Histidine Triad (HIT) Superfamily

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Advanced article

Article Contents

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Histidine triad (HIT) enzymes are an ancient superfamily of nucleotide hydrolases and transferases that catalyse mechanistically similar but biologically distinct reactions on nucleotide-containing substrates in pathways important for cellular growth, apoptosis, deoxyribonucleic acid, ribonucleic acid, vitamin and carbohydrate metabolism. Four branches of HIT enzymes function as nucleotide hydrolases. These enzymes include homologues and paralogues of histidine triad nucleotide-binding protein, fragile histidine triad protein, APRATAXIN and scavenger decapping protein, which act in various cellular compartments. One diverse branch of the HIT superfamily contains nucleotide transferases and phosphorylases related to galactose-1-phosphate uridylyltransferase, AppppA phosphorylase, adenylylsulfate: phosphate adenylyltransferase, adenosine diphosphate-glucose phosphorylase and VTC2, the guanosine diphosphate-L-galactose phosphorylase. This review provides tools to discern the identities and the probable functions of HIT enzymes from their sequences.

Introduction

Histidine triad (HIT) proteins are typically dimeric, soluble intracellular enzymes that contain a binding site for a nucleoside 5′ monophosphate linked to an amino acid, nucleotide, oligonucleotide or carbohydrate leaving group (Brenner, 2002). Five branches of HIT enzymes have evolved to perform distinct reactions using similar chemistry. In the case of the four branches of HIT hydrolases (histidine triad nucleotide-binding protein (HINT), fragile histidine triad protein (FHIT), Aprataxin and scavenger decapping protein (DCPS)), each enzyme forms a covalent, nucleotidylylated intermediate with the second histidine of the His-Ø-His-Ø-His-Ø-Ø (Ø, a hydrophobic amino acid) motif attacking the substrate α phosphate, releasing the leaving group. The nucleotidylylated intermediates of these enzymes are then turned over by hydrolysis. In the case of HIT transferases/phosphorylases including galactose-1-phosphate uridylyltransferase (GALT), each enzyme forms a covalent, nucleotidylylated enzyme intermediate with the second histidine of the related His-X-His-X-Gln motif, releasing the leaving group. Nucleotidylylated intermediates of HIT transferases, stable in water, are resolved by transfer to a second substrate containing a phosphate (**Figure 1**). Phosphorylases are those enzymes for which the second substrate is simply phosphate, such that the second product is always a nucleoside diphosphate.

The roles of particular HIT enzymes have been defined by a combination of genetics, enzymology and structural biology. GALT, which is conserved from *Escherichia coli* to humans as the second enzyme in the Leloir pathway of galactose utilisation, has the most clearly understood function at levels of analysis that span from enzyme mechanism to human development. This review attempts to fill gaps in understanding between biochemical specificity and cellular and organismal function.

The existence of branches within a superfamily indicates that family members, for example, sequences within a branch, are derived from an ancestral sequence that differed in function from the ancestral sequences giving rise to other branches. Although no ancestral sequences are extant, experience allows us to define prototypical members of each branch and sub-branch. **Table 1** is provided to define reference sequences and, when available, published protein structures in an informative, liganded form. Basic local alignment search tool (BLAST) searches of new HIT proteins against nonredundant sequence data often identify homologous sequences that are incorrectly annotated. In contrast, determining which of the reference sequences is most closely related to a new sequence of interest is expected to identify the branch of most HIT proteins

