The evolution of new structures

clues from plant cytoskeletal genes

How large numbers of genes were recruited simultaneously to build new organ structures is one of the greatest puzzles in evolutionary biology. Here, we present data suggesting that the vegetative and reproductive classes of actins and other cytoskeletal proteins arose concurrently with the macroevolutionary divergence of leaves and reproductive structures in the earliest land plants. That the cytoskeleton is essential for physically programming the development of organs and tissues is well established. Thus, we propose that this regulatory dichotomy represents an ancient landmark event in the global regulation of hundreds of higher-plant genes, an event that is linked to the macroevolution of plant vegetative and reproductive organs. The recent availability of sequence and expression data for large numbers of plant genes should make it possible to dissect this and other major macroevolutionary events.

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Perhaps one of the most complex questions in plant evolutionary biology is how to link the rapid macroevolutionary changes that occurred during vascular plant evolution with the molecular evolution of the genes that are necessary to control their development[1]. Remarkable new organ structures have evolved in the past 500 million years since land plants split from a common ancestor with green algae[2–5]. For example, it is widely accepted that there was a macroevolutionary divergence of leaves and reproductive structures early in vascular plant evolution (~400 million years)[3,6]. However, it has been difficult to model the evolution of any set of genes with the macroevolutionary origin of an organ, such as the leaf. Large numbers of interacting genes are required to regulate and physically direct the development of cell, tissue and organ structures. Distinct sets of genes have been uncovered that regulate the development of vegetative and reproductive organs[7–10]. Here, we propose that the macroevolution of vegetative and reproductive plant structures was linked to the molecular evolution of two large, differentially regulated classes of cytoskeletal genes that physically direct development.

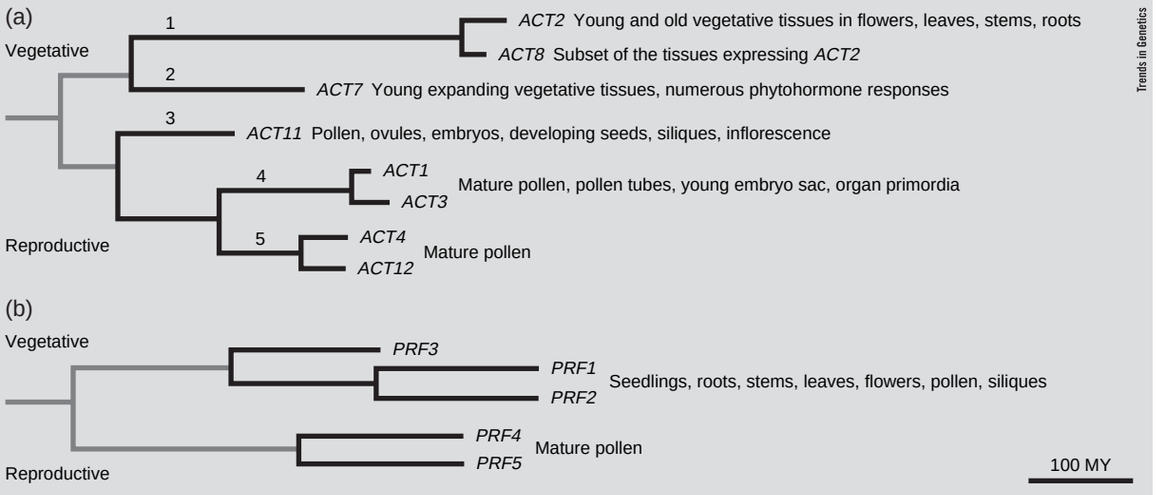
Actin is a cytoskeletal protein that is expressed in all eukaryotes. Higher plant actins are encoded by a relatively ancient and diverse family of genes, whose phylogeny was initiated with the emergence of vascular plants[1,11]. The earliest phylogenetic divergence resulted in separate vegetative and reproductive classes of actin genes, and these have been preserved as separate classes for 350–500 million years[12]. Because duplicate gene copies are genetically less stable than single-copy genes[13,14], two logical and non-exclusive arguments have been given for the preservation of ancient actin subclasses[12,15]. The first argument says that the amino acid sequence diversity found in the actin protein family is required for different cellular- or tissue-specific protein functions. Thus, biochemically dissimilar actin isovariants are required for distinct protein–protein interactions[16]. The second argument says that the differential regulation of the different gene family members is required for the quantitative expression of actin in complex temporal and spatial patterns. According to this molecular-genetic view, multiple genes are required to encode the various complex patterns and levels of gene expression required by all the diverse plant cells, tissues and organs.

