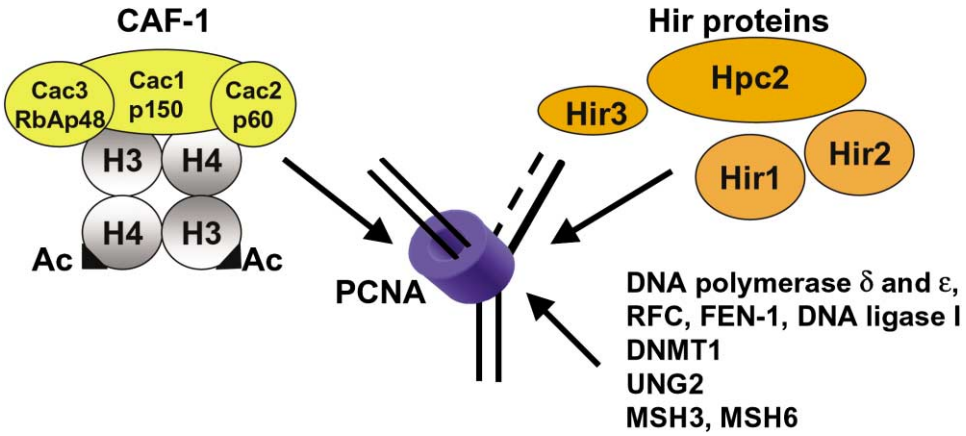

Two Parallel Pathways for Nucleosome Assembly Converge on PCNA at the Replication Fork

The names of the CAF-1 subunits in *S. cerevisiae* and human cells are shown. Yeast Hir1 and Hir2 are related to human HIRA. Many replication proteins (DNA polymerases, FEN-1, RF-C, ligase I) and enzymes involved in maintenance of DNA methylation (DNMT1), uracil excision repair (UNG2), and mismatch repair (MSH3 and MSH6) also contain PCNA binding motifs similar to that of CAF-1.

may also influence the conversion of stalled replication forks into damaged DNA.

The discovery that histone deposition must occur rapidly behind the fork has important ramifications. It is known that histone overexpression has deleterious effects on mitotic chromosome segregation (Meeks-Wagner and Hartwell, 1986) and that inhibition of DNA replication leads to disappearance of histone mRNAs. This implies that cells maintain a very delicate balance between histone and DNA synthesis. This is not a trivial task given that replication origin usage and S phase duration can vary dramatically among cell types. For example, some embryonic cells and activated B cells can replicate their DNA at a furious pace. In addition, most cell types are likely to experience a drastic decline in total rates of DNA synthesis as they progress from early S phase (when many replication forks are active) to late S phase (when fewer replication forks are active). The problem is compounded by the fact that many forms of DNA damage are known to slow down rates of elongation and prevent initiation of new replication forks (Tercero and Diffley, 2001). How cells balance histone and DNA synthesis in response to such abrupt, but accidental, changes in rates of DNA replication is not known. Future research will no doubt reveal a wealth of regulatory mechanisms that enable cells to maintain an optimal balance between histone and DNA synthesis during both normal S phase progression and in response to DNA damage.

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Plant Defense: One Post, Multiple Guards?!

Arabidopsis **RIN4 is a key bacterial virulence target that is guarded by the resistance (R) protein RPM1. Two recent studies suggest that another R protein, RPS2, also guards RIN4. Bacterial avirulence (Avr) effectors AvrB, AvrRpm1, and AvrRpt2 alter this key protein. R proteins RPM1 and RPS2 recognize the altered status and initiate a defense-signaling response. The guard hypothesis is in!**

The recognition of pathogen-derived avirulence (Avr) effectors by plant resistance (R) proteins triggers a defense response in the host that often results in rapid

cell death, the hypersensitive response (HR). This R-Avr interaction forms the basis of plant innate immunity. If either of the pair is missing then disease occurs.

The exact nature of the R-Avr interaction leading to recognition of pathogens is a mystery. The basic assumption is that R proteins behave like receptors for the effector ligands. Structural features of the R proteins support this model, as a majority of the R proteins have well-conserved leucine rich repeat (LRR) domains (Dangl and Jones, 2001). LRR domains are usually involved in mediating protein-protein interactions, and various studies indicate that the pathogen specificity resides in the LRR domain. Some specificity is also defined outside of the LRR domains. However, a direct physical interaction between LRR-containing R proteins and corresponding Avr effectors has only been shown for the rice protein Pita and the corresponding AvrPita of the rice blast fungus *Magnaporthe grisea* (Jia et al., 2000). Attempts with dozens of other similar R-Avr pairs have failed to identify such an interaction.