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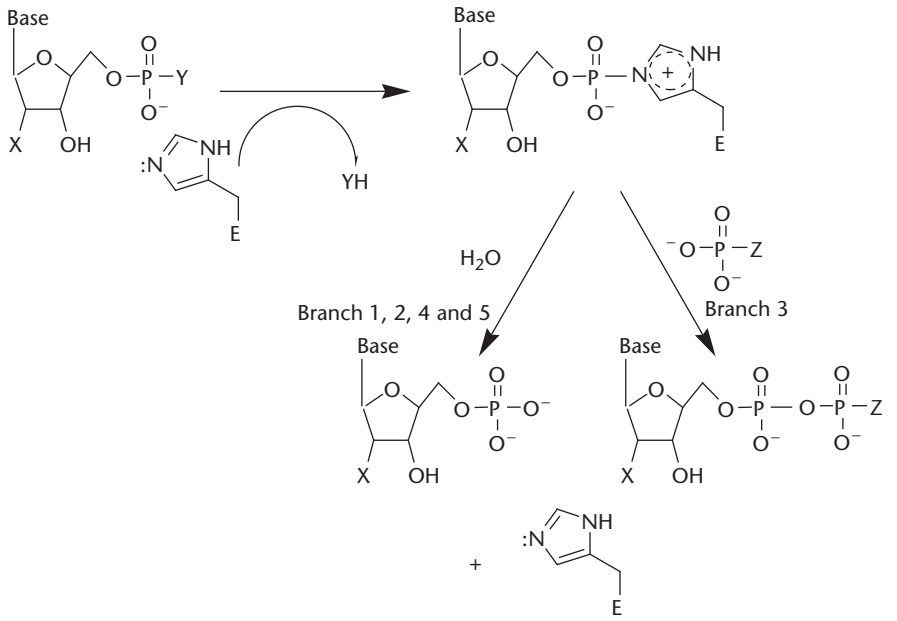

Figure 1 Catalysis by HIT enzymes. HIT substrates consist of nucleoside monophosphates varying in the base, 2′ substituent (X=OH or H) and leaving group (Y). In the first step, the enzymes form a covalent nucleotidylylated active site His intermediate with the α phosphate of the substrate, releasing the leaving group, YH. In the case of HIT hydrolases (branch 1, 2, 4 and 5 enzymes), the intermediate is hydrolysed to produce the nucleoside monophosphate (product 1). In the case of HIT transferases (branch 3 enzymes), the enzyme transfers the nucleoside monophosphate to a specific second substrate, containing phosphate (Z=O or a phosphate monoester substituent) to form product 2.

Table 1 Reference sequence and structures for histidine triad (HIT) enzyme

Branch	Gene	Protein	UniProt ID	PDB ID
1.1	*HINT1*	Rabbit HINT1	P80912	1RZY
1.2	*HINT2*	Human HINT2	Q9BX68	4INC
1.3	*HINT3*	Human HINT3	Q8N0Y9	
1.4	*ASW*	Chicken ASW	Q9I870	
2	*FHIT*	Human FHIT	P49789	2FHI
3.1	*galT*	*Escherichia coli* GALT	P09148	1HXQ
3.2	*APA2*	*Saccharomyces cerevisiae* Apa2	P22108	4I5T
3.3	*apt*	*T.d.* APAT[a]	Q9LA72	
3.4	At5g18200	*A.t.* ADP-Glc P'ase[b]	Q9FK51	1Z84
3.5	*VTC2*	*A.t.* VTC2	Q8LKQ7	
4	*APTX*	Aprataxin	Q7Z2E3	4NDF
5	*DCPS*	DCPS	Q96C86	1ST0

[a]*Thiobacillus denitrificans* adenosine monophosphate-sulphate adenylyltransferase.
[b]*Arabidopsis thaliana* adenosine diphosphate-Glc phosphorylase is widely mis-annotated as GALT, despite experimental analysis.
Abbreviations: ASW, avian sex-specific W-linked; DCPS, scavenger decapping protein; FHIT, fragile histidine triad protein; GALT, galactose-1-phosphate uridylyltransferase; HINT, histidine triad nucleotide-binding protein.

definitively. However, new sub-branches are almost sure to be identified, and two new major branches have been identified since the last superfamily review (Brenner, 2002).