Based on estimates that vegetative and reproductive structures and actin gene classes arose at about the same time, and given that the actin cytoskeleton is required for the development of these structures, we focus this article on differential gene regulation as the selective force that preserves divergent cytoskeletal gene family members. More particularly, we hypothesize that the vegetative and reproductive expression patterns of the actins and several other families of cytoskeletal protein genes are required for the development of the corresponding organ structures. Thus, we are proposing an ancient link between the evolution of cytoskeletal gene regulation and the macroevolution of organs. We begin by briefly reviewing the origin and regulation of different plant actin classes and subclasses, and then go on to discuss the regulation of other cytoskeletal gene families, the role of the cytoskeleton in development, the macroevolution of vegetative and reproductive structures, and the availability of extra gene copies.

An ancient split in the plant actin gene tree

The actin gene family of the crucifer *Arabidopsis thaliana* has been established as a valuable model system for the study of plant actins. There are only ten actin genes in *Arabidopsis*; all have been cloned, sequenced and characterized in detail[12].

FIGURE 1. *Arabidopsis* actin and profilin gene trees



(a) *Arabidopsis* actin genes in the ancient vegetative and reproductive classes differ by 4–7% in replacement nucleotide substitutions (RNS) that result in amino acid changes. In addition, the family is divided into five subclasses, numbered 1–5. The two most closely related subclasses, 4 and 5, differ by 2.5% RNS. This neighbor-joining tree is derived similarly to that described in McDowell *et al.*[12] The predominant expression patterns of each subclass are shown on the right. (b) The vegetative and reproductive classes of *Arabidopsis* profilin genes differ by as much as 28% in amino acid sequence, whereas the most closely related pairs of profilins in the same class are diverged in only 12% of their amino acids. This neighbor-joining tree was derived similarly to that described in Huang *et al.*[23] Available information on the predominant profilin expression patterns for four profilins are shown on the right. See profilin gene designations in Table 1.

Eight of the ten actin genes appear to be strongly expressed at some time and place during plant development[17–21]. The remaining two members of the *Arabidopsis* family appear to be pseudogenes[12]. The functional *Arabidopsis* actin genes are individually dispersed in the genome[22].

The evolution and diversity of *Arabidopsis* actin genes has been quantified and studied in detail[12]. A gene tree based on changes in the replacement nucleotide substitutions (i.e. those nucleotide changes that change amino acids) is shown in Fig. 1. The eight expressed members of the family can be divided into two ancient classes that are expressed predominantly in vegetative or reproductive organs and tissues (Tables 1 and 2). Using evolution rates as a measure, these two actin-gene classes appear to have diverged from a single common ancestral plant actin sequence some 350–500 million years ago. The gene tree and gene expression data suggest that over the past 150–350 million years both classes of actin have further subdivided, resulting in a total of five actin subclasses (1–5 in Fig. 1). Additional support for the topological and age relationships of subclasses in the tree comes from a recent immunochemical characterization of an ancient, pollen-specific epitope, centered on Asn79, which is found in all angiosperms and the most recently evolved gymnosperms[64]. This epitope is shared by the two most-recently derived reproductive actin subclasses (4 and 5) but not found in any other plant or animal actins. It is reasonable to assume that this amino acid change arose in a common ancestral gene. Thus, the branch of the actin tree that contains these pollen-specific actin subclasses traces its origin to late gymnosperms, about 220 million years ago.

Vegetative and reproductive actin classes

An analysis of steady-state RNA levels and the expression of actin translational fusions to a β-glucuronidase (GUS) reporter in transgenic plants has been performed on the eight functional *Arabidopsis* actin genes[12,17–21]. Actin-encoding gene and mRNA regulatory patterns can be categorized clearly as being one of two complementary types, either vegetative or reproductive (Table 1). For example, three vegetative actins, *ACT2*, *ACT7* and *ACT8*, are strongly expressed in roots, stems and leaves of germinating seedlings, young plants and mature plants[21] (Figs 2 and 3, Table 2). Vegetative gene expression includes some organs of the floral organ complex (e.g. sepals, petals, filaments, stigma, style). For example, see *ACT2*- and *ACT7*-driven reporter expression in Fig. 3 (Plates b and d, respectively). Strong *ACT2* activity continues to be expressed, even in older tissues, suggesting an important role in maintaining the plant cytoskeleton in mature vegetative tissues. *ACT7* is the primary actin responding to phytohormones, has exceptionally high activity in young, rapidly growing tissues and, by contrast with *ACT2*, its activity is low in mature tissues. Little or no expression of the vegetative genes has been observed in mature pollen sacs, ovules, embryos or seeds.

