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The importance of recycling

Generating the nuts and bolts of nucleic acids from scratch for DNA and RNA synthesis is a metabolically expensive business, so much so that it usually occurs only when the genome is replicating itself. What of the rest of the time? The use of RNA as a transient messenger between the nucleus and cytoplasm potentially generates a lot of polynucleotide 'waste' and, rather than discarding this energetically valuable resource, mRNA (and other nucleic-acid trash) is recycled for further use.

The so-called 'salvage pathway' for rescuing nucleotides is of vital importance to humans. One of the enzymes that operate on the pathway is hypoxathine-(guanine)-(xanthine) phosphoribosyltransferase—H(G)(X)PRTase for short; whether it is G and/or X depends on whether the enzyme can utilize guanine and/or xanthine as substrates (HGPRTase in the case of humans). H(G)(X)PRTase catalyses reactions in which the ribose phosphate of α-D-5-phosphoribosyl-1-pyrophosphate (PPRP) is transferred to a purine base (hypoxathine, guanine, xanthine) to form the corresponding (salvaged) purine ribonucleotide (IMP, GMP or XMP) and pyrophosphate (PPi).

The effects of the absence of HGPRTase activity in humans results in Lesch-Nyhan's disease. As well as causing hyperuricemia, spasticity and severe mental and growth retardation, the condition results in a bizarre pattern of self-mutilation: at the age of two or three, children with the disease begin compulsively biting their fingers and lips and become aggressive towards others. Reduced efficiency of the enzyme spares the patient devastating neurological and behavioural abnormalities, but results in a severe form of gouty arthritis or uric-acid nephrolithiasis in early adulthood.

The salvage pathway is also critical for various protozoan parasites that are unable to synthesize their own nucleotides and depend on the host as their sole source of these essential building materials. Included among these disease microorganisms are *Plasmodia* (malaria), *Trypanosoma* (sleeping sickness), *Leishmania* (leishmaniasis), *Entamoeba* (amoebic dysentery), *Giardia* (traveller's diarrhoea), and *Toxoplasma* (toxoplasmosis): the potential benefits of being able to block the salvage pathways of these parasites, and therefore to halt the disease process, are considerable, to say the least.

There has been a particular resurgence of interest in the last of these protozoans, *Toxoplasma gondii*. This is an unusually promiscuous parasite found in a great variety of vertebrates: it is capable of infecting, and causing extensive damage to, multiple organ systems, including the brain, skin, lungs,

heart, eyes, muscle and liver—in particular, the entire gastrointestinal tract is often severely affected. *T. gondii* has recently emerged as the most common opportunistic pathogen of the brain in patients with AIDS, and is one of the causes of the AIDS dementia complex. Of the 10–40% of AIDS patients infected with the parasite in the United States, a third will go on to develop toxoplasmic encephalitis. This narcotizing encephalitis may develop over a period of weeks, resulting in a diverse range of symptoms including an acute state of confusion, paralysis, visual field defects, cranial nerve palsies, as well as rapid death.

Clinical treatment of toxoplasmic encephalitis involves combination chemotherapy designed to block the folic-acid metabolism of the parasite: pyrimethamine (a dihydrofolate reductase inhibitor) and sulphadazine (a dihydrofolate synthase inhibitor). But the treatment is plagued by toxicity rates that can preclude its use in up to 40% of patients. Alternative therapeutic strategies are much sought after: the nucleotide salvage pathway provides a potential point of intervention and HGXPRTase a target of structure-based drug design.

A paper from Richard Brennan and colleagues in this issue[1] probes the structure and reaction mechanism of HGXPRTase from *T. gondii*. Previous structures of the HGPRTase from humans[2] (in complex with the reaction product GMP) and the HGXPRTase–GMP complex from the protozoan parasite *Tritrichomonas foetus*[3] have helped to define the general architecture of this family of proteins. The enzyme consists of two lobes, a larger 'core' domain and the smaller 'hood', with a deep—but solvent-exposed—cleft between them that harbours the active site. Access of water to the active site would result in the immediate hydrolysis of the chemically sensitive oxocarbonium transition-state intermediate formed during catalysis, with concomitant loss of product.

Comparison of the apo structure of *T. gondii* with that of the human HGPRTase–GMP complex shows that the highly conserved loop in the *T. gondii* structure is poised over the active site cleft in a position where it may protect the transition-state intermediate from the destructive attention of solvent. Once the products have formed, the loop would then move away from the active site, as seen in the human HGPRTase–GMP product complex, allowing the salvaged purine ribonuclotide to be released from the active site. Not everything is quite so straightforward, though: the specificity of the enzyme for xanthine, one of the features that distinguishes it from the human enzyme for example, is still unclear. Even so, details of the structures will be critical for any drug design effort.

The omens for tackling *T. gondii* and toxoplasmosis appear to be good: the protozoan is amenable to *in vitro* cell-culture techniques and methods for molecular transformation of the parasite are being developed. Nevertheless, although the HGXPRTase represents a potentially attractive point of intervention—there are many pre-existing purine base analogues (including xanthine) that might be explored as leads and synthesis of further analogues is a relatively straightforward business—perhaps the most critical test of for the development of such a treatment strategy has yet to be performed: it has not been clearly demonstrated that HG(X)PRTase has an essential nutrition function in any of the protozoan parasites. But, even if HG(X)PRTase proves not to be a good therapeutic target, the dependence of the protozoan parasites on nucleotide salvage pathways for nucleic acids leaves researchers plenty to aim at.

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2. Somoza, J.R., Chin, M.S., Focia, P.J., Wang, C.C. Fletterick, R.J. *Biochemistry* **35**, 7032–7040 (1996).
3. Eads, J.C., Scapin, G., Xu, Y., Grubmeyer, C. & Sacchettini, J.C. *Cell* **78**, 325–334 (1994).