Incorporating Mouse Genome
Mammalian Genome
Genes and Phenotypes

A radiation hybrid map of chicken Chromosome 4

Tarik S.K.M. Rabie,[1]* Richard P.M.A. Crooijmans,[1] Mireille Morisson,[2] Joanna Andryszkiewicz,[1] Jan J. van der Poel,[1] Alain Vignal,[2] Martien A.M. Groenen[1]

[1]Wageningen Institute of Animal Sciences, Animal Breeding and Genetics Group, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands
[2]Laboratoire de génétique cellulaire, Institut national de la recherche agronomique, 31326 Castanet-Tolosan, France

Received: 15 December 2003 / Accepted: 16 March 2004

Abstract

The mapping resolution of the physical map for chicken Chromosome 4 (GGA4) was improved by a combination of radiation hybrid (RH) mapping and bacterial artificial chromosome (BAC) mapping. The ChickRH6 hybrid panel was used to construct an RH map of GGA4. Eleven microsatellites known to be located on GGA4 were included as anchors to the genetic linkage map for this chromosome. Based on the known conserved synteny between GGA4 and human Chromosomes 4 and X, sequences were identified for the orthologous chicken genes from these human chromosomes by BLAST analysis. These sequences were subsequently used for the development of STS markers to be typed on the RH panel. Using a logarithm of the odds (LOD) threshold of 5.0, nine linkage groups could be constructed which were aligned with the genetic linkage map of this chromosome. The resulting RH map consisted of the 11 microsatellite markers and 50 genes. To further increase the number of genes on the map and to provide additional anchor points for the physical BAC map of this chromosome, BAC clones were identified for 22 microsatellites and 99 genes. The combined RH and BAC mapping approach resulted in the mapping of 61 genes on GGA4 increasing the resolution of the chicken–human comparative map for this chromosome. This enhanced comparative mapping resolution enabled the identification of multiple rearrangements between GGA4 and human Chromosomes 4q and Xp.

Comparative genomics plays an important role in the understanding of genome dynamics during evolution and as a tool for the transfer of mapping information from species with gene-dense maps to species whose maps are less well developed (O'Brien et al. 1993, 1999). For farm animals, therefore, the human and mouse have been the logical choice as the model species used for this comparison. Medium-resolution comparative maps have been published for many of the livestock species, including pig, cattle, sheep, and horse, identifying large regions of conserved synteny between these species and man and mouse. More detailed analyses subsequently showed the presence of many internal rearrangements resulting in altered gene orders within these syntenic blocks (Sun et al. 1997, 1999; Rink et al. 2002; Larkin et al. 2003). In chicken, the first comparative maps indicated an extraordinary conservation of synteny between this species and mammals, even though these species diverged around 300–350 Myr ago (Smith et al. 1997; Groenen et al. 1998; Nanda et al. 1999; Burt et al. 1999). However, subsequent detailed mapping studies on a number of chicken chromosomes indicated that the number of intrachromosomal rearrangements was considerably higher then thus far anticipated (Suchyta et al. 2001; Crooijmans et al. 2001; Buitenhuis et al. 2002 and Jennen et al. 2002, 2003), clearly showing the need for an increased gene density on the chicken maps. Although a considerable number of genes have been mapped on the chicken linkage map, achieving the required high gene density necessary to identify the different conserved blocks within the regions of conserved synteny is not very practical because of the required polymorphism in the markers used. An alternative mapping approach that circumvents this problem is by using the radiation hybrid mapping technique

**Present address:* Suez Canal University Faculty of Agriculture, Biotechnology Research Institute, Ismailia, 41522, Egypt
Correspondence to: T.S.K.M. Rabie; E-mail: Tarik@egypt.net

 DOI: 10.1007/s00335-004-2362-8 • Volume 15, 560–569 (2004) •

(Walter et al. 1994). Originally, Goss and Harris (1975) first developed a technology for physical map generation using irradiation and fusion gene transfer (IFGT). This technique, however, was rarely used until advances in molecular genetics allowed efficient polymerase chain reaction (PCR) screening of the RH panels. Therefore, it was recently rediscovered (Cox et al. 1990; Walter et al. 1994) as an effective approach to building ordered maps of sequence-tagged sites. Since then radiation hybrid (RH) cell lines have proven to be a powerful resource for gene mapping, particularly in mammals, and they have been used to develop detailed physical gene-dense maps in human (Gyapay et al. 1996), zebrafish (Geisler et al. 1999), mouse (McCarthy et al. 1997), pig (Yerle et al. 1998), and horse (Kiguwa et al. 2000). Recently, a RH panel has also been constructed for chicken (Morisson et al. 2002), which has been used in the present study to improve the gene density on chicken Chromosome 4 (GGA4).

Genes mapped on the chicken linkage map for GGA4 (Groenen et al. 2000; Schmid et al. 2000) indicated that most of this chromosome showed synteny with human Chr 4 and the q arm of the human X chromosome. In addition, genes located on a number of different human chromosomes (HSA2, 3, and 5) mapped to the end of the linkage group of GGA4, most likely representing the tip of the q arm of this chromosome. These results were further confirmed by zoo-FISH experiments between HSA4 and GGA4 (Chowdhary and Raudsepp 2000). These results indicated that the region from GGA4q1.1 to GGA4q2.6 is syntenic with HSA4.

Recently, large collections of chicken gene sequences have become available in the form of expressed sequenced tags (EST) (Tirunaguru et al. 2000; Abdrakhmanov et al. 2000; Boardman et al. 2002). Clustering of these ESTs followed by sequence comparisons to human genes indicates that the chance of finding a chicken ortholog for a particular human gene is around 2 out of 3. This resource of chicken EST sequences was used in the current study to improve the gene density on GGA4 both by using the ChickRH6 panel and the chicken BAC library constructed in Wageningen (Crooijmans et al. 2000).

