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Original article

A gene-based radiation hybrid map of chicken microchromosome 14: Comparison to human and alignment to the assembled chicken sequence

Mireille MORISSON[a]*, Sophie LEROUX[a], Carine JIGUET-JIGLAIRE[a], Sirine ASSAF[b], Frédérique PITEL[a], Sandrine LAGARRIGUE[b], Suzanne BARDES[a], Katia FEVE[a], Thomas FARAUT[a], Denis MILAN[a], Alain VIGNAL[a]

[a] Laboratoire de génétique cellulaire, Institut national de la recherche agronomique, 31326 Castanet-Tolosan, France
[b] Laboratoire de génétique animale, Institut national de la recherche agronomique, École nationale supérieure d'agronomie de Rennes, 35042 Rennes Cedex, France

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Abstract – We present a gene-based RH map of the chicken microchromosome GGA14, known to have synteny conservations with human chromosomal regions HSA16p13.3 and HSA17p11.2. Microsatellite markers from the genetic map were used to check the validity of the RH map and additional markers were developed from chicken EST data to yield comparative mapping data. A high rate of intra-chromosomal rearrangements was detected by comparison to the assembled human sequence. Finally, the alignment of the RH map to the assembled chicken sequence showed a small number of discordances, most of which involved the same region of the chromosome spanning between 40.5 and 75.9 cR_{6000} on the RH map.

chicken / comparative mapping / radiation hybrids / microchromosome 14 / intrachromosomal rearrangement

1. INTRODUCTION

The numerous efforts made these last years in the field of chicken genomics [2, 12] come from the importance of this species in agriculture and its great value for research in virology, developmental biology, oncology and immunology [6]. Thus, a large genomic toolset was developed including

* Corresponding author: mmorisso@toulouse.inra.fr

a detailed consensus linkage map of the genome including over two thousand markers [15, 16]. Large collections of chicken expressed sequence tags (EST) were released [1, 4, 37] and bacterial artificial chromosome (BAC) libraries [9, 21] were constituted and used to assemble both local and genome-wide chicken BAC contig maps [11, 29]. BAC contigs are usually used as a platform on which full genome sequences are assembled. They also serve as a bridge between the genome sequence and the linkage map, the essential tool for QTL analysis [30]. In March 2004, a first draft assembly of the chicken genome sequence was released by the Washington University Genome Sequencing Center (WUGSC) and the National Human Genome Research Institute (http://www.genome.gov/11510730). Due to a significantly lower rate of interspersed repetitive elements, this draft chicken genome sequence is probably more accurate than the first draft human genome sequences originally published three years ago [18, 38]. Nevertheless, the integration of all chicken genomic resources such as the BAC contigs, the genetic and the radiation hybrid (RH) maps, will be essential for the assembly of the whole genome sequence data to a reliable and more informative resource. Thus, in addition to the BAC contig map, an RH map will provide an independent platform to assist the chicken genome sequence assembly process towards a finished quality sequence. RH mapping is a powerful tool for locating genes since it uses the simple polymerase chain reaction (PCR): contrariwise to genetic markers, RH markers do not require the development of polymorphism. The whole genome radiation hybrid (WGRH) panel we produced [24] has already been used to build radiation maps for chicken microchromosome 15 [20], macrochromosome 4 and 7 [25, 28], and maps of other chromosomes are under construction.

The first comparisons of chicken gene maps with those of humans have revealed an unexpectedly high level of conserved syntenies [8, 14, 27, 32, 33]. However, subsequent and more detailed mapping studies have revealed high levels of intra-chromosomal rearrangements within them [7, 10, 19, 34, 36]. The expectations are therefore, that the number of segments of the conserved gene order will increase with the number of genes mapped in the chicken.

To develop a dense RH map of chicken microchromosome GGA14, we adopted a strategy based on the use of markers from the genetic map to check the validity of the RH map and from EST or genes whose location on GGA14 could be predicted from known data on conserved syntenies. The human/chicken comparative data published in 2000 by Schmid *et al.* [31] showed that two genes (*HBA* and *NTN2*) localised on GGA14 were both located on HSA16p13.3, suggesting that this human chromosome should be used as a base for developing gene-based markers. More recently, the gene *SREBP1*,

orthologous to *SREBF1* localised on HSA17p11.2 in human, was also shown to be located on GGA14 by segregation analysis in the East Lansing reference family [3], providing another source for the development of markers.

The GGA14 RH map obtained was compared to the assembled human sequence to detect the chromosome rearrangements that have occurred in the lineages leading to humans and chickens. Finally, the RH map was compared to the newly available assembled chicken genome sequence in order to detect discordances pointing to potential assembly problems of the sequence.

2. MATERIALS AND METHODS

2.1. Radiation hybrid panel

The production of the RH panel has already been described [24]. Briefly, normal diploid female chicken fibroblasts irradiated at 6000 rads by gamma rays from a Cesium-137 source were fused to the hypoxanthine guanine phosphoribosyl transferase (HPRT)-deficient hamster cell line, Wg3hCl2 [13]. The hybrid cells were selected on HAT (hypoxanthine-aminopterin-thymidine) media, tested for marker retention and subjected to large-scale culture for DNA extraction. The final panel was composed of 90 clones with a mean marker retention frequency of 22%.

2.2. Markers from the genetic map

Nine microsatellites (*ADL0118*, *ADL0263*, *LEI0066*, *LEI0098*, *MCW0123*, *MCW0136*, *MCW0225*, *MCW0296* and *ROS0005*) and 2 SSCP (*GCT0903* and *GCT0908*) markers from the two known genetic linkage groups attributed to GGA14 were used. In the course of the whole genome RH mapping work going on in the laboratory, the microsatellite marker *ADL0205* was found linked to GGA14. More information for these 12 markers is available at http://www.thearkdb.org/browser or https://acedb.asg.wur.nl/.

2.3. EST (expressed sequence tag) and gene markers

All publicly available chicken EST (>420 000) from Genbank and other sources [1, 4, 37] were collected in a local database, after which EST selection and primer design were performed after comparison to the human genome sequence using the Iccare web server [26]. EST markers corresponding to the *COQ7* and *DREV1* genes had been previously developed in our laboratory and

were found to be linked to the GGA14 RH linkage group. Finally, an additional gene marker was developed to map the chicken *SREBP1* gene, orthologous to human *SREBF1* [3] (Tab. I).

2.4. Markers from the sequence assembly

Eight markers (*SEQ0168*, *SEQ0170*, *SEQ0171*, *SEQ0172*, *SEQ0173*, *SEQ0174*, *SEQ0175* and *SEQ0177*) were designed directly from a portion of the GGA14 sequence assembly for which no markers existed on the GGA14 RH map. Primers are given in Table II.

2.5. PCR conditions

PCR were performed in 15 μL, containing 25 ng hybrid DNA, concentrations of $MgCl_2$ ranging from 1 to 3 mM as determined by test experiments, 0.3 U Taq DNA polymerase (Life technologies: Carlsblad, CA, USA), 1 X buffer (Life technologies), 200 μM of each dNTP, 0.2 μM of each primer and 1X loading buffer composed of 350 mM sucrose and 0.2 mM cresol. After denaturation for 10 min at 94 °C, 33 PCR cycles of 30 s at 94 °C, 30 s at the specific annealing temperature, 30 s at 72 °C, and a final elongation step of 10 min at 72 °C were performed. PCR products were analysed using 2% agarose gels and visualised using ethidium bromide staining. Chicken DNA was used as the positive control and Wg3hCl2 DNA and TE (Tris-EDTA) buffer as negative controls. Each marker was genotyped twice and a third genotyping experiment was performed when discrepancies between the first two experiments were found.

