Alterations in Chromatin Conformation During Transcription

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In the interphase nucleus of eukaryotes, DNA is organized as dynamic nucleoprotein complexes, termed chromatin. The basic unit of chromatin is the nucleosome, in which about 160 base pair DNA is wrapped twice around a histone octamer. This chromatin, not DNA itself, serves as a substrate for various biological processes such as replication, transcription, and recombination. Until recently, many scientists have considered nucleosomes as the structural component of nucleus. The idea has been, however, gradually changed (Grunstein, 1990; Kornberg and Lorch, 1991) and it seems clear that there are several biological concepts to be reestablished.

A wide variety of experimental approaches indicate that transcriptionally active genes are associated with nucleosomes, both upstream and downstream of traversing RNA polymerase II molecules. Electron micrographic study directly visualizing the nucleosome beads upstream and downstream of polymerase molecules is one of the very convincing evidences for nucleosomal structure in the active genes (McKnight *et al.*, 1978). While it is possible that histones become transiently displaced at the point of RNA polymerase II molecules (Lorch *et al.*, 1988; Jackson, 1990), it seems clear that the surrounding environment exhibits a nucleosomal structure. Then, what is the fate of nucleosomes during transcription ? What is the biological significance of chromatin in gene expression ?

"INACTIVE" AND "ACTIVE" CHROMATIN

In given eukaryotic cell, only small protions of the genome are capable of being actually transcribed into RNA. It is belived that chromatin structure plays an important role in gene expression. With respect to gene expression, two conformational states in chromatin which are "inactive" and "active" have been operationally used. The chromatin structure of active or potentially active genes appears to differ in many biochemical aspects to that of inactive genes (see Gross and Garrard, 1987; Gross and Garrard, 1988). For example, nucleosome structure, histone modification, HMG protein binding, topoisomerases, general nuclease sensitivity, localized nuclease hypersensitivity, and torsional stress are major parameter to distinguish between inactive and active chromatin. However, which are the result of gene expression and which are necessary prior to gene expression are in most cases not fully established. One important feature is the difference in *general nuclease sensitivity* between inactive and active chromatin, which has been extensively studied and is relevant to this study.

Since the toplogically constrained DNA associated with histone octamers within nucleosomes would impede RNA polymerase movement, the chromatin fiber itself would be expected to undergo conformational alterations to facilitate transcription. In fact, it has been known for many years that transcriptionally active or potentially active genes have an increased DNase I sensitivity in chromatin (Weintraub and Groudine, 1976), suggesting that their corresponding chromatin fibers are conformationally more accessible to nuclease

than those of inactive genes. Such an increased DNase I sensitivity also appears to be associated with torsional stress (Villeponteau *et al.*, 1984; Villeponteau and Martinson, 1987). It is, however, still ambiguous whether such changes are a cause or an effect of transcription. Furthermore, the nature of these alterations at the level of nucleosome structure is not understood. These long standing questions led me to address the roles of transcription and torsional stress on generating DNase I sensitivity and nucleosome alteration in chromatin.

YEAST AS A MODEL SYSTEM TO STUDY CHROMATIN STRUCTURE

The yeast *Saccharomyces cerevisiae* as a model system to study the chromatin structure-function relationship will be discussed here. It possesses several advantages over other eukaryotes. For example, it genome size is about 200-fold smaller than that of a mammalian cell, thus facillitating detection of specific nucleotide sequences by hybridization. It can be grown in the haploid state, thus facilitating mutant gene construction. In addition, one can insert a mutation into the yeast genome by site-directed gene replacement (Boeke *et al.*, 1987), thus allowing assessment of base changes in the normal chromosomal context on gene expression and chromatin structure. Studying the chromatin structure and gene expression in the natural chromosomal environment is most important because of the inherited nucleosome positioning by the given DNA sequences (see Gross *et al.*, 1986).

TRANSCRIPTIONALLY ALTERED ALLELE OF THE *HSP82* GENE

The constitutive expressed and further inducible heat shock gene, termed *HSP82* was chosen in my study. The *HSP82* gene can be induced about 20-fold simply by shifting the cells from 30 ℃ to 39℃ for 10 min. Since the relatively high basal level transcription in the control (non heat shocked) cells impeded the study, a promoter mutant was created by site-directed gene replacement technique (Boeke *et al.*, 1987). In this mutant, the basal level transcription was completely eleminated without markedly affecting heat induced transcription. This led to a situation where heat shock induction was now over 200-fold (McDaniel *et al.*, 1989; Lee and Garrard, 1992). This mutant strain proved to be extremely valuable for the resulting chromatin studies, since by heat shock treatment one can transcriptionally turn on the gene which was previously completely off. Therefore, the effect of the *HSP82* gene transcription on the chromatin structure can be directly examined.

