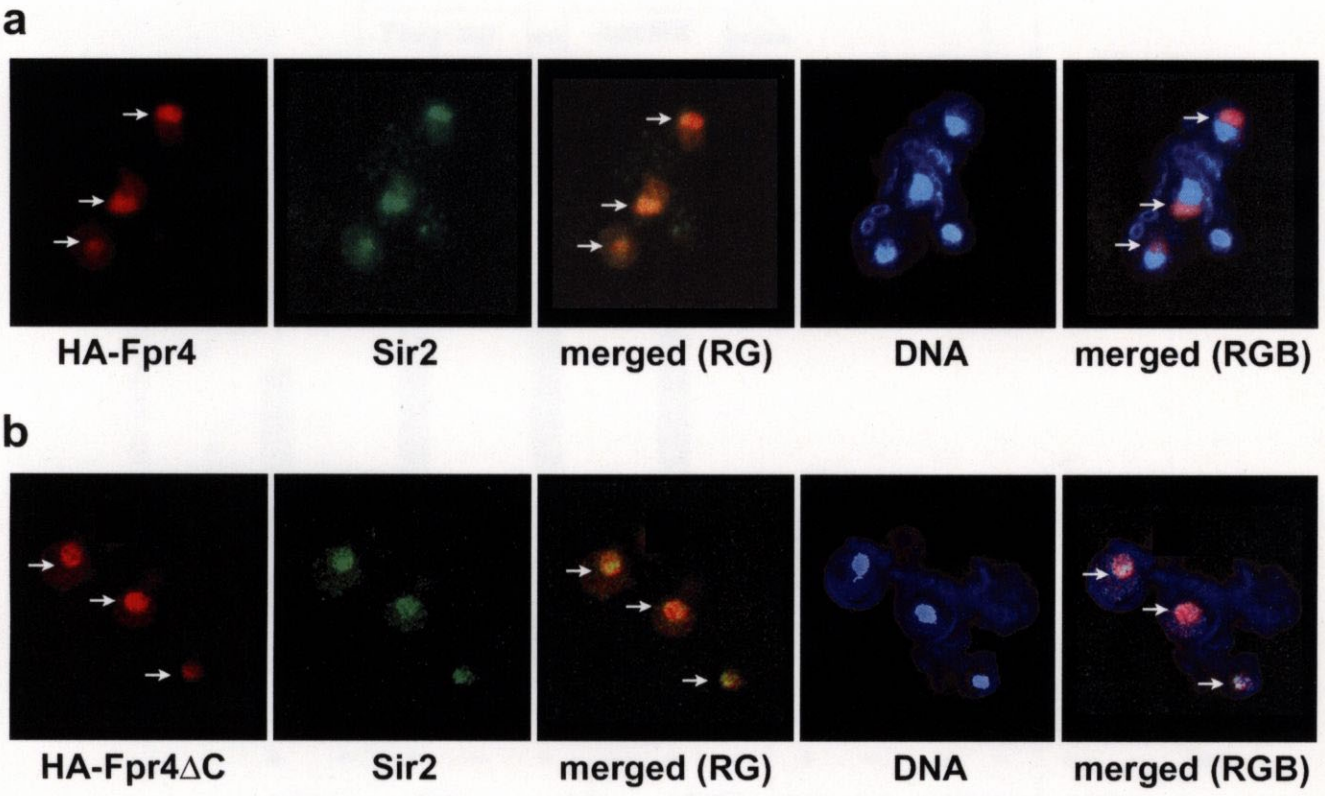


Figure 12: Nuclear FKBP is enriched in the nucleolus.
(a) The indirect immunofluorescent assay showing the localization of the Fpr4 protein.
Fpr4 protein localizes at the nucleus and is enriched at the nucleolus. The localization of HA-Fpr4 was tested by using anti-HA-tag mouse (12CA5) and anti-Sir2p goat (Santa Cruz) antibodies as the primary antibody, and TRITC-conjugated anti-mouse and FITC-conjugated anti-goat antibodies as the secondary antibody. Sir2 was used as a nucleolus marker because the major part of Sir2p has been reported to localize at the nucleolus. The merged image indicates the co-localization of the Fpr4 and Sir2 proteins as yellow colored area, the positions of which are indicated as white arrows. DNA was stained with Hoechst 33342 and was visualized by blue diode laser (Carl Zeiss). In the nucleolus, the DNA was excluded because of the richness of RNA in this region, and thus we can recognize the nucleolus as Hoechst-unstained patches, which are indicated as white arrows in the panel showing the DNA image.
(b) The localization of the histone chaperone domain of Fpr4 (Fpr4ΔC).
The histone chaperone domain of Fpr4p localizes at the nucleus and is enriched at the nucleolus, similar to the full length Fpr4p protein, as described in (a).