2.6. Map construction

All markers were scored as present or absent in each of the hybrids. Ambiguous results were also reported. Pairwise and multipoint data analysis was completed using RH2PT and RHMAXLIK programmes in the RHMAP3.0 software package [5, 22]. We assumed random breakage along the chromosomes and equiprobable retention of fragments. The RH map was constructed in three steps: (1) a two point analysis identified markers linked together with an LOD score greater than 6, thus defining linkage groups; (2) multipoint analyses were done with RHMAXLIK to define a framework map with the markers from the largest linkage group, using a stepwise marker addition strategy and

a LOD threshold of 3. The resulting framework map was further tested by removing one marker at a time and calculating the LOD score for all of its possible positions; (3) a comprehensive map was built by calculating the location of additional markers relative to the framework markers. Finally the map figure was created using MapChart, a software for the graphical presentation of linkage maps and QTL developed by Voorrips and colleagues [39].

2.7. Comparative mapping data – Alignment to the chicken sequence assembly

Data on human gene order were obtained from the Ensembl (http://www.ensembl.org/) or NCBI Mapview (http://www.ncbi.nlm.nih.gov/mapview/) browsers. Data on the location of chicken genes were obtained by BLASTN or BLAT searches using the Ensembl (http://www.ensembl.org/) or Golden Path (http://genome.ucsc.edu/) browsers.

3. RESULTS

3.1. Development of EST (expressed sequence tag) markers

Genes located in the regions of HSA16 and HSA17 identified as having a conservation of synteny with GGA14 were used to search for chicken orthologous EST with the Iccare software. Primers were then selected according to the constraints of RH mapping: species-specific amplification of an exonic region. Eighty such chicken EST could thus be used to design PCR primers suitable for RH mapping and 63 (80%) led to successful amplifications.

Markers corresponding to the *COQ7*, *DREV1* and *SREBF1* genes were added to our study (see Materials and Methods). Altogether, 66 EST or gene markers, as shown in Table I, were used to build the GGA14 RH map. However, mapping data concerning a few human genes have evolved since we designed the primers. Hence, *GCT1270* is similar to the open reading frame *Res4-22C* now localised on HSA4 and *UBC* (*GCT1264*) is now on HSA12. Therefore, only 64 of our EST markers were orthologous to genes located on HSA16p13.3 or HSA17p11.2.

3.2. Construction of the GGA14 RH map, marker retention

Genotyping data on the ChickRH6 panel for the 12 genetic and 66 gene markers were used to generate an RH map. After two point analysis at a LOD

Table I. Primer sequences for chicken markers corresponding to human genes and EST. Position on the genome sequence and chromosome information are from the Ensembl (http://www.ensembl.org/) or NCBI Mapview (http://www.ncbi.nlm.nih.gov/mapview/) genome browsers. Unknown: the genomic sequence exists but could not be assigned to a chromosome in the genome sequence assembly. No hits: the sequence was not found by BLASTN in the genome sequence.

Human				Chicken		
		Sequence data			Sequence data	
Gene	Genbank ID	Chromosome	Localisation (Mb)	Genbank ID	Chromosome	Localisation (Mb)
A2BP1	NM_145893	HSA16	7,383	AL588477	GGA14	13,241
ABAT	NM_000663	HSA16	8,736	BU384127	GGA14	12,243
ABCA3	NM_001089	HSA16	2,267	BI067240	GGA14	1,554
ACCN1	NM_001094	HSA17	31,487	BU281335	GGA27	0,277
AKAP10	NM_007202	HSA17	19,971	BU248455	GGA19	6,355
BLMH	NM_000386	HSA17	28,721	AJ445455	GGA19	5,818
BM045	NM_018459	HSA16	4,880	BI394462	unknown	
CACNA1H	AF223563	HSA16	1,204	BU313977	GGA14	7,320
CGTHBA	NM_012075	HSA16	0,074	AI979784	GGA14	13,944
CLCN7	NM_001287	HSA16	1,435	BI064676	GGA14	14,126
COQ7	NM_016138	HSA16	19,046	BU127240	GGA14	10,660
COX10	NM_001303	HSA17	14,173	BU297164	GGA18	2,459
CREBBP	NM_004380	HSA16	3,777	BU133172	GGA14	14,342
CRYBA1	NM_005208	HSA17	27,719	M15658	GGA19	5,568
DECR2	NM_020664	HSA16	0,392	BG713681	GGA14	2,063
DKFZp434F054	NM_032259	HSA16	0,675	BI392137	GGA14	1,942
DREV1	NM_016025	HSA16	21,577	AJ444698	GGA14	10,917
*E4F*1	NM_004424	HSA16	2,214	BU134214	GGA14	8,538
ELAC2	NM_018127	HSA17	13,096	BU481814	GGA18	0,988
FLJ14154	NM_024845	HSA16	3,494	BU446205	GGA14	14,176
FLJ20040	NM_018992	HSA16	2,732	AL587387	GGA14	9,409
GIT1	NM_014030	HSA17	28,046	BU365311	No Hits	
GRAP	NM_006613	HSA17	19,125	BU374370	GGA14	7,218
HAGH	NM_005326	HSA16	1,799	AJ396406	GGA14	8,085
HBZ	NG_000006	HSA16	0,220	AL586775	GGA14	13,962
HMOX2	NM_002134	HSA16	4,527	BI394804	GGA14	14,271
HSCARG	NM_020677	HSA16	4,512	BI390022	GGA14	14,573
HSPC055	NM_014153	HSA16	11,811	BU467982	GGA14	3,092
JJAZ1	NM_015355	HSA17	30,410	AJ443766	GGA18	6,405
KIAA0643	AB014543	HSA16	3,551	BG711879	unknown	
LLGL1	NM_004140	HSA17	18,329	BU222034	unknown	

Table I. Continued.

