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Journal of Nutritional Biochemistry 16 (2005) 411–415

Journal of Nutritional Biochemistry

The *Escherichia coli* biotin regulatory system: a transcriptional switch☆

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Received 28 March 2005; received in revised form 28 March 2005; accepted 28 March 2005

Abstract

The ability of any organism to survive depends, in part, on mechanisms that enable it to modify its patterns of gene expression in response to extra- and intracellular signals. In the classical response mechanisms, a small molecule signal impinges on either an extra- or intracellular receptor, and through a series of events the signal is ultimately transmitted to transcription regulatory proteins. An alternative to this classical mechanism is provided by multi-functional transcription factors. These proteins function directly in transcription as well as in at least one additional cellular process. An example of this class of proteins includes the dimerization cofactor of hepatocyte nuclear factor (DcoH), which serves as an enzyme involved in regeneration of the tetra-hydrobiopterin cofactor and as a factor that stabilizes the dimerization of the hepatocyte nuclear transcription factor (Mendel DB, Khavari PA, Conley PB, Graves MK, Hansen LP, Admon A, et al. Characterization of a cofactor that regulates dimerization of a mammalian homeodomain protein. Science 1991;254:1762–7; Citron BA, Davis MD, Milstien S, Gutierrez J, Mendel DB, Crabtree GR. Identity of 4a-carbinolamine dehydratase, a component of the phenylalanine hydroxylation system, and DCoH, a transregulator of homeodomain proteins. Proc Natl Acad Sci U S A 1992;89:11891–4). Another example is the protein PutA, a redox enzyme involved in proline utilization and a regulator of transcription of the genes involved in proline utilization (Ostrovsky de Spicer P, Maloy S. Puta protein, a membrane-associated flavin dehydrogenase, acts as a redox-dependent transcriptional regulator. Proc Natl Acad Sci U S A 1993;90:4295–8). While several proteins of this class have been identified, their mechanisms of functional switching remain to be elucidated.

Keywords: Biotin; Transcription regulation

1. The biotin regulatory system: communication between gene expression and "other cellular processes"

The ability of any organism to survive depends, in part, on mechanisms that enable it to modify its patterns of gene expression in response to extra- and intracellular signals. In the classical response mechanisms, a small molecule signal impinges on either an extra- or intracellular receptor, and through a series of events the signal is ultimately transmitted to transcription regulatory proteins. An alternative to this classical mechanism is provided by multi-functional transcription factors. These proteins function directly in transcription as well as in at least one additional cellular process. An example of this class of proteins includes the dimerization cofactor of hepatocyte nuclear factor (DcoH), which serves as an enzyme involved in regeneration of the tetra-hydrobiopterin cofactors and as a factor that stabilizes the dimerization of the hepatocyte nuclear transcription factor [1,2]. Another example is the protein PutA, a redox enzyme involved in proline utilization and a regulator of transcription of the genes involved in proline utilization [3]. While several proteins of this class have been identified, their mechanisms of functional switching remain to be elucidated.

Biotin utilization in prokaryotes and, more recently, in eukaryotes is functionally linked to transcription initiation through the multi-functional biotin holoenzyme synthetases (BHS). As the direct link between the two functions is best established for the BHS from *Escherichia coli*, this system will be the major subject of this discussion. Some brief comments on the relationship between results obtained in studies of this system to the eukaryotic systems will also be provided.

☆ This paper was presented at the "International Symposium: Vitamins as Regulators of Genetic Expression: Biotin as a Model," NAFTA Satellite Meeting to the XXV National Congress of Biochemistry held December 3–4, 2004, in Ixtapa, Zihuatanejo, Mexico. This meeting was sponsored by Sociedad Mexicana de Bioquimica A.C.; Programa de Doctorado en Ciencias Biomedicas, Universidad Nacional Autonoma de Mexico; Laboratorios Roche-Syntex, Mexico; and Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico.

