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How folate fights disease

We live in the era of molecular medicine — when it is common for people to ask 'what causes this disease?' or 'what does this drug do?', expecting an explanation that involves DNA, RNA and proteins. This era began officially in 1949, when Linus Pauling and colleagues pinpointed a difference between sickle cell hemoglobin and normal hemoglobin as the molecular basis for sickle cell anemia (see the History section on page 307 of this issue). It is particularly satisfying when molecular research not only yields in a better grasp of the causes underlying human diseases, but also points to possible therapies for those conditions. On page 359 of this issue of *Nature Structural Biology*, we gain insight into the cause and treatment of hyperhomocysteinemia[1], a condition associated with cardiovascular disease in adults and neural tube defects in newborns. We also discover a reason why folic acid — which can be obtained from dark green leafy vegetables, orange juice, dried beans, fortified grain products and, of course, multivitamin pills — is recommended to be part of our daily diet.

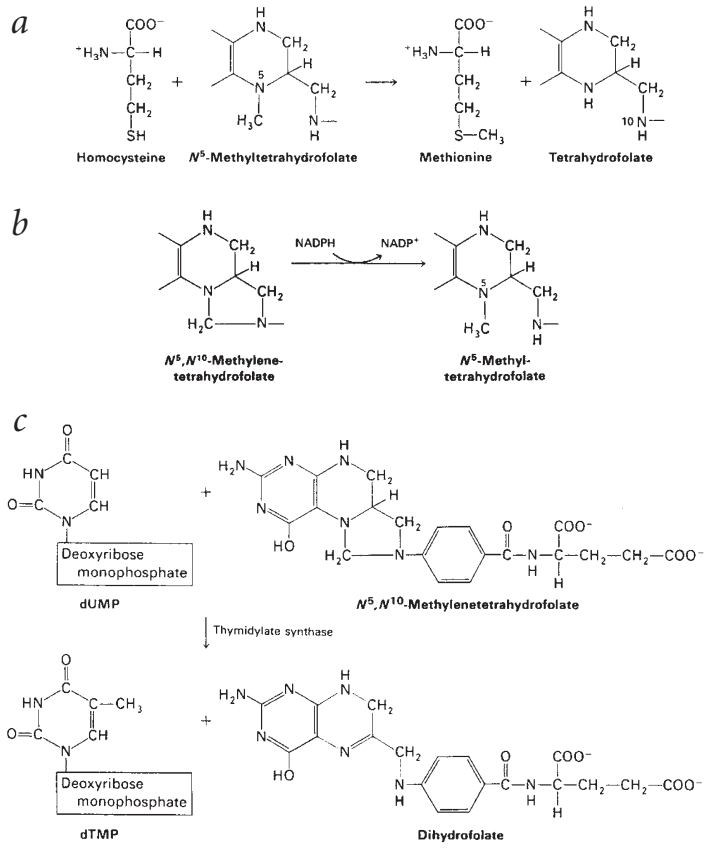

Fig. 1 Reactions involving folate derivatives. Note that in parts (*a*) and (*b*), only the reactive portions of the folates are shown. See part (*c*) for a diagram of a complete folate derivative. ***a***, Generation of methionine by transfer of a methyl group to homocysteine, a reaction catalyzed by methionine synthase. ***b***, Conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, a reaction catalyzed by methylenetetrahydrofolate reductase (MTHFR). ***c***, Methylation of dUMP to form dTMP, a reaction catalyzed by thymidylate synthase.

Elevated homocysteine levels, as measured in the blood plasma, are known to affect endothelial cells in negative ways[2], but homocysteine's exact roles in the various pathogenic conditions with which it is associated have not been determined. Severe and mild forms of hyperhomocysteinemia exist, resulting from enzymatic defects in different pathways that metabolize homocysteine. In one such pathway, homocysteine is converted into methionine by the enzyme methionine synthase (Fig. 1*a*). This reaction proceeds by donation of a methyl group from the carrier molecule 5-methyltetrahydrofolate, a compound that can be generated in only one way: conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate by the enzyme methylenetetrahydrofolate reductase (MTHFR) (Fig. 1*b*). MTHFR is composed of two domains: an N-terminal catalytic domain that performs the enzymatic reaction, and a C-terminal domain that regulates the activity in response to allosteric inhibitors. The MTHFR enzyme requires a flavin adenine dinucleotide (FAD) cofactor, and during the reaction NAD(P)H is converted to NAD(P)$^+$. Certain mutations in the catalytic domain of MTHFR result in lower yields of 5-methyltetrahydrofolate, and homocysteine levels are increased as a result since the homocysteine to methionine conversion (Fig. 1*a*) is slowed by lack of substrate.

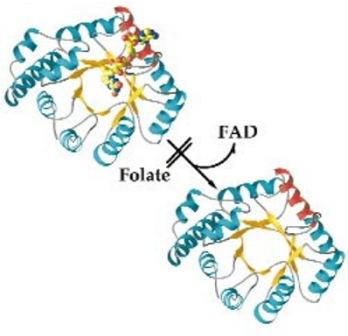

Fig. 2 Ribbon diagrams of the *E. coli* MTHFR structure indicating the effect of folate derivatives.

