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Current Topics

Functional Switches in Transcription Regulation; Molecular Mimicry and Plasticity in Protein−Protein Interactions†

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ABSTRACT: The pairwise interactions in which a protein participates can dictate the functional properties of the protein. Indeed, there are many biological regulatory processes in which protein function is orchestrated via exchange of one protein partner for another. Several transcription regulatory proteins that participate in functional switching have been identified and extensively studied. In the examination of the structural basis of the switch for four of these proteins, a common theme of mutually exclusive protein−protein interactions emerges. The ability of these proteins to utilize the same surface to form alternative interactions reflects a second characteristic of these systems of molecular mimicry. Finally, in two of the systems, plasticity in adoption of secondary structure is integral to the formation of alternative protein−protein interactions. Regulation of formation of the alternative parings occurs by a range of mechanisms. In the simplest systems, the outcome of the switch reflects the relative probability of encounter of one partner versus another. Alternatively, more complex mechanisms include regulation of protein availability and compartmentalization of protein partners.

Functional switching of macromolecules is an integral feature of many biological processes. A functional switch may involve a relatively subtle change in the efficiency of, for example, an enzyme via an allosteric mechanism, or alternatively, a macromolecule may undergo a more dramatic conversion to a completely distinct activity. Several proteins that function in transcription initiation have been shown to participate in additional cellular processes. The additional function(s) of each of these proteins can range from a closely related role in replication to an apparently unrelated activity in regeneration of an enzymatic cofactor. A mechanistic understanding of the regulatory biology of these proteins requires elucidation of the structural and energetic basis of the functional switches that they undergo. In this review, the state of our knowledge of functional switching of four transcription regulators, each of which possesses at least one additional biological function, is discussed. In each of these systems, the functional switch is correlated with a switch in protein−protein interactions, and in all cases, these distinct interactions are mutually exclusive. Structurally, these systems take advantage of molecular mimicry and plasticity in protein structure in forming these mutually exclusive complexes. However, the repertoire of mechanisms utilized for regulation of the switch is broad. Control of the formation of the alternative protein−protein complexes ranges from the simple relative probability of encounter of two partners to more elaborate processes including compartmentalization and chaperone-mediated remodeling of protein structure.

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Classifications of Protein−Protein Interactions

Regulation of function of many proteins, including those discussed in this review, occurs via switching of protein interaction partners. Thus prior to discussing the specific systems, classifications of protein−protein interactions will be reviewed. Thornton and colleagues have articulated a comprehensive classification scheme for these interactions as well as their regulation (*57*). Protein complexes can form either between identical protomers or nonidentical subunits with the former referred to as homooligomers and the latter as heterooligomers. Functional switching of transcription regulators is frequently characterized as trading off of a homotypic interaction for heterotypic. Protein assembly and protein folding can be interdependent or independent processes, which are termed obligate and nonobligate, respectively. In a nonobligate protein oligomer, each of the participating protomers can fold independent of complex formation. Alternatively, in obligate complexes, the individual protomers do not form stably folded structures. Rather, folding is coupled to assembly. Both obligate and nonobligate species are found in the systems that are the subject of this review. Moreover, the plasticity and apparent promiscuity observed in some of the protein−protein interactions associated with functional switching illustrate an advantage of obligate interactions for these switches. A third classification provided by Thornton is related to the lifetime of the protein−protein complexes, which may be transient or long-lived. Since functional switching occurs concomitant with switching of protein−protein interactions, the lifetime of each alternative complex can be significant in consideration of the mechanism of functional switching. Finally, the oligomeric state of a protein can be subject to control. The control can be at the level of the regulating the probability of encounter via mechanisms such as colocalization or coexpression. Local concentration effects can also influence the formation of protein−protein interactions and are particularly significant for proteins that interact with DNA. A third general mechanism for regulating protein−protein interactions is alteration of the local physicochemical environment. Shifts in concentrations of small ligand effectors such as ions, protons, or other small ligands as well as posttranslational modification of proteins can exert profound influences on assembly properties and, thus, biological function.

DCoH and Hepatocyte Nuclear Factor: Metabolic Cofactor Regeneration and Transcription Activation

The first example of a multifunctional transcription factor is a protein that serves both as a transcription coactivator and as an enzyme. The protein is referred to as the dimerization cofactor of hepatocyte nuclear factor or DCoH.[1] As an enzyme DCoH is a pterin-4a-carbinolamine dehydratase, which functions in regeneration of the cofactor biopterin (*1*). In transcription, the interaction of DCoH with the transcription factor, hepatocyte nuclear factor-1α, stimulates HNF-1α-controlled transcription in vivo (*2*). The HNF-1α protein functions in regulation of transcription of genes in liver, kidney, pancreatic islet cells, and stomach (*3*).

The dimerization cofactor, DCoH, was first identified as a factor that stabilizes dimers of hepatocyte nuclear factor, HNF-1α (*2*). The cofactor copurifies with HNF-1α and was demonstrated to stabilize the transcription factor dimers against exchange. DCoH by itself exhibits no DNA binding and its role in stabilizing the interaction of HNF-1α with DNA is unclear. It has been reported to provide no stabilization of the protein−DNA complex to competition but has been demonstrated to stabilize the complex of HNF-1α bound to the HNF site against salt-induced dissociation (*4*). As a coactivator, DCoH has been shown in transfection experiments to selectively enhance the transcriptional activity of HNF-1α (*2*). The protein was subsequently shown to be identical to the enzyme that functions in regeneration of the cofactor biopterin (*1*). In its enzymatic function, DCoH stimulates phenylalanine hydroxylase by catalyzing a dehyration step in the regeneration of the cofactor tetrahydrobiopterin, thereby preventing the formation of 7-BH_4, an inhibitory isomeric form of BH_4. With the exception of some evidence that DCoH functions in activation of transcription of the phenylalanine hydroxylase gene, no clear-cut physiological link between the two activities of DCoH has been firmly established (*5*).

Structural Studies

The structure of DCoH that was determined by X-ray crystallography is shown in Figure 1 (*6*, *7*). The enzyme is a dimer of dimers of four identical 12 kD subunits and contains four active sites. In the tetramer, each dimer forms by pairing of the β-sheet of each participating monomer. A striking feature of the dimer unit is the saddle-like structure associated with the extended β-sheet. The two dimers interact via α-helices to form a four-helix bundle. The resulting interface is so stable that the dimer−tetramer equilibrium for DCoH cannot be measured.