Materials and methods

Selection of markers and genes. For type II markers, 23 chicken microsatellite markers covering the p and q arms of GGA4 were selected from the published chicken genetic map (Groenen et al. 2000). Primer information for microsatellite markers located on GGA4, such as a primer sequence and PCR conditions, can be found at ARKdb farm animal database (http://www.thearkdb.org/) and ChickAce (https://acedb.asg.wur.nl/).

For type I markers, 127 primer pairs derived from EST sequences representing chicken orthologs to genes located on HSA4 (102 genes) and HSAX (25 genes) were selected. Potential chicken orthologous sequences were first identified by a BLAST database search (BLAST v2.0 software; http://www.ncbi.nlm.nih.gov/blast) with the human mRNA sequences representing all the genes known to be located on HSA4 and HSAX. The BLAST analysis was performed against a local chicken EST database containing all publicly available chicken EST sequences. Homologous chicken ESTs were subsequently used in a BLAST search against all human mRNAs (E-values at least e −50) to distinguish between orthologous and paralogous sequences. Only those chicken EST sequences that most likely represented the chicken ortholog of a gene located in human on HSA4 and HSAX were used for further analysis. Primer pairs were designed preferably within a single exon. For those cases where the resulting PCR product would be too small (<100 bp), primers were designed in adjacent exons spanning the intervening intron. In these cases, preferably the smaller introns were chosen. Primers were designed with the PRIMER3 program (http://frodo.wi.mit.edu/) (Table 1). Amplification conditions for each marker were optimized by varying the annealing temperature to produce a single amplicon of the predicted length with chicken genomic DNA and no amplification with genomic hamster DNA. Only primer pairs that gave a clear amplification product with the chicken and not with the hamster DNA were used for RH typing.

RH panel screening. The ChickRH6 panel (Morisson et al. 2002) consists of a total of 90 hybrids. Chicken and hamster genomic DNA and TE buffer were used as positive and negative controls, respectively. Ten to 25 ng of each panel DNA was amplified in a 384-well plate in a 6-μl mixture containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1 mM tetramethylammoniumchloride (TMAC), 0.1% Triton X-100, 0.01% gelatin, 0.2 mM of each dNTP, 0.125 U Silverstar polymerase (Eurogentec, Liege, Belgium) and 1.2 pmol of each primer. Amplification products were separated by electrophoresis on 1.5% ethidium bromide-stained agarose gels in 0.5 TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA, pH 8.3), and reactions were

Table 1. Primer information for genes used in this study

		Accession number					
Gene symbol[a]	*Human cytogenetic map position*	*Human*[b]	*Chicken*	*PCR product size (bp)*	*Temp*[c]	*Rf*[d]	*BAC clone*
ABCE1	4q31	002940	JMB20j18r1	118	50	0.27	bW028B23
ADH5	4q21–q25	000671	TC6849	168	50	—	bW029H01
AFAP	4p16	021638	NP344463	120	50	0.13	bW011H18
AGXT2L1	4q25	031279	46351.2	148	50	0.17	bW028C02
ALP	4q35	014476	345746.3	178	50	0.14	bW037A03
ANK2	4q25–q27	001148	47969.1	267	50	0.13	bW036M09
AP1S2	Xp22.31	003916	337157.1	140	50	—	bW003B19
APG-1	4q28	014278	JMB35g8r1	146	50	0.13	bW015H15
ARGBP2	4q35.1	003603	54771.8	104	50	0.13	bW020H21
ARHGEF9	Xq11.1	015185	334327.1	162	50	0.41	bW008I04
ATP7A	Xq13.2–q13.3	000052	34574.1	360	50	—	—
BCMP1	Xp11.4	031442	500086.1	115	50	—	bW039C07
BMPR1B	4q22–q24	001203	349923.1	157	50	0.26	bW015M22
BTK	Xq21.33–q22	000061	345575.2	154	50	—	—
CAMK2D	4q25	001221	346674	192	50	—	bW007B01
CCNA2	4q25–q31	001237	56394	104	50	—	—
CCNG2	4q13.3	004354	44206	145	50	—	bW013P18
CCNI	4q13.3	006835	TC6787	129	50	0.11	bW080M14
CDKL2	4q21.1	003948	56394	139	50	0.12	bW034M24
CENTG1	4q21.21	017593	47690.1	118	50	0.40	bW010H02
CLCN3	4q33	001829	JMB7c4r1	147	50	0.57	bW064B10
CLCN5	Xp11.23–p11.22	000084	346697.1	324	50	—	bW046O02
CLOCK	4q12	004898	TC7301	144	50	—	—
CMG2	4q21.22	058172	345485.2	328	45	—	—
CNGA1	4p12-cen	000087	NP346095	242	50	0.07	bW121F14
COPS4	4q21.3	016129	TC4353	121	50	0.13	bW104P05
COVA1	Xq25–q26.2	006375	334353.3	131	50	0.51	bW023C04
CPE	4q32.3	001873	TC4299	137	50	0.36	bW023N17
CRMP1	4p16.1–p15	001313	TC4156	147	50	—	—
CUL4B	Xq23	003588	38850.1	148	50	—	bW039K18
D4S234E	4p16.3	014392	JMB7123745	110	45	—	bW094K13
DC2	4q24	021227	TC7399	130	55	—	bW006A08
DCK	4q13.3–q21.1	000788	337391.2	131	50	0.10	bW095E06
DCX	Xq22.3–q23	000555	57758.2	196	50	—	bW012F01
DDX3X	Xp11.3–p11.23	001356	56851.1	126	50	—	—
DIAPH2	Xq22	006729	347324.1	184	50	0.73	bW012P24
DJ473B4	Xq26.3	019556	341971.1	121	45	—	—
DKC1	Xq28	001363	337406.3	150	50	0.58	—
DKK2	4q25	014421	342800.1	125	50	0.18	bW026B08
DLG3	Xq13.1	021120	58956.1	129	50	—	bW017B24
ED1	Xq12–q13.1	001399	337200.2	166	50	—	bW010A11
EIF2S3	Xp22–p22.1	001415	335006.2	157	55	—	bW037A22
EIF4E	4q21–q25	001968	TC6391	202	50	0.13	—
ELF2	4q28	006874	BI394288	183	50	—	bW031P04
ELOVL6	4q25	024090	AI981662	203	50	0.14	bW011K04
EPHA5	4q13.1	004439	334182.2	326	50	—	bW016J03
FACL2	4q34–q35	021122	339343.2	130	50	—	—
FAT	4q34–q35	005245	331914.2	422	50	0.31	bW061E08
FBXO8	4q34.1	012180	JMB30p13r1	184	50	—	bW048H08
FGB	4q28	005141	TC6789	204	50	—	bW013I12
FGF2	4q26–q27	002006	TC7431	280	50	0.16	bW014I21
FLNA	Xq28	001456	53860.1	107	50	0.18	bW041F20
FMR1	Xq27.3	002024	34198.1	138	45	0.60	bW010I17
GAB1	4q28.3	002039	1489.1	210	50	0.09	—
GABRA4	4p12	000809	17087.1	122	50	0.20	bW040D21
GALNT7	4q32.1	017423	5917	127	55	0.12	bW060F11
GDI1	Xq28	001493	TC6728	153	50	—	bW030F13
GLRB	4q31.3	000824	25861	171	50	0.50	—
GPM6A	4q34	005277	TC7231	109	50	0.18	—

