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Review

Glutathione transferases in the genomics era: New insights and perspectives

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Abstract

In the last decade the tumultuous development of ''omics'' greatly improved our ability to understand protein structure, function and evolution, and to define their roles and networks in complex biological processes. This fast accumulating knowledge holds great potential for biotechnological applications, from the development of biomolecules with novel properties of industrial and medical importance, to the creation of transgenic organisms with new, favorable characteristics.

This review focuses on glutathione transferases (GSTs), an ancient protein superfamily with multiple roles in all eukaryotic organisms, and attempts to give an overview of the new insights and perspectives provided by omics into the biology of these proteins. Among the aspects considered are the redefinition of GST subfamilies, their evolution in connection with structurally related families, present and future biotechnological outcomes.

Keywords: Omics; Protein families; Thioredoxin fold; Evolution; Forced evolution; Transgenics

Contents

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1. Introduction

Genome sequencing projects have started a new era in genetics and molecular biology, leading to the understanding of the sequence and organization of genes in many organisms, from the simplest bacteria to complex eukaryotes including man. With the development of high throughput technologies and sophisticated computational tools a change in perspective, from mere sequence description to the understanding of the structure and function of multiple genes and proteins in simple and complex organisms, took place. The ultimate goal is to go beyond the gene/protein level to elucidate the pathways leading to the organization and function of macromolecular complexes, organelles, cells, organs and whole organisms. All this is defined as systems biology and the new biotechnological tools that make it possible, genomics, transcriptomics, proteomics, functional/structural genomics, metabolomics, etc., go collectively under the name of ''omics''. Besides being invaluable in understanding structures and functions, omics are extremely powerful for detecting evolutionary relationships between sets of genes and, at a higher level, organisms. One field that can particularly benefit from omics is the study of gene families, often conserved across organisms, and of their evolution in terms of structure and functions. In this review the glutathione transferase gene/protein family is considered.

Glutathione transferases (GSTs) (EC 2.5.1.18) are a superfamily of multifunctional proteins with fundamental roles in the cellular detoxification of a wide range of exogenous and endogenous compounds. Although several of them are not transferases at all (see below), these proteins are still collectively called GSTs, probably because the first discovered ones were indeed transferases, and as such they will be referred to throughout this review. In plants and animals, GSTs are the principal phase II enzymes in metabolic detoxification processes. Their main chemistry is to catalyze the conjugation of the tripeptide glutathione (GSH: γ-Glu-Cys-Gly) with compounds containing an electrophilic centre, to form more soluble, non-toxic peptide derivatives, ready to be excreted or compartimentalized by phase III enzymes (Coleman et al., 1997). In addition, GSTs can serve as peroxidases, isomerases and thiol transferases (Jensson et al., 1986; Bartling et al., 1993; Fernandez-Cañon and Peñalva, 1998; Board et al., 2000), or have non-catalytic functions among which binding of non-substrate ligands and modulation of signaling processes (Listowsky, 1993; Marrs, 1996; Mueller et al., 2000; Smith et al., 2003; Axarli et al., 2004; Adler et al., 1999; Loyall et al., 2000; Cho et al., 2001). The original view of GSTs as solely detoxication enzymes has thus gradually changed, and their roles extended to non-stress metabolism, as leukotriene and prostaglandin biosynthesis (Tsuchida et al., 1987; Kanaoka et al., 1997; Jakobsson et al., 1999) and the catabolism of aromatic aminoacids (Fernandez-Cañon and Peñalva, 1998; Thom et al., 2001).

Three main subfamilies of GSTs are generally recognized, each encoded by distinct multigene families:

(1) the soluble or cytosolic GSTs (also termed canonical by some authors);
(2) the microsomal GSTs, now termed MAPEG (membrane-associated proteins involved in eicosanoid and glutathione metabolism);
(3) the plasmid-encoded bacterial fosfomycin-resistance GSTs.

Recent evidence suggests that Kappa GSTs, previously considered as just one class of the soluble GSTs, constitute a distinct subfamily instead (Jowsey et al., 2003; Ladner et al., 2004; Robinson et al., 2004). In addition, microsomal GSTs with primary and tertiary structure more similar to the Alpha class soluble GSTs than to MAPEGs have been identified (Prabhu et al., 2001, 2004; K.S. Prabhu and C.C. Reddy, personal communication). It is thus possible that the number of GST subfamilies is actually larger than so far thought. Furthermore, genomics and postgenomics studies are continuously highlighting the links with ''related families'' which borders with the GSTs can be quite thin and disputable. Such related families include glutaredoxins (GRX) (Xia et al., 2001; Collison and Grant, 2003), chloride intracellular channels (CLIC) (Harrop et al., 2001; Dulhunty et al., 2001; Cromer et al., 2002), dehydroascorbatereductases (DHAR) (Dixon et al., 2002a), selenocysteine glutathione peroxidases (SecGPX) (Epp et al., 1983), bacterial DsbA (Martin et al., 1993), eukaryotic protein elongation factors (eEF1Bγ) (Jeppesen et al., 2003), all of which share with GSTs a basic structural motif, the thioredoxin fold.

In this review I will summarize current knowledge on the main eukaryotic GST subfamilies (the soluble, the Kappa, the microsomal GSTs, and some related families), focusing on how structural and functional new acquisitions contributed to trace possible evolutionary scenarios of this protein superfamily. Bacterial GSTs are in some way a world apart, so far less characterized especially in terms of functions and diversification. For this reason here I will touch upon them only when appropriate to complete and explain general aspects of GSTs. For a more detailed description and discussion of bacterial GSTs readers are referred to specific reviews (Vuilleumier, 1997; Vuilleumier and Pagni, 2002).

Biotechnological perspectives, in terms of both transgenics and directed evolution, will also be discussed.

2. Cytosolic GSTs (cGSTs)

This subfamily, ubiquitously found in all aerobic organisms, is by far the most abundant, often counting tens of members in each species. For instance, in man and other mammalian species 15–20 different cGST genes have been identified (Hayes et al., 2005), 40–60 in plants (McGonigle et al., 2000; Wagner et al., 2002; Frova, 2003; Soranzo et al., 2004), 10–15 in bacteria (Vuilleumier and Pagni, 2002), over 10 in insects (Ranson et al., 1998 and refs. herein).

Based on several criteria, including aminoacid/nucleotide sequence identity, physical structure of the genes (i.e. intron number and position) and immunoreactivity properties, cGSTs have been grouped into numerous classes, some of which are ubiquitous throughout taxa and even kingdoms, while other are organism-specific. Presently, seven classes of cytosolic GSTs are recognized in mammals, namely the specific Alpha, Mu, Pi