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An evolutionary biochemist's perspective on promiscuity

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Evolutionary biochemists define enzyme promiscuity as the ability to catalyze secondary reactions that are physiologically irrelevant, either because they are too inefficient to affect fitness or because the enzyme never encounters the substrate. Promiscuous activities are common because evolution of a perfectly specific active site is both difficult and unnecessary; natural selection ceases when the performance of a protein is 'good enough' that it no longer affects fitness. Although promiscuous functions are accidental and physiologically irrelevant, they are of great importance because they provide opportunities for the evolution of new functions in nature and in the laboratory, as well as targets for therapeutic drugs and tools for a wide range of technological applications.

Introduction

Once upon a time, biology was (thought to be) simple. A gene encoded a single protein, which performed a single function with high specificity. However, biology is not simple. Genes can encode multiple proteins, proteins can perform multiple functions, and promiscuous functions abound. Our 'linear' thinking about biology is being supplanted by the recognition that understanding the complexity of living systems can best be accomplished by considering the function of the system as a whole in addition to the functions of the individual parts. From this viewpoint, the origins and implications of promiscuous functions are particularly challenging because we cannot predict every potential promiscuous function – and there may be many thousands – within the context of a particular proteome. This Opinion discusses the molecular- and system-level considerations necessary for an integrated perspective on the role of promiscuity in the function, evolution, and manipulation of biological systems.

'Promiscuity' means different things to different people

The term 'promiscuous' is used to describe enzymes that catalyze more than one reaction. Enzymes commonly display 'substrate promiscuity' (also known as substrate ambiguity), which is the ability to perform comparable chemical transformations using different substrates. Enzymes that are 'catalytically promiscuous' catalyze a secondary reaction that results in a chemical transformation different from that catalyzed with its canonical substrate. The term promiscuity is used in this article to encompass both catalytic and substrate promiscuity.

Protein biochemists and molecular biologists often use the term promiscuous to describe broad-specificity proteins that bind to multiple interaction partners, which may be ligands, substrates, or other macromolecules [1–3]. While this is a reasonable use of the term, evolutionary biochemists prefer to reserve the term promiscuous to refer to interactions that are not physiologically relevant [4]. This is a useful distinction, as it communicates not only the property of interest but also its relevance to physiology and the degree to which it is under selective pressure.

Broad substrate specificity is typical of detoxification enzymes such as glutathione S-transferases [2,5] and cytochrome P450s [6] and is critical to their ability to protect organisms from the numerous and unpredictable toxins to which they are exposed. Broad-specificity enzymes such as esterases, amidases, and phosphatases can allow microbes to initiate degradation of diverse compounds and can release products such as phosphate and ammonia that are useful to the organism even if the compound cannot be fully degraded. Cases in which broad specificity is important for fitness are clearly different from those in which enzymes catalyze reactions of nonphysiological substrates or of physiological substrates with extremely low efficiency.

When an evolutionary biochemist says that a particular enzyme is promiscuous, it is an operational definition meaning that, to the best of our knowledge, the promiscuous activity is physiologically irrelevant. This might be true because the substrate for the promiscuous activity is never encountered by the enzyme, or at least not in concentrations high enough to cause trouble. For example, many chemicals synthesized by humans for industrial or medicinal purposes have never before been present in the biosphere. Even the elaborate natural products synthesized by many organisms to communicate with or to kill other organisms may not be encountered outside the particular environmental niche in which they are produced.

A promiscuous activity may also be irrelevant because it is too inefficient to influence metabolism and therefore fitness. For instance, gamma-glutamyl phosphate reductase (ProA) from *Escherichia coli* has a low-level ability to reduce N-acetyl glutamyl phosphate, the substrate for

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ArgC [7,8]. However, ProA is unable to substitute for ArgC. The inefficiency of the promiscuous activity, which is several orders of magnitude below that of ArgC, is likely to preclude sufficient reduction of N-acetyl glutamyl phosphate to support arginine biosynthesis.

The past few decades have seen the assignment of genes to functions in metabolic pathways that degrade myriad organic compounds as well as synthesize the standard building blocks of macromolecules and a wide variety of secondary metabolites. However, every genome encodes numerous enzymes that do not participate in known metabolic pathways. Figure 1, which summarizes the activities of 23 haloacid dehalogenase-like phosphatases from *E. coli* with 80 physiological substrates, exemplifies the conundrum presented by such enzymes [9]. Only a few of the tested enzymes are highly specific. For example, two of the enzymes (HisB and SerB) are involved in amino acid biosynthesis. The substrate for HisB was not available, but, as expected, the enzyme did not have detectable activity with any of the tested substrates. SerB was quite specific for phosphoserine, its physiological substrate. Gph [10] and YniC [9] are involved in detoxification of 2-phosphogycolate and 2-deoxyglucose, respectively. However, what can we conclude about the remaining enzymes, many of which have weak activity with a number of substrates? One possibility is that these enzymes participate in as-yet-unidentified metabolic pathways and have high activity with a substrate that was not tested. If so, these lower-level activities would be promiscuous activities. Another possibility is that the broad activity profiles of these enzymes reflect a physiologically important function. For example, YbiV has high activity with fructose 1-phosphate and modest activity with ribose 5-phosphate and glucose 6-phosphate. Each of these sugar phosphates plays a role in primary metabolism and it seems wasteful to hydrolyze a high-energy phosphoester bond. However, this enzyme might play an important role under phosphate-limited conditions by making phosphate available for more critical processes. This situation may be more common than we appreciate. Not all enzymes need to fit neatly into metabolic pathways to contribute to fitness. The role of some enzymes may be to improve the function of the overall metabolic network and this role may require broad substrate specificity.

