Biochemical and Biophysical Research Communications **287,** 204–208 (2001)
doi:10.1006/bbrc.2001.5570, available online at http://www.idealibrary.com on IDEAL®

Theoretical 3D Model of Histamine *N*-Methyltransferase: Insights into the Effects of a Genetic Polymorphism on Enzymatic Activity and Thermal Stability

Yuan-Ping Pang,*,†,‡,[1] Xiang-E Zheng,* and Richard M. Weinshilboum*

**Department of Molecular Pharmacology and Experimental Therapeutics, †Mayo Clinic Cancer Center, and ‡Tumor Biology Program and Molecular Neuroscience Program, Mayo Foundation for Medical Education and Research, 200 First Street SW, Rochester, Minnesota 55905*

Received August 1, 2001

Histamine *N*-methyltransferase (HNMT) catalyzes the N-methylation of histamine in mammals. The experimentally determined HNMT three-dimensional (3D) structure is not available. However, there is a common genetic polymorphism for human HNMT (Thr105Ile) that reduces enzymatic activity and is a risk factor for asthma. To obtain insights into mechanisms responsible for the effects of that polymorphism on enzymatic activity and thermal stability, we predicted the 3D structure of HNMT using the threading method and molecular dynamics simulations in water. Herein, we report a theoretical 3D model of human HNMT which reveals that polymorphic residue Thr105Ile is located in the turn between a beta strand and an alpha helix on the protein surface away from the active site of HNMT. Ile105 energetically destabilizes folded HNMT because of its low Chou–Fasman score for forming a turn conformation and the exposure of its hydrophobic side chain to aqueous solution. It thus promotes the formation of misfolded proteins that are prone to the clearance by proteasomes. This information explains, for the first time, how genetic polymorphisms can cause enhanced protein degradation and why the thermal stability of allozyme Ile105 is lower than that of Thr105. It also supports the hypothesis that the experimental observation of a significantly lower level of HNMT enzymatic activity for allozyme Ile105 than that with Thr105 is due to a decreased concentration of allozyme Ile105, but not an alternation of the active-site topology of HNMT caused by the difference at residue 105.

Key Words: **histamine *N*-methyltransferase; single nucleotide polymorphisms; *S*-adenosyl-L-methionine; protein structure prediction; threading modeling; molecular dynamics simulations.**

Pharmacogenomics is the study of the genetic basis for individual variation in response to drugs and other xenobiotics. Successful prediction of the effects of genetic variations that change the encoded amino acid sequence on protein function and their consequent biomedical implications may be assisted by three-dimensional (3D) structures of the encoded amino acid sequences. Methyltransferases that catalyze the addition of a methyl group to biological molecules are a class of enzymes with functionally significant genetic variations (1). Unfortunately, only two small-molecule *S*-adenosyl-L-methionine (AdoMet)-dependent methyltransferase 3D structures have been determined experimentally (2, 3). The 3D structures of most small-molecule methyltransferase 3D structures have not been determined experimentally, nor are they readily available from homology modeling since their sequences share less than 30% sequence identity with known protein 3D structures. To facilitate pharmacogenomic studies of a large number of methyltransferases identified by the Human Genome Project (4), we have been determining computationally 3D structures of small-molecule AdoMet-dependent methyltransferases that are genetically remote from all methyltransferases whose 3D structures have already been determined experimentally.

One such methyltransferase is histamine *N*-methyltransferase (HNMT). Using a cosubstrate, AdoMet, as a methyl donor, this enzyme catalyzes the N-methylation of histamine, one of the two major pathways for the biotransformation of histamine in mammals, and the only process responsible for inactivation of the neurotransmitter actions of histamine in the mammalian central nervous system (5–9). The experimentally determined 3D structure of this enzyme is currently unavailable. However, there is a common genetic polymorphism for HNMT in humans (Thr105Ile) that results in decreased enzyme activity, decreased immu-

[1] To whom correspondence should be addressed. Fax: 507-284-9111. E-mail: pang@mayo.edu.

