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A class act

The discovery of the Theta class glutathione transferases

Two frequently cited papers published in the *Biochemical Journal* in the early 1990s highlight the importance of the glutathione transferase (GST) superfamily and the major contributions made to the field by Brian Ketterer and colleagues at University College London[1,2]. Over a span of 31 years, Brian Ketterer published over 45 papers in the BJ, the majority concerning GSTs.

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The GSTs are probably best known for their role in xenobiotic metabolism with its implications for protection against carcinogens and causing resistance to anticancer drugs. Brian Ketterer started out in this field purifying a protein that bound the glutathione conjugates of azo-dyes. In 1971, a paper by Litwack, Ketterer and Arias[3] revealed that the azo-dye-conjugate-binding protein was identical with corticosteroid-binder I and Y proteins that were also known to bind a large range of xenobiotics, steroid hormones and other endogenous metabolites. The protein was renamed ligandin, and, in a subsequent paper, Jakoby and colleagues identified ligandin as a GST that conjugated 1-chloro-2,4-dinitrobenzene (CDNB) to glutathione[4]. Looking back on these papers today, it is clear that they provided an early indication of the different metabolic roles played by members of the GST structural family. Recent studies have revealed the participation of GSTs in diverse metabolic pathways and regulatory processes including the catabolism of tyrosine[5], the synthesis of steroid hormones[6], the regulation of cell signalling kinases[7] and the formation and modulation of ion channels[8].

Early reports identified multiple GSTs based on their activities with a range of substrates (alkyl-glutathione transferase, aryl-glutathione transferase, etc.)[9]. However, subsequent studies revealed overlapping substrate specificities rendering the original classification system unworkable. Studies in Jakoby's laboratory at the NIH (National Institutes of Health) were the first to clearly purify multiple GST isoenzymes from rat and human liver and to associate identifiable proteins with particular substrate specificities[10]. This contribution generated considerable interest in the GSTs that was stimulated further by the introduction of glutathione-affinity-purification procedures[11]. By the mid-1980s, numerous laboratory groups were reporting the characterization of 'novel' GSTs purified from a variety of tissues and exhibiting a variety of properties. Although the 1980s was a period of great expansion in the study of GSTs, it was also a period of relative confusion, and at one stage it was remarked that there were more GST nomenclature systems in use than workers in the field. The use of N-terminal amino acid sequence analysis, immunological cross-reactivity and genetic analysis permitted the identification of some distinct isoenzyme groups[12-14]. Bengt Mannervik and colleagues defined these groups as the Alpha, Mu and Pi classes and this concept was subsequently refined and formed the basis of a widely accepted nomenclature for the GSTs that was published in the *Biochemical Journal* and has been cited over 500 times[15].

During the 1980s, most research into the GSTs

Professor Brian Ketterer

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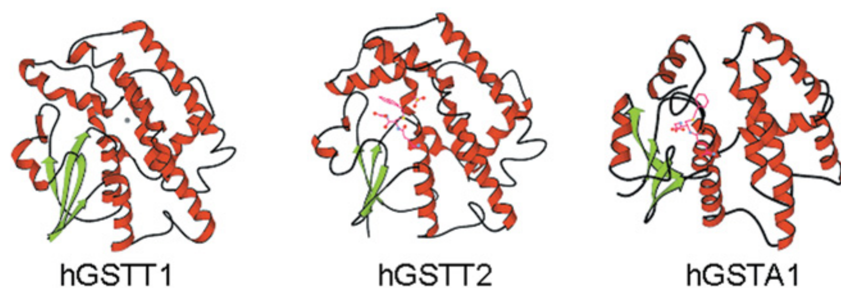

Figure 1. The Theta class GSTs exhibit a $\beta\alpha\beta\alpha\beta\beta\alpha\alpha_x$ fold that is a characteristic of the cytoplasmic GSTs. The structural representations were drawn from the following PDB files: hGSTT1, 2C3N; hGSTT2, 3LJR; hGSTA1, 1GUH.

was focused on isoenzymes that were members of the Alpha, Mu and Pi classes because of their ease of purification by glutathione-affinity chromatography and their facile spectrophotometric measurement of activity with CDNB as a co-substrate. So focused was this research that GSTs tended to be largely defined by these two properties. Astutely, Ketterer's team re-examined the early work of Jakoby and colleagues that had revealed a minor GST in rat liver that utilized 1,2-epoxy-3-(4-nitrophenoxy)propane (EPNP) as a co-substrate. This approach led to the purification of two enzymes from rat liver and one from human liver that exhibited significant activity with EPNP and little with CDNB. Strikingly, these isoenzymes failed to bind to glutathione-affinity matrices and had to be purified by other chromatographic procedures. In what was to become a classic paper in the *Biochemical Journal* that has been cited over 600 times, David Meyer, together with Brian Ketterer and colleagues, revealed that these rat and human isoenzymes had similar N-terminal amino acid sequences but, as a group, differed from other GST classes that were known at the time[1]. Ketterer's group defined these enzymes as members of a distinct new class that they termed Theta. In a separate study, Satoh's group in Japan identified a novel rat liver GST that conjugated sulfate

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esters of carcinogenic arylmethanols[16], and, as a result of subsequent sequence comparisons, this enzyme has been included in the Theta class. Later studies in several laboratories found that rats and humans have two Theta class GSTs: GSTT1-1 isoenzymes have distinct activity with EPNP, whereas GSTT2-2 isoenzymes catalyse a very specific reaction with 1-menaphthyl sulfate and related compounds[17,18]. In humans, the Theta class genes are clustered on the long arm of chromosome 22[19] and the crystal structure of hGST1-1 and hGST2-2 has been determined, confirming that the Theta class GSTs share a common fold with members of the Alpha, Mu and Pi classes (Figure 1)[20,21]. Despite the common structure, the Theta class GSTs utilize a serine residue in the active site in contrast with a tyrosine residue in the Alpha, Mu and Pi class GSTs[18].

