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Interstitial $(TTAGGG)_n$ sequences are not hot spots of recombination in the chicken lampbrush macrochromosomes 1–3

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Abstract

To study the role of telomere $(TTAGGG)_n$ sequences in promoting of crossing over in chicken female meiosis, we have localized telomere repeats by FISH and studied the distribution of chiasmata in the giant diplotene bivalents, the chicken lampbrush macrochromosomes 1–3. We show that all interstitial clusters of the $(TTAGGG)_n$ repeat in these chromosomes do not coincide with hot spots of genetic recombination (crossing over) in the chicken female. Moreover, terminal TTAGGG-positive chromomeres also are not chiasma hot spots. We conclude that, at least in chicken macrochromosomes in female meiosis, a role for canonical telomere sequences in promoting of crossing over is not confirmed.

Introduction

The chromosomes of all studied Vertebrata terminate with clusters of the telomere repeat sequence $(TTAGGG)_n$ (Kipling 1995). In the chicken, like many other avian species, clusters of this sequence are also found at intrachromosomal sites (Nanda & Schmid 1994, Solovei *et al*. 1994, Delany *et al*. 2000, Nanda *et al*. 2002). Several studies have shown that the interstitial telomeric repeats could promote chromosome rearrangement and fragility (Bouffler *et al*. 1993, Day *et al*. 1998, Desmaze *et al*. 1999, Kilburn *et al*. 2001, Peitl *et al*. 2002). There is also some evidence that, in some mammalian species, interstitial telomere sites (ITSs) function as recombinational hot spots (Ashley *et al*. 1993, Day *et al*. 1998). In particular, a study of Armenian hamster spermatocyte meiotic chromosomes has shown that ITS detected in a large metacentric autosome was the site of chiasma hot spot (Ashley & Ward 1993). Nanda and co-workers (2002) proposed that a high density of $(TTAGGG)_n$ repeats contributed to the exceptionally high meiotic recombination rate of avian microchromosomes.

In the present paper, to study the role of $(TTAGGG)_n$ sequences in promoting crossing over in birds, we have compared the location of telomere repeats and chiasma positions in the chicken lampbrush chromosomes (LBCs). LBCs are giant diplotene bivalents that appear in the growing oocytes of hatching fowls (Kropotova & Gaginskaya 1984, Hutchison 1987). At this stage, each chicken bivalent has a distinctive loop-chromomere pattern that

TTAGGG
100%
A
B
C1B1
TTAGGG
C
C1C51
D
cen
E
TTAGGG
F
G
C1G1
H
C1111
I
C1121
J
TTAGGG
K
L
TTAGGG
a
b
c
d

allows colocalization of both TTAGGG-positive chromomeres and chiasmata with high precision (Rodionov *et al*. 2002). Our results show that interstitial clusters of the $(TTAGGG)_n$ repeat are not hot spots of genetic recombination (crossing over) in the largest chicken macrochromosomes in female meiosis.

Materials and methods

Chromosome preparation and analysis of chiasma distribution

LBCs were isolated manually from previtellogenic or early vitellogenic oocytes of 0.5–2.5 mm in diameter of sexually mature chicken (commercial line) employing the standard lampbrush technique (Solovei *et al*. 1992) with few modifications. Preparations were stained with Coomassie Blue R 250 (Serva) in 50% methanol as described by Macgregor & Varley (1988). Identification of bivalents was carried out by using the cytological map of the chicken LBCs published by Chelysheva *et al*. (1990) and Rodionov *et al*. (2002, 2005). LBCs were identified by their length and by morphological characteristics such as marker loops and DAPI-positive chromomeres. Figures 1b, c, 2b, c and 3b, c present the cytological maps of the three largest LBCs. Each LBC is conveniently split into several regions, e.g. LBC1 is divided into 12 regions A–L (Figure 1b, c). All regions are characterized by a modal length of lateral loops, few marker loops and chromomeres. On the map, marker chromomeres are designated by the letter C; the next character of the marker symbols shows the chromosome number, then a letter referring to the chromosome region name. A final character in each LBC marker symbol is an individual number of the chromomere within the chromosome region; for example, C1B1—the first chromomere of region B of LBC 1.