TABLE 1. Cytoskeletal proteins encoded by vegetative and reproductive genes

Protein	Organism	Vegetative	Reproductive	Refs
Actin	*Arabidopsis*	*ACT2, -7, -8*	*ACT1, -3, -4, -11, -12*	12, 17–21
Profilin	*Arabidopsis*	*PRF1* (*PFN1*), *PRF2* (*PFN2*), *PRF3*	*PRF4* (*PFN3*), *PRF5* (*PFN4*)	23, 24
Profilin	Maize	*PRO4*	*PRO1, -2, -3*	25, 56, 57
ADF	Maize	*ADF3*	*ADF1, -2*	58, 59
α-Tubulin	*Arabidopsis*	*TUA2, -3, -4, -5*	*TUA1, -6*	60–62
β-Tubulin	*Arabidopsis*	*TUB1, -5, -6, -8*	*TUB2, -3, -7, -9*	63

Abbreviation: ADF, actin depolymerizing factor.

TABLE 2. Summary of vegetative and reproductive actin gene expression[a]

	Vegetative expression												Reproductive expression						
	Ger seed	Seed coat	Hypo	Cotyl	Leaf	Root tip	Root cort epid	Vas cyl	Meri stem	Sepal	Petal	Fila	Pol and tube	Carp	Trans tis	Endo	Ovule	Emb	Dev seed sil
ACT7	+++	+++	+++	+++	+	+++	+	++	+++	++	++	+++	+/–[c]						
ACT2	+++		+/–		+++	+++	+++	+++	+++	+++	+++	+++	+/–[d]						
ACT8	+/–			++	+++	++	++	++	++				+/–[d]						
ACT11			+[b]			+		+/–	++				++	+++	+++	+++	+++	+++	+++
ACT1						+		+/–	++				+++	+			++[e]	+[e]	
ACT3						+		+/–	++				++	+			++[e]	+[e]	
ACT4								+/–					++						
ACT12						+/–		+/–					++						

[a]Names and abbreviations of organs and tissues where actins are expressed: Carp, developing carpel; Cotyl, cotyledon; Dev seed sil, developing seed and silique; Emb, embryo; Endo, endosperm; Fila, filament at base of anther; Ger seed, germinating seed; Hypo, hypocotyl; Leaf, young and/or mature leaves; Meristem, floral or vegetative meristem; Ovule, young developing ovules; Petal; Pol and tube, mature pollen and pollen tube; Root tip; Root cort epid, root cortical and epidermal tissue; Sepal; Trans tis, transmittal tissue; Vas cyl, vascular cylinder.
[b]*ACT11* is strongly expressed in etiolated hypocotyls[19].
[c]*ACT7* is expressed for a 24 h period late in pollen development[21].
[d]*ACT2* and *ACT8* mRNA is detected but their promoter–reporter fusions are not expressed in transgenic plants[18].
[e]*ACT1* and *ACT3* reporter fusions are expressed in ovules of transgenic plants (Ref. 21 and A.V. Vitale and R.B. Meagher, unpublished).

By contrast, reproductive actins are characterized by expression in pollen, ovules, and/or seeds (Fig. 3e–k). For example, all five reproductive actins, *ACT1*, *ACT3*, *ACT4*, *ACT11* and *ACT12*, are strongly expressed in mature pollen (summarized in Fig. 2, Table 2)[17,19,20]. *ACT1*, *ACT3* and *ACT11* are expressed in developing floral meristems and the emerging carpel and ovules[19] (A. Vitale and R.B. Meagher, unpublished). *ACT11* predominates during embryo and seed development. Little or no reproductive-gene expression is detected in vegetative organs, such as root, stems, leaves, sepals and petals. Each actin gene shows significant tissue variability in steady-state RNA levels, ranging from eightfold for *ACT2* to 5000-fold for *ACT1*. There are a few exceptions to the distinct separation into vegetative and reproductive patterns (Table 2). For example, some of the vegetative actins are expressed early in pollen and ovule development and all five reproductive actins are expressed at low levels in vascular tissue development.

