Double agent: translational regulation by a transcription factor

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The homeodomain protein bicoid activates transcription of several target genes by binding to target sequences in DNA. It has recently been shown that bicoid is also an RNA-binding protein that regulates the translation of *caudal* mRNA. Several ways for a protein to acquire dual functions can be imagined.

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The homeodomain protein bicoid is a key regulator of pattern formation in the anterior half of the *Drosophila* embryo. Bicoid activates transcription of a set of target genes involved in segmentation of the embryo by binding to the target gene DNA. Bicoid is also involved in the accumulation of the homeodomain protein caudal, which forms a gradient inverse to that of bicoid in *Drosophila* embryos, and is also involved in transcriptional regulation of segmentation genes. Regulation of caudal by bicoid involves translational repression brought about by direct binding of the bicoid protein to the 3' end of the *caudal* (*cad*) mRNA. Thus the bicoid protein leads a double life, as both a DNA-binding transcription activator and a mRNA-binding translational repressor. There are now several proteins known to have dual functions, raising the question of how a protein acquires two seemingly unrelated functions.

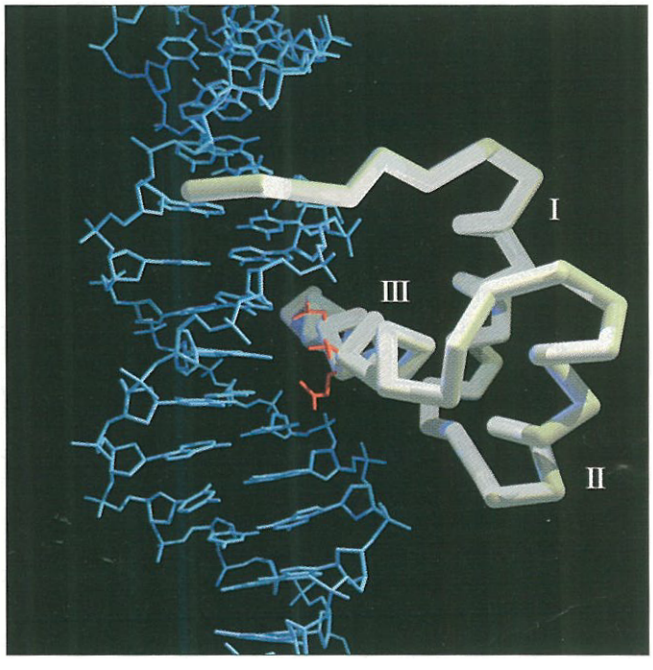
Transcriptional activation by bicoid

Bicoid is one of a family of proteins, known as homeodomain proteins, that are responsible for body plan formation and are key regulators of development in higher organisms (reviewed in [1]). In general, homeodomain proteins act as transcriptional activators of a set of developmentally regulated genes. Homeodomain proteins are defined by a central DNA-binding motif that consists of four α-helical regions contained within a 60 amino acid section of the protein. Helix III contains the main DNA-binding elements and interacts directly with bases in the major groove of the DNA. The structure of the *engrailed* homeodomain–DNA complex [2] is shown in Figure 1 (the structure of bicoid complexed to DNA has not been determined). The DNA sequences that are involved in high affinity binding with the homeodomain proteins are fairly well conserved and contain a central tetranucleotide core motif (ATTA). In addition to these high affinity binding sites there appear to be lower affinity binding sites (lacking the ATTA motif) that may be important in discriminating between the different homeodomain proteins for activation of specific genes. The bicoid protein accumulates in a gradient from the anterior to the posterior of *Drosophila* embryos and activates transcription of its target genes in a concentration-dependent manner [1,3].

