DNA is packaged into the procapsid, and requires the products of genes 4, 10, and 26, as shown in Fig. 1.

The protein composition of the fractions containing the isolated injection apparatus revealed five bands, whose mobilities corresponded to that of the gene 1 protein, gp9 tail spike polypeptide, the polypeptides specified by genes 4, 10, and 26, and the gene 5 major coat protein. Because we know that the shaft can only be assembled after DNA packaging, the collar must correspond to the structure within the capsid on which the shaft is assembled. In particles that have lost their chromosome but maintain the portal vertex, the collar can be observed within the capsid shell. This collar must be composed of the gene 1 product.

DISCUSSION

Though the mechanism of DNA transport into and out of the capsid remains obscure, features of the assembly of the machinery are becoming clearer. The procapsid of P22 is initiated in the cytoplasm without the participation of a membrane site (Lenk et al., 1975). Initiation must require interactions of coat, scaffolding, and gene 1 portal subunits, as well as the DNA injection proteins. We think the primary organizer is a complex of scaffolding protein, because certain mutants of the scaffolding protein exist that do not block shell assembly, but prevent incorporation of gp1 and the other minor proteins (unpublished results). However, some additional mechanism is needed to limit these interactions to one vertex. The scaffolding protein autoregulates its own synthesis, at the level of translation (Wyckoff and Casjens, 1984). Such a messenger RNA/scaffolding complex is an excellent candidate for an organizing center for assembling six different proteins into the portal vertex.

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NUCLEAR MAGNETIC RESONANCE STUDIES OF AN SV40 ENHANCER CORE DNA SEQUENCE

MARY DONLAN, SUSANNIE CHEUNG, AND PONZY LU
Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

We have observed that there is a correlation between the GTG/CAC sequence centered on the position corresponding to the sixth base of the messenger RNA of the *lac* operon with the observed biological properties of the *lac* operon DNA sequence (1) and the minimum imino proton spin lattice relaxation time in the region where the *lac* repressor binds. Position six also corresponds to the single base pair substitution leading to an operator mutation with the highest basal level of *lac* operon enzymes, i.e., the weakest repressor binding. The galactose operon, another inducible sugar utilization system, has two operators, an external operator with symmetrically located GTG/CAC sequences and an internal operator with one GTG/CAC sequence located homologously. Mutations have been found that affect the GTG/CAC sequences in the external operator. A number of interesting DNA sequences containing GTG/CAC where protein is expected to interact specifically are shown in Fig. 1. The selection of sequences

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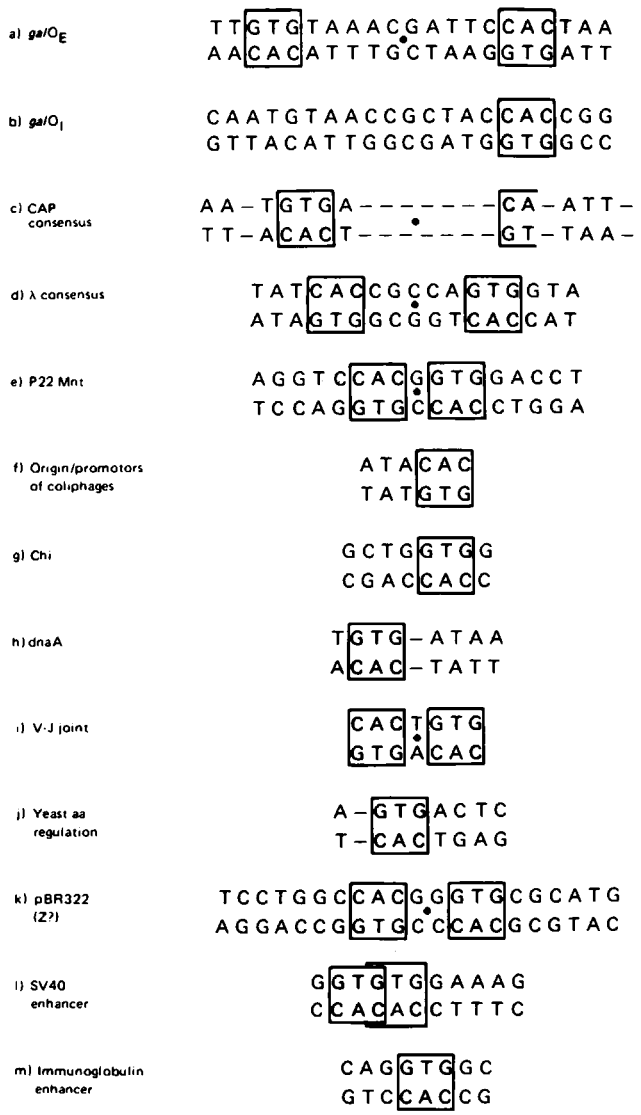