(continued)

Table 1. Continued

Gene symbol[a]	Human cytogenetic map position	Accession number: Human	Accession number: Chicken	PCR product size (bp)	Temp[c]	Rf[d]	BAC clone
GRIA2	4q32–q33	000826	43814.1	140	50	—	bW036I02
GRID2	4q22	001510	38183	249	50	0.19	bW056K10
GRPEL1	4p16	025196	JMB32n8r1	192	55	0.18	bW012N03
GUCY1A3	4q31.1–q31.2	000856	34342	269	50	0.52	bW010I19
HAND2	4q33	021973	TC7847	216	45	—	bW053D06
HD	4p16.3	002111	31503.1	195	50	0.17	bW017P21
HDAC8	Xq13	018486	335214.3	113	50	0.48	bW028E14
HERC3	4q21	014606	JMB7132077	110	55	0.10	bW035K18
HNRPD	4q21.1–q21.2	031370	AI981884	151	50	0.13	bW055D20
HNRPDL	4q13–q21	005463	TC7164	127	45	—	—
HNRPH2	Xq22	019597	TC4142	697	50	—	—
HSA6591	4	014487	345459.4	113	45	—	—
ING1L	4q35.1	001564	342599.1	260	50	—	bW012O02
KAL1	Xp22.32	000216	NP346520	139	50	0.07	—
KLHL2	4q21.2	007246	45313.2	125	50	—	bW023N17
LDB2	4p16	001290	NP345067	150	50	0.16	bW032K09
LPHN3	4q12	015236	26237	305	50	0.14	—
LGR3	4q31.3	021634	35188	127	55	0.67	bW024G14
LGR7	4q32.1	021634	35188.1	127	50	0.48	bW028J09
MAB21L2	4q31	006439	AL586521	441	50	—	bW056M04
MADH1	4q28	005900	TC5227	159	50	0.13	bW063C11
MGC10646	4q21.1	032693	TC6571	153	50	—	—
MGC11324	4q21.3	032717	336345.1	165	50	0.16	bW001O18
MID1	Xp22	000381	341078.2	137	50	—	bW129O12
MORF4	4q33–q34.1	006792	BI391102	222	50	0.12	bW045D14
NDST3	4q28.1	004784	19176	178	50	0.20	bW040N15
NDST4	4q25–q26	022569	58090.1	161	50	0.12	bW015N23
NFKB1	4q24	003998	TC4333	168	50	0.13	bW021B20
NR3C2	4q31.1	000901	349338	124	50	0.21	bW023A13
NUP54	4q21.1	017426	AI982010	135	50	0.11	bW096F06
PAPSS1	4q24	005443	34885	148	50	0.18	bW020L08
PCDH10	4q28.3	020815	508333	218	50	0.33	bW026F07
PCDH7	4p15	002589	4735.1	184	50	—	—
PDGFC	4q32	016205	NP344712	125	55	0.52	bW014N12
PDGFRA	4q12	006206	TC8274	223	50	0.20	bW029L03
PDHA2	4q22–q23	005390	332935.5	156	50	—	bW001E08
PDZGEF1	4q32.1	014247	5249	212	50	0.11	bW040J23
PGRMC2	4q26	006320	BI391557	152	55	—	bW015H15
PITX2	4q25–q27	000325	TC5315	106	50	—	—
PKD2	4q13.2	016457	AJ395970	250	50	0.14	bW016H13
PMSCL1	4q28.1	005033	JMB7120551	109	50	—	bW023K21
POLR2B	4q12	000938	TC7941	159	50	0.18	bW017P12
PPAT	4q12	002703	TC8440	187	50	0.13	bW018F15
PRSS12	4q28.1	003619	46899.1	166	50	0.13	bW014K04
RAB33B	4q28	031296	37969.1	282	50	0.29	bW039B02
RRAGB	Xp11.21	006064	335137.4	119	45	0.41	bW074F04
REST	4q12	005612	44465	292	50	—	bW017P12
RPS3A	4q31.2–q31.3	001006	TC4166	248	50	—	—
SEC3	4q11	018261	43272	127	50	—	bW016D22
SGCB	4q12	000232	TC6012	219	50	0.13	bW084D22
ShrmL	4q13.3	020859	331830.9	164	50	0.13	—
SLC25A4	4q35	001151	TC6688	183	50	—	—
SLC4A4	4q21	003759	346067	220	50	0.12	bW039D14
SLIT2	4p15.2	004787	2748.1	143	50	—	—
SMARCA5	4q31.1–q31.2	003601	TC6224	159	50	—	bW122E10
SMCIL1	Xp11.22–p11.21	006306	349580.1	150	50	—	—
SNX25	4q35.1	031953	33244	101	50	0.19	bW075K07