The hepatocyte nuclear factor, HNF-1α, a 631 amino acid residue protein, is composed of four functional regions (*3*). These include an N-terminal region, residues 1−32, that functions in dimerization, a DNA-binding motif composed of residues 98−280 that is homeodomain-like, a C-terminal transactivation domain made up of residues 281−631 and a flexible linker region of residues 33−97 that connects the dimerization and DNA binding regions. It is the dimerization region of the protein that interacts with the coactivator, DCoH, and is necessary for the transcription activation function of HNF-1α. Although HNF-1α can dimerize on its own, the interaction is weak. No structure of the intact HNF-1α is yet available. However, the protein segment encoding the dimerization domain can fold independently forming a dimer. Its structure has been determined by both NMR spectroscopy and X-ray crystallography and is shown in

[1] Abbreviations: DCoH, dimerization cofactor of hepatocyte nuclear factor; HNF, hepatocyte nuclear factor; BH_4, tetrahydrobiopterin; K_D, equilibrium dissociation constant; Wnt signaling, Wnt growth factor signaling pathway; Tcf/LEF, T-cell factor/lymphoid enhancer factor family of transcription factors; E-cadherin, epithelial cadherin; APC, adenomatous polyposis coli protein, a tumor suppressor; HMG, high mobility group box family of DNA binding proteins. HMG refers to the DNA binding domain; Rep proteins, replication initiator proteins; WH domains, winged helix-turn-helix domains; BirA, biotin repressor/biotin holoenzyme ligase; apoBirA, unliganded BirA; holoBirA, BirA bound to biotinyl-5′-AMP; BCCP, biotin carboxyl carrier protein domain of acetyl CoA carboxylase; BCCP87, 87-residue C-terminal domain of BCCP-functionally equivalent to BCCP in the biotin transfer reaction; apoBCCP, the BCCP subunit without the added biotin; holoBCCP, the BCCP subunit modified with biotin; bioO, the 40 basepair biotin operator sequence; bio-5′-AMP, biotinyl-5′ adenosine monophosphate.

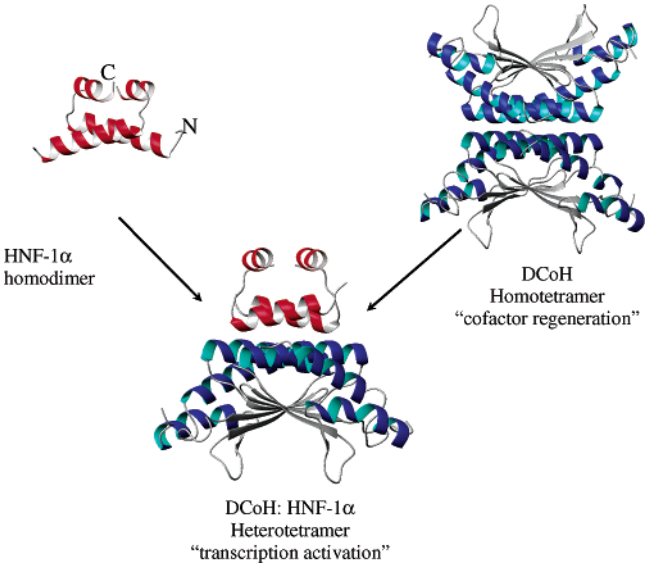

FIGURE 1: Models of the high-resolution structures of the HNF-1α dimerization domain (red; PDB file 1G2Y), DCoH (blue; PDB file 1DCH), and the complex of the HNF-1α dimerization domain bound to a DCoH dimer (PDB file 1F93). The models were generated using MolMol (*46*). N and C indicate the amino and carboxyl termini of one of the chains in the HNF-1α dimer.

Figure 1 (*8*, *9*). The dimerization domain forms an X-type antiparallel, four-helix bundle to which each monomer contributes two α-helices. In the crystal, these dimers, which interact very weakly in solution (K_D > 2mM), form a dimer of dimers. Because this homotetramer structure is not physiologically significant, only the HNF1-α dimer is shown in the figure. The sequence of the dimerization domain is rich in leucine residues, which pack into the hydrophobic core of each dimer and also present a surface for interaction with DCoH (see below).

Molecular Mimicry in the DCoH−HNF-1α Complex

The interaction between HNF-1α and DCoH has been defined through X-ray crystallographic studies (*10*). In the structure of the heterotetrameric complex shown in Figure 1, the interface formed between α-helices of the two proteins is composed of a hydrophobic core rimmed by complementary charged and polar interactions. The dimerization module of HNF-1α binds to the same surface of DCoH that mediates tetramerization of the coactivator. The helices of the two proteins in contact in the interface run in opposite directions. Despite the fact that no sequence similarity exists for DCoH and HNF-1α, chemical interactions that occur in the homooligomeric interface are satisfied by alternative interactions in the heteromeric interface. For example, residues of DCoH that form ion pairs with residues of HNF-1α in the heterotetramer form alternative hydrogen bonds in the DCoH homotetramer (*10*). The one conserved interaction in both complexes involves a leucine from each protein that fills a pocket both in the homotetramer and heterotetramer interfaces. Thus the trading off of a protein partner in this system results in very few net changes in the chemical nature of the interfacial interaction. Moreover, there is no extensive change in the secondary structure of either protein partner. Side chains do, however, undergo some conformational rearrangement. This side chain flexibility allows HNF-1α to serve as a reasonable mimic of DCoH with respect to the chemical groups that it offers for the protein−protein interaction.

Functional Switching of DCoH

The two interfaces formed in the DCoH/HNF-1α regulatory switch are mutually exclusive. The functional consequence in this case is, however, not immediately apparent. First, it appears that the enzymatic and transcriptional regulatory functions of DCoH occur in different cellular compartments. Thus, while the enzymatic function is cytoplasmic, transcription regulation occurs in the nucleus. Second, the formation of the heterotetramer between DCoH and HNF-1α does not appear to inhibit the enzymatic function of the coactivator (*4*). The mechanism of switching of interfaces in the DCoH−HNF-1α system is also not known. The DCoH homotetramer is very stable, and it has been shown that simple mixing of the two proteins results in no subunit exchange (*4*). Formation of heterotetramers in vitro requires denaturation of mixtures of the two proteins followed by renaturation (*10*). Therefore it is clear that no free exchange of DCoH dimers occurs between the homotetrameric and heterotetrameric assembly states. However, a homologue of DCoH, DCoH2, has recently been identified that forms a signficiantly weaker tetramer than does DCoH (*11*). Currently studies are focused on examining the exchange of homo- and heterotypic interactions of this species with HNF-1α. A second related issue is that of patterns of expression of the DCoH coactivator and the HNF-1α transcription factor. Results of measurements of the developmental and tissue specificity of DCoH expression in *Xenopus laevis* revealed that its location does not always coincide with that of HNF-1α. This has led to the suggestion that, in addition to HNF-1α, DCoH may function as a coactivator for other transcription factors (*12*). These authors speculate that, assuming that DCoH interacts with homeodomain transcription factors, members of the POU family may serve as an alternative partners.

β-Catenin Plasticity and Molecular Mimicry

A second example of multifunctionality in transcription factors is that of β-catenin, which functions both in cell−cell adhesion and as a transcriptional coactivator (*13*). In its cell adhesion function, β-catenin interacts with cadherins. The cadherins are components of adherens junctions that constitute sites of cell−cell contact to which the actin cytoskeletons of adjacent cells are linked. As a coactivator of transcription initiation β-catenin functions in the Wnt growth factor signaling pathway that is important in cell fate determination (*14*). Members of the T-cell factor (Tcf)/lymphoid enhancer factor (LEF) family of transcription factors interact with β-catenin to activate transcription initiation.

