The MutT Proteins or “Nudix” Hydrolases, a Family of Versatile, Widely Distributed, “Housecleaning” Enzymes

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Our studies on the biochemical basis of spontaneous mutations took an interesting and unexpected turn when we discovered that a small region of amino acid homology between the MutT protein of Escherichia coli and the MutX protein of Streptococcus pneumoniae was involved in their nucleoside triphosphatase as well as their antimutator activities (1–3). Computer searches of the data banks revealed that this same small conserved region was present in a number of other proteins in organisms ranging from viruses to humans (2, 4). Most of these proteins containing the signature are coded for by open reading frames (orf) whose products are either unidentified or of unknown function. We have been attempting, systematically, to identify and characterize enzymatic activities associated with these proteins, and it is now evident that nature has adopted this motif, originally identified as the active site of the nucleoside-triphosphate pyrophosphohydrolase of MutT (5, 6), and adapted it for use in many diverse reactions distinct from its function in the MutT protein. This short review summarizes our present knowledge of those reactions catalyzed by proteins harboring the MutT signature sequence and calls attention to a unique and versatile nucleotide binding and catalytic site. Although it might appear that the enzymes of this family act upon a wide variety of unrelated substrates, those characterized so far all hydrolyze a nucleoside diphosphate linked to some other moiety, X. For convenience, and to correct a misapprehension, we propose the mnemonic “nudix” hydrolase for this family of enzymes to replace the “MutT family.” Currently, this signature sequence is designated the “MutT pattern” in version 13.0 of the PROSITE data base of amino acid sequence motifs (7). This initial classification is misleading, because many, if not most, of these proteins are not involved directly in preventing mutations nor do they catalyze the archetypal nucleoside triphosphate pyrophosphohydrolysis reaction originally described for MutT itself (5, 6).

The MutT Prototype

Characterization of the E. coli MutT protein, the progenitor of the family, followed from studies designed to elucidate the biochemical basis of the mutT mutator phenotype. Treffers et al. (8) originally described a mutant strain of E. coli, mutT1, with spontaneous mutation frequencies ranging from 100- to 10,000-fold higher than normal. Yanofsky et al. (9) showed that unlike typical defective mutator genes causing a variety of derangements in DNA such as transitions, transversions, frameshifts, etc., mutT causes, exclusively, a single, unidirectional AT → TG transversion. This could be explained by either or both of the following base mispairing events during DNA replication.

We were persuaded by the structural arguments of Topal and Fresco (10) that the A → T mispair was the more likely event, and the experiments of Schaaper and Dunn (11) on in vitro DNA replication in extracts of mutT cells support this view. Cloning of the mutT gene by complementation of the mutT mutator phenotype (5, 12) and identification of the lesion in the original Treffers‘ mutT1 allele as an IS1 insertion in the mutT gene (5) established that mutT is directly involved in preventing the enormous increase in the frequency of AT → CG transversions. Expression, purification, and characterization of the cloned gene product (5, 6) uncovered a new enzyme, a nucleoside triphosphatase with a preference for dGTP but active on all eight canonical nucleoside triphosphates. The stoichiometry of the reaction is as follows: dGTP + H3O + → dGMP + PPi. Thus the enzyme is a nucleoside-triphosphate pyrophosphohydrolase, which most likely “sanitizes” the nucleotide pool (6) of a mutagenic form of dGTP having a proclivity to mispair with template adenine during replication. Maki and Sekiguchi (33) have reported that 8-oxo-dGTP is the mutagenic form of dGTP.

The MutT Signature

Studies on the structure and function of the MutT nucleoside triphosphatase were greatly enhanced by the discovery of MutX, a homologue of MutT present in S. pneumoniae (2). The mutX gene can complement a mutT strain of E. coli, and the purified MutX protein has similar nucleoside triphosphatase activity, yet the two enzymes differ markedly in their primary and quaternary structures (3). However, they do share a small region of homology involving about 10 conserved amino acids in a span of about 20. Site-directed mutants showed this region to be important for enzyme catalysis in vitro as well as for antimutator functions in vivo (2). When the human equivalent of E. coli mutT was cloned and sequenced (13), it was found to share this same homologous region as shown in Fig. 1A.