HINT Homologues in Growth and Differentiation Control

Homologues and paralogues of rabbit HINT, the first branch of the HIT superfamily, are found in nearly all cellular life forms and are thought to constitute the primordial HIT enzymes (Brenner *et al*., 1997). As shown in **Table 2** and **Figure 2**, the best biochemical substrates of Hint are peptides containing an adenylylated Lys residue (Parks *et al*., 2004), suggesting that HINT homologues function to remove nucleotidylylated posttranslational modifications of Lys on particular target proteins. Recent work has highlighted systems in which small guanosine triphosphatases are adenylylated, particularly by bacterial adenylyltransferases (Itzen *et al*., 2011).

Table 2 Classification of histidine triad (HIT) hydrolases and transferases by substrate specificity

Branch	Enzyme	Substrate 1	Substrate 2	Leaving group	Product 2
1	HINT	AMP-lysine	Water	Lysine	AMP
2	FHIT	ApppA	Water	ADP	AMP
3.1	GALT	UDP-Glc	Gal-1-P	Glc-1-P	UDP-Gal
3.2	AppppA phosphorylase	AppppA	Phosphate	ATP	ADP
3.3	APAT	AMP-sulphate	Phosphate	Sulphate	ADP
3.4	ADP-glucose phosphorylase	ADP-Glc	Phosphate	Glc-1-P	ADP
3.5	VTC2	GDP-L-Gal	Phosphate	L-Gal-1-P	GDP
4	Aprataxin	AppDNA	Water	pDNA	AMP
5	DCPS	7meGpppN	Water	NDP	7meGMP

Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; APAT, adenylylsulfate:phosphate adenylyltransferase; ATP, adenosine triphosphate; DCPS, scavenger decapping protein; FHIT, fragile histidine triad protein; GALT, galactose-1-phosphate uridylyltransferase; GDP, guanosine diphosphate; HINT, histidine triad nucleotide-binding protein; UDP, uridine diphosphate.

Human and bacterial HINT1 hydrolyse Lys-transfer ribonucleic acid (tRNA) synthetase-catalysed Lys-adenosine monophosphate (AMP) adducts (Chou and Wagner, 2007). The latter enzyme has been shown to be a positive regulator of D-alanine dehydrogenase *in vivo* (Bardaweel *et al*., 2011). However, D-alanine dehydrogenase has not yet been shown to be modified by adenylylation. HINT isozymes can also be assayed with substrates that link a *p*-nitroaniline group to AMP (Krakowiak *et al*., 2004).

In the yeast *Saccharomyces cerevisiae*, deletion of the *HNT1* gene or mutation of the active site His results in cells which are incapable of growing on galactose at elevated temperature and which are sensitive to hypomorphic mutations in components of general transcription factor TFIIH (Bieganowski *et al*., 2002).

As shown in **Table 1**, in mice and humans, there are at least three Hint isozymes. HINT1 (Su *et al*., 2003) and the mitochondrially targeted HINT2 isozyme (Martin *et al*., 2006) have been implicated as tumour suppressors. Several studies have implicated Hint1 as a cofactor in transcriptional regulation (Lee *et al*., 2004; Weiske and Huber, 2005), though the mechanism of this regulation remains unclear. More recently, work in the mouse has indicated that HINT1 plays a role in drug sensitivity, mood and anxiety (Barbier and Wang, 2009; Jackson *et al*., 2012; Varadarajulu *et al*., 2011). In people, HINT1 variants are associated with schizophrenia (Chen *et al*., 2008), nicotine dependence (Jackson *et al*., 2011) and autosomal recessive axonal neuropathy with neuromyotonia (Zimoń *et al*., 2012). HINT2 deletion in the mouse resulted in fatty liver, poor glucose regulation and defective thermoregulation on fasting (Martin *et al*., 2013). HINT3 remains less well characterised.