In light of such observations, the original receptor-ligand model was amended to add a new dimension to the R-Avr interaction. The R protein has been assigned the role of a sentinel of cellular machinery, guarding key virulence targets inside the cell (Van der Biezen and Jones, 1998; Dangl and Jones, 2001). This "guard hypothesis" proposes that Avr products interact with and modify non-R cellular factors. The R protein perceives the altered status of the virulence target and induces a rapid defense response.

The recent work of Mackey and colleagues (2002) provided support for the guard hypothesis. They identified RIN4, a protein that interacts with two *Pseudomonas syringae* effectors AvrB and AvrRpm1 and the R protein RPM1. Basal defense was elevated in plants with reduced expression of RIN4. AvrB and AvrRpm1 induced hyperphosphorylation of RIN4, possibly further suppressing basal defense. This latter presumption is based on the idea that *Avr* genes are maintained in the genomes of pathogens because they increase the pathogen's fitness by enhancing its virulence in a susceptible host. RPM1-mediated defense response was dependent on RIN4. Thus, it was proposed that the RPM1 protein monitored RIN4 activity and upon RIN4 phosphorylation triggered a defense response.

Two papers published in the February issue of *Cell* (Mackey et al., 2003; Axtell and Staskawicz, 2003) provide evidence that RIN4 is also the point at which another R protein RPS2 induces disease resistance against *P. syringae* effector AvrRpt2. The two groups report that infiltration of bacteria expressing AvrRpt2 into *Arabidopsis* leaves or expression of AvrRpt2 as a stable transgene causes the disappearance of RIN4 protein and that this occurs independent of the RPS2 locus.

AvrRpt2 is known to interfere with the RPM1-mediated resistance against the AvrRpm1 (Ritter and Dangl, 1996). When RPS2/RPM1 *Arabidopsis* plants are infiltrated with AvrRpm1 and AvrRpt2, only RPS2-specific responses are exhibited, while RPM1-mediated resistance is inhibited. A simple explanation for this observation, based on the above results, could be that RPM1 monitors phosphorylation of RIN4 (Mackey et al., 2002), but its function is inhibited when AvrRpt2 eliminates RIN4.

Though RPS2 responds to AvrRpt2, there is no physical interaction reported between them. Could RPS2 recognize AvrRpt2 by monitoring the disappearance of RIN4? When RIN4 was overexpressed in transgenic plants, RPS2 failed to respond to AvrRpt2 infiltrations and even RPM1 inhibition by AvrRpt2 was not seen (Mackey et al., 2003). The importance of RIN4 disappearance for RPS2 signaling is also established using a genetic approach. The authors report that the *rin4* null mutant is seedling lethal. This is perhaps because *rin4* mutations mimic infection by AvrRpt2 and trigger defense pathways early in development. This logic also agrees with the previous report (Mackey et al., 2002) that RIN4 antisense lines are constitutively induced for basal defense. If that is the case, inhibiting RPS2 should rescue *rin4* mutants. Indeed, a double mutant *rin4/rps2* is viable (Mackey et al., 2003). These experiments prove that RPS2 monitors and responds to the disappearance of RIN4 and not directly to AvrRpt2.

The conclusion is also supported by the demonstration of direct physical interactions in planta between RPS2 and RIN4 (Axtell and Staskawicz, 2003; Mackey et al., 2003). One point of contention was earlier reports that indicated RPS2 is a soluble protein while RIN4 is a membrane protein. Axtell and Staskawicz (2003), however, have empirically determined that RPS2 and AvrRpt2 are membrane-associated proteins. Since RPM1 is also a membrane protein, these results complement the model where RPM1 and RPS2 monitor RIN4 status in order to activate defense responses. It also indicates that recognition of bacteria takes place on the surface of the plasma membrane.

These two papers have extended the role of RIN4 to another *R* gene, suggesting that elements like RIN4 may be under surveillance by multiple R proteins. Molecules like RIN4 may play key roles in basal defense and are likely targets of multiple effector proteins. During the RPM1-mediated resistance response, RIN4 is phosphorylated and RPM1 is degraded while during the RPS2 interaction RIN4 is degraded and RPS2 is stable. Determining the mechanism and regulation of RIN4 and RPM1 degradation will be an important advance in the field and will shed light on other roles of protein degradation in disease resistance. A search of EST databases revealed that RIN4 is also present in soybean, tomato, and potato, indicating that it is a well-conserved protein. The bacterial effectors AvrB and AvrRpt2 also induce HR in soybean and tobacco, respectively (Ashfield et al., 1995; Peart et al., 2002), raising the possibility that RIN4 activity is necessary for these and perhaps other defense responses.

