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Brief report
Chromosomal localization of three GGA4 genes using BAC-based FISH mapping: a region of conserved synteny between the chicken and human genomes

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The chicken homologues of three genes spaced widely on HSA4q, *ANXA5* (annexin A5), *ALB* (albumin) and *EDNRA* (endothelin receptor type-A) have been mapped to the GGA4 linkage map but with some uncertainty as to their relative location (SCHMID et al. 2000; SUCHYTA et al. 2001). A cytogenetic map assignment has been made only for the *ALB* gene (SUZUKI et al. 1999). Here we report the chromosomal localization of all three genes on GGA4 by fluorescence in situ hybridization (FISH) using BAC clones as probes.

MATERIAL AND METHODS

Polymerase chain reaction (PCR) fragments representing chicken genes *ALB* (preproalbumin, accession number X60688), *ANXA5* (annexin A5, accession number M30971) and *EDNRA* (endothelin type A preceptor, accession number AF040634) were amplified from chicken genomic DNA using the following primer pairs:

ALB forward, 5′-GTACTCCCAAGGCAGGCT-3′
ALB reverse, 5′-GGGCTTGCGTTTAATGAGGT-TG-3′
ANXA5 forward, 5′-AGGGGCTGGCACTGATGA-TGATAC-3′
ANXA5 reverse, 5′-TCTGAGGAGGAGGGACTG-TT-3′
EDNRA forward, 5′-TACCACAATCTTCTTACCC-GACTG-3′
EDNRA reverse, 5′-GGCACTGGCATTTTGACC-TT-3′

Screening of gridded genomic Jungle Fowl BAC library (TAMU library code 031-JF256-BI; http://hbz.tamu.edu/bacindex.html) filters was performed as described (LEE et al. 2003; ROMANOV et al. 2003). Confirmation of BAC candidates positive for a certain gene was conducted by Southern blot hybridization and PCR tests. Southern blots of

Table 1. *Summary of mapping results.*

Locus	BAC clone ID	Confirmation*	Chromosomes analyzed	FLpter ± standard deviation	Band assignment	Location on HSA4**
ALB	b051J16	SH, PCR, FISH	25	0.36 ± 0.089	4q11–q12	4q11–q13 (~ 74.8 Mb)
ANXA5	b082B13	SH, FISH	14	0.72 ± 0.047	4q13–q21	4q28–q32 (~ 123 Mb)
EDNRA	b001H3	PCR, FISH	17	0.40 ± 0.033	4q11–q12	4q31.22 (~ 149 Mb)
EDNRA	b003M6	PCR, SH-OVERGO, FISH	10	0.37 ± 0.063	4q11	4q31.22 (~ 149 Mb)

*Tests used to confirm positive clones: SH, Southern blot hybridization using PCR-amplified DNA fragment; SH-OVERGO, Southern blot hybridization using OVERGO probe; PCR, polymerase chain reaction; FISH, fluorescence in situ hybridization.

**Cytogenetic location and, in parentheses, approximate DNA sequence coordinates (Build 34 Version 1) from the National Center for Biotechnology Information, NIH, Map View (http://www.ncbi.nlm.nih.gov/mapview).

putative BAC DNAs were hybridized to the amplified gene fragments used for the library screen, and hybridization patterns were compared to those observed with genomic DNA. In the case of the *EDNRA* gene, an OVERGO probe (Romanov et al. 2003) also was employed that had the following sequence: TTGCTGACTTTGCTCATTTGGGACTTCGTGA-TGGAAGCC (39-mer). Both conventional and OVERGO probes were hybridized to a blot containing a panel of candidate BACs.

Chicken metaphase spreads were prepared from female fibroblast cultures derived from white leghorn embryos. BAC clones b051J16, b082B13, b001H3 and b003M6 were biotinylated by nick-translation and hybridized to chicken mitotic chromosomes under conditions in which repetitive sequence binding is blocked (Masabanda et al. 1998). Hybridization signals were recorded with a fluorescent microscopic workstation including CCD camera and VideoTest-FISH software package (Ista, St Petersburg, Russia). Overlap of the DAPI (counterstain) and FITC (signal) grayscale images was achieved using the VideoTest-FISH software package (Ista, St Petersburg, Russia). The site of hybridization was assigned to a chromosome band based on the standard chicken karyotype (Ladjali-Mohammedi et al. 1999) by measuring the distance between the signal and the telomere of the p-arm in relation to the length of the entire chromosome and then calculating the fractional length (FLpter).

