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The Structural Basis of Gene Regulation for DNA Organized as Chromatin

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Abstract: DNA in the nucleus of eukaryotic cells is organized in chromatin, a nucleoprotein complex containing roughly half DNA and half protein. The nucleosome is the underlying DNA packaging element in chromatin, repeating approximately every 200 base pairs over essentially the entire genome. Our crystal structure of the nucleosome core particle explains in atomic detail how DNA in its first level of organization is kept untangled by the histone protein octamer and clarifies the unique role the nucleosome plays in the expression of genetic information. Dynamic assembly and disassembly of the chromatin fiber, the higher-order arrangement of nucleosomes, most probably defines the crucial step in controlling DNA access enabling efficient regulation of gene readout. Multiprotein complexes, here designated 'regulasomes', are bound at specific sites within chromatin to coalesce the histone modification and chromatin remodeling protein assemblies that affect the stability and structure of the chromatin fiber. The formation of a particular regulasome depends on cooperative interaction between the transcription factor proteins comprising it, and on their interaction with specific DNA sequences. Our crystal structures of selected transcription factor complexes bound to their target site DNA contribute to the structural basis of how specificity of gene expression is achieved.

Keywords: Chromatin · DNA transcription · Nucleosome · Protein crystallography · X-ray structure

1. Introduction

Recent announcements of the determination of the DNA sequence for the human genome [1][2], as well as those for yeast [3], nematode worm [4], and fruit fly [5] serve to emphasize that although the amount of information required for life as we know it is vast, it is nevertheless finite and potentially understandable as a whole. The human genome comprises about three billion nucleotide base pairs (bp) of DNA coding for roughly 30–40000 genes. Deciphering exactly where the sequence of a gene begins and ends is an immediate goal to be ascertained by genomic studies, to be followed by assignment of function to each of the RNA and protein gene products transcribed and translated from the genome. The larger challenge, however, is embodied in the emerging fields of functional genomics and proteomics (*e.g.* [6][7]). Knowledge of how the expression of one gene effects the readout of all other genes, how the activity of one protein modulates the activity of all other proteins, and how the genome and the proteome affect each other under any particular cellular circumstances are the long-term goals of these disciplines. A profound understanding of the interplay within and between these coordinated systems will require elucidation of the molecular mechanisms that underlie the modulation of gene expression and protein activities. In turn, the discovery of the underlying mechanistic principles will rely on a complete structural picture of the participating macromolecules caught in the act of interacting.

For eukaryotic or higher cells, the locale for interaction between the genome and the proteome is the cell nucleus, and more specifically, the chromosomes with their DNA packaged in chromatin. Chromatin is approximately half DNA and half protein for which the protein component in terms of its mass is almost entirely histone protein. But why chromatin and not simply bare DNA as effectively occurs for bacterial genomes? The amount of DNA in a eukaryotic nucleus compared to a bacterial cell is typically 1000-fold greater at least. The polymeric properties of DNA require that a DNA molecule of the size of the human genome would have an end-to-end distance of approximately 230 μm, whereas nuclei have diameters of 5–7 μm. Therefore, DNA is evidently efficiently packaged in chromatin [8]. However, since there is no obvious fundamental limitation to the size of a cell or its nucleus – giant cells do exist – packaging *per se* is probably not the *raison d'être* for chromatin. DNA must undergo replication with concomitant cell division as well as transcription, recombination and repair. DNA organized in chromatin helps to protect it from entanglement and the shear forces that must accompany these processes. In recent years, it has become clear that chromatin also acts as a reversible clamp, keeping the DNA and its information content hidden from all but a few protein regulatory factors when in the fiber form, then opening up under the appropriate signaling allowing the genetic code to be read [9]. Deciphering the molecular structures and mechanisms required for the chromatin template to function in the regulation of gene transcription is the principle aim of my laboratory.