On the basis of the dGTPase activity of the MutT protein, it was proposed (2, 4) that the conserved motif in the MutT family designated a new catalytic site for the hydrolysis of nucleoside triphosphates, with a preference for dGTP. We elected to test this hypothesis by cloning other open reading frames of unknown function containing the conserved sequence, expressing and purifying the proteins and characterizing their activities. The first of these was orf17, a close neighbor to rwc on the E. coli genome (14, 15) but not involved in the function of the rwc-encoded Holliday-junction endonuclease (14). The Orf17 protein was indeed found to be a nucleoside-triphosphate pyrophosphohydrolase, fulfilling the prophecy (3, 16), but its preferred substrate among the canonical nucleotides is dATP + H3O + → dAMP + PPi. dCTP is the least favored substrate for Orf17 (relative catalytic efficiency, dATP/dCTP = 10), whereas for MutT, the specificity is reversed (dGTP/dATP = 70). Thus, the original working hypothesis, which included a preference for dGTP as part of the catalytic site, was revised, and we concluded that the conserved signature sequence designated a general nucleoside-triphosphate pyrophosphohydrolase whose specificity was determined by other amino acids outside of the homologous sequence.
Minireview: MutT Proteins or “Nudix” Hydrolases

“A protein...”

“Substrate Specificity...”

"Additional Members of the Family..."

**A**

| MutT, coli | MutT, Human | MutT, Mm | MutT, Ss | MutT, X, Ss |
|------------|-------------|---------|---------|------------|
| NQRGQAVG... | PRLG... | LPAR... | LPAR... | LPAR... |
| MutT, Aa | MutT, Ks | MutT, Lp | MutT, Rl |
| NVNG... | KNYG... | LGF... | KNYG... |
| MutT, E. coli | MutT, Human | MutT, Human | MutT, Human |
| YPAE... | YPAE... | YPAE... | YPAE... |

**B**

| Protein | Accession | Sequence | Organism |
|---------|-----------|----------|----------|
| 1-MutT | P08317 | GKEIR... | E. coli |
| 2-MutT | P36030 | GKEIR... | S. vulgare |
| 3-MutX | P31514 | GKEIR... | S. lividans |
| 4-MutT | P36399 | GKEIR... | Human |
| 5-MutT | D49777 | GKEIR... | Ret |
| 6-MutT | D65607 | GKEIR... | Mouse |
| 7-Orf17 | Y42366 | GKEIR... | E. coli |
| 8-Orf257 | Y35664 | GKEIR... | E. coli |
| 9-Orf1.9 | Y22056 | GKEIR... | E. coli |
| 10-Orf156 | P45739 | GKEIR... | E. coli |
| 11-OrfAa | U13131 | GKEIR... | Human |
| 12-OrfBa | U13137 | GKEIR... | Human |
| 14-Orf154 | Q52191 | GKEIR... | S. cerevisiae |
| 15-Orf125 | Q82190 | GKEIR... | X. laevis |
| 18-Orf250 | Q18852 | GKEIR... | A. thaliana |
| 19-Orf190 | Q18852 | GKEIR... | E. coli |
| 20-Orf257 | Q18852 | GKEIR... | E. coli |
| 21-OrfAa | U13131 | GKEIR... | S. cerevisiae |

“Exceptio Probat Regulam”

“YSA1—Although several open reading frames from higher...”

“Substrate Specificity...”

**Additional Members of the Family**

"Orf1.9—This open reading frame was so named because..."
Minireview: MutT Proteins or “Nudix” Hydrolases

Each of the substrates listed in column 3 is composed of a nucleoside diphosphate linked to the compound shown in column 4.

| Enzyme* | Source | Substrate | Products |
|---------|--------|-----------|----------|
| MutT (5) | E. coli | (d)NTP | P, NMP + PP, |
| MutT (47) | Proteus vulgaris | (d)NTP | P, NMP + PP, |
| MutX (5) | S. pneumoniae | (d)NTP | P, NMP + PP, |
| 8-oxo-dGTPase (13) | Human | 8-oxo-dGTP | P, NMP + [PPi]b |
| 8-oxo-dGTPase (26) | Rat | 8-oxo-dGTP | P, NMP + [PPi]b |
| 8-oxo-dGTPase (27) | Mouse | 8-oxo-dGTP | P, NMP + [PPi]b |
| Orf17 (16) | E. coli | (d)NTP | P, NMP + PP, |
| Orf257 (19) | E. coli | NADH | NRH, NMP + AMP |
| Orf1.9 (21) | E. coli | GDP-mannose | Mannose, GDP + mannose |
| Orf186 (5) | E. coli | Ap, ADP | ADP, AMP + ADP |
| Orf209* | E. coli | ADP-ribose | Ribose, AMP + ribose-5-P |
| YSA1* | Saccharomyces cerevisiae | ADP-ribose | Ribose, AMP + ribose-5-P |
| ApA-Ase (29) | Human | ApA | ADP, AMP |

* The numbers in parentheses are references to the published work, and the superscripts refer to the footnotes in the body of the text.

