PIK-Related Kinases: DNA Repair, Recombination, and Cell Cycle Checkpoints

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The structural basis for the ability of kinase enzymes to transfer phosphate groups is sufficiently well understood that newly cloned members can usually be identified on the basis of their amino acid sequence alone. For the protein kinases, the sequence information can even indicate the preference of the enzyme for transferring phosphates to serine or threonine or to tyrosine. Another group of kinases (PIKs), which transfer phosphate groups to the phospholipid phosphatidylinositol (PI), are easily distinguished by their sequences from the protein kinases, and subtle sequence differences reliably indicate their preference for the 3- or 4-position on the inositol ring of PI.

Recently, a number of high molecular weight kinases have been cloned with sequences that elude easy classification as protein kinases or as PIKs, although they are related more closely to the lipid than to the protein kinases. The relevant substrates of these PIK-related kinases have not yet been identified, but recent results have provided insight into their cellular functions. Members of this new kinase family participate in meiotic and V(D)J recombination, chromosome maintenance and repair, cell cycle progression, and cell cycle checkpoints, and their dysfunction results in medical disorders ranging from a loss of immunological function to cancer.

The divergence of the PIK-related kinases from the standard PIKs is apparent in a number of ways. The dendrogram of the PIK-related and lipid kinases in Fig. 1 is derived from the amino acid residues in the postulated minimal kinase domain, predicted by using the classical protein kinases as a template. The clusterings reflect differences in sequences surrounding the absolutely conserved catalytic residues of the ATP-binding cleft, and to a lesser extent differences in the amino- and carboxyl-terminal lobes. The distinctness of the family becomes more pronounced as sequences outside the core kinase domain are included in the analysis. Most notably, a unique carboxyl-terminal region (Fig. 2) has no counterpart in the characterized PIKs (or in the serine-threonine or tyrosine protein kinases) and serves as a defining feature for the family. The long sequences that precede the kinase domain are less conserved throughout the family, although significant pairwise similarities are not uncommon. It is not known whether the divergence of these PIK-related kinases from the family of standard, biochemically defined PIKs reflects a difference in substrate specificity; however, to date only intrinsic protein kinase activity has been demonstrated for these enzymes.

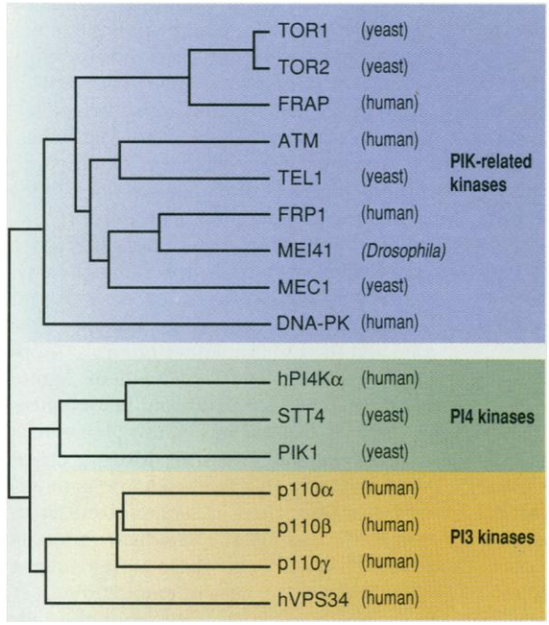

Fig. 1. PIK-related kinases: A distinct family. Phylogenetic tree of the PIK-related kinases (blue) and the biochemically defined PI4 kinases (green) and PI3 kinases (yellow), constructed on the basis of amino acid sequence in the kinase domain alone.

The *Saccharomyces cerevisiae* gene *MEC1/ESR1* (mitosis entry checkpoint mutant) encodes a 273-kilodalton member of this new kinase family and exemplifies the many possible functions of these kinases. Cells with mutations in *MEC1* cannot block entry into mitosis when DNA replication is incomplete or when they are exposed to certain genotoxic agents (*1*). As the name suggests, this is attributed to the loss of a "checkpoint," a regulatory mechanism whereby the order and completion of events of the cell cycle is enforced (*2*). *MEC1* is essential for the proper function of checkpoints that monitor the state of the genome at the G_1/S or G_2/M transitions, as well as within S phase itself (*3*). In addition, other evidence implicates *MEC1* in meiosis: The mutant strains show reduced meiotic recombination (*4*). In *Drosophila melanogaster*, the *mei-41* gene encoding a 270-kilodalton protein is required for these same processes and thus appears to be both a structural and functional homolog of *MEC1* (*5*). {A recently cloned human complementary DNA [FRAP-related protein (FRP1)] encoding a 301-kilodalton protein with considerable homology to MEC1 (*6*) is included in the analyses in the figures.}

The inability to respond appropriately to DNA-damaging agents is also seen in cells with mutations in another member of this family, DNA-PK_{cs}, the 465-kilodalton catalytic subunit of the DNA-dependent protein kinase (*7*). These cells are hypersensitive to irradiation with x-rays and are unable to repair the DNA damage it causes (*8*). The same mutation causes a form of severe combined immunodeficiency in mice, a disease characterized by the lack of a functional immune system (*9*). Specifically, V(D)J recombination (the process whereby antibody genes are rearranged) is defective in these animals (*10*). The underlying biochemical link between these phenotypes is an inability to rejoin double-strand breaks in DNA, in one case "naturally" occurring gaps between the coding regions of antibody genes, and in the other the "unnatural" DNA ends that x-rays can produce. This model is consistent with the in vitro behavior of DNA-PK, which is activated by DNA ends via the DNA-binding proteins Ku70 and Ku80 (*11*).

