Genomics & Informatics Vol. 4(1) 11-15, March 2006

Identification of hRad21-Binding Sites in Human Chromosome

Chur Chin and Byung Seon Chung*

Department of Biochemistry and Molecular Biology, College of Medicine, Pusan National University, Pusan 602-739, Korea

Abstract

The aim of this study is to identify hRad21-binding sites in human chromosome, the core component of cohesin complex that held sister chromatids together. After chromatin immunoprecipitation with an hRad21 antibody, it was cloned the recovered DNA and sequenced 30 independent clones. Among them, 20 clones (67%) contained repetitive elements including short interspersed transposable elements (SINE or Alu elements), long terminal repeat (LTR) and long interspersed transposable elements (LINE), fourteen of these twenty (70%) repeats clones had Alu elements, which could be categorized as the old and the young Alu Subfamily, eleven of the fourteen (73%) Alu elements belonged to the old Alu Subfamily, and only three Alu elements were categorized as young Alu subfamily. There is no CpG island within these selected clones. Association of hRad21 with Alu was confirmed by chromatin immunoprecipitation-PCR using conserved Alu primers. The primers were designed in the flanking region of Alu, and the specific Alu element was shown in the selected clone. From these experiments, it was demonstrated that hRad21 could bind to SINE, LTRs, and LINE as well as Alu.

Keywords: hRad21 cohesin repeated element, Alu, SINE, LINE, LTR, ChIP, sequence

Introduction

The linkage of sister chromatids, which is provided by a multi-subunit complex called cohesion (Riedel *et al.*, 2004) remains until every chromosome achieves amphitetic attachment, At the onset of anaphase, sister cohesion is eliminated by separase, a protease that cleaves the Scc1 subunit of the cohesion ring (Cairns, 2003) During DNA replication, cohesion molecules are laid down along the chromosome and their function is to hold sister chromatids together until anaphase. It is thought that cohesin forms a ring around the two sister chromatids, with Scc1 and Scc3 bridging the globular heads of SMC1 and SMC3. In human cells, a remodeling complex containing the ATPase SNF2h was found to co-purify with the Scc1 and SMC subunits, presumably due to a direct interaction between cohesin and this SNF2h complex. These results suggest that the remodeling complex might be required to recruit cohesion (Riedel *et al.*, 2004).

In addition, cohesin, which holds Sister chromatids together after DNA replication, has been known to be involved in both the repair of double strand breaks by homologous recombination and the intra S phase checkpoint. Phosphorylation of SMC1 is an important downstream signal required for mediating the intra S phase checkpoint and ether responses to DNA damage. Recently, it is known that Rad21 is one of the major cohesin subunits that holds sister chromatids together until anaphase when proteolytic cleavage by separase, a caspase like enzyme, allows chromosomal separation (Lehmann, 2005). In addition, Rad21 appears during apoptosis induced by diverse stimuli (Pati *et al.*, 2002). And nuclear phosphoprotein Rad21 is required for appropriate chromosomal cohesion during the mitotic cell cycle and double strand breaks repair after DNA damage (Strom *et al.*, 2005).

In this study, cloning and sequencing the ChIP products have been done to manifest the cohesin binding sites (Hakimi *et al.*, 2002).

Materials and Methods

Cell lines and cultural conditions

Human chronic myeloid leukemia K562 cells were grown in RPMI medium (Gibco, Life Technologies, Inc.) containing 10% fetal bovine serum (Gibco), streptomycin (100 μg /ml), and penicillin (100 units) in 4 plastic tissue culture plates in a humidified condition containing 5% CO2 at 37°C. The cells were grown to a density of 1×10^7 cells per dish before being harvested for 37% formaldehyde cross-linking experiments. The cell line was obtained from the American Type Culture Collection (CCL 243™).

*Corresponding author: E-mail chungbs@pusan.ac.kr,
Tel +82-051-240-7737, Fax +82-051-248-1118
Accepted 13 February 2006

