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Specific chromomeres on the chicken W lampbrush chromosome contain specific repetitive DNA sequence families

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Chromomeres 1 and 3 of the chicken W lampbrush chromosome contain most of the *Eco*RI and *Xho*I repeat sequence families respectively. These chromomeres were stained with DAPI and their sizes relative to other W chromomeres were observed. Their relative contents of *Eco*RI and *Xho*I repeats were determined using fluorescence *in situ* hybridization with genomic probes for each of the two repeat families. There were two types of W chromosome in the chickens (White Leghorn and Rhode Island Red) used in this study with respect to the amount of *Eco*RI repeat. A high-copy-number type has about 4000 copies of the 1.2-kb repeat per genome and shows a large fluorescence signal on W chromomere 1. A low-copy-number type has about 700 copies per genome and does not have a detectable chromomere 1 on W chromosome, nor does it show FISH labelling in the region normally occupied by chromomere 1. The genome of Fayoumi chickens has about one-sixth the amount of the *Xho*I sequence family of White Leghorns. W lampbrush chromomere 3 is much smaller and its FISH labelling with the *Xho*I probe is much weaker in Fayoumis than in White Leghorns. These results demonstrate that in the chicken W chromosome, specific chomomeres are occupied by specific DNA repeat sequence families.

Key words: chicken, chromomere, lampbrush chromosome, repetitive sequence family, W chromosome

Introduction

In the female chicken genome, the majority of C-band-positive constitutive heterochromatin is on the sex chromosomes. It occupies about two-thirds of the W chromosome (Saitoh & Mizuno, 1992) and one end of the Z chromosome (Hori *et al.* 1996). W-heterochromatin is largely made up of *Xho*I (Kodama *et al.* 1987) and *Eco*RI family (Saitoh *et al.* 1991) repetitive sequences, both of which are confined to the W chromosome. The overall sequence similarity of these two families is about 68%. Both families consist of tandem repeats of a basic unit of about 21 bp and both show remarkable curvature in solution (Saitoh *et al.* 1991; Suka *et al.* 1993). The *Xho*I and *Eco*RI family sequences are not intermingled with each other, at least within a distance of about 1 Mb (Saitoh *et al.* 1991). Fluorescence *in situ* hybridization (FISH) to mitotic W chromosome has shown that *Xho*I family sequences occupy the pericentric region widely, whereas *Eco*RI family sequences are distributed into two separate domains: the majority of them on the heterochromatic arm and the minor fraction near the middle of the other arm, which has a non-heterochromatic region at its end (Saitoh & Mizuno 1992). Cytological observations of the lampbrush W chromosome coupled with FISH have shown that the heterochromatic axis of the W chromosome consists of seven measurable chromomeres (chromomeres 1–7 as shown in Figure 1). The *Xho*I family sequences are contained in chromomere 3. The *Eco*RI family sequences are in chromomere 1 (the major fraction) and at the end of chromomere 5 (the minor fraction) (Solovei *et al.* 1993).

Repetition frequencies of the *Xho*I and the *Eco*RI family sequences can be widely variable within a chicken population. The *Xho*I family of White Leghorn chicken consists of about 30 000 copies of a 0.7-kb repeating unit, whereas only about one-sixth of them exist in the female genome of Fayoumi chicken, an Egyptian breed (Saitoh *et al.* 1991). For the *Eco*RI family, there seems to be two populations in the present-day chickens, one with about 4000 copies of the 1.2-kb repeating unit per genome and another with about 700 copies per genome (Saitoh *et al.* 1991).

In the present study, we have explored further the relationship between chromomeres and DNA repeat sequence families in the W chromosome of chickens. We have determined chromomere size using DAPI (4′,6-diamidino-2-phenylindole) staining, estimated relative sequence content using FISH and determined genomic sequence content using Southern hybridization.

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Materials and methods

White Leghorn and English Rhode Island Red chickens (*Gallus gallus domesticus*) were purchased from commercial suppliers. Fayoumi chickens were supplied from the National Institute of Animal Industry, Tsukuba, Japan.

Genomic DNA was purified from blood cells of an individual chicken as described previously (Saitoh *et al.* 1993). Southern blot hybridization was carried out at 65°C with a ^{32}P-labelled 0.7-kb *Xho*I family sequence from pUGD0600 (Kodama *et al.* 1987) or at 68°C with a ^{32}P-labelled 1.2-kb *Eco*RI family sequence from pUGD1202 (Saitoh *et al.* 1991) in the reaction mixture as described previously (Ogawa *et al.* 1997).