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2. The Nucleosome

The nucleosome is the fundamental repeating unit of chromatin and accounts for the first two levels of DNA organization in chromosomes – the nucleosome itself and the chromatin fiber. In the human cell nucleus, about 25 million nucleosomes are necessary to 'wrap up' the genome. The nucleosome core contains an octamer constructed from pairs of the four core histone proteins (H2A, H2B, H3, H4) and an approximately equal mass of DNA in 147 bp. Compared to the nucleosome, the nucleosome core is missing only the linker histone H1 and linker DNA – short stretches of DNA that connect the nucleosome cores to each other in chromatin. The linker DNA length and presence of H1 are variable throughout chromatin. The core histones are arranged in an octameric unit around which the DNA is wrapped in 1.65 left-handed superhelical turns (Fig. 1). This arrangement necessitates a substantial deformation of the DNA, bending the 22 Å diameter double helix to a mean radius of 42 Å in the nucleosomal superhelix. These features of the structure were first seen clearly in the crystal structure of the nucleosome core particle (NCP) at 2.8 Å resolution [10]. Some of the detailed insights gleaned from this atomic structure are described below and a brief history of the path to the structure follows in the next section.

The histone protein chains are divided into three types of structures: 1) rigid, folded α-helical domains named the 'histone-fold', 2) 'histone-fold extensions' which interact with each other and the histone-folds, and 3) flexible 'histone tails' [10] (Fig. 2). The primarily α-helical histone-fold domains are structurally highly conserved between the four types of core histones and have also been discovered in an increasing number of other molecules involved in the regulation of gene transcription [11]. They form crescent-shaped heterodimers in the pairings H3 with H4 and H2A with H2B, and are responsible for the construction of the twofold symmetric histone octamer having a central H3-H4 tetramer with H2A-H2B dimers bound on opposite faces. The histone-fold domains are responsible for organizing 121 bp of the DNA superhelix, not the entire 147 bp. The α-helical histone-fold extensions just prior to the H3 histone-folds are responsible for binding the first and last 13 bp of DNA in the NCP. The flexible tails of the histones reach out between and around the gyres of the DNA superhelix to contact neighboring particles in the crystals. About one-half of these flexible histone tails can be observed in the current electron density map, but the remainder are too disordered to be seen. The implication from the structure is that these flexible regions are meant to make inter-nucleosomal interactions, perhaps facilitating chromatin fiber formation (Fig. 3). The N-terminal tails of each histone contain the sites of methylation, acetylation and phosphorylation – post-translation modifications that correlate with different functional states of chromatin. These modifications can affect the stability and structure of chromatin directly as well as mediate the binding of factors that remodel chromatin. The histone tails are chromatin's likely arbiters of gene regulation.

Fourteen regions of contacts exist between the histone proteins and DNA: three from each of the four histone-fold dimers and two from histone-fold extensions. This construction allows the DNA molecule in a single nucleosome core to come loose over one-half of the superhelix while the histone proteins maintain their grip on the other half, permitting for example, the transcription of the genetic information stored in the DNA without complete dissociation of the histone octamer. The nucleosome core DNA was previously envisioned to be bound simply by electrostatic attraction: negatively charged DNA would be wound as yarn around a positively charged histone spool. Although this type of interaction appears essential, equally many interactions of other kinds, such as hydrogen bonds and hydrophobic interactions, are also important. The close spatial proximity of the nearly two superhelical turns of DNA and the periodic variation of double helix parameters with a mean of 10.3 bp per turn result in an alignment of major and minor grooves from one superhelical gyre to the next. The resulting narrow channels formed by the aligned minor grooves serve as the exit points for four of the eight basic histone tails, whereas the large pores formed by the aligned major grooves are in principle free to make base-specific contacts with other