Chromatin immunoprecipitation

K562 cells were treated with 37% formaldehyde for 10minutes incubation at 37°C to cross-link histones to DNA. After washing with cold phosphate-buffered saline containing 1 μg/ml pepstatin A and 1 mM phenylmethyl sulfonyl fluoride (PMSF), the cell pellets were resuspended in 300 μℓ of lysis buffer [150 mM NaCl, 25 mM Tris HCl (PH 7.5), 5 mM EDTA, 1% Triton X-100, 0,1% SDS, 0,5% sodium deoxycholate] and sonicated for 2×7 seconds in ice cold environment. The lysate (400 μℓ) was then divided into the fraction: One fraction (200 μℓ) was diluted with 18 ml ChIP dilution buffer [0.01% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris HCl, (PH 8.1), 150 mM NaCl], and other fraction (200 μℓf) was used for nonspecific background. The lysate was incubated with 2 μℓ of anti hRad21 antibody (Bethyl) at 4°C overnight or incubated with mouse IgG antibody (Abcam) at 4°C as a negative control. To collect the immunoprecipitated complexes, herring testis DNA/Protein A Agarose-50% surry beads were added and incubated for 1 hour at 4°C. After washing, the beads were eluted to 500 μℓ with elution buffer (1% SDS, 0.1 MNaHCO3). The DNAs cross-linked With histone were then reversed by heating the sample at 65°C for 4 h. The de-crosslinked DNAs were extracted by the phenol/chlorform method, ethanol-precipitated, and resuspended in 16 μℓ of distilled water. There was a total of four individual chromatin immunoprecipitations in the K562 cell line to obtain DNA enriched with anti-chyphone hRad21 antibody for cloning purposes (Yutaka *et al.*, 2003).

Cloning of hRad21-enriched DNA fragments

The ChIP products from K562 cells were treated with Klenow polymerase to fill the gaps into the making blunt ends and were ethanol-precipitated, then cloned into the zero blunt vector (Invitrogen). To optimize cloning efficiency, molar ratio of insert: vector has been keeping as 10:1. This ligation solution was stayed at room temperature overnight and could be gotten 30 colonies by transforming the ligation reaction into Top10 One shot competent E coli cells and cultured in S.O.C growth medium overnight.

Colony PCR

ChIP Products from K562 Cell were used to confirm the correct insertion of the DNA into the vector using the M13 primers as follows.

M13 forward primer, GTAAAACGACGGCCAG
M13 reverse primer, CAGGAAACAGCTATGAC

Colonies having inserts were sequenced at the core sequencing facility.

ChiP PCR

ChIP products from K562 were used for confirmation PCR using the oligonucleotide Primers as follows.

Alu18F, GCCTAGGGAGGCCTGTAAAC
Alu18R, GTCCATGCTGCTGTTCTCAT
p21F, GGTGTCTAGGTGCTCCAGGT
p21R, GCACTCTCCAGGAGGACACA
MLH1F, OTfGCTfCTTlfGGGCGTCAT
MLH1R, GGCTfGTGTGCCTCTGCTGA

The PCR products were visualized by agarose gel electrophoresis.

To identify genes bind to hRad21, ChIP using an anti hRad21 antibody is performed and cloned the immunop recipitated DNA. To confirm the binding sites, PCR confirmation of ChIP using anti hRad21 antibody was done.

Result

To identify genes linked to hRad21, ChIP using an anti hRad21 antibody was performed and cloned the immuno precipitated DNA.

Cell pellets were sonicated several times in various inputs and amplitudes. As optimal condition, the showed homogenous, about 500 bp sizeed nucleosomal particles were gained. As a first step toward reconstitution of cohesin activity *in vitro*, the minimal size of chromatin fragments that would be bound to cohesin in living cells was estimated. Same parameter has not been determined, as relevant ChIP data on cohesin-binding sites available have a resolution of no finer than 1 kb (Glynn *et al.*, 2004).

In addition, these fragments were inserted into zero blunt vector using Zero Blunt PCR Cloning Kit which was transformed to One Shot Top 10 chemically competent E-coli competent cells and cultured. After that 30 clones were randomly selected (Table 1), sequenced and characterized using REPBASE (girinst.org/) and BLAT (genome.ucsc.edu/) searches. It was revealed that the primer sets which had been derived from the selected clone (clone 1 8) was originated from that selected clone using PCR technique (Fig. 1 and Fig. 2). Twenty of thirty clones (67%) contained repeated elements of the human genome. Among them, fourteen of the twenty repeated clones (70%) had Alu elements that were categorized as the old and the young Alu subfamily. Eleven of fourteen (78%) AlU elements belonged to the old Alu Subfamily, and only three Alu elements were categorized as young Alu subfamily. All Alu elements were in or under 200 bp.

However, some of sequences could begin or end in the middle of the elements, and the range of Alu element length was 100-150 bp. Considering that AlU elements from about 10% of the human genome, these results Indicate significant enrichment of Alu elements, suggesting high density of chromatin remodeling complexes in these retroelements.

One clone (MIR) related to a short interspersed transposable element (SINE) other than Alu was obtained. Another clone (LIMEC) could be classified as a Long Interspersed Transposable Element (LINE) which is belonged to subclass2 retroelement. Four additional clones were belonged to subclass3 retroelement named as Long Terminal Repeats (LTR).

