ISSN 1022-7954, Russian Journal of Genetics, 2010, Vol. 46, No. 10, pp. 1173–1177. © Pleiades Publishing, Inc., 2010.
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Chicken Lampbrush Chromosomes: Transcription of Tandemly Repetitive DNA Sequences

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Received January 28, 2010

Abstract—The transcribed part of the genome includes both protein-coding sequences and a variety of sequences with unknown functions. Amphibian and avian lampbrush chromosomes represent a convenient experimental system for studying cell functions and the regulation of transcription of protein-noncoding DNA. Taking lumpy loops formed on chicken (*Gallus gallus domesticus*) chromosome 2 at the lampbrush stage as an example, we applied an approach allowing RNA sources to be identified in the lateral loops of lampbrush chromosomes. This approach involves a bioinformatic analysis of data from the chicken genome sequencing project and a high-resolution mapping of transcripts on microsurgically isolated bivalents. As a result, a novel tandemly repetitive DNA sequence, LL2R (lumpy loop 2 repeat), of ~440 bp in size was identified in the chicken genome, its transcripts taking part in the formation of lumpy loops with a massive RNP matrix on chromosome 2 in growing oocytes.

DOI: 10.1134/S1022795410100078

Realization of the projects on deciphering genomes of several model organisms has led to the understanding that the more complicated is the organism the less is the portion of protein-coding sequences in the genome, while the number of functional protein-noncoding sequences increases [1]. Only a small part of transcribed DNA sequences encodes proteins in animal and plant cells [2], and among long and short RNAs synthesized by the cell numerous transcripts do not code for protein products, but are important for the realization of genetic information and regulation of gene expression [3–5].

Studying the cell functions of regulatory protein-noncoding RNAs and RNA organizers of nuclear domains in developing female germline cells is particularly important regarding the possibility of involvement of maternal RNA in functional compartmentalization of the germinal vesicle and in the regulation of the embryo genome [6]. It is convenient to analyze the functions of protein-noncoding DNA sequences in oogenesis using as a model system lampbrush chromosomes (LBCs) (Fig. 1a) that are characteristic of growing oocytes in many animal species [7, 8]. The main advantages of this system are giant sizes of LBCs, a relatively simple procedure of their microsurgical isolation from oocyte nuclei, possibility of mapping DNA sequences with an unprecedentally high resolution, thousands of morphologically distinguishable long transcriptional units, a high rate of RNA synthesis, and, what is particularly important, the feasibility of cytological analysis of cotranscriptional stages of RNA processing.

The classical studies of LBCs from oocytes of tailed and tailless amphibians made an essential contribution to the understanding of many problems concerning the organization and functions of the eukaryotic genome [9, 10]. It is with the use of lampbrush chromosomes that initial cytological evidence for satellite DNA transcription was obtained [11, 12]. The technique of LBC isolation adapted for avian oocytes (http://projects.exeter.ac.uk/lampbrush/protocols.htm) [13, 14] facilitated the introduction of the new object and the expansion of comparative studies in this field of cell biology. Not only unique, but also many tandemly repetitive DNA sequences are transcribed on LBCs. Irrespective of activation of the unique sequences, LBCs of many species are characterized by repression of transcription of some housekeeping genes (e. g., genes of ribosomal RNAs) [15, 16]. Thus, the significance of the phenomenon of chromosome transformation in growing oocytes into giant transcriptionally active LBCs has no adequate explanation as yet.