Lampbrush chromosome spreads from chicken oocytes were prepared as described previously (Solovei *et al.* 1993). Digoxigenin (DIG)-labelled pUGD1202 and biotinylated pUGD0600 were used as probes to detect *Eco*RI and *Xho*I family sequences respectively. The plasmid DNA was labelled by nick translation with DIG-11-dUTP (Boehringer Mannheim) or biotin-16-dUTP (Boehringer Mannheim), as described previously (Saitoh & Mizuno, 1992). FISH was performed according to Saitoh & Mizuno (1992). Preparations were observed under a Leica DRMB fluorescence microscope coupled with a CCD camera and a Cyto Vision image processing system (Applied Imaging).

Results

A chromomere consisting of the major fraction of *Eco*RI family sequences

Lampbrush ZW bivalents prepared from 10 indivdual English Rhode Island Red chickens, numbered from 1 to 10, were stained with DAPI and observed under the fluorescence microscope. A typical chromomere profile of the chicken W lampbrush half-bivalent (Figure 1) and those of the above 10 individuals (Figure 2) are compared. It is noted that chromomere 1, which contains the major fraction of *Eco*RI family (Solovei *et al.* 1993), is present in six (3, 4, 6, 7, 9 and 10) but undetectable in the other four (1, 2, 5 and 8) individuals. FISH was performed with a probe for *Eco*RI family sequences (DIG-labelled pUGD1202) and with a probe for *Xho*I family sequences (biotinylated pUGD0600) to the lampbrush chromosome preparation from a White Leghorn chicken, in which chromomere 1 is present (Figure 4, left chromosome), and to that from an English Rhode Island Red, in which chromomere 1 is undetectable (Figure 4, right chromosome). Intensities of FISH signals on chromomere 3, representing the *Xho*I family sequence, and at the end of chromomere 5, representing the minor fraction of *Eco*RI family sequences, are the same for both preparations, but the FISH signal for the major fraction of the *Eco*RI family is undetectable for the preparation lacking chromomere 1.

Genomic DNAs were purified from blood samples of the above 10 female individuals of English Rhode Island Red chickens and subjected to Southern blot hybridization with a ^{32}P-labelled 1.2-kb *Eco*RI family probe. The results show clearly that individuals 1, 2, 5 and 8 belong to the low-copy-number type and that the other six individuals belong to the high-copy-number type, as shown by Saitoh *et al*. (1991) (Figure 3). These two types correlate exactly with the cytological types of chromomere 1 shown in Figure 2.

Figure 1. A typical chromomere pattern of chicken W lampbrush chromosome after DAPI-staining. Seven chromomeres are numbered; arrow points to a chiasma between W and Z chromosomes; a bright chromomere in the proximal part of Z lampbrush chromosome is marked as Z. Bar indicates 10 μm.

Figure 2. W lampbrush chromosomes from 10 English Rhode Island Red chicken individuals (numbered by red figures) after DAPI staining. Chromosomes from chickens 1, 2, 5 and 8 do not have the brightest terminal chromomere 1, which is marked with yellow arrowheads in other six chickens. Green arrows show the position of chromomere 2.

Figure 3. Southern blot hybridization of *Eco*RI-digested genomic DNA with *Eco*RI repetitive family probe. The DNA was extracted from blood of the same individuals (numbered as on Figure 2) from which preparations of W lampbrush chromosomes were made. The chickens possess either high (3, 4, 6, 7, 9, 10) or low (1, 2, 5, 8) copy numbers of the repeat.

Figure 4. Results of FISH with probes for *Eco*RI and *Xho*I families on W lampbrush chromosomes with 7 (left pair) and 6 (right pair) chromomeres. The *Eco*RI family probe (red) hybridizes to two sites on W lampbrush chromosome, the major one on chromomere 1 and the minor one on the proximal end of chromomere 5. The W lampbrush chromosome lacking chromomere 1 has only the minor site. The *Xho*I family probe (light blue) hybridizes along the whole length of chromomere 3. Black and white images show chromomere pattern after DAPI staining; labelled chromomeres are numbered.

Figure 5. Southern blot hybridization of chicken genomic DNA with the *Xho*I repetitive family probe. Genomic DNA was extracted from the blood of White Leghorn (11) and Fayoumi (12–15) chickens, *Xho*I digested and loaded onto a gel at a concentration of 0.5 μg per lane for chicken 11, and at 2 μg per lane for chickens 12–15. Note that chicken 12 has a slightly lower amount of the repeat.

