

The chicken β_2 -microglobulin gene is located on a non-major histocompatibility complex microchromosome: A small, G+C-rich gene with X and Y boxes in the promoter

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ABSTRACT β_2 -Microglobulin is an essential subunit of major histocompatibility complex (*Mhc*) class I molecules, which present antigenic peptides to T lymphocytes. We sequenced a number of cDNAs and two genomic clones corresponding to chicken β_2 -microglobulin. The chicken β_2 -microglobulin gene has a similar genomic organization but smaller introns and higher G+C content than mammalian β_2 -microglobulin genes. The promoter region is particularly G+C-rich and contains, in addition to interferon regulatory elements, potential S/W, X, and Y boxes that were originally described for mammalian class II but not class I α or β_2 -microglobulin genes. There is a single chicken β_2 -microglobulin gene that has little polymorphism in the coding region. Restriction fragment length polymorphisms from *Mhc* homozygous lines, *Mhc* congenic lines, and backcross families, as well as *in situ* hybridization, show that the β_2 -microglobulin gene is located on a microchromosome different from the one that contains the chicken *Mhc*. We propose that the structural similarities between the β_2 -microglobulin and *Mhc* genes in the chicken are due to their presence on microchromosomes and suggest that these features and the microchromosomes appeared by deletion of DNA in the lineage leading to the birds.

The avian genome is small and G+C-rich relative to typical mammalian and amphibian genomes. In addition, most birds have very similar karyotypes compared with mammals and amphibians, with a few chromosomes near mammalian size (so-called macrochromosomes) and around 30 smaller microchromosomes. By cytological staining, many birds have similar banding patterns on the macrochromosomes, indicating a conserved pattern of G+C-rich and A+T-rich isochores, with most microchromosomes staining G+C-rich. Variations in karyotype among birds have been ascribed to chromosomal fusions during evolution, as though all birds derived from an ancestor with many microchromosomes (1–6).

The major histocompatibility complex (*Mhc*) of chickens (or *B* complex) is a genetic region that is located on a microchromosome (number 16 in size) (7). Overall, the chicken *Mhc* appears to be smaller and simpler than the *Mhc* of mammals, with the few polymorphic class I α and class II β genes being closely spaced, compact, and G+C-rich (8–12).

We have suggested that the small introns, small intragenic distances, and high G+C content of chicken *Mhc* genes are due to the fact that they are located on a microchromosome. We have further proposed that during evolution, some unknown agent deleted DNA at random in the lineage that led to the

birds, that some chromosomes were affected to the point of becoming microchromosomes, and that this process effectively ended in the genomes of the few survivors that actually gave rise to the birds (12–14).

Mhc class I molecules are composed of an *Mhc*-encoded polymorphic class I α chain noncovalently associated with a small nonpolymorphic protein called β_2 -microglobulin (β_2m). In mammals, the β_2m gene is a relatively large gene with moderate G+C content and is encoded on a non-*Mhc* chromosome (15–18). We have reported the isolation of a chicken β_2m cDNA clone; as in chicken *Mhc* genes, the sequence was highly G+C-rich (11). Here we examine the chicken β_2m gene in order to determine which features are similar to and different from mammalian β_2m genes, in particular to determine whether the high G+C content found in β_2m cDNA correlates with short introns and microchromosomal location. §§

MATERIALS AND METHODS

cDNA and Genomic Clones. A partial cDNA clone for chicken β_2m (pRA5 from H.B19 spleen; ref. 11) was used to isolate longer cDNA clones (JB1b, JB6a, and JB15b from H.B19 intestine λ ZAP library and p34a from CB bursa λ ZAP-II library; chickens from the Basel Institute farm, Gipf-Oberfrick, Switzerland) and genomic clones [RG5 and RG6 from H.B15 library using blood cell DNA partially digested with *Sau3A*, size fractionated by sucrose-density gradient centrifugation, and ligated in *Bam*HI-digested EMBL3 vector by standard techniques (19); chickens from Copenhagen].

