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Reorganization of Chromatin Conformation from an Active to an Inactive State After Cessation of Transcription

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Taking advantage of the heat inducible *HSP82* gene in yeast, chromatin structure after transcription cessation was investigated. Alteration of chromatin conformation within the *HSP82* gene transcription unit into an active state has been shown to correlate with its transcriptional induction. It was thus of interest to examine whether the active chromatin state within the *HSP82* gene body could be maintained or erased after cessation of the transcription. Based on *HSP82* mRNA analysis, the gene ceased its transcription within a few hours of cultivation at a normal condition after heat induction. In this condition, an active chromatin conformation in the *HSP82* gene body was changed into an inactive state which was revealed by DNase I resistance and by typical nucleosomal cutting periodicity in the corresponding chromatin. These results thus ruled out the possibility of a long-term maintenance of the DNase I sensitive chromatin after transcription cessation. DNA replication may be a critical event for the chromatin reprogramming.

Key words: ***Saccharomyces cerevisiae*, *HSP82*, chromatin, DNase I sensitivity, nucleosome**

DNA in the interphase nucleus is organized as a dynamic nucleoprotein complex termed as chromatin. Recently, chromatin has been known to serve as not only structural basis of DNA organization, but functional negative or positive regulator of gene expression (9, 10, 40). The basic unit of chromatin is the nucleosome which contains about 200 base pairs (bp) DNA wrapping twice around a histone octamer. In yeast *Saccharomyces cerevisiae*, a nucleosome contains about 165 bp (16). The chromatin can be gradually condensed in a precise manner into a mitotic chromosome by about 10,000-fold. Even in interphase, the level of chromatin condensation seems to depend primarily on the transcriptional state (6). It is still unknown how such a highly organized chromatin exhibits precise DNA replication and transcription. In fact, only small portions of genome are being transcribed into RNA in eukaryotic cells, suggesting that accessibility of chromatin by various protein factors may be structurally limited. Structural studies of chromatin have thus focused on the event related to transcription initiation, activation, and regulation.

Based on the structural studies, two states of chromatin conformation are operationally defined as active and inactive with respect to gene expression (28). Differences in biochemical and morphological features between the two states are still elusive despite numerous characteristics have been reported in the corresponding chromatin (8). Among these characteristics, one important feature for active genes within domains of chromatin is a high DNase I sensitivity in the corresponding active chromatin compared to inactive ones when they are digested with the enzyme (39).

It has been known for many years that such a high DNase I sensitivity in domain of chromatin is accompanied by induction of gene transcription (7, 13, 21, 32). However, other evidences suggest that this sensitivity does not represent ongoing transcription *per se*. For example, during transcription, some actively transcribed genes in chromatin are not sensitive to DNase I, and others are sensitive when the genes are inactive (4, 15). This suggests that something other than transcription may be involved in establishment of the DNase I sensitive chromatin. Furthermore, the sensitivity to DNase I and pro perties of genes in an active chromatin state appear to persist for long time after transcription has ceased (22, 27, 37). Importantly, low level of transcripts could have been detected in these experiments. Weak transcription which occurred along the corresponding chromatin region prior to the experimental analysis may result in preservation of the DNase I sensitivity. The

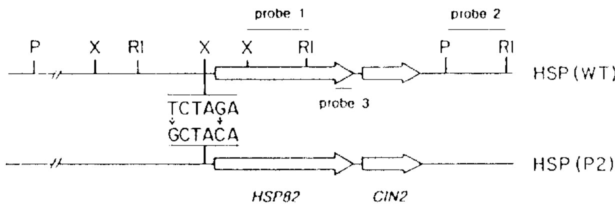
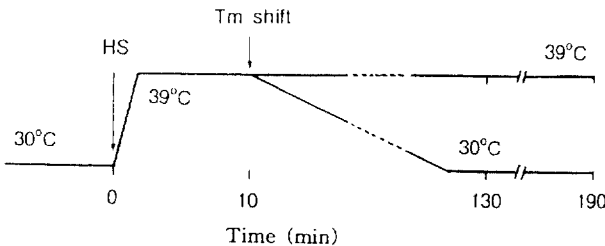
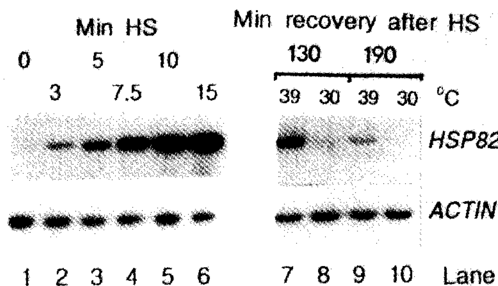