In birds, HINT is encoded on the Z chromosome, such that ZZ males have two *HINTZ* genes and heterogametic ZW females have one. Remarkably, females encode approximately 40 copies of a W-chromosome-localised molecule termed avian sex-specific W-linked (ASW) gene or HINTW that appears to inhibit and/or alter HINT activity by heterodimerization (Parks *et al*., 2004). Thus, localised inhibition and/or alteration of HINTZ enzymatic activity in the developing urogenetical ridge may play a role in avian feminisation. Although HINTW remains a powerful marker of feminity in birds (Nagai *et al*., 2014), gene transfer experiments do not support the idea that HINTW is sufficient for feminisation (Smith *et al*., 2009).

FHIT Homologues in Tumour Suppression

The *FHIT* gene, encoded at 3p14.2, the most fragile site in the human genome, is frequently inactivated early in epithelial carcinogenesis. Relative to HINT, FHIT homologues, the second branch of the HIT superfamily, appeared late in evolution, potentially in a protist that predated the emergence of plants, fungi and metazoa. In invertebrates, FHIT is encoded as a fusion protein with a member of the nitrilase superfamily (Pace *et al*., 2000). Loss of murine *fhit* predisposes to a range of solid and blood-borne tumours. Infection with viruses encoding human FHIT protects against the development of *fhit* tumours by initiating a programmed cell death pathway (Ishii *et al*., 2004). **See also**: Chromosome 3

As shown in **Table 2** and **Figure 2**, the best biochemical substrate of FHIT is the diadenosine polyphosphate, ApppA (Barnes *et al*., 1996), such that FHIT products are adenosine diphosphate (ADP) plus AMP. FHIT and homologues are conveniently assayed with GpppBODIPY (Draganescu *et al*., 2000). Mutant forms of FHIT that bind substrates well but hydrolyse them poorly retain good cellular activity in induction of apoptosis, whereas mutants that reduce substrate binding lose cellular activity (Trapasso *et al*., 2003). These data indicate that the pro-apoptotic activity of Fhit is limited by substrate binding. Although it is known that heat shock induces dinucleoside polyphosphate synthesis in yeast (Rubio-Texeira *et al*., 2002), it is not known what the proximal effectors of FHIT are in proapoptotic tumour suppression. Knockdown of