Other vegetative and reproductive classes of cytoskeletal genes

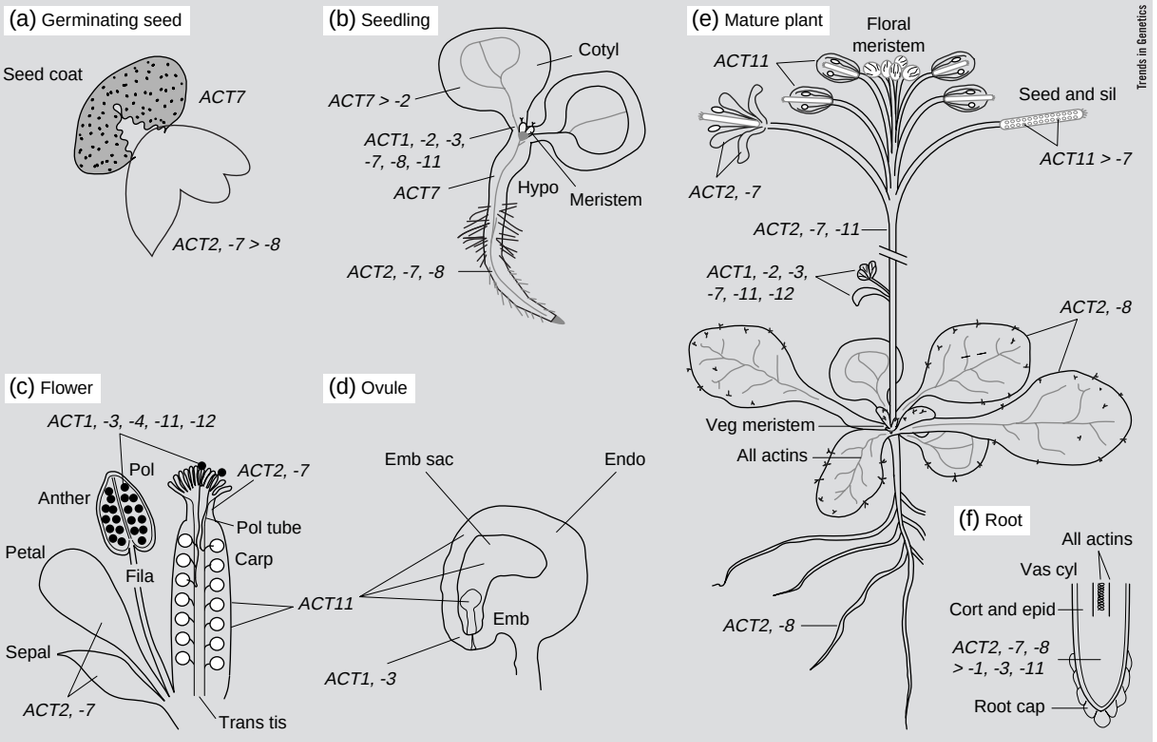
Dozens of interacting proteins are required for the plant cytoskeleton to function properly. Thus, it would not be surprising to find that genes that encode additional cytoskeletal proteins co-evolved with the actins. In fact, in the few cases where gene families for non-actin plant cytoskeletal proteins have been described thoroughly, vegetative and reproductive gene classes are emerging. Relatively detailed descriptions of expression patterns are available for the proflins, actin depolymerizing factors (ADFs) and the α- and β-tubulins (see Table 1). Profilin is a major actin-binding protein with a variety of effects on the actin cytoskeleton, including sequestration of monomers, addition of monomers to the barbed end of actin filaments and nucleotide exchange. The profilin gene family in *Arabidopsis* appears to be as large as, and even more diverse in sequence than, the *Arabidopsis* actin family[23,24]. Like actins, plant profilins can be placed into either vegetative or reproductive classes, based on sequence and expression patterns (Fig. 1b; Table 1). *PRF1*, *PRF2* and *PRF3* are expressed in most vegetative tissues, while *PRF4* and *PRF5* are expressed strongly in pollen. Detailed examination of the tissue-specific expression of promoter–reporter fusions from one vegetative (*PRF2*) and one reproductive (*PRF4*) profilin show patterns that are quite similar to their possible actin counterparts, *ACT2* and *ACT1*, respectively[24]. Maize profilins have also been reported that are closely related in sequence to the *Arabidopsis* reproductive sequences (Table 1), and these maize sequences are also specifically expressed in pollen[23,25] and ovules[26]. These observations have led us to suggest that the evolution of the vegetative and reproductive classes of actins and profilins was concordant[23].