(continued)

Table 1. Continued

Gene symbol[a]	Human cytogenetic map position	Accession number: Human[b]	Accession number: Chicken	PCR product size (bp)	Temp[c]	Rf[d]	BAC clone
STIM2	4p15.2	020860	AI981296	148	50	0.18	bW056N05
TEC	4p12	003215	JMB38k23r1	135	50	0.17	bW082I06
TLL1	4q32–q33	012464	20997	176	50	0.37	bW034I19
TPARL	4q12	018475	340757.2	129	50	0.19	bW022H06
TRIM2	4q31.23	015271	21084	215	50	—	—
TUBB4Q	4q35	020040	TC4138	212	50	0.11	bW088E02
UBE2D3	4q22.2	003340	TC6035	159	45	0.20	bW012N06
UGDH	4p15.1	003359	TC5502	147	50	0.13	bW001M13
UGT8	4q26	003360	33803.1	276	50	0.16	bW015N23
USP38	4q31.1	032557	JMB21p18r1	207	50	0.29	bW068F11
VEGFC	4q34.1–q34.3	005429	44227.1	188	50	0.19	bW027L11

[a] Primer information such as a primer sequence can be found at (https://acedb.asg.wur.nl/).
[b] Accession number (NM_) of human genes used in BLAST, searching for identifies chicken orthologous genes.
[c] Annealing temperature in PCR.
[d] Rf: retention frequency.

scored for the presence or absence of the specific amplification product. Each marker was typed independently in duplicate.

BAC library screening. The BAC library was screened for type I and type II markers by two-dimensional PCR (Crooijmans et al. 2000). In the first round, positive plates were identified followed by the PCR analysis of the row and column pools of the positive plates. At least one BAC clone from each of the markers was identified.

PCR conditions. For both RH panel and BAC screening, the PCR reactions were started with 5 min at 95°C followed by 35 cycles for 30 sec at 95°C, 45 sec at 45°C, 50°C or 55°C annealing temperature, and 60 sec at 72°C, followed by a final elongation step at 72°C for 10 min, and finally stop step at 4°C. PCR reactions were performed in PCR system Biometra® using 384-well plates.

Statistical analysis and map construction. The CarthaGene program (Schiex and Gaspin 1997) was used to analyze and construct the RH map for GGA4. CarthaGene is a maximum-likelihood multipoint RH and genetic data-mapping tool (available at http://www.inra.fr/bia/T/CarthaGene/). Markers disrupting good map ordering were identified in the best sets of map orders produced and by examining the consistency of patterns for two-point LOD scores (higher than 5) of markers in their assigned order against each other across the chromosome to get the final frame work groups (Fig. 1). Therefore, final map distances were calculated by using CarthaGene order as input for the RH map for maximum likelihood.

Results

RH mapping. We initially started with the 127 STS markers representing likely orthologs of genes from human Chrs 4 and X and 23 chicken microsatellite markers that had previously been mapped to GGA4. These markers were tested for successful amplification on chicken genomic DNA and on the DNA from the RH panel. Successful amplification was defined as a single amplification product as visualized on agarose gel electrophoresis and the absence of that fragment using the hamster genomic DNA as a control. This was eventually achieved for 77 genes (60.63%) and 15 of the microsatellite markers (65.22%). These markers were typed on the RH panel and used for further linkage analysis.

The first step in building the chicken Chr 4 RH map was to group markers within separate linkage groups using a LOD threshold of 5.0. This allowed us to create an initial set of 9 RH linkage groups, containing 61 of the genes and 11 of the microsatellite markers. For 22 markers (randomly distributed along HSA4 and HSAX), no linkage was observed with any of the other markers, even after lowering the LOD threshold to 2.0. For the linkage groups 1–9 (Fig. 1), the locus order was investigated by using the build option of the CarthaGene program. The retention frequency (*Rf*) was calculated for all markers used and was found to vary enormously. Although the average *Rf* for the markers was 24%, the lowest *Rf* observed was 7% for the *KAL1* gene whereas the