Among birds, LBCs are best characterized in members of the order Galliformes. Detailed cytological maps of the chromomere–loop pattern for all macrochromosomes and a scheme of the structure of microchromosomes of *Gallus gallus domesticus* at the LB stage have been constructed, and the centromeric regions have been identified, which is necessary for cytogenetic analysis [17–19]. Transcription of pericentromeric, subtelomeric, and interstitial arrays of tandemly repeated DNA sequences by hyperphosphorylated RNA polymerase II was revealed on chicken LBCs [18, 20, 21]. Based on analysis of transcription

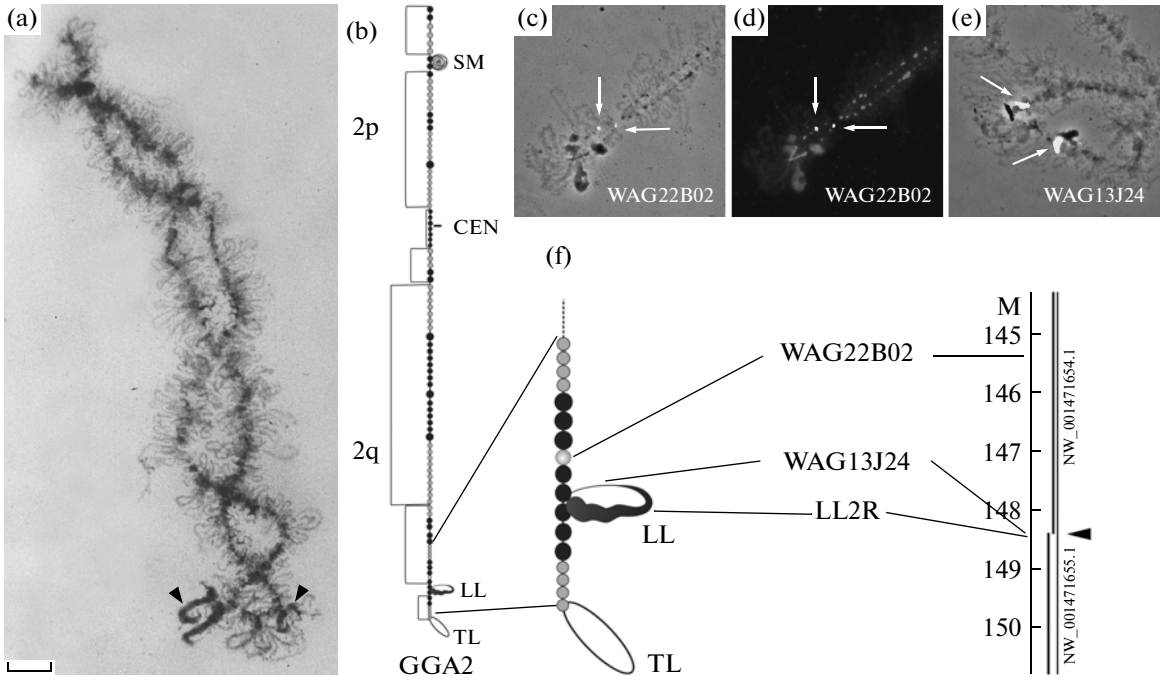

Fig. 1. The locus of lumpy loop formation on chicken chromosome 2 (GGA2). (a) chicken bivalent 2 at the lampbrush stage. Triangles indicate lumpy loops 21 (LL21) with a massive RNP matrix. Scale bar 10 μm. (b) the cytological map of chicken lampbrush chromosome 2 according to [17], with changes. Grey and black circles are DAPI-positive chromomeres. SM, spaghetti marker; CEN, centromere; LL, lumpy loop; TL, terminal loops. (c–e) fragments of chicken bivalent 2. Fluorescence DNA/(DNA + RNA) in situ hybridization with BAC clones WAG22B02 (145.4 Mb) (c, d) and WAG13J24 (148.4 Mb) (e), the arrows indicate hybridization signals. (f) a fragment of the cytological map of chicken lampbrush chromosome 2 with the mapped BAC clones and LL2R (at the left) and a scheme of contigs in the 145–150 Mb region of chicken chromosome 2 (at the right, information from the site http://www.ncbi.nlm.nih.gov/). The locus of LL21 formation in GGA2 corresponds to the gap between two contigs (triangle).

of tandemly repetitive noncoding DNA sequences on LBCs in members of the order Galliformes, tentative conclusions were made on the role of this transcription in oogenesis. Also, new details were added to the classical hypothesis of the lampbrush chromosome phenomenon [8, 18]. Transcripts of satellite repeats synthesized in oocytes may serve as the sources of short regulatory RNAs needed for heterochromatin formation in early embryogenesis.

