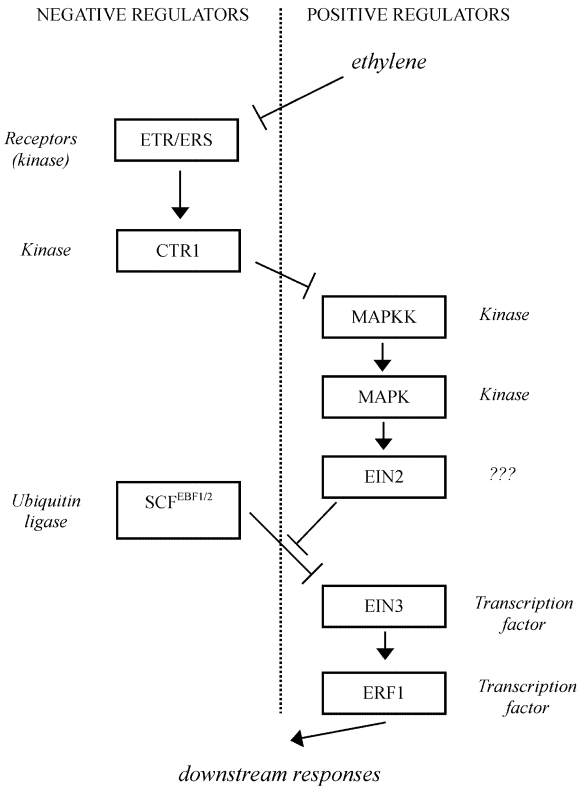

Figure 1. The Basics of Ethylene Signaling

Ethylene acts through a series of both positive and negative regulators to influence gene expression. Ethylene inactivates the ETR/ERS family of ethylene receptors, releasing the repression of downstream components which contribute to the accumulation of the transcription factor EIN3 (see text). In the absence of ethylene, EIN3 is constitutively degraded via a novel component, $SCF^{EBF1/2}$.

they are further elevated by ethylene treatment. This indicates the existence of a new CTR1-independent section of the pathway.

These discoveries further strengthen two, now fully emerged themes in plant signaling: the prevalence of negative regulation and of regulated protein degradation. There are more than 700 F-box proteins in *Arabidopsis* (Risseeuw et al., 2003), many more than in similarly complex organisms from other kingdoms. Why so many? Of course it is far from certain that these will all be involved in proteolysis, yet it seems likely that many of these 700 are involved in the imposition of, or release from, negative regulation though degradation of regulatory proteins.

This seems evolutionarily counter-intuitive because it is apparently so energetically wasteful. While it is always impertinent to question evolution, it prompts consideration of why negative regulation through protein destruction is so pervasive in higher plant signaling. Where many animals can literally side-step adverse environmental fluctuations by shuffling off to a more favorable elsewhere, the sessile plant must endure. It is therefore essential to their fitness that they respond to changing conditions quickly and quantitatively. Negative regulation allows this, and since plants are less energy-limited than animals, it would appear to be an evolutionarily affordable compromise. Thus, although upon casual observation plants look as though they are not doing very much, within, innumerable pathways are working overtime to keep things as they are, while maintaining a constant state of readiness: not quite as passive as they look.

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Another Cytoskeleton in the Closet

Many eukaryotic cells contain up to three families of cytoskeletal proteins that are responsible for the spatial organization of the cell. Although the prokaryotic origins of the actin and tubulin families have now been established, the origin of the third was unknown. In this issue of *Cell*, Ausmees et al. (2003) provide evidence that the third family, comprising the intermediate filaments, also has origins in bacteria and is responsible for producing curved cells.

Most eukaryotic cells (animal but not plant cells) contain three cytoskeletal filament systems that are involved in spatial organization of the cell. The three types were originally differentiated by the diameter of the filament and include thick microtubules (25 nm), thin actin filaments (7 nm), and intermediate filaments (10–12 nm)

(Alberts et al., 2002). The bacterial origin of the proteins that constitute microtubules and actin filaments has been well established in the past few years (van den Ent et al., 2001). This took some time, as the limited amino acid identity between the prokaryotic and eukaryotic counterparts needed structural information for confirmation of inferences about ancestry based on functional similarities. In this issue of *Cell*, Ausmees et al. (2003) provide very strong evidence that intermediate filaments, the least-studied filament system, also have a bacterial origin, and that these filaments are used by a limited number of bacteria to produce cells with a curved morphology.