One hundred well-spread LBCs 1, 100 LBCs 2 and 57 LBCs 3 were selected and photographed to study chiasma distribution within bivalents.

Chiasmata were defined as criss-crosses and/or tight conjunction sites of homologous chromosomes delineated on either side by an identical loop-chromomere pattern. A total of 1883 chiasmata was observed in 257 macrobivalents 1–3. To estimate the mean distribution of chiasmata along chromosomal arms for each bivalent, LBC 1 was divided into 99 segments of equal size, LBC2 into 83, LBC3 into 70 segments. The results of chiasma counting were represented histogrammatically. Hot spots of recombination were defined as chromosome regions where the difference between the chiasma frequency and mean chiasma frequency was statistically significant according to the chi-squared test.

FISH

The sites of the $(TTAGGG)_n$ repeat in chicken LBCs were detected by using the plasmid pHuR 93 carrying 40 copies of the TTAGGG unit (Moyzis *et al*. 1988). It was labelled with digoxigenin-11-dUTP by the nick-translation kit (both from Boehringer Mannheim). The hybridization mixture contained 20 ng/μl probe DNA in 50% formamide, $2 \times$ SSC, pH 7.0 ($1 \times$ SSC is 0.15 mol/L NaCl, 0.015 mol/L sodium citrate), 10% dextran sulfate, 100 μg/ml *Saccharomyces cerevisiae* tRNA. Prior to hybridization, slides with LBCs were pretreated with pepsin (0.0002%) and Triton X100 as reported by Saifitdinova *et al*. (2003). Both probe and chromosomal DNA were denatured by heating at 82°C for 5 min. Hybridizations were performed overnight and the hybridization sites were visualized by rabbit anti-digoxigenin (Sigma) and Fluorolink™ Cy™3 labelled goat anti-rabbit IgG (Amersham Pharmacia Biotech). LBCs were counterstained with DAPI.

Slides were examined using a Leica DMRXA fluorescence microscope equipped with a black-and-white Cohu CCD camera and appropriate filter cubes. Leica QFISH software was used to acquire and process multicolour images.

Figure 1. Chicken lampbrush chromosome 1. **(a)**. FISH of $(TTAGGG)_n$ telomere probe on chicken lampbrush chromosome 1 stained by DAPI. The $(TTAGGG)_n$-positive sites (red signals) are indicated by arrowheads. Cytological **(b)** and chromomere **(c)** maps of the chicken lampbrush chromosome 1 show the landmarks of chromosome regions, some marker loops and chromomeres. Arrowheads indicate the $(TTAGGG)_n$ positive sites. **(d)**. Histogram of chiasma distribution along the bivalent divided into 2-μm intervals. The mean chiasma number is indicated in each segment. Arrows indicate chiasma hot spots, arrowheads—$(TTAGGG)_n$-positive sites. Scale bar represents 10 μm.

Results and discussion

Localization of (TTAGGG)$_n$ repeats in the chicken lampbrush chromosomes

As expected from telomere sequence mapping in chicken mitotic chromosomes (Nanda & Schmid 1994, Nanda *et al*. 2002), the TTAGGG-positive chromomeres were observed not only at the ends of all LBCs, but also in a few internal sites of LBCs 1, 2 and 3 (Figures 1a, 2a, 3a, respectively). Positions of both telomere and interstitial (TTAGGG)$_n$ sites have been mapped on the cytological LBC maps (Figures 1b, c, 2b, c, 3b, c).