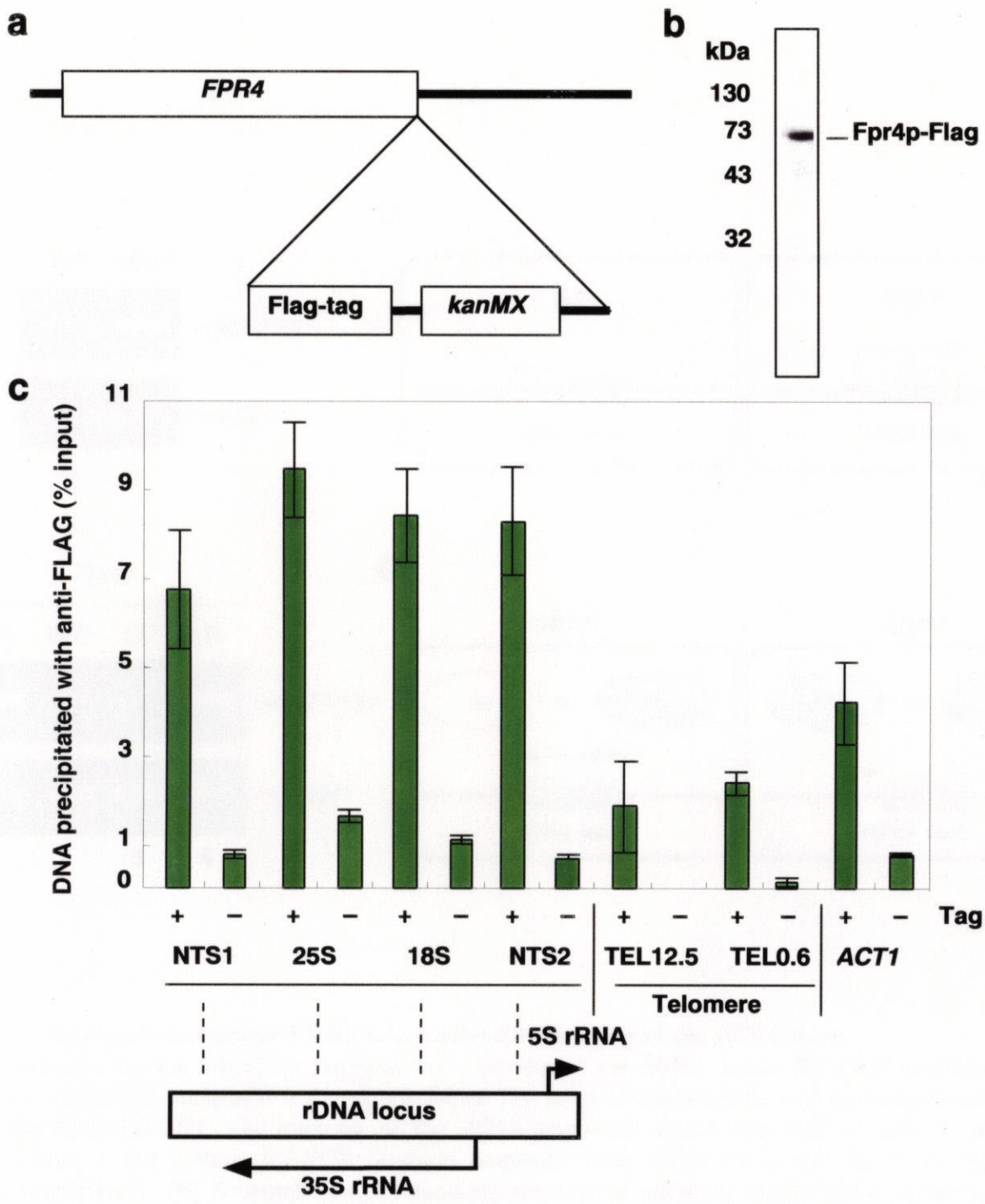


Figure 13: The association of nuclear FKBP with chromatin at rDNA loci *in vivo*.
The association of nuclear FKBP was demonstrated by ChIP experiments. (**a**) Schematic depiction of the Flag-tagging of Fpr4p encoded by the endogenous *FPR4* gene. The Flag-tag was integrated at the native *FPR4* locus and *KanMX* was used as the marker to select the tagged strain. (**b**) Expression of Fpr4-Flag protein. The specificity of the anti-Flag antibody for the Fpr4-Flag protein in cell extracts was shown by Western blot analysis. Fpr3p, which is an isolog of Fpr4p, is known to be phosphorylated in cells and to be detected as a 70 kDa protein20. (**c**) Association of Fpr4p with chromatin at rDNA loci. Fpr4-Flag protein was precipitated from formaldehyde-crosslinked whole-cell extracts prepared from Fpr4-Flag and control strains. The immunoprecipitated DNA was analyzed by PCR using primer pairs directed against rDNA loci. Real-time quantitative PCR was performed by using primers corresponding to rDNA regions. TEL12.5 and TEL 0.6 are the regions that are 12.5 kbp and 0.6 kbp from the chromosomal terminus. The ratio of the DNA precipitated relative to the input DNA is plotted. The data are presented as means ± 1 standard deviation of duplicates. The positions of the primers are schematically indicated. NTS1 and NTS2 are non transcribed spacer sequences 1 and 2, respectively. 25S and 18S are ribosomal genes that are transcribed and processed at rDNA loci.