Genomic Southern Blots. Genomic erythrocyte DNA was isolated as described (20) from chickens at the Basel Institute farm and from H.B21 and H.B15 backcrossed onto CB chickens at the Danish State Serum Institute (kind gift of Claus Koch). Restriction enzyme digestion, agarose gel electrophoresis, transfer in 0.4 M NaOH to nylon filters (Zeta-Probe, Bio-Rad), hybridization, and detection using x-ray film were by standard methods; the pRA5 insert was twice isolated from low-melting-point agarose and labeled by random priming (19).

Abbreviations: β_2m , β_2 -microglobulin; RFLP, restriction fragment length polymorphism; UT, untranslated region.

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^{§§}The sequences reported in this paper have been deposited in the GenBank data base [accession nos. Z48922 (genomic clone RG5), Z48931 (genomic clone RG6), and Z48921 (cDNA)].

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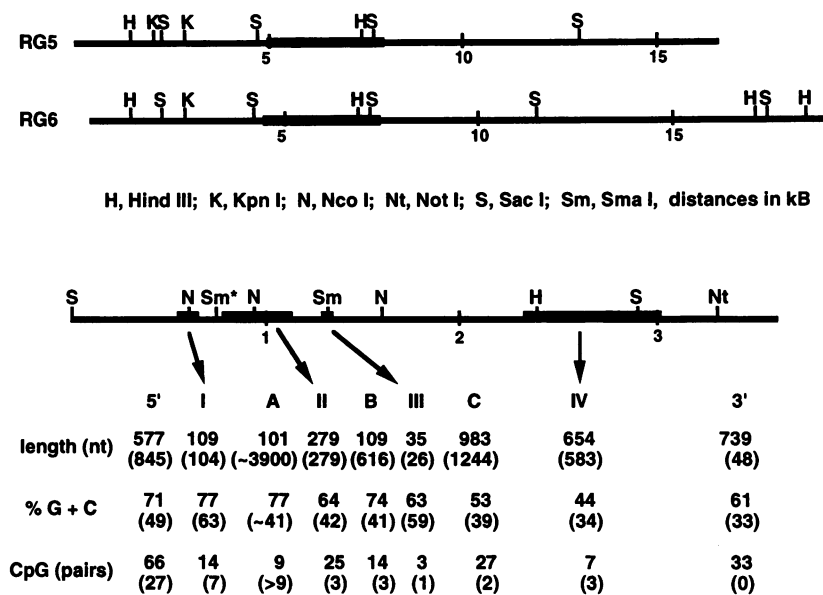


FIG. 1. Restriction enzyme maps of the genomic clones RG5 and RG6 and a subclone that represents the sequenced region. Sm* indicates a *Sma* I site present in RG5 but not RG6. Boxes in the genomic clones indicate hybridizing fragments; boxes in the subclone indicate exons. Indicated below for each region is length in nucleotides, % G+C nucleotides, and the number of CpG dinucleotides, with the values for the corresponding regions in human β_2m [ref. 17 and analysis of sequences in GenBank/European Molecular Biology Laboratory (EMBL) data base] in parentheses.

Subcloning and Sequencing. Restriction enzyme digestion and Southern blot analysis of the genomic clones (Fig. 1) were used to select subclones for further sequence analysis. RG5 and RG6 *Sac* I fragments were subcloned in Bluescript plasmid (Stratagene) and sequenced as double-stranded DNA by dideoxy chain termination using modified T7 DNA polymerase (Sequenase 2.0 kit, United States Biochemical), dATP [35 S], and specific primers. The 5' *Sac* I subclones were further subcloned as *Sma* I fragments, single-stranded DNA was produced by coinfection of XL-1 Blue with the helper phage VCSM13 (Stratagene), and *Taq* polymerase, fluorescent dye terminators, a thermocycler, and a 373A DNA sequencer (Applied Biosystems) were used for automatic sequencing. The sequences were analyzed using the GCG software (Genetics Computer Group, Madison, WI) and the Transcription Factor Database (21) on a VAX cluster, and SeqEd (Applied Biosystems) on a Macintosh Quadra 900.

