PERSPECTIVES: CELL CYCLE

Rad9 Comes of Age

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Normal cells that have sustained damage to their DNA wisely stop dividing, halted at the entry point of mitosis. But budding yeast (*Saccharomyces cerevisiae*) cells with mutations in the gene *RAD9* are exceedingly imprudent. They ignore the presence of breaks in the double-stranded DNA of their genome and proceed unchecked into mitosis (*1*)—a life-threatening situation roughly akin to embarking on a long car journey with holes in the tires. Even before the impetuosity of the *rad9* mutants became apparent 10 years ago (*1*), it was known that eukaryotic cells delayed their entry into mitosis when their DNA was damaged, but the *rad9* mutant pointed to a specific extrinsic mechanism for this effect. As the basis of this so-called checkpoint has been genetically dissected, we have learned much about mitosis and DNA repair in eukaryotic cells. We have, however, discovered surprisingly little about the Rad9 protein itself. A report on page 272 of this issue (*2*) begins to correct that shortcoming.

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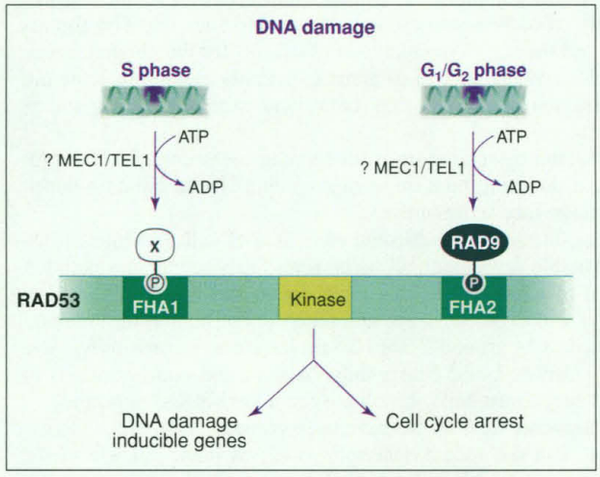
Proteins with some similarity to Rad9 have been identified in species from human to *Schizosaccharomyces pombe*. A region of homology at the COOH-terminus of Rad9—the BRCT domain—is shared between Rad9 and at least two other gene products, BRCA1 and 53BP1, both of which have intriguing connections to human cancer (*3*). BRCA1, a putative tumor suppressor, is linked to breast cancer, and 53BP1 is a protein that binds to the tumor suppressor p53 (*4*), although neither of these proteins are true homologs of budding yeast *RAD9*. The *S. pombe* homolog of *RAD9*, called both *rhp9* (*5*) and *crb2* (*6*), functions in much the same way as the *S. cerevisiae* Rad9 protein (*5, 6*): Mutants lacking *rhp9/crb2* function fail to arrest in the cell cycle when DNA is damaged or when DNA replication is impaired by inactivating DNA polymerases or DNA ligase.

Sun *et al.* encountered Rad9 in a search for proteins that could interact with the protein kinase encoded by *RAD53*. Rad53 is an essential protein kinase in *S. cerevisiae* required for DNA replication and for cell cycle arrest in response to replication blocks and DNA damage (*7*). Rad53 is phosphorylated when DNA is damaged, and this phosphorylation depends on a number of other gene products (*8*). Rad9 function is necessary for phosphorylation of Rad53 when cells in G_1 or G_2 are exposed to DNA damage (*9*). Using a kinase-defective allele of Rad53, Sun *et al.* performed a two-hybrid protein interaction screen and isolated Rad9 (*2*). They show that Rad9 is modified in response to DNA damage (probably by phosphorylation) and that the modified form of Rad9 is selectively bound by Rad53. Furthermore, Rad9 interacts with the COOH-terminal domain of Rad53.