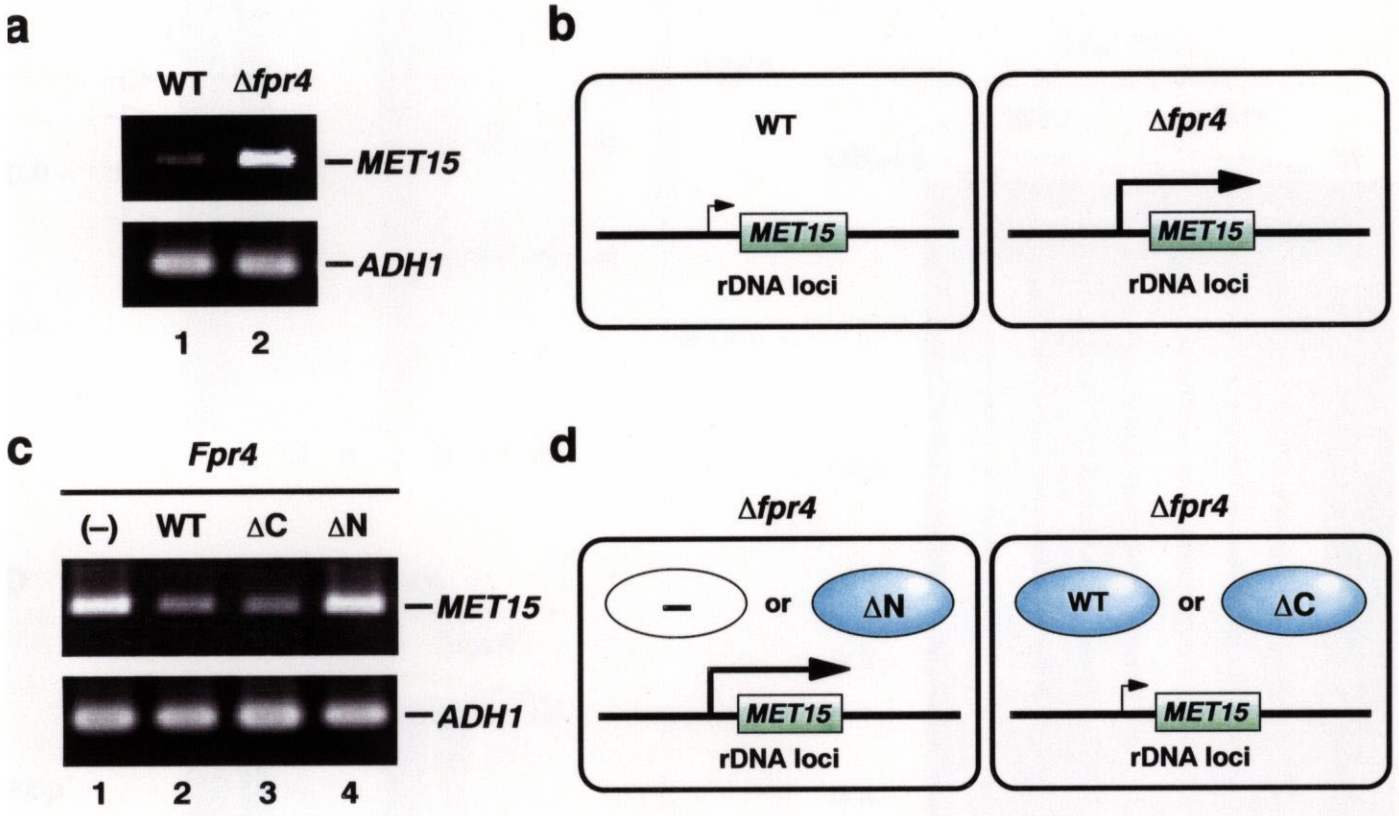


Figure 14: Endogenous nuclear FKBP is essential for silencing at the rDNA locus.
(**a**) Endogenous nuclear FKBP is required for silencing at the rDNA locus. RT-PCR analysis of the expression of *MET15* integrated at the rDNA locus. The level of transcription was normalized relative to that of the control *ADH1*. The intensity of the cDNA produced reflects the level of *MET15* or *ADH1* mRNA. Lanes 1 and 2 show RT-PCR products amplified from JS237 (WT) and the *FPR4* disruptant (Δ*fpr4*), respectively. (**b**) Schematic model showing the loss of silencing at the rDNA locus by a null mutation in the endogenous FKBP gene. *MET15* expression is indicated by arrows. (**c**) The N-terminal domain of nuclear FKBP is sufficient to suppress the loss of silencing at the rDNA locus. rDNA silencing was monitored by RT-PCR as shown in Figure 4d. Equivalent amounts of RNA extracted from cells expressing full length (WT) Fpr4, or ΔN and ΔC Fpr4 derivatives were analyzed. Lanes 1, 2, 3 and 4 show products amplified from cells transformed with the vector alone, or from cells expressing full length (WT) Fpr4, the N-terminal histone chaperone domain (ΔC) and the C-terminal PPIase domain (ΔN), respectively. Quantitative analyses are summarized in Table 1. (**d**) Model describing the effect that the expression of full length Fpr4 or of Fpr4 domains has on silencing at the rDNA locus in Δ*fpr4* cells.

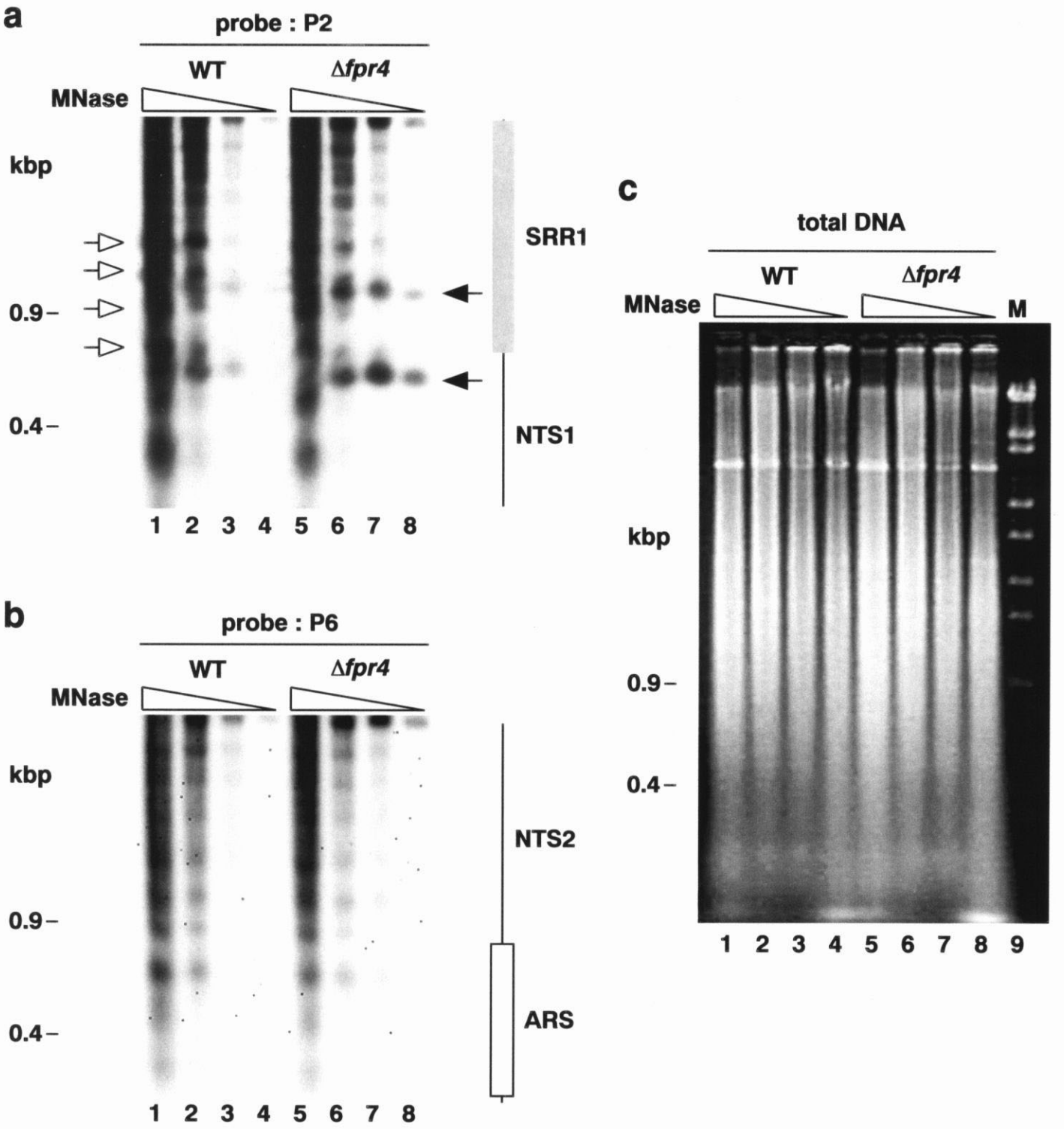


Figure 15: Endogenous nuclear FKBPs alter chromatin structure at the rDNA locus.
(**a**) Alteration of the chromatin structure at the NTS1 region of the rDNA locus. Isolated nuclei of wild type or Δ*fpr4* cells were treated with MNase. The extracted genomic DNA was digested with *Eco*RI, separated by 1.5 % agarose electrophoresis and analyzed by Southern blot analysis using the P2 probe that encodes NTS1 of the rDNA locus. Lanes 1-4 and 5-8 represent results of wild type or Δ*fpr4*, respectively. The amounts of MNase used are 10, 3, 1 or 0 units for (lanes 1, 5), (lanes 2, 6), (lanes 3, 7) or (lanes 4, 8), respectively. Lane 9 contains λ-DNA digested with *Eco*T14I and serves as the molecular weight marker. Open and closed arrows indicate wild type- and Δ*fpr4*-sensitive sites, respectively. SRR1 is the SIR2-responsive region 1, named by Fritze *et al.*38, who originally used the probes P2 and P6 for NTS1 and NTS2, respectively. (**b**) Alteration of the chromatin structure at NTS2 region of the rDNA locus was not observed. The same membrane of panel **a** was re-hybridized using probe P6 for NTS2. The lane contents and the method used are as described in **a**. (**c**) The total digested DNA stained by EtBr before blotting. Lanes 1 - 8 are the same as described in **a**, and lane 9 contains the λ–DNA digested marker.