In Situ Hybridization of Chromosomes. The genomic clone RG5 was biotin-labeled by nick-translation through incorporation of biotin-11 dUTP and separated from the unincorporated nucleotides using Sephadex G-50 spin columns in the presence of 0.1% SDS. Primary fibroblast cultures were established from the peritoneum and lung tissue of a young male chicken and grown at 37°C in minimum essential medium (GIBCO) supplemented with 16% fetal calf serum and antibiotics. Metaphase chromosome spreads were prepared by standard procedures. The *in situ* hybridizations and the fluorescent detection of the bound probe were performed basically as described (22, 23). Microphotographs were taken on Kodak Ektachrome 160 tungsten color slide film developed to enhance sensitivity to 320 ASA.

RESULTS

The Single β_2m Gene of Chickens Has a Similar Genomic Organization but Smaller Introns than Mammals. The sequence of the β_2m gene was determined from subclones of two complete genomic clones (Fig. 1) and compared to cDNA clones in order to establish the intron-exon structure and the encoded protein sequence (Fig. 2).

Four exons were found with an open reading frame of 342 nucleotides (114 amino acids) that give a mature protein

sequence that matched the cDNA and protein sequences reported for chicken β_2m (11, 24). The exons are roughly the same length as in mammalian genes (Fig. 1 and data not shown), but only exon II has significant sequence identity (43% identity in 279 nt for human, 46–48% for different mouse species, 36–38% for different fish species found in EMBL/GenBank). Exon IV contains a single poly(A) site but is otherwise not very similar in sequence to mammalian genes (best match is 55% identity in 124 nt with human exon IV).

All three introns of the chicken gene are much shorter than those in the mammalian β_2m genes (Fig. 1 and other comparisons not shown). The chicken β_2m introns contain the appropriate signals for splicing (in phase 1 like most *Mhc* genes) but are otherwise virtually unrelated in sequence to the mammalian β_2m introns (best match is 67% identity in 48 nt with human intron C).

The β_2m Gene of Chickens Has a Much Higher G+C Content than Mammals. In contrast to mammalian β_2m sequences, the cDNA of chicken β_2m contains many more G and C residues, with the 5'UT consisting of GGAGC repeats and the wobble bases of the protein coding region being 97% G+C (11). In the chicken β_2m gene, there is a gradient from high G+C content at the 5' end to low at the 3' end, but in all parts of the gene there is a higher G+C content than mammalian β_2m genes (Fig. 1). The number of CpG dinucleotide pairs was also very high at the 5' end and low at the 3' end, particularly in comparison to mammalian β_2m genes (Fig. 1).

The Chicken β_2m Gene Has a G+C-Rich Promoter Region Similar to Chicken *Mhc* Class II β Genes. Unlike mammalian β_2m and *Mhc* gene promoters (16–18, 25–30), the chicken β_2m upstream region has no potential TATA box. Instead, an Sp1 site and a potential initiator site (Inr; ref. 31) were found just upstream of the potential transcriptional start site(s). Of the functionally defined elements of mammalian β_2m and class I gene promoters [interferon response element (IRE), PAM box, NF- κ B/rel box, and retinoic acid response element; refs. 16–18 and 25–28], only several tandem repeats of the IRE were found in the chicken β_2m upstream region (Fig. 2).

Unexpectedly, the 5' flanking region of chicken β_2m gene picked out chicken class II β genes from the EMBL/GenBank database, rather than mammalian β_2m genes. These chicken genes are all G+C-rich and share many sequence identities

