

行政院國家科學委員會專題研究計畫 成果報告

著絲點蛋白 H 連結的著絲點 DNA 之特性分析(第 3 年) 研究成果報告(完整版)

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中文摘要：

著絲點蛋白 H 是位於著絲點 Kinetochores 內層的結構性蛋白。先前的研究顯示著絲點蛋白 H 有助於著絲點蛋白 A 和 C 聚集在著絲點上以形成有活性著絲點。然而，著絲點蛋白 H 如何幫助著絲點蛋白 A 和 C 聚集在著絲點上是值得探討的課題。我們假設著絲點蛋白 H 是藉由連結一特定的著絲點 DNA 而聚集著絲點蛋白 A 和 C 在著絲點上。為了研究著絲點蛋白 H 連結的著絲點 DNA，我們利用抗著絲點蛋白 H 抗體所抓取的染色質絲(也就是染色質絲免疫沉澱法)建立了一個小型染色質絲資料庫，叫做 CHIP-H。我們利用細胞外蛋白質-DNA 鍵結分析法篩選小型染色質絲資料庫 CHIP-H 菌株，其中有五個菌株的 DNA 出現較強的連結訊號，為了更加確立菌株 DNA 在細胞內著絲點蛋白 H 的連結能力，我們將這五個菌株的 DNA 送入細胞中，培養 48 小時，之後將細胞固定，用免疫螢光染色及原位螢光雜交分析，菌株 DNA 和細胞內著絲點蛋白連結的能力，結果顯示這五個菌株 DNA 確實具有與著絲點蛋白連結的能力。我們再次利用膠體滯留結合西方吸漬法更加證實這五個菌株 DNA 具有著絲點蛋白 H 連結的能力。除此之外，我們更進一步探討著絲點蛋白 H 連結 DNA 在整體基因組中的關係，因此我們利用篩選到的菌株 DNA 和已知的著絲點蛋白 A 連結之特定的著絲點 DNA 當作探針篩選 BAC DNAs，結果有三個 BAC DNAs 同時具有著絲點蛋白 A 連結之特定的著絲點 DNA 和著絲點蛋白 H 連結之特定的著絲點 DNA 的訊號。接下來，我們利用有系統地定序法定序這些候選的 BAC DNAs，雖然這些候選的 BAC DNAs 含有大量的重複性 DNA，比一般的定序需要耗費更多的時間，困難度也相當大。不過目前我們已完成定序這些候選 BAC DNAs 的限制酶片段，這些片段的序列也做了相互的比對及和基因資料庫的比對，有趣的是，著絲點蛋白 A 連結之特定的著絲點 DNA 和著絲點蛋白 H 連結之特定的著絲點 DNA 都和先前找到的著絲點衛星 DNA 有 80% 以上的相似度，這樣的證據顯示，著絲點蛋白 H 是藉由連結上著絲點 DNA，再幫助著絲點蛋白 A 和 C 聚集在著絲點 DNA 上以形成有活性著絲點。所以我們已完成這一計畫所要探討的主要目的了。另外，在執行計畫的三年中，我們已有 5 篇論文發表，兩篇已被接受，一篇已送出接受審查，兩篇論文正在撰寫中。

關鍵詞: 著絲點、著絲點蛋白 H 連結 DNA、染色質絲免疫沉澱

英文摘要:

Centromere protein H (CENP-H) is a constituted protein and located within the inner layer of Kinetochore. The previous studies showed that the CENP-H contributes gathering the CENP-A and -C together at the centromere to form active centromere. However, it is not clear how the CENP-H to help the CENP-A and -C gather at the centromere. We assumed that the CENP-H is associated with a specific centromeric DNA to recruit the CENP- A and C on the centromere. To study the CENP-H associating DNA, we used the anti-CENP- H antibodies to catch down the CENP-H associating chromatin filament (that is, the chromatin immuno-precipitation) and then constructed a mini-library, designated as CHIP-H. We obtained five positive clones by screening this mini-library with multi-well protein-DNA binding assay. Ex-vivo protein assay and gel-retardation combing western blot assay showed that the isolated clones have CENP-H binding activity. Furthermore, we screened the centromeric BAC DNA using these five clones and CENP-A associating DNA as probes for characterizing the genomic organization of CENP-H associating DNA. Three positive BAC clones obtained were further sequencing. Because the BAC DNA clones contain the significant repetitive DNA, it is not easy to complete sequence BAC DNA by shot-gun sequencing or second-generation sequencing. Even so, we sequenced three BAC DNAs by the systemically hierarchical sequencing. We had finished all sequences of subclones. We had compared all sequences of subclones each other and compared them with the nucleotides collected in GeneBank of NCBI. The sequences comparison result showed that the CENP-H associating DNA and CENP-A associating DNA shared more 80% identity to centromeric satellite II. These evidences indicated that the CENP-H recruited CENP-A and C through associating centromeric satellite II to form an active centromere. Therefore, we had achieved the main purpose what we want to explore in this project. During the term of carrying out this project, we had 5 papers published, two papers accepted in press, one paper submitted and two papers in preparation.

Key words: centromere, CENP-H associating DNA, chromatin immunoprecipitation

報告内容:

Introduction:

The centromere mediates normal mitotic and meiotic process including kinetochore assembly, spindle attachment, sister chromatid cohesion and subsequently proper separation of sister chromatids during mitotic anaphase or segregation of synaptic homologous chromosomes during meiosis I (Choo 1997). The centromeric function is mediated by the kinetochore, which is a proteinaceous structure that assembles onto the centromeric DNA (Cleveland et al. 2003; Amor et al. 2004). Therefore, the maintenance of centromere identity is critical importance, since the loss of a centromere identity results in the gain or loss of a chromosome (aneuploidy) (Hook 1985). However, it is not clear so far what determines the identity of the centromere and maintains its function?

An active prekinetochore loading on the centromeric DNA is the primary step of centromeric identity. The prekinetochore is mainly composed of CENP-A, -B, -C, -H, -I, and Mis12. The results of the CENP protein-depletion experiment indicated that CENP-H-I complex could assist the incorporation of the nascent CENP-A into centromere to form the prekinetochore (Nishihashi et al. 2002; Okada et al. 2006). The CENP-A is a key loader of the prekinetochore onto the centromeric DNA to determine the centromere identity (Sugata et al. 1999; Sugata et al. 2000). In contrast to the conservation of the protein components, the centromeric DNAs are highly variable among species (Kitagawa and Hieter 2001; Sullivan et al. 2001; Cleveland et al. 2003; Mellone and Allshire 2003). Several studies showed that the centromeric heterochromatin is mainly composed of the highly repeated satellite DNA in higher eukaryotes. Interestingly but ambiguously, how these conserved fundamental centromeric proteins assemble on the diverse centromeric heterochromatin. CENP-A, B, C and H had been found to associate with the centromeric DNA. However, what is the essential DNA sequence for the loading of the prekinetochore onto the centromere? It is not clear so far.

Specific Aims:

The CENP-H loading is essential for the efficient loading of the nascent CENP-A (Okada et al. 2006). These suggested that the loading of CENP-H onto centromeric chromatin play an important role in de novo forming centromere. The question is how the CENP-H loads to the centromeric chromatin? Therefore, our aims are to explore (1) the CENP-H associating centromeric DNA, (2) the spatial relationship of genomic organization between CENP-A and CENP-H associating DNA for explaining the mechanism of the loading of CENP-C onto the centromere, (3) the sufficiency of the CENP-A and CENP-H associating DNA together to form a centromere.