Fig. 1. Structure of *HSP82* alleles and positions of probes. *HSP82* alleles are schematically represented with the arrows depicting the *HSP82* and *CIN2* gene transcription units. HSP(WT) is the wild type allele. HSP(P2) is a mutant where 2 bases were mutated in the HSE element closest to the TATA box as indicated. Pertinent restriction sites shown are *Pst*I (P), *Xba*I (X), and *Eco*RI (RI), Probes used in this study are: 1. A 753 bp *Xmn*I-*Eco*RI fragment for indirect end-labelling; 2. A 1 kb *Pst*I-*Eco*RI fragment for indirect end-labelling 3. A 100-mer spanning +2190 to +2289 used for Northern analysis of *HSP82* mRNA.



(a)



(b)

Fig. 2. Northern analysis of *HSP82* transcripts. Panel A. Simplified diagram of the experimental procedure for RNA analysis. One of two cultures of exponentially growing yeast cells was heat shocked (t=0) and maintained the temperature at 39°C. Samples were taken at the indicated times in Panel B. The other was heat shocked for 10 min and transferred to the 30°C shaking incubator (Tm shift), such that temperature of the culture would gradually decrease to 30°C. Samples were taken at the indicated times in Panel B. Panel B. Northern analysis of *HSP82* transcripts. RNA samples were isolated from HSP(P2) cells at indicated times (min) after heat shock (lane 1~6) or indicated time of incubation at either 39°C or 30°C after heat shock (lane 7~10). Northern analysis are performed as described in the materials and methods.

above uncertainty led me to investigate DNase I sensitivity in chromatin and structure of nucleosome within the corresponding chromatin after transcription cessation. For this purpose, a tightly regulated gene must be provided, so that one can turn on and off the gene by any means.

Here, I utilized *S. cerevisiae* carrying point mutations in the promoter region of the heat shock inducible gene, termed as *HSP82* (5). In this mutant, basal transcription was abolished at a normal temperature without a significant change in induced transcription after brief heat shock treatment. The primary goal of this study was to determine whether the active, DNase I sensitive chromatin could be maintained or be changed into an inactive, DNase I resistant state when *HSP82* transcription had been ceased at normal temperature. As a result, the active conformation was reorganized into an inactive chromatin state when the *HSP82* gene had ceased its transcription within a few hours of cultivation at normal temperature after heat shock induction. Possible implication and mechanism for chromatin reprogramming will be discussed.

Materials and Methods

Yeast strains and growth conditions

The *S. cerevisiae* haploid strain W303-1A (*Mata ade2-1 ura3-2 his3-11, 15 leu2-3, 112 trp1-1 can1-100*) was used as a parental strain termed as HSP(WT). To generate the mutant strain termed as HSP(P2), two bases were changed in the heat shock regulatory element (HSE) within the upstream region of the *HSP82* gene by site-directed gene replacement (2, 18, 24).

Cells were grown in 1% yeast extract/ 2% Bacto-peptone/ 1% dextrose (YPD) medium at 30°C to a logarithmic phase (~5×10^7 cells per ml). For heat-shock treatment, the temperature was shifted rapidly from 30°C to 39°C by addition of equal volume of prewarmed YPD medium to 51°C and maintained at 39°C in a gyrotary shaking waterbath. For recovery experiment after heat shock, 10 min heat shocked culture was maintained for indicated times at either 30°C or 39°C. Simplified diagram of the experimental design is shown in Figure 2A.