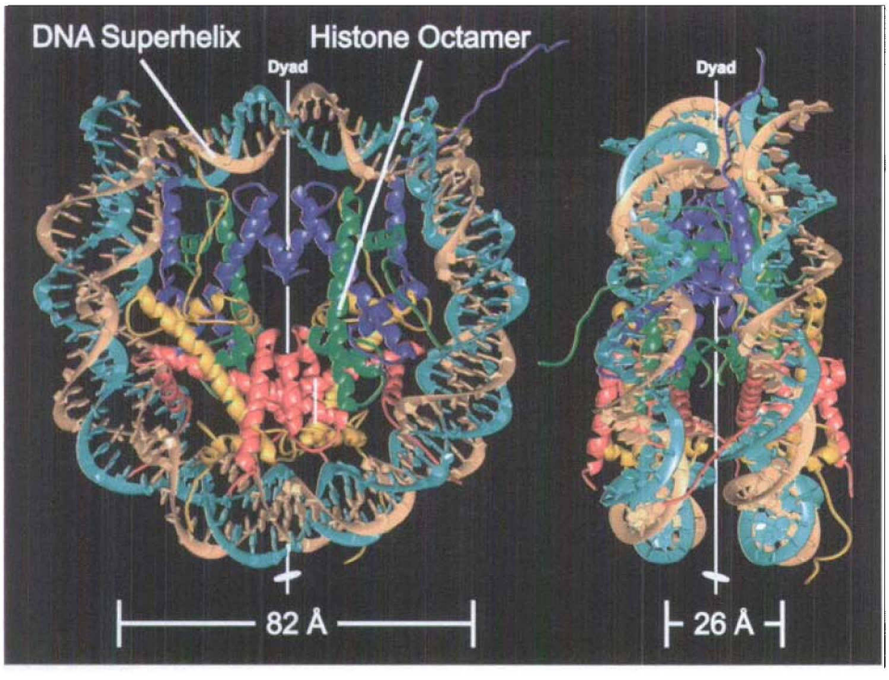

Fig. 1. Crystal structure of the nucleosome core particle (NCP) at 2.8 Å resolution (adapted from [10]). The DNA double helix (146 bp in two chains: turquoise and brown) is wound around the protein histone octamer (two copies each of H2A: yellow, H2B: red, H3: blue, and H4: green) in 1.65 left-handed superhelical turns. This is the predominant form of DNA in the cells of higher organisms. The left view is down the superhelix axis. The right view is orthogonal to the superhelix rotated around the overall pseudo-twofold axis (dyad). The ribbons show the path of the phosphodiester chain for the DNA strands, and the course of the main chain for the protein α-helical secondary structural elements. The radius and pitch of the DNA superhelix are indicated.