Control of β-catenin function is at the level of protein availability. In the absence of Wnt signaling, the protein functions only in cell adhesion. In adherens assembly, the cadherins associate with β-catenins shortly after biosynthesis while still in the endoplasmic reticulum. In the absence of Wnt signaling, excess β-catenin is targeted for degradation via glycogen synthase β-catalyzed phosphorylation (*15*, *16*). This phosphorylation occurs in the context of a multiprotein complex that includes the tumor suppressor adenomatous

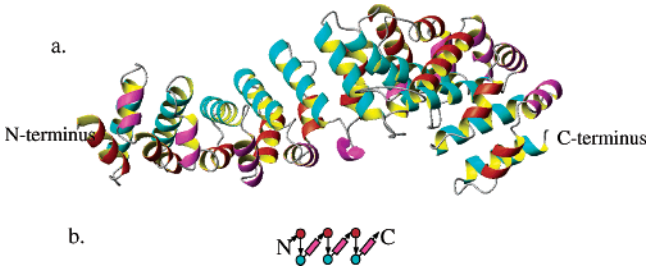

FIGURE 2: Three-dimensional structure of (a) the β-catenin armadillo repeat (PDB file 3BCT). The arrangement of the three helices in each armadillo repeat are shown in panel b with the following color code: H1, violet; H2, orange; H3, cyan.

polyposis coli (APC) and axin. Both of these latter proteins bind directly to β-catenin (*17*−*19*). In response to Wnt signaling the GSK-3β catalyzed phosphorylation of β-catenin is inhibited, the protein accumulates in the cytoplasm and interacts with transcription factors of the LEF/Tcf family, and complexes translocate to the nucleus to function in transcription activation. Thus, multiple protein−protein interactions contribute to regulation of the activity of β-catenin. First, interaction with the APC protein and axin regulates phosphorylation and thus degradation of the protein; second, interactions with cadherins are associated with its function in cell adhesion; finally, direct interaction with the LEF/Tcf family of transcription factors mediate its role in transcription initiation. Although the multiple binding partners share no obvious sequence homology, competition among them for binding to β-catenin has been observed (*18*, *20*).

Structures of β-Catenin and Its Complexes

The β-catenin polypeptide folds into three domains: an amino-terminal domain of 149 amino acids, a central domain of 515 residues, and a carboxy-terminal 108 residue domain (*21*). The central domain is structurally characterized by 12 armadillo repeats (arm). It is this arm repeat segment that binds to β-catenin, cadherins, APC, axin, and the LEF/Tcf family of transcription factors. High-resolution structures of the arm repeat have been determined by X-ray crystallography (Figure 2a) (*22*). Each repeat consists of three α-helices designated H1, H2, and H3, and the repeats are packed against each other in a manner that results in a superhelical structure in the entire domain. The groove of the superhelix, the floor of which is composed of the H3 helices, is positively charged. In the following sections, the description of high-resolution structures of the complexes formed between β-catenin and three of its binding partners illustrates how protein plasticity and molecular mimicry enable the multiple protein−protein interactions that are essential for the multiple functions of β-catenin.

The β-catenin binding partner in cell adhesion, cadherin, is a single-pass transmembrane protein. In adhesion, the extracellular domains of cadherins of adjacent cells interact in a Ca^{2+}-dependent manner. The cytoplasmic domain of cadherin binds to β-catenin, which in turn binds to the actin-associated protein, α-catenin (*23*). These assemblies are referred to as adherens, sites of cell−cell contact that link the actin cytosketons of adjacent cells. The C-terminal cytoplasmic region of epithelial cadherin (E-cadherin) consisting of 72 residues is sufficient for binding to β-catenin

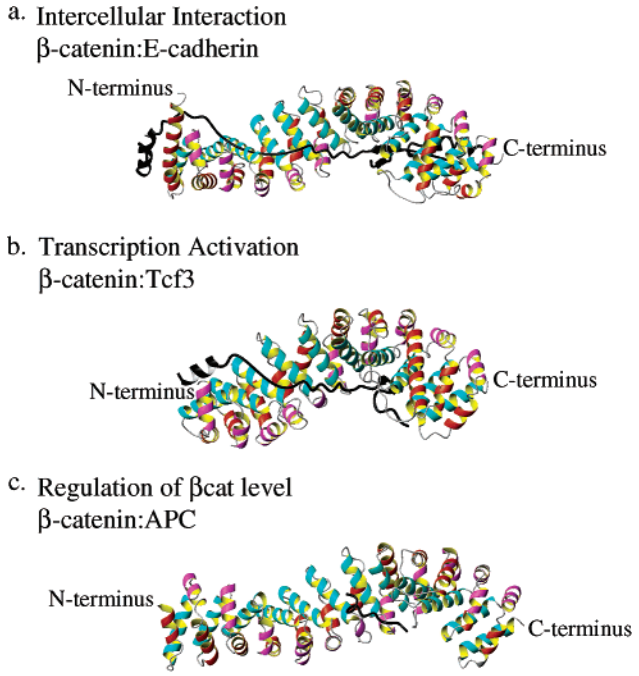

FIGURE 3: Models of complexes of the β-catenin armadillo repeat bound to (a) the cytoplasmic domain of E-cadherin (PDB file 1I7W), (b) the N-terminal segment of the transcription factor Tcf-3 from *Xenopus laevis* (PDB file 1G37) and (c) a 15 residue peptide from the adenomatous polyposis coli protein (PDB file 1JPP). The models were generated using MolMol (*46*). Color coding for the β-catenin arm repeat is as indicated in Figure 2. The binding partners are shown in black.

(*24*). A structure of a complex of the armadillo repeat of β-catenin bound to the cytoplasmic domain of E-cadherin from mouse has been determined by X-ray crystallography and is shown in Figure 3a (*25*). The E-cadherin interacts with the β-catenin, in large part, via the groove formed by H3 of each arm repeat. The interface formed between the two proteins is extensive with 6100 $Å^2$ of surface area buried in the complex.

The Tcf/LEF1 family of transcription factors all share homology in their DNA binding domains as well as at their amino termini (*26*). The DNA binding domains are of the high mobility group or HMG-box family that bind in the minor groove of DNA, while the N-termini interact with β-catenin. A structure of a complex of the β-catenin armadillo repeat region bound to the N-terminal region of *Xenopus* Tcf-3 transcription factor has been determined by X-ray crystallography (Figure 3b) (*27*). As expected from the results of competition studies mentioned above, there is considerable overlap between the sites on β-catenin where E-cadherin and the transcription factor bind. Moreover, close inspection of the interfaces reveals a considerable degree of conservation with respect to the chemical nature of the protein−protein contacts.