There was no CpG island within these selected clones (data not shown). (www.med.ualberta.ca/tutorials/synthgene/doc/cpgplot./)

Colony PCR was done to confirm that the blunted end products from ChIP were inserted into near EcoRI cutting sites on pCR blunt vector, 500 bp sized particles were shown on UV gel transillumination. These tests have been repeated in each 30 selected colonies. After that, the products were sequenced at the were sequenced facility.

To test this hypothesis, one Alu clone of 30 clones was selected and prepare primer sets encompassing the Alu elements. The primer was located in the flanking region of the specific Alu element in the selected clone. ChIP-PCR by these primers are shown in Fig. 1. DNA of clone 18 combined with hRad21 antibody was found, suggesting that clone 18 might be the binding site of hRad21 antibody.

Clone 18(Alu Sq)

AAGACTGATTCGCCAGCTATTTAGGTGACGCGTTAGAATACTCAAGCTATGCATCAAGCTTG
GTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCAGG**GTAATTTATA**
AAGGAAACAGGTTTAATTGACTCACAGCTCTGCATGCCTAGGGAGGCCTGTAAACA
GGAAATTTACAATTGTGGCAGAAGGGGAAGCAAACACATCTTTCTTCTCAGCTCAG
ACAGGATCTGAGCTGAGATGGCAGGAAGGAGAACTGCTGAGCAAAGGAGAGAAAA
GCCCCTTATAAAACCATCAAATCTCCTGAGAGCTCATTTACTATCATGAGAACAGC
AGCATGGACCCTGAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTA
GAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGT
CGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGC
CAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTAT
ACGTACGGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGAT
GTACAGAGTGATATTATTGACACGCCGGGGCGACGGATGGTGATCCCCCTGGCCAGTGCAC
GTCTGCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGC
TGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGTGG
CTGAGTAAA

Fig. 1. A profile of one (clone 18) of 30 selected clones. Bold characters reveals the Alu sequence.

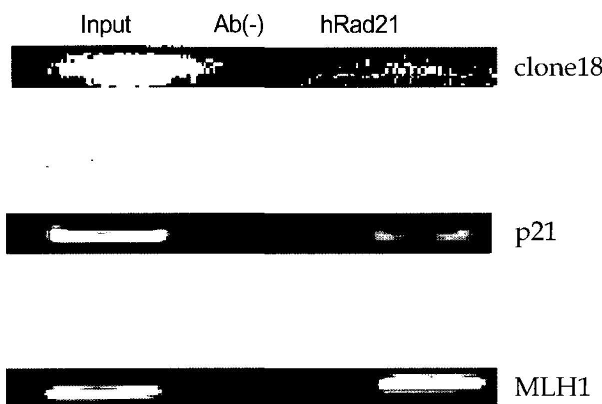

Fig. 2. Confirmation of hRad21 targets. Using ChIP-PCR DNA *of clone 18 combinewith* hRad21 antibody was immunoprecipitation and detected by PCR. p21 and MLH1 promoter regions were used for control. p21 showed a low degree of hRad21, whereas MLH1 showed a high degree of hRad21.

Discussion

It is important to identify the coheSin binding sites in human genome to explore what is happening when DNA damage is occured nearby (Strem *et al.*, 2005). In this study, Alu elements were identified as major seats for cohesin using chromatin immunoprecipitation. It was

Table 1. Identification of DNA fragments enriched for hRad21

Clone	Site (bp)	GC content	Locus	Gene	Location
1	428	50	10q25	MIR (SINB)	94,537,772-94,538,299
2	159	50	22q12	AluY	35,596,737-35,596,875
3	109	49	14q21	Alu	49,506,171-49,506,279
4	504	50	5p22	AluSp	37,184,041-37,184,544
5	140	47	17q11	Alusq	25,396,420-25,396,567
6	186	45	10q11	Alus	43,195,656-43,195,841
7	186	44	10q11	Alusq	43,185,656,43,195,841
8	146	47	17q11	Alus	25,396,422-25,396,567
9	127	48	19p15	Alus	8,445,733-8,445,959
10	159	49	22a12	AluY	35,596,737-35,596,875
11	656	46	1p55	THE1 (LTR)	48,285,298-48,285,933
I2	125	54	5p15	AluYbsal	7,871,628-7,871,752
13	120	44	15q54	LIMEC (LINE)	112,548,698-112,548,817
14	558	50	1q52	MLT1 (LTR)	201,993,238-201,993,775
I5	107	42	1q21	Alujo	103,747,061-103,747,167
I6	487	48	20p11	LTR7B	18,445,431-18,445,917
17	257	45	5q22	THE1C (LTR)	138,647,570-138,647,806
I8	585	46	10p11	AluSc	38,758,880-38,759,262
19	515	50	15q12	AluS	30,073,354-30,073,666
20	282	48	17q12	AluSq	25,393142-25393423
21	254	46	17p15	unknown	2,141,495,2,141,728
22	587	52	15q55	unknown	101,182,859-101,183,245
23	469	44	20a15	unknown	42,389,020-42,389,478
24	121	40	1q12	unknown	220,074,430-220,074,550
25	159	46	22q12	unknown	35,596,737-35,596,875
26	125	49	5q22	unknown	135,560,262-135,560,384
27	260	49	21q22	unknown	42,600,127-42,600,386
28	120	46	1p22	unknown	91,719,727-91,719,846
29	404	60	6q22	unknown	116,635,531-116,635,934
30	149	40	18q25	unknown	74,719,689-74,719,837