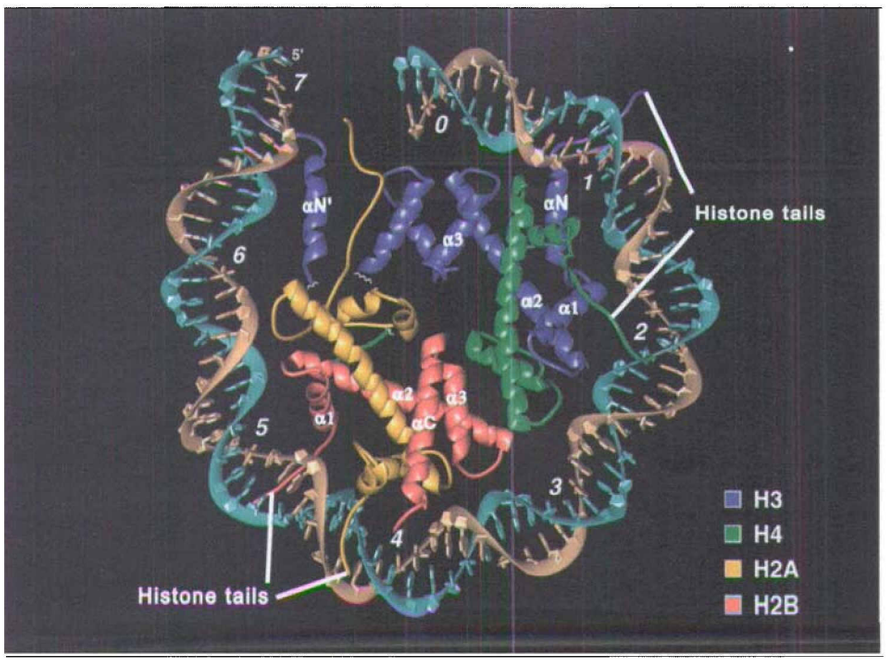

Fig. 2. One DNA superhelical gyre and associated histone protein elements (adapted from [10]). The view down the DNA superhelix axis showing essentially one-half the NCP structure makes visible the approximately three double helical turns of DNA associated with both the H3-H4 and the H2A-H2B histone-fold pairs. Successive turns of the DNA double helix are labeled 0-7, where the center of the DNA with the major groove facing the histone proteins is labeled 0 (dyad position). The three α-helices contained within a histone-fold domain are labeled α1, α2 and α3 for histones H3 and H2B. The segments of histone tails closest the histone octamer core are shown. Much of the rest of the tails, containing the post-translational modification sites, are not well-ordered in the electron density map (not shown).

proteins. The path of the DNA around the histone octamer deviates from that of an ideal superhelix, displaying strong bends in some regions, while being nearly straight in others. Whether this path is determined predominantly by histone-DNA contacts or is dependent on the DNA nucleotide sequence is the question we find currently most engaging.

3. Solving the Nucleosome Core Particle Structure

The X-ray crystallographic structure of the 206 kD NCP was published at 7 Å resolution in 1984 [12]. Although this early stage of the study preceded general use of synchrotron radiation in protein crystallography, the limited spatial resolution was not due to the X-ray source but instead to disorder within the NCP crystals themselves. The particles used were heterogeneous in composition owing to endogenesis modification of the histone proteins and to DNA sequences representing the entire nuclear content. DNA inhomogeneity created the largest problem as the endonuclease enzyme used to cut chromatin into NCP cleaves imprecisely based on sequence preferences near the borders defining a particle. Taking advantage of recombinant genetics as it was being developed, an exact 146 bp defined-sequence twofold-symmetric DNA could be made in bacterial cells and then assembled with core histone proteins into a NCP [13]. The core histones were made individually in their unmodified form again using bacterial cultures [14]. Particles assembled from these materials eventually yielded crystals that diffracted to high resolution, albeit only very weakly. Fortunately, using undulator beamlines at a third generation X-ray source, the E.S.R.F. in Grenoble, diffraction data measured in sessions over a two-year period led to the NCP structure at 2.8 Å resolution [10].

The multiple isomorphous replacement (MIR) method using metal-containing compounds was used to phase the diffraction intensity data measured at the synchrotron. Because of the large size of the structure and weak diffraction, we had earlier introduced the use of multi-heavy atom compounds for MIR [15]. One such compound, tetrakis-(acetoxymercuri)-methane (TAMM) used in the original study could again be used at higher resolution. To guarantee sufficient suitable sites for heavy metal binding, we substituted cysteine into the amino-acid sequence through recombinant 'genetics until we found combinations of sites that gave good MIR crystal derivatives on addition of mercurial compounds. Refinement of the structure from these crystals has resulted in a description of the NCP at 2.0 Å resolution [16].

Our best NCP crystals have now yielded a structure at 1.9 Å (C. Davey & T.J.R., in preparation). An undulator X-ray source at the E.S.R.F. was again used. As before, data was collected at –180 °C to slow the process of radiation damage, but nevertheless it was necessary to use 44 crystals to obtain a complete data set because of the weak diffraction intensities and rapid decay of the diffraction pattern. In all of these studies, we have used controlled dehydration of the crystals by post-growth addition of non-volatile alcohols to extend the limits of the Bragg diffraction.

4. Signposts Along Chromatin Guide Gene Activity

Cells of higher organisms must respond to a variety of different molecular signals impinging on their surface as well as those generated from within. The flow of this information regulates cell division, differentiation and metabolic state. In most cases, signals are transmitted through the cytoplasm where cross-talk occurs, and then pass into the nucleus to affect gene activity. Genes are turned on and off *via* proteins which bind specifically to regulatory regions along the DNA, thereby enabling or disabling gene readout by the DNA transcription machinery. These transcription factor proteins work in concert by assembling in multi-protein complexes on adjacent and overlapping DNA sites [17]. The transcription pre-initiation, regulatory complexes formed are probably best termed 'regulasomes', since in general terms, they not only can enhance gene transcription, but also can silence it. The protein factors comprising any particular regulasome may undergo chemical modification, such as phosphorylation by a signal transducing protein kinase that in turn alters the local transcription activation/repression potential.