Bicoid regulates caudal protein accumulation

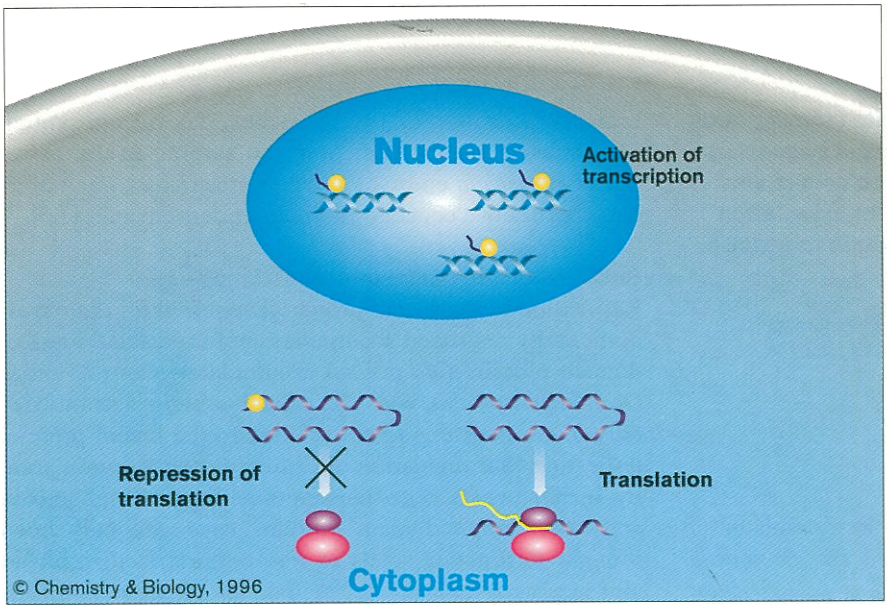
Expression of the bicoid protein is required for the developmentally regulated accumulation of the caudal homeodomain protein. *Cad* mRNA accumulates over the entire *Drosophila* embryo, but the caudal protein accumulates only in an inverse gradient relative to the bicoid protein, indicating that regulation of caudal protein accumulation by bicoid occurs post-transcriptionally. Although bicoid and other homeodomain proteins have generally been thought to function solely as transcription factors, bicoid also acts as a translational suppressor of the *cad* mRNA [4,5] (see Fig. 2). The functional domain of bicoid required for caudal regulation is located within the homeodomain region of the protein. Site-directed mutagenesis of helix III of the bicoid protein results in loss of *cad* translational regulation *in vivo*, but this mutant retains DNA binding and transcriptional activation activities.

Figure 1



The crystal structure of the *engrailed* homeodomain–DNA complex [2]. The helices of the protein are numbered. The highlighted amino acids are those that interact directly with the bases in the major groove of the DNA.

Figure 2



The dual functions of the bicoid protein in gene regulation. Within the nucleus the bicoid protein binds to the promoter region of a set of developmentally regulated genes and activates transcription. In the cytoplasm binding of bicoid to the 3' UTR of the *cad* mRNA results in suppression of translation initiation and caudal protein does not accumulate. When bicoid is absent from the cytoplasm the *cad* mRNA undergoes normal translation initiation and caudal protein accumulates.

Thus, both the DNA- and RNA-binding sites are located within the homeodomain of the bicoid protein, but the DNA- and RNA-binding activities appear to be separable functions within this domain [4].

Defining the bicoid response element in *cad* mRNA

The region of the *cad* mRNA required for bicoid regulation was mapped using fragments of the *cad* gene fused to a reporter gene [4,5]. A small section of the 3' untranslated region (UTR) of the *cad* mRNA is required for regulation by bicoid. Adding this RNA element to a reporter mRNA results in translational repression of the reporter by bicoid.

The bicoid protein was found to bind to the 3' UTR of the *cad* mRNA *in vitro* with high affinity. This binding is resistant to competition with other mRNAs [4,5], suggesting that the bicoid protein/*cad* mRNA interaction is specific. A truncated bicoid protein containing only the homeodomain was also shown to bind the 3' UTR of *cad* mRNA, confirming that the region of bicoid responsible for *cad* mRNA binding is contained completely within the homeodomain. No structural information is available on the bicoid/*cad* mRNA complex. We do know, however, that double-stranded RNA and DNA are structurally quite different, and that the major groove of RNA is too narrow for easy insertion of an α-helix, as pictured in Figure 1 for the binding of a homeodomain to DNA. In any case, RNA-binding proteins, in general, do not recognize RNAs as double-stranded helices but rather as more complex tertiary structures. The bicoid homeodomain does have a large number of arginine residues, as do many RNA-binding proteins.