Methods:

Chromatin immunoprecipitation and construction of mini-library of CENP-H associating DNAs:

This protocol is mainly referred to Lo et al. 2001. About 10^7 Indian muntjac fibroblast cells were incubated in TBS (0.01M Tris-HCl, pH7.5, 3 mM CaCl₂, 2 mM MgCl₂ with 0.1 mM phenylmethylsulphonyl fluoride [PMSF] and proteinase inhibitors [complete, proteinase inhibitor cocktail tablet, Roche]) with 0.25% Tween 40 at 4°C on a roller stirrer for 2 hours before extruding the nuclei using 30 strokes with the “Tight” or “A” pestle on a Dounce homogenizer (Wheaton). Nuclei of Indian muntjac cells were separated from cytoplasmic debris by centrifugation at 1500 g for

20 min at 4°C through a 25%/50% discontinuous sucrose gradient. Oligonucleosomes are produced by digesting the nuclei with micrococcal nuclease (USB) in digestion buffer (0.32 M sucrose, 50mM Tris-HCl at pH7.5, 4 mM MgCl₂, 1mM CaCl₂, 0.1mM PMSF) at a concentration of 80 U/mg DNA at 37°C for 10 min. The supernatant contains mainly mononucleosomes. The pellet fraction is further processed by incubation with lysis buffer (1 mM Tris-HCl at pH7.5, 0.2 mM EDTA, 0.2 mM PMSF, and proteinase inhibitors) on ice for 1 hour. The final supernatant containing oligonucleosomes is then obtained by centrifugation at 15,000 g for 5 min at 4°C. The two supernatant fractions were pooled and precleared by the incubation with 1:1000 dilution of the preimmunized rabbit serum and 1% protein A-sepharose (Amersham-Pharmacia) at 4°C. After preclearing, the supernatant is obtained by centrifugation at 250 g for 5 min at 4°C. This fraction is used immediately for immunoprecipitation (input fraction). Equal volumes of the supernatant and incubation buffer (50 mM NaCl, 20 mM Tris-HCl at pH 7.5, 5 mM EDTA, 0.1 mM PMSF, and protease inhibitors) were incubated with 1:500 anti-CENP-H at 4°C overnight. The immune complexes were captured by incubating in 12.5% protein A-sepharose then washed extensively in a stepwise manner in buffer A (50mM Tris-HCl at pH7.5, 10 mM EDTA) containing 50, 100, and 150 mM NaCl. Bounded immune complexes were then eluted with 2 vol of 1% SDS. DNA (bound fraction) is extracted from the eluate by phenol/chloroform/isoamyl alcohol extraction. The extracted DNAs were blunted by Klenow fragment and introduced a nucleotide “A” to 3’-end. The treated DNA was ligated into pGEMT vector and the recombinant plasmids were used to transform XL1-Blue *Escherichia coli* competent cells. The transformation mixture was plated onto Luria–Bertani (LB) agar plates containing 100 µg/ml of ampicillin , 40 µg/ml of X-Gal, and 0.05 mmol/l IPTG to construct a CHIP-H mini-library.

Chromosome preparation:

The HeLa cells and the fibroblast cell lines of Indian muntjac (*Muntiacus muntjak vaginalis*) (male cell line, CCL-157, American Type Culture Collection) were grown in DMEM medium supplemented with 10% FBS, 1% penicillin and streptomycin, and 1% L-glutamine at 37°C, 5% CO₂ incubator. Metaphase chromosome spreads were prepared from the growing cells and fixed in 3:1 methanol: Acetic acid. The fixed cells were drop-splashed onto glass slide and stand at room temperature for two days aging. The detailed protocol for chromosome preparation has been described elsewhere (Li et al 2000).

Fluorescence in situ hybridization

The immunoprecipitated DNA was labeled with biotin by nick translation. The biotin –labeled DNA is hybridized to metaphase chromosomes. The biotin labeled probe is observed by means of incubating with Cy3-conjugated avidin, biotinylated anti-avidin and Cy3-conjugated avidin, sequentially. The metaphase chromosomes were counterstained with DAPI in antifade mounting medium (vector). Fluorescent signals were captured under an Olympus BX51 fluorescence microscope equipped with appropriate filter sets and a cooled CCD camera (Photometrics, Sensys). The images were normalized and enhanced using the FISH software (Applied Spectral Image, Israel), and processed in Photoshop (Adobe, San Jose, CA). The detailed FISH protocol had been established in our laboratory (Li et al. 2002).

Screening the CHIP-H mini-library by multi-well protein-DNA binding assay:

All inserts of constructs were amplified by PCR and labeled with biotin. Each biotin-labeled construct binds with the streptavidin-coating well in 96 multi-wells. Subsequently, each well was incubated with the nuclear extract of Indian muntjac. Immunoassay with anti CENP-H was performed.

Ex-vivo protein-DNA binding assay:

Briefly, 40% confluent HeLa cells were seeded onto 18x18mm coverslip in 35mm petri dish 24 hours before the transfection experiment. The next day, transfection mixture combining 1 μ g of DNA (the five CHIP-H clones) and 3 μ l of Fugene 6 transfection reagent (Roche) in 100 μ l of OptiMEM was added to 70% subconfluent HeLa cells in 2 ml of culture medium. Transfection medium was replaced with normal growth medium 16 hours after transfection. 48 hours after transfection, the transfected cells were fixed with 1% formaldehyde/1XPBS. After fixation, the fixed cells were treated with 0.2% Triton X-100 in KCM buffer for 15 min. Centromeric proteins were detected by incubating the anti-CENPs antibodies with fixed cells in KB buffer for 30 min at 37°C. Subsequently the FITC conjugated goat anti-rabbit antibodies was applied to fixed cells in KB buffer for 30 min at 37°C for visualizing the centromeric proteins. After immunofluorescent experiment, cells on the slide was immediately fixed in 10% formalin/KCM buffer (120mM KCl, 20 mM NaCl, 10 mM Tris-HCl pH 7.6, 0.1 % Triton X-100) for 10 min and then in 3:1 methanol/acetic acid for another 15 min. The following FISH experiment was carried out. Biotin-labelled CHIP-H DNA probes will be hybridized with the fixed cells that transfected with the CHIP-H DNA. After hybridization, the non-specific binding probes were washed out by 50% formamide/2XSSC at 37°C for 15 min. The transfected CHIP-H DNA will be observed by incubating with the AF568-conjugated streptavidin. Subsequently, samples were mounted in antifade mounting medium with DAPI (vector). The colocalizaing signals of immunofluorescence and FISH were analyzed using the confocal microscope (Zeiss LSM 510 META). The detailed procedure of simultaneous immunofluorescence and FISH was described in Li et al. 2002.

non-isotopic gel retardation:

Briefly, a non-isotopic DNA fragment with 250bp-size was incubated with the crude nuclear extract in 1X binding buffer for 20min at room temperature. After incubation, the incubated protein-DNA mixtures were run on 2% agarose at 50V on ice for 70 min. The agarose was performed with the regular western blot and the protein bands were detected by anti-CENP-A, CENP-B, CENP-H antibodies and visualized by chemiluminisence assay.

Dot blot hybridization:

The centromeric BAC DNAs were spotted in high density onto Hybond N+ filters with the Genetix Q-Bot (Genetix Ltd.). The filters were hybridized with p³² labeled probes prepared from CHIP-H DNA clones and CENP-A associating cervid centromeric DNA (cervid satellite II; Vafa et al. 1999). The conditions and procedure of filter hybridization were described elsewhere in detail (Li et al. 2000b). The clones with positive hybridization signals were picked up for further characterization.