MutT enzyme, its functional homologues, and also Orf17 make up one subset of the general class in which X is a phosphate group. The other six enzymes listed in Table I adhere to this pattern. They are all specific for nucleoside diphosphates but differ in their requirement for X. For convenience, we will refer to all of these enzymes as nudix hydrolases and to their homologous amino acid region as the nudix motif or nudix signature sequence.

Structure-Function Relationships

In Fig. 1B, a check marks the nudix signature sequences for those proteins so far identified with enzymatic activities. The availability (through genetic engineering) of large quantities of these proteins in a highly purified state provides the opportunity for comparative studies aimed at dissecting out their mechanisms of catalysis and their modes of substrate binding. Most of the studies, so far, have been done with E. coli MutT, because it was the first of the nudix hydrolases purified (5, 6).

Tertiary Structure of MutT

The small size of MutT (129 amino acids) and its relative stability (3–6 days at 32 °C) along with its abundance in engineered cells (35 mg of pure protein per liter of culture) make it ideal for analysis by heteronuclear multidimensional NMR spectroscopy. The three-dimensional solution structure of MutT has recently been completed, and one view of it is shown in Fig. 2 (31). It consists of a 5-stranded, mixed β-sheet sandwiched between two α-helices connected by long loops. Of special interest are the positions of the amino acids of the nudix signature sequence. Situated mostly in loop I and helix I, they are closely arranged spatially and are readily accessible to the external environment. An examination of Fig. 1B reveals that of the several amino acid identities common to most of the proteins, only 4 amino acids are absolutely conserved in all of them. These are (for MutT) Gly-38, Glu-44, Arg-52, and Glu-57. Recently, the site-directed mutant, E57Q, has been constructed and shown to have at least 105-fold lower activity than wild type (32).

It will be of interest to see if other members of the nudix hydrolase family share the structural and chemical features of the E. coli MutT prototype, especially the architecture of the nudix motif. Koomba (4) has noted that these proteins share a common loop-helix-loop motif, and Thorne et al. (29) point out that a predictive analysis of human ApA-Ase hydrolase shows that the sequence ^55^ALRETQE^AEG^94 (see ApA-Ase in Fig. 1B) is solvent-accessible and has a probability of 71% of being α-helical and flanked by loops. Two other members of the nudix family have been crystallized, the Orf17 nucleoside triphosphatase,5 and the Orf1.9 GDP-mannose hydrolase,5 and so our information on the generality of the structural features of the nudix motif should be forthcoming.

Physiological Function of the Nudix Hydrolases, “Housecleaning” Enzymes?

The enzymes discussed in this review have two features in common. They share a small region of homology herein referred to as the nudix signature sequence, and they all hydrolyze X-linked nucleoside diphosphates. Besides these two similarities, they seem to be widely disparate in their substrate preferences, which include nucleoside triphosphates, coenzymes, nucleotide sugars, and dinucleoside polyphosphates. This would suggest that these enzymes are involved in diverse metabolic pathways. An important clue in establishing the function of a biologic agent is an observable change, a phenotype, associated with its under-or overproduction, and that has been done for only one of the nudix hydrolases, MutT itself. In this case it seems fairly well established that the MutT pyrophosphohydrolase inactivates a potentially mutagenic form of dGTP, thus “sanitizing” (5) the nucleotide pool at the site of DNA synthesis. The very recent discovery of the rest of these nudix hydrolases has precluded extensive studies of their roles in metabolism, and mutants have not been identified that might be tied to some phenotype. In lieu of this, it is interesting to note a common feature of these enzymes; they all seem to hydrolyze potentially hazardous materials, or they prevent the unbalanced accumulation of normal metabolites. In the former category, we may include, in addition to MutT itself, Orf17, Orf257, Orf186, and ApA-Ase hydrolase. For example, the Orf17 dATPase could possibly play a similar role to that suggested for MutT’s action on 8-oxo-dGTP (33) by hydrolyzing the recently reported, 2-hydroxy-dATP (34), that is remove a potentially mutagenic nucleotide from the pool. On the other hand, its role could be to prevent the accumulation of dATP.
Minireview: MutT Proteins or “Nudix” Hydrolases

