al., 1998).

In highly networked systems like the cytoskeleton, where the central proteins have multiple activities, it will not be easy to identify those molecular parameters most affected by the coexpression of isovariants. Some molecular biological properties of isovariants can be more easily dissected by suppression of yeast mutants (Belostotsky and Meagher, 1996) because yeast usually has only a single homologous actin gene. Microinjection into living cells has proved to be a very powerful tool for testing specific protein functions and differences among isovariants (Gibbon et al., 1997; Valster et al., 1997; Hussey et al., 1998). Physical chemical techniques, generally applied in vitro, can dissect those parameters that vary significantly in a protein familiy (e.g., affinity and rate constants for substrate or cofactors and for hetero- or homomultimer formation). Many of these complex variables have been elegantly determined for animal and protist actins (Taylor et al., 1981; Pollard and Cooper, 1982; Perelroizen et al., 1994; Wachsstock et al., 1994; Petrella et al., 1996).

Isovariant-specific antibodies would be required to demonstrate different subcellular localization of coexpressed isovariants (De Nofrio et al., 1989), and in situ hybridization analyses of mRNAs within single cells could be used to determine if protein localization is due primarily to RNA or protein trafficking (Kislauskis et al., 1994; Lucas et al., 1995; Bouget et al., 1996). Finally, all the above approaches combined with crystallographic analysis of mutant and isovariant structures can further define those protein subdomains most likely to participate in these interactions (Puius et al., 1998). This three-dimensional information can then be used to refine molecular genetic and physical approaches.

CONCLUSION

The role that multiple isovariants for actin and ABPs plays in constructing the cytoskeleton and in enhancing cytoskeletal dynamics adds a fascinating depth to the study of plant systems. However, understanding the dynamic interactions of protein isovariants coexpressed in the same cells will be an extremely challenging task. To elucidate the significance of isovariant dynamics for actin or other protein families, we will need to understand these systems at the evolutionary, genetic, cell biological, biochemical, and biophysical levels.

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