Plasmid DNA and BAC DNA Sequencing

CHIP-H DNA clones were sequenced from the vector-specific primers (M13-F

and M13-R) by the automated sequencing. The automated sequencing was carried out using ABI Prism cycle sequencing and electrophoresed on an ABI377 system according to the manufacturer's instruction. For full sequencing candidate centromeric BAC DNAs, candidate centromeric BAC DNAs were first subcloned into smaller fragments generated by two different restriction enzymes. The vector-specific primers (M13-F and M13-R) and internal primers designed from the generated sequences were used for sequencing subclones with more than 1.5kb. The subclone with an insert > 2.0 kb and highly repetitive sequences was sequenced through the ExoIII-nested deletion clones. Briefly, the M13-F side of the insert of the subclone was cleaved with *HindIII* and *KpnI* at 37°C for one hour. A linearized DNA with a 5'-protruding and 3'-protruding end was digested from the 5'-end by ExonucleaseIII at 37°C (theoretically, the digestion rate is about 300bp/min). The nested deleted DNAs at three given digestion time intervals (2 mins/4mins/8 mins) were subcloned into pBluescript II SK(-) plasmid vectors. The exodeleted subclones with the appropriate insert size were picked up for further DNA sequencing from the M13-F end of the vector. The detailed protocol was based on the Erase-a-Base System (Promega) protocol. All the subcloning strategies were referred to *Molecular cloning* (Maniatis et al. 1982) and have been routinely performed in our laboratory.

DNA sequences analysis:

DNA sequences of CHIP-H clones and centromeric BAC DNA were aligned with GenBank database using the BLAST programs for searching the identified satellite DNAs elements. The monomer of repetitive sequences was analyzed by Tandem repeat finder software <http://tandem.bu.edu/trf/trf.html> (Benson 1999). Palindromic and mirror repeats were identified by self comparison using BLAST 2 program. The interspersed repeats and low complexity DNA sequences were screened using RepeatMasker program from <http://www.repeatmasker.org/>. The composition of sequences will be determined using nucleic acid statistics programs from Biological Workbench (<http://workbench.sdsc.edu/>).

Results and discussions:

1. Establishing a CENP-H-associating DNAs mini-library:

The CENP-H associating DNA was probed by the way of chromatin immunoprecipitation using CENP-H specific serum incubated with the isolated and sonicated the Indian muntjac nuclear. The immunoprecipitated CENP-H DNAs were identified by FISH. The FISH result showed that the immunoprecipitated DNAs localized on centromere regions of Indian muntjac (Figure 1). It suggested that the chromatin-immunoprecipitation method could successfully isolate the specific centromeric DNA associated with CENP-H from genomic DNA. The immunoprecipitated CENP-H DNAs were purified and were introduced into pGEMT vector to construct a mini-library. More than 300 constructs were obtained in this mini-library, designated as CHIP-H mini-library.

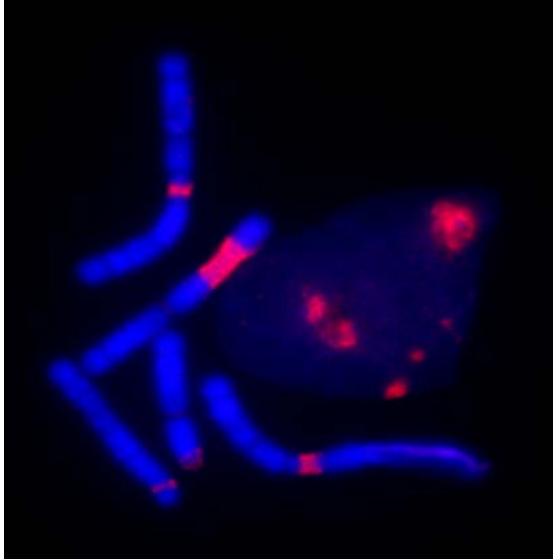


Fig 1: The FISH result showed that the CENP-H associating DNAs localized on centromere regions of Indian muntjac. The kinetochore binding DNA, which is immunoprecipitated by anti-CENP-H antibodies.

2. Screening the mini-library:

All constructs were assayed using the multi-well protein-DNA binding assay. There are five candidate clones with stronger positive signals in this assay.

3. DNA sequences analysis of CHIP-H DNA clones:

The insert of five candidate clones is 107 bp in #52, 538 bp in #53, 333 bp in #65, 330 bp in #66, and 654 bp in #75. After repeatmasker analysis, full length of #53, 98~333 bp of #65, and 51~330 bp of #66 contain LINE or SINE elements. After comparing the sequence similarity with the nucleotides in GeneBank of NCBI, #75 shared 79% identity with Formosan muntjac satellite II (Fig. 2). Clone #52 didn't significantly match any collected nucleotides or contain any known repetitive sequences. After self-comparison, #75 has inverted repeat in 148-326 bp. This sequence could form a loop structure with a stem of 12bp and a loop of 154 bases.

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CHIP-H#75 1      GGGGCCCTGTCCACTGCAAGGGTTTGGAGAACGGATGGCCTGGTCAGAGGGAAGAGGC 60
FM-satII 428    GGGGCTCTCTTCACACTGCAGGGGACTGGAGCCCGGACGGTCTGGTCAGAGGGAAGAGGC 487
CHIP-H#75 61      CTGCAAGGGGGTTGATGCCCAAGGCCTCTTTGTCCCAGGAGCCTCAGGGCAGCAGGGTGA 120
FM-satII 488    CAGCAAGGGGGTTTGATCCCCAAGGCCTCTTTGGCCCAGCAGCCTCAGGGCAGCAGGAAGA 547
CHIP-H#75 121     GCAAGTGGCCCACTGTGCCCTAGGACGGCCTTGCCCTTCACACACAGGGCCTGCTGCT 180
FM-satII 548    GCAAGTGGCC-ACTGTGCCCAAGGTTGGCCCTGCCCTTCACACACAGGGGCTGCTGCT 606
CHIP-H#75 181     GGGCTTGACC-AGGAGCAGCCTGACAGACTCTGGCTCACCCGAGCTCTCTGATGGTGCAG 239
FM-satII 607    GGGTTTTTCCCATGAGCTGCCTGACATACTCTGGATCACCTGAGCTCTCTGACGGTGCAG 666
CHIP-H#75 240     GGGGCAGGAAGATTCTGTGTGCATGTGGTTCTGCCTTGGCCTGGGAGTTTGGAGCCCCTAG 299
FM-satII 667    AGGGCCGGAAGAGACGTGGGCATGTGGTTCTGCCTTGGCCTGGGAGTGTGGATCCCCTAG 726
CHIP-H#75 300     CTGTCACCCAGGCTCAGGGGCAAGGCCCTGAGAGGGGACCA--CACAAAGCACTTTTCTCC 357
FM-satII 727    TTGTACCCAAAGCAATTGGCCAAGGGCCTGAGAGCGGACGAACCTGATGCGCTATTGTCC 786
CHIP-H#75 358     TGGGCTTGGGAGCAGAGGGCATGGCTTGCGGGAAAAGCGGGCCTCCCTCGGTGAGGGAGA 417
FM-satII 787    TTGGCTTGGGAGCAGAGTGCCTGGCTTGCGGGAAATGCGGGTCTCCCTCG----- 836
CHIP-H#75 418     GGTAGCCAACCAGCATGAGTGTCTGCAAACCGGCCCTTGGAAAGCTGCTGGTTAAGTCTTC 477
FM-satII 837    GGTA-----GAGTGTCTGAAACTGGCCTTGGAGGCAGCCGGGGTGGTCTTC 884
CHIP-H#75 478     CAACAGCACAAATGCTCCTTCTGTTCGAAAGCCCC-GAGGAAAGGGGAGGTCCCAGCAAG 536
FM-satII 885    CAGCGCCAGAGTGTGGATTTGCTCTGTGGCCCCACAGTCTGGGGCAGGTCCCAGCATG 944
CHIP-H#75 537     GATCCTGCCCCAGGCCAGCCTTGACAAGACCTTCTGGCCTCCGGGGGTACAGCCCCATG 596
FM-satII 945    GATACTGCTCCAGACCAGCCTTGCCGAGACCTACCTGGCTTCCGGGGGTACTGCCCCATG 1004
CHIP-H#75 597     AAGGGGCAGGCAGCAGGGACTGTGTCCAGACACGCCCAAGCACCTCCCCTGTATCATT 654
FM-satII 1005   AAGGAGCTGGCTGTGGGGTGTGTGTCCATACATAACCAGGCGCCTCCCAGGCTCATT 1062