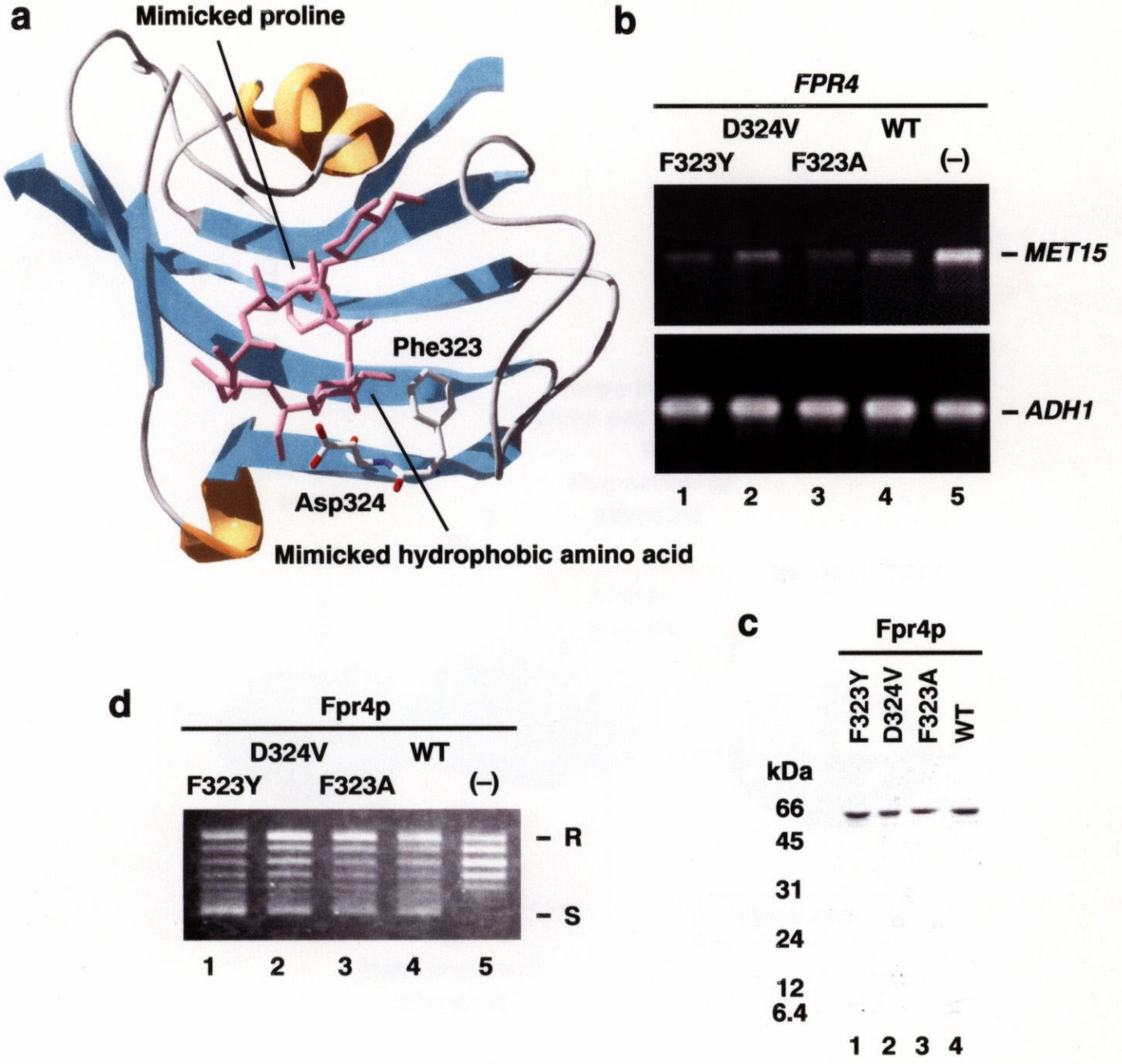


Figure 16: Regulation of rDNA silencing by the C-terminal PPIase pocket *in vivo*.
(a) Positions of mutated amino acids are indicated on the tertiary structure54-56 of the FKBP domain-FK506 (a FKBP PPIase substrate analogue) complex. The coordinates for the tertiary structure were obtained from the NCBI protein database (1FKF) and were visualized by using the SwissPdbViewer program (URL: http://kr.expasy.org/spdbv/). The ribbon structure indicates the FKBP domain, and the FK506 (shown in purple) molecule is bound to the PPIase catalytic pocket. The side chains of mutated amino acids are indicated as Phe323 and Asp324. FKBP isomerizes imide bonds between hydrophobic amino acids and proline residues. Since the side chain of Phe323 interacts with a hydrophobic amino acid analogue of FK506, this residue is important for substrate recognition. (**b**) Induction of hyper-silencing by two mutations affecting Phe323. RT-PCR analyses were performed for Δ*fpr4* cells carrying a single-copy vector expressing WT Fpr4 or the Phe323Tyr (F323Y), Asp324Val (D324V) or Phe323Ala (F323A) mutant proteins. rDNA silencing was assayed by the expression of *MET15* integrated at the rDNA locus. Expression of *ADH1* serves as a control for normalization. Lanes 1, 2, and 3, and 4 show the effect of the F323Y, D324V, and F323A point mutants and WT Fpr4 on silencing, respectively; lane 5, control strain with vector alone. Quantitative analyses are summarized in Table 1. (**c**) Purity of WT and point mutated Fpr4 proteins. Proteins (0.2 μg) were analyzed on 12 % SDS-polyacrylamide gels and stained by Coomassie brilliant blue. Lanes 1, 2, 3 and 4 represent the purified F323Y, D324V, F323A and WT proteins, respectively. (**d**) Histone chaperone activities of point-mutated nuclear FKBPs. After the nucleosome assembly reaction, the DNA was extracted and analyzed by 1 % agarose gel electrophoresis. Lanes 1, 2, 3, 4 and 5 show the F323Y, D324V, F323A, and WT proteins and reactions with buffer alone (14 pmol), respectively. R, relaxed and S, supercoiled DNA.