Figure 6. Chromomere patterns of W lampbrush chromosomes from four Fayoumi individuals (chickens 12–15) after DAPI-staining. Green arrows indicate a small chromomere 3; yellow arrowheads point to remarkably bright part of chromo- mere 1.

Figure 7. A typical chromomere pattern of Fayoumi W lampbrush chromosome after DAPI-staining. Note the presence of subchromomere 1a. The arrow points to a chiasma between the W and the Z chromosomes. A bright chromomere in the proximal part of Z lampbrush chromosome is marked as Z. Bar indicates 10 μm.

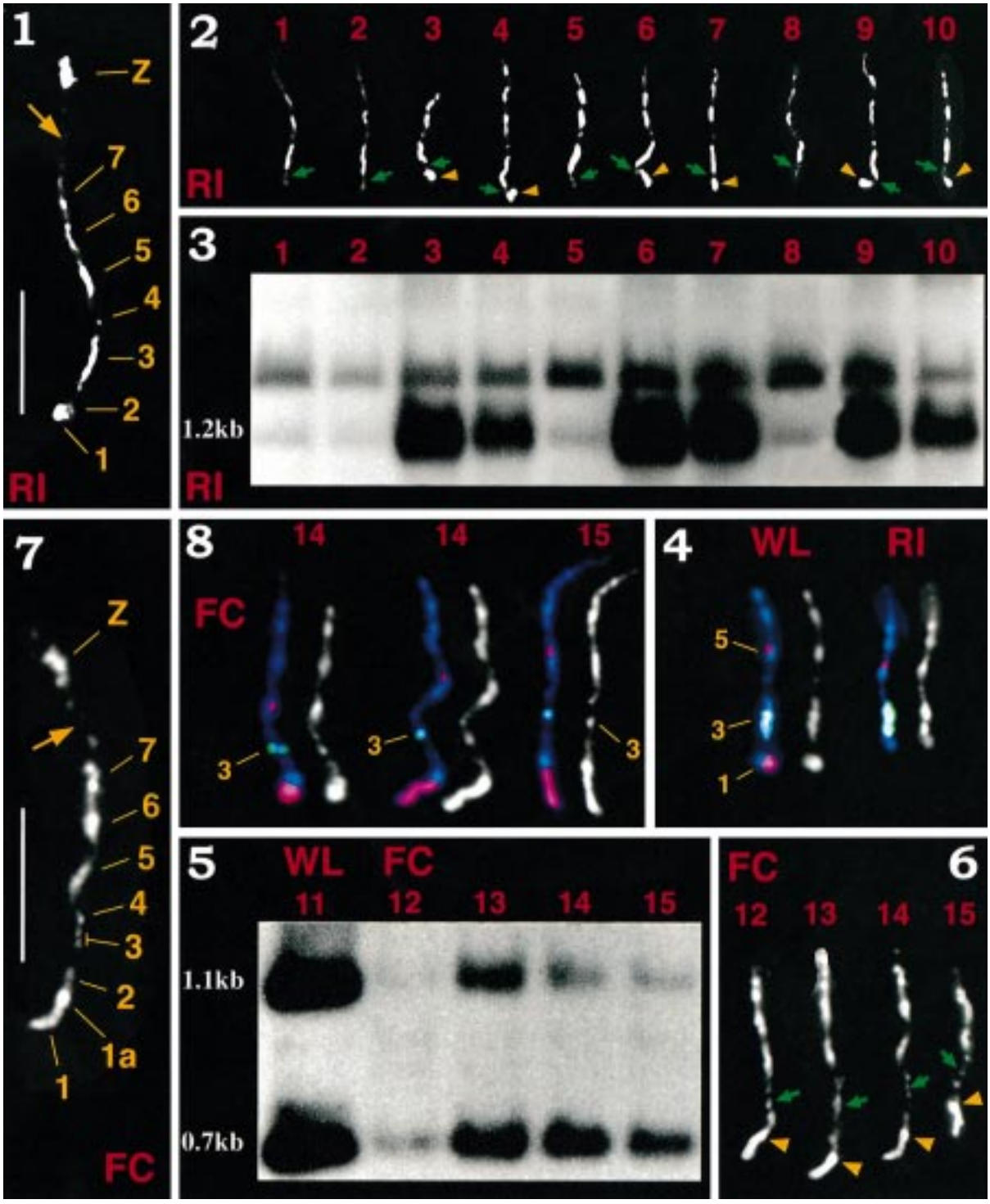

Figure 8. Results of FISH with probes for *Eco*RI and *Xho*I families on Fayoumi W lampbrush chromosomes isolated from chickens 14 and 15. The *Xho*I family probe (green) hybridizes to small chromomere 3 (compare with label of chromomere 3 on Figure 4); The *Eco*RI family probe (red) hybridizes to two sites, proximal part of chromomere 5 and distal part of chromomere 1; note that distal part of chromomere 1a is not labelled. Black and white images show chromomere patterns of W lampbrush chromosomes after DAPI staining. The source of chromosomes or DNA samples is marked as follows: RI, English Rhode Island Red chickens; WL, White Leghorn chickens; FC, Fayoumi chickens.

A chromomere consisting of the *Xho*I family sequence

When DAPI-stained lampbrush W chromosomes from four Fayoumi chickens (12–15) are observed under the fluorescence microscope, chromomere 3, which is conspicuously large in the preparations from White Leghorn and English Rhode Island Red chickens (Figures 1 & 4), is remarkably small in Fayoumi chickens (Figures 6–8). FISH with *Xho*I and *Eco*RI family probes on lampbrush chromosome preparations indicates that the intensity of the signal representing the *Xho*I family sequence in chromomere 3 is substantially lower in Fayoumi than in White Leghorn chickens (Figure 8 vs. Figure 4). Intensities of signals for the major and minor fractions of the *Eco*RI family in chromomeres 1 and 5, respectively, are similar between Fayoumi and White Leghorn (Figures 8 & 4). It is noted in Fayoumi that a brightly DAPI-stained chromomere 1a is present at the centromere proximal side of chromomere 1, to which the *Eco*RI family probe does not hybridize (Figures 7 & 8).

Southern blot hybridization to genomic DNAs from a female White Leghorn and from the four female Fayoumi individuals, which were used for the observation in Figures 6–8, demonstrates that the genomic contents of *Xho*I family sequence are substantially lower in these Fayoumi individuals (Figure 5), as noted by Saitoh *et al*. (1991).

Discussion

