Minireview

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The MutT Proteins or "Nudix" Hydrolases, a Family of Versatile, Widely Distributed, "Housecleaning" Enzymes*

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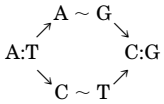
Our studies on the biochemical basis of spontaneous mutations took an interesting and unexpected turn when we discovered that a small region of amino acid homology between the MutT protein of *Escherichia coli* and the MutX protein of *Streptococcus pneumoniae* was involved in their nucleoside triphosphatase 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*s)[1] 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 **nu**cleoside **di**phosphate 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*, *mutT*1, 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 → CG 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 ~ G 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' *mutT*1 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 + H_2O → dGMP + PP_i.** Thus the enzyme is a nucleoside-triphosphate *pyro*phosphohydrolase, 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. 1*A*.

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 *ruvC* on the *E. coli* genome (14, 15) but not involved in the function of the *ruvC*-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: **dATP + H_2O → dAMP + PP_i.** dGTP is the *least* favored substrate for Orf17 (relative catalytic efficiency, dATP/dGTP = 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.

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[1] The abbreviations used are: Orf, open reading frame (protein); *orf*, open reading frame (gene); Ap_3A, diadenosine 5′,5‴-P^1,P^3-triphosphate (other diadenosine oligophosphates are abbreviated in an analogous manner). The names of the Orfs described in this review, as they appear in the data banks, derive from the following: Orf257, Orf209, Orf186 are proteins having 257, 209, and 186 amino acids, respectively; Orf17 is a 17-kDa protein; Orf1.9 is a locus on a physical map of a DNA fragment.

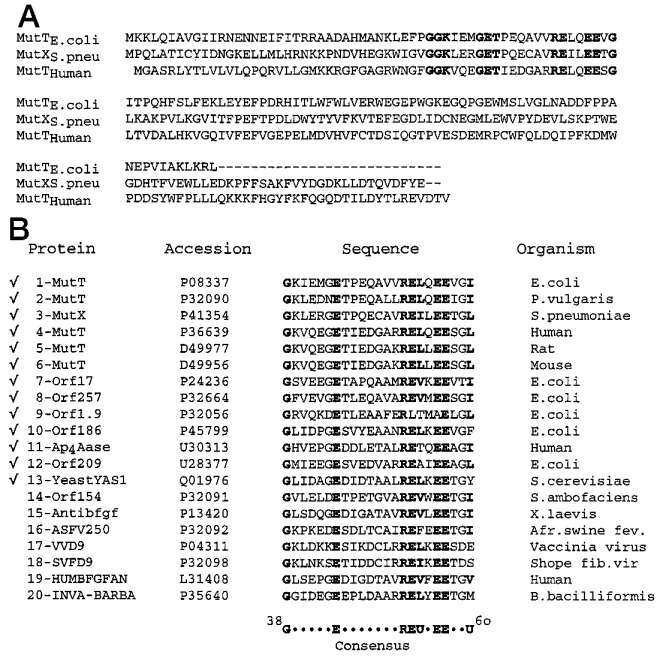

FIG. 1. **Consensus of the nudix hydrolases.** *A*, total sequences of the *E. coli* (11), MutX, (2), and human (13) MutT-related proteins showing the small region (*boldface*) of identity. *B*, part of a BLAST search (17) against the 23 amino acids of the MutT signature sequence. In the consensus sequence, U represents the bulky alphatic amino acids, I, L, or V. The *check marks* indicate those proteins so far identified with enzymatic activities.

"Exceptio Probat Regulam"

"The exception tests the rule." Fig. 1*B* depicts part of a recent BLAST (17) search of the SWISSPROT data base using the 23 amino acids spanning the $MutT_{E.\ coli}$ conserved sequence as the query. The broad distribution of the signature sequence, from viruses to humans, is clearly displayed. In addition to the MutT and Orf17 enzymes, four other *E. coli* open reading frames are present in the list: Orf257, Orf209, Orf186, and Orf1.9. Are they all nucleoside-triphosphate pyrophosphohydrolases?