In fact, to date, all well-characterized cytoskeletal-protein gene families have vegetative and reproductive members. The three plant ADFs, whose transcript levels have been carefully assayed, are easily distinguished by strong expression, either in reproductive or vegetative tissues (Table 1). The members of the *Arabidopsis* α- and β-tubulin gene families can each be divided easily into vegetative and reproductive classes (Table 1) based both on expression patterns and on sequence relatedness. Thus, from these data, one might anticipate that the plant gene families that encode many other cytoskeleton-related proteins that interact with the actin- and tubulin-based systems directly, or that signal changes in cytoskeletal structures, will all have members that separate into vegetative and reproductive classes. Indeed, the hundreds of cytoskeletal genes already found in animals, protists and fungi[27] suggest that similar numbers of cytoskeletal-related genes and gene families are yet to be discovered in plants. When the possible transacting regulatory proteins and signal-transduction proteins[28] that must control the differential expression of these cytoskeletal proteins are taken into account, thousands of plant genes might be split between ancient vegetative and reproductive classes.

Role of the actin cytoskeleton in directing plant development

The plant actin cytoskeleton is required to direct numerous subcellular functions and the development of complex

FIGURE 2. Summary of actin expression patterns

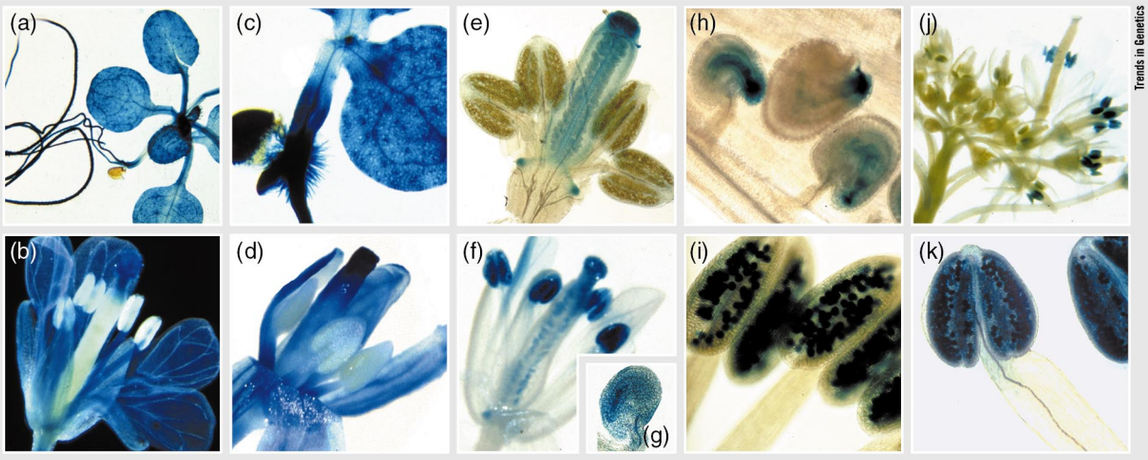


The two ancient classes of *Arabidopsis* actin-encoding genes are distinguished easily based on vegetative and reproductive expression patterns. Vegetative actins (*ACT2*, *ACT7*, *ACT8*) predominate in germinating seeds (a), seedlings (b), mature plants (e) and root tips (f); whereas reproductive actins (*ACT1*, *ACT3*, *ACT4*, *ACT11*, *ACT12*) predominate in the ovule (d) and in pollen sacs in the flower (c). A few patterns of expression for each gene class do not fit neatly into these categories. For example, vegetative actins (*ACT2*, *ACT7*) are strongly expressed in parts of the floral organ complex, such as sepals, petals, stigma and style (c, e). Three reproductive actins (*ACT1*, *ACT3*, *ACT11*) are expressed in meristems (b, e). All eight actins are expressed at some level during vascular tissue development (e, f). A summary of these data and the abbreviations used is presented in Table 2.