in the cell. dATP is an important effector at a pivotal control point, because it turns off ribonucleoside-diphosphate reductase by feedback inhibition and thereby effectively shuts down the synthesis of all four deoxynucleotides (35). Orf257 has a 100-fold higher catalytic efficiency on NADH compared with NAD⁺. Under anaerobic conditions, the accumulation of NADH would inhibit NAD⁺-linked dehydrogenases, which are essentially reversible metabolic reactions. The hydrolysis of NADH by Orf257 would allow oxidation to proceed. Both Orf186E.coli and Ap₄A hydrolasehuman α mutant members of the diadenosine polyphosphates, compounds having the structure of Ap₄A, when n = 2–6. The first member of this family of compounds, Ap₄A, was discovered by Zamenick et al. (36) and since that time representatives of this class have been implicated in the initiation of DNA replication, as cell-signaling molecules in response to stress or heat shock (“alarmones”), as neurotransmitters, as effectors of the cardiovascular system, etc. (for review, see Ref. 37). The removal of these compounds would be part of the normal regulatory processes in the cell. It is noteworthy that the human FHit gene (for fragile histidine triade) has been implicated in digestive tract and lung cancers. It is 69% homologous to a gene that the nudix signature sequence had the “right chemistry” and widespread distribution and its diversity of application, it appears hard part; specificity is relatively easy to deal with later. As for its tasks. The state “Getting the chemistry right, it would seem, is the most likely the homologue of bacilliformis (42) and humans(43). Also noteworthy is protein 20, from basic fibroblast growth factor and have been identified in metabolic pool. deoxyribosylation. The authors hypothesize that the hydrolase is necessary to inactivate the toxic intermediate, 3'-deoxy-ATP, by hydrolyzing it to the monophosphate, another example of sanitizing the metabolic pool. Thus, by analogy to the “housekeeping” or maintenance genes, those coding for the nudix hydrolases could be considered “house-keeping” genes whose function is to cleanse the cell of potentially deleterious endogenous metabolites and to modulate the accumulation of intermediates in biochemical pathways.

Several as yet unspecified proteins are of special interest and on further investigation could reveal novel activities as augured by our experience with those members of the family identified so far. For example, attention is called to proteins 15 and 19 (Fig. 1B). These Orfs are encoded for by the antisense strand of the gene for basic fibroblast growth factor and have been identified in Xenopus (42) and humans (43). Also noteworthy is protein 20, from Bartonella bacilliformis, the causative agent of human Oroya fever. This protein is associated with the ability to invade erythrocytes (44). Orf154 (protein 14) from pSAM2, an integrating plasmid in Streptomyces ambofaciens capable of autonomous replication (45), is most likely the homologue of purT involved in purinobiosynthesis as mentioned previously. We have cloned the gene and purT of the protein and are currently examining its properties. In addition to its metabolic significance, its presence in an autonomously replicating and integrating plasmid may provide some clue to the widespread dissemination of the nudix genes.

In their interesting article on the origin and evolution of enzymatic species, Petsko et al. (46) argue that nature recruits pre-existent catalytic motifs and modifies them to perform related tasks. They state “Getting the chemistry right, it would seem, is the hard part; specificity is relatively easy to deal with later.” From its widespread distribution and its diversity of application, it appears that the nudix signature sequence had the “right chemistry” and was one of the primordial catalytic motifs selected and adapted during evolution.