	S/W box	S/W box	pyr box	X box	TGASTCA (TRE-like)	(enhancer B)	matches with	
	GGACCTY	GGACCTY	YYYYYY	CCYAGMRACAGATG	TGACGTCA (CRE-like)	Y box	consensus	
							S X Y	
HLA-DQ B	tgaGACCTTcaca	aaaaaaaaa	TCTy	CCGAGGACAGATG	AGGTC	ttcaagctcag tg	CTGATTGGTY	6 14 10
HLA-DR B	aaaGACCTTc	atacagca	TCTy	CCGAGGACAGATG	ATGATG	ctcattgta ctcagatg	CTGATTGGTY	7 12 10
HLA-DR A	cctGGACCTT	tgcagaag	CCCTT	CCCTTAGCAACAGATG	GCTCA	ctcctcaaatattttt	CTGATTGGCT	6 14 10
B-L BII	gaaGGAGC	Ccggcgca	gaaCTCTy	CCTyGAGACgGg	TGACGCC	cgcccgccg	CTcATTGGCCctcc	5 11 9
chick B2M	ctgtaACCTT	ctactcgc	CgCTCTy	CCCGGAGACTGg	TGACGC	gggggttaccgcgc	gccATTGGCCgcgc	5 11 7
mouse B2M	ttgtaACCT	Tagtt cagca	TTaaCag	CtagGAGACTGg	TGACG	GaCctccggatctgagtc	CgGATTGGCTgtga	4 9 9
human B2M	ctctaACCT	Tgg cactcgc	TCgCTy	GtTgGAGACAGg	TGAGGT	CTcgcgggctgttc	CTGATTGGCTgggc	4 11 10
B-F IV	tcacaACCT	tyagGGAgCgC	aTTCTy	CCTyGCGcCc	GATGACGTCA	acataaaactccaact	accATTGGCggaga	4-5 11 6
H-2 Kb	acctaACCT	Tgggtcagg	tcCTTCTy	tCCGAcACTGt	TGACGC	gcagtc agctcttacc	CccATTGGTgagcg	4 9 7
HLA-A2	tcccaACCT	Tatgtaggg	tcCTTCTCTy	GAtACTcAc	GACCG	Gggaaccagttctcact	CccATTGGGtTgctg	4 9 7

FIG. 3. Comparison of the 5' region of β_2m , *Mhc* class I α , and class II genes from chickens and mammals. Blanks indicate gaps introduced to maximize alignment. Sequence elements demonstrated to bind factors or to be functionally important are underlined, with consensus sequences (29) indicated above. Alignments of upstream regions indicate possible transcription factor binding elements; the residues that are identical to a consensus sequence are in uppercase letters. Sequences are class II of humans (HLA-DQ and DR; refs. 29 and 30) and chicken (B-L BII; ref. 9), β_2m of mouse and human (17, 26), and class I of chicken (B-F IV; ref. 10), mouse (H-2 K^b; ref. 26), and human (HLA-A2; ref. 27). Nucleotide ambiguity symbols are Y, C or T; R, A or G; W, A or T; S, C or G; M, A or C.

coding region (CCA/pro in most strains versus CCG/pro in CB and CC strains, nt 855–857). However, some differences (roughly 1%) were found outside of the coding region, most appearing to be point mutations. There were a few deletions and insertions, of which the most interesting was near the transcription start site, with one GGAGC repeat absent in one of the genomic clones (after nt 577). The expansion and contraction of these repeats in different alleles may be responsible for sequence differences found at the 5' ends of different cDNAs (data not shown). Another site of sequence variation was after a long string of Gs in the 3'UT, with five patterns represented among the genomic and cDNA clones (nt 2436–2440).

There are some differences between the restriction maps of the two genomic clones isolated from the H.B15 chicken in Copenhagen (Fig. 1). DNA preparations from chicken strains at the Basel Institute for Immunology were digested with a number of restriction enzymes and probed with the pRA5

cDNA, 5' flanking sequences, and 3' flanking sequences (Fig. 4 and data not shown). In most strains, two or three different patterns (corresponding to two alleles and the heterozygote at a single locus) were found for each enzyme. All of the data are consistent with a single β_2m gene with only moderate restriction fragment length polymorphism (RFLP).

The Chicken β_2m Gene Is Located on a Microchromosome Different from the One That Encodes the Chicken *Mhc*. We examined RFLPs of different chicken strains to determine whether the chicken β_2m gene is located in the chicken *Mhc*. Most *Mhc*-homozygous partially inbred chicken strains had more than one β_2m RFLP pattern (Fig. 4A and data not shown). In contrast, *Mhc* congenic strains (different at the *Mhc* but bred to be genetically identical elsewhere) had the same β_2m RFLP pattern (Fig. 4B).