organ structures. The different plant processes with demonstrated or proposed roles for actins include the following: (1) establishing cell polarity[29]; (2) determining the location of the division plane (by positioning the preprophase band); (3) preprogramming of development and cell wall deposition; (4) cell elongation; (5) tip growth (pollen tubes); (6) positioning of receptors and transmembrane transport; (7) transporting mRNAs within the cell; (8) streaming of cytoplasm; and (9) orienting chloroplasts to light and positioning of the nucleus[11,30–32]. Because cell death and migration do not play a significant role in plant development, plant organ structure depends primarily upon the control of periclinal and anticlinal cell divisions in the apical meristem[33,34], and the subsequent expansion and maturation of these cells in the developing organ. Thus, the first four of these actin functions (establishing polarity, determining the plane of division, cell elongation and directing cell-wall deposition) are critical to all of plant development and morphology. As an alternative view, positional information in the cell provides spatial cues that lead to organ shape[35,36]. Based on this view of development, polarity maintained in parent cells by the actin cytoskeleton is paramount and the other three actin-mediated processes (e.g. cell division, elongation and wall formation) follow. It would not be surprising if these two interpretations represent partially redundant mechanisms of plant development in which the determination of the plane and the polarity of cell division are both capable of some independent control. The role for the tubulin-based cytoskeletal system in these processes is similarly complex. Thus, the development of vegetative and reproductive structures in plants requires the expression of multiple actin and tubulin genes, and an enormous cadre of genes encoding binding and signal proteins that act in concert.

Based on patterns and timing of expression, some predictions can be made about which actins might program the development of particular vegetative and reproductive structures. For example, strong expression of two vegetative actins, *ACT2* and *ACT7*, in germinating seedlings and in the expanding apical and root meristems might help to program the development of cotyledons, leaves and roots (Table 2, Figs 2, 3). Five genes, *ACT1*, *ACT3*, *ACT4*, *ACT11* and *ACT12*, are expressed strongly in mature pollen and probably direct the growth of the pollen tube. Insertion mutations in *Arabidopsis* actin genes have been identified using a sequence-based screening procedure[37] that avoids making assumptions about expected phenotypes. Starting with a heterozygous parent plant, multigenerational studies on *ACT2*, *ACT4* and *ACT7* mutants demonstrate that each mutation is extremely deleterious to the survival of *Arabidopsis* (Refs 38, 39). When plants are grown and seeds are harvested as a population, it is already possible to measure the loss of the mutant allele in each population by the T_2 generation. Thus, these three

FIGURE 3. Histochemical localization of actin–reporter fusions



Fusions of actin and β-glucuronidase (GUS) reporter, expressed in transgenic *Arabidopsis* plants, reveal detailed differential regulation that distinguishes the vegetative and reproductive subclasses of genes. The 5′ flanking regions (i.e. the promoter, an exon encoding most of the 5′UTR, an intron in the 5′UTR, and a portion of the first exon encoding the first 19 codons) of each actin gene were coupled to a reporter gene encoding GUS, and transformed into *Arabidopsis*. These data represent the expression patterns observed in over 90% of the 11–20 independent transgenic plant lines examined for each construct. Each column of frames represents the expression pattern for one of the five *Arabidopsis* actin subclasses. (a, b) *ACT2–GUS*. The reporter fusion for the other member of this subclass, *ACT8–GUS* (not shown), shows weaker and less constitutive expression than *ACT2–GUS*, particularly in flowers, where *ACT8–GUS* is not significantly expressed. (a) A four-week-old plant shows strong expression in all tissues. (b) A stage 14 flower shows strong expression in sepals, petals, filaments, stigma and style, but no staining in the anther or ovary. (c, d) *ACT7–GUS*. (c) A five-day-old seedling, showing staining in the seed coat, root, hypocotyl and cotyledons; (d) a stage 12 flower shows staining in sepals, petals, filaments, stigma and style, but no staining in the anther or ovary, similar to that observed for *ACT2–GUS* fusions. (e, g) *ACT11–GUS*. (e) A stage 12 flower, showing strong expression in all tissues of the carpel, including the ovules. (f) A stage 14 flower shows strong expression in mature pollen and ovules. (g) A mature, fertilized ovule showing strong expression in all tissues. (h, i) *ACT1–GUS* fusion. Data shown for *ACT1–GUS* fusions accurately resembles the pattern obtained for *ACT3–GUS* fusions. (h) Fertilized ovules, showing strong expression in the embryo sacs and developing embryos. (i) Strong expression of *GUS* in the mature pollen of dehiscing anthers. (j, k) *ACT12–GUS* and *ACT4–GUS*. (j) Inflorescence of an *ACT12–GUS* fusion showing expression in mature anthers. (k) Staining of mature pollen in anthers of an *ACT4–GUS* transformant. The pollen-specific expression of *ACT4* and *ACT12* is indistinguishable.