Mechanism of translational repression by bicoid

Although bicoid binds to the 3' UTR of the *cad* mRNA, it seems that translational repression operates by inhibiting the initiation of translation [4], a process normally thought to involve the 5' UTR of mRNAs (reviewed in [6]). Inhibition of normal 5' translation initiation by bicoid binding was demonstrated using a chimeric mRNA containing two coding sequences and the 3' UTR from *cad* on a single mRNA. Binding of bicoid represses translation of the first coding region of this mRNA, which is 5' UTR dependent, but not that of the second coding region, which is not 5' UTR dependent because it contains an internal ribosome entry site [4]. The internal ribosome entry site is an RNA sequence that allows ribosomes to initiate translation independent of the 5' UTR of the mRNA [7]. The *cad* 3' UTR also acts as a translational repressor when placed in the middle of the 3' UTR of an unrelated reporter mRNA [5]. Protein binding to the 3' UTR of other mRNAs has been shown to influence translation initiation [8], indicating that 3' UTRs of mRNAs can be functional components of translation initiation. Thus, binding of bicoid to the *cad* 3' UTR could block binding of another protein to the 3' UTR that is required for *cad* mRNA translation initiation, or alternatively, could directly interfere with ribosome association with the mRNA.

Other proteins with dual functions in gene regulation

Is bicoid a unique type of protein, or just one of many examples of dual function proteins that act in gene regulation? Another protein that has both DNA- and RNA-binding activities is the transcription factor IIIA (TFIIIA) [9]. The DNA-binding activity of TFIIIA is required for transcription of the 5S ribosomal RNA (rRNA) genes. In

Xenopus, the same protein also binds to 5S rRNA in the cytoplasm of immature oocytes and stabilizes the rRNA until it is used in ribosome assembly.

The nucleic acid binding domain of TFIIIA consists of nine zinc fingers, with the DNA-binding activity located within the three amino-terminal fingers of the protein, and the high affinity RNA-binding activity located within fingers four through seven [10]. Thus, unlike bicoid, the DNA- and RNA-binding sites of TFIIIA are located within distinct regions of the protein. TFIIIA binds to a DNA site within a 50-base-pair region located within the coding region of the rRNA gene. Mutation at many sites of the 5S rRNA gene affect binding of TFIIIA to the RNA, and binding of TFIIIA to the RNA appears to be dependent on secondary and tertiary structure rather than primary sequence, at least in several critical regions of the rRNA [10]. TFIIIA is thus a dual function protein like bicoid, although it does not regulate translation of RNA as bicoid does, but rather stabilizes the RNA after transcription and prior to ribosome assembly.

Another system in which a protein appears to have a dual role within the cell involves the regulated expression of ferritin mRNA. Ferritin is an iron-chelating protein whose expression is regulated in response to cellular iron concentration. An RNA element, termed the iron responsive element (IRE), is located within the 5' UTR of ferritin mRNA and is bound by a specific protein, the iron responsive protein-1 (IRP-1), and it is this binding that inhibits translation of the ferritin mRNA (reviewed in [11]). The binding affinity between IRP-1 and the IRE is sensitive to cellular iron levels. When iron is available, the IRP-1 has low affinity for the IRE and ferritin mRNA is translated. When iron levels are low, the IRP-1 has high affinity for the IRE and binds to the 5' UTR of ferritin mRNA, suppressing translation (reviewed in [11]). Thus IRP-1 acts much like bicoid; binding of the protein to the mRNA suppresses translation. Unlike bicoid, however, the activity of IRP-1 is not regulated by asymmetric distribution of the protein in the cell; rather it is regulated by the availability of iron within the cell.