0006-291X/01 $35.00

noreactive protein and decreased thermal stability (10). Functional genomic association studies have led to evidence that this genetic polymorphism might contribute to the role of inheritance in risk for asthma (11). Computational prediction of the 3D structure of HNMT which may shed light on mechanisms responsible for the effects of genetic polymorphisms on HNMT function would be desirable.

Herein, we report a theoretical 3D model of human HNMT predicted by a heuristic approach using the threading method and molecular dynamics simulations. This model provides insights into a structural mechanism for the effects of a common single nucleotide polymorphism in HNMT on enzymatic activity and thermal stability.

METHODS

The all-atom 3D model of truncated human HNMT (Met1-Asn249) was generated by the MaxSprout program (12) using the corresponding alpha-carbon 3D model which was first generated according to the folding patterns of catechol *O*-methyltransferase (COMT) (2) and NAD^+ synthetase (13) using the 3D-PSSM program (14). Gaps in the all-atom 3D model were closed manually by inserting the residue(s) that were not determined in the α-carbon 3D model by the 3D-PSSM program because of the low similarity between HNMT and the folding templates. The backbone and side chain conformations of each inserted residue were manually adjusted according to the secondary structure of Met1-Asn249 predicted by the Psi-Pred program (15). All inserted residues were energy minimized with a positional constraint applied to the entire system except for the inserted residues using the Sander module of the AMBER 5.0 program (16). Manual adjustments were also made to enforce that all the residues from Met1 to Asn249 except for Gly were in the L-form and that all peptide bonds were in the *trans* configuration. The protein was then immersed in a box of water molecules (TIP3P) (17). The resulting system was energy minimized for 500 steps, gradually heated to 298 K over 30 ps, and then refined by a molecular dynamics (MD) simulation using the Sander module of the AMBER 5.0 program. All MD simulations were carried out by employing the Cornell *et al.* force field (18), and used (i) a dielectric constant of 1.0; (ii) the Berendsen coupling algorithm (19); (iii) a periodic boundary condition in the NPT ensemble at constant temperature of 298K and constant pressure of 1 atm with isotropic molecule-based scaling; (iv) the Particle Mesh Ewald (PME) method to calculate the long-range electrostatic interactions (20); and (iv) default values of all other inputs of the SANDER module.

RESULTS AND DISCUSSION

The Heuristic approach. As a result of the genetically remote relationship of HNMT to COMT (2) and glycine *N*-methyltransferase (GNMT) (3), two small-molecule methyltransferases whose crystal structures have already been reported, the homology approach, which was applicable to the prediction of the 3D structures of thiopurine *S*-methyltransferase and indolethylamine *N*-methyltransferases (21, 22), failed to yield a 3D structure of HNMT that could sustain a 1.0 ns (1.0 fs time step) MD simulation, a process which served as a self-consistency test. The 3D structure of HNMT, generated from homology modeling using the crystal structure of COMT as a template, partially unfolded, resulting in a collapse of the active site during the 1.0 ns MD simulation.

The threading method (23, 24) was then used to predict the 3D structure of HNMT. Although low similarity was found between the HNMT sequence and any known 3D protein structure, two threading-based programs (3D-PSSM and INBGU (14, 25)) predicted that the folding of the human HNMT amino acid sequence (NCBI Entrez Protein code: NP_008826) was similar to those of NAD^+ synthetase (13) and COMT (2). Use of the crystal structures of NAD^+ synthetase and COMT as the folding templates in predicting the HNMT 3D structure was encouraged by the fact that all these enzymes have an adenine-containing cosubstrate in the active site (2, 10, 13), and the fact that the secondary structure of the all-atom 3D model of the truncated human HNMT (Met1-Asn249), which was constructed by the MaxSprout program using the α-carbon coordinates generated by the 3D-PSSM program, was consistent with the secondary structure of human HNMT predicted by the Psi-Pred program (15) from the human HNMT amino acid sequence. Since the similarities of HNMT to COMT and NAD^+ synthetase were 9 and 19%, respectively, suggesting that HNMT had regions in its 3D structure markedly different from those of NAD^+ synthetase and COMT, the β strand and coils in the *C*-terminus of HNMT were manually adjusted to adopt the seven-strand β sheet which is commonly seen in the 3D structures of methyltransferases (26–28). The resulting structure was refined by a 1.0 ns (1.0 fs time step) MD simulation in water.