Human				Chicken		
		Sequence data			Sequence data	
Gene	Genbank ID	Chromosome	Localisation (Mb)	Genbank ID	Chromosome	Localisation (Mb)
LOC51061	NM_015914	HSA16	11,739	BU117620	GGA14	3,096
LUC7L	NM_018032	HSA16	0,179	AJ397480	GGA14	14,119
Magmas	AL833954	HSA16	4,371	BI392448	GGA14	14,524
MAP2K3	NM_002756	HSA17	21,364	BU215684	GGA14	6,604
MGC15416	BC001912	HSA16	0,638	BG712373	unknown	
MGC2605	NM_032304	HSA16	0,717	BI394017	GGA14	8,081
MRPS34	NM_023936	HSA16	1,762	AL585124	unknown	
MYH11	NM_002474	HSA16	15,763	X06546	GGA14	9,602
NCOR1	NM_006311	HSA17	16,136	BU143568	GGA19	6,068
NF1	NM_000267	HSA17	29,568	S62087	GGA19	8,557
NLK	NM_016231	HSA17	26,515	AJ443268	GGA19	8,826
NT5M	NM_020201	HSA17	17,407	BI394839	GGA14	6,862
NTHL1	NM_002528	HSA16	2,030	BU249652	GGA14	8,303
NUBP2	NM_012225	HSA16	1,777	BG711301	GGA14	1,889
PDPK1	NM_002613	HSA16	2,528	AL587372	GGA14	9,385
PM5	NM_014287	HSA16	16,293	BU315883	GGA14	9,790
PMP22	NM_000304	HSA17	15,334	BU408850	GGA18	2,175
PPL	NM_002705	HSA16	4,933	BU284851	GGA14	1,838
PRPSAP2	NM_002767	HSA17	18,962	BU389510	GGA14	7,179
Rab11	NM_014700	HSA16	0,416	AL585583	GGA18	6,198
RAB26	NM_014353	HSA16	2,139	BU205196	GGA14	8,454
RES4-22C	AB000461	HSA4	2,659	BU140047	GGA4	82,790
RGS11	NM_003834	HSA16	0,258	BU460973	GGA14	14,083
SDOS	NM_032349	HSA16	4,744	AF095446	GGA14	14,180
SOX8	AK024491	HSA16	0,972	BU451000	GGA14	7,800
SREBF1	NM_004176	HSA17	17,917	AY029224	GGA14	6,985
STUB1	NM_005861	HSA16	0,670	BU449476	unknown	
TRAF4	NM_004295	HSA17	27,217	BU258287	GGA19	5,446
TRAP1	NM_016292	HSA16	3,708	BE139917	GGA14	14,326
UBC	NM_021009	HSA12	123,749	BU409506	GGA15	4,481
UBE2I	AK024172	HSA16	1,289	AJ396786	GGA14	9,438
UBN1	NM_016936	HSA16	4,898	BU390306	GGA14	1,865
ULK2	BC034988	HSA17	19,862	BU204959	GGA19	6,388
USP7	NM_003470	HSA16	8,810	BQ038106	GGA14	12,143
WSB1	NM_015626	HSA17	25,767	BU318529	GGA19	8,635

Table I. Continued.

Marker				
Human gene	Name	Forward primer (5′-3′)	Reverse primer (5′-3′)	Product size
A2BP1	*GCT1062*	GGAAGCTACAGCACCTCC	TGCTCTGGAACAGTGGTTTG	147
ABAT	*GCT1348*	CAGGAAAATTCTGGGCACAC	GGCCTGAACTCCTCTTTGTG	112
ABCA3	*GCT1054*	CATTGTCCTGCACCTTGAAC	AAGATGATGGATTTGCCACAC	160
ACCN1	*GCT1344*	TCGTCAGACATGGGCTTAGAC	GCATGTGAACCATTTTGCAG	112
AKAP10	*GCT1265*	TCAAGCCACACATCCTCTTG	TGCCGTAAGGGAGTAGTGAAAC	111
BLMH	*GCT1341*	AATGCCTTTGTGGAAACAGC	ATATCCCACTGGCCTCCATC	101
BM045	*GCT1067*	GAGCAGAGCAGAGGTCGTTC	TAAAAGGACCCGACCGAGAC	245
CACNA1H	*GCT1243*	GCTTTTTCTCCAGGCGTC	TCATCGTCAGCTTCTTCGTG	135
CGTHBA	*GCT1047*	AACAATTTTGGCTACAAAGTCAG	AACGTAGGGTGACCAACAAAG	87
CLCN7	*GCT1052*	GTGGAGGCAGGGAGAGGAC	ACTGGAGAAGGCAGCAACAC	107
COQ7	*GCT1469*	GCACAGTGGCGGTGGAAG	GCATCAGTTCTTTGTACTTTTC	100
COX10	*GCT1259*	CCCTGCTTGCTGTATGCTTC	GTGTTGGCGATGCTCATTC	157
CREBBP	*GCT1271*	CACTAAGAGCCATGACCACAAG	GCACAACCCGTTTCATCTTC	206
CRYBA1	*GCT1339*	CACAAGGAATCCAAGATCACTG	ATCTTCATGGAGCCCACTTC	128
DECR2	*GCT1227*	ACATCCGAGTGAACAGCTTG	GACCAGTGTGGTGCCAGTC	200
DKFZp434F054	*GCT1050*	CGAGCTTTGGCCTCTCTG	CTGCATCCAAGCCCCAAC	122
DREV1	*GCT1470*	GGGCTCAATGTTTGTGTTTTC	GACCTCTCCATCTCCAGCTC	109
*E4F*1	*GCT1248*	AGTCATTGTGGCAGGAGACC	TCATGTGAGCTTCGAGGATG	214
ELAC2	*GCT1255*	TGGGATGAAGATGAATGCTG	AATTCCAACCTTCTCACTGAAGTC	103
FLJ14154	*GCT1346*	CCATCTACCTGCACGTCCTC	GGTGGGTGTCCACCATTG	160
FLJ20040	*GCT1055*	TGCAGTGAGTTGTCACGTTG	GCCAGTTGACACCAGCTTATAG	117
GIT1	*GCT1340*	CTCAGGCCAACTTCTTCCAC	CGTACACCACCAGCAGCTC	99
GRAP	*GCT1260*	CAGCACGTCCAGCACTTC	CCTGTAGAAGTCCACCAGCTC	102
HAGH	*GCT1066*	TCAAAGACAGTCGCACAAGC	CCACTATTGCAGCCTCCTTC	109
HBZ	*GCT1239*	CCGAGGACAAGAAGCTCATC	GGTCGAAGTGGGGGAAGTAG	132
HMOX2	*GCT1060*	CTGGAAGAGGAAATGGATCG	GCTTCTTTCCGGTGAAGCTC	83
HSCARG	*GCT1232*	AATGTGCCCACCACAACC	TGGCCTATGTACTGCTCTGG	200
HSPC055	*GCT1355*	AGAAACAGTGGCAGGGTCAC	AAACATCCGGTTGGAAAGC	106
JJAZ1	*GCT1342*	CCAAAGCGTACGAAAGCAAG	ATTTCTTGTGGACGGAGAGG	140
KIAA0643	*GCT1057*	AGATGGCTATGCGGTGAGAG	TGAATTTGCTGCTGTCTTCC	113
LLGL1	*GCT1257*	GCTGGGGAAGGAGATCCAG	AGGAGATGAGCATGGAGTGG	155
LOC51061	*GCT1354*	TGAATACAAAGGCCCAATGAG	TTTTGATCGAGACGAGATGTAGAG	91
LUC7L	*GCT1048*	ATCCTTCATTGCGGAGTGTG	CAGCACTGATCTCTTCCTGTG	80
Magmas	*GCT1059*	AAAGCCGAGACGTGACTCC	GAGGACACGGCGTTTATTG	83
MAP2K3	*GCT1262*	CGGGGATGACTCTTAACTGG	AAGGTGTTGGCTTCCAAGG	192
MGC15416	*GCT1063*	GGCCAAAATTGAAAACTGCTC	TGGGGTTGTTGTTGTTGTTG	150
MGC2605	*GCT1064*	GGAAGGAGGATGTGGTTCTC	CTCATCCGCACCGTACAC	203
MRPS34	*GCT1065*	GAAGACCCCCTTGCCTACTG	CGACCAGGAGTTCCAAGTTC	185
MYH11	*GCT1353*	TGAAGAGAAGAACCTCCTCCAG	TCAATTCTGGCCTCCATCTC	138
NCOR1	*GCT1263*	GAAAATAATCCACGCAGGAAAG	GAAATCGCTCTTGCTGTTCTC	103

Table I. Continued.