genes and perhaps all eight expressed actins are required individually for normal growth and development. A more detailed analysis on the kinetics of loss of the *ACT2* mutant allele from these populations revealed that the deleterious effects are on the viability or fertility of the plant, as expected for a vegetative gene, and are not from lower fitness of mutant gametes. Under the right growth conditions, *ACT2* and *ACT7* mutants exhibit severe root phenotypes (L. Gilliland and R.B. Meagher, unpublished). Finally, the co-expression of multiple actin isovariants enables some extremes in the dynamic behavior of the actin cytoskeleton that might play additional roles in plant development[16]. A continued analysis of mutants should help to define the roles for each of the various actins and other cytoskeletal genes in the development of vegetative and reproductive structures.

A link between the evolution of cytoskeletal genes and the evolution of organs

The ancient classes and subclasses of plant actins were evolving during the same period as the rapid emergence of numerous tissues and organs in vascular plants[2,4]. In particular, the separation of vegetative leaf-like organs from reproductive structures was an early landmark macroevolutionary event that distinguished land plants from their algal ancestors[3,40]. Based on molecular evolutionary data on extant species and geological dating of fossil plant species, this event probably occurred some 400 million years ago. From the phylogenetic relationships of these species it is argued that leaves of some early vascular plants, like the lycopsids, were derived from sporangia (spore-bearing organs on seedless vascular plants) that were sterilized and altered for more efficient photosynthesis. The apical meristems of most members of Lycopodophyta can give rise both to reproductive structures and to leaves, as they can in other more recently evolved plant phyla (e.g. Equisetophyta, Pteridophyta, Cycadophyta)[41,42]. The apical meristems of plants in more ancient phyla (e.g. Bryophyta, Psilophyta) develop primarily into reproductive structures. A developmental subroutine in the apical meristem that produces leaf-like structures requires not only complex signaling and cell–cell communication[33] but also changes in the cytoskeleton to execute this program. The split between the two ancient classes of vegetative and reproductive actins occurred during the same time period that this new developmental pathway, which gives rise to leaves, split from that for sporangia. Hence, we are proposing that the molecular evolution of plant cytoskeletal-gene regulation and the macroevolution of separate vegetative and reproductive organs were contingent upon one another. The mechanisms of this linkage or contingency between macroevolution and gene evolution have been discussed elsewhere[1].

Origin of reproductive and vegetative cytoskeletal genes

The ready availability of extra gene copies increases the chance of accumulating the regulatory mutations that are necessary in order to link a gene to the macroevolution of new organ structures. As leaves and reproductive structures evolved from a common ancestral organ type, extra

actin-encoding gene copies (and genes encoding other cytoskeletal proteins) acquired the appropriate changes for novel expression patterns and functions. It is widely recognized that gene duplication plays a central role in evolution[43] and it must have been an essential early event in this global split in the regulation of actin and other cytoskeletal gene families. Extra gene copies are less constrained against mutation and more easily altered to perform new functions, enabling pre-existing and appropriately regulated genes to survive undamaged. Tandem duplications and local endo-redundancy[14,44,45], nomadic duplications[22,46], and gene duplications resulting from increases in ploidy[47–49] all occur frequently in higher plants. In angiosperms, these duplication mechanisms have undoubtedly contributed to the high frequency of gene families[16] and the enormous variation in genome sizes[50]. Relatively recent increases in ploidy probably generated several closely related pairs of actin genes in soybean[15,51] and *Arabidopsis* (Refs 12, 22) and tandem duplications gave rise to dozens of petunia actin genes[45,52]. Thus, it is likely that extra actin-encoding gene copies were available to participate in the macroevolution of vegetative and reproductive organs in many ancient plant species.