Dynamic assembly/disassembly of the chromatin fiber (nucleosomal higher-order structure) is emerging as an essential process in the mechanism of eukaryotic gene regulation [18][19]. Most probably, specific DNA-binding sites for one or more of the factors forming a regulasome

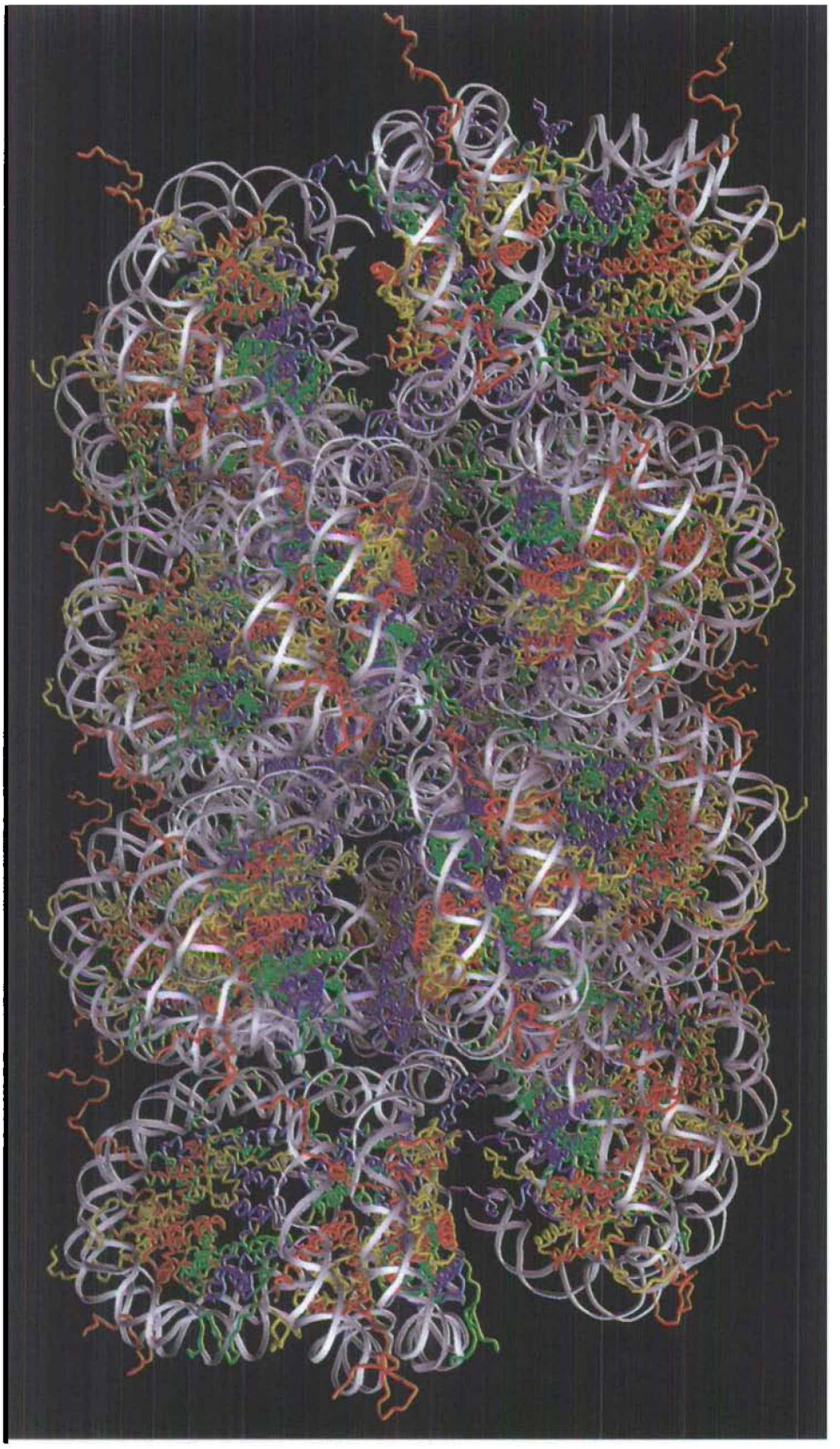

Fig. 3. A possible model of the chromatin fiber based on the NCP X-ray structure and the 'solenoid' proposal of Finch and Klug [35]. Structural details for the linker DNA, linker histone H1 and N-terminal histone tails are not known.