In addition to so-called normal (or simple) loops, LBCs also carry loops with a specific morphology that are reliable markers in constructing cytological chromomere–loop maps of LBCs of different animals. A prominent example of such loops are interstitial, so-called lumpy loops, which may represent loop accumulators of certain RNP complexes in the oocyte nucleus. Lumpy loops (LL21) with an ample dense RNP matrix are consistently detected on the long arm of chicken chromosome 2 (GGA2) at the LB stage (Figs. 1a and b) [14, 17, 22]. The DNA sequences involved in the formation of lumpy loops on avian lampbrush chromosomes are still unknown, despite the fact that these structures are formed at strictly definite chromosomal loci [8]. We assumed that the unusual morphology of the lateral loops at these loci and(or) the composition of the RNP matrix of the transcriptional units in them may be associated with transcription of repeated sequences.

To elucidate the nature of lumpy loops that are formed on chicken giant LBCs, we studied the loci of their formation by mapping BAC clones containing fragments of chicken genomic DNA. We chose from the chicken BAC library (http://www.bioinformatics.nl/gbrowse/cgi-bin/gbrowse/) [23] the WAG22B02 clone containing DNA sequences of chromosome 2 (gene *v-MYC*) presumably located not far from the LL21 locus (~145.4 Mb in the identified GGA2 sequences, http://www.ncbi.nlm.nih.gov/). Hybridization on preparations of microsurgically isolated LBCs was carried out in accordance to standard protocols of DNA/(DNA + RNA) hybridization with the exception of pepsin treatment. The results of FISH with the WAG22B02 probe showed that on chicken lampbrush chromosome 2 the *v-MYC* gene is localized in a chromomere separated by a distance of two chromomeres (~3.0 Mb) from the lumpy loop (Figs. 1c, d, f). The next to be mapped on lampbrush chromosome 2 was the BAC clone WAG13J24 containing DNA fragments located in the same GGA2 contig in position ~148.39 Mb (Fig. 1e), i. e., at the same measured distance from the *v-MYC* gene. The DNA sequences cloned in this vector were detected with the FISH

(a)

AGATGGCTGTCAGAAACACTGCATCAGGCTTGGATAT**CCTGCAAGTAATGCGGTGAGC**AGCGTGCATGAGGGAGGGC
AGGGCAGTGAAGCCACGGGCGGCCTTTAGGGATGTTGGTGGCTGCTTCTCTGTGGTTGGTTTGTGTGTCTCTTGCAG
CCTGGCAGATGGATGCATGAGTTGACGTGTTTGTTGCAAGTCCTTGTCCTCTGCCTGAAAGAGTAAGGAAATGGGAG
ACCTGAGCCTTCTCTACTGGTAAGGTCATTTATGCCCACCCGTTTTTCATGCTGTTTTTCCTGGGGCTGAAATCTAC
ATTCTTGTGCTTTGAAGGGAGCTCTTTTGTTAGTGTGTGCAATCCACTGTGGCTCCTGCATCCCAAGTACTGGCCCA
GATACAGTGATGAATTTGCC**GGTGTTGGCCGCTGTCT**CTACATTTTTGACCAGGGTTTCCC