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Fig. 2: The DNA sequences of CHIP-H#75 aligned with the satellite II DNA sequences of Formosan muntjac (FM-satII)

4. Ex-vivo protein-DNA binding assay.

The five candidate CHIP-H DNA clones was introduced into HeLa cells for 48 hours incubation; subsequently, simultaneously immunofluorescence with CENPs and FISH with satellite DNA would be performed to visualize whether the signals of immunofluorescence and FISH colocalized. The result showed that the FISH signals of these five candidate CHIP-DNA and CENP-B box colocalized with the immunofluorescence signals of the human centromeric proteins; while the FISH signals of the pBSK (vector only) doesn't colocalize with the immunofluorescence signals of the human centromeric proteins (Figure 3). These suggested that the CHIP-H DNAs could associate with human centromeric proteins ex-vivo.

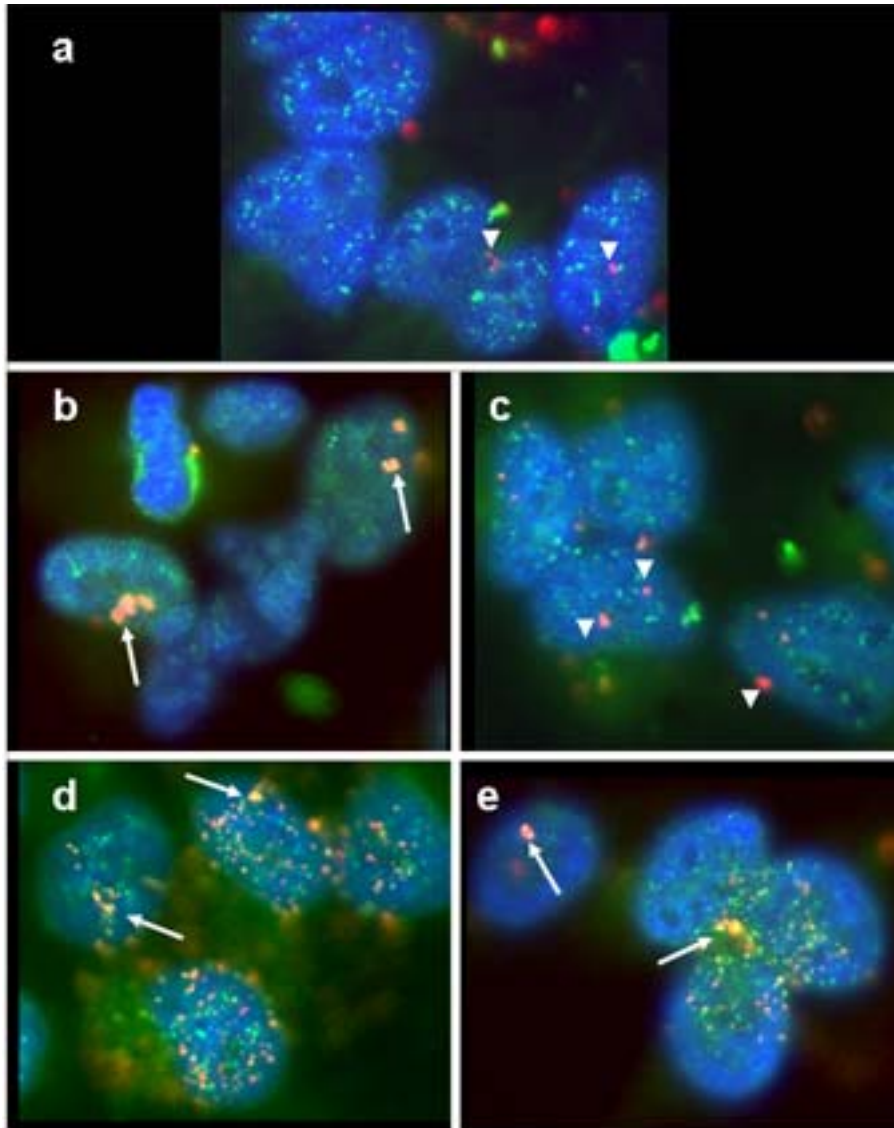


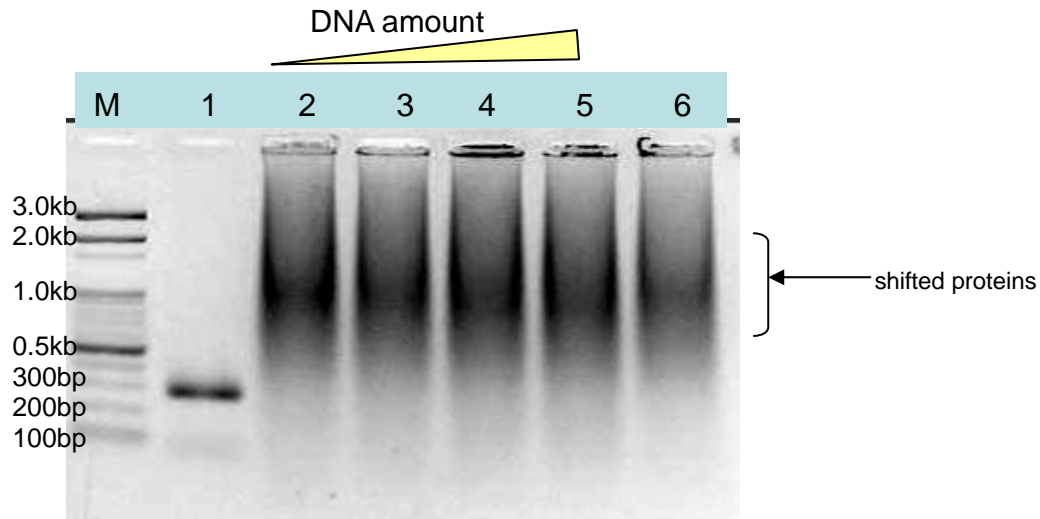
Figure 3: Ex-vivo protein-DNA binding assay: Briefly, the DNA clones pBSK (a); pBSK-CENP-B box (b); CHIP-H-65 (c); CHIP-H-66(d); and CHIP-H-75 (e) was each introduced into HeLa cells for 48 hours incubation; subsequently, simultaneously immunofluorescence of CENPs (human centromeric proteins) and FISH of introduced DNA were performed. In (a), the arrow heads indicate that the FISH signals (red signals) doesn't colocalize with the immunofluorescence signals (green signals). In (b), (c), (d) and (e), yellow hybridization signals were observed (denoted by arrows). They represented that the FISH signals colocalized with the immunofluorescence signals.