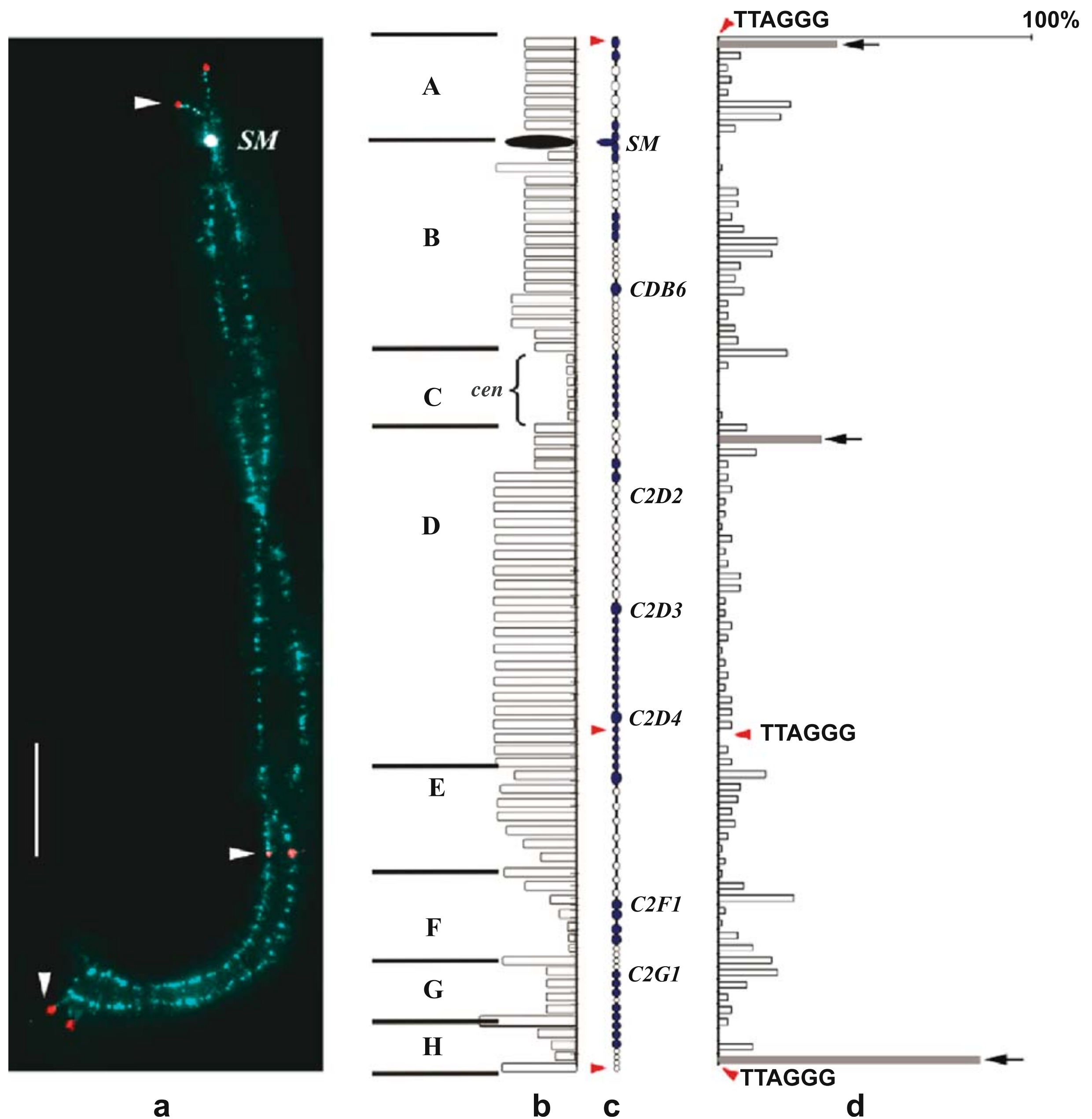

Figure 2. Chicken lampbrush chromosome 2. All indications as for Figure 1. SM: spaghetti marker.

Chicken LBC 1 has three prominent interstitial telomere sites in the regions 1C (ITS11), 1J (ITS13), and in the putative centromere region 1E (ITS12) (Figure 1a–c). Both LBC 2 and LBC 3 have only single clearly defined ITS-ITS21 in the region 2E, and ITS31 in region 3A, respectively (Figures 2a–c, 3a–c).