orf257 was cloned, and the expressed protein was purified to homogeneity. To our surprise (and discomfort), it was inactive on all eight canonical nucleoside triphosphates. We therefore considered the possibility that this family of enzymes catalyzed a general pyrophosphoryl *transfer*, wherein water was the acceptor for the previous enzymes (18), and that the proper acceptor was missing for Orf257. Accordingly, we set up an NADH-linked indicator assay to follow, indirectly, the disappearance of nucleoside triphosphates and found that NADH was rapidly degraded in the absence of any added nucleoside triphosphates or putative acceptor. On further investigation (19), it was shown that Orf257 represents a new member of the dinucleotide pyrophosphatases (EC 3.6.1.9) with a unique specificity for the *reduced* form of the co-factor (NADH/NAD^+ = 100) and with an absence of activity on any of the nucleoside triphosphates. The reaction may be represented as follows: **NADH + H_2O → NMNH + AMP.** In addition to its unusual specificity, this is the only known metabolic pathway for the generation of NMNH. Thus, Orf257 was not only an interesting enzyme, but it was also the harbinger of a series of new enzymes, since it broadened the scope of the reactions identified with the conserved sequence.

Additional Members of the Family

Orf1.9—This open reading frame was so named because of its map position in a fragment of DNA from the *cps* region of *E. coli* K12 (20). The *cps* region is involved in the synthesis of the capsular polysaccharide known as M-antigen, or colanic acid, and *orf1.9* is near *cpsB* and *cpsG*, which code for GDP-mannose pyrophosphorylase and phosphomannomutase, respectively. Orf1.9 was purified from an expression system (21), and it, too, did not hydrolyze nucleoside triphosphates. Instead, it did have an atypical, sugar-nucleotide hydrolase activity catalyzing the following reaction: **GDP-mannose + H_2O → GDP + mannose.** The release of the free sugar from sugar nucleotides is rare in metabolism. Only one other enzyme, yeast GDP-glucose glucohydrolase (EC 3.2.1.42), catalyzes a similar reaction (22), but its properties clearly distinguish it from the Orf1.9 GDP-mannosyl hydrolase.

Orf186—This open reading frame was identified as a hypothetical 21.2-kDa protein in the *E. coli* genome-sequencing project. It was cloned, expressed, and purified[2] and is most active on Ap_3A, hydrolyzing it as follows: **Ap_3A + H_2O → ADP + AMP.** It has substantial activity on NADH as well but does not hydrolyze nucleoside triphosphates. Rat liver cytoplasmic extracts have an active Ap_3A triphosphatase (EC 3.6.1.29) (23), and *E. coli* has a related enzyme (24), but both can be distinguished from Orf186 by their substrate specificities. *E. coli* contains an active Ap_4A tetraphosphatase as well, the product of the *apaH* gene (25), but it, too, is distinct from Orf186.

Orf209—This protein was also identified as part of the *E. coli* sequencing project and is located in the 65–68-minute region of the genome. It was cloned, expressed, and purified[3] and is highly specific for ADP-sugars, with its highest activity on ADP-ribose. Unlike Orf1.9, Orf209 catalyzes the hydrolysis of the pyrophosphate linkage: **ADP-ribose + H_2O → AMP + ribose 5-P.** It hydrolyzes ADP-glucose and ADP-mannose at 80 and 40% of the rate, respectively, but it has little or no activity on the corresponding GDP- or UDP-sugars and no activity on the nucleoside triphosphates.

This concludes the list of *E. coli* genes bearing the conserved sequence uncovered so far. Of the six enzymes identified, two are nucleoside triphosphatases, and the remaining four catalyze the hydrolysis of a variety of substrates. How do these enzymes relate to those in higher organisms?

YSA1—Although several open reading frames from higher organisms harboring the conserved sequence had been deposited in the data banks (see Fig. 1*B*), the only non-bacterial members of this family for whom enzymatic activities had been described were the nucleoside triphosphatases from human (13), rat (26), and mouse (27), all homologues of *E. coli* MutT. We have recently cloned and expressed a gene, *YSA1*, from chromosome II of yeast, having the conserved sequence and which we find is closely related, in function, to Orf209 of *E. coli*.[4] It prefers ADP-ribose but hydrolyzes ADP-glucose and ADP-mannose at about half the rate. Nucleoside triphosphates, Ap_4A, and Ap_3A are not substrates for the enzyme.