RNA extraction and Northern analysis

RNA analysis was performed as essentially described elsewhere (11). After cells were grown at the indicated conditions as shown in Figure 2A, samples were taken out at the indicated time. Sodium azide was then treated to the taken samples (20 mM final concentration) to inactivate ATP generating metabolism (34) and the cells were harvested for RNA analysis. Total RNA was isolated as described elsewhere (14). Resulting purified RNA was quantified colorimetrically by the orcinol meth-

od. Ten microgram of each RNA sample was treated with formamide, incubated briefly at 65℃, and separated on 1.4% agarose-formaldehyde gel (29). RNA was then transferred to a Zeta-Probe membrane (BioRad), and hybridized with a radiolabelled 100-mer probe as described elsewhere (probe 3 in Figure 1) (9). Autoradiography was performed by exposing pre-flashed Kodak XAR-5 film with intensifying screens (DuPont Cronex) at −70℃ for 1-4 days and by developing it with an automatic developer (Pharmacia). The same filter was hybridized with a radiolabelled antisense RNA probe transcribed *in vitro* from pGem-actin as described (23). Hybridization signal was scanned and quantified densitometrically, using a beta-scope. The levels of *HSP82* gene transcripts were normalized relative to those of *Actin* gene transcripts.

Nuclei isolation and nuclease digestion

For the control state, one liter of HSP(P2) mutant cells were grown in YPD medium at 30℃. For the heat shocked state, same cells were heat treated by adding equal volume of YPD medium prewarmed to 51℃ followed by incubation at 39℃ for 10 min. For the recovered state from the heat shocked, 10 min heat shocked cells were grown at 30℃ for 180 min, such that temperature would be gradually decreased to 30℃. Nuclei were isolated as described elsewhere (18) with the following modifications. Cells cultured at these conditions were harvested and incubated with oxalyticase (Enzogenetics, USA) in spheroplast buffer (1.4 M sorbitol, 40 mM Hepes, pH 7.4, 0.5 mM $MgCl_2$, 20 mM NaN_3) for 60 min at 30℃. All solutions contained 20 mM sodium azide up to nuclear pelleting stage. Nuclei and naked DNA controls were digested with either DNase I or MNase as indicated and purified as described (34). For naked DNA controls, genomic DNA was purified from nuclei of heat-shocked cells.

Chromatin and nucleosome analysis

For nucleosomal structure analysis of the bulk chromatin, 10 μg of MNase digested DNA were separated on a 1.2% agarose gel by electrophoresis in 1× TPE buffer (29). For the size marker, *Hin*dIII and *Eco*RI digested λ DNA were used for calibration. For chromatin analysis, 20 μg of DNA samples were digested with *Eco*RI or *Kpn*I and *Eco*RV, and separated on a 1.5 or 2.0% agarose gel by electrophoresis. For the size marker, *Hin*dIII and *Eco*RI digested λ DNA were used for calibration. DNA was transferred to a Zeta-Probe membrane (BioRad), and prehybridized for 2 h at 65℃, hybridized with a radiolabelled 753 bp *Xmn*I-*Eco*RI (probe 1 in Figure 1) or 1 kb *Pst*I-*Eco*RI DNA fragment (probe 2 in Figure 1) for 15 h at 65℃ as described elsewhere (26). The probes were labelled with [p^{32}]dCTP by random hexamer primer synthesis (Pharmacia), and were purified by cetylpyridinium bromide precipitation methods (29). Filters were washed once at room temperature for 20 min, and three times for 20 min each at 65℃ with a washing buffer (0.1× SSC, 20 mM sodium phosphate buffer pH 7.0, 2% SDS) (29). Autoradiography was performed by exposing Kodak XAR-5 film with intensifying screens at −70℃ for 24~36 h.