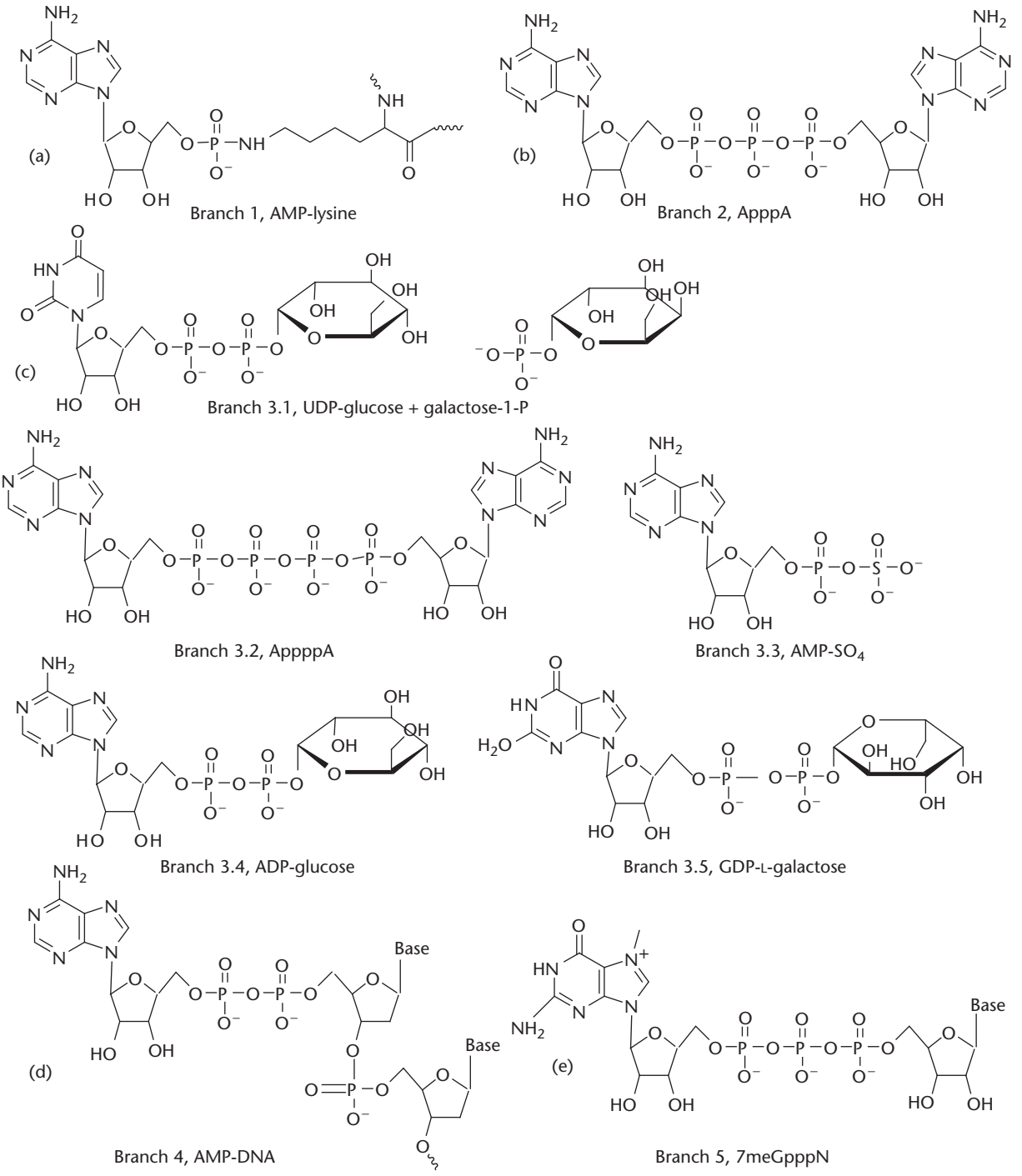

Figure 2 Preferred substrates of HIT enzymes. (a) HINT substrate, AMP-lysine residue; (b) Fhit substrate, ApppA; (c) GALT substrates, UDP-glucose and galactose-1-phosphate; AppppA phosphorylase substrate, AppppA; APAT substrate, AMP-SO_4; ADP-glucose phosphorylase substrate, ADP-glucose; VTC2 substrate, GDP-L-galactose; (d) APRATAXIN substrate, 5′ adenylylated 5′ phosphorylated DNA; and (e) DCPS substrate, 7-methyl GpppN.

FHIT expression in developing zebrafish has been shown to induce expression of a small set of genes, including the N transcript of p53 (Robu *et al*., 2007). More recently, FHIT loss has been shown to cause replication stress, genome instability, accumulation of further genetic alterations (Miuma *et al*., 2013), and when combined with cigarette smoke cause superinduction of heme oxygenase 1 (Boylston and Brenner, 2014).

GALT-related Transferases and Phosphorylases in Carbohydrate Metabolism

The best characterised member of the third branch of the HIT superfamily, GALT (branch 3.1) (Frey, 1996), is a model for all HIT transferases, which include AppppA phosphorylase (yeast Apa1 protein, branch 3.2) (Plateau *et al*., 1990), adenylylsulfate: phosphate adenylyltransferase (*Thiobacillus denitrificans* APAT, branch 3.3) (Bruser *et al*., 2000), ADP-glucose phosphorylase (*Arabidopsis thaliana*, branch 3.4) (McCoy *et al*., 2006) and VTC2 (*A. thaliana*, branch 3.5), the guanosine diphosphate (GDP)-L-galactose phosphorylase (Linster *et al*., 2007). Although branch 1 and 2 enzymes conserve a His-Ø-His-Ø-His-Ø-Ø motif and substantial similarity outside of the HIT motif, the transferases conserve an alignable His-X-His-X-Gln motif and limited primary sequence similarity with HIT hydrolases. In fact, sequence similarity between GALT and HINT is so low that structural superposition of a GALT monomer with a HINT dimer preceded the identification of primary sequence similarity between the enzymes (Brenner *et al*., 1997).