5. Identifying the protein-binding activity of candidate CHIP-H clones by gel-retardation:

First, we would like to set up the standard protocol of non-isotopic gel-retardation combination with western blotting. After try and error many times, we finally find an appropriate method for non-isotopic gel-retardation combination with western blotting. This method is able to present more precisely the specific shift DNA-protein band than the previous non-isotopic gel-retardation combination without western blot (Fig. 4). It also benefit without the isotopic usage. Our data showed that the specific shifted bands were presented in the specific DNA fragment incubated

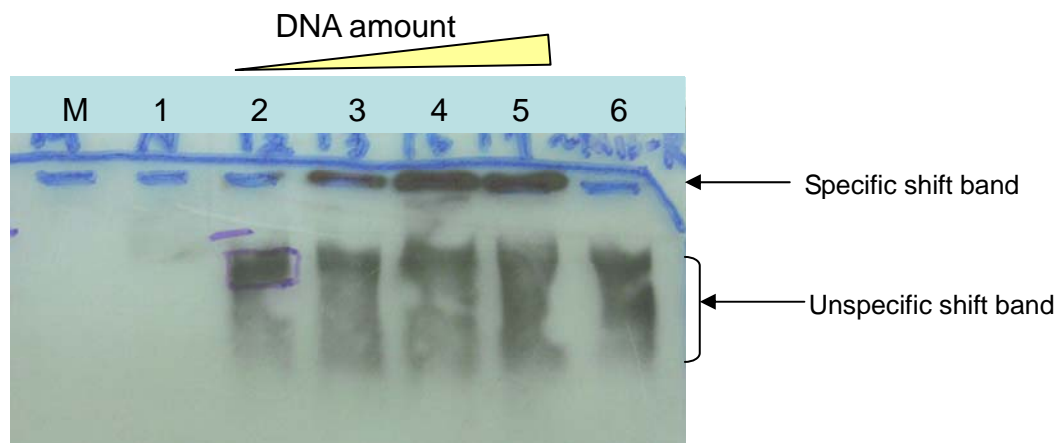
with the nuclear extract by method of the non-isotopic gel-retardation combination with western blotting (Fig.5).

Fig 4: non-isotopic gel-retardation combination without western blot:



The non-isotopic DNA fragment with 250bp-size was incubated with the crude nuclear extract in 1X binding buffer for 20min at room temperature. After incubation, the incubated protein-DNA mixtures were run on 2% agarose at 50V on ice for 70 min. The visualizing bands were stained with Ethidium bromide dye. Lane M denoted as the standard DNA marker. Lane 1 : only 30ng of CENP-B DNA; lane 2 : 30ng of CENP-B DNA was incubated with crude nuclear extract 12.6 μ g; lane 3 : 45ng of CENP-B DNA was incubated with crude nuclear extract 12.6 μ g; lane 4 : 75ng of CENP-B DNA was incubated with crude nuclear extract 12.6 μ g; lane 5 : 105ng of CENP-B DNA was incubated with crude nuclear extract 12.6 μ g; lane 6 : 30ng of vehicle DNA was incubated with crude nuclear extract 12.6 μ g. The result showed that the protein was shifted in lane 2 to lane 6. It doesn't matter with what kind of DNA fragment was incubated with crude nuclear extract. It suggested that the shifted protein bands is a non-specific shift band.

Fig 5: non-isotopic gel-retardation combination with western blot:



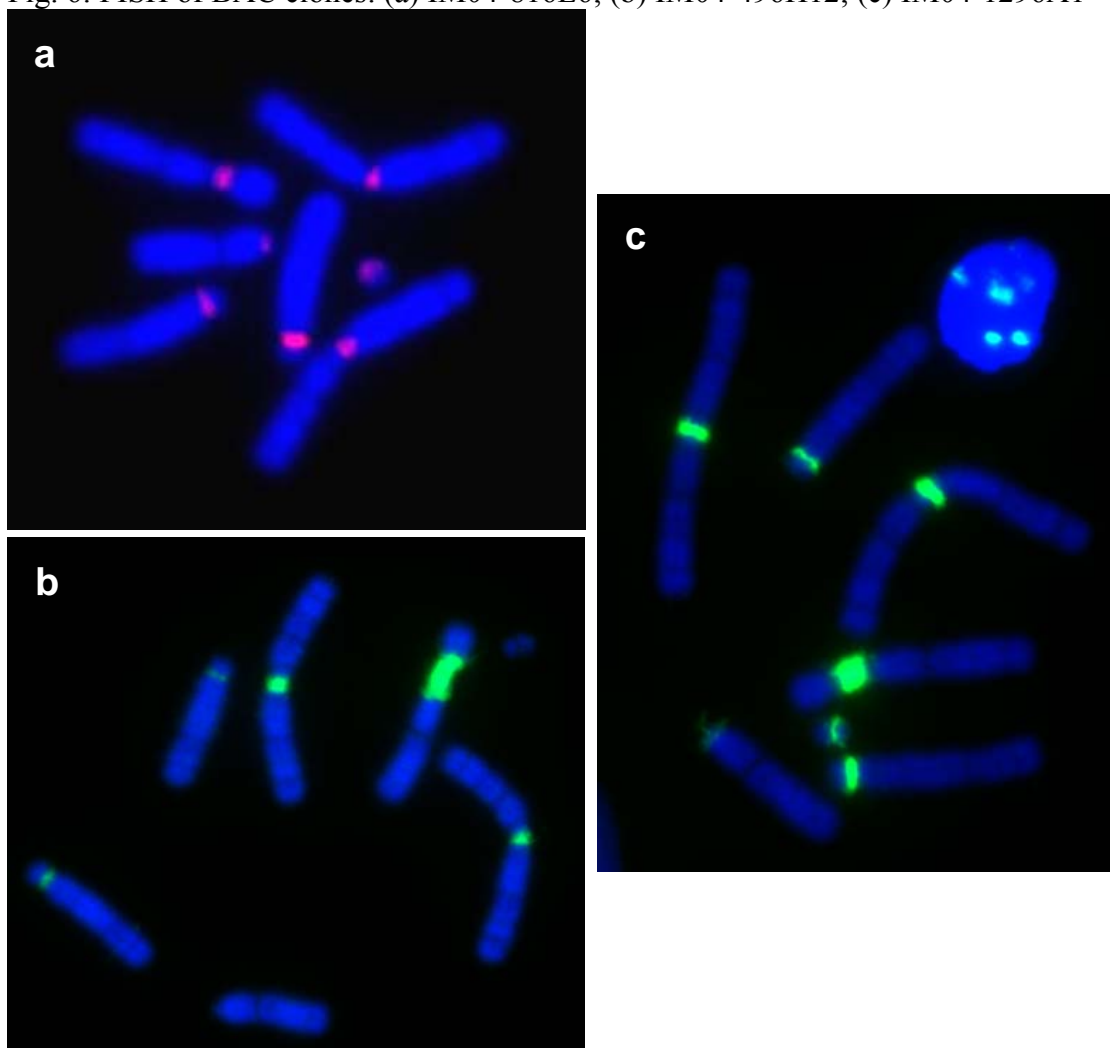
The non-isotopic DNA fragment with 250bp-size was incubated with the crude nuclear extract in 1X binding buffer for 20min at room temperature. After

incubation, the incubated protein-DNA mixtures were run on 2% agarose at 50V on ice for 70 min. The agarose was performed with the regular western blot and the protein bands were detected by CENP-B antibodies and visualized by chemiluminescence assay. Lane M denoted as the standard DNA marker. Lane 1 : only 30ng of CENP-B DNA; lane 2 : 30ng of CENP-B DNA was incubated with crude nuclear extract 12.6 μ g; lane 3 : 45ng of CENP-B DNA was incubated with crude nuclear extract 12.6 μ g; lane 4 : 75ng of CENP-B DNA was incubated with crude nuclear extract 12.6 μ g; lane 5 : 105ng of CENP-B DNA was incubated with crude nuclear extract 12.6 μ g; lane 6 : 30ng of vehicle DNA was incubated with crude nuclear extract 12.6 μ g. The result showed that the specific shift band is only presented in lane 2 to lane 5 which the CENP-B DNA was incubated with crude nuclear extract. The signals of specific shift bands were stronger as the amount of CENP-B DNA was increased.