are left accessible even in repressive, compact chromatin (*e.g.* [20]). For activation or enhancement of transcription, the regulasome would localize complexes containing histone transacetylase and chromatin remodeling activities. Current evidence suggests that these additional factors open up the chromatin fiber, facilitating the assembly of the transcription machinery at the transcription initiation site for a gene. Conversely, the regulasome under other circumstances would repress or silence gene expression by recruiting histone deacetylase activity to the gene and inducing chromatin fiber formation excluding the transcription apparatus.

A second principle area of research in my laboratory resulting in completed X-ray structures concerns the transcription factor proteins that make up selected regulasomes [21–25]. We also have contributed atomic structures of transcription factors that are essential to most genes [26][27]. Many different families of transcription activation proteins have arisen *via* evolution as defined by the type of DNA-binding domain they contain (see *http://transfac.gbf.de/* for a complete list). With regard to gene-specific com-

plexes, we have concentrated mainly on the MADS family of transcription factors and have determined the atomic structures of the following factors all bound to DNA: human serum response factor (SRF) both with and without the additional SRF associated protein-1 (SAP-1) factor bound; human myocyte enhancer factor-2 (MEF2A); and yeast mini-chromosome maintenance protein-1 (MCM1) in a complex with the homeodomain protein MATα2 [22–25] (Fig. 4). The X-ray structures of the two protein complexes SAP-1/SRF/DNA and MATα2/MCM1/DNA reveal an important feature of the interactions between proteins largely responsible for their cooperative binding to DNA. For both SAP-1 and MATα2, a segment of a long flexible region of polypeptide chain binds to the edge of a β-sheet secondary-structural element in the respective MADS-domain protein. Further elaboration of the MATα2/MCM1/DNA structure follows.