Rad53 has two FHA (forkhead-associated) domains, one in the NH_2-terminal region (FHA1) and one in the COOH-terminal region (FHA2). The FHA domain, originally described as a region of homology in a subset of the family of forkhead-type transcription factors (*10*), lies outside of the DNA binding domain conserved in all forkhead transcription factors and consists of a stretch of 55 to 75 amino acids with three highly conserved blocks of residues. This motif is also found in several proteins that are not transcription factors, including Rad53 and its fission yeast homolog, Cds1. Mutations in FHA2 of Rad53 diminish its capacity to associate with Rad9, abolish DNA damage–dependent phosphorylation of Rad53, eliminate G_2/M arrest, and increase RNR3 transcription (*2*). Thus, the ability of Rad53 to associate with phosphorylated Rad9 correlates with its ability to function in the DNA damage response, suggesting that this association is physiologically relevant and that phosphorylation of Rad9 is a necessary component of the response.



Two ways to stop. In G_1/G_2 phase of the cell cycle, DNA damage triggers arrest of the cell cycle via Rad9's interaction with one FHA domain of the kinase RAD53. In S phase the same arrest occurs via interaction of an unknown, protein (x) with Rad53's other FHA domain.

There is precedence for the recognition of a phosphorylated partner by an FHA domain–containing protein. The *Arabidopsis thaliana* protein KAPP (kinase–associated protein phosphatase) binds to the serine-threonine receptor–like protein kinase RLK5 (*11*). The domain of KAPP that binds to RLK5 (originally called the KI domain for "kinase interacting") includes an FHA domain (*10*). Like Rad9, RLK5 must be phosphorylated to bind to this region of its partner (*11*). Thus, FHA domains may be analogous to SH2 (Src homology 2) domains that recognize tyrosine-phosphorylated residues to mediate signal transduction through cell surface receptors. It will be of interest to know whether mutations in the conserved residues of KAPP FHA abolish binding to RLK5, and to confirm that conserved residues within FHA domains of other proteins are important for function and that phosphorylation of the binding partner is necessary for FHA-mediated association.

Mutations in the COOH-terminal FHA2 domain prevent Rad53 from responding to some types of DNA damage—alkylation by methyl methane sulfonate or production of single-stranded DNA by inactivation of *cdc13*. FHA2 mutations, however, do not prevent Rad53 phosphorylation or cell cycle arrest when DNA replication is inhibited by hydroxyurea (*2, 12*), nor does it confer sensitivity to ultraviolet (UV) light (*12*). Therefore, this domain is not solely responsible for Rad53's role in the DNA damage response; the NH_2-terminal FHA1 domain may participate as well. Consistent with a role for FHA1, deletion of the NH_2-terminal FHA1 domain confers sensitivity both to hydroxyurea and to UV light (*12*). Perhaps the two domains mediate Rad53 function at different points in the cell cycle—FHA1 during S phase and FHA2 during G_1 and G_2 (see the figure). This hypothesis is consistent with the observation that Rad53's response to UV light depends on Rad9 dur-

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ing G_1 and G_2, and on DNA polymerase ε during S phase. A model presents itself: Rad9 binds to FHA2 to mediate the Rad53 response during G_1 or G_2 while another protein, perhaps polymerase ε, binds to FHA1 to mediate the Rad53 response during S phase (see the figure). If this is the case, then the sensitivity of the FHA1 deletion mutant and resistance of the FHA2 deletion mutant to UV light may be explained by the fact that cells in S phase are more sensitive to UV light. This analysis is complicated by the fact that the FHA1 deletion mutant also reduces the catalytic activity of Rad53 (*12*). Point mutations in the FHA1 domain (that do not affect catalytic activity) will determine whether FHA1 specifically confers sensitivity to UV light.

The gene *cds1* encodes a homolog of Rad53 in fission yeast (*13*), but its function is not entirely parallel. Cds1 has only a single FHA domain, and mutants lacking cds1 function have only some of the phenotypes of *rad53* mutants in budding yeast. Like *rad53* mutants, cells without *cds1* function lose viability when exposed to either replication blocks or DNA damage; unlike *rad53* mutants, however, they arrest the cell cycle in either case (*14*). Thus, Cds1 does not share Rad53's checkpoint function. Even so, both proteins clearly respond to DNA damage in a cell cycle–specific fashion. Cds1 activity is increased by DNA damage, but only during S phase (*14*). Rad53 requires Rad9 to function during G_1 and G_2, but not during S phase. The FHA domain in Cds1 may be analogous to the FHA1 domain of Rad53 in conferring S phase–specific regulation on these kinases. The second function of Rad53, to mediate arrest in response to DNA damage, is provided by the fission yeast protein kinase Chk1 (*15*). The fission yeast homolog of Rad9, Crb2, binds Chk1 (*6*). Although Crb2 is phosphorylated in response to DNA damage, it is not yet known whether phosphorylation of Crb2 or its association with Chk1 is necessary for Crb2 function in fission yeast. Chk1 does not have an obvious FHA domain, suggesting that the Crb2-Chk1 interaction may be mediated by another mechanism.