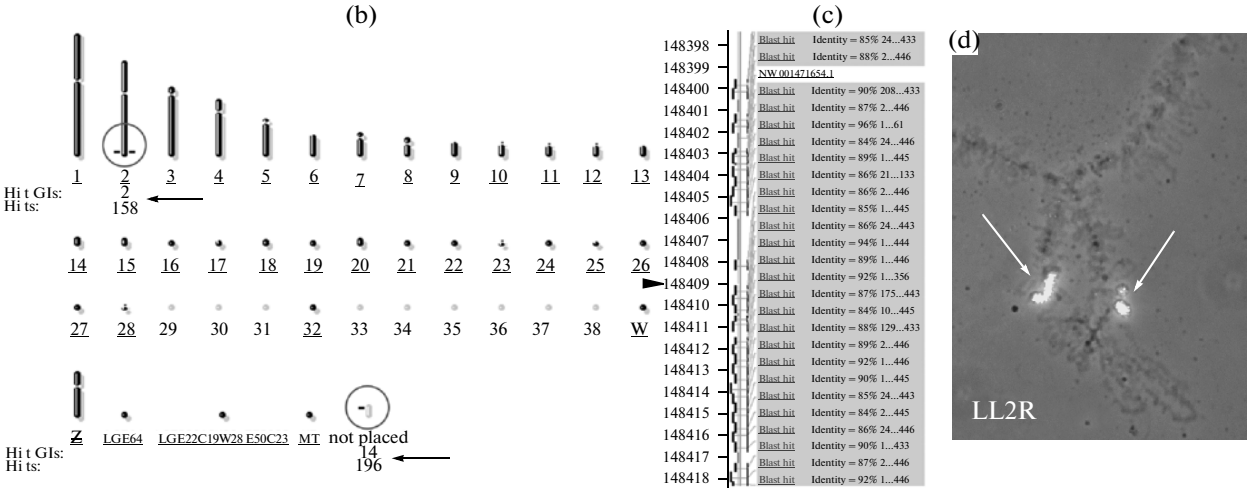

Fig. 2. The noncentromeric tandem repeat LL2R (lumpy loop 2 repeat) in the chicken genome. (a) LL2R consensus sequence. Fragments of the sequence corresponding to the primers are shown in bold type. (b) Regions of homology with LL2R (enclosed in a circle) in the identified sequences of chicken chromosomes (version 2.1) found using the BLAST program (Assembled Genomes). (c) Genome organization of the LL2R cluster on GGA2 (148397.5–148418.2 kb region). Regions of homology with LL2R are shown by short black lines. The gap between two contigs is indicated by a triangle. (d) Identification of LL2R transcripts in lumpy loops (arrows) on chicken bivalent 2 using fluorescence DNA/RNA in situ hybridization.

method directly at the basis of LL21 on chicken lampbrush chromosome 2; they were also found to be transcribed in the course of oogenesis (Fig. 1f).

The mapping results indicate that to the locus of LL21 formation on the GG2A sequence map there corresponds a gap in position 148.4 Mb (Fig. 1f). It might be suggested that this segment of chicken chromosome 2 consists of tandem repeats that are difficult to sequence. This suggestion is based on the fact that difficulties connected with cloning, determination of the primary DNA sequence, and reconstruction of extended fragments enriched with tandem repeats are the causes for the existence of gaps in the deciphered genome sequences. Thus, despite the fact that in December of 2004 the first step of the chicken genome sequencing was successfully completed [24], the primary structure of the centromeric, telomeric, and some other chromosomal segments remains to be determined.

Our subsequent experiments were aimed to identify primary sequences whose transcription seems to entail the formation of lumpy loops on chicken lampbrush chromosome 2. The bioinformatic analysis of the deciphered chicken genome revealed about 50 copies of a tandemly repeated ~446-bp DNA sequence in the contigs bordering the gap in position 148.4 Mb on GGA2 (Fig. 2b). In the only cluster of repeats of this sequence located on the long arm of the deciphered sequences of chromosome 2 (148395.5–148435.3 kb) all repeated elements are oriented in the same direction (Fig. 2c). The identified repeat was not found in the supercontigs of other chromosomes by means of alignment, but 14 clusters of the repeat are contained in the deciphered sequences that are not assigned to any definite chromosome (chromosome unknown, ChrUn) (Fig. 2b); the degree of similarity of the repeats within all clusters is 87.25%.