Ap_4A Tetraphosphatase—This enzyme, an asymmetrical Ap_4A hydrolase producing ATP and AMP from Ap_4A, was purified from human placenta and characterized by Lazewska *et al.* (28). It has recently been cloned from placenta by Thorne *et al.* (29) and shown to have the conserved motif. Its high specificity for Ap_4A and inactivity on Ap_3A clearly distinguish it from Orf186.

From these last two examples, it is evident that the members of this family are not confined to the bacteria and that additional activities will be identified with other Orfs from the eukaryotes listed in Fig. 1*B*.

Substrate Specificity

As pointed out in the Introduction, attention was focused on the small region of homology between the MutT and MutX proteins, because biochemical and genetic experiments identified this sequence of amino acids as important for enzymatic activity *in vitro* and also for the maintenance of normal mutation rates *in vivo* (2). From these two enzymes, the list of proteins has grown to 13, and many more, no doubt, will follow. Those identified, so far, are shown in Table I, along with their preferred substrates and the reactions they catalyze. All of these enzymes belong in the category, EC 3.6.1, the phosphoanhydrases (30). This large group of enzymes ranges from inorganic pyrophosphatase to plasma membrane calcium-transporting ATPase and includes all enzymes hydrolyzing a pyrophosphate linkage. The enzymes listed in Table I, although seemingly eclectic in their selection of substrates, are, in fact, closely related to each other by their requirement for a particular class of compounds as pointed out earlier, *i.e.* a nucleoside diphosphate linked to some other component, *X*. The original *E. coli*

[2] S. F. O'Handley, D. N. Frick, and M. J. Bessman, work in progress.
[3] D. N. Frick and M. J. Bessman, work in progress.
[4] C. Dunn, D. N. Frick, S. F. O'Handley, and M. J. Bessman, work in progress.

TABLE I
Substrate specificities of the 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[a]	Source	Substrate	*X*	Products
MutT (5)	*E. coli*	(d)NTP	P_i	NMP + PP_i
MutT (47)	*Proteus vulgarus*	(d)NTP	P_i	NMP + PP_i
MutX (3)	*S. pneumoniae*	(d)NTP	P_i	NMP + PP_i
8-oxo-dGTPase (13)	Human	8-oxo-dGTP	P_i	NMP + $[PP_i]^b$
8-oxo-dGTPase (26)	Rat	8-oxo-dGTP	P_i	NMP + $[PP_i]^b$
8-oxo-dGTPase (27)	Mouse	8-oxo-dGTP	P_i	NMP + $[PP_i]^b$
Orf17 (16)	*E. coli*	(d)NTP	P_i	NMP + PP_i
Orf257 (19)	*E. coli*	NADH	NRH[c]	NMNH + AMP
Orf1.9 (21)	*E. coli*	GDP-mannose	Mannose	GDP + mannose
Orf186[2]	*E. coli*	Ap_3A	AMP	AMP + ADP
Orf209[3]	*E. coli*	ADP-ribose	Ribose	AMP + ribose-5-P
YSA1[4]	*Saccharomyces cerevisiae*	ADP-ribose	Ribose	AMP + ribose-5-P
Ap_4Aase (29)	Human	Ap_4A	ADP	ATP + AMP

[a] The numbers in parentheses are references to the published work, and the superscripts refer to the footnotes in the body of the text.
[b] Not reported.
[c] Reduced nicotinamide ribonucleoside.