The β-catenin protein also interacts with the APC protein in regulation of its turnover. The APC protein possesses three 15 amino acid repeats and seven 20 amino acid repeats that interact with the armadillo repeats of β-catenin (*28*). A structure of the arm repeat region of β-catenin bound to a peptide with a sequence corresponding to the first 15 amino acid repeat has been determined (Figure 3c) (*29*). As observed for Tcf and E-cadherin, the peptide binds in the groove defined by H3 of each armadillo repeat, and the

contacts that it makes in the region of the groove are virtually identical to those formed between the other ligands and that same segment of the groove.

Molecular Mimicry and Protein Plasticity in the β-Catenin Interactions

Comparison of the structures of the complexes of the β-catenin armadillo repeat bound to protein ligands reveals a remarkable level of molecular mimicry in the system. This is most evident in a comparison of the interfaces formed between β-catenin in its complexes with the cytoplasmic domain of cadherin and the N-terminal domain of the transcription factor, Tcf-3. Considerable overlap exists between the binding sites for the two ligands, and although the secondary structures of the Tcf-3 and cadherin segments in the complexes are distinct, the chemical nature of the contacts formed between the two proteins in each complex is well conserved. The Tcf-3 interactions with β-catenin constitute a subset of the interactions formed between β-catenin and cadherin (Figure 3a,b). The structures of the ligands in the cores of the two interfaces, the extended regions that constitute the center of the each ligand in Figure 3a,b, are virtually superimposable. To the C-terminus of this region, although the two polypeptides adopt divergent secondary structures, the chemistry of the interactions between β-catenin and the two ligands is conserved. The β-catenin system, thus, like the DCoH/HNF1-α switch, provides an example of molecular mimicry. The striking distinctive feature of this system is that the apparent promiscuity that characterizes the β-catenin protein−protein interactions is achieved via the plasticity that characterizes all of its binding partners. The three segments of polypeptide chain that interact with the β-catenin armadillo repeats are unstructured when free in solution and acquire structure concomitant with binding to β-catenin. Despite a lack of significant sequence similarity among the ligands, they all adopt structures that present similar chemical groups for interaction with β-catenin. This system is thus an excellent example of how obligate protein−protein interactions can be exploited by a system in which multiple binding partners must adapt to a single receptor protein.

The Functional Switch

The β-catenin system is another example of a system in which the function of a transcriptional regulator is dictated by the pairwise protein−protein interactions in which it participates. At a structural level, the system has evolved so that the different ligands adapt, using different secondary structures, to the features of the receptor groove. Thermodynamic studies of the interaction of one binding partner, T-cell factor 4 (Tcf-4), with β-catenin indicate that the interaction is very tight (*30*, *31*). One might conclude that this would serve as a kinetic barrier to functional switching. However, the system utilizes compartmentalization and phosphorylation to control availability of β-catenin and thus its function. In the absence of the extracellular signaling, excess β-catenin is retained in the cytoplasm via interaction with APC, phosphorylated, and targeted for degradation. Release of this constraint via Wnt signaling allows for accumulation of β-catenin, its interaction with transcription factors, and translocation to the nucleus to function in transcription activation.

The known association of disruption of the β-catenin interactions with APC and LEF with certain forms of cancer renders this interaction a potential target for pharmaceuticals. However, the similarity of the chemistries of the interfaces formed between β-catenin and each of its partners suggest that agents designed to interfere with any one interaction will affect all three.

Replication Initiator Proteins: Functional Switching via Chaperone-Mediated Remodeling of Protein Structure

Initiation of replication of many circular bacterial plasmids occurs at specific sequences referred to as origins of replication (*32*). For some plasmids, the origin sequence is characterized by direct repeat sequences or iterons that function as binding sites for the plasmid-encoded replication initiator, or Rep, protein. The assembly of multiple Rep proteins on these sequences in concert with host-encoded proteins is necessary for initiation of plasmid replication. Formation of the multiprotein complex on the DNA is a prerequisite to unwinding of the DNA and synthesis of daughter molecules. In addition to their function in DNA replication the Rep proteins regulate transcription initiation in an autoregulatory mechanism. The protein binds to an inverted repeat sequence at the promoter region for the gene that encodes Rep thus regulating its own biosynthesis.

The functional switch of a plasmid Rep protein from transcription repression to DNA replication occurs via a switch in assembly properties. In general, these proteins bind cooperatively as monomers to the replication initiation regions of plasmids (*32*). For example, binding of RepA encoded by the *Pseudomonas* plasmid pPS10 to the direct repeats of the origin of replication iteron occurs by cooperative association of multiple Rep protein monomers (*33*−*35*). By contrast, the dimer binds to the inverted repeat sequence of the promoter region of the *repA* gene. The stability of the Rep dimer appears to be high (*33*), and consequently, a central question related to the functional switch is how dissociation of the dimer occurs to shift RepA function from transcription repression to replication initiation.

The functional switch of Rep from transcription regulation to DNA replication is a facilitated process. Rep dimers are very stable in solution, and at concentrations in the low micromolar range, the protein is 100% dimer. This result is consistent with an equilibrium dissociation constant for the dimerization reaction in the nanomolar range of concentration (*33*). Thus, the shift of the protein from the dimeric species that functions in transcription repression to the monomeric species that functions in replication cannot occur by a simple dissociation process. Results of biochemical studies indicate that either chaperones or the iteron site itself can drive the RepA dimer to its monomeric state. The chaperones DnaK-DnaJ-GrpE, ClpA, and ClpX have all been shown to activate Rep proteins for function in replication of a number of plasmids (*36*−*38*). Coupled to this dissociation is conformational conversion to a species that is competent to bind iteron. Furthermore, addition of double-stranded DNA oligomers characterized by the sequence of the iteron site to the RepA dimer results in dissociation to monomer with the same conformational transitions as those observed in the chaperone-mediated process (*33*). Results of circular dichroism measurements of the conformational transitions that the

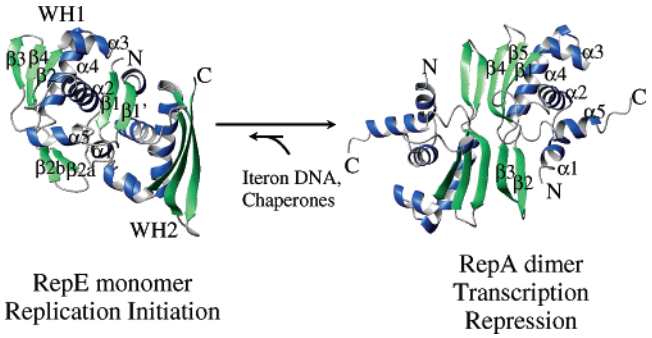

FIGURE 4: Interconversion of the dimeric Rep protein that functions in transcription regulation and the monomeric species that functions in replication initiation. The dimer and monomer models were generated using the PDB files 1HKQ and 1REP, respectively. Models were constructed using graphics program MolMol (*46*). The numbering of secondary structure elements for only the WH1 domain is shown in both models. N and C indicate amino and carboxy termini of the polypeptide chain.

protein undergoes upon chaperone- or iteron DNA-induced dissociation indicate a loss in α-helical structure accompanied by an increase in β-strand structure (*33*). However, the fraction of the secondary structure estimated to participate in this change is small and constitutes only 4% of the total secondary structure.