3D model of HNMT. The MD simulation-refined 3D structure of the truncated human HNMT (Met1-Asn249) had a typical α/β fold with nine helices and six β strands (Fig. 1). Helices α4-α5 and the parallel beta-strands beta1-beta3 were similar to the Rossmann folds found in the crystal structures of GNMT and COMT. This structure was considered as a putative 3D model of human HNMT for a series of reasons. First, one pocket as a potential active site of HNMT was found in the 3D model, although it was not built intentionally during the 3D model building process. Second, the conserved methyltransferase region I (Ile56-Gly64) in HNMT, which was reportedly part of the AdoMet binding pocket in the enzyme (2, 3, 29), was located in the pocket of the HNMT 3D model. Third, the conserved methyltransferase region III (Leu155-Thr164) in HNMT (29) was located away from the pocket, consistent with the X-ray structures of GNMT and COMT in which region III was also away from the active site (2, 3). Fourth, the 3D structure sustained a 1.0 ns MD simulation and no unfolding or pocket collapse were observed during the MD simulation.

Biological implications. On the basis of Western blot immunoreactivity experiments, we previously pro-

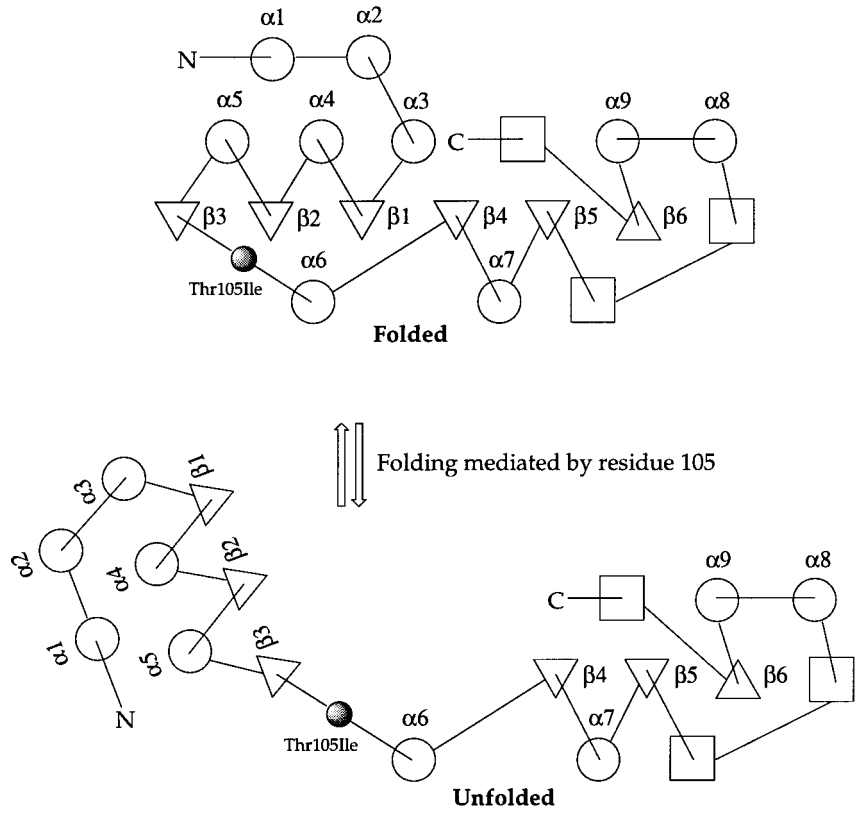

FIG. 1. Schematic topology diagram of the 3D structure of the truncated human HNMT (Met1-Asn249) refined by a 1.0 ns (1.0 fs time step) MD simulation and its folding process mediated by residue 105 (triangle, β strand; circle, α helix; and square, β strand-like).