GALT homologues are found in many eubacteria, fungi and metazoa and in some archaea. Other HIT transferases are largely fungal or eubacterial enzymes or enzymes found in particular plant and animal species. As shown in **Table 2** and **Figure 2**, GALT first attacks uridine diphosphate (UDP)-glucose, forming a uridylylated enzyme intermediate with release of glucose-1-phosphate. However, unlike a HIT hydrolase, whose uridylylated intermediate would be hydrolysed to enzyme plus uridine monophosphate (UMP), the GALT-uridylyl intermediate awaits collision with a specific second substrate. In the case of GALT–UMP, the second substrate is galactose-1-phosphate, resulting in formation of UDP-galactose (Frey, 1996).

The Leloir pathway of galactose utilisation is a three-enzyme scheme to flip the configuration a single hydroxyl group to make galactose available for glycolysis. Cells initiate galactose metabolism with a galactokinase activity to produce galactose-1-phosphate. GALT then converts UDP-glucose to UDP-galactose with consumption of the galactose-1-phosphate and production of glucose-1-phosphate, which is convertible to the glycolytic intermediate, glucose-6-phosphate. UDP-galactose 4-epimerase completes the Leloir pathway via nicotinamide adenine dinucleotide (NAD^+)-dependent epimerization of the four carbon of UDP-galactose to produce UDP-glucose for utilisation by GALT (Frey, 1996). Loss of function mutations in human GALT results in galactosaemia, which causes failure to thrive in infants. Although total elimination of dietary galactose permits development, learning disabilities and ovarian failure are frequent complications. **See also**: Chromosome 9; Genetic Disorders; Metabolism: Hereditary Errors

For characterised branch three enzymes other than GALT, the second substrate is simply phosphate, such that phosphorolysis of an enzyme-nucleoside monophosphate intermediate produces the nucleoside diphosphate as product two (**Table 2**). This is the case for AppppA phosphorylase (Plateau *et al*., 1990), APAT (Bruser *et al*., 2000) and for ADP-glucose phosphorylase (McCoy *et al*., 2006), an *Arabidopsis* enzyme whose structure and activity were determined as a part of a structural genomics effort. It is significant to note that, because of sequence similarity to GALT, *Arabidopsis* ADP-glucose phosphorylase and the majority of its apparent orthologues are annotated as putative or probable GALTs. In fact, the enzyme prefers simple phosphorolysis of ADP-glucose to the GALT reaction by almost 1000-fold (McCoy *et al*., 2006). Although APAT, the *Thiobacillus* enzyme that phosphorylyses AMP-sulfate to form sulfate and ADP, is thought to play a role in bacterial sulfite oxidation (Bruser *et al*., 2000), the function of AppppA phosphorylase is not clear. ADP-glucose phosphorylase functions in plant starch biosynthesis (Bahaji *et al*., 2011).

GALT-related HIT transferases have been identified as responsible for the first committed step in the Smirnoff–Wheeler pathway of plant ascorbate synthesis. In this step, the products of the *Arabidopsis VTC2* and *VTC5* genes produces L-galactose-1-phosphate from GDP-L-galactose in a reaction that requires phosphate and produces GDP (Linster *et al*., 2007, 2008). Surprisingly, VTC2 has homologues not only in plants but also in worms, flies and vertebrates (Linster *et al*., 2007). By analogy with the enzyme-uridylyl intermediate of GALT and the enzyme-adenylyl intermediates of AppppA phosphorylase, APAT and ADP-glucose phosphorylase, VTC2 is expected to proceed through a covalent guanylylated intermediate through the second His of the HIT-related motif. Plant *VTC2* mutants are ozone-sensitive and make little ascorbic acid (Conklin *et al*., 2000). Animal VTC2 homologues appear to control levels of GDP-D-glucose formed by GDP-D-mannose pyrophosphorylase, which would limit misincorporation of glucose into protein glycosylation (Adler *et al*., 2011).