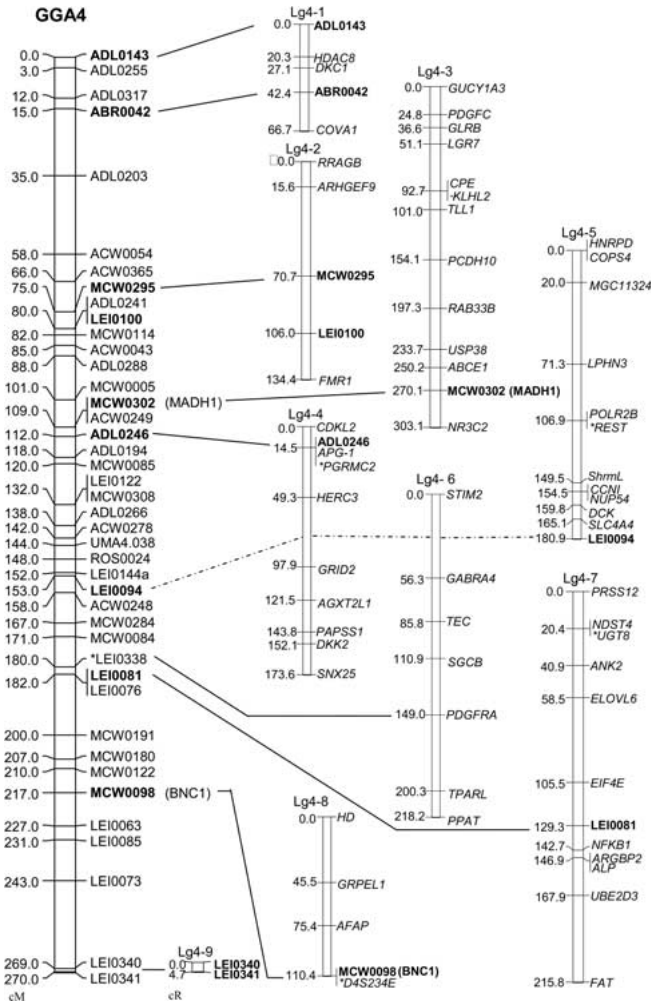

Fig. 1. Alignment of RH maps of chicken Chr 4 with the framework genetic linkage map of GGA4 map. Microsatellites that are also located on the genetic linkage map are indicated in bold. Genes indicated by an asterisk have been mapped by using BAC information. RH linkage groups shown are supported by a two-point LOD score >5. The map within each group was identified as the best order using the ''Nice-map'' analysis within CarthaGene.

highest *Rf* (82%) was observed for marker *ADL0317* (Table 1).

BAC library. To further increase the number of genes on GGA4 and to increase the number of anchor points between the RH maps of Chr 4 and the BAC contig map currently under construction, the Wageningen BAC library (Table 1) was screened with 127 of the STS markers described above. For 99.99 markers at least one BAC clone was identified (77.95%). Because several of the STSs appeared to be positive for the same BAC(s), an additional 5 genes could be assigned to the RH linkage maps (linkage groups 3, 4, 5, 7, and 8). Furthermore, an additional RH linkage 180 group (linkage group 6) could be

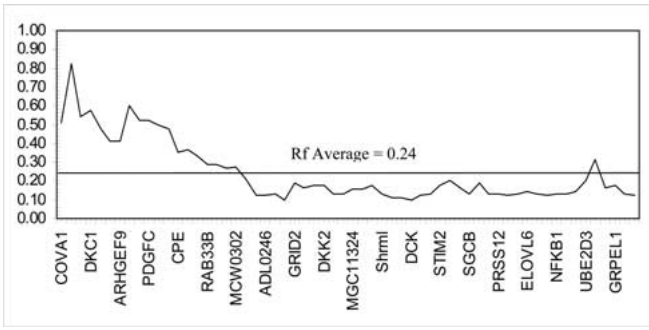

Fig. 2. Distribution of retention frequency along Chromosome 4 for the markers used. The average retention frequency was 24%, the highest and the lowest values (82% and 7%) were observed for the marker ADL0317 and for *KAL1* gene, respectively.

anchored to the genetic linkage map of GGA4 (Fig. 1). The BAC clone bW040D21 identified for microsatellite marker *LEI0388* was also positive for the marker developed within the gene *GABRA4*, allowing the positioning of RH linkage group 6 containing the genes *STIM2*, *GABRA4*, *TEC*, *SGCB*, *PDGFRA*, *TPARL*, and *PPAT* to the region around position 180 cM on the genetic linkage map for GGA4.

The five genes (*KLHL2*, *PGRMC2*, *REST*, *UGT8*, and *D4S234E*) that were added to RH linkage groups 3, 4, 5, 7 and 8, respectively, based on the identified BACs are labeled in Figure 1 by an asterisk. In order to avoid inflation of the map size of these linkage groups, we chose to project these additional markers at their most likely location without altering the multipoint distance between framework markers (Fig. 1).

Discussion

RH mapping. Because GGA4 showed conservation of synteny mainly to HSAX and HSA4 (Schmid et al. 2000), chicken ESTs orthologous to genes located in man on HSAX and HSA4 were identified. The combined RH mapping and BAC identification approach eventually resulted in the successful mapping of 61 genes to GGA4, 54 of which were homologous to genes located on HSA4 and 7 of which were homologous to genes located on HSAX. Although the resulting RH map consists of 9 independent linkage groups, all are linked by a marker to the genetic linkage map of GGA4 and therefore could be ordered with respect to each other. However, since most of these linkage maps are connected to the genetic linkage map by only a single marker, the relative orientation of them is not known.