Structures of the Rep Monomer and Dimer

Structures of a monomeric and dimeric species of plasmid replication initiator proteins are available. It should be noted that the proteins used in the two structural studies are encoded by different plasmids and are characterized by different primary structures. The monomer structure is of the protein RepE of F factor, a plasmid involved in sexual conjugation in *Escherichia coli* (*39*). The 251 residue monomer is characterized by a structure that possesses pseudo-dyad symmetry with N and C terminal domains of similar topology (Figure 4). Each domain is comprised of four α-helices and four β-strands. The N-terminal domain possesses an additional helix and two β-strands. The tertiary structure adopted by each domain is similar to that of the winged helix-turn-helix (WH) structure that characterizes the catabolite activator protein with the N-terminal domain fold bearing closer similarity to CAP. In the structure, which was determined for the protein−DNA complex, each of the WH domains interacts with consecutive major grooves of the 19 base pair iteron sequence with which the protein was cocrystallized.

The structure of a Rep dimer, RepA protein from the plasmid pPS10, has been obtained by X-ray crystallography (*40*). The primary structure of this protein bears 25% identity plus 46% conservation with that of RepE. The dimer structure was, moreover, determined for residues 8−132 of the 230 RepA polypeptide chain. Thus the structure corresponds to the segment of RepE that folds to form the WH1 domain in the monomer. In the dimer, both protomers adopt nearly identical structures, and the interface is formed by hydrogen bonding of β-strands from each monomer (Figure 4). The resulting interface is a five-stranded antiparallel β-sheet. Comparison of the folds of the monomeric and dimeric Rep polypeptide has been used to infer the structural transitions that accompany conversion of the dimer to monomer. The WH1 segment is nearly identical in the two structures. However, outside of this structurally conserved region, there are changes in the secondary structures of both the N- and C-termini of the protein. The extreme N-terminus of the protein, which is disordered in the dimer structure, forms a β-strand in the monomer. In addition, segments of α-helices at both termini undergo conversion to loop and β-strand conformation in the transition from dimer to monomer. In total, 7% of the residues of each monomer participate in the secondary structure transformation that accompanies dissociation of the dimer. In critically evaluating the significance of the results of these high-resolution structural studies, one must keep in mind that the structure of the dimer corresponds to that of a fragment of RepA, while that of the monomer is for an intact and distinct, albeit homologous, initiator protein, RepE. Nonetheless, the excellent agreement of the structural differences between the monomer and dimer observed in the crystal structures with the changes deduced from results of solution measurements helps to mitigate this concern (*33*).

The structural changes and dissociation that accompany the transition of Rep protein from transcription regulation to replication initiation are facilitated processes. Either the chaperones DnaK-DnaJ-GrpE, ClpA, and ClpX or the iteron DNA itself can facilitate the process. In the conversion from the transcription repressor to the replication initiator, the stable monomer−monomer interface and the fold associated with this interface are altered. The chaperone is thought to facilitate both dimer disruption and "remodeling" of the secondary structure. In assembly of the replication initiator complex, the Rep protein binds cooperatively to multiple sites in the origin. This cooperativity is mediated by a protein−protein interaction that, because it is formed from a head-to-tail arrangement of protein monomers, must be distinct from that observed in the dimeric repressor structure. Based on the known structure of the RepE monomer−iteron complex, the interaction is assumed to be mediated by the β-sheets of WH1 and WH2 domains of adjacent monomers (Figure 4). Thus Rep is an example of a protein in which the same polypeptide chain adopts two distinct structures that result in different protein−protein interactions thereby yielding different functions. The chaperone or the iteron DNA in this system reduces the kinetic barrier associated with the conversion.

The Biotin Regulatory System: A Switch from Essential Metabolic Enzyme to Transcriptional Repressor

The biotin regulatory system provides a final example of a system in which a protein functions both in repression of transcription initiation and in an essential metabolic reaction (Figure 5) (*41*, *42*). This is a system for which the solution biophysical studies are very well developed. While the structural details of interactions associated with each function are not fully elucidated, the accumulated data are consistent with a switching involving formation of mutually exclusive protein−protein interactions. The enzymatic and transcription repression functions of the biotin repressor are at first consideration unrelated. However, the bifunctionality makes physiological sense. The enzymatic function of the biotin repressor, BirA, is catalysis of posttranslational linkage of biotin to the biotin carboxyl protein subunit (BCCP) of the biotin-dependent carboxylase, acetyl-CoA carboxylase. The transcriptional repression function occurs at the biotin biosynthetic operon at which BirA binds to the 40 base-pair

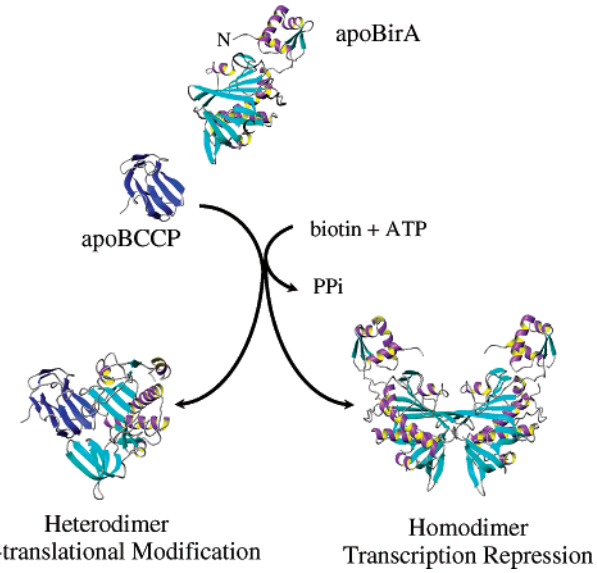

FIGURE 5: Schematic representation of the functional switch of the *E. coli* protein, BirA, between its biotin ligation and transcription repression functions. The models were generated using MolMol (*46*) with the Protein Data Bank files 1BIB (BirA monomer), 1HXD (BirA dimer), 1BIA (BCCP), and 1K67 (BirA−BCCP). For simplicity, the DNA binding domain is absent in the model of the heterodimer structure that forms between the BirA and BCCP87 monomers. N and C indicate the amino and carboxy termini of the BirA polypeptide chain.

biotin operator sequence to repressor transcription initiation at two divergent promoters. Thus a single protein both funnels biotin into metabolism via its biotin ligation function and regulates biotin biosynthesis via its transcription regulatory function. The biotin ligation function occurs in two steps involving initial synthesis of the intermediate, biotinoyl-5′-AMP, from substrates biotin and ATP followed by transfer of the biotin moiety to a single lysine residue of BCCP (*43*). Remarkably, the adenylated biotin intermediate serves a second function in allosteric activation of the BirA for binding to the biotin operator DNA (*44*).