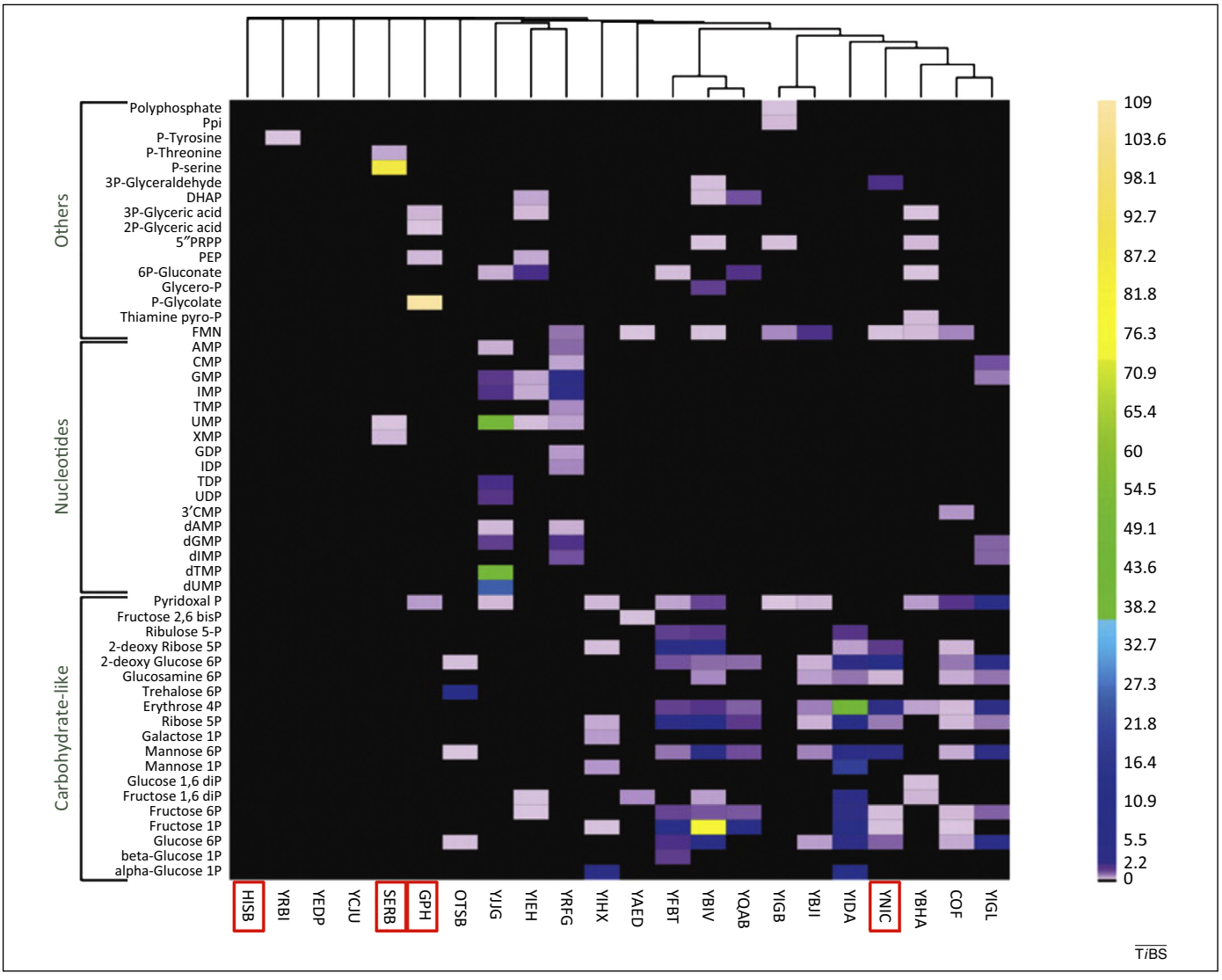

Figure 1. Heat map summarizing the activities of 23 haloacid dehalogenase-like proteins from *Escherichia coli* with 80 physiological substrates. Activities are given in units of μmol/min/mg of protein. Red boxes indicate proteins that are discussed in the text. Reproduced from [9].