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Chromosomal localization of seven HSA3q13→q23 *Not*I linking clones on chicken microchromosomes: orthology of GGA14 and GGA15 to a gene-rich region of HSA3

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Abstract. Double-color fluorescence in situ hybridization was performed on chicken chromosomes using seven unique clones from the human chromosome 3-specific *Not*I linking libraries. Six of them (NL1-097, NL2-092, NL2-230, NLM-007, NLM-118, and NLM-196) were located on the same chicken microchromosome and NL1-290 on another. Two chicken microchromosome GGA15-specific BAC clones, JE024F14 containing the *IGVPS* gene and JE020G17 containing the *ALDH1A1* gene, were cytogenetically mapped to the same microchromosome that carried the six *Not*I linking clones, allowing identification of this chromosome as GGA15. Two GGA14-specific clones, JE027C23 and JE014E08 containing the *HBA* gene cluster, were co-localized on the same microchromosome as NL1-290, suggesting that this chromosome was GGA14. The results indicated that the human chromosomal region HSA3q13→q23 is likely to be orthologous to GGA15 and GGA14. The breakpoint of evolutionary conservation of human and chicken chromosomes was detected on HSA3q13.3→q23 between NL1-290, on the one hand, and six other *Not*I clones, on the other hand. Considering the available chicken-human comparative mapping data, another breakpoint appears to exist between the above *Not*I loci and four other genes, *TFRC*, *EIF4A2*, *SKIL* and *DHX36* located on HSA3q24→qter and GGA9. Based on human sequences within the *Not*I clones, localization of the six new chicken coding sequences orthologous to the human/rodent genes was suggested to be on GGA15 and one on GGA14. Microchromosomal location of seven *Not*I clones from the HSA3q21 T-band region can be considered as evidence in support of our hypothesis about the functional analogy of mammalian T-bands and avian microchromosomes.

The avian genome is characterized by an extremely high level of karyotype conservation within the class Aves and specific for warm-blooded animals structural compartmentalization (presence of few macrochromosomes and numerous, morphologically almost indistinguishable microchromosomes) (Shetty et al., 1999; Guttenbach et al., 2003). Due to this, the avian genome is considered as a very promising model for comparative functional genomics analyses. Exploration of features in the structural and functional organization of chicken microchromosomes could be useful for both revealing minimally required elements of eukaryotic chromosomes and understanding evolutionary karyology of vertebrates. On the other hand, an unexpectedly high level of orthology between human and chicken chromosomes, which is comparable even with evolutionary conservation within mammals (Burt et al., 1999; Schmid et al., 2000), enables us to apply much more

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detailed data on mammalian genome organization to avian species.

Among birds, chicken undoubtedly represents the best-studied organism, for which the intrachromosomal localization of about 250 type I markers is known (Schmid et al., 2000; International Chicken Genome Sequencing Consortium, 2004). Additionally, many physically assigned large insert clones may contain coding sequences, which are applicable for comparative genome anchoring. Recent completion of the first draft of the chicken whole genome sequence opens new research avenues and opportunities in this respect (International Chicken Genome Sequencing Consortium, 2004).

Studies of functional genome organization could be accelerated by the application of universal gene markers capable to distinguish coding from non-coding regions of the genome. A good example of such markers are CpG islands that are related to the presence of genes (Bird, 1987). In turn, it was also demonstrated that *Not*I restriction sites are associated with CpG islands (Larsen et al., 1992; Allikmets et al., 1994; Protopopov et al., 1996). So, unique sequences flanking *Not*I restriction sites (*Not*I-STSs) could serve as universal markers for the major portion of vertebrate genes. *Not*I linking clones include pairs of sequences flanking a single *Not*I recognition site, while *Not*I jumping clones contain DNA sequences spanning between neighboring *Not*I restriction sites. Such clones were shown to be tightly associated with CpG islands and genes (Bird, 1987; Kashuba et al., 1999). Three libraries of genome clones with high gene density were established for human chromosome 3 (HSA3) based on monosomic hybrid cells containing HSA3 as the only source of human DNA (Kashuba et al., 1995, 1999). It was experimentally confirmed that there is a certain statistical connection between CpG islands, *Not*I sites and expressed sequences in the human genome (Zabarovsky et al., 2000). The contigs of *Not*I clones were created for HSA3p21→p14 and HSA3q13→q23 by means of FISH and RH mapping (Kashuba et al., 1999; Sulimova et al., 2002). Maximal densities of *Not*I clones within the contigs were shown in T-bands on HSA3p15, HSA3p21.3, HSA3q21 and HSA3q29 (Protopopov et al., 1996).