FIGURE 1 Sources of sequences *a–l* are in the references found in references 1 and 4. Sequence *m* is from reference 31.

shown is chosen for the sake of illustration and is not exhaustive. Most of the sequences are consensus sequences, making the occurrence of the triplet GTG/CAC of greater significance. As a consequence of the frequent occurrence of these sequences, which has also been noted by others (2, 3), we have proposed the possibility that they serve an indexing function for proteins searching the DNA sequence for sites of interaction (4). They function as thumb indexes in dictionaries or as detents in mechanical systems. We have chosen the term "detent" because it describes function without any geometric prejudice (4).

GTG/CAC AT A DNA ANTIBODY COMBINING SITE

To explore the generality of the imino proton exchange rates observed at these A·T pairs between two GTG/CAC pairs, we have synthesized two fragments corresponding to sequences (*k*) and (*l*) of Fig. 1 because of their potential interest in other DNA polymorphism studies. Rich and colleagues have introduced the idea that left-handed DNA in Z form is potentially a structural variant in regulatory regions of biological relevance (5, 6). The localization of Z-form DNA in regulatory regions of a biologically relevant system was based on the use of antibodies against Z-form DNA (7, 8). Control experiments show that sequence (*l*) in pBR322 is a binding site for antibodies against Z DNA (7). This sequence contains a pair of GTG/CAC sequences symmetrically arranged. It has potential for Z formation because of a run of alternating purine-pyrimidines that span the center part of the sequence. We conclude that this area of Z DNA is based on the protection of this fragment against restriction enzyme digestion in the presence of anti-Z–DNA antibodies (5, 7, 8).

GTG/CAC AT TRANSCRIPTIONAL ENHANCERS

These same antibodies, in turn, were used to probe the genome of SV40 DNA in the absence of cellular proteins (4, 6). It was found that a region spanning the SV40 transcriptional enhancer is bound by anti-Z–DNA antibodies (6). Examination of that sequence shows several runs of alternating purine-pyrimidine sequences. Further localization of the exact binding site of Z DNA has not been reported. However, in the same sequence there are several interesting features of SV40 that have genetically been established to be essential to early transcription in SV40 (9). This is indicated by sequence (*l*) of the figure, a core sequence with two overlapping GTG sequences which has homology with enhancers found in other systems. In addition to being in the enhancer "core" sequence (10, 11) three GTG/CAC sequences have been demonstrated to occur as necessary bases in an comprehensive mutational analysis of the bovine papilloma virus enhancer (12).

We synthesized two 17-base pair DNA double-helical fragments corresponding to the sequences (*k*) and (*l*) of the figure. The saturation recovery experiment was done to estimate the imino proton exchange rates as we did for the fragments from the *lac* operon system. The data show the interesting local maxima associated with the A·T base pairs of interest. They serve as an incentive to examine the structure of these specific sequences in greater detail. We are not suggesting that the dynamic properties of this DNA sequence are the features leading to protein recognition but that they are a measurable hint of the uniqueness of the sequences.

ARE EPITOPES ON DNA ALSO MOBILE REGIONS?

Recent analysis of the interaction between antibodies and their sites of interaction on protein antigens, the epitope, suggests that more mobile regions may be preferential interactants for the antibody (13, 14). Although there is controversy (15) as to the Z-DNA structure or absence

thereof in sequence (*I*), illustrated by Fig. 1, it is clear that it is antigenically unique. A natural consequence of the recent observations with mobility of epitopes on the surface of proteins suggests that perhaps this region is also more locally mobile than other portions of the DNA, lending qualitative consistency to our suggestion of higher exchange rate of imino protons at the T between the pair of G's in the GTG/CAC sequence.