The second function of the IRP-1 is not as a regulatory protein, but as an enzyme. When assembled with an iron–sulfur cluster, the IRP-1 is a cytosolic aconitase that catalyzes the conversion of citrate to isocitrate. In the presence of iron the IRP-1 has enzymatic aconitase activity but low RNA affinity. In the absence of iron the IRP-1 is disassociated from its iron–sulfur cluster and has high RNA affinity but is inactive as an enzyme. The RNA-binding site of IRP-1 has been mapped to the active-site cleft of aconitase [12].

Development of a dual function protein.

Why would a translational regulatory protein have another unrelated cellular function? Neither bicoid nor the IRP-1 appear to have intrinsic structural or functional properties that strongly predispose them to acting in translational regulation. Perhaps these proteins came to function as translational regulators not because of a particular biochemical function that they normally perform, but rather because they could readily be adapted to function in translational regulation.

Achieving the correct pattern of translational repression of *cad* requires a protein that accumulates in the proper anterior to posterior gradient, a requirement that bicoid fills very well. For translational regulation of ferritin the most important aspect of the regulatory protein is that it be able to sense the iron content of the cell, a task for which aconitase happens to be well suited given that it has an iron-containing cofactor. In addition to accumulating in the proper gradient or sensing cellular iron content, these proteins need to be capable of repressing translation. As described above, however, translational repression might simply involve binding of the protein to a key position within the mRNA, blocking the action of another translation factor that normally functions by binding to either the 5' or 3' UTR. Once a protein with the correct primary attributes is present, therefore, it could simply be adapted to its secondary role of binding to the relevant RNA.

How did these specific RNA–protein interactions arise? Two possibilities can be imagined. First, the ability to bind specifically to an RNA may have evolved in a protein with another function. This would require the accumulation of mutations within the protein that do not disrupt its existing function. Alternatively, mutations might accumulate in an RNA that make it capable of binding to the appropriate protein. There is experimental evidence that this second mechanism is, at least, feasible. From diverse pools of RNA, individual RNAs have been selected *in vitro* that have high affinity for a variety of proteins, including those that do not normally bind nucleic acids (reviewed in [13]). A similar process might have operated *in vivo* to generate an RNA element that is capable of binding either the bicoid or aconitase protein. Variants of the 3' UTR (*cad* mRNA) or the 5' UTR (transferrin mRNA), with high affinity for the bicoid or aconitase proteins, respectively, would have been selected. It seems reasonable to assume that 3' and 5' UTRs of mRNAs have fewer constraints on their exact sequence, as compared to coding regions, and these reduced constraints would allow for the exploration of greater diversity in these UTRs. In contrast, the 5S rRNA lacks the 5' and 3' UTRs of mRNAs and might not be very amenable to mutations which may disrupt the functional domain of the rRNA. In this case, tolerance of mutation exists in the protein (TFIIIA), where the DNA- and RNA-binding domains occur within separate regions.

If variation in the 5' and 3' UTR led to the formation of specific protein-binding domains, and the proteins were

then able to exert their effects by sterically blocking the binding of other proteins, there would be no specific requirement for a biochemical function for the bound protein in order for it to participate in translational regulation. In the case of bicoid the proteins would simply have to be in the right place at the right time to bind the *cad* RNA. Binding of the bicoid protein would displace some component of the apparatus normally required for translation of the mRNA. In the case of IRP-1, the ferritin mRNA has evolved to recognize the aconitase protein in the iron-depleted state in preference to the iron-bound state. In this way, translation of the ferritin mRNA is responsive to the level of cellular iron based on differential binding to the IRP-1/aconitase protein, independent of aconitase activity.

Is bicoid really the double agent?

Bicoid appears to be an example of a protein that has dual functions within the cell. Its first function is to activate transcription of a set of genes in a graduated manner across the developing *Drosophila* embryo. Bicoid is also required for the regulated accumulation of the caudal protein in an inverse gradient relative to bicoid accumulation. This inverse gradient is established by translational repression of the *cad* mRNA by bicoid. Perhaps credit for this clever design should be bestowed not upon bicoid, but rather on the *caudal* mRNA, that used the unwitting bicoid protein for its own design. Perhaps, as suggested by the additional example of the ferritin mRNA, we may find dual function molecules to be quite common in nature, and we simply have to look at the correct macromolecular players to find them.

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