The *Not*I linking clones were used in several investigations on human chromosomes to characterize functional features of genomic regions (Kashuba et al., 1999; Zabarovsky et al., 2000; Sulimova et al., 2002). In the present study, we applied seven *Not*I linking clones from HSA3q13→q21 for heterologous in situ hybridization on chicken chromosomes to detect (1) regions of orthology to HSA3q13→q21 and (2) chromosomal regions with high density of CpG islands and coding genes.

Materials and methods

Probe description

The *Not*I linking clones used in this study were NL1-097, NL1-290, NL2-092, NL2-230, NLM-007, NLM-118, and NLM-196 (http://www.mtc.ki.se/groups/zabarovsky/base/bindex.htm; Table 1). They include human genomic fragments that were cloned into SK17 and SK22 vectors using *Sal*I restriction enzyme to construct the plasmids (Zabarovsky et al., 1994). DNA transformation of the *E. coli* strain DH5α and DNA preparation were done according to standard protocols (Sambrook and Russell, 2001).

By using the overgo probe hybridization approach (Romanov et al., 2003) to screen the chicken genomic BAC library TAM32 (Lee et al., 2003; Ren et al., 2003), we previously detected the following BAC clones: JE014E08, JE027C23 (*HBA@*, GGA14), JE024F14 (*IGVPS*, GGA15) and JE020G17 (*ALDH1A1*, GGA15) (http://poultry.mph.msu.edu/resources/Resources.htm#bacdata). These BACs were used here for double-color FISH.

The HSA3-specific *Not*I clones NL1-097, NL1-290, NL2-092 and NLM-196 were biotinylated (bio), and the clones NL2-230, NLM-007 and NLM-118 as well as the BACs JE027C23, JE014E08, JE024F14 and JE020G17 were digoxigenin labeled (dig) by nick translation.

Slide preparation

Preparations of mitotic chromosomes were obtained from cells of the 96-hour embryos of Brown Leghorn chickens by consecutive incubation with colchicine, hypotonic treatment, and fixation. Colchicine (0.05%; Acros Organics, Geel, Belgium) was injected into the air chambers (20 µl per egg), followed by incubation at 37°C for 1 h. The embryos were removed from the eggs to hypotonic solution (1.25% sodium citrate), gently suspended and incubated at 37°C for 20 min. The cells were collected and treated three times with ice-cold fixative solution (methanol and glacial acetic acid, 3:1), and then air-dried on glass slides.

Double-color FISH

This procedure was performed essentially as described by Florijn et al. (1995). Chromosome slides were hardened at 55°C for 2 h, treated with 100 µg RNase A 100 (Sigma-Aldrich, St. Louis, USA) at 37°C for 30 min and washed three times with 2× SSC. Then, the slides were treated with 0.001% protein kinase K solution (Serva, Wichita Falls, USA) at 37°C for 10 min, and rinsed in PBS and in 2× SSC. The slides were treated with 4% paraformaldehyde (pH 7.0) at room temperature for 10 min and washed three times in 2× SSC, dehydrated in an ethanol series and air-dried. Afterwards, the chromosome slides were denatured at 70°C for 2 min in 70% formamide, 2× SSC, and dehydrated in an ethanol series at 4°C. The labeled DNA probes were ethanol precipitated with a 20- to 100-fold excess of competitive alkali sheared total chicken genomic DNA and 1000-fold excess of sonicated herring DNA (Sigma-Aldrich), mixed with an equal volume of hybridization solution to yield a final concentration of 50% formamide, 2× SSC, 10% dextran sulfate, 1 mg/ml BSA (Roche, Basel, Switzerland), denatured at 95°C for 5 min and prehybridized at 37°C for 20 min. A 20-µl mixture containing 200 ng each of biotinylated and digoxigenin-labeled probe was placed on the denatured slides, covered with cover slips, and incubated at 37°C for 36 h.