APRATAXIN Homologues in Single-strand DNA Break Repair

Children with biallelic mutations in the *APTX* gene at 9p13.3 suffer from a disease termed ataxia-oculomotor apraxia, consisting of early-onset cerebellar ataxia coupled with oculomotor apraxia and hypoalbuminemia (Date *et al*., 2001; Moreira *et al*., 2001). The mutant genes encode loss of function enzymes in APRATAXIN that impair protein stability and enzymatic activity (Seidle *et al*., 2005). The APRATAXIN polypeptide contains a forkhead-associated (FHA) domain that is highly similar to that of human deoxyribonucleic acid (DNA) kinase, which has 5′ kinase and 3′ phosphatase activity, and a HIT hydrolase domain, which constitutes the fourth branch of the HIT superfamily. Branch four enzymes are found in metazoa as

well as some fungi, plants and protists. However, invertebrate, fungal, plant and protist APRATAXIN homologues appear to lack the FHA domain.

Presence of the FHA domain in human APRATAXIN suggested involvement in DNA repair. Protein associations with XRCC1, XRCC4 (Clements *et al*., 2004), DNA ligase III, DNA kinase and DNA polymerase β (Luo *et al*., 2004) suggested a specific involvement in repair of single-strand breaks. In the course of ligating a 5′ phosphate to a 3′ hydroxyl group, the phosphate becomes transiently adenylylated. When ligation is aborted, the unsealed DNA strand contains a 5′ adenylylated adduct as shown in **Figure 2** and **Table 2**. APRATAXIN deadenylylates such molecules, thereby creating a clean substrate for ligation, the final step in single-stranded break repair (Ahel *et al*., 2006). More recently, it was realised that ribonucleotide excision repair produces millions of nicked RNA–DNA junctions per cell cycle in mice (Reijns *et al*., 2012) and that this may be the RNA–DNA deadenylylation reaction of greatest importance for APRATAXIN-dependent repair (Tumbale *et al*., 2014). The genetics of *APTX* mutations suggest that resolution of such nonligated adducts is important for proper cerebellar development. **See also**: Genetic Disorders

DCPS Homologues in messenger RNA (mRNA) and microRNA (miRNA) Turnover

The fifth branch of the HIT superfamily contains homologues and paralogues of human DCPS, a HIT enzyme that hydrolyses the residual mRNA cap structure, 7-methyl GpppN (**Figure 2** and **Table 2**). DCPS cleaves 7 meGpppN or short oligonucleotides containing the cap that remain after 3′ to 5′ exonucleolytic degradation of deadenylylated mRNA, producing a 7-methyl GMP product (Liu *et al*., 2002) plus either a nucleoside diphosphate or a short oligoribonucleotide with a diphosphorylated 5′ end. Absent from monera or plants and extremely rare in protists, DCPS homologues appear to have emerged just before the common ancestor of fungi and metazoa. DCPS is termed the scavenger decapping activity because it has little activity on capped mRNA (Gu *et al*., 2004; Liu *et al*., 2002), which is the substrate of Dcp2 (Wang *et al*., 2002), an unrelated decapping enzyme whose activity precedes 5′ to 3′ mRNA degradation. More recently, the worm DCPS has been shown to control miRNA levels via interaction with XRN1 (Bossé *et al*., 2013). **See also**: RNA-binding Proteins: Regulation of mRNA Splicing, Export and Decay

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