Identification of the Female-Specific DNA Fragments in Chickens

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It was previously demonstrated that when the genomic DNA of the chicken was digested with restriction enzyme, *XhoI*, and analyzed by agarose gel electrophoresis with ethidium bromide as a staining agent, a DNA fragment of about 0.7 kbp was detected specifically in the female (KAGAMI and TOMITA, 1990). In the present study, the W chromosome-specific DNA probe was found to hybridize with the initially detected female specific DNA fragment mentioned above.

Consequently, it was concluded that the initially detected female specific DNA fragment of about 0.7 kbp was homologous to the W chromosome-specific DNA probe.

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Key words : Chickens, Female-specific DNA fragments, W-specific DNA probe

Introduction

Since the W chromosome is female specific in chickens, it has been considered that the genes on the W chromosome play important roles in primary sex determination. Scientific works have been performed to clarify the role of the W chromosome. Direct cytological evidence for the W chromosome was presented as an unpaired acrocentric chromosome of the size class of No. 7-8 in female chicken cells¹⁾. And entire regions of the W chromosome in metaphase were found to be stained with C-banding method²⁾.

Recently, the W chromosome-specific DNA fragments were cloned³⁾. However, the meaning of the repetitive DNA units in the W chromosome is still uncertain. And the knowledge about the W chromosome at molecular level is scanty as compared with that of the mammalian Y chromosome⁴⁾.

In the present study, detection of the female-specific DNA fragments was performed by hybridization of the cloned DNA probe to the genomic DNA of the chicken digested with a restriction enzyme, *XhoI*.

Materials and Methods

Blood samples of 1 ml were taken with a heparin-wetted syringe from the wing vein of White Leghorn chickens aged eleven months. High molecular DNA was prepared and digested with *XhoI*. The DNA was subjected to electrophoresis in

agarose gel, stained with ethidium bromide and photographed through a conventional UV transilluminator at 254 nm^{5,6}. The DNA was transferred from the gel to a sheet of nylon membrane (Amersham, Hybond-N+) by capillary method⁷.

One ng of plasmid DNA (pUGD 06000) which contained the W chromosome-specific 0.7 kbp DNA unit was transformed to competent cells (*Escherichia coli* DH 5 α). The transformed bacteria was incubated overnight at 37°C in 3.5 ml of LB medium containing Ampicilin. Lysis of the bacteria was done by adding 200 μ l of alkaline solution (0.2 N NaOH and 1% SDS) and the plasmid DNA was harvested. The DNA was double digested with restriction enzymes *Bam*HI and *Pst*I, and the insert of the 0.7 kbp W chromosome-specific DNA fragments were recovered. The DNA fragments were labelled with horseradish peroxidase (Amersham) and used as W-specific DNA probe.

Pre-hybridization of the membrane was done for 1 hour at 42°C. Initially labelled DNA probe was added and the hybridization was carried out at 42°C overnight. After washing the membrane with primary wash buffer (0.4% SDS and 0.1 xSSC) and secondary wash buffer (2 xSSC), 0.0625 ml/cm²-membrane of the detection solution (Amersham) was added and incubated precisely 1 minute at room temperature. Exposure to a sheet of film (Hyperfilm-ECL, Amersham) was carried out for 1 hour.

Results

When the genomic DNA of the chickens were digested with *Xho*I and stained with ethidium bromide, the electrophoretic patterns of the DNA were easily distinguished between the male and female. In the female, a DNA fragment was clearly detected as a dense band at about 0.7 kbp (Fig. 1; lanes 6, 7 and 8). In the male the fragment

Fig. 1. Digestion of the chicken genomic DNA with *Xho*I and detection of the female specific DNA fragments. The male genomic DNA is in the lanes 2, 3, 4 and 5. The female genomic DNA is in the lanes 6, 7 and 8. The DNA size marker (ϕ x 174-*Hae*III digest) is shown in lane 1.