The slides were washed three times: in 50% formamide, 2× SSC at 37°C for 20 min, and then at room temperature in 2× SSC and 0.1× SSC for 20 min each. After being rinsed in 4× SSC, 1% Tween 20, the slides were incubated at 37°C for 30 min using 3% blocking reagent (Roche), 4× SSC, 1% Tween 20, and washed with 2× SSC at 37°C for 15 min. Under each cover slip, 20 µl of 5-µg/ml solution of conjugate avidin-FITC (Sigma-Aldrich) and 50 µl of 1:250 diluted solution of anti-digoxigenin-rhodamine conjugate (Roche) were added, and slides were incubated at 37°C for 30 min. The slides were washed three times (5 min each) with 4× SSC, 1% Tween 20. After that, 20 µl of 5-µg/ml anti-avidin-FITC antibody solution (Sigma-Aldrich) and 50 µl of 1:250 diluted antibody against anti-digoxigenin-rhodamine conjugate (Roche) were added under each cover slip and the slides were incubated at 37°C for 30 min. The slides were subsequently washed three times (5 min each) with 4× SSC, 1% Tween 20 and DAPI-counterstained in Vectashield antifade solution (Vector, Burlingame, USA).

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 (blue counterstain), rhodamine (red) and FITC (green) images was achieved using the VideoTest-FISH software package (Ista).

Results

Hybridization of the NotI linking clones

Following co-hybridization of the pairs NLM-118 dig/NL2-092 bio, NLM-007 dig/NL1-097 bio, NLM-007 dig/NLM-196 bio, NL2-230 dig/NLM-196 bio and NL2-230 dig/NL2-092 bio, strong signals were observed for each probe pair on one

Table 1. Human NotI clones used for FISH on chicken chromosomes and the appropriate sequence homology and comparative mapping information