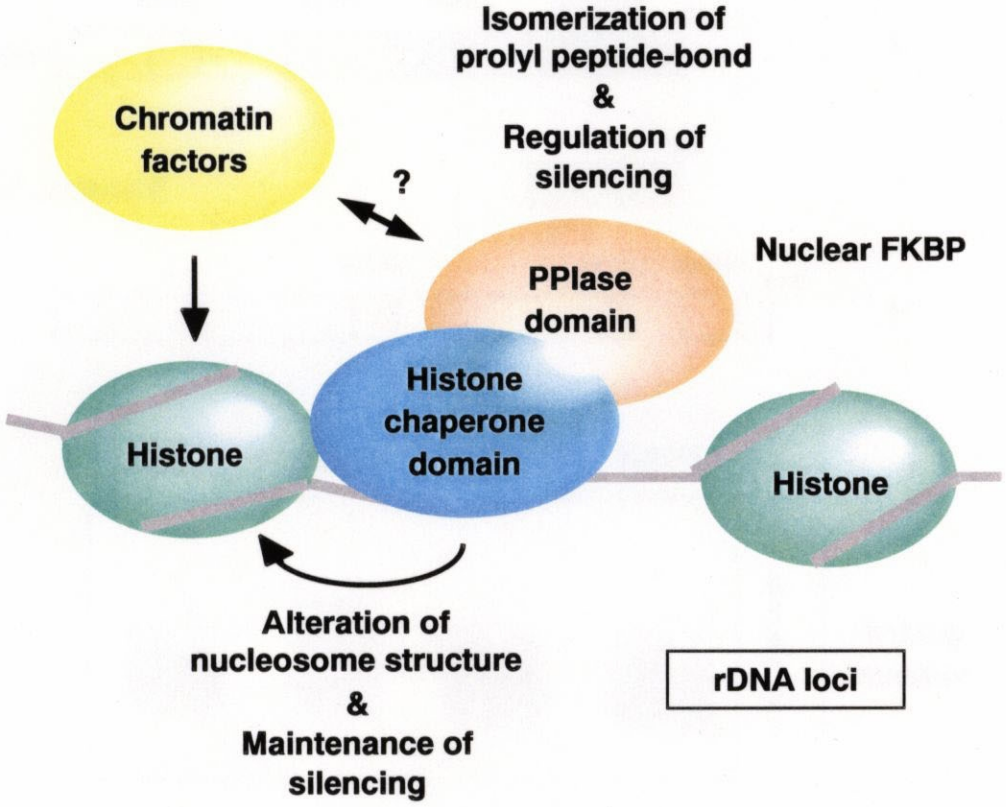


Figure 17: Model for a molecular network of transcriptional regulation centered on nuclear FKBP.

Nuclear FKBP effects rDNA silencing through its N-terminal histone chaperone domain. The C-terminal PPIase domain is not essential but plays a regulatory role in rDNA silencing *in vivo*. As described in the Discussion, other chromatin factor(s), silencing factors, histone deacetylases, ATPases, transcription fators, proline-rich factors, etc. may be the putative targets of the C-terminal PPIase domain. Cooperative action between the N-terminal histone chaperone domain and the C-terminal PPIase domain would organize chromatin efficiently *in vivo*. We propose that nuclear FKBP is a novel type of chromatin factor that consists of a histone chaperone domain and a PPIase domain.

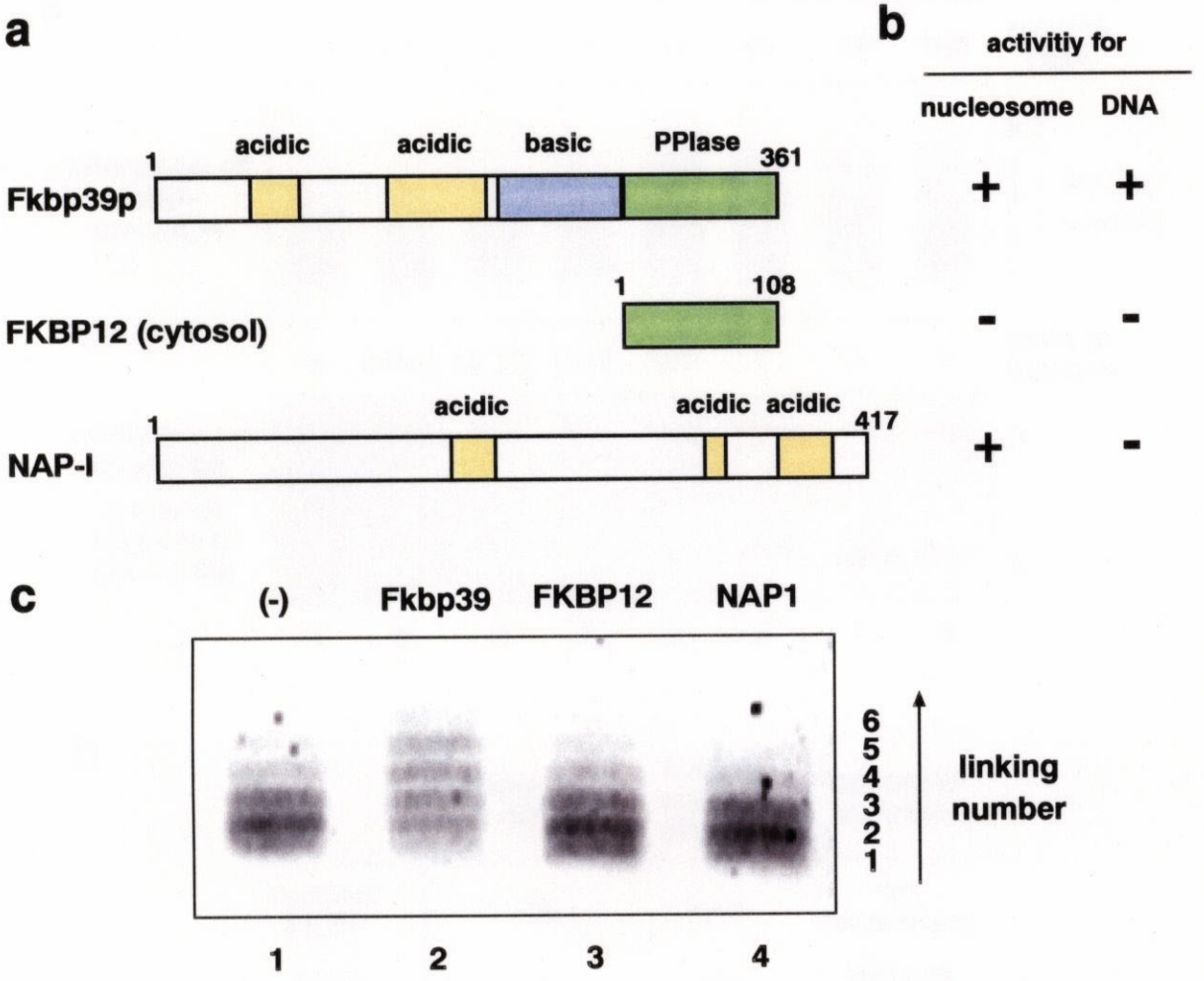
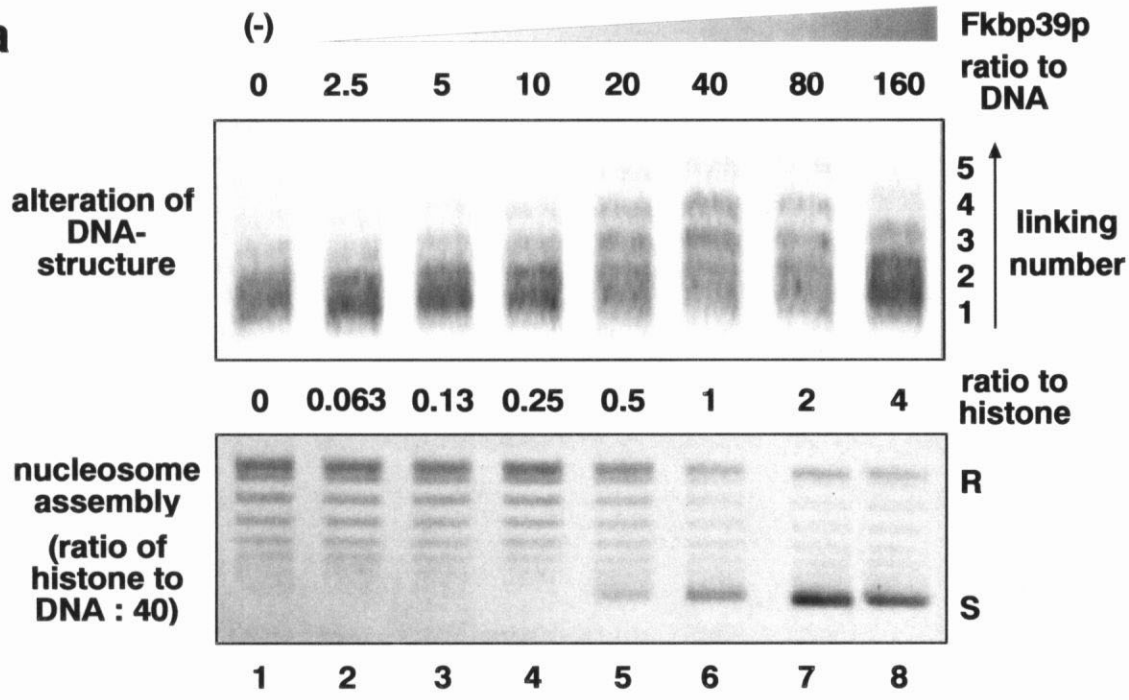