The discovery of the Theta class of GSTs outside of the Alpha, Mu and Pi class paradigm represented a significant turning point in the development of the field and carried with it the exciting prospect that there may be a range of undiscovered members of this family that have novel substrates and functions. This proved to be the case, and the availability and expansion of the expressed sequence tag (EST) database, together with the development of improved sequence alignment tools, has subsequently allowed the identification of several additional members of the GST superfamily, including the Zeta and Omega classes that have novel glutathione-dependent activities[22,23]. Although this bioinformatic approach to gene discovery has been productive, it presents additional problems in identifying the catalytic activities and substrates, if any, of proteins that have only been identified by sequence homology. As shown in Table 1, there are now a number of members of the GST structural family, including several that may not be enzymes.

A second *Biochemical Journal* classic paper concerning the Theta class emerged from Brian Ketterer's laboratory in 1994[2]. This paper (cited over 600 times) was largely the work of Sally Pemble and John Taylor and involved a timely collaboration with a team at the Universität Dortmund in Germany. The German team had previously reported significant differences in the

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Table 1

General properties of members of the human cytosolic GST family

GST class	*Defined enzymatic activity*	*Bind to glutathione agarose*	*Dimeric structure*
Alpha	Yes	Yes	Yes
Mu	Yes	Yes	Yes
Pi	Yes	Yes	Yes
Theta	Yes	No	Yes
Sigma	Yes	Some	Yes
Zeta	Yes	No	Yes
Omega	Yes	Some	Yes
CLIC	No	No	Monomeric
GDAP	No	No	Yes

CLIC, chloride intracellular channel protein[28]; GDAP, ganglioside-induced differentiation protein[29]

capacity of individuals to conjugate monohalomethanes and that the population could be subdivided into 'conjugators' and 'non-conjugators'[24]. Although it was known that the conjugation of monohalomethanes and dichloromethane was glutathione-dependent and probably the result of GSTT1-1 activity, the methods for determining monohalomethane-conjugator status were cumbersome and did not lend themselves to genetic analysis. Pemble and colleagues cloned the cDNA encoding human GSTT1 and showed that some individuals appeared to lack the *GSTT1* gene[2]. They were also able to correlate the presence or absence of the *GSTT1* gene with the monohalomethane-conjugator status and concluded that the inability of some individuals to conjugate monohalomethanes or dichloromethane was the result of a genetically determined deficiency of the *GSTT1* gene. This finding was of immediate interest because it had recently been shown that the commonly used industrial solvent dichloromethane was a substrate for rat GST5-5 (now termed rGSTT1-1) and that a product of the reaction was mutagenic[25]. Thier et al.[25] had measured the mutagenicity of dichloromethane in the Ames test strain *Salmonella typhimurium* TA1535 and reported a significant increase if rGSTT1-1 was co-expressed in the tester strain. Subsequent studies in humans using PCR revealed that the frequency of homozygotes for hGSTT1-1 deficiency was about 16% in Europeans[26]. Because this polymorphism could have a direct effect on the mutagenicity of a commonly used industrial chemical, the study of Pemble and colleagues attracted further interest in the Theta class GSTs, especially in the fields of pharmacogenetics and genetic epidemiology.

Complete gene deletions causing enzyme deficiencies are not common, but a deletion of the gene encoding the Mu class isoenzyme, now termed hGSTM1-1, was reported in 1981 to be homozygous in 50–60% of European subjects[12]. This deficiency has also been the subject of considerable epidemiological investigation and several reports have confirmed the association of GSTM1-1 deficiency with relapse-free survival of children with acute lymphoblastic leukaemia[27]. Many additional polymorphisms have now been reported in GSTs from most classes, and the burgeoning interest in toxicogenomics and the potential of personalized medicine continues to generate interest in this area.

Over the last 25 years, research into the GSTs has been strongly supported by the *Biochemical Journal*. In addition to the two classic papers discussed here and the numerous papers from Brian Ketterer's laboratory, many other excellent contributions to the field from Bengt Mannervik and John Hayes (among many others) have been recorded in the pages of the Journal. Arguably, the *Biochemical Journal* has been the single most influential journal in this field.

Philip Board gained a PhD at the University of New England in Australia and completed a postdoc with Joseph Smith at the Department of Pathology at Kansas State University. Philip returned to the John Curtin School of Medical Research at the Australian National University in 1978, where he is currently Professor and leader of the Molecular Genetics Group. Philip's research interests are focused on the structure, function and genetics of enzymes involved in glutathione metabolism.

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