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. 1*B*, 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. 1*B* 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 10^5-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. Koonin (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 Ap_4A hydrolase shows that the sequence ^{55}ALRETQEEAG^{64} (see Ap_4Aase in Fig. 1*B*) 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,[6] 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 *over*production, 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 Ap_4A 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

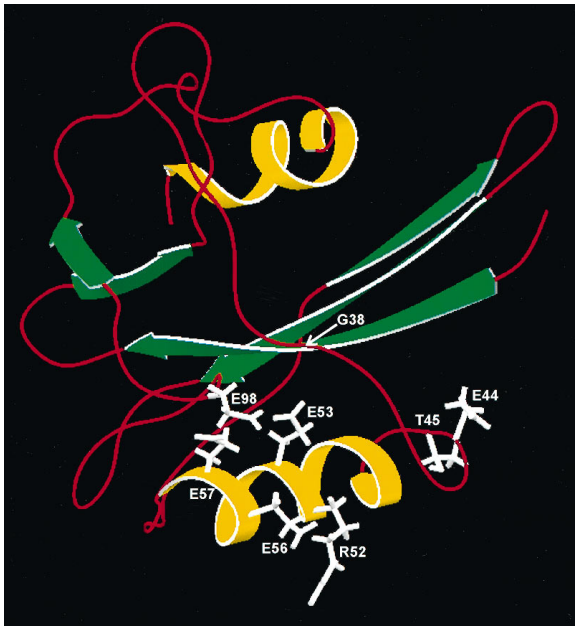

FIG. 2. **Ribbon diagram of *E. coli* MutT showing amino acids of the conserved sequence.** Reprinted from Ref. 31 with permission. Copyright (1995) American Chemical Society.

[5] S. Gabelli, M. J. Bessman, and L. M. Amzel, work in progress.
[6] J. J. Habel, M. J. Bessman, and S. Quirk, work in progress.

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 anaerobiosis, 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 **Orf186**$_{E.\ coli}$ and **Ap$_4$A hydrolase**$_{human}$ act on members of the diadenosine polyphosphates, compounds having the structure of Ap_nA, when $n = 2–6$. The first member of this family of compounds, Ap_4A, was discovered by Zamecnik *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 triad) has been implicated in digestive tract and lung cancers. It is 69% homologous to a gene from the yeast, *Schizosaccharomyces pombe*, which codes for an Ap_4A hydrolase (quoted in Ref. 38). The remaining three members of the nudix hydrolase series identified so far, **Orf1.9**, **Orf209**, and **YSA1**, are sugar-nucleotide hydrolases. Glaser (39) and Sonnino *et al.* (40) in their studies on sugar-nucleotide metabolism point out that the hydrolases play an important role in preventing the accumulation of the nucleoside diphosphate-sugar intermediates and in diverting them for reutilization as the different classes of polysaccharides are synthesized during cell maturation. The latter two enzymes, Orf209 and YSA1, having ADP-ribose as their preferred substrate could play a role in the ubiquitous pathways of ADP-ribosylation. Recently, Tercero *et al.* (41) cloned and sequenced the genes involved in the puromycin biosynthetic pathway in *Streptomyces alboniger*. One of them, *pur7*, has the nudix signature sequence. The authors hypothesize that the hydrolase is necessary to inactivate the toxic intermediate, 3′-keto-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 "housecleaning" 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. 1*B*). These Orfs are coded 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 *pur7* involved in puromycin biosynthesis mentioned previously. We have cloned the gene and purified the protein and are currently examining its properties.[7] 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 preexisting 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.

[7] C. Dunn and M. J. Bessman, work in progress.