Figure 18: Alteration DNA structure by nuclear FKBP.
Nuclear FKBP alters DNA structure too. Nuclear alters the linking number of circular DNA. (**a**) Comparison of the primary structures of SpFkbp39p, cytosolic FKBP and NAP-I. Numbers indicate amino acid positions. The acidic/basic regions of SpFkbp39p were originally described by Himukai et al. (**b**) Comparison of the activities of SpFkbp39p, cytosolic FKBP and NAP-I for nuclesome and DNA. (**c**) Alteration of DNA structure as determined by the supercoiling assay. DNA was extracted and analyzed by 1 % agarose gel electrophoresis. Lanes 1, 2, 3 and 4 show reactions with buffer alone, the activity of SpFkbp39p, FKBP12, NAP-I ,respectively. Linking number is indicated.



b

	molar ratio to ccDNA	linking number altered	essential cofactor
nuclear FKBP	40	1 - 3	not necessary
core histone	40	>10	histone chaperone

Figure 19: Alteration DNA structure and histone chaperone activities by FKBP.
Alteration of DNA correlates with nucleosome assembly by nuclear FKBP.
(**a**) Comparison of dose curves of nucleosome assembly and alteration DNA structure by SpFkbp39p. Lanes 1, 2, 3, 4, 5, 6, 7 and 8 show reactions with 0, 0.13, 0.25, 0.5, 1, 2, 4, 8 nmol of SpFkbp39p, respectively. (**b**) Comparison of SpFkbp39p and core histone for alteration of DNA structure.

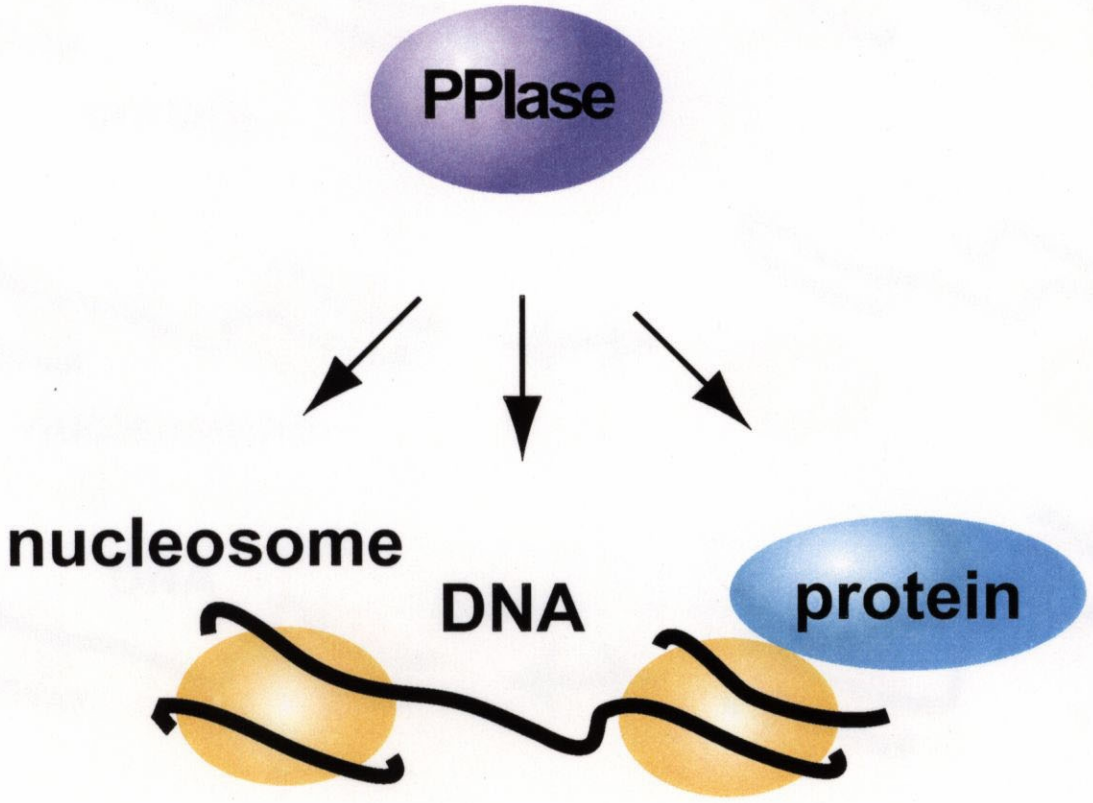


Figure 20 Model of chromatin structural change by PPIase
Nuclear FKBP alters the three types of structures, nucleosome, DNA and protein. These activities should efficiently alter chromatin structure.