Marker				
Human gene	Name	Forward primer (5′-3′)	Reverse primer (5′-3′)	Product size
NF1	*GCT1343*	GTCATAGCATTCCGCTCCAG	TCCATGATCTCCAGCAAAGC	119
NLK	*GCT1337*	GGCCAGGGTGGAAGAATTAG	TGCAAAGATGCAGCCTACAG	142
NT5M	*GCT1261*	GCTGGTGGACATGGATGG	AGGGCAATGTAGGGCTTGTC	93
NTHL1	*GCT1246*	TCTCTGGAGGGAGATAAACTGG	TGCTCAAAGAAGCCAGAATG	146
NUBP2	*GCT1053*	GGAAGAGCACCATCAGCAC	CATTGTCCTGCACCTTGAAC	221
PDPK1	*GCT1056*	TGCTACCAAAAGATTAGGCTGTG	TGCTACCAAAAGATTAGGCTGTG	151
PM5	*GCT1352*	TGGGAAAGCAGGCTTATTTATG	ACTGTGATGAGAGGGGAAGG	102
PMP22	*GCT1258*	CAGTCTGTCCAAGCAATGATG	CTTGGTGAGCGTGAAGAGC	90
PPL	*GCT1251*	CTCCCTCTACAGCCCTACCC	AACATGGAGCTGACGTACCC	212
PRPSAP2	*GCT1256*	TCAGAAGGAAATACAGGGCTTC	TTCTTGGATGTACTGTAGTAAAAATGG	82
Rab11	*GCT1049*	GCAGGCGGAAGTTGATCTC	CAGCCTCCTTCTCTGAATCC	226
RAB26	*GCT1240*	CGGCCTCAATGTGGAATTAG	GCAGCTACAAAAACGGTTGC	177
RES4-22C	*GCT1270*	TTGTAGCTGTATGCCGTTGG	AGGTCCATTCCTTTGTCAGG	195
RGS11	*GCT1247*	CAGGTGAAATCATCCATCTGC	TAGCCCAGTAGGTGGTGTCG	130
SDOS	*GCT1061*	ACGCAGATAGCGGAGAGAAC	ATTACAAACACGGGGGCATC	218
SOX8	*GCT1268*	GACCCCCAAAACTGACCTG	CGAAGGTCTCCATGTTGTTG	152
SREBF1	*EXT0022*	CAGCCCCACTTCATCAAGGC	GGCCAGGATGGTCCCTCCAC	249
STUB1	*GCT1242*	CGCTGTGCTACCTGAAGATG	GGCCTCGTCGTAGTTCTCCA	140
TRAF4	*GCT1338*	ACATCACCGAGACCTTCCAC	CCTCGTGGGAGATGAACTTG	123
TRAP1	*GCT1058*	CCAAGGACATCGGTGAGTG	GCTCAGGCACATAGAAGATGC	135
UBC	*GCT1264*	AAGACCCTCACTGGCAAGAC	GGACCAGATGGAGAGTGGAC	196
UBE2I	*GCT1051*	GCACAAGCCAAGAAGTTTGC	GTGGCGAATATGGAAAATGG	178
UBN1	*GCT1252*	GCTGTCGCTCACTCTCTTTG	AGAGCCTCCGCTTTATGTCTC	168
ULK2	*GCT1266*	AGTGTTCCTCCAGCAGATCG	GGAACCACACAGAGTTGCAG	205
USP7	*GCT1349*	GATAACAGTGAGCTGCCAACAG	TCGTTGGGGATGGTTTTATC	92
WSB1	*GCT1336*	CATGGCACAAAGAATGTTGC	GTCCTGCCCAAATTTGAAAC	201

threshold of 6, a large linkage group containing 60 markers (49 genes or EST, plus 11 genetic markers) was defined. By lowering the LOD threshold down to 4, the microsatellite marker *ADL0118* could be added. A framework map 342 cR_{6000} long, containing 23 markers and covering the entire chicken chromosome 14 was constructed (Fig. 1). Thirty-eight additional markers linked to GGA14, but whose positions were not supported by a LOD score greater than 3, are indicated on the side of the map to avoid size inflation and to keep track of different possible local orders.

The average retention rate for the markers was 23.7%, in accordance with the first estimates for the panel [24]. The retention frequency along the chromosome, as estimated by plotting the retention of the markers from the framework map against their positions, demonstrates that the higher retention rates were