Results and Discussion

Expression of the *HSP82* gene and manipulation of its promoter region

To investigate DNase I sensitivity in chromatin and structure of nucleosome within the corresponding chromatin after transcri ption cessation, a tightly regulated gene must be provided, such that one can turn immediately on and off the gene. For this purpose, heat shock inducible genes and yeast provide us with a suitable model system. No other heat shock genes but the yeast *HSP82* gene (formerly termed *HSP90*) are well studied both in regulation of gene expression and changes in chromatin structure with respect to transcription (3, 5, 12, 13, 14, 18, 34). At the normal temperature of 30℃, the *HSP82* gene exhibits significant basal level of transcription; at higher temperature of 39℃ the gene, however, induces its transcription by about 20-fold, where translation products of the transcripts represent about 4.5% of the total cell's proteins (17). The chromatin domain of the transcription unit of the *HSP82* gene exhibits a DNase I sensitive, active conformation because of the active transcription in both normal and heat shocked states (34). Previously, we have made a *HSP82* gene mutant carrying 2 base changes within the HSE element of the promoter region of the *HSP82* gene (18). In this mutant, basal transcription of the *HSP82* gene was abolished at a normal temperature without a significant change in induced transcription after brief heat shock treatment. Furthermore, this mutation eliminated DNase I sensitivity within the chromatin of the *HSP82* gene at the normal temperature, clearly demonstrating that transcriptional process induces chromatin conformation from an inactive to an active state (13).

Since the above mutant was constructed within an α-mating type strain, thus α-factor mediated arrest of the cell cycle would not be possible (see below for the reason). Thus, I have introduced the same mutations in an a-mating type by site-directed gene integration (2). The resulting mutant exhibited similar regulation of the *HSP82* gene expression as did in that of α-mating type

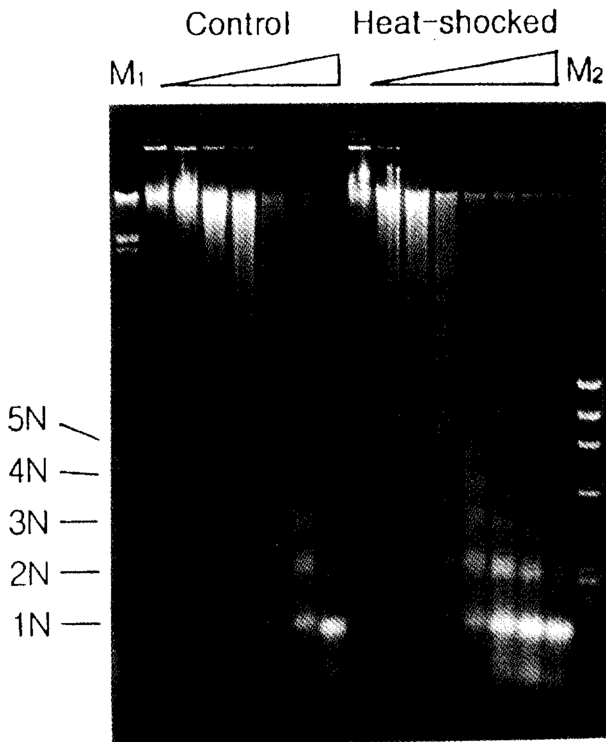


Fig. 3. Nucleosome structural analysis of the heat shocked cells. Nuclei isolated from control and 10 min heat shocked cells were digested with increasing concentration of Micrococcal Nuclease. The purified DNA was separated by electrophoresis and visualized. *Eco*RI-*Hin*dIII restricted lambda DNA (M_1) and *Hae*III restricted ϕX174 (M_2) were used as molecular size markers. N-mers on the left represent oligomeric nucleosomal DNA fragments.