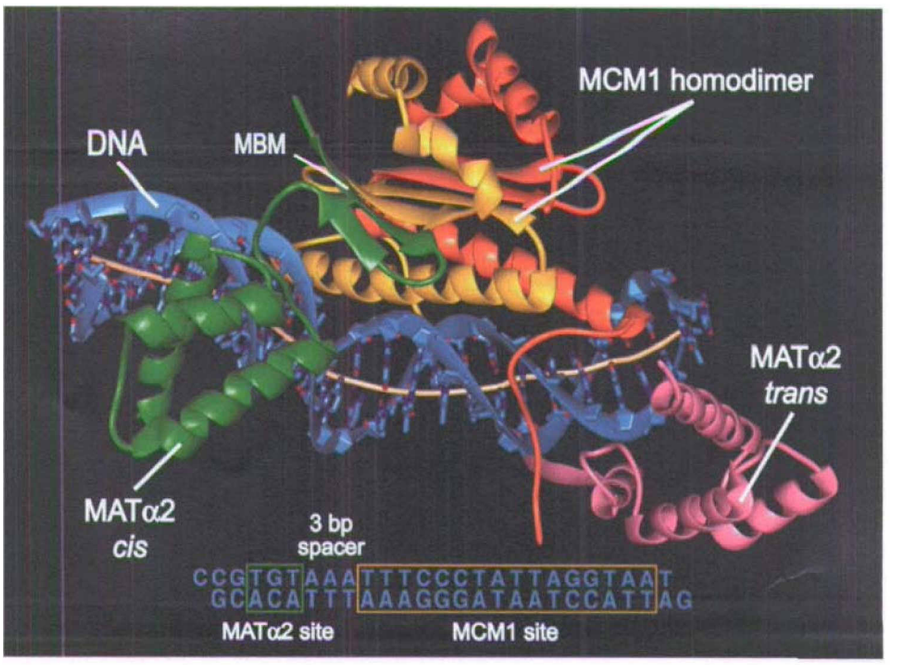

Fig. 4. Crystal structure of the MATα2/MCM1/DNA complex at 2.2 Å resolution (adapted from [25]). The MATα2 and MCM1 proteins are the DNA-binding components of a 'regulasome' that plays a fundamental role in yeast cell-type determination. The MATα2 homeodomain protein and MCM1 MADS-domain protein bind to their specific sites on nearly opposite faces of the DNA. A flexible linker polypeptide from MATα2 which includes the MCM1-binding motif (MBM) adds to the four-stranded α-sheet of MCM1 extending it to six strands. This interaction significantly increases the affinity of the two proteins for DNA, thereby promoting the formation of this regulasome.

5. A Regulasome in Yeast Determines Mating Type

Regulation of mating type in Baker's yeast (*S. cerevisiae*) consists of selection of **a** or α haploid cell types and provides a relatively simple example of combinatorial control of gene expression by transcription factors. The essential protein MCM1 is central to the determination of whether **a** or α-cell specific genes are active. Whereas MCM1 is sufficient for this discrimination in **a** cells, turning on only **a**-cell specific genes, it associates with two accessory factors in α cells which reverse its function – MATα2 represses **a**-cell specific genes and MATα1 activates α-cell specific genes [28]. MCM1 is 286 amino acids long overall, but 80 amino acids near its N-terminus are sufficient for DNA-binding, dimerization, accessory factor interaction and gene regulatory function. MATα2 comprises 210 amino acids containing a 60 amino acid homeodomain connected to an N-terminal arm, both of which bind DNA only weakly on their own. We solved the X-ray structure of these DNA-binding/intermolecular interaction domains bound to DNA to unveil the details of their sequence-specific affinity for DNA and the basis of their cooperative interaction [25]. MATα2 has a 100 amino acid domain at its N-terminus that mediates dimerization and interaction with the corepressor TUP1 [29]. MCM1 and MATα2 together with the SSN6-TUP1 complex can specify an arrangement of nucleosomes in the surrounding chromatin that blocks access to the transcription start site [30]. The direct interaction of TUP1 with the nucleosome is dependent on the histone H3 and H4 N-terminal tails [31]. The lessons learned from these yeast factors are to a large degree applicable generally as the functional domain of MCM1 is highly homologous to a domain in the human serum response factor, and homeodomain proteins such as MATα2 are an important class of developmental regulators in all animals.

The **a**-cell specific, DNA regulatory sequences are 30–31 bp in length – a 16 bp MCM1 binding site flanked by MATα2 binding sites spaced 2 and 3 bp from the edges of the MCM1 site (Fig. 4). The cooperativity effect arising from both proteins binding together is 50–500 fold and requires the natural arrangement of the DNA binding sites [28]. The MCM1 binding motif (MBM) of MATα2, mainly responsible for the higher affinity association, is only eight amino acids in length and separated from the homeodomain by 16 amino acids [32]. This stretch of chain is part of a longer linker connecting the C-terminal homeodomain and N-terminal TUP1 binding domain in the intact protein. The DNA used in the MATα2/MCM1/DNA crystal structure was designed to have one MATα2 homeodomain and flexible linker bound to a dimer of MCM1 with binding sites separated by 1.5 double helical turns (3 bp spacer). The structure reveals that in this molecular context the MATα2 linker forms two strands of antiparallel β-sheet with the MBM portion adding in the parallel orientation to the four-stranded antiparallel β-sheet in the middle of MCM1 [25].