The RIN4 story makes a strong case in support of the guard hypothesis. Three different effectors, AvrB, AvrRpm1, and AvrRpt2, modify RIN4 and two R proteins, RPM1 and RPS2, sense this perturbed state of RIN4, activating disease resistance pathways to combat the intrusion. Even if it is argued that the plants' basal defense system protects against a majority of pathogens, the number of potential pathogens capable of causing disease still easily outnumbers the limited number of R-like proteins deduced from the *Arabidopsis* genome. Instead of the costly approach of one sentry per intruder, a simpler strategy is to guard the virulence targets.

One implication of this guard strategy for plant biologists is that inter-species transfer of *R* genes may not be sufficient to transfer resistance if the virulence target

is absent. *R* genes from tobacco, for instance, can function in tomato but not in rice or *Arabidopsis*. How well conserved are the virulence targets for a given pathogen in different host plant species? Is it practical to transfer a suite of genes or "resistance complex" from one plant species to another? Does the guard system apply to nonbacterial pathosystems? Is the R-Avr interaction of Pita-AvrPita sufficient to trigger a resistance response? In any case, these findings will undoubtedly lead researchers working on disease resistance to hunt for their favorite "guardee."

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The Kin I Kinesins Are Microtubule End-Stimulated ATPases

The Kin I kinesins are microtubule-destabilizing enzymes important for neuronal transport, spindle assembly, and chromosome segregation. Hunter et al. (2003) now show that the Kin I MCAK is a microtubule end-stimulated ATPase that can catalytically depolymerize MT's.

Molecular motors are enzymes that couple the energy of ATP hydrolysis to force production. The kinesin-related proteins (KRP's) are a family of motors that generally translocate unidirectionally down a microtubule (MT) polymer. In contrast to many KRP's, the Kin I kinesins, including MCAK and XKCM1, are unique because rather than walk on MT's, they catalytically depolymerize them (Desai et al., 1999; Maney et al., 2001; Moores et al., 2002; Niederstrasser et al., 2002; Ovechkina et al., 2002). The Kin I kinesins specifically target to the ends of MT's, where they induce a conformational change in the lattice that results in MT catastrophe. It has been proposed that Kin I could be released in a complex with tubulin dimer, and then ATP hydrolysis would release the Kin I from the tubulin dimer and allow for recycling (Figure 1A) (Desai et al., 1999). These studies established the basic biochemistry of the Kin I family and provided a comparison between them and conventional kinesin, but many unanswered questions remain: What features of the enzyme are essential for MT destabilization? Are they MT-stimulated ATPases like conventional kinesin? How do they reach the end of MT's? Are they processive enzymes? Several of these interesting questions are addressed by Hunter and coworkers in this issue of *Molecular Cell* (Hunter et al., 2003).

The authors developed a series of assays to simultaneously monitor the kinetics of MT depolymerization and ATP hydrolysis by MCAK, including real-time microscopy, a spectrophotometric assay to measure the kinetics of MT depolymerization, and standard ATPase assays. They found that the depolymerization rate of GMPCPP-stabilized MT's by MCAK was 0.88 μm/min, $\sim$100 times faster than the rate that GMPCPP MT's depolymerize on their own. Under similar conditions, MCAK ATPase activity could be stimulated $\sim$350-fold by MT's to a rate of 5 s^{-1}. Surprisingly, MCAK activity could also be stimulated $\sim$10-fold by tubulin dimer to a rate of 0.15 s^{-1}. While stimulation of ATPase activity by MT's is similar to that of other KRP's, stimulation by tubulin dimer is unique to Kin I kinesins and could be due to unique binding sites on tubulin dimers that are exposed at the end of the MT, or to interaction with tubulin dimer during part of the ATP catalytic cycle.

A series of creative experiments revealed another key finding of Hunter and colleagues: MCAK ATPase activity was stimulated by MT ends. Addition of increasing amounts of MCAK to a fixed amount of MT's showed saturation of the depolymerization rate at an MCAK concentration of 4 nM. This is substantially lower than the MT lattice concentration of 300 nM but similar to the concentration of MT ends in the assay (2.4 nM). A second and very convincing experiment involved shearing a population of MT's, which creates more MT ends while keeping the concentration of tubulin polymer constant, and assaying ATPase activity. Sheared MT's had ATPase activity approximately 2-fold higher than unsheared MT's, suggesting that the increased number of ends contributes to enhanced ATPase activity. These data suggest that ATP hydrolysis occurs at the end of the MT and not on the tubulin dimer as originally proposed (see Figure, panel A) (Desai et al., 1999). Consistent with the end-stimulated ATPase activity, the authors estimate that MCAK has one high-affinity binding site/protofilament end, which may be predicted for an enzyme that depolymerizes MT's from the end. Given that the cellular concentration of MCAK is $\sim$100 nM compared to a tubulin concentration of $\sim$20 μM, if MCAK did not have a higher affinity for MT ends the MT lattice would act as a sink.