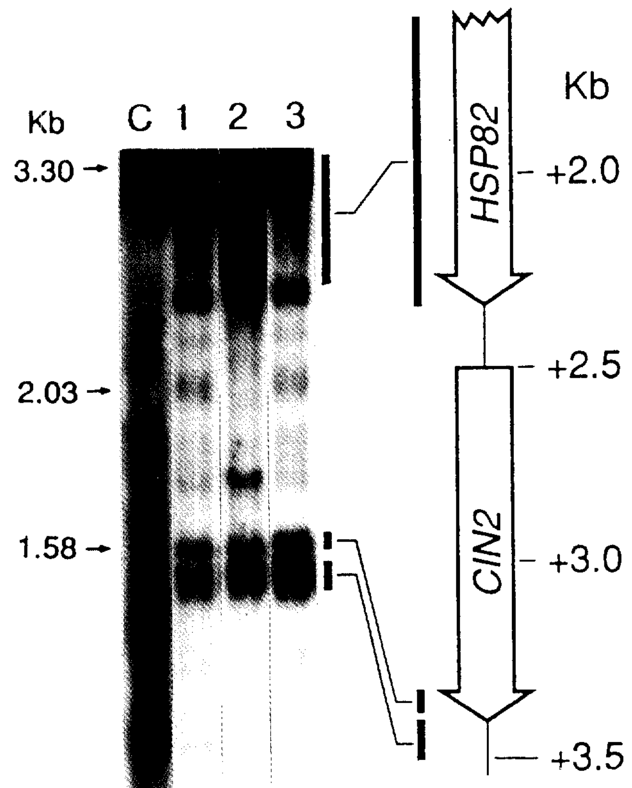


Fig. 4. DNase I sensitivity in the chromatin of the *HSP82* gene at various conditions. Nuclei isolated from control (lane 1), heat shocked (lane 2), and recovered cells from heat shock (lane 3) were digested with DNase I. Resulting purified DNA were digested with *Eco*RI (ref. 13), separated by electrophoresis, transferred to membrane filter, and indirectly end-labelled with the probe 2 in Fig. 1. The region subsequently hybridized with the probe corresponds to 3.3 kb *Eco*RI fragments (+1601~+4900 from the *HSP82* transcription start site). For naked DNA control (C), DNA isolated from the heat shocked cells was digested with DNase I. The open vertical arrows depict the *HSP82* and *CIN2* gene transcription units. The difference in DNase I sensitivity of chromatin between the lanes is observed within the *HSP82* transcription unit (see upper filled vertical bar on the figure). Calibration on the left is absolute DNA length and on the right is map position with respect to the *HSP82* gene transcription start site. Filled vertical bars depict hypersensitive sites.

strain (data not shown; see Fig. 2B). Thus, I have established a new mutant in which basal transcription of the *HSP82* gene was abolished at a normal temperature without a significant change in induced transcription after heat shock treatment.

Transcriptional "on and off" the *HSP82* gene in the mutant yeast strain

The relationship between transcriptional induction and chromatin structure has been studied by many researchers, providing various lines of evidence that the chromatin conformation in which gene transcription had been induced has been correlated with the transcription (7, 13, 21, 32). Here, I have thus focused my study on examining DNase I sensitivity in chromatin and structure of nucleosome within the corresponding chromatin after transcription cessation. To do so, it is prerequisite to determine whether and when *HSP82* gene transcription would cease after its transcription has been induced. For this purpose, I have taken an advantage of the mutant strain HSP(P2), which lacks basal-level transcription (lane 1 in Fig. 2B). As shown in the Figure 2B, *HSP82* gene transcripts still persisted when 10 min heat shocked cells grown for 120 min at either non heat shocked or heat shocked conditions (lane 7 and 8). However, the transcripts disappeared when the heat shocked cells were grown for 180 min at 30℃ at a non heat shocked condition (but not at the heat shocked condition) (compare lane 9 and 10 in Fig. 2B). Therefore, I have decided to determine whether growth of 10 min heat-shocked cells at a non heat shocked condition for 180 min changes chromatin conformation from an active to an inactive state.

DNase I sensitivity in the 3′ region of the *HSP82* gene at various conditions

To examine relationship between cessation of *HSP82* gene transcription and DNase I sensitivity in the corresponding chromatin, I prepared nuclei from the non heat shocked, 10 min heat shocked, and non heat shocked, 180 min recovered cells from heat shocked condition as shown in Figure 2B. I first looked at the quality of the isolated nuclei by examining the nucleosome structure of the total bulk chromatin, since heat shock treatment itself may affect nucleosomal structure. As expected, there was about 165 bp cutting nucleosomal periodicity of the MNase digested nuclei, showing no differences in nucleosome structure between non heat shocked and heat shocked conditions (Fig. 3; see also ref. 13).