6. Screening the centromeric BAC clones by dot blot hybridization

We screened 135 centromeric BACs clones by dot blot hybridization using a mix probe of five CHIP-H clones. We obtained three positive BAC clones. These three BAC clones were identified by FISH experiments. The FISH results showed the centromeric location of BACs (Fig.6).

Fig. 6: FISH of BAC clones: (a) IM04-816E6; (b) IM04-496H12; (c) IM04-1296A1



7. Sequencing the candidate centromeric BAC clones

Because the centromeric BAC clones contain significant repetitive DNA sequences, it is not easy to completely sequencing using the shot-gun sequencing or directly walking sequencing. Three candidate centromeric BAC clones were sequenced by the hierarchical sequencing strategy using restriction enzyme. One of three BACs' sequences was assembled 95% complete. However, it has some problems in assembling the sequence of subclones, because considerable repetitive DNA presented. Other two BACs were sequenced 80% completely.

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Self-evaluation:

Our major aim is to characterizing the genomic organization of the CENP-H associating centromeric DNA sequence. We had successfully constructed a mini-library of CENP-H associating DNA by chromatin immunoprecipitation. We obtained five clones, designated as CHIP-H#53, 55, 65, 66, and 75. Ex-vivo protein assay showed that the isolated clones have CENP-H binding activity. Additionally, we also established a method of non-isotopic gel-retardation combination with western blot that is more efficient and more precise. It is very valuable to identify the CENP-binding activity of CHIP-H DNA clones. Furthermore, we screened the

centromeric BAC DNA using these five clones and CENP-A associating DNA as probes. Three positive BAC clones were further sequencing. Because the BAC DNA clones contain the significant repetitive DNA, it is not easy to complete sequence BAC DNA by shot-gun sequencing or second-generation sequencing. We had finished all sequences of subclones. It takes us a lot of time to assemble the sequences of subclones. Once we finished sequences assemble of the candidate BAC DNA, it will shed more light on the genomic organization of the CENP-H associating centromeric DNA sequence. In addition, we compared all sequences of subclones each other and compared them with the nucleotides collected in GeneBank of NCBI. The sequences comparison result showed that the CENP-H associating DNA and CENP-A associating DNA shared more 80% identity to centromeric satellite II. These evidences indicated that the CENP-H recruited CENP-A and C through associating centromeric satellite II to form an active centromere. Therefore, we had achieved the purpose what we want to explore in this project.

During the term of carrying out this project, we had 5 papers published, two papers accepted in press, one paper submitted and two papers in preparation.

Appendix:

Zoological Studies 47(3): 282-292 (2008)

*Zoological
Studies*

Construction of an Indian Muntjac BAC Library and Production of the Most Highly Dense FISH Map of the Species

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Chyi-Chyang Lin, Pei-Ching Hsu, Tzai-Shiuan Li, Shu-Ju Liao, Ya-Ming Cheng, Lie-Jiau Hsieh, and Yueh-Chun Li (2008) Construction of an Indian muntjac BAC library and production of the most highly dense FISH map of the species. *Zoological Studies* 47(3): 282-292. Following completion of the genome sequences of some mammalian species, comparative genomic studies in mammals have been actively conducted to assess gene changes or to identify syntenic conservation during evolution. The Indian muntjac (*Muntiacus muntjac vaginalis*) ($2n = 6$ in the female and 7 in the male) may have evolved from an ancient deer species with a karyotype $2n = 70$ through extensive chromosome rearrangements creating the lowest chromosome number of a mammalian species. Therefore, the species has become a good resource for studying syntenic conservation among deer species. An Indian muntjac bacterial artificial chromosome (BAC) library that contains 126,336 individual BAC clones with an average insert size of 80 kilobases was obtained in this study. The frequency of clones with inserts was 88%, and thus this library corresponds to approximately 4x coverage of the Indian muntjac genome. Individual chromosomal locations of 1619 BAC clones on the Indian muntjac metaphase chromosomes were identified by fluorescence *in situ* hybridization (FISH). Among these clones, 1517 BAC clones were mapped onto specific loci, and 102 BAC clones were mapped onto the centromeric region. This provides the most highly dense FISH BAC clone map for the species. This densely ordered map can be used as a blueprint for comparative FISH mapping studies of other deer species in order to investigate the mechanism of genomic rearrangement and karyotypic evolution. Moreover, centromeric BAC clones will provide an excellent resource for studying the structure and function of mammalian centromeres.
<http://zoolstud.sinica.edu.tw/Journals/47.3/282.pdf>

Key words: BAC library, FISH mapping.

Complex genomic organization of Indian muntjac centromeric DNA

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Abstract A 69-kb Indian muntjac bacterial artificial chromosome (BAC) clone that screened positive for Cervid satellites I and IV was selected for complete sequence analysis and further characterization. The sequences of this BAC clone were found in the centromeres and in some interstitial sites of Indian muntjac chromosomes. Sequence analyses showed that the BAC clone contained a 14.5 kb Cervid

satellite I-like DNA element and a 9 kb Cervid satellite IV-like DNA element. In addition, it contained 51 regions each organized in a complex fashion, with sequences homology to intersperse repetitive sequences such as LINES, SINEs, LTRs, other published DNA elements, and unassigned sequences. The FISH patterns of seven non-satellite sequence elements generated from the BAC clone showed mainly specific to centromeres of the Indian muntjac representing novel centromeric DNAs of the species. Furthermore, FISH signals and Southern blot patterns of these elements suggest the existence of a not yet identified repetitive sequence with giant repeated monomers. Positive FISH signals of these elements were also detected in the centromeric regions of Fomosan muntjac. This suggests that these newly identified non-Cervid satellite DNA sequences have been conserved in the centromere of the Fomosan muntjac.

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Keywords centromere · centromeric DNA · satellite
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muntjac

Abbreviations

BAC Bacterial artificial chromosome
FISH Fluorescence in situ hybridization
LINES Long interspersed nucleotide elements
SINEs Short interspersed nucleotide elements
LTRs Long terminal repeats

Small Supernumerary Marker Chromosome Originating From Chromosome 10 Associated With an Apparently Normal Phenotype

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Small supernumerary marker chromosomes (sSMC) originating from chromosome 10 are rare. Only seven cases have been documented, and among those three cases were diagnosed prenatally. We reported on another prenatal diagnosis of a de novo mosaic sSMC in an apparently normal female fetus whose mother had conceived with assisted reproductive technology (ART) procedures. G-banding analysis of amniotic cells was performed. Spectral karyotyping (SKY) and fluorescence in situ hybridization (FISH) studies with chromosome 10-specific alphoid satellite DNA probe were used to identify the chromosome 10 origin of the sSMC. Further FISH study with telomeric sequence probes showed that the sSMC lacked a hybridization signal, suggesting that the marker could be a ring chromosome. FISH studies using BAC clone probes specific for the regions within 10p11.2, 10q11.1, and 10q11.2 showed that the short arm breakpoint was located between 29.8 and 30.7 Mb from the 10p telomere, and that the long arm breakpoint was located less than 43.6 Mb from the 10p telomere. The karyotype of the fetus was 47,XX,+mar.ish der(10)(SKY+ CEP 10+, CTD-2130I7+, RP11-89J23-)/46,XX. Oligonucleotide microarray-based copy number variations (CNV) analysis was also performed and showed a 6.7 Mb duplication from 10p11.2 to 10q11.2 (36.2–42.9 Mb) with Affymetrix SNP-array 6.0 genotype: arr cgh. 10p11.2q11.2(CN_519687→CN_541524) X 3. At the 1-year follow-up, the baby did not have any findings of the trisomy 10p syndrome. This observation provided further credence to the concept that additional chromosome material of proximal 10p11.2 may not contribute to the trisomy 10p syndrome phenotype. © 2009 Wiley-Liss, Inc.