Rad9 joins a growing list of proteins implicated in the cell cycle checkpoint pathway that are phosphorylated in response to DNA damage. Which kinases are responsible for these events? Certainly a number of protein kinases function along the checkpoint pathways, but we are still a long way from understanding how they are regulated by DNA damage or replication blocks and what their in vivo substrates actually are. The protein kinases thus far implicated in regulating the damage response in *S. cerevisiae*—Mec1, Tel1, and Rad53—are thought to function downstream of Rad9. The results of Sun *et al.*, however, inform us that Rad9 phosphorylation may in fact be dependent on these kinases (*2*), implying that they act upstream of Rad9. Either way, an additional as yet unidentified kinase could be involved, and the pathways are more complicated than we have thought.

How best to dissect this complex pathway of interacting proteins, kinases, and substrates? Forge ahead with open minds. A combination of genetics, cell biology, and biochemistry has gotten us into this tangle. Let us hope that these approaches can eventually lead us out.

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NOTA BENE: IMMUNOLOGY

Monie a Mickle Maks a Muckle

For the first time, we have a set of benchmark figures for the prokaryotes in the planet's active biosphere. In a paper in the *Proceedings of the National Academy of Science*, Whitman *et al.* calculate the number and location of the world's prokaryotes and the amount of carbon sequestered in their biomass (*1*). The figures are large, staggeringly so, and these new data have implications for the understanding of global geochemical cycles and the control of genetic diversity. Seldom has the old Scots saying (which means many small things combined can make a big thing) seemed so appropriate.

The numbers were calculated by scaling up from existing measurements in representative habitats, making a daunting task quite manageable. Three habitats dominate—seawater, soil, and subsurface sediment.

For marine environments, the several published estimates of cell densities in different localities are in reasonable agreement, allowing for a fairly secure computation of 1×10^{29} cells, one-third of which are in the upper ocean and two-thirds in deep water. Counts for freshwater and in polar ice are several orders of magnitude smaller.

In soil there are estimated to be around 2.5×10^{29} prokaryotes. Surprisingly, most soils, including grassland, cultivated, and desert soils, have similar concentrations of prokaryotes, an exception being forest soils, which are considerably less populous.

A decade ago, soil and seawater data would have been considered sufficient to assess prokaryote abundance. But the recent descriptions (*2*) of bountiful life in the subsurface sediment (below 8 m) lead Whitman *et al.* to estimate that these populations may dwarf all others: There may be in excess of 4×10^{30} subsurface prokaryotes, accounting for more than 90% of the global population. Other notable but numerically minor populations occur in animals (for example, around 10^{14} per human), on leaves (1×10^{11} per square meter), and in the air itself.

These figures were used to calculate that the amount of carbon allocated to prokaryotes is about 5×10^{17} g (assuming that carbon is half of the dry weight of cells). This is half the amount found in plants, whereas for nitrogen and phosphorous the prokaryotic pool may actually rival that of plants (because a large proportion of plant material is extracellular). These huge numbers are significant in the global carbon, nitrogen, and phosphorus cycles.

Although subsurface prokaryotes dominate numerically, their metabolism is constrained because of limited access to nutrients. Their total productivity is merely equivalent to that of the rapidly growing population associated with domestic animals. Far and away the greatest productivity occurs in marine environments, with upward of 10^{30} generations per annum. This frantic replication provides enormous scope for mutation and speciation. The factors that constrain these processes are yet unknown—just one more set of questions to add to the list on prokaryotes. **–RICHARD GALLAGHER**

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