The most common known cause of mild hyperhomocysteinemia is an alanine to valine substitution at residue 222 in MTHFR (for which an estimated 10% of the population is homozygous)[3]. Previously, the only information available about this mutation was that it rendered MTHFR more thermolabile and that detectable MTHFR activity in patients with this mutation was only ~30% that of the wild-type[3]. Here, we are presented with the crystal structure of *Escherichia coli* MTHFR, which is highly homologous to the N-terminal catalytic domain of the human enzyme, and the biochemical properties of the wild type *E. coli* enzyme and a mutant (A177V) in which the position that is homologous to the human Ala 222 residue has been mutated to valine. The authors chose to work on the homologous *E. coli* system because the human enzyme could not be expressed sufficiently.

Somewhat surprisingly, the most obvious phenotypes that one might expect — poor substrate binding or catalysis — were not observed. The A177V MTHFR mutant binds folate derivatives and NAD(P)H with near wild-type K_m values, and the k_{cat} of the mutant is also similar to that of wild type. However, the A177V *E. coli* mutant is thermolabile, as had been observed for the human A222V mutant. In addition, the A177V mutant enzyme is ~10 times more likely than the wild-type enzyme to dissociate from its required FAD cofactor. The particular details of their experiments were critical for uncovering the phenotypes of the A177V mutant: the defect in FAD binding was not apparent in the k_{cat} assays because they were performed at such low enzyme concentrations that FAD was always bound. These results suggest that, together, decreased stability and lowered affinity for FAD account for the low enzyme activity detected in patients with the A222V mutation in the human protein. The structure indicates that the A177V mutation (and, by extension, A222V in the human enzyme) affects the FAD binding site indirectly — substitution of the larger valine likely disrupts packing of a particular α-helix that is proximal to the site of the mutation. Residues in this α-helix contribute to the FAD binding site, and thus structural changes that compensate for the larger valine side chain probably propagate through the protein and disrupt MTHFR–FAD interactions in a subtle way.

In recent years, an effective treatment that lowers homocysteine levels — supplementing the diet with folic acid — has become widespread[1]. Studies have suggested that hyperhomocysteinemia is a causal factor in cardiovascular disease[2]. Moreover, ~4,000 pregnancies per year are affected by neural tube defects, such as spina bifida, and it has been suggested that a majority of these result from elevated homocysteine levels[2,4]. These statistics have prompted the US Food and Drug Administration to recommend that 'enriched' flour or grain products, such as bread, rice and pasta (which already contain extra amounts of other vitamins) also be fortified with folic acid[4]. Despite these precautions, women are still encouraged to take supplements of concentrated folic acid four weeks before conception and at least four weeks after becoming pregnant, as the homocysteine level in the mother during early fetal development is a critical variable.

Dietary supplements of folic acid are likely to increase the levels of all folic acid derivatives in the body, including the substrate of MTHFR. Now, the paper on page 359 clearly demonstrates that the likely target of folic acid treatment is MTHFR: addition of folates to MTHFR *in vitro* stabilizes the binding of FAD to the wild-type and mutant *E. coli* enzymes[1]. Moreover, the wild-type and mutant *E. coli* enzymes are stabilized by folates against heat inactivation. To show that their results with the *E. coli* enzyme are representative of the human case, these researchers also demonstrate that addition of folates stabilizes the human wild-type and mutant enzymes present in crude lymphocyte extracts. Thus, both factors combined — stabilization of the wild-type and mutant enzymes and increasing affinity for FAD — enhance the activity of MTHFR and likely result in lower levels of homocysteine.

An interesting question is: why is the A222V defect so prevalent in the population (~10% are homozygous)? Is the A222V mutation advantageous in some way? One suggestion has been that reducing the activity of MTHFR by mutation increases the concentration of its 5,10-methylenetetrahydrofolate substrate, which is also a substrate for the conversion of dUMP to dTMP by thymidylate synthase (Fig. 1*c*). Folate deficiency is known to lead to uracil misincorporation into DNA and chromosome breakage. Therefore, increasing the concentration of the folate derviative required for the thymidylate synthase reaction may lessen the concentration of dUMP, resulting in fewer incidences of misincorporation. It may be more advantageous to reduce the activity of MTHFR to 30% of its normal level than to tolerate uracil misincorporations. Selective advantages of particular mutations in intertwined networks of biosynthetic reactions are probably not unusual, but they may be harder to detect than selective advantages that result from external pressures, such as the well known case of the sickle cell mutation, which is thought to confer malarial resistance to heterozygous carriers.

The structural and biochemical studies of MTHFR presented in this issue yield a satisfying scenario. Now, we can understand the basis for a condition that 10% of the population is likely to experience and grasp how a known effective treatment alleviates the problem (Fig. 2). Rarely do we gain such molecular insight into human disease in one small dose.

1. Guenther, B.D., Sheppard, C.A., Tran, P., Rozen, R., Matthews, R.G. & Ludwig, M.L. *Nature Struct. Biol.* **6**, 359–365 (1999).
2. Boushey, C.J., Beresford, S.A.A., Omenn, G.S. & Motulsky, A.G. *JAMA* **274**, 1049–1057 (1995).
3. Frosst, P. *et al. Nature Genet.* **10**, 111–113 (1995).
4. Center for Disease Control and Prevention: frequently asked questions about spina bifida and anencephaly. http://www.cdc.gov/nceh/programs/infants/brthdfct/prevent/ntd_faq.htm#