Bacteria come in an array of shapes, and this feature has been a useful phenotypic trait in classification. Why bacteria come in these different shapes is not clear, but we are beginning to understand the factors that determine the shape of bacterial cells. Most bacteria are surrounded by a rigid cell wall (peptidolglycan) that retains the shape of the bacterium when it is isolated, leading to the idea that the shape of the bacterium is dictated by the enzyme systems that lay down new cell wall (Young, 2003). In the most well-studied rod-shaped bacteria, there are two complex morphogenetic systems composed of peptidoglycan biosynthetic enzymes and other assorted proteins. One system dictates the rod shape and the other is required for cell division. Work over the past few years indicates that these two systems for laying down new cell wall track along cytoskeletal filaments comprised of the ancestors of the proteins that constitute the actin (bacterial MreB) and microtubule filaments (bacterial FtsZ).

Cell division of rod-shaped bacteria requires the reorientation of cell wall synthesis from diffuse insertion along the cylindrical wall to centripetal insertion at the division site resulting in formation of a septum. This is accomplished by the Z ring assembled from the ancestral tubulin homolog FtsZ, which recruits the components of the septal peptidoglycan complex (Errington, 2003). Even the diffuse lateral insertion in the cylindrical wall is more organized than first thought. By using a fluorescent derivative of the antibiotic vancomycin, which labels nascent cell wall material, a helical mode of insertion that resembles the helical filaments formed by actin homologs of the MreB family was revealed, raising the possibility that the lateral synthetic machinery is linked to filaments of the MreB family (Jones et al., 2001; Daniel and Errington, 2003). Interestingly, coccal-shaped bacteria lack MreB family members and rely on the septal mode afforded by the Z ring. However, when FtsZ is removed, these cells go to a dispersive mode of growth, which increases the cell volume but ultimately leads to cell lysis (Pinho and Errington, 2003).

However, some bacteria reject simple geometric shapes for a more curved morphology. What is responsible? A few years ago, it was shown that the spiral shape of a spirochete, *Borrelia burgdorferi*, was due to flagella located in the periplasmic space between the cytoplasmic and outer membranes (Motaleb et al., 2000). Inactivation of the major flagellin gene resulted in the organism defaulting from spiral to rod morphology. However, many other bacteria are curved and lack a periplasmic flagellum, indicating something else is responsible.

Screening transposon insertion libraries of the naturally curved bacterium *Caulobacter crescentus*, Ausmees et al. (2003) found mutants that had lost their crescent shape and had assumed the more common rod shape. These mutants also prevented *C. crescentus* cells from assuming a more extended helical shape seen after a prolonged period in stationary phase. Tracking down the locations of the transposons led to an ORF encoding a predicted protein, designated crescentin, consisting mostly of heptad repeats, suggesting that the protein had mostly a coiled coil structure. Although the coiled coil is a widespread structural motif and therefore of limited use in blast searches, it led the investigators to examine the possibility that crescentin was a component of a cytoskeletal system. Immunofluorescence microscopy revealed a dramatic result: a single filamentous structure along the concave side of the cell and along the shortest helical path in the stationary phase induced helical bacteria. A GFP fusion was unable to complement the insertions, but behaved as a dominant negative when introduced into a wild-type, disrupting the membrane localization of the filament and producing rod-shaped bacteria. This result argued that the proper localization of the filament was required for the curved morphology.

The localization results led to a further examination of the amino acid sequence of crescentin and comparison to intermediate filament proteins. Intermediate filament proteins have been divided into 5 families: 4 cytoplasmic types and the nuclear lamin family (Strelkov et al., 2003). The common features include an extended, central rod domain composed of the coiled coil with characteristically positioned interruptions flanked by globular domains. Crescentin has $\sim$25% identity to cytokeratin and nuclear lamin A largely due to the heptad repeat pattern. However, the common domain principles shared by the eukaryotic intermediate filament proteins are also present in crescentin. These similarities led to in vitro studies to examine assembly of crescentin. The conditions for in vitro assembly of intermediate filament proteins are quite different than for actin or tubulin family proteins. These latter proteins are globular and undergo nucleotide-dependent assembly to form polar filaments under physiological conditions. In contrast, intermediate filaments are solubilized and purified by using strong denaturing agents and are assembled into nonpolar filaments without nucleotide by dialysis to remove the denaturant. Following the established protocols for intermediate filaments, Ausmees et al. found that crescentin behaved similarly and produced 10 nm filaments similar to intermediate filaments. These results led to the conclusion that crescentin filaments are responsible for inducing the curved morphology of *Caulobacter crescentus* cells. This study further begs the question why some bacteria are curved. There must be some selective advantage, and the authors speculate that the shape has something to do with *Caulobacter*'s wandering lifestyle in dilute aquatic environments in which the cell's geometry may aide in sensing the environment. Also, how crescentin filaments effect curvature of the cell remains to be determined; however, it is likely to be a

general mechanism, as other curved bacteria, such as *Helicobacter pylori*, contain similar proteins.

