biochemical analyses in *Drosophila* have revealed that multiple proteins associated in large multiprotein complexes participate in the process. These *Polycomb complexes* are discussed further in Chapter 15. As in the case of SIR proteins binding to yeast telomeres (see Figure 11-29), these *Drosophila* Polycomb proteins can be visualized binding to the genes they repress at multiple, specific locations in the genome by in situ binding of specific-labeled antibodies to salivary gland polytene chromosomes.

Repressors Can Direct Histone Deacetylation at Specific Genes

The importance of *histone deacetylation* in chromatin-mediated gene repression has been further supported by studies of eukaryotic repressors that regulate genes at internal chromosomal positions. These proteins are now known to act in part by causing deacetylation of histone tails in nucleosomes that bind to the TATA box and promoter-proximal region of the genes they repress. In vitro studies have shown that when promoter DNA is assembled onto a nucleosome with unacetylated histones, the general transcription factors cannot bind to the TATA box and initiation region. In unacetylated histones, the N-terminal lysines are positively charged and interact strongly with DNA phosphates. The unacetylated histone tails also interact with neighboring histone octamers, favoring the folding of chromatin into condensed, higher-order structures whose precise conformation is not well understood. The net effect is that general transcription factors cannot assemble into a preinitiation complex on a promoter associated with hypoacetylated histones. In contrast, binding of general transcription factors is repressed much less by histones with hyperacetylated tails in which the positively charged lysines are neutralized and electrostatic interactions with DNA phosphates are eliminated.

The connection between histone deacetylation and repression of transcription at nearby yeast promoters became clearer when the cDNA encoding a human *histone deacetylase* was found to have high homology to the yeast *RPD3* gene, known to be required for the normal repression of a number of yeast genes. Further work showed that RPD3 protein has histone deacetylase activity. The ability of RPD3 to deacetylate histones at a number of promoters depends on two other proteins: UME6, a repressor that binds to a specific upstream regulatory sequence (URS1), and SIN3, which is part of a large, multiprotein complex that also contains

▶ EXPERIMENTAL FIGURE 11-31

The chromatin immunoprecipitation method can reveal the acetylation state of histones in chromatin. Histones are lightly cross-linked to DNA in vivo using a cell-permeable, reversible, chemical cross-linking agent. Nucleosomes with acetylated histone tails are shown in green. Step 1: Cross-linked chromatin is then isolated and sheared to an average length of two to three nucleosomes. Step 2: An antibody against a particular acetylated histone tail sequence is added, and (step 3) bound nucleosomes are immunoprecipitated. Step 4: DNA in the immunoprecipitated chromatin fragments is released by reversing the cross-link and then is quantitated using a sensitive PCR method. The method can be used to analyze the in vivo association of any protein with a specific sequence of DNA by using an antibody against the protein of interest in step 2. [See S. E. Rundlett et al., 1998, *Nature* **392**:831]