The Biotin Repressor: Homodimerization and Ligand Binding

The structures of both the biotin ligase and the biotin carboxyl carrier protein domain that functions in biotin transfer are known. The biotin ligase is a 35.3 kD protein, and the structure of the monomeric unliganded protein is shown in Figure 5 (*45*, *46*). The protein consists of three domains, the N-terminal winged helix-turn-helix DNA binding domain, the central domain that is composed of a core β-sheet surrounded by α-helices, and the C-terminal domain that is entirely β-sheet structure. A notable feature of the central domain structure is the existence of four partially disordered surface loops.

A structure of the biotin repressor bound to the substrate, biotin, is also available. Biotin, however, does not simply function as a substrate in bio-5′-AMP synthesis but is also a weak allosteric activator of the DNA binding function of BirA (*44*, *47*). The structure determined for this liganded form of the protein is dimeric (*48*). Interestingly, the major structural differences between the protein in its dimeric and monomeric forms are localized to three of the four central domain surface loops mentioned above. Electron density for each of the three loops is clearly visible in the structure of the dimer. While one of the loops directly participates in biotin binding, the other two appear to function only in the monomer−monomer interface. Additionally, the biotin-binding loop contributes side chains to the homodimer interface.

Extensive functional studies of the mechanism of allosteric activation of BirA resulting from binding of bio-5′-AMP have been performed. First, binding of bio-5′-AMP significantly enhances the energetics of BirA dimerization (*49*). Moreover, results of studies of mutants of BirA in the three loops found in the dimer interface indicate that they are defective in dimerization (*50*). Taken together, the results of solution and structural studies of the system are consistent with a model in which binding of the allosteric effector induces a disorder-to-order transition in one or all of the three loops. This preorganization process presumably reduces the entropic barrier to dimerization of BirA.

The homodimerization of BirA is critical for tight binding of the protein to the biotin operator sequence. Mutation of three residues in the interfacial loops not only compromises dimerization but also renders these proteins defective in site-specific binding to bioO and transcription repression in vivo (*42*, *50*). These combined results suggest a model for allosteric activation of BirA in which effector binding enhances dimerization, which enhances the total free energy of assembly of the repression complex. Indeed, a direct correlation between the extent to which a small ligand enhances dimerization energetics and the total assembly energetics of the protein−DNA complex has been found (*47*). These results emphasize the importance of modulation of the stability of protein−protein interactions in dictating the level of occupancy and thus the level of transcription initiation at any transcription control region.

Functional Switching in the Biotin Regulatory System

The level of transcription initiation at the biotin operon is dictated by the occupancy of the biotin operator by holoBirA. This, in turn, is regulated by the demand for biotin. However, the signal for this demand is not biotin but, rather, the intracellular level of the acceptor protein, apoBCCP (*51*). Indeed, induction of expression of the apoBCCP results in derepression at the transcription control region of the biotin operon. The structure of the biotinylated domain of BCCP has been determined by X-ray crystallography and multidimensional NMR spectroscopy and is shown in Figure 5 (*52*, *53*). The protein is all β-strand with the lysine residue that accepts biotin protruding from the tip of a type II β-turn. No structure of the complex of holoBirA bound to apoBCCP is yet available. However, the availability of the BirA−biotin and apoBCCP structures prompted construction of a model of the complex shown in Figure 5 (*54*). In the model, BCCP, like the second monomer in a BirA dimer, extends the central β-sheet of a repressor monomer. Moreover, in the model, the acceptor lysine residue is ideally positioned for biotin transfer. Furthermore, the model is consistent with results of low-resolution NMR and mutagenesis studies (*55*, *56*).

The known and proposed structures of the homodimer and heterodimer provide the basis for the prediction that functional switching in the biotin regulatory system reflects competition between apoBCCP and a second holoBirA

monomer for interaction with a holoBirA monomer. Thus, a homodimeric interaction is replaced by a heterodimeric interaction. Moreover, the interactions of the proteins in the hetero- and homodimers are relatively weak or transient. Therefore, the control of function in this system is most likely simply at the level of the probability of encounter between two proteins. When apoBCCP concentration is high, the probability of heterodimer formation is commensurately high and holoBirA functions as an enzyme. Alternatively, depletion of the apoBCCP pool results in accumulation of holoBirA, homodimerization, and site-specific DNA binding. Questions that remain to be addressed in the system relate to how a single surface on BirA can be utilized for both homodimerization and the heterologous interaction with BCCP. Superficially the interactions are structurally similar as both involve hydrogen bonding of β-strands. However, in the homodimerization, an antiparallel strand interaction is formed, while in the heterodimer, a parallel strand interaction is predicted. Moreover, the loops are clearly involved in stabilizing the homodimer interface. Results of biotin transfer studies performed on the loop mutants suggest that a subset of the loop residues that are involved in the homodimer interface are also utilized for the interaction with apoBCCP (*54*). Direct experimental determination of the heterodimer structure as well as thorough analysis of structure−energetic relationships for both complexes will be required to resolve these questions regarding molecular mimicry in this system.

Summary

The proteins discussed in this review all undergo switching from participation in transcription control to some other function. At a structural level, the switch of each protein involves a change in protein−protein interaction. For the biotin repressor, DCoH, and β-catenin, one partner is actually swapped for a distinct protein partner. Among the replication initiators, a rearrangement of a single polypeptide chain leads to a switch from tight homodimerization to the relatively weak protein−protein interactions associated with cooperative binding to multiple iteron sites. The pathway to repartnering can occur by a range of mechanisms. In the biotin repressor system, the alternative protein−protein interactions are relatively weak and both interactions involve association of prefolded protein units. In this case, the switch appears to be kinetically regulated and depends simply on the probability of encounter of like versus distinct protein partner. In the DCoH system, it is difficult to know how the switch in regulated. The coactivator DCoH forms a very stable tetramer, and in vitro, assembly of the heterotetramer occurs only if the two proteins are mixed in denaturing conditions. The β-catenin system, despite the fact that all three protein−protein interactions associated with the switch are competitive, overcomes the switching dilemma via limiting availability and localization. An extracellular signal initiates the events that allow expansion of the repertoire of β-catenin function to transcriptional activation. Finally, the switch of the replication initiation protein is facilitated by "remodeling" chaperones. Perhaps a similar system functions in the DCoH switch. In terms of the classification of protein−protein interactions discussed at the outset, the transcription switches employ the range from nonobligate to obligate and short-lived to very stable. Given this range of physical−chemical properties associated with the alternative protein−protein complexes, a number of mechanisms have evolved to regulate these functional switches.