The identification and partial cloning of the defective gene in individuals with ataxia telangiectasia [*ataxia telangiectasia mutated* (ATM)] confirmed the phylogenetic link of ATM to DNA-PK_{cs} and MEC1, a link that was anticipated on the basis of the phenotypes associated with the disease (*12*). Cultured cells from AT patients show increased x-ray sensitivity and suboptimal induction of p53-dependent signaling in response to a wide range of DNA-damaging agents (*13*). In addition, mitotic recombination is increased in these cells (as it is in yeast strains with mutations in *MEC1*), accounting in part for their diminished genomic integrity (*14*). These observations suggest a role for

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ATM in checkpoints or cell cycle control, possibly via its action in a p53-dependent process. As in DNA-PK$_{cs}$ mutants, V(D)J recombination is affected, likely accounting for the immune deficiencies in AT patients (*15*). However, other defects in AT patients (for example, cerebellar degeneration) cannot be explained by analogy to MEC1 and DNA-PK$_{cs}$.

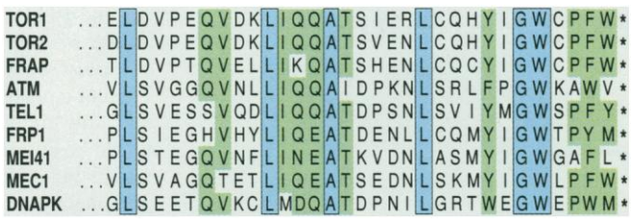

Fig. 2. The unique carboxyl-terminal region. The PIK-related kinases are distinguished by the presence of a conserved, carboxyl-terminal domain. Blue, identical amino acids; green, conservative changes.

The *S. cerevisiae* gene *TEL1* encodes a 322-kilodalton protein that is the closest homolog to human ATM (*16*). Originally identified in a screen for strains with abnormal telomere lengths, the *TEL1* mutant has other deficiencies that might be expected given its membership in this family (*17*). In addition to shortening of telomeres, mitotic recombination is increased (albeit to a lesser extent than in cells lacking MEC1 or ATM), and entire chromosomes are lost at a higher rate. Curiously, AT cell lines also show shortened telomeres and decreased chromosome stability (*18*). *TEL1* apparently is also functionally related to *MEC1*, because increased expression of *TEL1* complements the essential and checkpoint functions of *MEC1* (*19*).

The first mammalian member of this family to be cloned was the 289-kilodalton protein FRAP (FKBP rapamycin–associated protein), a direct target for the FKBP-rapamycin complex in humans and an apparent homolog of the *S. cerevisiae* TOR proteins (*20*). Growth factor–stimulated progression through the G_1 phase of the cell cycle in many cell types is sensitive to rapamycin. Recently, FRAP's function in this pathway was investigated directly (*21*); FRAP is required for p70 S6 kinase activation (resulting in increased translation of certain messenger RNAs), and conservative mutations in the kinase domain abolish this activation. When these mutations were made in the kinase domain of TEL1, telomere shortening was observed, again indicating a loss of function (*16*). Although FRAP can catalyze phosphotransfer to serine in an autophosphorylation reaction, no FRAP-specific PI phosphorylation has been measured so far. Likewise, only serine and threonine phosphorylation have been observed with DNA-PK$_{cs}$ (*7*).

Another apparent role for this family in cell-cycle control may be the modulation of amounts of cyclin-dependent kinase inhibitors (CDIs). FRAP is presumed to mediate interleukin-2–induced elimination of $p27^{Kip1}$ and increases in $p21^{Waf1/Cip1}$ (rapamycin-sensitive events) (*22*), and $p21^{Waf1/Cip1}$ induction in response to ionizing radiation is impaired in ATM-deficient cells (*13*). No doubt the future will reveal further functional similarities among members of the PIK-related kinase family, possibly including roles for the FRAP/TOR proteins in DNA repair, recombination, and in cell cycle checkpoints.

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Exciting Resonances

In an insulator or semiconductor, absorption of external energy produces an excited electron and a corresponding positively charged hole. Instead of recombining, the electron hole pair can be bound together by Coulombic attraction into a stable hydrogen-like complex known as an exciton. Excitons have the unusual property of being able to transport energy while remaining charge neutral. Energy transport by excitons has been used to explain energy transfer in organic crystals, polymers, liquids, and biological systems such as the photosynthetic reaction center. A method for producing excitons in quantum wells has been reported in the 7 August issue of *Physical Review Letters* by Cao *et al.* of Stanford University (*1*).

Quantum wells, layered structures made from materials with carefully chosen band gaps, offer an ideal way to confine excitons for study. Excitons have already been shown to tunnel through quantum wells as single entities (*2*), but Cao *et al.* have devised a way to create excitons directly in the quantum well region. Their approach involves a cleverly engineered structure: with voltage bias off, free holes from a *p*-doped AlGaAs layer diffuse into the quantum well region. Under an applied voltage, the free electrons in an *n*-doped GaAs layer can tunnel into the quantum well and combine with the trapped holes to form excitons. Because the electron energy can be adjusted so that the tunneling is resonant (that is, the energy of the hole and the tunneling electron become matched to that of the exciton they will create), the combination rate can be enhanced.

Now that excitons can be directly created inside quantum wells, some interesting fundamental phenomena in quantum electrodynamics may come under experimental scrutiny (*3*). For instance, just as the effect of vacuum fluctuations on single atoms can be studied in microcavities, it may be possible to examine how the fluctuations affect exciton resonance. On the practical side, because excitons can emit radiation when they go to the crystal ground state, the method of Cao *et al.* may also lead to a new class of optoelectronic devices.

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