THE EFFECT OF TRANSCRIPTION ON CHROMATIN STRUCTURE

To address the question of how transcription of the *HSP82* gene affects chromatin structure, nuclei from control and heat shocked mutant cells were prepared and digested lightly with DNase I. The resulting purified genomic DNA was cleaved with a restriction endonuclease, separated by agarose gel electrophoresis. The DNA was transferred to a membrane and the chromatin structure surrounding the *HSP82* gene locus was analyzed via Southern blot hybridization with a radioactive DNA probe chosen to about the restriction site. This technique is known as indirect end-labelling (Wu, 1980), and is analogus to *in vivo* genomic DNA footprinting technique used to study various protein-DNA complexes. Using this technique, it was possible to focus my attention to a specific region of the genome by carefully selecting the appropriate restriction enzyme(s) to generate about 2 to 4 kb long DNA fragment containing a region of the *HSP82* gene that I was interested in. A probe was carefully chosen not to cross-hybridize with other gene family, termed *HSC82*. As shown in Fig. 1A, the 3′ region of the *HSP82* gene transcription unit before heat shock (−) lacks

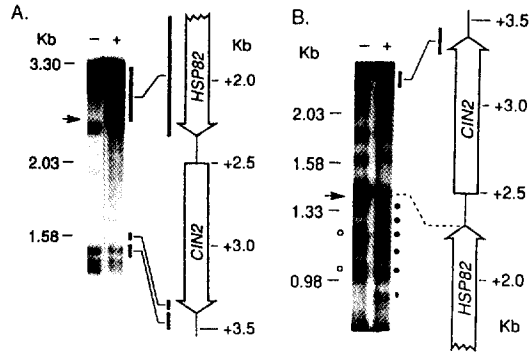

Fig. 1. Transcription induces DNase I sensitivity and nucleosome "splitting". The chromatin structure of the 3′ region of the *HSP82* gene and the immediate downstream *CIN2* gene is shown. The experimental procedures for the chromatin footpringting are described in the text. Panel A is low resolution nucleosome mapping, while Panel B is higher resolution. The open vertical arrows depict the *HSP82* and *CIN2* gene transcription units. Calibration on the left is absolute DNA length on the gel and on the right is the map position on a linear scale with respect to the start site of the *HSP82* gene transcription unit. Filled vertical bars and arrows depict DNase I hypersensitive regions, open circles refer to internucleosomal cleavage sites, and closed circles depict cleavage sites that exhibit a half-nucleosomal periodicity.

DNase I sensitivity but possesses a DNase I hypersensitive site at the end of the gene (arrow). After heat shock (+), the *HSP82* gene body exhibits marked sensitivity to DNase I (filled bars). Furthermore, this sensitivity exhibits a sharp boundary at the end of the gene since immediately downstream the *CIN2* gene lacks such DNase I sensitivity (Fig. 1A). This result suggests that there is a chromosome anchorage site at the end of the gene which may serve as an independent chromatin domain. Thus, from these results I conclude that transcription induces DNase I sensitivity in the 3′ region of the *HSP82* gene.

NUCLEOSOME STRUCTURE IN THE DNase I SENSITIVE CHROMATIN

What is the nature of alterations at the level of nucleosome structure within the underlying DNase I sensitive chromatin? Does this chromatin still possess typical nucleosome? To examine the nature of alterations in the chromatin structure of the *HSP82* gene in more detail, indirect-end labelling experiments at higher resolution were performed. As shown in Fig. 1B, before heat shock (−) when the corresponding region of the *HSP82* gene lacks pronounced DNase I sensitivity it exhibits a "whole-nucleosomal" cleavage periodicity of about 160 bp (open circles). However, strikingly, about an 80 bp DNase I cutting interval is observed within the same region after heat shock (+) induction (filled circles). As an important control experiment to demonstrate that such cleavage sites in the 3′ region reflect a specific chromatin structure, a similar experiment was performed with naked DNA. As expected, the naked DNA was digested randomly, and exhibited a smear along the corresponding DNA sequences. This novel periodicity is approximately half of the nucleosomal repeat length of yeast chromatin and thus corresponds to a "half-nucleosomal" cleavage periodicity. Therfore, the term, "split nuclesome" was used to define the structure associated with these regions (Lee and Garrard, 1991). Fig. 2 schematically summarizes the results of the above experiments. Taken together, I conclude that nucleosomes split within the 3′ region of the *HSP82* gene by observing a transcription-associated change from a whole to a half-nucleosomal DNase I cleavage periodicity. These results led me to suggest that a split nucleosomal structure represents one of the underlying structures of DNase I sensitive chromatin, in which genes are being transcribed.

