CHAPTER 5

Functional Divergence of the $L$-Fucose System in Mutants of *Escherichia coli*

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1. Introduction

The catabolic system for $L$-fucose of *Escherichia coli* has given rise to a number of novel metabolic functions. Studies on this system have yielded illustrations of three concepts for biochemical evolution: (1) a pyridine nucleotide-linked oxidoreductase can be elected by the cell to serve as either a dehydrogenase or a reductase, depending primarily on the mode of regulating the expression of the structural gene and on the nature of the preceding and following reactions in the pathway; (2) genetic mobilization of components of an established metabolic systems for a novel function can lead to the extinction of the remaining genes that become superfluous; and (3) once the expression of a structural gene is liberated from its normal regulatory constraint to provide a new service, the gene product can act as steppingstone for the elaboration of other novel metabolic pathways—the principle of preadaptation (Lin et al., 1976; Wu, 1978; Lin, 1979, 1981). The evolutionary studies of the fucose system, as well as metabolic systems derived from or related to it, will be presented partly in a historical framework, since the ways in which knowledge unfolds and converges are often of heuristic value themselves.
2. Reversibility of NAD-Linked Reactions

Interconversions of alcohols and their corresponding aldehydes and ketones at neutral pH are overwhelmingly in favor of alcohol formation when coupled with pyridine nucleotides [typically by a factor of $10^4$ (Krebs and Kornberg, 1957)]. Because of this thermodynamic bias, it is a simple matter for enzymes catalyzing this class of reactions to serve physiologically as carbonyl group reductases. Classical cases are the reduction of acetaldehyde to ethanol and of pyruvate to lactate during anaerobiosis. (In this context, alcohol dehydrogenase and lactate dehydrogenase, the enzymes that catalyze these respective reactions, are functionally misnomers.) On the other hand, many pyridine nucleotide-linked enzymes do serve a reverse physiological role as hydrogenases of alcohols. The conversion of D-arabitol or xylitol to D-xylulose and of ribitol to D-ribulose by such enzymes are good examples (see Chapter 1). Cellular NAD/NADH ratios are not radically different in aerobically or anaerobically growing cells (Wimpenny and Firth, 1972), which is not surprising in view of the fact that under any growth conditions there are numerous metabolic reactions that require either one or the other form of the coenzyme. Therefore, drastic changes in the relative concentrations of the oxidized and reduced forms of this coenzyme are not available as a general mechanism for determining whether an enzyme is to act as a dehydrogenase or a reductase. In the utilization of the pentitols, the cell partially surmounts the energetic obstacle of the dehydrogenation step by active transport of the substrate to an elevated intracellular concentration, thus providing a chemical “push.” More importantly, a “pull” for the reaction is provided by using an ATP-dependent kinase in the subsequent step to trap the pentose as a phosphorylated product. The equilibrium constant of the pulling reaction is about $10^3$ in favor of pentose phosphate formation. Because pyridine nucleotide-linked enzymes can function in opposite manners, they are often referred to as oxidoreductases.

3. A Mutant That Uses an NAD-Linked Dehydrogenase to Grow on L-1,2-Propanediol

Wild-type *E. coli* strains K12, B, ML, and W do not utilize L-1,2-propanediol (propylene glycol), but mutants that do can be readily isolated. In a pilot experiment, about $10^{10}$ *E. coli* K12 cells derived from a previous population treated with a chemical mutagen (ethyl methanesulfonate) were inoculated into 1 liter of mineral medium containing 0.2% of DL-1,2-propanediol (13 mM of the L isomer) as the sole potential source...