posed that a significantly lower level of HNMT activity for allozyme Ile105 than allozyme Thr105 was due to a reduction of the concentration of allozyme Ile105, but not an alternation of the active-site topology of HNMT caused by the difference at residue 105 (10). This hypothesis was supported by the reports that common polymorphisms for another methyltransferase, thiopurine *S*-methyltransferases (TPMT), resulted in enhanced protein degradation, through an ATP-dependent proteasomal pathway, leading to low or undetectable levels of immunoreactive TPMT protein and enzymatic activity (30, 31). However, the mechanism(s) by which genetic polymorphisms that alter encoded amino acids lead to enhanced protein degradation was unclear.

The present work supports, at the structural level, our hypothesis with regard to HNMT, and explains, for the first time, how genetic polymorphisms might lead to enhanced protein degradation. The theoretical 3D structure of human HNMT shows that polymorphic residue Thr105Ile is located on the protein surface away from the active site of HNMT (Fig. 2). This structural information suggests that the enzymatic activity for allozyme Ile105 be not likely decreased by residue 105 residing in the active site of HNMT. In contrast, it might be altered by the folding of HNMT which is, in part, governed by residue 105 because this residue is critically located in the turn between β3 and α6 (Figs. 1 and 2). Mutations involving residue 105 can thus affect the structural stability of folded HNMT. When a mutation destabilizes a folded protein, it promotes the formation of misfolded proteins that are prone to the clearance by proteasomes that have evolved, in part, for folding-quality control (32–35). Therefore, residue 105 plays an important role in controlling the amount of folded HNMT and its enzymatic activity. According to the theoretic model of HNMT, Ile105 energetically destabilizes the folded enzyme because of its low Chou–Fasman score (0.47) for forming a turn conformation in comparison to that of Thr (0.96) and because of the exposure of its hydrophobic side chain to aqueous solution, which results in decreased thermal stability and misfolding which in turn leads to decreased immunoreactive protein and decreased enzymatic activity.

The present work also suggests that the involvement of a polymorphic residue in the control of the folding of HNMT, which controls the amount of folded HNMT and the enzymatic activity, may be applicable to other small-molecule methyltransferases such as TPMT. In a reported theoretical model of TPMT, polymorphic residue Ala154Thr (TPMT*2) is situated in the loop between a β strand and an α helix on the surface of the