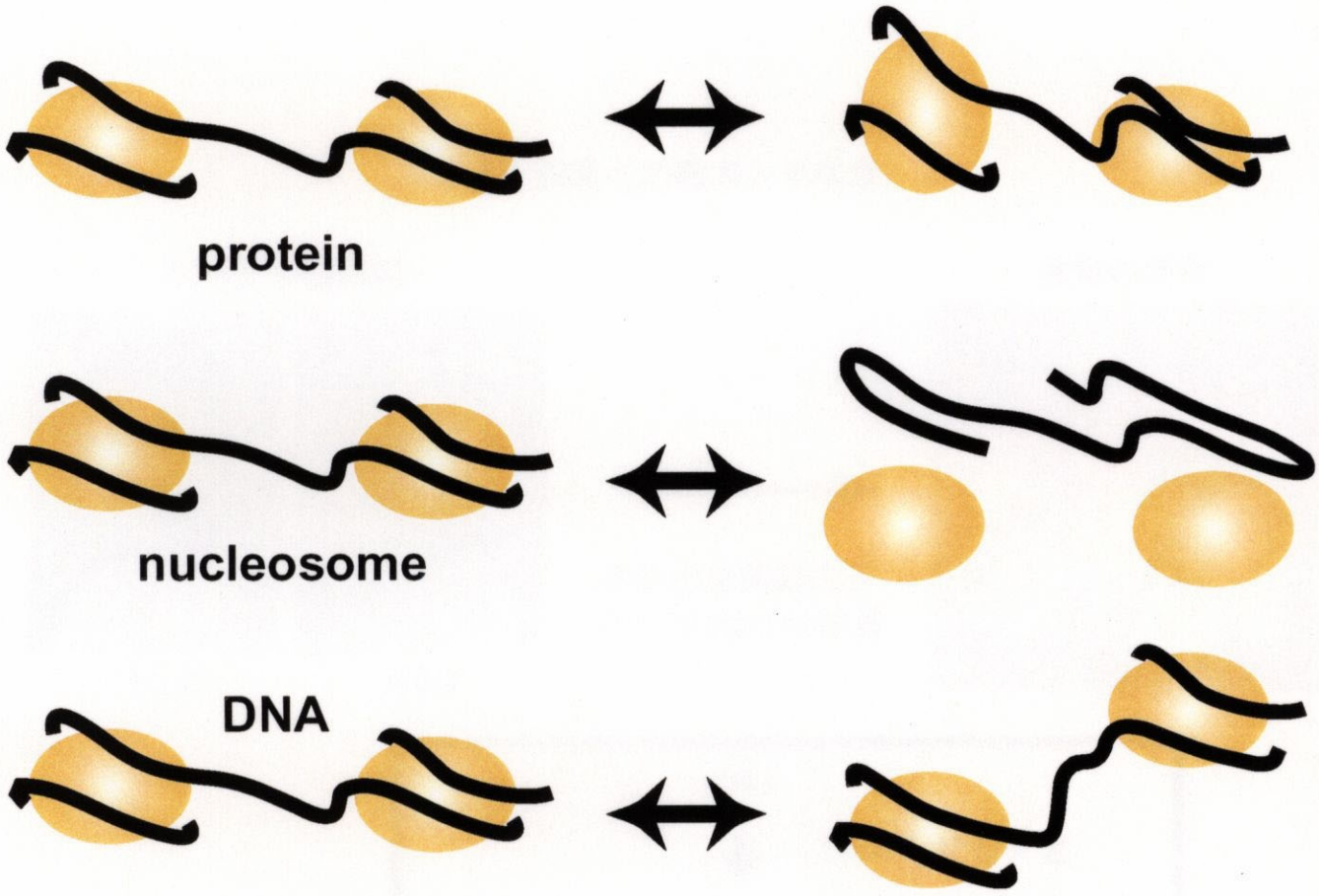


Figure 21 Structural changes of chromatin
Major components of chromatin are DNA and proteins.
Nucleosome, a minimum unit of chromatin, is composed of histone and DNA.
Nuclear FKBP alters the structures of these three types of components.
Therefore, nuclear FKBP would efficiently alter chromatin structures.