RESULTS AND DISCUSSION

Lee et al. (2003) described the isolation of one BAC containing the *ALB* gene, four for the *ANXA5* gene, and four for the *EDNRA* gene. Subsequently, BACs b082B13 and b001H3 were also confirmed as positives for *ANXA5* and *EDNRA*, respectively. Specificity of the BAC clones was verified by Southern blot and/or PCR tests (Table 1). Clones b051J16 (*ALB*), b082B13 (*ANXA5*) and b001H3 and b003M6 (*EDNRA*) were assigned to GGA4 by FISH (Table 1, Fig. 1). All three genes were mapped to the general area of the 4q11–q24 segment. Conserved synteny of GGA4q11–q24 with HSA4p16–q28 was previously observed by both chromosome painting and linkage map analysis (Juneja et al. 1982; Chowdhary and Raudsepp 2000; Schmid et al. 2000; Suchyta et al. 2001). Several chicken QTLs were also mapped to GGA4q11–q24 (Tuiskila-Haavisto et al. 2002). Previously, the *ALB* gene was mapped to GGA4q16–q21 by Suzuki et al. (1999) using a cDNA FISH probe. With the much larger BAC probe employed here, a location of GGA4q11–q12 was obtained. *ALB* (158 cM) and *ANXA5* (157 cM) map

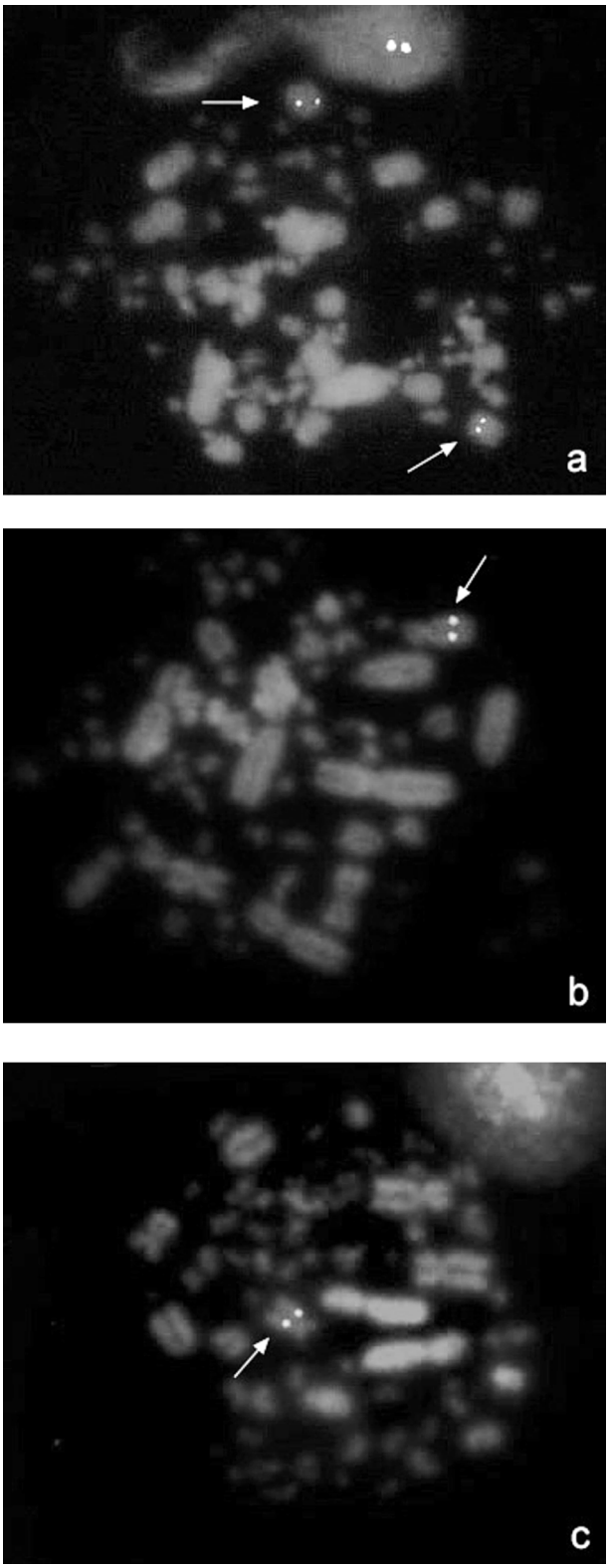

Fig. 1. Fluorescence in situ hybridization of BAC clones on chicken mitotic chromosomes. (**a**) b051J16 (*ALB*). (**b**) b082B13 (*ANXA5*). (**c**) b001H03 (*EDNRA*). Arrows indicate the sites of specific hybridization. Scale bar: 10 μm.

to essentially the same location in the consensus linkage map of SCHMID et al. (2000), but could not be positioned very precisely, due to a limited number of informative meioses. SUCHYTA et al. (2001) placed *ALB* (132 cM) and *EDNRA* (109 cM) on the East Lansing reference linkage map, but *ANXA5* has not been mapped in this population. Thus, the relative order of these three genes has been uncertain. Although the *EDNRA* and *ALB* genes are close to one another by FISH analysis alone, our results are consistent with an order of *EDNRA-ALB-ANXA5* in the centromere to telomere direction on GGA4q. On HSA4q, the order is *ALB-ANXA5-EDNRA*. Thus, there has been at least one inversion in the separate evolution of this region, consistent with the comparative map of SUCHYTA et al. (2001).

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