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doi:10.1016/j.jnutbio.2005.03.019

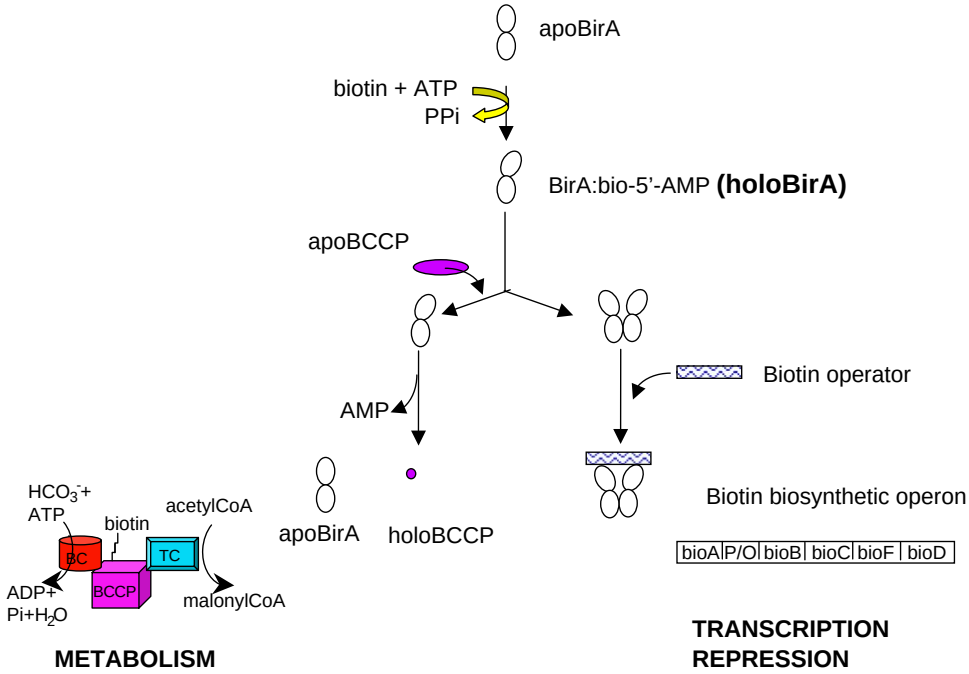

Fig. 1. Schematic diagram of the *Escherichia coli* Biotin Regulatory System. See text for description.

Biotin utilization by any biotin-dependent carboxylase requires covalent linkage of the cofactor to the biotin carboxyl carrier protein (BCCP) subunit or domain of the enzyme (Fig. 1). In addition to the biotin carrier protein activity, these carboxylases also possess biotin carboxylase and transcarboxylase activities. While the BC activity is required for loading carboxylate onto the biotin cofactor, the TC activity is utilized for subsequent transfer of the carboxylate to an acceptor molecule. In *E. coli* acetyl-CoA carboxylase (ACC), the enzyme responsible for synthesis of malonyl CoA from acetyl-CoA is the only biotin-dependent carboxylase. Synthesis of the ACC enzyme is linked to growth rate and increases with increased rates of cell division [4]. Thus, since ACC is the only biotin acceptor in *E. coli*, the demand for biotin is also growth rate controlled.

In order to function as a cofactor in carboxylate transfer, biotin must be covalently linked to the transcarboxylases. The biotin holoenzyme ligases catalyze this reaction in two steps (Fig. 1). In the first step the biotin and ATP react to form an adenylated intermediate, biotinoyl-5′ -AMP [5]. In *E. coli*, the enzyme–intermediate complex interacts with the BCCP subunit of ACC, resulting in the formation of an amide bond between biotin and the epsilon amino group of a single lysine residue of BCCP and release of AMP. A second function of the enzyme–intermediate complex in *E. coli* is to bind site-specifically to the biotin operator sequence in the transcription control region of the biotin biosynthetic operon (Fig. 1) [6,7]. The physiological consequence of this binding event is repression of transcription initiation at the operon. Thus, the biotin holoenzyme ligase both funnels biotin into metabolism and regulates its biosynthesis. Significantly, it is the intermediate-bound enzyme that functions in site-specific DNA binding. The adenylate serves as both the intermediate in the biotinylation reaction and the allosteric activator of the protein for DNA binding [8].