The definition for nucleosome splitting is operational, and the actual structure of split nucleosomes remains to be determined. However, previous studies by others have revealed that major structural changes occur within nucleosomes associated with active genes (Prior *et al.*, 1983) and "half-nucleosomes" have been directly observed by electron micrograpy under specialized conditions (Ou-

det *et al.*, 1977). Moreover, the path or DNA about the histone octamer exhibits diad axis of symmetry, and it has been previously suggested that conformational alterations might generate DNase I cleavage sites near this dyad axis (Weintraub *et al.*, 1976; Altenburger *et al.*, 1976).

MECHANISM FOR TRANSCRIPTION-INDUCED ALTERATIONS IN CHROMATIN

While the above data convincingly demonstrate that transcription induces DNase I sensitivity in chromatin and alterations in nucleosome structure, these observations raise yet another important question. What is the precise mechanism for transcription-induced DNase I sensitivity in chromatin and nucleosome splitting?

Physical disruption of nuclesomes during transcription elongation by RNA polymerase movement might induce DNase I sensitivity in chromatin and nucleosome splitting. However, this idea is not favored since studies by others have revealed that DNase I sensitive chromatin often extends far upstream and downstream of the boundary of transcription units (see Jentzen *et al.*, 1986). Furthermore, chromatin is still sensitive to neclease digestion when only a few RNA polymerase II molecules are enggaged in low level of transcription or during the first round of induced transcription (Lee and Garrard, 1991). On the other hand, such changes in chromatin might be induced by signals that transiently spread down the chromatin fiber ahead of the transcription complex, such as waves of DNA supercoiling.

Experimental evidence supports the hypothesis that the DNA template rotates relatively to traversing RNA polymerase molecules (Giaever and Wang, 1988), leading to twin domains of DNA supercoiling: positive supercoiling downstream and negative supercoiling upstream of the transcription complex (Liu and Wang, 1987). Although DNA topoisomerases relax these structures, this relaxation may kinetically lag behind supercoil generation.

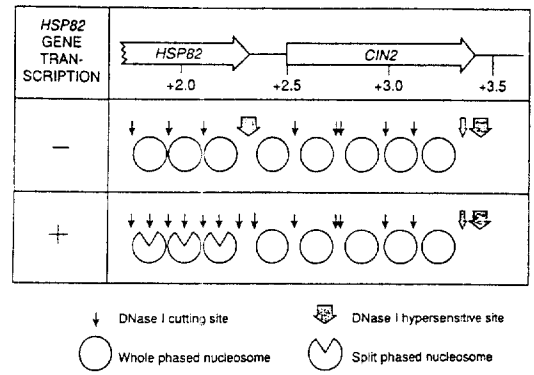

Fig. 2. Chromatin structure of the 3′ region of the *HSP82* gene. Schematic diagram summarizing the results of the chromatin structural analysis of Fig. 1.

The twin domain model of DNA supercoiling has an important implication at the level of chromatin structure. If positive supercoils are introduced into a topologically fixed chromosomal loop, they would alter the higher order of chromatin structure, since the 30 nm chromatin fiber is the supercoiled form of the nucleosome repeat and may itself be negatively supercoiled (Williams *et al.*, 1986). Positive DNA supercoils would theoretically decondense the negatively supercoiledd 30 nm fiber and the remaining positive stresses may uncoil the negatively supercoiled nucleosomal DNA around a histone octamer, thereby causing alterations in typical nucleosomal structure (Thoma, 1991). Thus, taken together with the observations described as above, transient positive DNA supercoiling downstream of RNA polymerase movement may induce DNase I sensitivity in chromatin and alterations in nucleosome structure. How can we test the positive DNA supercoiling hypothesis?