Fig. 2. Hybridization of the W chromosome-specific DNA probe to the *XhoI* digested chicken genomic DNA. The male genomic DNA is in the lanes 1, 2, 3 and 4. No hybridization could be detected. The female genomic DNA is in the lanes 5, 6 and 7. Hybrid bands with the probe are detected at about 0.7 kbp.

could not be detected (Fig. 1 ; lanes 2, 3, 4 and 5).

Hybridization of the W chromosome-specific DNA probe to the digested and electrophoretically separated genomic DNA fragments showed a clear difference between male and female. In the female, a major hybridization band was detected at about 0.7 kbp (Fig. 2 ; lanes 5, 6 and 7). In the male no hybridization band could be detected (Fig. 2 ; lanes 1,2,3 and 4).

Discussion

In the previous study, we have reported that digestion of the chicken genomic DNA with restriction enzyme, *XhoI*, and staining with ethidium bromide led to the detection of a specific DNA fragment at about 0.7 kbp in female (KAGAMI and TOMITA, 1990). Hybridization of the W chromosome-specific DNA probe to the digested DNA also showed a major hybrid band at about 0.7 kbp in female. Consequently, it was identified that the initially detected band at about 0.7 kbp correspond to the W chromosome-specific DNA fragment.

In chickens, entire region of the W chromosome was stained with C banding

method²⁾. Generally, this method is considered to detect constitutive heterochromatin which include satellite or highly repetitive DNA. Thus, it was considered that the detected female specific DNA fragments constitute the heterochromatic region on the W chromosome^{3,8)}.

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References

- 1) SCHMID, W. (1962) DNA replication patterns of heterochromosomes in *Gallus domesticus*. Cytogenet., **1** : 344-352.
- 2) STEFOS, K. and F.E. ARRIGHI (1971) The heterochromatic nature of the W chromosome in birds. Exp. Cell Res., **68** : 228-231.
- 3) TONE, M., Y. SAKAKI, T. HASHIGUCHI and S. MIZUNO (1984) Genus specificity and extensive methylation of the W chromosome-specific repetitive DNA sequences from the domestic fowl, *Gallus gallus domesticus*. Chromosoma, **89** : 228-237.
- 4) KOOPMAN, P., J. GUBBAY, N. VIVIAN, P. GOODFELLOW and R. LOVE-BADGE. (1991) Male development of chromosomally female mice transgenic for *Sry*. Nature, **351** : 117-121.
- 5) KAGAMI, H., H. NAKAMURA and T. TOMITA (1990) Sex Identification in Chickens by Means of the Presence of the W Chromosome-Specific Repetitive DNA Units. Jpn. Poult. Sci., **27** : 379-384.
- 6) KAGAMI, H. and T. TOMITA (1990) Sexing of Chickens by Detection of the W Chromosome-Specific Highly Repetitive DNA Units. Tokai J. Anim. Prod., **1** : 43-47.
(in Japanese with English Summary)
- 7) SOUTHERN, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol., **98** : 503-517.
- 8) KODAMA, H., H. SAITOH, M. TONE, S. KUHARA, Y. SASAKI and S. MIZUNO (1987) Nucleotide sequences and unusual electrophoretic behavior of the W chromosome-specific repeating DNA units of the domestic fowl, *Gallus gallus domesticus*. Chromosoma, **96** : 18-25.

ニワトリにおける雌特異的 DNA 断片の同定

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著者らはすでに、ニワトリのゲノムの DNA を制限酵素 *XhoI* で切断し、アガロースゲル電気泳動法により DNA 断片の分離を行ったところ、雌特異的な約 0.7 kbp の DNA 断片が検出されることを確認した (KAGAMI and TOMITA, 1990)。この論文では、この DNA 断片をナイロンメンブレンに固定し、既にクローニングされているニワトリの W 染色体特異的 DNA プローブを用いてハイブリダイゼーションを行ったところ、

約 0.7 kbp において雌特異的にハイブリダイズすることを確認した。以上の結果から、制限酵素 *XhoI* の切断により検出された約 0.7 kbp の DNA 断片は、クローニングされたニワトリの W 染色体特異的 DNA プローブと相同なフラグメントであることが確認された。

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キーワード: ニワトリ, 雌特異的 DNA 配列, W 染色体特異的 DNA プローブ