Chromomeres on the axis of lampbrush chromosomes in newts and salamanders have been shown to contain the majority of genomic DNA and to be transcriptionally inactive (Callan, 1986). Vlad & Macgregor (1975) counted chromomeres on the lampbrush chromosomes of two species belonging to the salamander genus *Plethodon* that had genome sizes in a ratio of approximately 2:1, although they had the same number and shapes of mitotic chromosomes. They found that the chromomere number was directly related to genome size. In these species, Cot curves of genomic DNA are nearly identical and the basis of the large difference in genome size seems to be accounted for by an increase in the amount and diversity of middle repetitive sequences (Mizuno & Macgregor 1974). One implication of this study was that individual chromomeres may represent clusters of individual families of moderately repeated DNA sequences.

The results described here support this general idea. The chromomere containing the *Xho*I family (chromomere 3) is small in Fayoumi chickens whose genomic content of *Xho*I family is about one-sixth or less than that in White Leghorn. The size of the chromomere containing most of the *Eco*RI family (chromomere 1) is likewise related to the genomic content of this family. These observations, together with an earlier report that a single macrosatellite family occupies almost the whole of the uniformly small chromomere and loops region near the end of the free arm of the Z lampbrush chromosome (Hori *et al*. 1996), demonstrate that a particular DNA repetitive sequence family may be confined to a particular lampbrush chromomere or set of chromomeres, and that the size of the chromomere is determined by repetition frequency of the sequence. Furthermore, our observations support the notion that amplification of new sequence elements in a genome leads to the formation of new sequence families and new chromomeres.

One of the remaining unanswered questions is why individual chromomeres in lampbrush chromosomes remain completely separate and discrete from one another, whereas in metaphase chromosomes adjacent sequence families are merged into a single rod of more or less uniform thickness. The question of the relationship between a chromomere and a pair of loops – which represent one or more active transcription units – also remains unanswered and is not assisted by our studies on the W lampbrush chromosome that seems to have distinct chromomeres but no loops.

Nevertheless, the chicken W chromosome has allowed us to demonstrate that the chromomeric pattern of a lampbrush chromosome is constant, locus-specific, species-specific and related to the nature and repeat frequencies of individual families of repeated DNA sequences.

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