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Coupled Folding during Translation Initiation

The structure of the eukaryotic initiation factor eIF4E bound to a cognate domain of eIF4G and m^{7}GDP in this issue of *Cell* shows that these factors undergo coupled folding to form a stable complex with high cap binding activity that promotes efficient ribosomal attachment to mRNA during translation initiation.

An early step in the initiation of translation on most eukaryotic mRNAs is the attachment of a 43S preinitiation complex, comprising a 40S ribosomal subunit, initiator tRNA, and eukaryotic initiation factors (eIFs) 1, 1A, 2, and 3 to the 5′ end of mRNA. The complex then scans downstream to the initiation codon. Eukaryotic mRNAs have a 5′ terminal "cap" structure (m^{7}G[5′]ppp[5′]N, where N is any nucleotide) and a 3′ poly(A) tail that synergistically enhance ribosomal recruitment to mRNA.

Ribosomal attachment begins with recognition of the cap by the 25 kDa subunit (eIF4E) of the cap binding complex eIF4F, which also contains eIF4A and eIF4G subunits. eIF4G has binding sites for eIF4E, eIF4A, eIF3, the poly(A) binding protein PABP, and RNA. Mammalian eIF4G is larger than its yeast counterpart, and has additional carboxy-terminal residues that bind Mnk protein kinases and contain a second eIF4A binding site (Figure 1). eIF4A is an ATP-dependent helicase that, as part of eIF4F, unwinds the cap-proximal region of mRNA. Association of eIF4E with eIF4G, which directs the helicase activity of eIF4A to the cap-proximal region of mRNA, is essential for initiation. mRNA unwinding and binding of eIF4G to the eIF3 component of the 43S complex allow ribosomal attachment to mRNA.

The activity of eIF4F is a focal point for the regulation of protein synthesis (Gingras et al., 1999). Mammalian eIF4E binding proteins (4E-BPs) and yeast p20 inhibit cap-dependent initiation by blocking the interaction of eIF4G with eIF4E. Inhibition by 4E-BP is related to its phosphorylation state, so that hyperphosphorylated 4E-BP has the lowest affinity for eIF4E. Mammalian eIF4E also undergoes regulated phosphorylation by the eIF4G-associated Mnk kinases. Phosphorylation of eIF4E is increased in response to stimuli that activate translation. Its significance for eIF4E's function is a topic of current interest (Scheper and Proud, 2002).

The structures of mammalian and yeast eIF4E bound to the cap analogs m^{7}GDP, m^{7}GTP, and m^{7}GpppA are similar (Marcotrigiano et al., 1997; Matsuo et al., 1997). In these structures, eIF4E consists of an unstructured N terminus and an antiparallel β sheet backed by three helices on its convex surface. Binding of cap analogs to a hydrophobic slot on eIF4E's concave surface is stabilized by interactions that include π-π stacking of the m^{7}G base between tryptophan residues, a hydrogen-bonding network that fixes the triphosphate moiety, and additional interactions with the second RNA base. A conserved Tyr-X-X-X-X-Leu-ϕ recognition motif (where ϕ is Leu, Met, or Phe) in eIF4G binds to a phylogenetically invariant site on the convex surface of eIF4E; 4E-BPs contain the same motif and bind to the same site on eIF4E, thereby preventing eIF4E-eIF4G interaction (Marcotrigiano et al., 1999). 4E-BP and eIF4G peptides containing this recognition motif are disordered but assume a helical conformation when they bind to eIF4E, whereas the structure of eIF4E remains unaltered (Marcotrigiano et al., 1999).

Biochemical data suggested that association of eIF4G with eIF4E significantly enhances eIF4E's affinity for the cap (Haghighat and Sonenberg, 1997; Ptushkina et al., 1998). However, interaction of eIF4E with an eIF4G-peptide containing this recognition motif did not enhance eIF4E's cap binding activity, whereas a larger yeast eIF4G fragment containing this motif did (von der Haar et al., 2000). Moreover, mutations outside this motif influenced eIF4G's interaction with eIF4E (Hershey et al., 1999). The observed enhanced affinity of eIF4E for the cap and stabilization of the interaction would significantly increase the efficiency of attachment of 43S complexes to capped mRNAs.

How the interaction between eIF4G and eIF4E led to the enhanced association of eIF4E with the cap remained unknown. Now, Gross et al. (2003) in this issue of *Cell* report the solution structure of yeast eIF4E/cap bound to eIF4G (393–490), which shows that eIF4E's interaction with eIF4G is not limited to the recognition motif but extends over a large (4400 Å^2) interface area. Protein binding results in coupled folding of part of the previously unstructured N terminus of eIF4E (amino acid residues 23–38) to form a "wrist" and a protruding "fist" on the convex surface of eIF4E, and of eIF4G (393–490), which forms a "bracelet" of five helices (with the recognition motif in helix α4) that encircles the "wrist." Kinetic analyses indicated that the initial binding of eIF4E to the