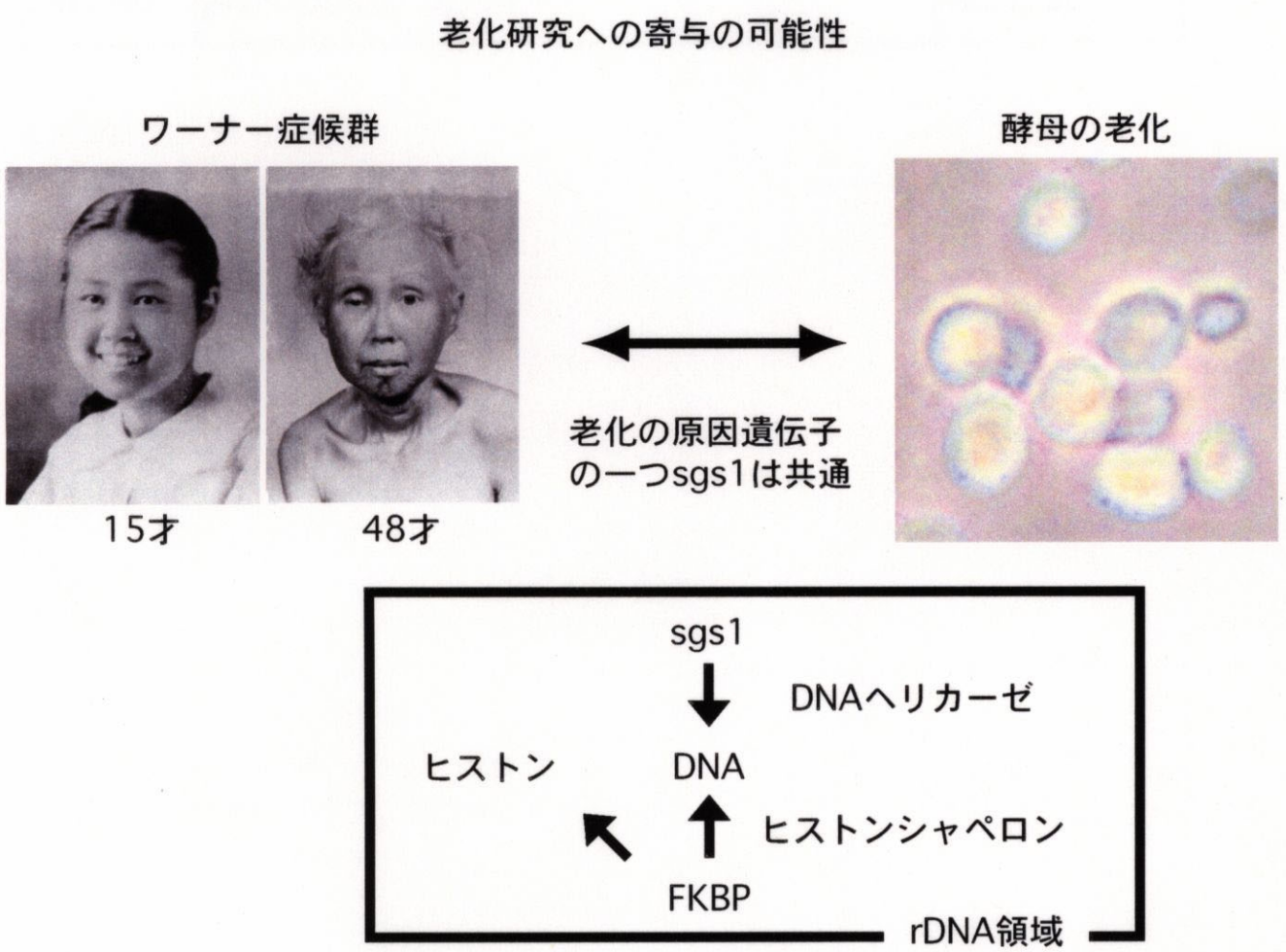


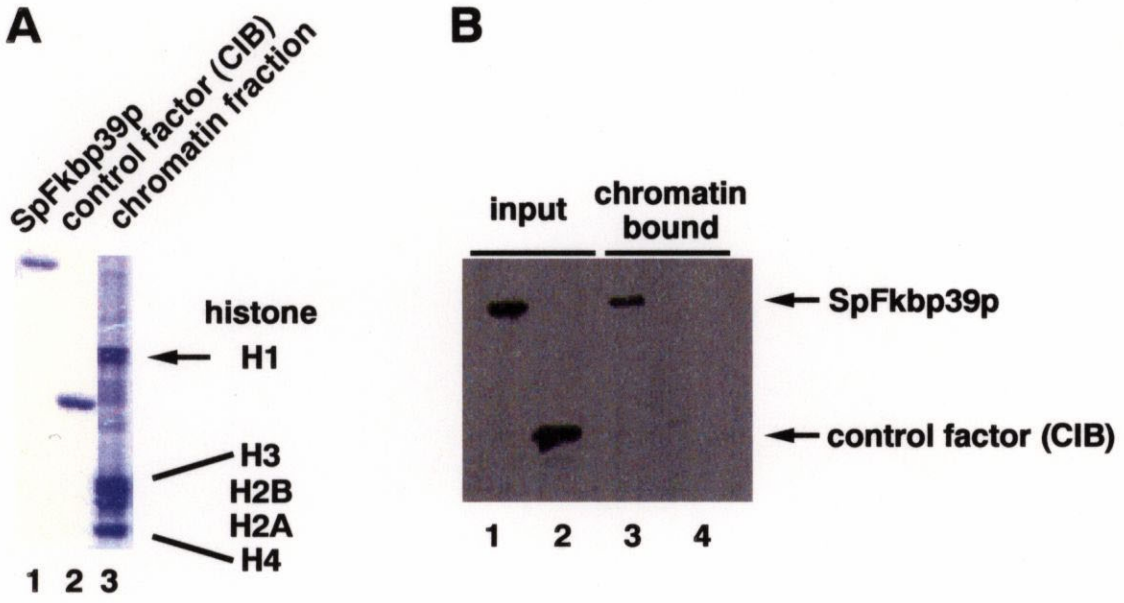
Fig. 22 Perspective of nuclear FKBP for aging study

Supplementary Table 1: Yeast strains used in this study

Strain	Genotype	Reference
JS237	*MATα his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 RDN1::Ty1-MET15*	Smith, *et al.*[36]
Δfpr4	*fpr4*:: *LEU2* (JS237)	This study
FPR4-Flag	*fpr4::FPR4*-Flag (JS237)	This study

Supplementary Table 2: Primers used for analyses of nuclear FKBP in yeast cells

Name	Sequence	Experiment
Primer 1	5'-TGA AAA TTT TAG CTG GTG CAT CGA AAA GAG AAG GGC ATT GTC GAG GTC GAC GGT ATC-3'	*fpr4* disruption
Primer 2	5'-TAT TAT AGA TAC ATA TAT CAA TAC GTA TGC ATT AAG GAC CCG CTC TAG AAC TAG TGG ATC-3'	
Primer 3	5'-TTA TCT CAA ACC CGA GGC AG -3'	PCR analysis for *fpr4* disruption
Primer 4	5'-TAG CTG GGC GGC TAT ATC AC -3'	
Primer 5	5'-ATG CCA TCT CAT TTC GAT ACT -3'	RT-PCR analysis of *MET15* mRNA
Primer 6	5'-CAA AAG ATT GCT GGA AGT CTG C -3'	
Primer 7	5'-ACC AAA TGC CGA CAA GGA AA -3'	Quantitative RT-PCR analysis of *MET15* mRNA
Primer 8	5'-GTT AGA GGC AAG CTT TAA ATT GTC AA -3'	
NTS1 forward	5'-TGC GGA AAA AAC TGC TCC AT-3'	ChIP analysis
NTS1 reverse	5'-AAG CTT CCC GAG CGT GAA-3'	
NTS2 forward	5'-AAT ACT GCC GCC GAC ATT CT-3'	
NTS2 reverse	5'-CGG ATG CGG GCG ATA AT-3'	
25S forward	5'-GGA CGG TGG CCA TGG AA-3'	
25S reverse	5'-CAT TCG GCC GGT GAG TTG-3'	
18S forward	5'-TGG TGC ATG GCC GTT CTT A-3'	
18S reverse	5'-GGT CTC GTT CGT TAT CGC AAT T-3'	



Supplement figure

A **Component of chromatin fraction. Chromatin fraction includes histones H1, H2A, H2B, H3 and H4 as major components (lane 3). Lanes 1 and 2 indicate the purities of SpFkbp39p and control factor (CCG1 interacting factor B: CIB, Padmanabhan, B., et al., *Acta Crystallogr D* (2000) 56, 1479-1481) proteins, respectively. Proteins were detected by CBB-staining.**

B **Binding of SpFkbp39p to chromatin fraction *in vitro*. The input proteins (20 pmol) were mixed with chromatin fraction in 500 ml of buffer A (20 mM HepesK, 150 mM NaCl, 10 mM $MgCl_2$, 10 % glycerol, 2 mM b-mercaptoethanol, 0.1 % NP-40), incubated at 4 ^{o}C 1hr and washed three times with 1 ml of buffer A. The bound fraction was separated in SDS-PAGE and analyzed by western blot analysis using an anti-his-tag antibody. Lanes 1 and 2 indicate the input proteins. Lanes 3 and 4 indicate chromatin-bound fractions. Lanes (1,3) and (2,4) indicate SpFkbp39p and control factor (CIB) proteins, respectively.**