REFERENCES

1. Bullions, L. C., Mejean, V., Claverys, J. P., and Bessman, M. J. (1993) FASEB J. 7, A1384 (abstr.)
2. Mejean, V., Salles, C., Bullions, L. C., Bessman, M. J., and Claverys, J. P. (1994) Mol. Microbiol. 11, 223–230
3. Bullions, L. C., Mejean, V., Claverys, J. P., and Bessman, M. J. (1994) J. Biol. Chem. 269, 12339–12344
4. Koonin, E. V. (1993) Nucleic Acids Res. 21, 4847
5. Bhatnagar, S. K., and Bessman, M. J. (1988) J. Biol. Chem. 263, 8953–8957
6. Bhatnagar, S. K., Bullions, L. C., and Bessman, M. J. (1991) J. Biol. Chem. 266, 9050–9054
7. Baczko, A., Bucher, P., and Hoffman, K. (1995) Nucleic Acids Res. 24, 189–196
8. Treffers, H. P., Spinelli, V., and Belder, N. O. (1954) Proc. Natl. Acad. Sci. U.S.A. 40, 1064–1071
9. Yanofsky, C., Cox, E., and Horn, V. (1966) Proc. Natl. Acad. Sci. U.S.A. 55, 274–281
10. Topal, M. D., and Fresco, J. R. (1976) Nature 263, 285–289
11. Schaeper, B. R., and Dunn, R. L. (1987) J. Biol. Chem. 262, 16267–16270
12. Akutsu, M., Horiiuchi, T., and Sekiguchi, M. (1987) Mol. Gen. Genet. 206, 9–16
13. Sakuni, K., Furuchi, M., Tsuzuki, T., Kakuma, T., Kawabata, S. I., Maki, H., and Sekiguchi, M. (1993) J. Biol. Chem. 268, 23524–23530
14. Takahagi, M., Iwasaki, H., Nakata, A., and Shinagawa, H. (1991) J. Bacteriol. 173, 5747–5753
15. Sharples, G. J., and Lloyd, G. R. (1991) J. Bacteriol. 173, 7711–7715
16. O’Hare, S. F., Frick, D. N., Bullions, L. C., Mildvan, A. S., and Bessman, M. J. (1996) J. Biol. Chem. 271, 24649–24654
17. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 205, 403–410
18. Weber, D. J., Bhatnagar, S. K., Bullions, L. C., Bessman, M. J., and Mildvan, A. S. (1992) J. Biol. Chem. 267, 16939–16942
19. Frick, D. N., and Bessman, M. J. (1996) J. Biol. Chem. 270, 1529–1534
20. Anyama, K., Haase, A. M., and Reeves, P. R. (1994) J. Biol. Chem. 270, 829–838
21. Frick, D. N., Townsend, B. D., and Bessman, M. J. (1995) J. Biol. Chem. 270, 64–65
22. Sonnio, S., Carminatti, H., and Cabib, E. (1966) J. Biol. Chem. 241, 1009–1010
23. Singh, R., A. G., Villalba, R., Moreno, A., Quintinilla, M., Lobaton, C. D., and Siliero, A. (1977) Eur. J. Biochem. 76, 351–377
24. Hurtado, C., Ruiz, A., Siliero, A., and Siliero, M. A. G. (1987) J. Bacteriol. 169, 1718–1722
25. Mechala, Y., Fromant, M., Pallet, M., Patureau, A., Blanclin, G., and Blanquet, S. (1985) J. Bacteriol. 164, 63–69
26. Cai, J. P., Kakuma, T., Tsuzuki, T., and Sekiguchi, M. (1995) Cancer Genes Cancer 5, 133–150
27. Kakuma, T., Hishida, J. I., Tsuzuki, T., and Sekiguchi, M. (1995) J. Biol. Chem. 270, 25942–25948
28. Lawaska, D., Starzyńska, E., and Guranowski, A. (1993) Protein Expression Purif. 4, 45–51
29. Thorne, M. H., Hankin, S., Wilkinson, M. C., Cuneo, C., Barraclough, R., and McLennan, A. G. (1995) Biochem. J. 311, 717–721
30. International Union of Biochemistry (1984) Enzyme Nomenclature, pp 362–368, Academic Press, Inc., New York
31. Abeygunawardana, C., Weber, D. J., Gittis, A., Frick, D. N., Lin, J., Miller, A. J., Bessman, M. J., and Mildvan, A. S. (1995) Biochemistry 34, 14997–15005
32. Lin, J., Abeygunawardana, C., Frick, D. N., Gittis, A., Frick, D. N., Lin, J., Miller, A. J., Bessman, M. J., and Mildvan, A. S. (1996) Biochemistry 35, 6715–6726
33. Maki, H., and Sekiguchi, M. (1992) Nature 355, 271–275
34. Kamath, A. V., and Yanofsky, C. (1993) Gene (Amst.) 134, 99–102