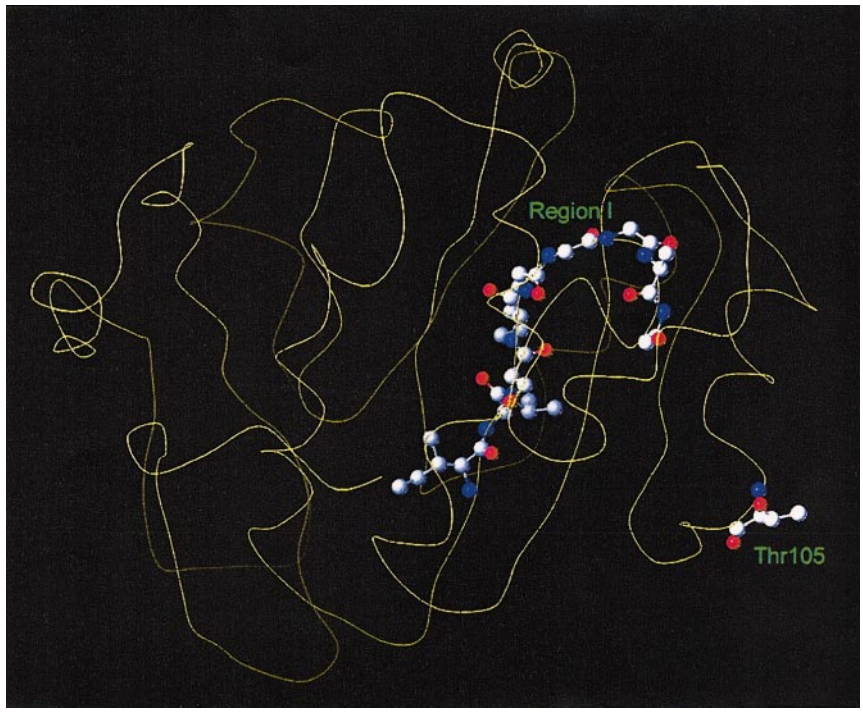

FIG. 2. Overview of the truncated human HNMT (Met1-Asn249) showing the spatial relationship of Thr105 located in a turn to the conserved methyltransferase region I (Ile56-Gly64) in the active site.

protein away from the TPMT active site (21). This structural arrangement is very similar to that of Ile105Thr in the 3D model of HNMT. Therefore, Ala154Thr might be responsible for the folding of TPMT and subsequently for the decreased concentration of TPMT protein which has been reported for this allozyme, although TPMT residue 154 was reportedly predicted to be indirectly involved in the binding of AdoMet (21).

On the basis of this chain of logic, it would be predicted that the degradation half-life for HNMT with Ile105 might be significantly shorter than that for Thr105 allozyme. That hypothesis should be tested experimentally using a pules-chase experiment which was employed in the studies of degradation half-lives of TPMT allozymes (30, 31). If that hypothesis were confirmed experimentally, it would be logical to propose the use of thermal stability (T_{50}) as a potential measure of the degradation half-lives of this class of methyltransferases with functionally significant genetic variations and this thermal stability parameter might be useful in determining risk factors in clinical practice.

Conclusion. We have predicted the 3D model of a truncated human HNMT (Met1-Asn249) using a heuristic approach. The theoretical 3D structure of human HNMT reveals that polymorphic residue Thr105Ile is located in the turn between β3 and α6 on the protein surface away from the active site of HNMT, and plays a role in the folding of HNMT. This information explains, for the first time, how genetic polymorphisms that alter encoded amino acids might result in enhanced protein degradation and why the thermal stability of allozyme Ile105 is lower than that of Thr105. It also supports the hypothesis that the experimental observation of a significantly lower level of enzyme activity for allozyme Ile105 than the one encoded with Thr105 is due to a reduction of the concentration of allozyme Ile105, but not an alternation of the active-site topology of HNMT caused by the difference at residue 105.

Coordinates deposition. The coordinates of the 3D model of the truncated human HNMT (Met1-Asn249) was deposited to the Protein Data Bank on April 2, 2001 (PDB code: 1ICZ).

ACKNOWLEDGMENTS

This work was supported by the Mayo Foundation for Medical Education and Research and in part by the U01 GM61388-01B (R.M.W. and Y.P.P.) and the R01 GM28157 (R.M.W.).

REFERENCES

1. Weinshilboum, R. M., Otterness, D. M., and Szumlanski, C. L. (1999) Methylation pharmacogenetics: Catechol *O*-methyltransferase, thiopurine methyltransferase, and histamine *N*-methyltransferase [Review]. *Ann. Rev. Pharmacol.* **39,** 19–52.
2. Vidgren, J., Svensson, L. A., and Liljas, A. (1994) Crystal structure of catechol *O*-methyltransferase. *Nature* **368,** 354–358.