Key words: marker chromosome 10; small supernumerary marker chromosomes (sSMCs); spectral karyotyping (SKY); fluorescence in situ hybridization (FISH); genome-wide oligonucleotide microarray; trisomy 10p syndrome

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INTRODUCTION

Supernumerary small marker chromosomes (sSMC) are defined as extra, structurally abnormal chromosomes in which no part can be identified unambiguously by conventional cytogenetic techniques [Shaffer and Tommerup, 2005]. The incidence of all SMC was reported to range from 0.4/1,000 to 1.5/1,000 in live birth [Ferguson-Smith and Yates, 1984; Sachs et al., 1987; Hook and Cross, 1987; Warburton, 1991]. Small supernumerary marker chromosomes (sSMCs), which are equal in size or smaller than a chromosome

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Rare Rearrangements: A “Jumping Satellite” in One Family and Autosomal Location of the SRY Gene in an XX Male

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A satellited short arm of the Y chromosome (Yps) is rare. Only one *de novo* case of Yps has been documented. Here we report the prenatal diagnosis of Yps in a male fetus with a karyotype, 46,XYps. Family chromosome study showed that the father and a sister had a satellited short arm of the X chromosome (Xps). A phenotypically normal male child with the Yps was delivered. This is the first familial case showing a satellite “jumping” from Xp to Yp. We propose that it resulted from a crossover within the pseudoautosomal region 1 (PAR1) on the distal Xp and Yp during paternal meiosis. In addition to the rare translocation mentioned above, relocation of the SRY gene onto an autosome in XX males is also a rare event. Herein we report a phenotypically normal male fetus with a 46,XX karyotype. Fluorescence in situ hybridization (FISH) study showed that the SRY locus had been transferred to the terminal short arm of a chromosome 3. The terminal short arm deletion of this chromosome 3 was also confirmed by FISH study with a subtelomeric probe and the breakpoint of the terminal deletion was estimated between 446 and 664 kb from the 3p telomere by real-time qPCR study with a gene sequence and STS markers in this region. A healthy boy was delivered at 37 weeks of gestation. At 1-year follow-up, the child’s growth pattern and development were appropriate for age. © 2009 Wiley-Liss, Inc.

Key words: familial “jumping translocation”; satellited Xp; satellited Yp; XX male; Y^{SRY+}; autosome translocation; Fluorescence in situ hybridization (FISH); multiplex ligation-dependent probe amplification (MLPA); real-time quantitative-PCR (RT-qPCR)

INTRODUCTION

In general, translocation of chromosome segments occurs sporadically and randomly. Pseudoautosomal regions (PAR1) located on

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the tips of the short arms of human X and Y chromosomes are known to achieve X and Y chromosome pairing and recombination (crossing-over) during meiosis [Rappold, 1993; Mangs and Morris, 2007]. Some duplicated DNA elements at centromeric/pericentromeric regions and telomeric/subtelomeric regions can also facilitate chromosome rearrangement in these regions involving different human chromosomes [Bailey et al., 2002; Samonte and Eichler, 2002; Linardopoulou et al., 2005]. Recently, a translocation breakpoint hot spot in human chromosome region 22q11, was isolated and found to have a palindromic structure which leads to

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A New Familial Insertion, ins(18;9)(q12.2;q33.1q31.1) With a 9q31.1–9q33.1 Deletion in a Girl With a Cleft Lip and Palate

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TO THE EDITOR:

Interchromosomal insertion (insertion between two non-homologous chromosomes) is a rare chromosomal rearrangement with an incidence of 1 in 80,000 live births [Van Hemel and Eussen, 2000]. At least three breaks are required for the generation of the insertion. This may account in part for its rareness. A carrier of an interchromosomal insertion has a theoretical risk of 50% of producing offspring with genomic imbalance (deletion or duplication of chromosome material) in each pregnancy. Herein we report on a patient with a 9q deletion due to a familial insertion (18;9)(q12.2;q33.1q31.1). The proposita presented at the age of 6 to the pediatric clinic with mental retardation and dysmorphic craniofacial features including a repaired cleft lip and palate, hypertelorism, strabismus, low-set ears, downslanting palpebral fissures, and tapered fingers (Fig. 1A). She is the first child of nonconsanguineous parents and was born when the mother was 19 years and the father was 26 years of age. Her body weight is 18 kg and her height is 95 cm (<3rd centile). The mother denied exposure to teratogens or drugs during pregnancy. The results of a second-trimester screening for Down syndrome were normal. A mid-trimester ultrasound detected a female fetus with unilateral cleft lip and palate but the mother did not undergo any invasive prenatal diagnostic testing procedures. The proposita was born at 38 weeks' gestation with a body weight of 2,750 g. During delivery, meconium aspiration syndrome with respiratory distress, facial dysmorphism, unilateral cleft lip and palate, and cephalohematoma over biparietal regions were diagnosed. Echocardiography revealed mitral valve prolapse. Unilateral cleft lip and palate was repaired at

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the age of 1 year. The patient has a history of delayed motor development. She began to sit without support at 12 months and could stand unaided at 20 months. Hearing impairment was identified at 20 months. At the age of 2 years, the patient underwent correction of strabismus. At present, she can only speak single

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Abstract: Mosaicism with an isodicentric 8 with a breakpoint at p23.3 [idic(8)(p23.3)] is very rare. We report the first prenatal case on a male fetus in which obstetric ultrasound revealed multiple congenital anomalies at 28 weeks of gestation. Cytogenetic analysis of amniocytes showed mos 45,XY,-8,psu idic(8)(p23.3)[16]/ 46,XY,psu idic(8)(p23.3)[4] and that of cord blood lymphocytes revealed mos 45,XY,-8,psu idic(8)(p23.3)[13]/ 46,XY, psu idic(8)(p23.3)[37]. FISH studies revealed that the break-reunion occurred at the cytoband 8p23.3 within the physical position 2.08 Mb from the 8p telomere. Chromosomal microarray analyses further assigned the duplication/deletion breakpoint at 2.16 Mb (Agilent 244K) and at 2.19 Mb (Affymetrix SNP6.0). Analysis of microsatellite DNA indicated that the psu idic(8)(p23.3) was derived from the maternal chromosome 8. Together, these findings indicate that the fetus was nullisomic for ~2.2 Mb from 8pter, trisomic for the rest of chromosome 8 in mosaic condition and likely had breaks in MYOM2 repeats of the maternal chromosome 8.