We also examined the segregation of β_2m RFLP in a large backcross family that has been used to construct a linkage map of the chicken genome (32). We found that the β_2m gene failed to segregate with markers defining the chicken *Mhc*; instead it segregated with a single marker (the random cDNA COM101) that defined a new linkage group (data not shown).

Finally, the β_2m genomic clone RG5 was used for *in situ* hybridization of chromosome spreads from chicken lymphocytes (Fig. 5). In several experiments, the clones hybridized to a large microchromosome, apparently number 10 or 11 in size. The *Mhc* microchromosome is much smaller (7).

DISCUSSION

Like mammalian and zebrafish β_2m genes (17, 18, 33), the chicken β_2m gene has four exons: exon I encodes a short 5'UT and signal sequence, exon II encodes the bulk of the mature β_2m protein, exon III encodes the last 4 amino acids of the protein and 20 nt of the 3'UT, and exon IV encodes the rest of the 3'UT. As in mammals (but unknown for other vertebrate groups), there is a single β_2m gene in chickens that is neither highly polymorphic nor located on the same chromosome as the *Mhc*. It has been suggested that the β_2m escaped from the *Mhc* in order to avoid cycles of expansion and contraction of multigene families (33, 34). We favor the idea that the *Mhc* represents a region enriched in polymorphic genes and that the β_2m gene was lost from the *Mhc* because there was no advantage to it being polymorphic (12).

In contrast to mammalian β_2m genes, the chicken β_2m gene has smaller introns, a very high G+C content, and a very G+C-rich promoter with apparent S/W, X, and Y boxes. All of these features are shared with chicken but not mammalian *Mhc* class I α and class II β genes. These structural features at the genomic level have consequences at other levels of organization, such as structure and function of protein and promoter. Therefore, the origin and maintenance of these features are of interest.

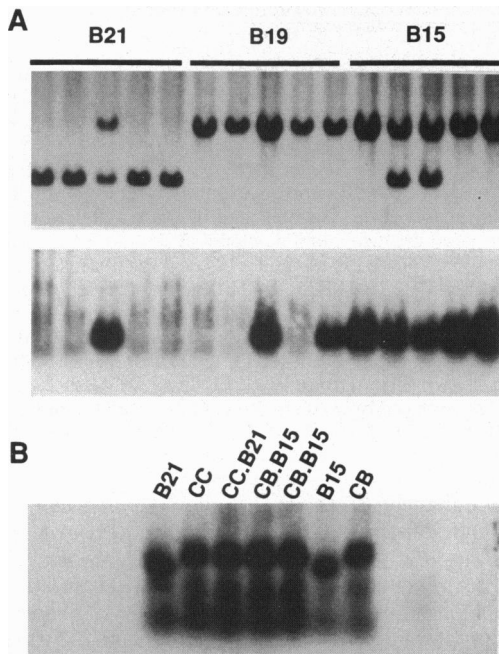


FIG. 4. Southern blots of genomic DNA probed with the pRA5 cDNA. (A) DNA from five chickens each of strains H.B21b, H.B19ov⁺, and H.B15b were digested with *Sac* I (Upper) (bands of roughly 3 and 1.4 kb) and with *Sma* I (Lower) (band of roughly 0.5 kb). (B) Individual chickens from strains H.B15, H.B21, CB, CC, and the xxth backcross of CC.B15 \times CB and CC.B21 \times CB; DNA digested with restriction enzyme *Sau*96A (polymorphic upper bands are roughly 3 and 3.5 kb, lower bands are between 1.5 and 2.5 kb). CB and CC are *Mhc* congenic strains.