*Not*I clone name [alias]	Insert size (kb)	Clone location on HSA3[a]	Sequenced fragment size (bp) [GenBank acc. no.]	ENSEMBL BLASTView alignment location[b]	Homology and comparative mapping information (GenBank acc. no.; identity percentage for longest alignment, if more than one)[c]
NL1-097 [HSNL197D, HSNL197R]	4.8	3q21-q23	248 [X87512], 327 [X87513]	HSA4p16.1; 9869150 to 9869203 (X87512) and 9869364 to 9869477 (X87512)	HSA4 CSA:38490 contig 38490 scaffold 9536 (AADC01038491; 86%) HSA4 WGA:25521 contig 25521 scaffold 1010 (AADB01025522; 86%) HSA4 WGSA:42995 contig 42995 scaffold 75 (AADD01042996; 92%) HSA4p16.1 *WDR1*, mRNA (NM_017491; 99%) MMU5 clone rp23-115a12 strain C57BL/6J (AC093570; 99%) MMU5 clone rp23-268d19 strain C57BL/6J (AC084070; 99%) MMU5 rp23-376e18 strain C57BL/6J (AC084322; 99%) MMU5 *Wdr1*, mRNA (AK004644; 99%) RNO14 LOC360950 similar to Wdr1 protein, mRNA (XM_341228; 95%) GGA4 Cont31.191 (AADN01046670; 100%) GGA4 LOC422842 similar to WDR1 protein, mRNA (XM_420788; 100%)
NL1-290 [HSNL1290D, HSNL1290R]	8.0	3q13.3-q21	419 [X87500], 255 [X87501]	HSA3q21.3; 127563870 to 127564125 (X87501) and 127564118 to 12756465 (X87500)	HSA3 CSA:33308 contig 33308 scaffold 8140 (AADC01033309; 98%) HSA3 WGA:22032 contig 22032 scaffold 854 (AADB01022033; 98%) HSA3 WGSA:37384 contig 37384 scaffold 66 (AADD01037385; 98%) HSA3q21.3 *CHST13*, mRNA (AY120869; 96%) GGA12 cDNA clone ChEST275f7, mRNA (BU465241; 90%)
NL2-092 [HSDNASEAQ, HSDNASEAP]	9.5	3q21	379 [Z22356], 431 [Z22357]	HSA3q21.1; 124066973 to 124067327 (Z22356) and 124067319 to 124067507 (Z22357)	HSA3 CSA:33063 contig 33063 scaffold 8050 (AADC01033064; 93%) HSA3 WGA:21881 contig 21881 scaffold 847 (AADB01021882; 93%) HSA3 WGSA:37140 contig 37140 scaffold 66 (AADD01037141; 93%) HSA3q21.1 *SEMA5B*, mRNA (AB040878; 95%) GGA7 cDNA clone ChEST904g7, mRNA (BU224821; 80%)
NL2-230 [NL2A230R, HSZ94789; HSDNASAAU]	9.0	3q21	303 [Z94789], 351 [Z22257]	HSA3q21.3; 128712709 to 128712921 (Z94789) and 128712913 to 128713259 (Z22257)	HSA3 CSA:33348 contig 33348 scaffold 8140 (AADC01033349; 96%) HSA3 WGA:22006 contig 22006 scaffold 852 (AADB01022007; 96%) Human chromosome U WGA:135109 contig 135109 scaffold 38143 (AADB01135110; 94%) HSA3q21 *ABTB1*, mRNA (NM_172027; 87%) GGA12 Cont16.220 (AADN01027940; 89%) GGA12 LOC416026 similar to *ABTB1*, mRNA (XM_414366; 88%)
NLM-007 [HSDNASJAD, HSDNASJAC]	5.6	3q21	257 [Z22474], 299 [Z22473]	HSA3q21.3; 129765846 to 129766057 (Z22474), 129766103 to 129766128 (Z22473) and 129766168 to 129766395 (Z22473)	HSA3 WGA:22135 contig 22135 scaffold 857 (AADB01022136; 98%) HSA3 WGSA:37508 contig 37508 scaffold 67 (AADD01037509; 98%) HSA3q21.3 *RAB7*, mRNA (AK094449; 87%) GGA12 cDNA clone ChEST950l22, mRNA (BU412189; 85%)
NLM-118 [NLMA118D, HSZ95363; NLMA118R, HSZ95362]	7.0	3q21	313 [Z95363], 312 [Z95362]	MMU11 A3.3; 26491585 to 26491895 (Z95363) and 26491894 26492207 (Z95362)	MMU11 clone RP23-69H10 (AL929272; 97%) MMU11 *Vrk2*, mRNA (AK089825; 98%) GGA3 cDNA clone ChEST918e4, mRNA (CD217613; 85%)
NLM-196 [HSDNASIAS; NLMA196S, HSZ95365]	4.3	3q21	344 [Z22463], 332 [Z95365]	HSA3q21.3, 129591279 to 129591615 (Z95365), 129594915 to 129595138 (Z22463) and 129595160 129595258 (Z22463)	HSA3 WGA:22124 contig 22124 scaffold 855 (AADB01022125; 98%) HSA3 WGSA:37493 contig 37493 scaffold 67 (AADD01037494; 98%) HSA3 CSA:33417 contig 33417 scaffold 8140 (AADC01033418; 98%) HSA3q21.3 DKFZp547K1610 (hypothetical protein), mRNA (AL713779; 98%) GGA5(?) cDNA clone ChEST648d12 5', mRNA (BU257582; 87%)

[a] http://www.mtc.ki.se/groups/zabarovsky/base/bindex.htm.
[b] http://www.ensembl.org/Multi/blastview.
[c] http://www.ncbi.nlm.nih.gov.

pair of microchromosomes of comparatively large size (Fig. 1). Co-hybridization of NLM-118 dig/NL1-290 bio and NL2-230 dig/NL1-290 bio resulted in signals on two different microchromosomes for each probe pair (Fig. 1). This allowed us to conclude that the clones NL1-097, NL2-092, NL2-230, NLM-007, NLM-118, and NLM-196 were located on the same microchromosome, but separately from NL1-290.