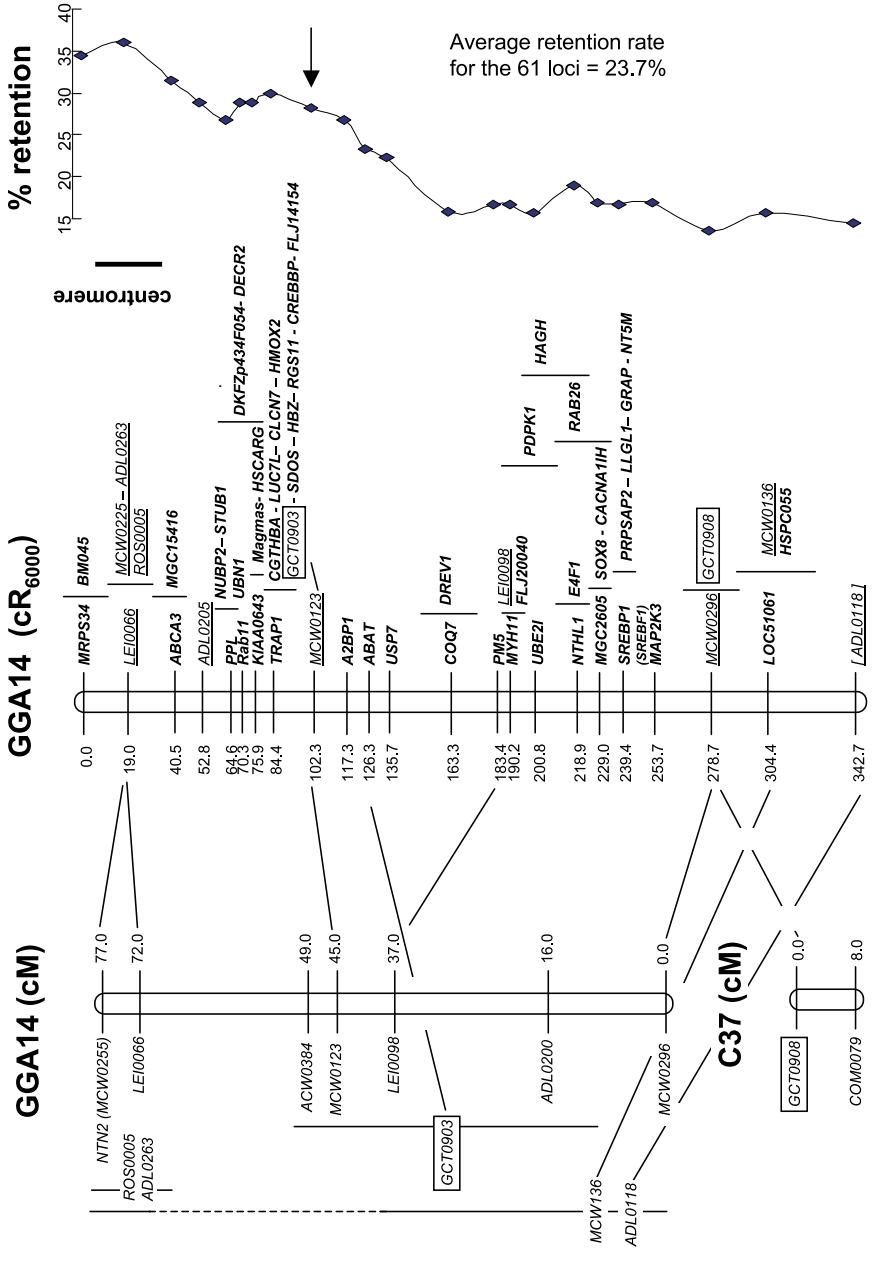
% retention
15 20 25 30 35 40
Average retention rate
for the 61 loci = 23.7%
centromere
GGA14 (cR6000)
GGA14 (cM)
C37 (cM)
0.0 MRPS34 BM045
19.0 LEI0066 MCW0225 – ADL0263 ROS0005
40.5 ABCA3 MGC15416
52.8 ADL0205
64.6 NUBP2– STUB1
70.3 PPL UBN1 DKFZp434F054- DECR2
75.9 RAb11 KIAA0643 Magmas- HSCARG CGTHBA - LUC7L– CLCN7 – HMOX2
84.4 TRAP1 GCT0903 - SDOS – HBZ– RGS11 - CREBBP- FLJ14154
102.3 MCW0123
117.3 A2BP1
126.3 ABAT
135.7 USP7
163.3 COQ7 DREV1
183.4 PM5 LEI0098
190.2 MYH11 FLJ20040 PDPK1 HAGH
200.8 UBE2I
218.9 NTHL1 E4F1 RAB26
229.0 MGC2605 SOX8 - CACNA1IH
239.4 SREBP1 (SREBF1) PRPSAP2 – LLGL1– GRAP - NT5M
253.7 MAP2K3
278.7 MCW0296 GCT0908
304.4 LOC51061 MCW0136 HSPC055
342.7 LADL0118
77.0 NTN2 (MCW0255)
ROS0005
ADL0263
72.0 LEI0066
49.0 ACW0384
45.0 MCW0123
37.0 LEI0098
GCT0903
16.0 ADL0200
MCW136
ADL0118
0.0 MCW0296
0.0 GCT0908
8.0 COM0079

Figure 1. Alignment of the chicken chromosome 14 genetic and radiation hybrid maps. The genetic maps on the left of the figure are from Groenen *et al.* [15]. For clarity, only the framework markers and additional markers present both on the genetic and radiation hybrid (RH) maps are shown. The RH map is in the middle. Markers positioned on the vertical double bars of the RH map are the framework markers and additional markers are indicated to the right of the map. Microsatellite markers are underlined. The microsatellite marker *ADL0118* in brackets was added to the map by lowering the twopoint lod threshold from 6 to 4. *GCT903* and *GCT908* (boxed) are located on the same microchromosome by FISH [23]. The chart to the right indicates the retention rate of the framework markers. The marker *MCW0123* which was used for selecting the clones for the panel is indicated by an arrow on the retention chart. The suggested centromeric region is indicated by a vertical bar to the right of the RH map.

←

located around the microsatellite marker *LEI0066*, suggesting that the centromere could be located in this area (Fig. 1). The marker *MCW0123*, which was used for selecting the clones while constructing the panel [24] did not show a particularly high level of retention, when compared to the surrounding markers.

3.3. Colinearity between genetic and RH maps, resolution of the panel

Eleven loci (*ADL0118*, *ADL0263*, *GCT0903*, *GCT0908*, *LEI0066*, *LEI0098*, *MCW0123*, *MCW0136*, *MCW0225*, *MCW0296* and *ROS0005*) were shared between the genetic and the RH maps (the microsatellite marker *ADL0205* is not on the genetic map; *MCW0225* corresponds to *NTN2* on the genetic map).

Comparing both maps indicates a good overall agreement with several improvements in marker ordering. One major change comes from the localisation of the centromere around *LEI0066*, suggesting a reverse orientation for the GGA14 genetic map. Markers *MCW0136*, *ADL0118* and *GCT0903* are mapped with a higher precision on the RH map. Finally, the localisation of *GCT0908* close to *MCW0296* indicates that the genetic linkage group C37 is a part of GGA14.

The part of the RH map between *LEI0066* and *MCW0296* was 259.7 cR_{6000} long while the genetic distance between these two markers was 72 cM. The ratio between the two maps was thus 3.6 cR_{6000} to 1 cM.

3.4. Comparison to the assembled GGA14 sequence

When compared, the GGA14 genome sequence assembly and the RH map presented an overall good colinearity (Fig. 2), although two major discrepancies could be detected.

The first concerned a region from positions 1.554 to 2.063 Mb, terminal on the sequence assembly, but found in position 40.5 to 75.9 cR_{6000} of the RH map, shown in red in Figure 2. This region includes six genes (*DECR2*, *DKFZp434F054*, *NUBP2*, *UBN1*, *PPL* and *ABCA3*) on the sequence map, as well as the microsatellite marker *ADL0205*. On the RH map, it contains additionally *Rab11*, located on the sequence contig of GGA18, and the three markers *STUB1*, *KIAA0643* and *MGC15416*, matching all three with sequence data of unknown location in the chicken genome sequence assembly. The second main discrepancy concerns the region between 14.573 Mb (*HSCARG*) and 20.310 Mb (*MCW0225*) of the sequence assembly, for which no marker could be found on the RH map, despite a high density. To test this region, we developed new markers: *SEQ0168*, *SEQ0170*, *SEQ0171*, *SEQ0172*, *SEQ0173*, *SEQ0174*, *SEQ0175* and *SEQ0177*, directly from the genomic sequence. As a result, marker *SEQ0177* was the only one to be linked to the GGA14 RH map, close to *LEI0066*. Markers *SEQ0172*, *SEQ0173*, *SEQ0174* and *SEQ0175* were linked by RH mapping to markers from GGA3 and markers *SEQ0168*, *SEQ0170* and *SEQ0171* were linked to markers not yet positioned on our RH maps.

Finally, a few improvements over the sequence assembly concerned three markers corresponding to an existing genomic sequence of unknown location. One (*LLGL1*) was at position 239.4 cR_{6000}. The two others (*MRPS34* and *BM045*) at position 0 cR_{6000}, extended the RH map further than the sequence assembly.

4. DISCUSSION

4.1. Development of the EST markers

The first constraint on the choice of primers for RH mapping was to avoid the presence of introns, whose positions in the chicken were predicted on the basis of the human genomic sequence. The second was to design primers in the most divergent regions of the human-chicken sequence alignments so as to avoid cross-amplification with the hamster DNA present in the hybrids. Using Iccare proved to be very efficient, with 80% of the primer pairs designed yielding usable RH mapping data, enabling the mapping of a high number of

Table II. Primer sequences for chicken markers designed from the sequence assembly. Positions on the genome sequence are from the Ensembl (http://www.ensembl.org/) genome browser.