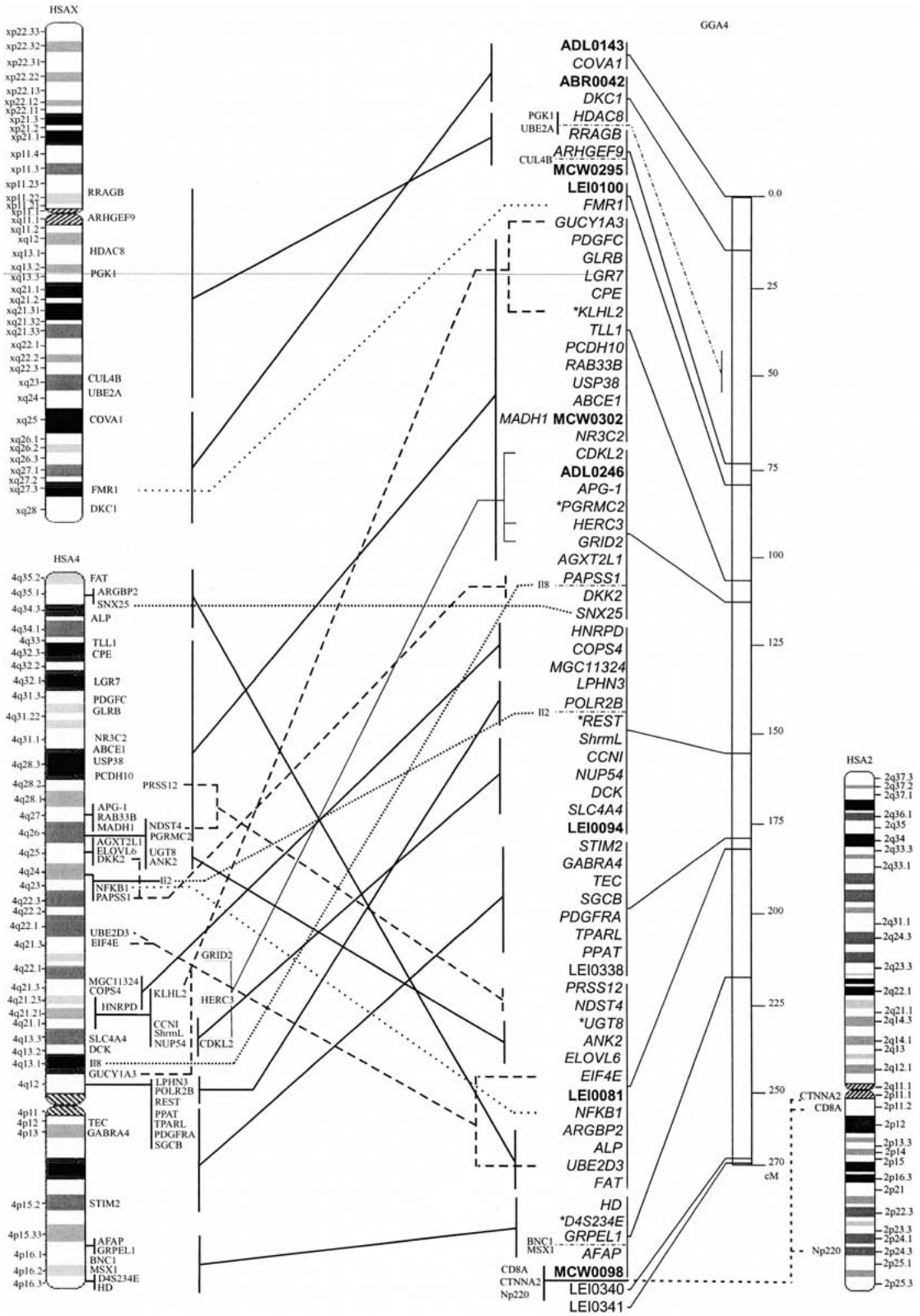

Fig. 3. Comparative map between chicken Chr 4 and human Chr 2, 4, and X. Estimated positions for human genes are given in cM, according to the Map Viewer from Entrez Genomes (http://www.ncbi.nlm.nih.gov/). The numbers located on the right of the vertical bar of GGA4 represent the relative positions in cM of the chicken loci. Microsatellite markers are in bold; genes labeled with an asterisk are mapped based on the BAC screening. Genes mapped by RH are in italics.

There were some initial difficulties at arriving at the gene order for the 9 radiation hybrid maps for GGA4. For example, although *PDGFRA* has a low two-point LOD score with *SGCB*, *GABRA4*, and *PPAT* (only 2.0, 0.2, and 3.3, respectively), it has a high two-point LOD score with *TEC* and *TPARL* (7.0 and 8.0, respectively), clearly indicating that it is located on the same linkage group. The average observed *Rf* for the markers mapped to GGA4 is 24%, which is similar to the results described by Morisson et al. (2002) who used two microsatellite markers from GGA4 (*MCW0085* and *MCW0099*). The *Rf*s for these two markers were 20% and 7%, respectively. In another study using this RH panel (Jennen et al. 2004), which focused on GGA15, the observed average *Rf* was also found to be in the same range (18%). However, the observed *Rf* of the markers used in this study varied considerably along the chromosome (Fig. 2). The *Rf* was particularly high in the region around position 15 and 80 cM of the consensus linkage map (around map positions 27 and 134 cR of linkage groups 1 and 2, respectively). The differences in *Rf* are observed for markers that are not just randomly distributed along GGA4 but that are located in the same region on the chromosome. This indicates that the observed retention frequencies reflect the actual retention of these chromosomal fragments in the cell lines from the radiation hybrid cell panel and are not caused by nonspecific amplification of the marker.

BAC library. The BAC clones that were identified by screening the Wageningen BAC library resulted in the mapping of several additional genes on GGA4, i.e., *KLHL2*, *PGRMC2*, *REST*, *UGT8*, and *D4S234E*. Furthermore, the identification of BAC bW040D21 for markers *LEI0338* and *GABRA4* resulted in the positioning of RH linkage group 6 around position 180 on the genetic linkage map of GGA4, thereby placing another 7 genes on this chromosome (*STIM2*, *GABRA4*, *TEC*, *SGCB*, *PDGFRA*, *TPARL*, and *PPAT*) (marker details can be found at https://acedb.asg.wur.nl/). BACs that have been identified with markers mapped on GGA4 are also key elements that help to integrate the cytogenetic and linkage maps with the physical BAC contig map. BAC end sequencing of the Wageningen BAC clones is currently in progress and this will aid in the further integration of the RH, linkage, and BAC mapping data with chicken genome sequence contigs.