Determination of chiasma hot spots

According to our data, the mean chiasma frequency is 8.0, 6.3, and 4.5 chiasmata per LBCs 1–3, respectively. The cumulative distributions of all chiasma observed on each bivalent are presented in Figures 1d,

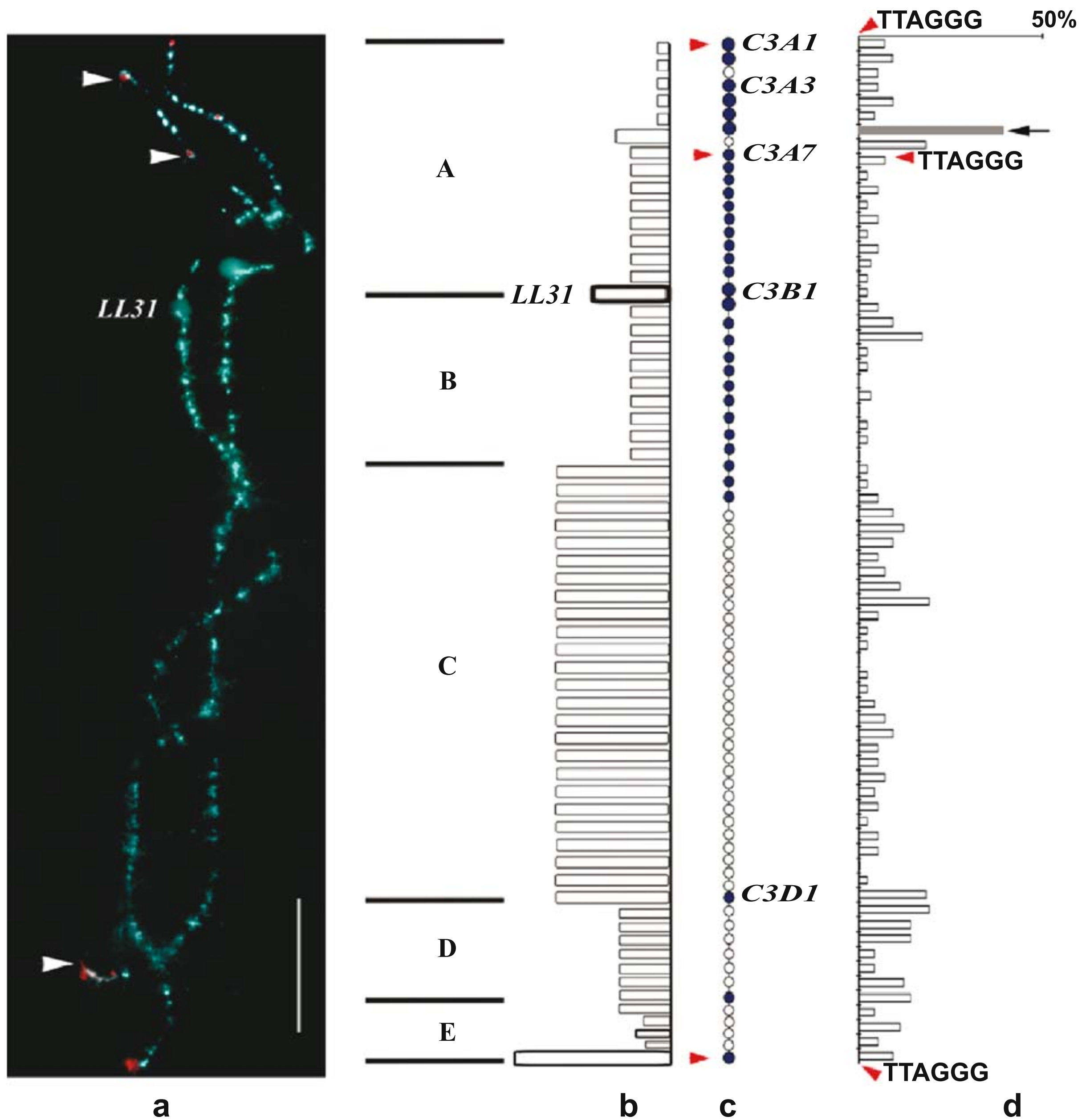

Figure 3. Chicken lampbrush chromosome 3. All indications as for Figure 1. LL31: marker lumpy loop.