Name	Seq. loc. (Mb)	Forward primer (5′-3′)	Reverse primer (5′-3′)
SEQ0168	14.6	TGGAGAAGAGAAGGCTGTGG	TCCCTCTCACATCCAACCTC
SEQ0170	15.0	TTCAGGCAGCACAAGTTCAG	AGCATTTCCACCAGATCGAC
SEQ0171	16.0	GTGATTTCACCCCGATCTTC	GTAGCTGAGGGGCAGCTATG
SEQ0172	17.0	AAAAGAAGGCAAGCAACACG	AACAGCTCAAACTTGCATGG
SEQ0173	18.0	GTAGAGACGTGGCGCTGAG	AGCAATTTGCAACTGCTCTG
SEQ0174	19.0	ACTGTGCTCACCATTTGCAG	TTTGGGTTCCCTAAAAGTGC
SEQ0175	19.5	ATTCCCCACACCTTGTGTTC	TCGAGGAAGATAGGCAGGTG
SEQ0177	20.2	TCATTCCTGGCCCTACTCTG	AGCGTGCTAAAGACCACCTC

EST and genes on the GGA14 RH map. Moreover, the development of EST through the use of Iccare minimises the chances of choosing wrong ortholog genes, since it performs a BLASTN comparison of all chicken EST against the complete set of human Unigene clusters.

4.2. Marker retention and position of the centromere

Preferential retention in RH clones of chromosome fragments from pericentromeric regions of donor cells has been shown in various species, including humans [17, 35] and chickens [25]. The retention frequency of markers shown in Figure 1 indicates a drop from 35% down to 15% over the first 200 cR_{6000} from one end of the map, after which the retention of the markers varies only slightly, with values comprised between 15 and 20%. This data suggests a position for the centromere towards one end of the RH map and is therefore compatible with an acrocentric microchromosome. As a result of our observations and so as to position the centromere conventionally towards the top of the figures, we suggest to reverse the orientation of the genetic map and of the Mb counting on the sequence assembly.

A similar trend, with a drop of 45% down to 15% over a similar distance of 200 cR_{6000} from the centromere was observed for GGA7, although this chromosome is twice the size of GGA14 [25]. This can explain the higher retention rate observed for microchromosome markers when constructing the panel [24], since they have a higher chance than macrochromosome markers of being close to the centromere.

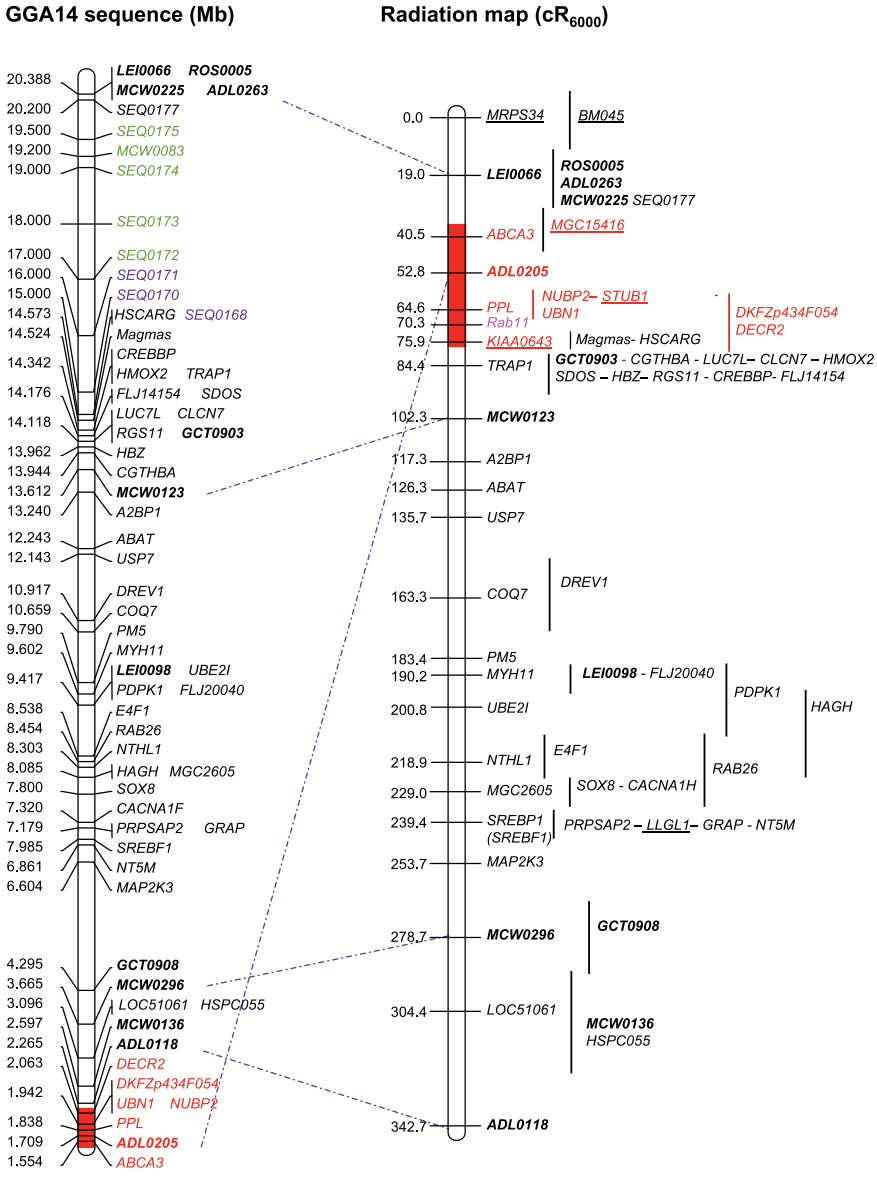
GGA14 sequence (Mb)
Radiation map (cR6000)
20.388 LEI0066 ROS0005
MCW0225 ADL0263
20.200 SEQ0177
19.500 SEQ0175
19.200 MCW0083
19.000 SEQ0174
18.000 SEQ0173
17.000 SEQ0172
16.000 SEQ0171
15.000 SEQ0170
14.573 HSCARG SEQ0168
14.524 Magmas
14.342 CREBBP
HMOX2 TRAP1
14.176 FLJ14154 SDOS
14.118 LUC7L CLCN7
RGS11 GCT0903
13.962 HBZ
13.944 CGTHBA
13.612 MCW0123
13.240 A2BP1
12.243 ABAT
12.143 USP7
10.917 DREV1
10.659 COQ7
9.790 PM5
9.602 MYH11
9.417 LEI0098 UBE2I
PDPK1 FLJ20040
8.538 E4F1
8.454 RAB26
8.303 NTHL1
8.085 HAGH MGC2605
7.800 SOX8
7.320 CACNA1F
7.179 PRPSAP2 GRAP
7.985 SREBF1
6.861 NT5M
6.604 MAP2K3
4.295 GCT0908
3.665 MCW0296
3.096 LOC51061 HSPC055
2.597 MCW0136
2.265 ADL0118
2.063 DECR2
1.942 DKFZp434F054
UBN1 NUBP2
1.838 PPL
1.709 ADL0205
1.554 ABCA3
0.0 MRPS34 BM045
19.0 LEI0066 ROS0005 ADL0263 MCW0225 SEQ0177
40.5 ABCA3 MGC15416
52.8 ADL0205
64.6 PPL NUBP2– STUB1 UBN1 DKFZp434F054 DECR2
70.3 Rab11
75.9 KIAA0643 Magmas- HSCARG
84.4 TRAP1 GCT0903 - CGTHBA - LUC7L– CLCN7 – HMOX2 SDOS – HBZ– RGS11 - CREBBP- FLJ14154
102.3 MCW0123
117.3 A2BP1
126.3 ABAT
135.7 USP7
163.3 COQ7 DREV1
183.4 PM5
190.2 MYH11 LEI0098 - FLJ20040 PDPK1 HAGH
200.8 UBE2I
218.9 NTHL1 E4F1 RAB26
229.0 MGC2605 SOX8 - CACNA1H
239.4 SREBP1 (SREBF1) PRPSAP2 – LLGL1– GRAP - NT5M
253.7 MAP2K3
278.7 MCW0296 GCT0908
304.4 LOC51061 MCW0136 HSPC055
342.7 ADL0118