Comparative map. A comparison of the genes mapped on GGA4 to the chromosomal location of the orthologous genes in human is shown in Figure 3. The available comparative mapping data clearly show that the major part of GGA4 shows conserved synteny to the q arm of HSAX and the q arm of HSA4. In addition, regions syntenic to the p arm of HSA4 and to small regions on HSA21q, HSA3p, HSA2p, and HSA5q have also been described (Groenen et al. 2000; Schmid et al. 2000; Matsishima et al. 2000). However, in those cases where such conserved synteny is based on only a single gene, one has to pay particular caution as many of these links may be the result of incorrect previous identifications of orthologs versus paralogs. The conserved synteny between GGA4 and HSA5, for example, is based on the FISH mapping of the *CTNN1* gene to the distal tip of the q arm of GGA4 (Suzuki et al. 1999). Interestingly, the related *CTNN2* gene has also been mapped to HSA2p12–11.1, already indicating that the gene mapped in chicken probably is the ortholog of *CTNN2* (Groenen and Crooijmans 2003). This led us to reexamine the FISH mapping results by Suzuki et al. (1999) and a BLASTN search was done with the sequence of the cDNA clone used in the FISH experiment (accession number D11090). The results clearly showed that this cDNA was the orthologous gene of the human *CTNNA2* gene that is located on GGA4 (with a sequence identity of 82%). The other example on GGA4 is the *TGFBR2* gene, which is located on HSA3 and on MMU9. No other genes currently mapped on GGA4 are located on these chromosomes in man and mouse. A close reexamination of the data for the consensus linkage map showed that the localization of this gene on GGA4 on the consensus map (Groenen et al. 2000) has been erroneous. Finally, the link between the distal part of GGA4p to HSA21q needs to be regarded as doubtful. This presumed syntenic link was established by mapping the microsatellite *MCW0047* to position 23 cM on the linkage map of GGA4 (Crooijmans et al. 1995; Groenen et al. 2000). This microsatellite was derived from a genomic clone (accession number M20817) containing part of the *HMGN1* gene. However, a close examination of a BLASTN database search using this sequence showed that this sequence also has sequence identity of 94–97% to several other human clones derived from a number of different human chromosomes (11, 12, 13, 14, 15, 17, 21, and 22) including HSAX.

Although the exact map location of the genes that were mapped in this study to GGA4 is not known yet, the approximate positions of these genes already clearly indicate that multiple rearrangements have occurred within the syntenic regions on chicken Chr 4 and human Chr 4, X, and 2 (Fig. 3). The comparative data that are currently available for GGA4 indicate that this chromosome contains at

least 20 CSOs (Conserved Segments Ordered) when compared to human. These findings are in agreement with previous studies on chicken Chr 10 and 15 where a large number of rearrangements were observed within regions of conserved synteny between man and chicken (Crooijmans et al. 2001; Jennen et al. 2003). In these studies the average size of these CSOs was between 4 and 6 cM, which is even smaller than that observed for GGA4. This, however, is due to the density of the genes currently mapped on these chromosomes, and it is expected that the number of conserved segments will increase as the number of genes mapped in chicken increases. Based on the currently available data, we expect that the number of conserved regions between chicken and human might be as high as 300 (20 CSOs with a size of GGA4 approximately 7% of the chicken genome) to 1000 (19 CSOs with a size of GGA15 approximately 1.8% of the chicken genome; Jennen et al. 2004) with an average size of just a few cM. A more accurate estimation of this number has to await the completion of the physical and sequence maps of the chicken genome expected to become available in 2004.

Acknowledgments

The authors would like to thank the Egyptian Ministry of Higher Education and Scientific Research for financial support (Project No. 29110/416000).