Three samples of nuclei were digested with DNase I and DNase I sensitivity of the chromatin was determined by chromatin footprinting (termed indirect-end labelling technique) developed by Wu (41). As shown in Figure 4, at a control state the chromatin within the *HSP 82* gene exhibited a low DNase I sensitivity (lane 1), whereas it showed a high sensitivity at the heat shocked condition (lane 2). The remaining chromatin region of the *HSP82* gene could not be examined because of a cross-hybridization with the cognate *HSC82* gene (34). When I looked for DNase I sensitivity of chromatin prepared from non heat shocked, recovered cells after heat shocked condition, where *HSP82* transcription had been ceased, its sensitivity was erased as it was at the control state (lane 3). No changes were observed in the *CIN2* chromatin region at the above conditions. I also performed similar experiments with 120 min (instead of 180 min) recovered cells, where *HSP82* mRNA were detected (lane 7 in Fig. 2B). In this condition, the DNase I sensitivity in the chromatin still persisted due to the active state of *HSP82* transcription (data not shown). The DNase I cutting pattern around the *HSP82* region reflects chromatin specific since it was not observed when naked DNA was digested as a control (lane C). Thus, I conclude that a high DNase I sensitivity of chromatin is eliminated after transcription has been ceased.

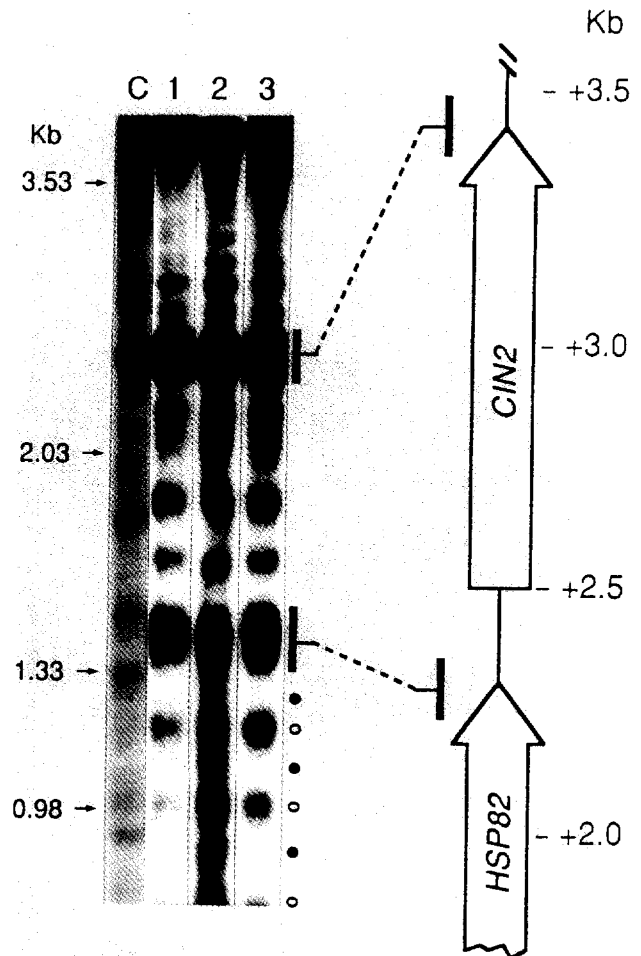


Fig. 5. Mapping DNase I cutting sites in the chromatin of the *HSP82* gene at various conditions. Nuclei isolated from control (lane 1), heat shocked (lane 2), and recovered cells from heat shock were digested with DNase I. Resulting purified DNA were digested with *Eco*RV plus *Kpn*I (ref. 13), separated by electrophoresis, transferred to membrane filter, and indirectly end-labelled with the probe 1 in Fig. 1. The region subsequently hybridized with the probe corresponds to 3.6 kb fragments (+906~+4500 from the *HSP82* transcription start site). For naked DNA control (C), DNA isolated from the heat shocked cells was digested with DNase I. The open vertical arrows depict the *HSP82* and *CIN2* gene transcription units. The difference in nucleosome structure within the corresponding chromatin between the lanes is observed within the *HSP82* transcription unit (see open and closed circles in lower part of the figure). Calibration on the left is absolute DNA length and on the right is map position with respect to the *HSP82* gene transcription start site. Filled vertical bars depict hypersensitive sites, open circles refer to internucleosomal linker cleavage site, and filled circles depict additional cutting site within the nucleosomal DNA.

Nucleosome structure underlying chromatin of the *HSP82* gene at various conditions

To determine nucleosome structure of the *HSP82* gene, nucleosomal cutting sites within the chromatin were mapped by high resolution (13). As shown in Figure 5, at a control state the chromatin of the *HSP82* gene exhibited about 160 bp cutting periodicities which correspond nucleosomal sized DNA fragments (lane 1, open circles). As shown previously, at the heat shocked condition the underlying chromatin exhibited about 80 bp cutting periodicities which represent an alteration of the nucleosome structure (lane 2, see additional closed circles). This altered nucleosomal structure corresponds to the DNase I sensitive chromatin shown previously in Figure 4. Half-nucleosomal cutting periodicity clearly demonstrates disruption of nucleosomal structure during movement of RNA polymerase complex (36). Although the structural basis of the altered nucleosomes has not