The tandem organization of the ~446-bp repeat in the chicken genome was confirmed by PCR with specific primers (LL2R_F: 5'-CCTGCAAGTAATGCGGTGAGC-3'; LL2R_R: 5'-AGACAGCGGCCAACACC-3'). Physical mapping of the amplified repeat on chicken LBCs microsurgically isolated from oocyte nuclei permitted the localization of its cluster on the long arm of chromosome 2 at a distance of 7 to 8 chromomeres from the terminal region, namely in the lumpy loops (Figs. 1f and 2d). We concluded that the identified tandemly repetitive ~446-bp DNA sequence is responsible for the formation of lumpy loops on lampbrush chromosome 2 in growing chicken oocytes and therefore called this repeat LL2R (lumpy loop 2 repeat).

When performing fluorescence DNA/RNA transcript in situ hybridization with the LLR2 probe with-

out denaturation of chromosomes, we discovered LL2R transcripts in the RNP matrix of lumpy loops on lampbrush chromosome 2 (Fig. 2d). Thus, the sources of RNA transcripts in special transcriptional units described over 20 years ago (forming lumpy loops) on chicken lumbrush chromosomes were identified for the first time. In addition, five sequences containing LL2R fragments were found in the chicken EST (expression sequence tags) library consisting of short sequenced cDNA fragments (SRA database; Specialized BLAST; http://ncbi.nih.gov) (similarity with consensus from 85 to 92%), which confirms the possibility of transcriptional activation of LL2R and suggests that the transcripts obtained are polyadenylated. At the same time, LL2R does not have open reading frames.

The mechanism of initiation of LL2R transcription is unknown at present. It was shown in early biochemical studies carried out by Davidson et al. [6] that a high proportion of polyadenylated transcripts synthesized in the oocyte during the LB stage was accumulated in the cytoplasm and remained stable up to the oocyte maturation. Most (70%) of RNA molecules in the oocyte cytoplasm are represented by extremely long chimeric transcripts containing a coding sequence and a dispersed repeat. These transcripts are unlikely to be translated and may have the regulatory function in early embryogenesis [6]. We suppose that transcription of tandem repeats occurs as a result of activation of retrotransposons or their long terminal repeats that are detected in the arrays of satellite DNA by the in silico analysis [21].

The computer-aided analysis of the primary sequence of RNA molecules encoded by satellite repeats with the use of the program GENSCAN (http://genes.mit.edu/GENSCAN.html) [25] showed that the products of LL2R transcription contain potential binding sites for the spliceosome components (small nuclear RNAs U1 and U2). On the contrary, all presently known transcribed pericentromeric sequences in the genomes of chicken (CNM, PO41), Japanese quail (*Bgl*II repeat, PO41), and rock pigeon (PR1) lack canonic 5'- or 3'-sites of splicing.

The cotranscriptional stages of processing of newly synthesized transcripts, including noncoding RNA, can be judged from the components of the RNP matrices of corresponding lateral loops on LBCs [26]. It was shown earlier with the use of immuno-FISH that transcripts of tandem 41-bp repeats (CNM, *Bgl*II repeat, and PO41) on chicken and Japanese quail LBCs do not recruit mature small nuclear RNAs (snRNAs) and the splicosome Sm proteins binding to them [21]. Using the same approach, we found that in contrast to RNA transcribed on LBCs from 41-bp repeats, LL2R transcripts in lumpy loops having an ample RNP matrix are colocalized with snRNAs containing a trimethylguanosine cap, with the snRNP Sm proteins, and with the SR protein SC35 that are involved in splicing. Hence, the noncoding RNA of the satellite LL2R sequence on LBCs is associated with the RNA splicing factors. We suppose that analogously to the noncoding NEAT RNA, LL2R transcripts can comprise a population of structural RNA responsible for the formation of special loops on chromosomes and recruitment of the splicing factors.

ACKNOWLEDGMENTS

We thank Professor M. Groenen and Professor R. Crooijmans for providing BAC clones from the Wageningen chicken BAC library (Wageningen, Netherlands).

The work was carried out with the use of the equipment of the Chromas Core Facility and supported by the Federal Education Agency (SC P1367 of September 2, 2009), and the Russian Foundation for Basic Research (project no. 08-04-01328).

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