References

1. Abdrakhomanov I, Lodygin D, Geroth P, Arakawa H, et al. (2000) A large database of chicken bursal ESTs as a resource for the analysis of vertebrate gene function. Genome Res 10, 2062–2069
2. Aerts J, Crooijmans RPMA, Cornelissen S, Hemmatian K, et al. (2003) Integration of chicken genomic resources to enable whole-genome sequencing. Cytogenet Genome Res 102, 297–303
3. Boardman PE, Sanz–Ezquerro J, Overton JM, Burt DW, et al. (2002) A comprehensive collection of chicken cDNAs. Curr Biol 12, 1965–1969
4. Buitenhuis AJ, Crooijmans RPMA, Bruijnesteijn van Coppenraet L, Veenendaal A, et al. (2002) Improvement of the comparative map of chicken chromosome 13. Anim Genet 33, 249–254
5. Burt DW, Bruley C, Dunn IC, Jones CT, et al. (1999) The dynamics of chromosome evolution in birds and mammals. Nature 402, 411–413
6. Chowdhary BP, Raudsepp T (2000) HSA4 and GGA4: Remarkable conservation despite 300-Myr divergence. Genomics 64, 102–105
7. Cox DR, Burmeister M, Price ER, Kim S, Myers RM (1990) Radiation hybrid mapping: A somatic cell genetic method for constructing high-resolution maps of mammalian chromosomes. Science 250, 245–250
8. Crooijmans RPMA, van der Poel JJ, Groenen MAM (1995) Functional genes mapped on the chicken genome. Anim Genet 26, 73–78
9. Crooijmans RPMA, Verbalove J, Dijkhof RJM, Van Der Poel JJ, Groenen MAM (2000) Two-dimensional screening of the wageningen chicken BAC library. Mamm Genome 11, 360–363
10. Crooijmans RPMA, Dijkhof RJM, Veenendaal T, van der Poel JJ, et al. (2001) The gene orders on human chromosome 15 and chicken chromosome 10 reveal multiple inter- and intrachromosomal rearrangements. Mol Biol Evol 18, 2102–2109
11. Geisler R, Rauch G-J, Baier H, Van Bebber F, et al. (1999) A radiation hybrid map of the zebrafish genome. Nat Genet 23, 86–89
12. Goss S, Harris H (1975) New method for mapping genes in human chromosomes. Nature 255, 680–684
13. Groenen MAM, Crooijmans RPMA (2003) Structural genomics: integrating linkage, physical and sequence maps. In *Poultry genetics, breeding and technology,* Muir WM, Koehn RK, eds. (Cambridge, MA: CAB International), pp 497–536
14. Groenen MAM, Crooijmans RPMA, Veenendaal A, Cheng HH, et al. (1998) A comprehensive microsatellite linkage map of the chicken genome. Genomics 49, 265–274
15. Groenen MAM, Cheng HH, Bumstead N, Benkel B, et al. (2000) Consensus linkage map of the chicken genome. Genome Res 10, 137–147
16. Gyapay G, Schmitt K, Fizames C, Jones H, et al. (1996) A radiation hybrid map of the human genome. Hum Mol Genet 5, 339–346
17. Jennen DGJ, Crooijmans RPMA, Kamps B, Acar A, et al. (2002) A comparative map of chicken chromosome 24 and human chromosome 11. Anim Genet 33, 205–210
18. Jennen DGJ, Crooijmans RPMA, Kamps B, Açar R, et al. (2003) Comparative map between chicken chromosome 15 and human chromosomal region 12q24 and 22q11-q12. Mamm Genome 14, 629–639
19. Jennen DGJ, Crooijmans RPMA, Morisson M, Grootemaat AE, et al. (2004) A radiation hybrid map of chicken chromosome 15. Anim Genet 35, 63–65
20. Kiguwa SL, Hextall P, Smith AL, Critcher R, et al. (2000) Characterization of whole genome radiation hybrid panel: Chromosomes 1 and 10 preliminary maps. Mamm Genome 11, 803–805
21. Larkin DM, Everts–van der wind A, Rebeiz WM, Schweitzer PA, et al. (2003) A cattle–human comparative map built with cattle BAC-ends and human genome sequence. Genome Res 13, 1966–1972
22. Matsishima Y, Matsumura K, Ishii S, Suzuki Y, et al. (2000) Chromosomal assignment of the chicken NP220 gene encoding a large DNA-binding nuclear protein and its targeted disruption in chicken DT40 cells. Cytotechnology 33, 109–115
23. McCarthy LC, Terrett J, Davis ME, Smith AL, et al. (1997) A first-generation whole-genome radiation hy-

brid map spanning the mouse genome. Genome Res 7, 1153–1161
24. Morisson M, Lemière A, Bosc S, Galan M, et al. (2002) ChickRH6: a chicken whole-genome radiation hybrid panel. Genet Sel Evol 34, 521–533
25. Nanda I, Koehler M, Schartl M, Burt DW, et al. (1999) 300 million years of conserved synteny between chicken Z and human chromosome 9. Nat Genet 21, 258–259
26. O'Brien SJ, Womack JE, Lyons LA, Moore KJ, et al. (1993) Anchored reference loci for comparative genome mapping in mammals. Nat Genet 3, 103–112
27. O'Brien SJ, Raymond MM, Murphy WJ, Nash WG, et al. (1999) The promise of comparative genomics in mammals. Science 286, 458–481
28. Rink A, Santschi EM, Eyer KM, Roelofs B, et al. (2002) A first generation EST RH comparative map of porcine and human genome. Mamm Genome 13, 578–587
29. Schiex T, Gaspin C (1997) CARTHAGENE: constructing and joining maximum likelihood genetic maps. Proc Int Conf Intell Syst Mol Biol 5, 258–267
30. Schmid M, Nanda I, Guttenbach M, Steinlein C, et al. (2000) First report on chicken genes and chromosomes 2000. Cytogenet Cell Genet 90, 169–218
31. Smith EJ, Lyons LA, Cheng HH, Suchyta SP (1997) Comparative mapping of the chicken genome using the East Lansing reference population. Poultry Sci 76, 743–747
32. Suchyta SP, Cheng HH, Burnside J, Dodgson JB (2001) Comparative mapping of chicken anchor loci orthologous to genes on human chromosomes 1, 4 and 9. Anim Genet 32, 12–18
33. Sun HS, Cai L, Davis SK, Taylor JF, et al. (1997) Comparative linkage mapping of human chromosome 13 and bovine chromosome 12. Genomics 39, 47–54
34. Sun HS, Yerle M, Pinton P, Chardon P, et al. (1999) Physical assignments of human chromosome 13 genes on pig chromosome 11 demonstrate extensive synteny and gene order conservation between pig and human. Anim Genet 30, 304–308
35. Suzuki T, Kurosaki T, Shimada K, Kansaku N, et al. (1999) Cytogenetic mapping of 31 functional genes on chicken chromosomes by direct R-banding FISH. Cytogenet Cell Genet 87, 32–40
36. Tirunaguru VG, Sofer L, Cui J, Burnside J (2000) An expressed sequence tag database of T-cell-enriched activated chicken splenocytes: sequence analysis of 5251 clones. Genomics 66, 144–151
37. Walter MA, Spillett DJ, Tomas P, Weissenbach J, Goodfellow PN (1994) A method for constructing radiation hybrid maps of whole genomes. Nat Genet 7, 22–28
38. Yerle M, Pinton P, Robic A, Alfonso A, et al. (1998) Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. Cytogenet Cell Genet 82, 182–188