been delineated, several models for such a disrupted nucleosome structure were proposed (1, 19, 25, 35, 36, 42). Depending on the experimental approach, some examples of structure of the disrupted nucleosome have been interpreted as severely disturbed, whereas others have been interpreted as in someway intact. When DNase I cutting intervals were examined within the DNase I sensitive chromatin from non heat shocked, recovered cells after heat shocked condition, its 80 bp cutting interval was changed again to 160 bp as it was at the control state (lane 3). No changes were observed in the *CIN2* chromatin region at the above conditions. Taken together, I concluded that immediately after transcription has been ceased, the altered nucleosomal structure of DNase I sensitive chromatin is reorganized into a typical nucleosomes which represent a DNase I resistant chromatin. In these experimental conditions with an yeast system, I argue against the possibility that the sensitivity to DNase I and properties of genes in an active chromatin state persist for long time after transcription has ceased. Thus, the DNase I sensitive chromatin state does not appear to be memorized for long time as a stable heritable complex after gene transcription.

Mechanisms for reorganization of chromatin structure after transcription cessation

What are the mechanisms of erasing a DNase I sensitive, active chromatin conformation into an inactive DNase I resistant state? The answer may be DNA replication. On the other hand, it may be just cessation of transcription itself. In fact, replication dependent or independent changes in chromatin structure have been proposed by many researchers (30, 33). These contradictory ideas deal with the memory of chromatin conformation after transcription cessation. Thus, the answer to the above question would be what happens in chromatin structure right after transcription cessation. Studies of replication dependent or independent changes in chromatin structure have been limited primarily within the promoter and other regulatory regions of genes, whereas the chromatin structural changes in the gene transcription unit by DNA replication has not been investigated. The above two possibilities could not be distinguished in this work although the hypothesis for replication dependent reorganization of chromatin conformation is favored. Since 180 min of cultivation after heat shock treatment corresponds to about two rounds of DNA replication in the yeast strain used in this work, replication dependent chromatin reprogramming cannot be ruled out. Furthermore, I do not know exact time point when *HSP82* transcription has ceased even though *HSP82* transcripts disappeared after 180 min growth of the cells (lane 10 in Fig. 4). Investigation is in progress to determine chromatin structure of the *HSP82* gene in which DNA has or has not been replicated by utilizing synchronized yeasts with α-factor. With this synchronized a cells, the newly synthesized DNA can be density-labelled with 5-bromodeoxyuridine. Differences in chromatin structure between replicated and unreplicated form can be determined.

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