REFERENCES

1. Citron, B. A., Davis, M. D., Milstien, S., Gutierrez, J., Mendel, D. B., Crabtree, G. R., and Kaufman, S. (1992) 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. 89*, 11891−11894.
2. Mendel, D. B., Khavari, P. A., Conley, P. B., Graves, M. K., Hansen, L. P., Admon, A., and Crabtree, G. R. (1991) Characterization of a cofactor that regulates dimerization of a mammalian homeodomain protein, *Science 254*, 1762−1767.
3. Mendel, D. B., and Crabtree, G. R. (1991) HNF-1, a member of a novel class of dimerizing homeodomain proteins, *J. Biol. Chem. 266*, 677−680.
4. Rhee, K. H., Stier, G., Becker, P. B., Suck, D., and Sandaltzopoulos, R. (1997) The bifunctional protein DCoH modulates interactions of the homeodomain transcription factor HNF1 with nucleic acids, *J. Mol. Biol. 265*, 20−29.
5. Lei, X. D., and Kaufman, S. (1998) Identification of hepatic nuclear factor 1 binding sites in the 5′ flanking region of the human phenylalanine hydroxylase gene: Implication of a dual function of phenylalanine hydroxylase stimulator in the phenylalanine hydroxylation system, *Proc. Natl. Acad. Sci. U.S.A. 95*, 1500−1504.
6. Endrizzi, J. A., Cronk, J. D., Wang, W., Crabtree, G. R., and Alber, T. (1995) Crystal structure of DCoH, a bifunctional, protein-binding transcriptional coactivator, *Science 268*, 556−559.
7. Ficner, R., Sauer, U. H., Stier, G., and Suck, D. (1995) Three-dimensional structure of the bifunctional protein pcd/DCoH, a cytoplasmic enzyme interacting with transcription factor HNF1, *EMBO J. 14*, 2034−2042.
8. Rose, R. B., Endrizzi, J. A., Cronk, J. D., Holton, J., and Alber, T. (2000) High-resolution structure of the HNF-1α dimerization domain, *Biochemistry 39*, 15062−15070.
9. Narayana, N., Hua, Q., and Weiss, M. A. (2001) The dimerization domain of HNF-1alpha: Structure and plasticity of an intertwined four-helix bundle with application to diabetes mellitus, *J. Mol. Biol. 310*, 635−658.
10. Rose, R. B., Bayle, J. H., Endrizzi, J. A., Cronk, J. D., Crabtree, G. R., and Alber, T. (2000) Structural basis of dimerization, coactivator recognition and mody3 mutations in HNF-1alpha, *Nat. Struct. Biol. 7*, 744−748.
11. Bayle, J. H., Randazzo, F., Johnen, G., Kaufman, S., Nagy, A., Rossant, J., and Crabtree, G. R. (2002) Hyperphenylalaninemia and impaired glucose tolerance in mice lacking the bifunctional DCoH gene, *J. Biol. Chem. 277*, 28884−28891.
12. Pogge yon Strandmann, E., and Ryffel, G. U. (1995) Developmental expression of the maternal protein xDCoH, the dimerization cofactor of the homeoprotein lfb1 (HNF1), *Development 121*, 1217−1226.
13. Yap, A. S., Brieher, W. M., and Gumbiner, B. M. (1997) Molecular and functional analysis of cadherin-based adherens junctions, *Annu. Rev. Cell Dev. Biol. 13*, 119−146.
14. Polakis, P. (2000) Wnt signaling and cancer, *Genes Dev. 14*, 1837−1851.
15. Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. (1997) Beta-catenin is a target for the ubiquitin-proteasome pathway, *EMBO J. 16*, 3797−3804.
16. Orford, K., Crockett, C., Jensen, J. P., Weissman, A. M., and Byers, S. W. (1997) Serine phosphorylation-regulated ubiquitination and degradation of beta-catenin, *J. Biol. Chem. 272*, 24735−24738.
17. Behrens, J., Jerchow, B. A., Wurtele, M., Grimm, J., Asbrand, C., Wirtz, R., Kuhl, M., Wedlich, D., and Birchmeier, W. (1998) Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta, *Science 280*, 596−599.
18. Hulsken, J., Birchmeier, W., and Behrens, J. (1994) E-cadherin and APC compete for the interaction with beta-catenin and the cytoskeleton, *J. Cell Biol. 127*, 2061−2069.
19. Ikeda, S., Kishida, S., Yamamoto, H., Murai, H., Koyama, S., and Kikuchi, A. (1998) Axin, a negative regulator of the wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin, *EMBO J. 17*, 1371−1384.