A high-resolution three-dimensional structure of the *E. coli* biotin holoenzyme synthetase has been determined by X-ray crystallography [9]. In this structure the 321 amino acid protein monomer folds into three domains: an N-terminal-winged helix-turn-helix DNA binding domain, a central domain composed of a core β-sheet flanked by α-helices and a C-terminal domain that is entirely β-sheet. The central domain is also characterized by four surface loops that are partially disordered. Thus far, three-dimensional structural information is only available for the *E. coli* biotin holoenzyme synthetase.

The multi-functional property of the biotin holoenzyme synthetases has been conserved in many bacteria and archae [10]. Comparison of the N-terminal sequences of the putative biotin holoenzyme synthetases from a range of organisms indicates a high probability that they encode DNA binding domains. However, since not all of these organisms encode the enzymes required for biotin biosynthesis the targets of regulation vary. Sequence analysis reveals that the targets of transcription regulation by these biotin holoenzyme ligases include genes that encode biotin permeases and biotin-dependent carboxylases.

2. Assembly of the transcription regulatory complex: the importance of repressor dimerization

Studies of the mechanism of assembly of the transcription regulatory complex of the *E. coli* biotin regulatory system have provided insight into the mechanism of control

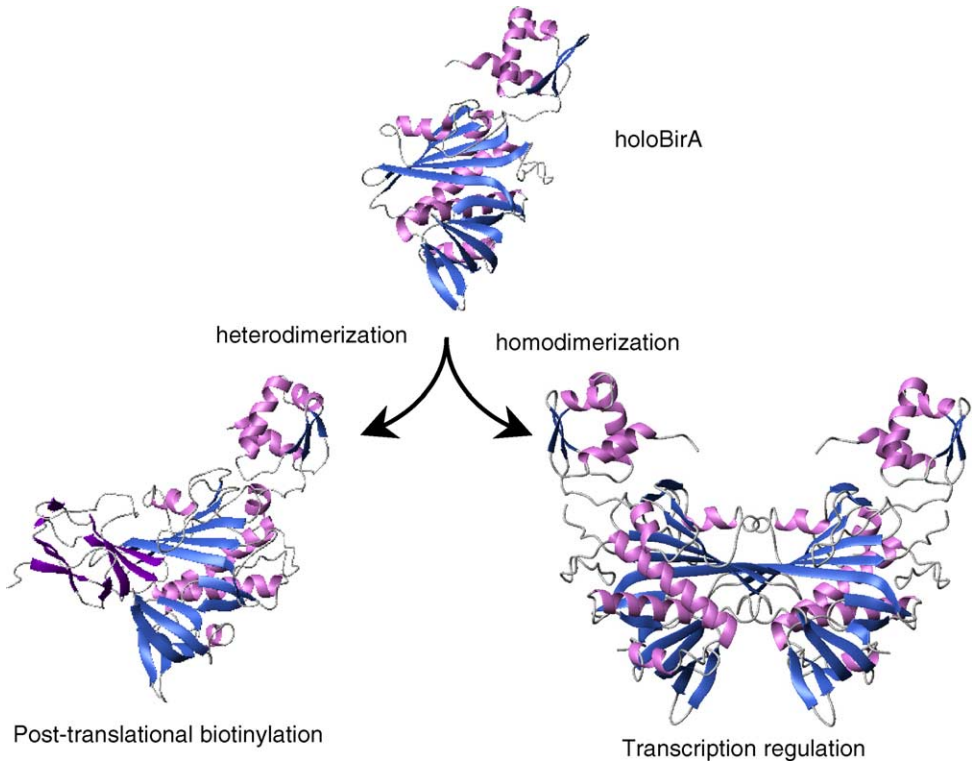

Fig. 2. Structural models of the BirA monomer, the BirA homodimer, and the BirA: apoBCCP87 heterodimer. The models were created in MolMol [23] with the following PDB files as input: BirA monomer (1BIB), BirA homodimer (1HXD), and BirA: apoBCCP heterodimer model (1K67).