Chromosome identification

Two large insert DNA probes, specific for chicken microchromosomes of comparable size to those observed (GGA14 and GGA15), were selected for chromosome identification. Successive co-hybridizations of the pairs NL1-290 bio/JE024F14 (GGA15) dig and NL1-097 bio/JE027C23 (GGA14) dig resulted in signals on different microchromosomes. In case of co-hybridizations of the probe pairs NLM-196 bio/

JE024F14 (GGA15) dig and NL1-290 bio/JE027C23 (GGA14) dig the signals were observed on the same microchromosome for each pair (Fig. 1). GGA15 specificity of the *Not*I linking clones NL1-097, NL2-092, NL2-230, NLM-007, NLM-118, and NLM-196 was confirmed by co-localization of the BAC clone JE020G17 (GGA15) with NL1-097 and GGA14 specificity of the clone NL1-290 by its co-localization with the BAC clone JE014E08 (GGA14) (Fig. 1).

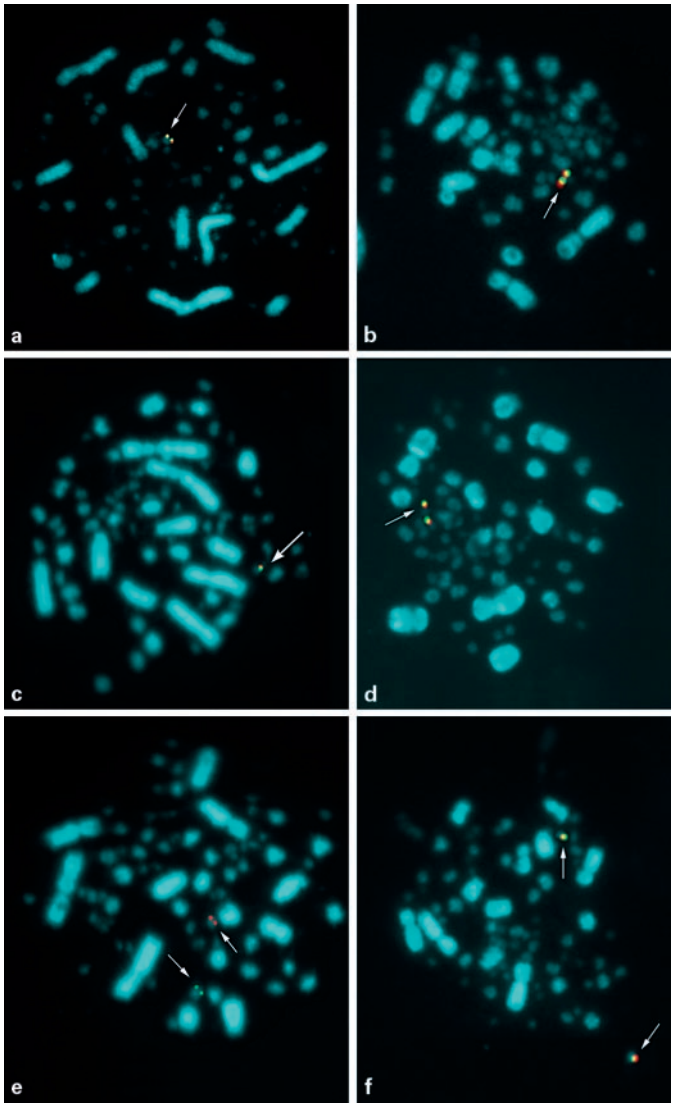

Fig. 1. Double-color FISH of two *Not*I clones or *Not*I and BAC clones on chicken mitotic chromosomes. (**a**) Biotinylated NLM-196 (green) and digoxigenin-labeled NLM-007 (red); (**b**) biotinylated NLM-196 (green) and digoxigenin-labeled JE024F14 (red); (**c**) biotinylated NLM-196 (green) and digoxigenin-labeled NL2-230 (red); (**d**) biotinylated NL1-097 (green) and digoxigenin-labeled JE020G17 (red); (**e**) biotinylated NL1-290 (green) and digoxigenin-labeled NL2-230 (red); (**f**) biotinylated NL1-290 (green) and digoxigenin-labeled JE014E08 (red). Arrows indicate the sites of specific hybridization. Superimposition of both signals results in yellow hybridization signals.