THE EFFECT OF POSITIVE DNA SUPERCOILING ON CHROMATIN STRUCTURE

To test the hypothesis that positive DNA supercoiling induces DNase I sensitivity in chromatin,

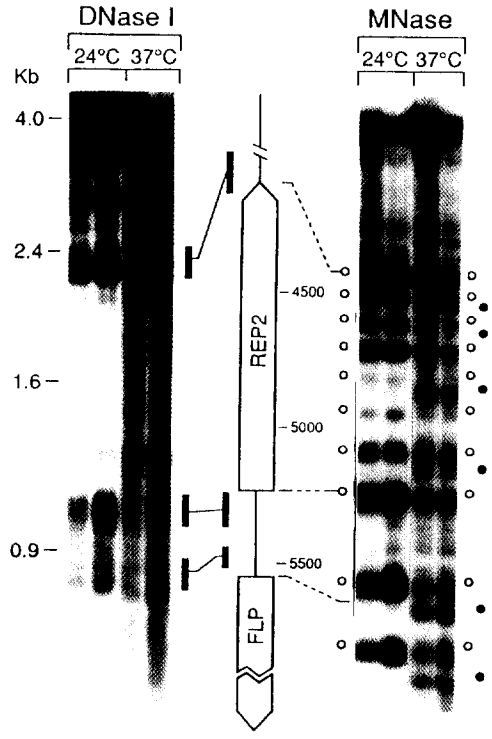

Fig. 3. Positive DNA supercoiling induces DNase I sensitivity and nucleosome alterations in 2 μ minichromosomes. The chromatin structure of the *REP2* gene and its flanking region is shown. The experimental procedure for the chromatin footpringting are described in the text. Left Panel is nucleosome mapping by DNase I (DNase I), while Micrococcal nuclease (MNase) is used in the right Panel. The open vertical arrows depict the open reading frames. Calibration on the left is absolute DNA length on the gel and on the center is the nucleotide map position on a linear scale. Filled vertical bars depict DNase I hypersensitive regions, open circles refer to internucleosomal cleavage sites, and closed circles depict cleavage sites within nucleosomes.

a conditional topoisomerase mutant expressing *E. coli* topoisomerase I was utilized (Lee and Garrard, 1991). At non permissive temperature of 37 ℃ where there is no known relaxing activity, *E. coli* topoisomerase I preferentially relaxes negative DNA supercoils generated by RNA polymerase movement during transcriptional elongation, thus resulting in gradual accumulation of positive DNA supercoils. Therfore, the effect of this positive

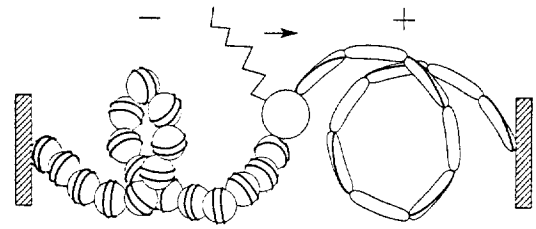

Fig. 4. Positive DNA supercoils downstream of the transcription complex clear the path for RNA polymerase II. The schematic diagram depicts a chromosomal loop with fixed ends, composed of a polynucleosomal array that are being traversed from left to right by RNA polymerase II. The upstream nucleosomes become tightly packed and downstream nucleosomes split as a consequence of the twin-domain model of DNA supercoiling of Liu and Wang (1987). Topoisomerases will offset these processes. Thus, they will be transient.

DNA supercoiling on the chromatin structure of the yeast 2 μ endogenous minichromosomes has been examined (Circular chromosomes should be studied to demonstrate the DNA supercoiling state). As expected, converting the DNA supercoiling state from negative to positive *in vivo* by shifting the temperature from 24℃ to 37℃ generated the chromatin of the *REP2* gene and its flanking region to become DNase I sensitive (Fig. 3, DNase I). When studied with Micrococcal nuclease (Fig. 3, MNase), it also induced alterations in nucleosome conformation from a whole (open circles) to a half-nucleosomal (closed circles) cleavage periodicity within the DNase I sensitive chromatin. Such alterations in chromatin structure are characteristic of transcriptionally active genes. Therefore, transient positive DNA supercoiling during transcription may induce the transition from an inactive to an active conformation in chromatin.

BIOLOGICAL IMPLICATIONS OF POSITIVE SUPERCOILING FOR TRANSCRIPTION

Based on the above results and other studies, positive DNA supercoiling downstream of transcription complex is believed to decondense the ch-

romatin fiber and pave the way for RNA polymerase passage. Previous electron micrograpic studies have visualized chromatin decondensation downstream of traversing RNA polymerase molecules (Bjorkroth *et al.*, 1988). Furthermore, torsional stress appears to be required for transcription, since circular, but not linearized or nicked, DNA molecules are transcriptionally active *in vivo* (Harland *et al.*, 1983; Weintraub *et al.*, 1986: Luchnik *et al.*, 1986). Recent studies reveal that nucleosomes are potent inhibitors of transcriptional elongation *in vitro* (Izban *et al.*, 1991). Thus, as shown schematically in the Fig. 4, conformational transition in chromatin may serve a crucial function in paving the way for RNA polymerase passage.

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