Future directions

Genome- and cDNA-sequencing projects and transcriptional profiling[53,54] will very shortly identify hundreds of plant gene families and separate their members based on patterns of regulation. We would predict that hundreds of gene families with vegetative and reproductive gene classes will be uncovered. When groups of genes like the vegetative and reproductive actins have distinct spatial and temporal expression patterns that are conserved over long evolutionary time periods, it is reasonable to argue that selective constraint has preserved the gene family. Presumably, different gene copies are needed to express novel protein isovariants and/or needed for extremes in gene regulation in particular organs and tissues. However, the selective-constraint model does not alone explain how complex cell, tissue and organ structures evolved in concert with the necessary newly regulated sets of genes in the first place. Perhaps one of the most biologically meaningful approaches to understanding the origin of these global expression patterns will be to link gene-family phylogeny and expression patterns to the macroevolutionary origins of the organs, tissues and cellular phenomena that they support[1]. Analysis of mutants and ectopic gene-expression experiments[55] can be used to test if the regulation of these genes, or expression of particular protein isovariants, or both, are essential to appropriate organ development and plant viability. If the hypothesis linking the emergence of vegetative and reproductive organs with corresponding cytoskeletal-gene family members is robust, quantitative molecular phylogenies of many gene families will identify classes of genes similar in age to the two actin classes. Dissecting the contingent relationships between the evolution of global patterns of gene regulation and the macroevolution of organ structures should deepen our understanding of the genome and development.

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Making effective use of human genomic sequence data

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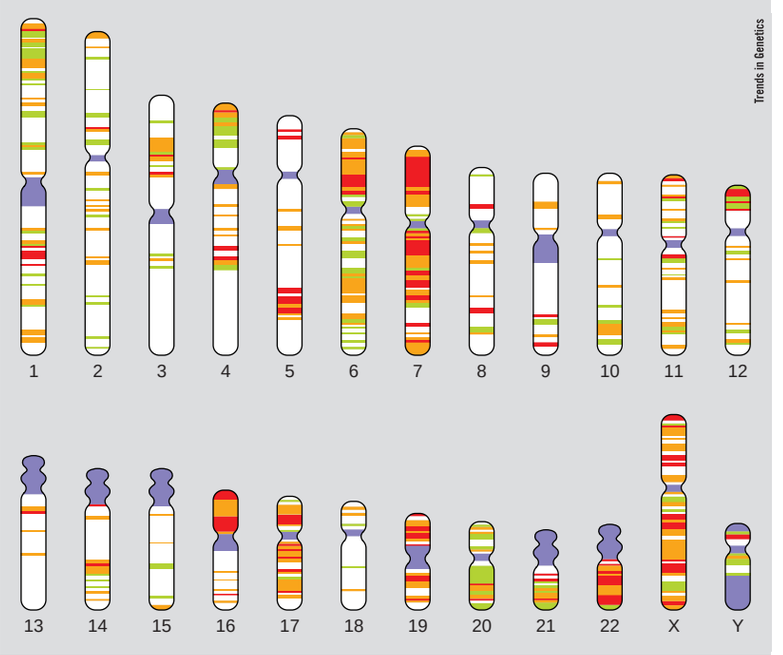
The Human Genome Project has completed a successful three-year pilot project that has resulted in 280 875 kb of accurate, non-redundant human genomic sequence data being deposited in the public database (about 9% of the genome, shown graphically in Fig. 1). Recently, it has been announced that, as a first step towards accelerated completion of the genome, a 'working draft' sequence will be produced early in 2000 that will account for about 90% of the approximately three billion total bases[1]. This strategy might be likened to the authoring of a book – an initial working draft will be produced, followed by cycles of revisions in which details are added and errors corrected, ultimately leading to the final reference work. While it will be interesting to watch the developments in this fast-paced field over the next year or two, the practical benefit for most of us is the fact that huge amounts of DNA sequence will be pouring into the public databases. It is important for researchers to have early access to the genome because of its tremendous potential for accelerating biomedical research. We have developed an Internet resource (www.ncbi.nlm.nih.gov/genome/seq) to help make effective use of these data, by providing a simple means of browsing and searching of the data, together with background

FIGURE 1. Progress in human genome sequencing



Images of the 24 human chromosomes have been subdivided into segments of ~1 Mb in size and shaded to indicate available sequence data (at the end of March 1999). Additionally, completed regions are given different shades of orange and red to indicate the degree of continuity. Sequence data that are only of draft quality are shown in a contrasting light-green shade. Sequences were placed into segments based on sequence-tagged site (STS) content and combined radiation hybrid map data that has been converted to a uniform base-pair scale.