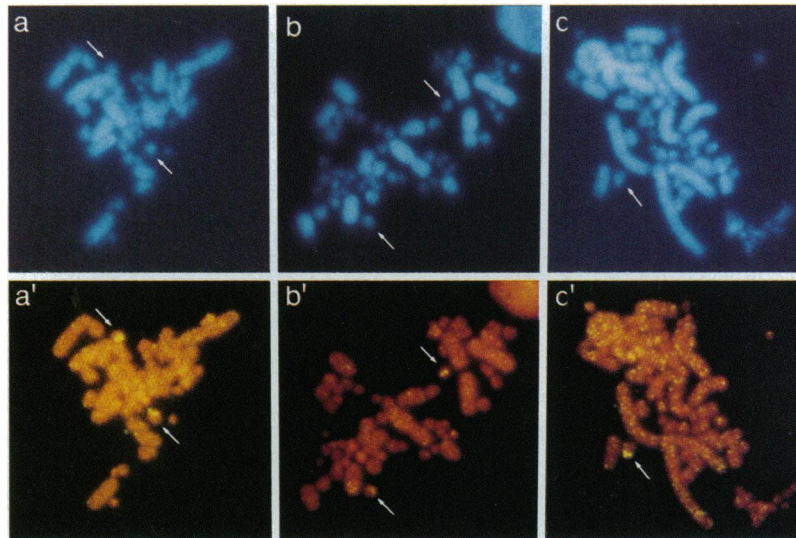


FIG. 5. *In situ* hybridization of genomic clone RG5 to chicken metaphase spreads. Identical metaphase spreads under light microscopy to show counterstaining with 4',6-diamidino-2-phenylindole (*a-c*) and under fluorescent microscopy to show propidium iodide counterstaining and fluorescein fluorescence representing hybridization (*a'-c'*). The hybridization signals are clearly distinguishable on a larger pair of microchromosomes (white arrows), tightly linked to the primary constriction (see *c* and *c'*).

The high G+C content in coding regions correlates with a very biased codon usage and a shift to those amino acids with G+C-rich codons. For *Mhc* molecules and β_2m , this appears to be related to the rate of divergence between chickens and mammals, since there is an inverse correlation between G+C richness and percentage amino acid identity (11). This divergence affects the structure but also allows some functionally important features to be recognized as highly conserved. The surface residues of the G+C-rich β_2m and class I $\alpha 3$ domain are nearly completely diverged, with $\alpha 3$ coevolving to bind the coreceptor CD8 (11, 35). However, some surface features that are important for structure and/or function are conserved, including contact sites with other domains and two residues in $\alpha 3$ that interact with CD8. On this basis, there is an exposed patch in β_2m which might be functionally significant (11, 36–38).

A similar situation is found for the structure and function of the promoters. On one hand, the TATA boxes in the mammalian genes are replaced with G+C-rich Sp1 and Initiator sites in the chicken genes. On the other hand, the IRE, S/W, X, and Y boxes in the highly G+C-rich chicken genes show up as conserved blocks when compared with the less G+C-rich mammalian homologs. The level of sequence identity for the S/W, X, and Y boxes in all of the chicken and mammalian homologs implies that they are functional, but there is no evidence outside of mammalian class II genes. It would be interesting if the chicken β_2m and class I α genes were regulated differently than their mammalian homologs.

The chicken karyotype is composed of a few macrochromosomes with alternating G and R bands, as well as many microchromosomes, most of which stain as G+C-rich (1–6). Macrochromosomes bear both large A+T-rich genes like ovalbumin and small G+C-rich genes like ϵ and ρ globin (39), which presumably are located in G and R bands, respectively, as in mammals (40). Microchromosomes also bear large genes without G+C bias like ovomucoid and nerve growth factor as well as small genes with high G+C bias like α^A and α^D globins, β_2m , and *Mhc* class I and class II molecules (7–11, 22, 39); perhaps these genes are located on microchromosomes that stain like, and originated from, G and R bands, respectively.

The chicken genome is overall smaller and more G+C-rich than mammals, and it is tempting to speculate that this is due to the presence of many microchromosomes. Holmquist (4) envisions a dynamic equilibrium of transposons that keeps

certain avian chromosomes small. We have proposed that the lineage leading to birds was afflicted with an unknown agent that led to the random deletion of DNA over evolutionary time, which left only a few survivors bearing the relatively stable avian karyotype consisting mostly of G+C-rich microchromosomes (12–14). While the available data may be considered too fragmentary or at too low a resolution to choose between the various possibilities, the chicken β_2m and *Mhc* genes certainly provide evidence that changes at the level of the genome can have important evolutionary consequences at other levels of the organism.

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