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Cervid satellite DNA and karyotypic evolution of Indian muntjac
--Manuscript Draft--

Manuscript Number:	
Full Title:	Cervid satellite DNA and karyotypic evolution of Indian muntjac
Article Type:	Review
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Abstract:	Five satellite DNA families (designated as satellite I-V) have been identified in the Cervidae so far. Among those, satellite I, II and IV are centromere specific. Satellite I and II are shared by large number of deer species, where satellite IV is highly conserved among several deer species examined. Satellite III was initially thought to be roe deer specific but later identified in Chinese water deer as well. Satellite V is Y-chromosome specific for several Asian deer species examined but also found in the pericentric region of Indian muntjac chromosome 3 and in X chromosome. The observation of interstitial hybridization sites on Indian muntjac chromosomes with satellite DNA I probe generated from Chinese muntjac provides the first molecular evidence supporting the tandem fusion theory that $2n=6Q/7Q$ of Indian muntjac karyotype could derive from an ancestral Chinese muntjac-like species with $2n=46$. Interspecies chromosome painting study and the maximum number of interstitial hybridization detected with satellite I and satellite II DNA probes lend support to the hypothesis that the Indian muntjac karyotype could evolve directly from an ancestral Chinese water deer-like species with $2n=70$. Such hypothesis is further substantiated by the finding of satellite V signals presented in specific chromosome regions between the Chinese water deer and the Indian muntjac chromosomes.
Suggested Reviewers:	

國科會補助專題研究計畫項下出席國際學術會議心得報告

日期：100年7月8日

計畫編號	NSC 97-2311-B-040-003-MY3		
計畫名稱	著絲點蛋白 H 連結的著絲點 DNA 之特性分析		
出國人員 姓名	李月君	服務機構 及職稱	中山醫學大學生物醫學科學系 教授
會議時間	100年7月2日至 100年7月5日	會議地點	葡萄牙奧波多
會議名稱	(中文)第8屆歐洲細胞遺傳學會議 (英文)8 th European cytogenetics conference		
發表論文 題目	(中文)台灣本土三個鹿種的 G-顯帶之核型比較及印度山羌 BAC 的染色體定位 (英文) Comparative G-banded karyotype and chromosomal mapping of Indian muntjac BAC clones to three Taiwan indigenous deer species		

一、參加會議經過

第一天上午辦理報到及海報的張貼，下午參與有關比較基因體學在臨床細胞遺傳學上應用的討論會及比較細胞遺傳學在動物學上的應用討論會，晚餐則參與大會舉辦的歡迎會與來自美國的學者做了一些學術及文化交流。

第二天早上聽了兩場有關微小結構異常在癌症及早期發育中的形成及影響。下午聽了二場染色體端粒與疾病的關連性，及以細胞模式及腫瘤研究提出染色體端粒的假說。

第三天上午聽了有關動物細胞遺傳學領域的研究報告，下午參觀海報。

二、與會心得

The European Cytogenetics Conference is an annual congress which has gathered high level experts in chromosomal biology. This conference has reached a worldwide dimension and plays a paramount role in the international scientific life: allowing the gathering of high level specialists, it encourages the exchange of ideas and stimulates the works of the researchers all through the world.

This is my first time to attend this meeting. This meeting is different from the ASHG annual meeting that I used to attend before. Although this meeting is smaller than ASHG annual meeting, it mainly focuses on the chromosomal biology related topics, including clinical cytogenetics session that discuss diseases related to telomeres, epigenetics and DNA repair, 3D nucleus, and genomic instability and animal cytogenetics session. In this conference, I learned what has been achieved in clinical cytogenetics by the new molecular cytogenetic technique, aCGH. This new powerful tools would become available in clinical cytogenetic diagnosis in the future. Another lectures in the animal cytogenetic session also stimulated my new ideas in animal comparative cytogenetics studies.

In this meeting, I found that the animal cytogenetics study was more encouraged in European than in Taiwan. This is positive thinking, studying the genome in various organism not only in human species

would realize how the gene works in different organism and would further shed more light on the mechanism of disease-gene in human

三、考察參觀活動(無是項活動者略)

無

四、建議

無

五、攜回資料名稱及內容

1. Chromosome Research 2011 vol.19 Supplement 1: a publish journal for all abstracts of lectures, oral presentation, poster presentation in 8th European Cytogenetics Conference

2.Molecular Syndromolgy 2010 Vol.1:159-210: a publish journal for research articles, short reports and reviews on the molecular basis of genetic syndromes, genotype-phenotype correlations, natural history, strategies in disease management and novel therapeutic approaches linked to molecular basis.

六、其他

無

國科會補助計畫衍生研發成果推廣資料表

日期:2011/07/11

國科會補助計畫	計畫名稱: 著絲點蛋白H連結的著絲點DNA之特性分析
	計畫主持人: 李月君
	計畫編號: 97-2311-B-040-003-MY3 學門領域: 生物學之生化及分子生物
無研發成果推廣資料	

97 年度專題研究計畫研究成果彙整表

計畫主持人：李月君		計畫編號：97-2311-B-040-003-MY3				計畫名稱：著絲點蛋白 H 連結的著絲點 DNA 之特性分析	
成果項目		量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）	
		實際已達成數（被接受或已發表）	預期總達成數（含實際已達成數）	本計畫實際貢獻百分比			
國內	論文著作	期刊論文	2	0	100%	篇	
		研究報告/技術報告	0	0	0%		
		研討會論文	0	0	0%		
		專書	0	0	0%		
	專利	申請中件數	0	0	0%	件	
		已獲得件數	0	0	0%		
	技術移轉	件數	0	0	0%	件	
		權利金	0	0	0%	千元	
	參與計畫人力（本國籍）	碩士生	1	0	100%	人次	
		博士生	0	0	0%		
博士後研究員		0	0	0%			
專任助理		0	0	0%			
國外	論文著作	期刊論文	5	0	100%	篇	
		研究報告/技術報告	0	0	0%		
		研討會論文	3	0	100%		
		專書	0	0	0%	章/本	
	專利	申請中件數	0	0	0%	件	
		已獲得件數	0	0	0%		
	技術移轉	件數	0	0	0%	件	
		權利金	0	0	0%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	0%	人次	
		博士生	0	0	0%		
博士後研究員		0	0	0%			
專任助理		0	0	0%			

<p>其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)</p>	<p>無</p>
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	成果項目	量化	名稱或內容性質簡述
科 教 處 計 畫 加 填 項 目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與(閱聽)人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

達成目標

未達成目標（請說明，以 100 字為限）

實驗失敗

因故實驗中斷

其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文： 已發表 未發表之文稿 撰寫中 無

專利： 已獲得 申請中 無

技轉： 已技轉 洽談中 無

其他：（以 100 字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限）

Our major aim is to characterizing the genomic organization of the CENP-H associating centromeric DNA sequence. We had successfully constructed a mini-library of CENP-H associating DNA by chromatin immunoprecipitation. We obtained five clones, designated as CHIP-H#53, 55, 65, 66, and 75. Ex-vivo protein assay showed that the isolated clones have CENP-H binding activity. Additionally, we also established a method of non-isotopic gel-retardation combination with western blot that is more efficient and more precise. It is very valuable to identify the CENP-binding activity of CHIP-H DNA clones. Furthermore, we screened the centromeric BAC DNA using these five clones and CENP-A associating DNA as probes. Three positive BAC clones were further sequencing. Because the BAC DNA clones contain the significant repetitive DNA, it is not easy to complete sequence BAC DNA by shot-gun sequencing or second-generation sequencing. We had finished all sequences of subclones. It takes us a lot of time to assemble the sequences of subclones. Once we finished sequences assemble of the candidate BAC DNA, it will shed more light on the genomic organization of the CENP-H associating centromeric DNA sequence. In addition, we compared all sequences of subclones each other and compared them with the nucleotides collected in GeneBank of NCBI. The sequences comparison result showed that the CENP-H associating DNA and CENP-A

associating DNA shared more 80% identity to centromeric satellite II. These evidences indicated that the CENP-H recruited CENP-A and C through associating centromeric satellite II to form an active centromere. Therefore, we had achieved the purpose what we want to explore in this project. This result will shed more light on the function of centromere and might provide more information on constructing an artificial chromosome for gene therapy.