REFERENCES

1. Bullions, L. C., Méjean, V., Claverys, J. P., and Bessman, M. J. (1993) *FASEB J.* **7,** A1384 (abstr.)
2. Méjean, V., Salles, C., Bullions, L. C., Bessman, M. J., and Claverys, J. P. (1994) *Mol. Microbiol.* **11,** 323–330
3. Bullions, L. C., Méjean, 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. Bairoch, A., Bucher, P., and Hoffman, K. (1995) *Nucleic Acids Res.* **24,** 189–196
8. Treffers, H. P., Spinelli, V., and Belser, N. O. (1954) *Proc. Natl. Acad. Sci. U. S. A.* **40,** 1064–1071
9. Yanofsky, C., Cox, E. C., 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. Schaaper, R. M., and Dunn, R. L. (1987) *J. Biol. Chem.* **262,** 16267–16270
12. Akiyama, M., Horiuchi, T., and Sekiguchi, M. (1987) *Mol. Gen. Genet.* **206,** 9–16
13. Sakumi, K., Furuichi, 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, R. G. (1991) *J. Bacteriol.* **173,** 7711–7715
16. O'Handley, 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., Meyers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* **203,** 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. (1995) *J. Biol. Chem.* **270,** 1529–1534
20. Aoyama, K., Haase, A. M., and Reeves, P. R. (1994) *Mol. Biol. Evol.* **11,** 829–838
21. Frick, D. N., Townsend, B. D., and Bessman, M. J. (1995) *J. Biol. Chem.* **270,** 1–6
22. Sonnino, S., Carminatti, H., and Cabib, E. (1966) *J. Biol. Chem.* **241,** 1009–1010
23. Sillero, M. A. G., Villalba, R., Moreno, A., Quintinilla, M., Lobaton, C. D., and Sillero, A. (1977) *Eur. J. Biochem.* **76,** 331–377
24. Hurtado, C., Ruiz, A., Sillero, A., and Sillero, M. A. G. (1987) *J. Bacteriol.* **169,** 1718–1723
25. Mechulam, Y., Fromant, M., Mallot, P., Plateau, P., Blancin-Roland, S., Fayat, G., and Blanquet, S. (1985) *J. Bacteriol.* **164,** 63–69
26. Cai, J. P., Kakuma, T., Tsuzuki, T., and Sekiguchi, M. (1995) *Carcinogenesis* **16,** 2343–2350
27. Kakuma, T., Hishida, J. I., Tsuzuki, T., and Sekiguchi, M. (1995) *J. Biol. Chem.* **270,** 25942–25948
28. Lazewska, D., Starzynska, E., and Guranowski, A. (1993) *Protein Expression Purif.* **4,** 45–51
29. Thorne, M. H., Hankin, S., Wilkinson, M. C., Nunez, 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.-F., Bessman, M. J., and Mildvan, A. S. (1995) *Biochemistry* **34,** 14997–15005
32. Lin, J., Abeygunawardana, C., Frick, D. N., Bessman, M. J., and Mildvan, A. S. (1996) *Biochemistry* **35,** 6715–6726
33. Maki, H., and Sekiguchi, M. (1992) *Nature* **355,** 273–275
34. Kamiya, H., and Kasai, H. (1995) *J. Biol. Chem.* **270,** 19446–19450
35. Thelander, L., and Reichard, P. (1979) *Annu. Rev. Biochem.* **48,** 133–158
36. Zamecnik, P. C., Stephenson, M. L., Janeway, C. M., and Randerath, K. (1966) *Biochem. Biophys. Res. Commun.* **24,** 91–97
37. Baxi, M. D., and Vishivanatha, V. K. (1995) *J. Pharmacol. Toxicol. Methods* **33,** 121–128
38. Sozzi, G., Veronese, M. L., Negrini, M., Baffa, R., Cotticelli, M. G., Inoue, H., Tornielli, S., Pilotti, S., De Gregorio, L., Pastorino, V., Pierotti, M. A., Ohta, M., Huebner, K., and Croce, M. (1996) *Cell* **85,** 17–26
39. Glaser, L. (1965) *Biochim. Biophys. Acta* **101,** 6–15
40. Sonnino, S., Carminatti, H., and Cabib, E. (1966) *Arch. Biochem. Biophys.* **116,** 26–33
41. Tercero, J. A., Espinosa, C., Lacalle, R. A., and Jiménez, A. (1996) *J. Biol. Chem.* **271,** 1579–1590
42. Volk, R., Köster, M., Pöting, A., Hartmann, L., and Knöchel, W. (1989) *EMBO J.* **8,** 2983–2988
43. Murphy, P. R., and Knee, R. S. (1994) *Mol. Endocrinol.* **8,** 852–859
44. Mitchell, S. J., and Minnick, M. F. (1995) *Infect. Immun.* **63,** 1552–1562
45. Hagége, J., Pernodet, J.-L., Friedmann, A., and Guerineau, M. (1993) *Mol. Microbiol.* **10,** 799–812
46. Petsko, G., Kenyon, G. L., Gerlt, J. A., Ringe, D., and Kozarich, J. W. (1993) *Trends Biochem. Sci.* **18,** 372–376
47. Kamath, A. V., and Yanofsky, C. (1993) *Gene* (*Amst.*) **134,** 99–102