Discussion

Microchromosomal localization of the NotI linking clones

Seven *Not*I linking clones with high density of transcriptionally active regions were cytogenetically assigned to chicken microchromosomes. The latter constitute about 30% of the avian genome (Smith et al., 2000). They are not only much shorter than macrochromosomes, but also possess specific cytological and biochemical features (Smith et al., 2000; Burt, 2002). The recombination frequencies in macro- and microchromosomes are one crossover per 30 and 12 Mb, respectively, that is two and five times less than in mammals (Rodionov, 1996). Microchromosomes are R-positive and GC-rich (Schmid et al., 2000). Microchromosomes contain more CpG islands than macrochromosomes, as shown by DNA–DNA in situ hybridization, which indirectly testifies gene enrichment in microchromosomes (McQueen et al., 1996). In chicken, this fraction of the genome proved to be rich in previously mapped genes (Smith and Burt, 1998). Another distinctive feature of microchromosomes is a lower level of methylation of the GC base pairs and a higher level of histon acetylation as compared to macrochromosomes, which is also typical for functionally active chromatin (McQueen et al., 1998). Thus, the avian genome is characterized by structural and, presumably, structural–functional compartmentalization. Previously, we have shown that avian microchromosomes possess a major portion of heavy isochores and could be considered as functional analogs to mammalian T-bands (Andreozzi et al., 2001; Sazanov et al., 2003). The T-bands on HSA3p15, HSA3p21.3, HSA3q21 and HSA3q29 contain sites with high concentrations of *Not*I clones (Protopopov et al., 1996). Since the *Not*I linking clones NL2-092, NL2-230, NLM-007, NLM-118, and NLM-196 are located in the HSA3q21 T-band and location intervals of the NL1-290 (HSA3q13.3→q21) and NL1-097 (HSA3q21→q23) clones also correspond to the T-bands, assignment of these clones to chicken microchromosomes is in good agreement with our hypothesis about coincidence of mammalian T-bands and avian microchromosomes.

Orthology

From the comparative genomics point of view, there may be 80 or more regions of evolutionary conservation between the human and the chicken chromosomes (Burt et al., 1999; Burt, 2002). In this study, six *Not*I linking clones (NL1-097, NL2-092, NL2-230, NLM-007, NLM-118 and NLM-196) were localized on GGA15, and clone NL1-290 on GGA14. That means that the human chromosomal region HSA3q13→q21 has homology to at least two chicken microchromosomes, GGA15 and GGA14. Orthology between HSA3q and GGA14 and GGA15 has not been reported before (Schmid et al., 2000; Burt, 2002). Judging from our analysis, the breakpoint of evolutionary conservation of human and chicken chromosomes should be considered in the HSA3q13.3→q21 region between the loci NL1-290 and the others. It is known that GGA14 is partially orthologous to HSA16 based on the location of *HBA@* and *NTN2L*, and GGA15 has homology to HSA22 due to the location of *CRYBA4*, *CRYBB1*, *MIF* and *IGL@* (Burt, 2002). Another case of evolutionary conservation of HSA3q and chicken chromosomes was revealed in the neighboring region

Table 2. Chicken BAC clones used for FISH

Clone	Chromosome, ChickFPC contig[a]	Locus symbol (alias)	Locus name	GenBank acc. no.	Ensembl BLASTView location[b]
JE014E08, JE027C23	GGA14, ctg1301	*HBA@*	Alpha globin region	AY016020	GGA14, Contig173.10 to Contig173.27
JE024F14	GGA15, ctg52701	*IGVPS* [*MCW0052*, *IGL@*]	Immunoglobulin V26 and V6 pseudogene segments	D13439	GGA15, Contig62.115 to Contig62.117
JE020G17	GGA15, ctg37501	*ALDH1A1* [*ALDH, ALDH1*]	Aldehyde dehydrogenase 1 family, member A1	X58869	GGAZ, Contig153.194 to Contig153.205

[a] http://www.bioinformatics.nl/gbrowse/cgi-bin/gbrowse/ChickFPC.
[b] http://www.ensembl.org/Gallus_gallus/.