3. Fu, Z., Hu, Y., Konishi, K., Takata, Y., Ogawa, H., Gomi, T., Fujioka, M., and Takusagawa, F. (1996) Crystal structure of glycine *N*-methyltransferase from rat liver. *Biochemistry* **35,** 11985–11993.
4. Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amanatides, P., Ballew, R. M., Huson, D. H., Wortman, J. R., Zhang, Q., Kodira, C. D., Zheng, X. Q. H., Chen, L., Skupski, M., Subramanian, G., Thomas, P. D., Zhang, J. H., Miklos, G. L. G., Nelson, C., and et al. (2001) The sequence of the human genome. *Science* **291,** 1304–1351.
5. Brown, D. D., Tomchick, R., and Axelrod, J. (1959) The distribution and properties of a histamine-methylating enzyme. *J. Biol. Chem.* **234,** 2948–2950.
6. Kahlson, G., Rosengsen, E., Swahn, D., and Thunberg, R. (1964) Mobilization and formation of histamine in the gastric mucosa as related to gastric secretion. *J. Physiol. (Lond.)* **174,** 400–416.
7. Wasserman, S. I. (1983) Mediators of immediate hypersensitivity. *J. Allergy. Clin. Immun.* **72,** 101–119.
8. Schwartz, J. C., Barbin, G., Garbarg, B. M., Pollard, H., Rose, C., and Verdiere, M. (1976) Neurochemical evidence for histamine acting as a transmitter in mammalian brain. *Adv. Biochem. Psychoph.* **15,** 111–126.
9. Hough, L. B., and Green, J. P. (1984) Histamine and its receptors in the nervous system. *Handb. Neurochem.* **6,** 145–211.
10. Preuss, C. V., Wood, T. C., Szumlanski, C. L., Raftogianis, R. B., Otterness, D. M., Girard, B., Scott, M. C., and Weinshilboum, R. M. (1998) Human histamine *N*-methyltransferase pharmacogenetics: Common genetic polymorphisms that alter activity. *Mol. Pharmacol.* **53,** 708–717.
11. Yan, L., Galinsky, R. E., Bernstein, J. A., Liggett, S. B., and Weinshilboum, R. M. (2000) Histamine *N*-methyltransferase pharmacogenetics: Association of a common functional polymorphism with asthma. *Pharmacogenetics* **10,** 261–266.
12. Holm, L., and Sander, C. (1991) Database algorithm for generating protein backbone and side-chain co-ordinates from a C alpha trace application to model building and detection of co-ordinate errors. *J. Mol. Biol.* **218,** 183–194.
13. Rizzi, M., Bolognesi, M., and Coda, A. (1998) A novel deamido-NAD+-binding site revealed by the trapped NAD-adenylate intermediate in the NAD+ synthetase structure. *Structure* **6,** 1129–1140.
14. Kelley, L. A., MacCallum, R. M., and Sternberg, M. J. E. (2000) Enhanced genome annotation using structural profiles in the program 3D-PSSM. *J. Mol. Biol.* **299,** 499–520.
15. Jones, D. T. (1999) Protein secondary structure prediction based on position-specific scoring matrices. *J. Mol. Biol.* **292,** 195–202.
16. Pearlman, D. A., Case, D. A., Caldwell, J. W., Ross, W. S., Cheatham, T. E., III, Debolt, S., Ferguson, D., Seibel, G., and Kollman, P. A. (1995) AMBER, a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules. *Comput. Phys. Commun.* **91,** 1–41.
17. Jorgensen, W. L., Chandreskhar, J., Madura, J. D., Impey, R. W., and Klein, M. L. (1982) Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* **79,** 926–935.
18. Cornell, W. D., Cieplak, P., Bayly, C. I., Gould, I. R., Merz, K. M., Jr., Ferguson, D. M., Spellmeyer, D. C., Fox, T., Caldwell, J. W., and Kollman, P. A. (1995) A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.* **117,** 5179–5197.
19. Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., Di Nola, A., and Haak, J. R. (1984) Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* **81,** 3684–3690.
20. Darden, T. A., York, D. M., and Pedersen, L. G. (1993) Particle Mesh Ewald: An N log(N) method for Ewald sums in large systems. *J. Chem. Phys.* **98,** 10089–10092.
21. Lysaa, R. A., Sylte, I., and Aarbakke, J. (1998) A three dimensional model of human thiopurine methyltransferase—Ligand interactions and structural consequences of naturally occurring mutations. *J. Mol. Model.* **4,** 211–220.
22. Thompson, M. A., Weinshilboum, R. M., ElYazal, J., Wood, T. C., and Pang, Y. P. (2001) Rabbit indolethylamine *N*-methyltransferase three-dimensional structure prediction: A model approach to bridge sequence to function in pharmacogenomic studies. *J. Mol. Model,* in press.
23. Jones, D. T., and Thornton, J. M. (1993) Protein fold recognition. *J. Comput.-Aid. Mol. Des.* **7,** 439.
24. Wodak, S. J., and Rooman, M. J. (1993) Generating and testing protein folds. *Curr. Opin. Struct. Biol.* **3,** 247–259.
25. Fischer, D. (2000) *in* Pacific Symp. Biocomputing, pp. 119–130, World Scientific, Hawaii.
26. Schluckebier, G., O'Gara, M., Saenger, W., and Cheng, X. (1995) Universal catalytic domain structure of AdoMet-dependent methyltransferases. *J. Mol. Biol.* **247,** 16–20.
27. Malone, T., Blumenthal, R. M., and Cheng, X. (1995) Structure-guided analysis reveals nine sequence motifs conserved among DNA amino-methyltransferases, and suggests a catalytic mechanism for these enzymes. *J. Mol. Biol.* **253,** 618–632.
28. Djordjevic, S., and Stock, A. M. (1997) Crystal structure of the chemotaxis receptor methyltransferase CheR suggests a conserved structural motif for binding *S*-adenosylmethionine. *Structure* **5,** 545–558.
29. Kagan, R. M., and Clarke, S. (1994) Widespread occurrence of three sequence motifs in diverse *S*-adenosylmethionine-dependent methyltransferases suggests a common structure for these enzymes. *Arch. Biochem. Biophys.* **310,** 417–427.
30. Tai, H. L., Krynetski, E. Y., Schuetz, E. G., Yanishevski, Y., and Evans, W. E. (1997) Enhanced proteolysis of thiopurine *S*-methyltransferase (TPMT) encoded by mutant alleles in humans (TPMT*3A, TPMT*2): Mechanisms for the genetic polymorphism of TPMT activity. *Proc. Natl. Acad. Sci. USA* **94,** 6444–6449.
31. Tai, H. L., Fessing, M. Y., Bonten, E. J., Yanishevsky, Y., d'Azzo, A., Krynetski, E. Y., and Evans, W. E. (1999) Enhanced proteasomal degradation of mutant human thiopurine *S*-methyltransferase (TPMT) in mammalian cells: Mechanism for TPMT protein deficiency inherited by TPMT*2, TPMT*3A, TPMT*3B or TPMT*3C. *Pharmacogenetics* **9,** 641–650.
32. Sippl, M. J. (1993) Recognition of errors in three-dimensional structures of proteins. *Proteins* **17,** 355–362.
33. Hammond, C., and Helenius, A. (1995) Quality control in the secretory pathway. *Curr. Opin. Cell Biol.* **7,** 523–529.
34. Lord, J. M., Davey, J., Frigerio, L., and Roberts, L. M. (2000) Endoplasmic reticulum-associated protein degradation. *Semin. Bell Dev. Biol.* **11,** 159–164.
35. Chevet, E., Cameron, P. H., Pelletier, M. F., Thomas, D. Y., and Bergeron, J. J. M. (2001) The endoplasmic reticulum: Integration of protein folding, quality control, signaling and degradation. *Curr. Opin. Struc. Biol.* **11,** 120–124.