found a correlation between modification of histone tails and association of the cohesin complex with chromatin. In this study, parts of human genome binding to hRad21 of cohesin compiex are identified (Fig. 3).

The SNF2h-NuRD-cohesin complex, which is a chromatin remodeling complex, was recently reported to specifically associate with some Alu elements, accompanied by histone H3-Lys 4 methylation. In those studies, the binding of the cohesin complex to chromatin around Alu elements could be increased by inhibition of DNA methylation.

Among 14 Alu clones recovered here, the frequency of old Alu elements (11clones, 78%) and young Alu elements (3 clones, 12%) and is consistent with the prevalence of old and young Alu elements in the human genome. This study shows that hRad21 is characteristic of both old and young Alu subfamilies. Alu repeats represent the most frequent repetitive element in the human genome.

It was found another repetitive elements, LINE, in one clone (5%). LINE elements comprise about 5% of human genome and are concentrated in A-T rich regions. In this study LINE elements were less specifically targeted than Alu elements, although the number of clones overall in this study was small. In addition, 1 SINE (5%) and 4

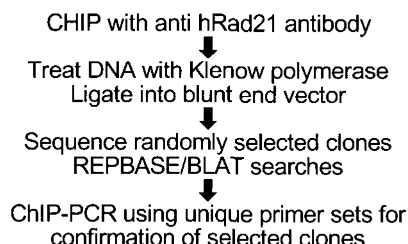

Fig. 3. A procedure for the identification of DNA fragments enriched in hRad21.

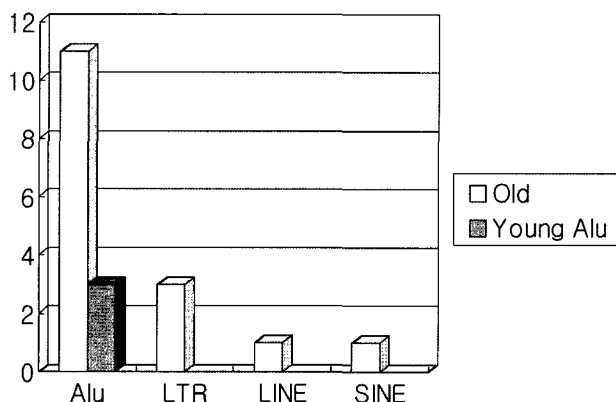

Fig. 4. Alu elements are the main binding sites of hRad21.

LTRs (20%) was also found (Fig. 4).

The chromatin remodeling complex, SNF2h-NuRD was recently reported to specifically associated with some Alu elements, accompanied by Histone Lysine methylation (Campbell *et al.*, 2002).

In this study it is revealed cohesin itself is bound to Alu elements and contribute to genomic stability with H3-Lysine methylation (Nasmyth and Haering., 2005). An obvious functional significance of these findings is to provide a molecular mechanism for the known silencing of Alu elements (Hogue *et al.*, 2002). This silencing is thought to be essential in reducing the mutational load associated with active Alu elements in dividing cells (Jessberger, 2002). During DNA replication, hRad21 is binding to the SMC heads, consisting of cohesin complex and recruits Scc3 to strengthen the structure at the Alu sequences of the two sister chromatids (Volkov, 2005)

References

Riedel, C.G., Gregan, J., Gruber, S., and Nasmyth, K. (2004). Is chromatin remodeling required to build sister chromatid cohesion? *Trends Biochem. Sci.* 8, 389-392.

Morrison, C., Vagnarelli, P., Sonoda, E., Takeda, S., and Earnshaw, W.C. (2003). Sister chromatid cohesion and genome stability in vertebrate cells. *Biochem. Soc. Trans.* 31, 263-265.

