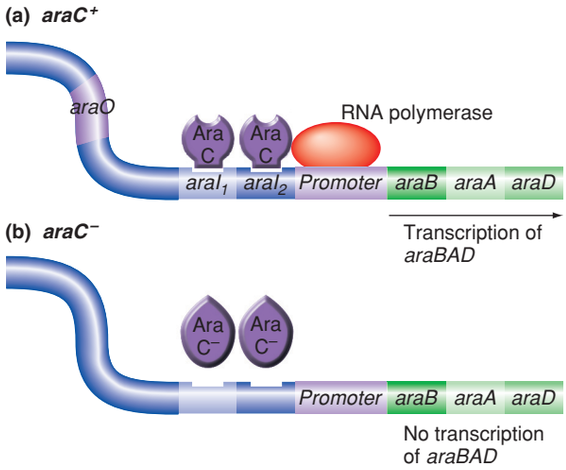

Figure 16.12 Loss-of-function mutations show that AraC is a positive regulator. Expression of the arabinose genes in *E. coli* requires the AraC protein to be bound next to the promoter. In an *araC*$^-$ mutant, the defective protein cannot bind and RNA polymerase will not transcribe the genes.

contrast, loss of function of a negative regulator causes constitutive production of the operon's gene products.)

Summary of How DNA-Binding Proteins Control the Initiation of Transcription at the Lactose and Other Operons

In bacteria, the initiation of transcription by RNA polymerase is under the control of regulatory genes whose products bind to specific DNA sequences in the vicinity of the promoter. The binding of negative regulatory proteins prevents the initiation of transcription; the binding of positive regulators assists the initiation of transcription. Regulation of the *lac* operon depends on at least two proteins: the repressor (a negative regulator) and CRP (a positive regulator). Maximum induction of the *lac* operon occurs in media containing lactose but lacking glucose. Under these conditions, the repressor binds inducer and becomes unable to bind to the operator, while CRP complexed with cAMP binds to a site near the promoter to assist RNA polymerase in the initiation of transcription.

Operons that function in the breakdown of other sugars are also under the control of negative and positive regulators. Transcription of the arabinose operon, for example, which is induced in the presence of arabinose, receives a boost from two positive regulators: the CRP–cAMP complex and AraC.

Thus, proteins that bind to DNA affect RNA polymerase's ability to transcribe a gene. The activity of multiple regulators that respond to different cues increases the range of gene regulation.

Molecular Studies Help Fill in the Details of Control Mechanisms

In 1966, Walter Gilbert and coworkers purified the *lac* repressor protein and determined that it is a tetramer of four *lacI*-encoded subunits, with each subunit containing an inducer-binding domain as well as a domain that recognizes and binds to DNA. (Note that we use the term "domains" for the functional parts of proteins but the term "sites" for the DNA sequences with which a protein's DNA-binding domain interacts.) Gilbert and colleagues then used radioactively labeled repressor protein and a bacterial virus DNA that contained the *lac* operon to show that the repressor binds to operator DNA. When they combined the labeled protein and viral DNA and centrifuged the mixture in a glycerol gradient, the radioactive protein cosedimented with the DNA (Fig. 16.13). If the viral DNA contained a *lac* operon that had an *lacO*^{c} mutation, the protein did not cosediment with the DNA, because it could not bind to it. Subsequent sequence analysis of the isolated DNA revealed that the *lac* operator is about 26 bp in length, and it includes the first nucleotides used as a template for the mRNA.

With the development of cloning, DNA sequencing, and techniques for analyzing protein-DNA interactions in the 1970s, researchers increased their ability to isolate specific macromolecules, determine the structure of each molecule, and analyze the interactions between molecules. This new experimental potential revolutionized the study of genetics even though researchers still used mutants isolated in earlier genetic analyses as the bases of many of their studies. The new generation of gene-regulation analyses confirmed the basic principles of the operon theory and elucidated compelling details of their components. Such mutants continue to be valuable research tools today.

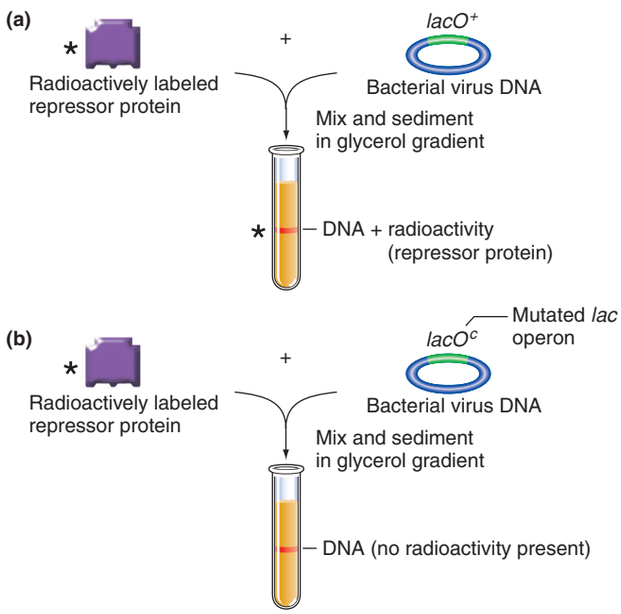

Figure 16.13 The *lac* repressor binds to operator DNA. A radioactive tag is attached to the *lac* repressor protein so it can be followed in the experiment. **(a)** When repressor protein from *lacI*$^+$ cells was purified and mixed with DNA containing the *lac* operator (on bacterial virus DNA), the protein cosedimented with the DNA. **(b)** When wild-type repressor was mixed with DNA containing a mutant operator site, no radioactivity sedimented with the DNA.