Although unanticipated, one further copy of MATα2 bound to the junction of DNA fragments in the crystals (Fig. 5). In this case, the MBM binds the MCM1 molecule on an adjacent DNA fragment, so we have called this MATα2 molecule the *trans* copy and the first molecule the *cis* copy. The outer β-strand formed by the eight amino acids of the *cis* MATα2 linker is no longer present in the *trans* linker, but instead has become a 2.5 turn α-helix. Therefore, the same sequence (QDMINKS) takes on two different secondary structural conformations in the same crystal, depending on restrictions imposed by the relative positions of MCM1 and MATα2. Otherwise, the parallel β-strand interaction with MCM1 is essentially identical for the *cis* and *trans* copies of the MBM. The *cis* copy shows directly the arrangement for a 3 bp spacer

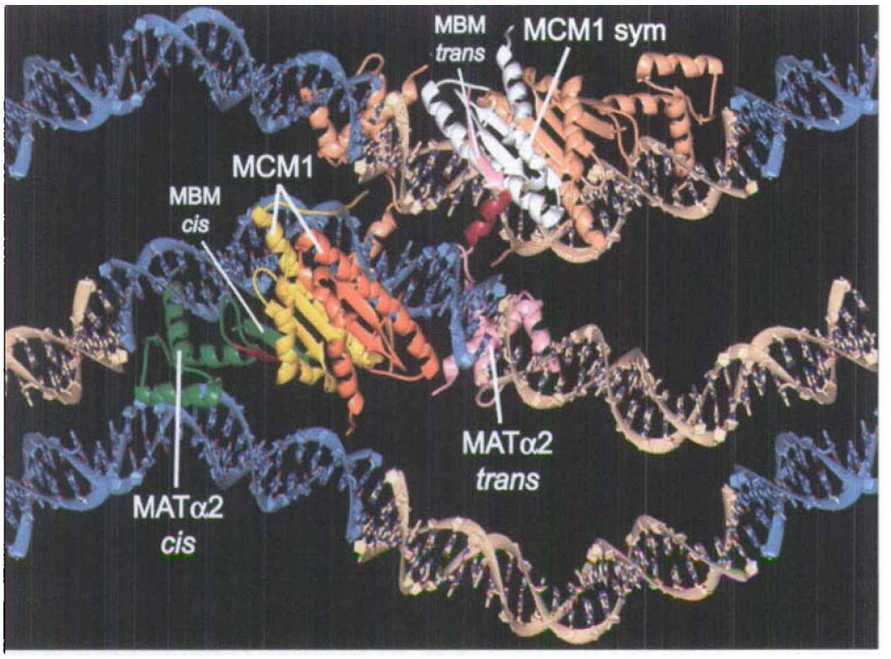

Fig. 5. Alternate conformation of the flexible linker between the MATα2 homeodomain and MBM (adapted from [25]). The *cis* copy MATα2 interacts with the MCM1 molecule adjacent to it on the same DNA double helix as shown in Fig. 4. The *trans* copy of MATα2 binds adventitiously to the end-to-end junction of DNA fragments in the crystal and the corresponding MBM binds to the MCM1 molecule (MCM1 sym) bound to an adjacent DNA fragment in the crystal. An eight amino acid region (purple) accommodates the different orientations of the MATα2 and MCM1 molecules by adopting either β or α conformation in the respective *cis* or *trans* copies.

between sites, but the polypeptide chain spanning between the homeodomain and MBM is not sufficiently long to be applicable to the 2 bp spacing. These units would be further rotated away from each other by approximately 34°. However, the *trans* copy serves as a workable model for the 2 bp separation of sites.

6. New Directions

Our improved knowledge on the number and kinds of factors involved in gene regulation gained over the last decade reveals the enormous complexity of the transcription process in eukaryotic cells where chromatin must be taken into account. To understand the mechanisms underlying gene expression, we must attempt to determine even larger macromolecular structures by crystallographic means yielding atomic descriptions. Our current ability to measure X-ray data and solve crystal structures is not limiting. Very high molecular weight structures, such as those for virus particles and ribosome subunits, have already been determined using the facilities at third generation synchrotrons [33][34]. The challenge now is to prepare biological macromolecular assemblies in a functional state and to produce crystals diffracting to atomic resolution. In my laboratory, we endeavor to elucidate the structures of the chromatin fiber, chromatin remodeling and modification complexes, as well as regulasome and general transcription factor assemblies.

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