

FHIT-Substrate Complexes: a New Paradigm in Reversible Protein Phosphorylation

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Fhit is a tumor suppressor protein encoded at the most fragile site in the human genome that is inactivated by genetic deletions early in the development of many cancers. A member of the Histidine Triad (HIT) superfamily of nucleotide-binding proteins, Fhit binds diadenosine triphosphate (ApppA) and cleaves it to produce AMP + ADP. The His96Asn mutation of Fhit, which reduces k_{cat} by more than a million-fold, does not reduce tumor suppressor activity in a nude mouse assay. Thus, genetic and biochemical evidence suggest that ApppA binding but not cleavage is required for tumor suppression. Crystal structures of Fhit bound to a nonhydrolyzable ApppA analog revealed that Fhit binds two substrates per dimer, presenting all of the phosphates and two of the adenosines on the surface of the protein in place of a deep, positively charged groove in the empty Fhit protein dimer. It is proposed that signaling by Fhit is mediated by presentation of nucleotide substrates to cytosolic effectors.

Keywords: cancer; molecular switch; X-ray crystallography

Overview

Cancer is characterized by multiple genetic changes that lead to hyperactivity of proliferative signaling pathways and reduced commitment to differentiated states and apoptosis. Because many

important regulatory proteins are controlled post-translationally, changes in the activity of protein kinases and phosphatases are frequently associated with cancer. Discovery of the *FHIT* gene^[1], encoding an ApppA hydrolase^[2], as a frequent, early target in epithelial tumors^[3] has led to the cell biological problem of explaining the connection between the metabolism of little known dinucleotides and cancer. A multidisciplinary approach that has made use of X-ray crystallography, mutagenesis, biochemical analysis, and assays of tumor suppression has led to the hypothesis that Fhit functions as a molecular switch, with the substrate-bound form signaling to antiproliferative or proapoptotic effectors^[4]. Crystallographic analysis of Fhit revealed a potentially new paradigm in reversible protein phosphorylation. As the substrate-bound form of Fhit displays all of the phosphates in two diadenosine polyphosphate substrates on the protein surface, the intrinsic hydrolase activity of Fhit would be expected to release phosphates from the surface and terminate the proposed signaling state of the protein.

Hit Superfamily of Nucleotide-Binding Proteins

HIT proteins are unique among nucleotide-binding proteins in binding substrates on the solvent-exposed surface of a 10-stranded β -sheet, termed the GalT half-barrel^[5]. Two main branches of HIT proteins are recognized: Hint homologs are found in all organisms, whereas Fhit homologs are found in animals and fungi. The most highly conserved residues in the superfamily mediate nucleotide-binding. Whereas Hint homologs are mononucleotide-binding proteins, Fhit homologs are dinucleotide-binding and hydrolyzing proteins. In fact, most of the residues specifically conserved in the Fhit branch of the superfamily were identified around the second nucleotide-binding sites^[5]. HIT proteins are also unique among nucleotide-binding proteins in not undergoing significant *protein* conformational change upon binding or hydrolyzing nucleotides. However, because they bind nucleotides on a solvent-exposed surface, the shape and electrostatic properties of the dimer complex are highly influenced by nucleotide occupancy.

Evidence for the proposed mechanism of signaling by a Fhit-substrate complex is summarized as follows. 1) Re-expression of Fhit in cancer cell lines with *FHIT* deletions suppressed tumor formation in

nude mice^[6]. 2) The His96Asn allele of Fhit, designed from Hint-nucleotide structures and predicted to affect *cleavage* of ApppA but not *binding*, was also functional in the nude mouse assay^[6]. 3) Biochemical analysis of the His96Asn mutant proved that it reduced k_{cat} more than a million fold without significantly increasing K_m , excluding the idea that the important function of Fhit is to catabolize ApppA^[4]. 4) Biochemical analysis of the mutant demonstrated that it is greatly retarded in the first step of the catalytic reaction, eliminating the idea that signaling might be mediated by wild-type and mutant Fhit proteins in complex with a catalytic intermediate^[4]. 5) Crystal structures of wild-type and mutant Fhit bound to nonhydrolyzable ApppA revealed that a deep, positively charged groove (Fig. 1, left) becomes filled with phosphates and adenosines^[4] in dinucleotide-bound complexes (Fig. 1, right). Substrate complexes, the putative active form of Fhit, are thus transiently and noncovalently phosphorylated forms of Fhit. As in the case of G-protein signaling, accessory proteins that modulate intrinsic rates of nucleotide-binding, hydrolysis or release may play important roles in signal transmission.

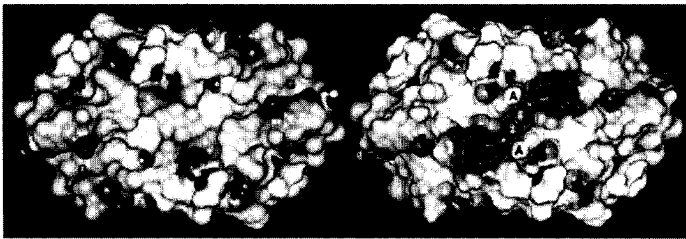


FIG. 1 Surface potential of apo and ApppA analog-bound Fhit^[4]

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(See Color Plate I at the back of this issue)

Downstream of the Fhit-ApppA complex

Whereas the literature indicates that biologically relevant signals like interferon stimulation produce ApppA signals^[7], little is known about the biological effectors that might mediate Fhit-substrate complex signaling. Recent identification of nitrilase homologs as fusion proteins with Fhit in invertebrates suggests that the Fhit signal transduction

pathway may be a small molecule-generating pathway yet to be characterized in animals^[8].

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