Figure 2. Alignment of the RH map to the assembled sequence of GGA14. The numbering in Mb of the genomic sequence assembly (left) from http://www.ensembl.org/, is in inverse order to that of the cR_{6000} on the RH map (right), as the latter takes into account the suggested position of the centromere. Markers localised on the genetic maps are in bold and loci not yet found on the sequence assembly are underlined on the RH map. Markers placed in a different position on the GGA14 RH map and GGA14 genome sequence assembly are in red. *Rab11* in purple is on the GGA18 in the genome sequence assembly. Markers in the GGA14 genome sequence assembly mapped to other chromosomes by RH are in green (linked to GGA3 markers) or blue (linked to markers not yet assigned to a chromosome on the current RH maps). The dashed lines indicate the relative position of fragments on the two maps.

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4.3. Comparison to the genetic map

Fluorescent *in situ* hybridisation (FISH) experiments with the BAC clones P1-8 and P6-V11 from which the genetic SSCP markers *GCT0903* and *GCT0908* are derived, suggested that the small linkage group C37 could be linked to GGA14 [23]. Here we confirm this result by the inclusion of both *GCT0903* and *GCT908* on the RH map. The reason why these two linkage groups are independent on the genetic map is that *GCT908* and *COM0079* were only mapped in the Compton population and the nearby marker *MCW0296* only on the Wageningen population. Due to the history of its development using three independent populations, the chicken genetic map still contains a number of small linkage groups, whose chromosome assignment has to be determined. The RH map also enables a greater precision for the mapping of the two markers *MCW0136* and *ADL0118*. The ratio of 3.6 cR_{6000} to 1 cM was close, although slightly lower, to the previous observation ($4cR_{6000}$ to 1 cM) for GGA7 [25]. The recombination rate of microchromosomes being higher than that of macrochromosomes, a lower cR_{6000} to cM ratio was expected for GGA14 than for GGA7.

4.4. Comparison to the genome sequence assembly

Although the genomic sequence assembly for GGA14 covers a total of 20.4 Mb (Fig. 2), a few discrepancies were found with the RH map, one of them suggesting that a portion of 5 Mb from the sequence assembly (between *HSCARG* and *MCW0225*) belongs in fact for a large part to GGA3 and possibly to other chromosomes. As a confirmation, the microsatellite marker

MCW0083, from the GGA3 genetic linkage group was also found at position 19.2 Mb of the GGA14 sequence assembly. This brings the length of the GGA14 sequence down to 15 Mb instead of 20 Mb. On the contrary, the RH map extends 19 cR further than the available sequence, with the addition of *MRPS34* and *BM045*. The length of the RH map to be compared to the sequence was thus 324 cR_{6000} and not 343 cR_{6000}. By using these figures, the ratio between the two maps was 46 kb/cR_{6000}. The previously published figure of 61 kb/cR_{6000} for GGA7 used size estimations for this chromosome based on cytogenetic data [25]. The updated value for GGA7 using the genomic sequence assembly was thus 56 kb/cR_{6000}. Similarly, a value of 37 kb/cR_{6000} can be calculated for GGA15 [20]. The breaking of chromosomes by radiation is a physical process, suggesting similar ratios to be expected for the different chromosomes. Future studies will indicate if the differences between chromosomes observed here are due to structural differences, or to errors in the RH maps and/or the sequence assembly. Given the mean retention frequency of 23.7% of GGA14 in the ChickRH6 panel containing 90 clones, we expect an average of 21.3 observations of such breaks per marker and thus a mean expected resolution power of 215 kb. However, since the retention frequency varies along the chromosome from 35% close to the centromere down to an average of 15% elsewhere, the expected resolution of the panel will vary accordingly from 146 to 340 kb.

It is noteworthy, that the two main discrepancies between the RH map and the genome sequence assembly involves the region close to the centromere: a portion of GGA14 sequence from this region was moved to the telomere region and was replaced by sequence fragments from other chromosomes, mainly GGA3. It is noticeable, that the proportion of markers corresponding to existing sequence fragments of unknown location in the sequence assembly was higher in this region of the RH map than in the other regions and that the only marker of the GGA14 RH map found on another chromosome in the genomic sequence assembly (*Rab1* on GGA18), was also mapped there. One explanation for the difficulties to assemble the sequence in this region could be a similarity between subtelomeric and pericentromeric repeats creating false joining of sequences.

4.5. Comparative mapping

By increasing the number of genes assigned to GGA14 to 48, we greatly improved the comparative mapping data available for this chromosome. If we consider a group of conserved gene segments as containing at least

two genes, 10 groups of global conservation were found with the human region HSA16p13.3 and one with the human region HSA17p11.2, indicated by coloured boxes in Figure 3. This comparison also showed eight genes, indicated in black in Figure 3, which could not be assigned to the conserved gene segments we defined. Apart for *MAP2K3* located on HSA17, all other seven genes (*ABCA3*, *BM045*, *CLCN7*, *MGC15416*, *MRPS34, NUBP2* and *UBE21)* are located in the region between 1.2 and 4.9 Mb on HSA16, in which the highest density of genes and EST were developed. The higher level of resolution thus obtained may partly account for the detection of such small segments of conserved gene order, but it is also possible that this region has undergone a higher number of rearrangements.

The development of EST markers based solely on the prior information of synteny conservation with HSA16p13.3 and HSA17p11.2 does not enable us to rule out the possibility that some small regions from GGA14 correspond to other HSA regions. However, as previously noted in other detailed comparative mapping studies, despite the high level of synteny conservation, a high number of intra-chromosomal rearrangements can be observed between the human and chicken genome.

Due to the lack of precision on the length of the regions of conserved synteny with humans available at the beginning of our work, we extended out of them when choosing EST markers and thus developed markers for other chicken chromosomes. This was particularly true in the case of markers from HSA17, for which the region of conserved synteny appeared to be quite small. As a result, in addition to the conservation between HSA17 and GGA14 demonstrated by *NT5M*, *SREBF1*, *LLGL1*, *PRPSAP2* and *GRAP*, small blocks homologous to regions located on GGA18 (in pink in Fig. 3) or GGA19 (in blue in Fig. 3) could be defined. Finally, *ACCN1* is located on GGA27 (in green in Fig. 3) and no hits for *GIT1* were found in the chicken genome sequence.