SUMMARY

Variation in the observed spin lattice relaxation rate (R_{obs}), interpreted as proton exchange dominated in sequences corresponding to part of promoters where RNA-polymerase initiates messenger RNA synthesis, has been observed by both Patel and coworkers (16) and Reid and coworkers (17). A higher R_{obs} was also seen in the T·A pair of the GTG/CAC in the sequence corresponding to the λ phage *cro* repressor binding site by Kyogoku and coworkers (18). In the one case where a three-dimensional structure for a turn of a helix was determined by Dickerson and coworkers (19) clear structural heterogeneities arise which has led to detailed considerations of geometries of regulatory regions. Nussinov and collaborators have generalized the details of the Dickerson dodecamer to note potential similarities in operators including the *lac* system (3) and the enhancer sequences described above (20). Like the steric considerations of Calladine (21) and Dickerson (22) and nearest neighbor structure analysis of Bubienko et al. (23), the focus is on the geometry of a sequence leading to base tilt angles and potential overlap since they are measurable parameters.

With the observation that the DNA molecule is both structurally (24–26) and dynamically flexible (27–30), there will no doubt be many other variables that can be measured as a function of DNA sequence.

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PROTEIN-PROTEIN INTERACTIONS IN DNA RECOGNITION

^{1}H-Nuclear Magnetic Resonance Studies of λ cI Repressors Genetically Altered by Site-Directed Mutagenesis

MICHAEL A. WEISS,*‡ ROBERT STEARMAN,§ ANNA JEITLER-NILSSON,‖ MARTIN KARPLUS,* AND ROBERT T. SAUER‖
**Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138; ‡Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology Cambridge, Massachusetts 02139; §Department of Biophysics, The Johns Hopkins School of Medicine, Baltimore, Maryland 21205; and ‖Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

Phage λ repressor contains two domains separable by proteolysis (1). The isolated NH_2-terminal domain recognizes operator DNA and provides a tractable model system for biophysical and genetic studies of macromolecular recognition (2–5). The active species in operator recognition is a dimer. We describe here one- and two-dimensional ^{2}H-NMR studies of the dimer interface in wild-type and genetically altered repressors.

The structure of the NH_2-terminal domain, a fragment of 92 residues, has been determined by x-ray crystallography (6). As shown in Fig. 1 *A*, the domain consists of five α helices. The first four form a globular region, which

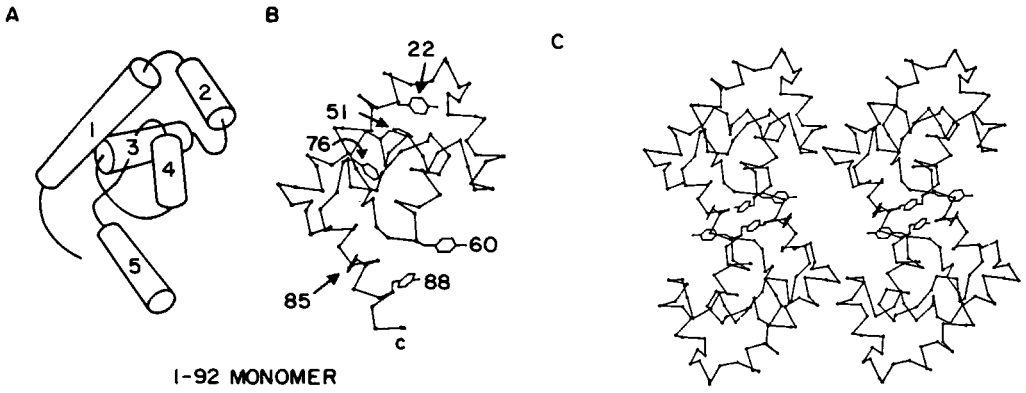

FIGURE 1 (*A*) Model of the 1–92 domain of phage λ repressor. The α helices are shown as cylinders. The first four helices form a globular region, and the fifth extends outward. (*B*) α-carbon representation showing the aromatic side chains. Tyr22 and Phe51, and Phe76 are buried in the hydrophobic interior and provide sensitive markers for changes in tertiary structure. Tyr60, Tyr85, and Tyr88 are on the surface of the monomer structure. Their environments are altered in the dimer. (*C*) Stereo pair, showing the helix 5–helix 5 interaction. This helix is amphipathic, and the interaction is primarily hydrophobic. In the crystallographic dimer the two DNA-binding surfaces are appropriately oriented to contact successive major grooves of B-DNA. The structures were calculated from the crystal coordinates of Pabo and Lewis (6).