of the functional switch. As indicated in Fig. 1, the adenylate-bound enzyme/repressor is the active species in both site-specific DNA binding and biotin transfer. Indeed, the apoBirA species binds with very low affinity to the biotin operator sequence [8,11]. Adenylate binding to BirA allosterically activates it for high-affinity bioO binding.

The mechanism of allosteric activation of BirA by bio-5′ -AMP has been subjected to extensive studies. The 40-bp bioO sequence is characterized by inverted dyad symmetry, and two repressor monomers bind to the site [12,13]. Earlier studies indicated that the adenylate-bound protein is predominantly monomeric under the conditions of DNA binding. Moreover, these results indicated that the two monomers function synergistically in binding to the operator. Thus, dimerization and DNA binding are coupled in assembly of the repression complex. Measurements of repressor self-assembly in the absence and presence of bio-5′ -AMP indicate that the dimerization reaction is promoted by corepressor binding [14]. In fact, the energetics of dimerization become more favorable by −4 to −5 kcal/mol as a consequence of bio-5′ -AMP binding [11]. Moreover, mutations that result in defects in repressor dimerization also lead to loss of repression in vivo [7,15]. Thus, the effector functions by promoting dimerization that is required for tight binding of the repressor to the operator site.

Structural studies have revealed, in part, how effector binding promotes dimerization. The structure of the biotin-bound repressor was solved by X-ray crystallography [16]. While biotin is not the physiological corepressor of DNA binding, it is a weak allosteric activator of transcription repression complex assembly. The structure reveals a dimerization interface formed by side-by-side alignment of the β-sheets of the repressor central domain (Fig. 2). In addition, three of the four loops that were partially disordered in the apo-structure are located in the interface. Mutations in each of these loops result in loss of repression in vivo and dramatically reduced dimerization in vitro [7,15]. These structural data, when combined with the functional studies, prompted formulation of a mechanism of allosteric activation. The activation of repressor binding to bioO occurs as a consequence of the increased dimerization of repressor resulting from bio-5′ -AMP binding. The increased dimerization energetics results from folding of the loops that accompanies effector binding. This ligand-mediated organization of the loops enhances the dimerization process by reducing the entropic penalty associated with dimerization.

3. Dimerization and the functional switch

The studies of the transcription complex assembly described above demonstrate that the central species in activation of the repressor for DNA binding is the adenylate-bound monomer. The adenylate, by binding to the repressor monomer, changes its properties so that it dimerizes with greatly enhanced energetics. The enhanced dimerization energetics leads to increased binding to bioO and resulting transcription repression. The activated or

holorepressor monomer is also the active species in biotin transfer to apoBCCP.

Results of in vivo studies and structural modeling have proven important in developing a model for functional switching of BirA. As indicated above, synthesis of ACC, the only biotin acceptor in *E. coli*, is growth-rate controlled. Moreover, Cronan [17] demonstrated that simple overexpression of the biotin acceptor protein in vivo results in derepression of transcription initiation at the biotin biosynthetic operon control region. This indicates that as the intracellular concentration of acceptor protein is increased, the repressor/holoenzyme ligase partitions away from DNA binding and toward its enzymatic function. The structure of the biotin-bound repressor homodimer suggests that the enzyme active site is tied up in the protein/protein interface. This conclusion is based on the observation that in the structure the reactive carbonyl of the biotin valerate chain is in close proximity to the interface [16]. This observation prompted development of a model of the complex formed between apoBCCP and BirA (Fig. 2) [18]. In this model, which is supported by results of biochemical studies [18,19], the heterodimeric BirA/BCCP interface employs the same surface of BirA as that utilized in the homodimer interface. Instead of the antiparallel sheet interaction observed in the homodimer, a parallel β-sheet interaction is formed in the heterodimer.

