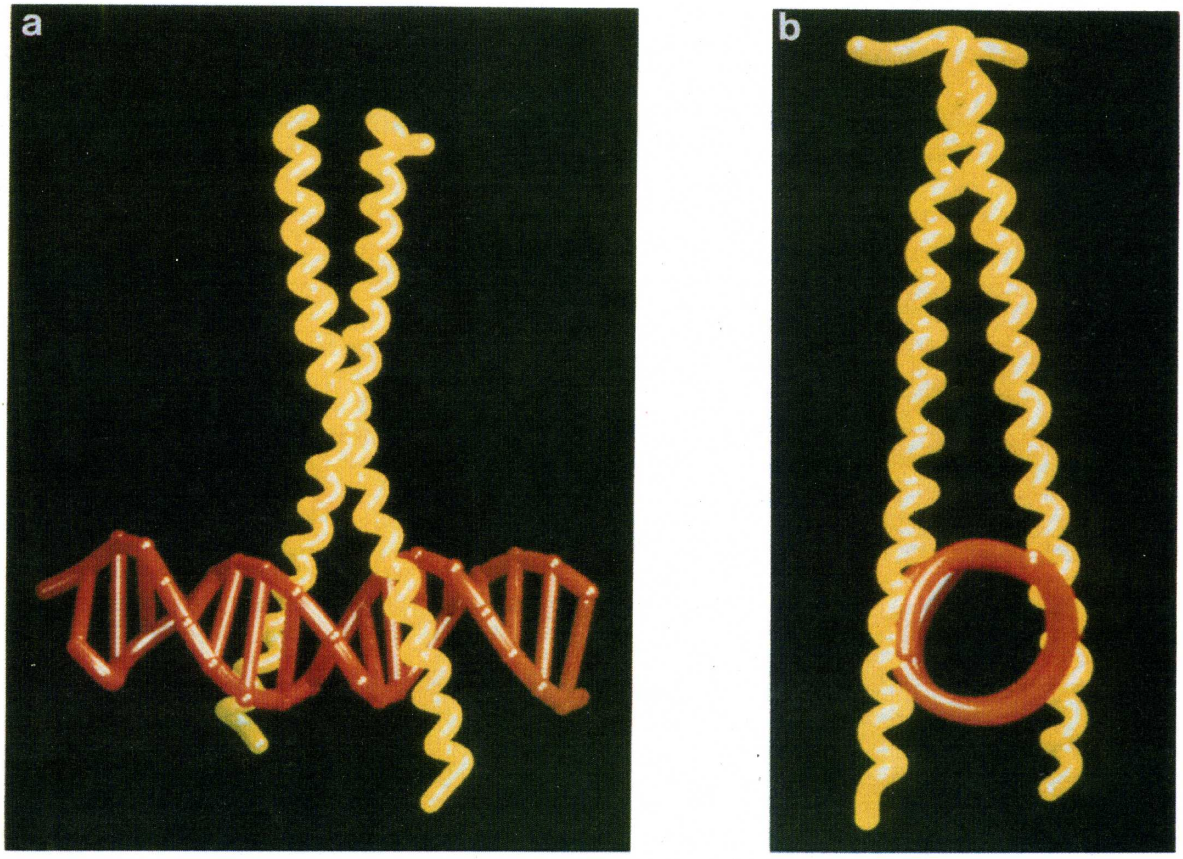

Figure 3. Structure of the GCN4 bZIP Complex

(a) The bZIP dimer (yellow) binds in the major groove of the DNA (red). Each bZIP protomer is a smoothly curved, continuous α helix. The carboxy-terminal residues of the monomers pack together as a coiled coil, which gradually diverges to allow the basic region residues to follow the major groove of either DNA half site. This divergence of the bZIP monomers corresponds to an unwinding of the coiled-coil super helix, with a slight righthanded rotation of basic region residues about the α-helical axis of each chain and a lateral displacement of each monomer along the helical axis of the DNA. The DNA in the complex is straight, and its conformation is in the B form across the region contacted by the protein.

(b) View down the DNA axis, with the DNA backbone represented as a red coil. The basic region residues amino-terminal to the point of DNA contact are in a straight, α-helical conformation. The amino-terminal residues of the basic region do not wrap around the back side of the binding site.

unesterified phosphate oxygen of Cyt-0 is contacted by Arg-243 of the right monomer, with the phosphate of Gua-0′ contacted by Arg-240 of the left monomer. The central asymmetry imposed by contacts to bases at position 0 is corrected by local contacts, which position both monomers over their respective half sites in an analogous way. This adjustment implies that the α-helical fork at the junction between the basic region and the leucine zipper coiled coil is somewhat flexible. Flexibility of GCN4 is likely to be a key component in its specific recognition of the activating transcription factor/cAMP response element–binding protein (ATF/CREB)-binding site (Sellers et al., 1990). The ATF/CREB site is a palindromic sequence homologous to the AP-1 sequence, but it contains an additional central guanine–cytosine base pair.

Structure of the DNA in the Complex

The AP-1 site in the GCN4 complex is straight, B-form DNA across the region contacted by the protein (see Figure 3). The central 15 bp of the AP-1 site DNA have an average helical rise and twist of 3.2 Å and 34°. No systematic variation in phosphodiester backbone or base pair geometry is evident across the binding site. DNAs in adjacent unit cells of the crystals pack end to end, forming a Watson–Crick base pair between the 5′ thymine of one complex and the 5′ adenine of the adjacent DNA (Figure 9a). The DNA is aligned along crystallographic unit cell axis c, which has a length (65.3 Å) commensurate with that of a B-form 20-mer oligonucleotide.

The DNA packing arrangement in the crystals changes markedly in response to crystal harvest conditions. Prior to collecting diffraction data from crystals at −160°C, the crystals were soaked in cryoprotectant to minimize crystalline ice formation during freezing (see Experimental Procedures). Following equilibration of the crystals in a cryoprotectant solution containing an elevated concentration of polyethylene glycol (molecular weight = 400) (PEG 400), the length of the crystallographic c axis is reduced by 6.1 Å (c = 59.2 Å). This shrinkage is due to a local rearrangement in base-stacking interactions that decreases