2d and 3d. They show that chromosome regions are significantly different in the chiasma frequency. There are chiasma hot spots with considerable increased chiasma frequency and there is a decrease in chiasma frequency in the putative centromeric regions (1E for LBC1 and 2C for LBC2) (Figures 1d, 2d). LBC 1 has only one chiasma hot spot at the pter (1A region, arrow on Figure 1d). On LBC 2, there are three hot spots, two of them locate terminally and the other is situated in the 2D region (arrows on Figure 2d). A single chiasma hot spot is presented on LBC 3 in the middle of the 3A region (Figure 3d).

Relationship between $(TTAGGG)_n$ sites and chiasma hot spots

We have observed that, in all cases, the positions of chiasma hot spots did not coincide with $(TTAGGG)_n$ sites. Figures 1d and 2d show a high recombination frequency in the 1pter, 2pter and 2qter regions. Each of these telomeric regions consists of a few chromomeres with an average size of about 2.0 Mb (Rodionov *et al*. 2005). The detailed analysis of telomeric regions demonstrates that, as a rule, the chiasma localizes near TTAGGG-positive chromomere but not in the immediate chromomere (Figures 1a, 2a).

It can be concluded that, in chicken female meiosis, positions of chiasma hot spots do not associate with TTAGGG-positive chromomeres at least in the largest macrochromosomes.

Our data on the relationship between chiasma hot spots and $(TTAGGG)_n$ sequence argue against a role of interstitial $(TTAGGG)_n$ sequence in promoting of recombination events and disagree with the conclusions of Ashley & Ward (1993), who studied the distribution of chiasmata and interstitial $(TTAGGG)_n$ sequences on a single pair of large autosomes, probably 2, of Armenian hamster. They found that, in 69% spermatocytes examined, ITSs were the sites of chiasmata and, in the remaining cells, the chiasmata were situated near the telomere sequence signals. Therefore, all the chiasmata observed were associated with $(TTAGGG)_n$ sequences.

The discrepancies can be explained by the fact that LBCs of diplotene oocytes are less condensed than pachytene chromosomes of mouse spermatocyte nuclei. In particular, chicken chromosome 1 at the lampbrush stage is about 185 μm in length, that is 33 times longer than in metaphase, and 15 times longer than the length of meiotic bivalents in spermatogenesis (Chelysheva *et al*. 1990). Here, $(TTAGGG)_n$ sequence sites have been registered within a LBC chromomere, whose average size was estimated as 1.8–2.3 Mb (Rodionov *et al*. 2005).

It can be speculated also that chicken ITS and hamster ITS carry different classes of $(TTAGGG)_n$ sequences, one of which is stable to double-strand breakage and the other is unstable. Indeed, in the human genome, three classes of ITSs differing in origin and composition have been identified (Azzalin *et al*. 2001). It has been hypothesized that one of them (short ITSs) is not fragile and simply marks sites of double-strand breaks that occurred within unstable regions, whereas very extended internal blocks of $(TTAGGG)_n$ repeats could be unstable.

In conclusion it should be noted that, to date, the recombination hot spots in the genomes of higher Vertebrata are not predictable from primary DNA sequence alone, and no shared sequence has been observed among all the hot spots of recombination (Jeffreys *et al*. 2000, Petes 2001). It therefore appears that hot-spot activity may reflect open chromatin domains, as in yeast (Wu & Lichten 1994) which allows access of the meiotic recombinational machinery.

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