5. CONCLUSION

The first purpose of our work on the GGA14 RH map was to develop a dense map including a high number of genes, in order to validate the use of the ChickRH6 panel for a microchromosome and to provide detailed comparative mapping information. At the end of our project, the first draft chicken genome sequence was released and we used our data to test the sequence contig of GGA14. Although the sequence assembly is globally in good agreement with our data, we show that RH mapping can detect some errors, demonstrating its usefulness as a contribution towards a high quality assembly of the sequence.

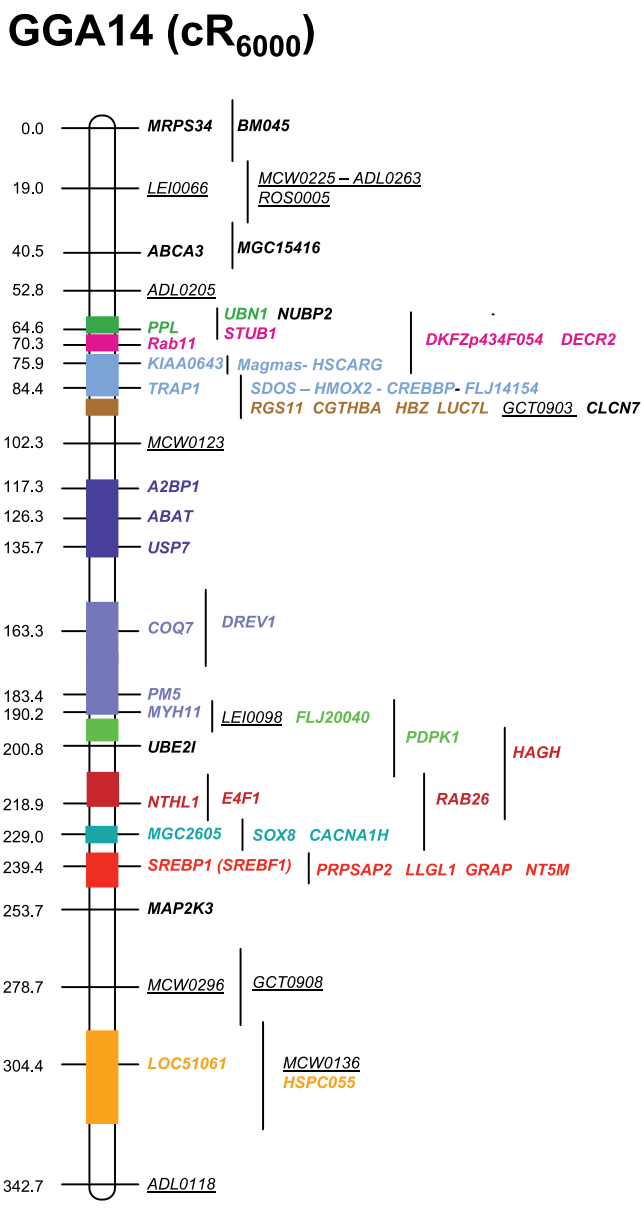

Figure 3. Comparative mapping between the GGA14 RH map and the sequence maps of human regions HSA16p13.3 and HSA17p11.2. Markers localised on the genetic map are underlined on the RH map. Groups of global conserved gene order between chickens and humans are identified by coloured boxes. Genes or EST markers that could not be assigned to any such group are in black.

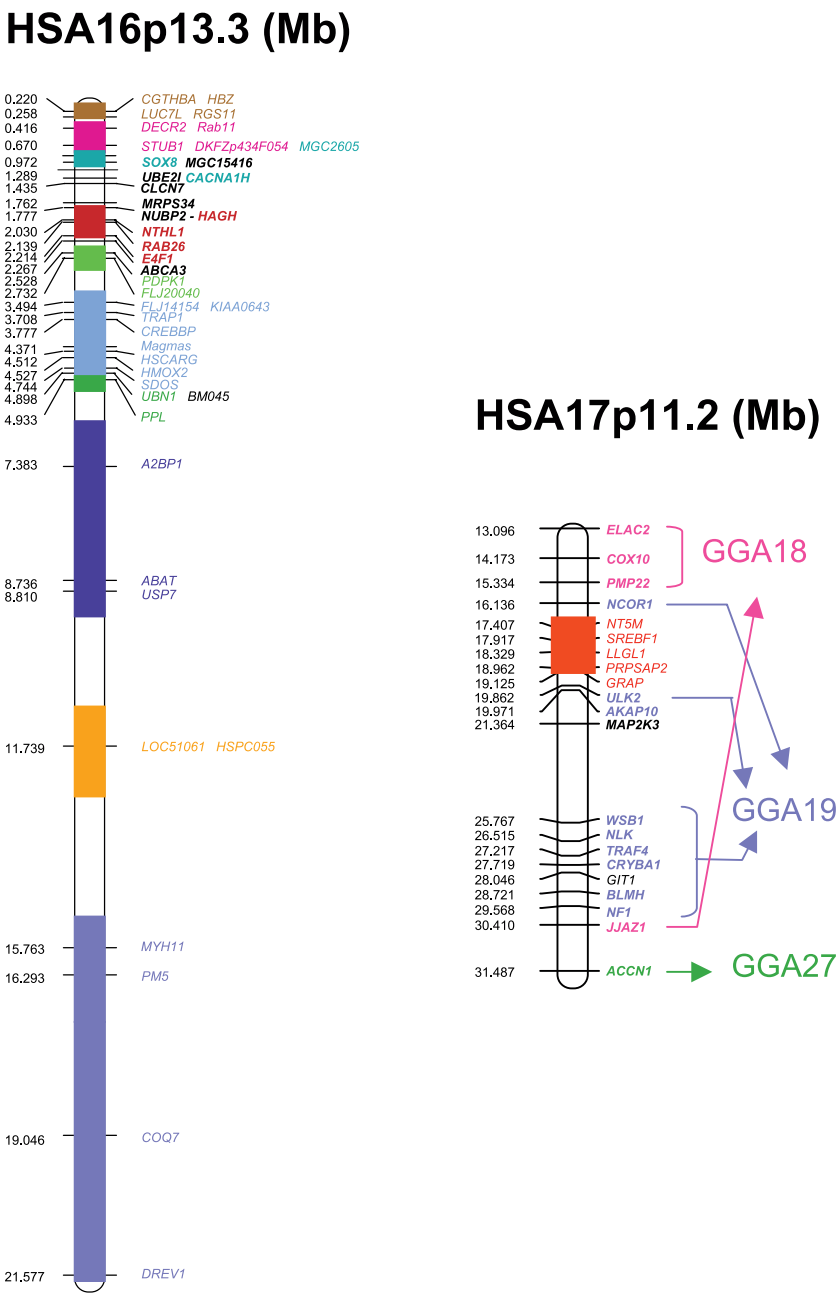

Figure 3. Continued.

Future developments towards a complete chicken RH framework map will now be based on the genomic sequence, using it for choosing STS markers regularly spaced along the chromosomes.

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