The combined structural model and in vivo studies have led to formulation of testable models for the mechanism of functional switching in the biotin regulatory system. The two structures of the hetero- and homodimeric interfaces involve a single surface of BirA and are, therefore, mutually exclusive. As indicated above, overexpression of the biotin acceptor protein in vivo results in derepression of transcription initiation at the biotin biosynthetic control region. Based on these two observations, the switch can occur either upstream of the two dimerization processes or the acceptor protein can act on the DNA-bound repressor, actively causing its dissociation from the biotin operator. In the first model, the switch occurs at partitioning of the repressor between homo- and heterodimerization. In the second, apoBCCP forms a ternary complex with the repressor and DNA, and forces dissociation of the holoBirA/bioO complex.

The two models for functional switching were tested using kinetic approaches. The following experiments were performed:

1. The effect of addition of apoBCCP on the dissociation of the holoBirA/bioO complex was measured.
2. The kinetics of assembly of the repression complex from holoBirA and bioO were measured.
3. Perturbations of the kinetics of assembly of the repression complex by addition of apoBCCP were investigated.

Results of measurements of dissociation of the holoBirA/bioO complex were sufficient to rule out a model involving forced dissociation of the repression complex. Measurement of the dissociation, which was monitored by DNaseI cleavage of the biotin operator DNA upon dilution of the holoBirA/bioO complex, revealed slow unimolecular dissociation. Moreover, the unimolecular dissociation rate is independent of the addition of the acceptor protein [20].

If the functional switch involves partitioning of the holorepressor monomer between homo- and heterodimerization, the dimer is expected to be an obligate intermediate in assembly of the holoBirA/bioO complex. The kinetics of assembly of the repression complex were measured by rapid quench-flow DNaseI footprinting. Results of these measurements indicate that the assembly process does occur through a holorepressor dimer intermediate [20]. While not conclusive, this result supports the idea that the functional partitioning of holoBirA occurs at kinetic partitioning of the protein between homo- and heterodimerization.

The effects of apoBCCP addition on the rapid kinetics of assembly of the holoBirA/bioO complex were also measured. Addition of the acceptor protein to the quench-flow measurements of bioO binding kinetics reveals that the major effect of acceptor protein is to reduce the final level of bioO binding (manuscript in preparation). Moreover, the magnitude of this reduction increased with increasing acceptor protein concentration. This result is consistent with a model in which the acceptor protein sequesters some fraction of the repressor in a complex thus rendering it unavailable for dimerization and bioO binding. The result is, moreover, consistent with a simple partitioning model in which the relative kinetics of the two dimerization reactions govern repressor function. At low acceptor protein concentrations (slow growth rate), homodimerization is favored and BirA functions as a transcription repressor. By contrast, as acceptor protein concentration increases (rapid growth rate), the rate of heterodimerization of holoBirA with acceptor protein dominates and BirA functions as an enzyme.

4. Summary

The mechanism of the biotin repressor functional switch appears to adhere to relatively simple kinetic rules. However, direct measurements of the kinetics of the relevant protein/protein binding reactions will be required to further test this model. Results of recent studies implicate the eukaryotic biotin holoenzyme ligases in regulation of transcription of several genes [21]. However, in these systems the biochemical activity of the holoenzyme synthetase is retained in both functions. While in its metabolic function, the BHS biotinylates biotin-dependent carboxylases, in its transcriptional regulatory role it transfers biotin to histones [22]. In elucidating the mechanism of this functional switch one must determine the process by which the target of biotin transfer is selected. Since one process occurs in the cytoplasm while the other occurs in the nucleus, understanding the control of compartmentalization of the eukaryotic enzymes will be crucial to understanding their functional switching.

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