3q24→qter. Four coding sequences (*TFRC*, *EIF4A2*, *SKIL* and *DHX36*) were shown to be located in this chromosomal region in human and on GGA9 (Schmid et al., 2000). Therefore, another breakpoint appears to exist between chicken orthologs of *Not*I linking clones NL1-097, NL2-092, NL2-230, NLM-007, NLM-118, NLM-196 (HSAq21→q23, GGA15), NL1-290 (HSA3q13.3→q21, GGA14) and *TFRC*, *EIF4A2*, *SKIL*, *DHX36* (HSA3q24→qter, GGA9).

Coding sequences

Homology to seven coding sequences was detected within the *Not*I linking clones under investigation (http://www.mtc.ki.se/groups/zabarovsky; http://www.ncbi.nlm.nih.gov; Table 1). Five of them – homologs of *CHST13*, *SEMA5B*, *ABTB1*, *RAB7* and DKFZp547K1610 – are located on HSA3q21 (Table 1). It means that these homologous chicken sequences should have the comparative anchoring tags on GGA14 or GGA15 that would correspond to the above human genes. The BLAST search using the original *Not*I linking clone sequences against the NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) and ENSEMBL (http://www.ensembl.org/Multi/blastview?species=Gallus_gallus) whole genome sequence databases, however, did not result in any significant hit on these two chicken microchromosomes. In two other cases, NL1-097 and NLM-118 did not produce any hits to HSA3. As discussed by Sulimova et al. (2002), non-coincidence of locations of original genes (ESTs) and corresponding *Not*I clones could be explained by various factors such as the large size of some genes and the occurrence of related genes from the same gene family, pseudogenes, or gene duplications. Interestingly, the sequences of HSA3q21→q23 *Not*I linking clone NL1-097 are homologous to the HSA4p16.1 *WDR1* gene and those of NLM-118 to the mouse *Vrk2* gene, the human homolog of which is located on HSA2p16→p15 (http://www.ncbi.nlm.nih.gov; Table 1). Hence, we may suppose homology of HSA3q21→q23 to HSA4p16.1 and HSA2p16→p15 or, in other words, presence of new paralogous regions within the human genome. Alternatively, presence of putative *WDR1* pseudogenes and a *Vrk2* homolog could be supposed on HSA3q21→q23. A similar observation was reported for three *Not*I clones from HSA3p21, which were shown to have homology to three different genes located on HSA12 (Sulimova et al., 2002).

Previously, by employing cytogenetic or linkage mapping, the chicken locus *HBA@* was assigned to GGA14 (Levin et al., 1994). Linkage mapping of both *IGVPS* (*MCW0052*, *IGL*) (Crooijmans et al., 1995) and *ALDH1A1* (Suchyta et al., 2001) suggested their location on GGA15. Using FISH, Guttenbach et al. (2000) controversially mapped *ALDH1A1* to GGA2q21→q22, while Smith et al. (2000) mapped it to a microchromosome. At the same time, conserved synteny existing between HSA9 and GGAZ supports location of *ALDH1A1* on GGAZ (http://www.ensembl.org/Multi/blastview; Table 2). We showed here the large insert BAC clones positive for *ALDH1A* and *IGVPS* to be on the same microchromosome.

In conclusion, we demonstrated the correspondence of human *Not*I clones from the HSA3q21 T-band region to the chicken gene-rich microchromosomes GGA14 and GGA15 as well as possible breakpoints of evolutionary conservation between HSA3q and chicken microchromosomes. Based on human sequences within the *Not*I clones, we can assume localization of the new chicken sequences orthologous to the six human/rodent coding genes on GGA15 and one on GGA14.

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