Cairns, B.R. (2003). Chromatin remodeling complexes: strength in diversity, precision through specialization. *Curr. Opin. Genet. Dev.* 15, 185-190.

Lehmann, A.R. (2005). The role of SMC proteins in the responses to DNA damage. *DNA Repair* 4, 309-314.

PaPi, M., Berdougo, E., Randall, C.L., Ganguly, S., and Jallepalli, P.V. (2005). MultiPle roles for separase auto-cleavage during the G2/M transition. *Nat. Cell Biol.* 7, 1029-1035.

Pati, D., Zhang, N., and Plon, S.E. (2002). Linking sister chromatid cohesion and apoptosis: role of Rad21. *Mol. Cell Biol.* 22, 8267-8277.

Strom. L. and Sjogren, C. (2005). DNA damage-induced cohesion. *Cell Cycle* 4, 536-539.

Hakimi, M.A., Bochar, D.A., Schmiesing, J.A., Dong, Y., Barak, O.G., Speicher, D.W., Yokomori, K., and Shiekhatter, R. (2002). A chromatin remodelling complex that loads cohesin onto human chromosomes. *Nature* 418, 994-998.

Xu, H., Beasley, M., Erschoor, S., Inselman, A., Handel, M.A., and Mckay, M.J. (2004). A new role for the mitotic Rad21/Sccl cohesin in meiotic chromosome cohesion and segregation in the mouse. EMBO J. 5, 378-384.

Kondo, Y. and Issa, J.P. (2003). Enrichment for histone H3

lysine methylation at Alu repeats in Human cells. *J. Biol. Chem.* 278, 27658-27662.

Glynn, E.F., Megee, P.C., Yu, H.G., Mistrot, C., Unal, E., Koshland, D.E., DeRish, J.L., and Gerton, J.L. (2004). Genome-wide mapping of the cohesin complex in the yeast Saccharomyces cerevisiae. *PLoS Biol.* 2, E259.

Mighell, A.J., Markham, A.F., and Robinson. P.A. (1997). Alu Sequences. *FEBS Lett.* 417, 1-5.

Presscott, L., Deininger, B., Batzer, M.A. (2002). Mammalian Retroelements., *Genome Res.* 12, 1455-1465.

Strom, L., Lindroos, H.B., Shirahige, K., and Sjogren, C. (2005). Postreplicative recruitment cohesion to double-strand breaks required for DNA repair. *Mol. Cell* 6, 1003-1015.

Huang, C.E., Milutinovich, M., Koshland, D. (2005). Rings, bracelet or snaps: fashionable alternatives for Smc complexes. *PhiloS. Trans R. Soc. Lond.* B. Biol. Sci. 360, 537-542.

Hirano, T. (2005). SMC proteins and chromosome mechanics: from bacteria to humans. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 360,507-514.

Uhlmann, F. (2004). The mechanism of sister chromatid cohesion. *Exp. Cell Res.* 296, 80-85.

Kagansky, A., Freeman, L., Lukyanov, D., and Strunnikov, A. (2004). Histone tail independent chromatin binding activity of recombinant cohesion holocompiex. *J. Biol. Chem.* 279, 3382-3388.

Campbell, J.L. and Cohen, F.O. (2002). Chromosome cohesion: ring around the sisters? *Trends. Biochem. Sci.* 27, 492-495.

Nasmyth, K. and Haering, C.H. (2005). The structure and function of SMC and kleisin complexes. *Annu. Rev. Biochem.* 74, 595-648.

Jessberger, R. (2002). The many functions of SMC proteins in chrmosome dynamics. *Nat. Rev. Mol. Cell Biol.* 3, 767-378.

Marston, A.L., Tham, W.H., Shah, H., and Amon, A. (2003). A gennome-wide screen identifies genes required for centromeric cohesion. *Science* 303, 1367-1370.

Hogue, M.T. and Ishikawa, F. (2002). Cohesin defects lead to premature sister chromatid separation, kinetochore dysfunction, and spindle-assembly checkpoint activation. *J. Biol. Chem.* 277, 42306-42314.

Jessberger, R. (2003). SMC proteins at the crossroads of diverse chromosomal Processes. *IUBMB Life* 55, 643-652.

Volkov, A., Mascarenhas, J., Andrei-Selmer, C., Ulrich, H.D., and Graumann, P.A. (2003). prokaryotic condensin/ cohesin-like complex can actively compact chromosomes from a single position on the nucleoid and binds to DNA as a ring-like structure. *Mol. Cell Biol.* 23, 5638-5650.

Jessberger, R. (2005). How to divorcer engaged chromsomes? *Mol. Cell Biol.* 25, 18-22.