20. von Kries, J. P., Winbeck, G., Asbrand, C., Schwarz-Romond, T., Sochnikova, N., Dell'Oro, A., Behrens, J., and Birchmeier, W. (2000) Hot spots in beta-catenin for interactions with LEF-1, conductin and APC, *Nat. Struct. Biol. 7*, 800−807.
21. Peifer, M., Berg, S., and Reynolds, A. B. (1994) A repeating amino acid motif shared by proteins with diverse cellular roles, *Cell 76*, 789−791.
22. Huber, A. H., Nelson, W. J., and Weis, W. I. (1997) Three-dimensional structure of the armadillo repeat region of beta-catenin, *Cell 90*, 871−882.
23. Ozawa, M., Baribault, H., and Kemler, R. (1989) The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species, *EMBO J. 8*, 1711−1717.
24. Ozawa, M., Ringwald, M., and Kemler, R. (1990) Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule, *Proc. Natl. Acad. Sci. U.S.A. 87*, 4246−4250.
25. Huber, A. H., and Weis, W. I. (2001) The structure of the beta-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by beta-catenin, *Cell 105*, 391−402.
26. Roose, J., and Clevers, H. (1999) Tcf transcription factors: Molecular switches in carcinogenesis, *Biochim. Biophys. Acta 1424*, M23−M37.
27. Graham, T. A., Weaver, C., Mao, F., Kimelman, D., and Xu, W. (2000) Crystal structure of a beta-catenin/Tcf complex, *Cell 103*, 885−896.
28. Rubinfeld, B., Albert, I., Porfiri, E., Munemitsu, S., and Polakis, P. (1997) Loss of beta-catenin regulation by the APC tumor suppressor protein correlates with loss of structure due to common somatic mutations of the gene, *Cancer Res. 57*, 4624−4630.
29. Eklof Spink, K., Fridman, S. G., and Weis, W. I. (2001) Molecular mechanisms of beta-catenin recognition by adenomatous polyposis coli revealed by the structure of an APC-beta-catenin complex, *EMBO J. 20*, 6203−6212.
30. Knapp, S., Zamai, M., Volpi, D., Nardese, V., Avanzi, N., Breton, J., Plyte, S., Flocco, M., Marconi, M., Isacchi, A., and Caiolfa, V. R. (2001) Thermodynamics of the high-affinity interaction of Tcf4 with beta-catenin, *J. Mol. Biol. 306*, 1179−1189.
31. Fasolini, M., Wu, X., Flocco, M., Trosset, J. Y., Oppermann, U., and Knapp, S. (2003) Hot spots in Tcf4 for the interaction with beta-catenin, *J. Biol. Chem. 278*, 21092−21098.
32. del Solar, G., Giraldo, R., Ruiz-Echevarria, M. J., Espinosa, M., and Diaz-Orejas, R. (1998) Replication and control of circular bacterial plasmids, *Microbiol. Mol. Biol. Rev. 62*, 434−464.
33. Diaz-Lopez, T., Lages-Gonzalo, M., Serrano-Lopez, A., Alfonso, C., Rivas, G., Diaz-Orejas, R., and Giraldo, R. (2003) Structural changes in Repa, a plasmid replication initiator, upon binding to origin DNA, *J. Biol. Chem. 278*, 18606−18616.
34. Ishiai, M., Wada, C., Kawasaki, Y., and Yura, T. (1994) Replication initiator protein Repe of mini-f plasmid: Functional differentiation between monomers (initiator) and dimers (autogenous repressor), *Proc. Natl. Acad. Sci. U.S.A. 91*, 3839−3843.
35. Garcia de Viedma, D., Giraldo, R., Ruiz-Echevarria, M. J., Lurz, R., and Diaz-Orejas, R. (1995) Transcription of Repa, the gene of the initiation protein of the pseudomonas plasmid pPS10, is autoregulated by interactions of the Repa protein at a symmetrical operator, *J. Mol. Biol. 247*, 211−223.
36. Wickner, S., Hoskins, J., and McKenney, K. (1991) Function of DNAj and DNAk as chaperones in origin-specific DNA binding by Repa, *Nature 350*, 165−167.
37. Wickner, S., Hoskins, J., and McKenney, K. (1991) Monomerization of Repa dimers by heat shock proteins activates binding to DNA replication origin, *Proc. Natl. Acad. Sci. U.S.A. 88*, 7903−7907.
38. Wickner, S., Gottesman, S., Skowyra, D., Hoskins, J., McKenney, K., and Maurizi, M. R. (1994) A molecular chaperone, clpa, functions like DNAk and DNAj, *Proc. Natl. Acad. Sci. U.S.A. 91*, 12218−12222.
39. Komori, H., Matsunaga, F., Higuchi, Y., Ishiai, M., Wada, C., and Miki, K. (1999) Crystal structure of a prokaryotic replication initiator protein bound to DNA at 2.6 Å resolution, *EMBO J. 18*, 4597−4607.
40. Giraldo, R., Fernandez-Tornero, C., Evans, P. R., Diaz-Orejas, R., and Romero, A. (2003) A conformational switch between transcriptional repression and replication initiation in the Repa dimerization domain, *Nat. Struct. Biol. 10*, 565−571.
41. Barker, D. F., and Campbell, A. M. (1981) The BirA gene of *Escherichia coli* encodes a biotin holoenzyme synthetase, *J. Mol. Biol. 146*, 451−467.
42. Barker, D. F., and Campbell, A. M. (1981) Genetic and biochemical characterization of the BirA gene and its product: Evidence for a direct role of biotin holoenzyme synthetase in repression of the biotin operon in *Escherichia coli*, *J. Mol. Biol. 146*, 469−492.
43. Lane, M. D., Rominger, K. L., Young, D. L., and Lynen, F. (1964) The enzymatic synthesis of holotranscarboxylase from apotranscarboxylase and (+)-biotin, *J. Biol. Chem. 239*, 2865−2871.
44. Prakash, O., and Eisenberg, M. A. (1979) Biotinyl 5′-adenylate: Corepressor role in the regulation of the biotin genes of *Escherichia coli* k-12, *Proc. Natl. Acad. Sci. U.S.A. 76*, 5592−5595.
45. Wilson, K. P., Shewchuk, L. M., Brennan, R. G., Otsuka, A. J., and Matthews, B. W. (1992) *Escherichia coli* biotin holoenzyme synthetase/bio repressor crystal structure delineates the biotin- and DNA-binding domains, *Proc. Natl. Acad. Sci. U.S.A. 89*, 9257−9261.
46. Koradi, R., Billeter, M., and Wuthrich, K. (1996) Molmol: A program for display and analysis of macromolecular structures, *J. Mol. Graphics 14*, 51−55, 29−32.
47. Streaker, E. D., Gupta, A., and Beckett, D. (2002) The biotin repressor: Thermodynamic coupling of corepressor binding, protein assembly, and sequence-specific DNA binding, *Biochemistry 41*, 14263−14271.
48. Weaver, L. H., Kwon, K., Beckett, D., and Matthews, B. W. (2001) Corepressor-induced organization and assembly of the biotin repressor: A model for allosteric activation of a transcriptional regulator, *Proc. Natl. Acad. Sci. U.S.A. 98*, 6045−6050.
49. Eisenstein, E., and Beckett, D. (1999) Dimerization of the *Escherichia coli* biotin repressor: Corepressor function in protein assembly, *Biochemistry 38*, 13077−13084.
50. Kwon, K., Streaker, E. D., Ruparelia, S., and Beckett, D. (2000) Multiple disordered loops function in corepressor-induced dimerization of the biotin repressor, *J. Mol. Biol. 304*, 821−833.
51. Cronan, J. E., Jr. (1988) Expression of the biotin biosynthetic operon of *Escherichia coli* is regulated by the rate of protein biotination, *J. Biol. Chem. 263*, 10332−10336.
52. Athappilly, F. K., and Hendrickson, W. A. (1995) Structure of the biotinyl domain of acetyl-coenzyme a carboxylase determined by mad phasing, *Structure 3*, 1407−1419.
53. Yao, X., Wei, D., Soden, C., Jr., Summers, M. F., and Beckett, D. (1997) Structure of the carboxy-terminal fragment of the apo-biotin carboxyl carrier subunit of *Escherichia coli* acetyl-CoA carboxylase, *Biochemistry 36*, 15089−15100.
54. Weaver, L. H., Kwon, K., Beckett, D., and Matthews, B. W. (2001) Competing protein: Protein interactions are proposed to control the biological switch of the *E. coli* biotin repressor, *Protein Sci. 10*, 2618−2622.
55. Polyak, S. W., Chapman-Smith, A., Mulhern, T. D., Cronan, J. E., Jr., and Wallace, J. C. (2001) Mutational analysis of protein substrate presentation in the posttranslational attachment of biotin to biotin domains, *J. Biol. Chem. 276*, 3037−3045.
56. Reche, P. A., Howard, M. J., Broadhurst, R. W., and Perham, R. N. (2000) Heteronuclear NMR studies of the specificity of the posttranslational modification of biotinyl domains by biotinyl protein ligase, *FEBS Lett. 479*, 93−98.
57. Nooren, I. M., and Thornton, J. M. (2003) Diversity of protein−protein interactions, *EMBO J. 22*, 3486−3492.

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