diluted in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and usually applied by a picospritzer (Parker) coupled to standard micropipettes or to double-barrelled theta glass capillaries (Clark). The application pipettes were placed at a distance of roughly 15 μm above the soma. When NTs were applied at intervals of 5 min or longer, the NT-elicited responses remained stable for up to 2 h. At shorter application intervals, probably because of desensitization, the responses were smaller or absent. In some experiments, BDNF was bath-applied. For this purpose it was directly dissolved in ACSF containing (in μM): 10 bicuculline, 5 CNQX, 100 APV. The protein kinase antagonists K-252a or K-252b (both diluted in ACSF; Calbiochem) were either applied to the somata by micropipettes or by a gravitation-based microperfusion system. All plastic and glassware were blocked twice with PBS containing 0.1% BSA before exposing the material to NTs to prevent binding to the storage and application ware. In some control experiments, BDNF was heat inactivated by incubating it for 30 min at 50 °C or protease inactivated by a 15-min treatment with papain (200 U, 0.5 mg ml^{-1}, Boehringer Mannheim).

Sodium imaging

Cells were loaded with the fluorescent Na^+ indicator dye sodium-binding benzofuran-isophthalate (SBFI, 1 mM, Molecular Probes) through the patch pipette. Fluorescence images were acquired in parallel to the whole-cell recordings by a variable scan digital imaging system (TILL Photonics) attached to an upright microscope (Zeiss Axioskop, 40× water immersion objective, NA 0.75). The fluorescence signals from the somata were obtained at excitation wavelengths of 345 nm (isosbestic point) and of 380 nm (Na^+-sensitive wavelength) and were background corrected. Data were expressed as changes in fluorescence ratio (345/380 nm) using Igor Pro software (Wavemetrics) for analyses.

Received 24 May; accepted 18 August 1999.

1. Lewin, G. R. & Barde, Y. A. Physiology of the neutrophins. *Annu. Rev. Neurosci.* **19,** 289–317 (1996).
2. Bothwell, M. Functional interactions of neurotrophins and neurotrophin receptors. *Annu. Rev. Neurosci.* **18,** 223–253 (1995).
3. Ibanez, C. F. Emerging themes in structural biology of neurotrophic factors. *Trends Neurosci.* **21,** 438–444 (1998).
4. Schuman, E. M. Neurotrophin regulation of synaptic transmission. *Curr. Opin. Neurobiol.* **9,** 105–109 (1999).
5. Bonhoeffer, T. Neurotrophins and activity-dependent development of the neocortex. *Curr. Opin. Neurobiol.* **6,** 119–126 (1996).
6. Cellerino, A. & Maffei, L. The action of neurotrophins in the development and plasticity of the visual cortex [published erratum in *Prog. Neurobiol.* **50,** 333 (1996)]. *Prog. Neurobiol.* **49,** 53–71 (1996).
7. Thoenen, H. Neurotrophins and neuronal plasticity. *Science* **270,** 593–598 (1995).
8. Knüsel, B. & Hefti, F. K-252 compounds: modulators of neurotrophin signal transduction. *J. Neurochem.* **59,** 1987–1996 (1992).
9. Edwards, F. A., Konnerth, A., Sakmann, B. & Takahashi, T. A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. *Pflügers Arch.* **414,** 600–612 (1989).
10. Ip, N. Y., Li, Y., Yancopoulos, G. D. & Lindsay, R. M. Cultured hippocampal neurons show responses to BDNF, NT-3, and NT-4, but not NGF. *J. Neurosci.* **13,** 3394–3405 (1993).
11. Kang, H. & Schuman, E. M. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science* **267,** 1658–1662 (1995).
12. Figurov, A., Pozzo-Miller, L. D., Olafsson, P., Wang, T. & Lu, B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature* **381,** 706–709 (1996).
13. Lessmann, V., Gottmann, K. & Heumann, R. BDNF and NT-4/5 enhance glutamatergic synaptic transmission in cultured hippocampal neurones. *Neuroreport* **6,** 21–25 (1994).
14. Levine, E. S., Crozier, R. A., Black, I. B. & Plummer, M. R. Brain-derived neurotrophic factor modulates hippocampal synaptic transmission by increasing *N*-methyl-D-aspartic acid receptor activity. *Proc. Natl Acad. Sci. USA* **95,** 10235–10239 (1998).
15. Patterson, S. L. *et al.* Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron* **16,** 1137–1145 (1996).
16. Suen, P. C. *et al.* Brain-derived neurotrophic factor rapidly enhances phosphorylation of the postsynaptic *N*-methyl-D-apsartate receptor subunit 1. *Proc. Natl Acad. Sci. USA* **94,** 8191–8195 (1997).
17. Canossa, M. *et al.* Neurotrophin release by neurotrophins: implications for activity-dependent neuronal plasticity. *Proc. Natl Acad. Sci. USA* **94,** 13279–13286 (1997).
18. Ringstedt, T., Lagercrantz, H. & Persson, H. Expression of members of the trk family in the developing postnatal rat brain. *Brain Res. Dev.* **72,** 119–131 (1993).
19. Yan, Q. *et al.* Immunocytochemical localization of TrkB in the central nervous system of the adult rat [published erratum in J. Comp. Neurol. **382,** 546–547 (1997)]. *J. Comp. Neurol.* **378,** 135–157 (1997).
20. Berninger, B., Garcia, D. E., Inagaki, N., Hahnel, C. & Lindholm, D. BDNF and NT-3 induce intracellular Ca^{2+} elevation in hippocampal neurones. *Neuroreport* **4,** 1303–1306 (1993).
21. Barbacid, M. Neurotrophic factors and their receptors. *Curr. Opin. Cell Biol.* **7,** 148–155 (1995).
22. Merlio, J. P., Ernfors, P., Jaber, M. & Persson, H. Molecular cloning of rat trkC and distribution of cells expressing messenger RNAs for members of the trk family in the rat central nervous system. *Neuroscience* **51,** 513–532 (1992).
23. Korte, M. *et al.* Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. *Proc. Natl Acad. Sci. USA* **93,** 12547–12552 (1996).
24. Berninger, B. & Poo, M. Fast actions of neurotrophic factors. *Curr. Opin. Neurobiol.* **6,** 324–330 (1996).
25. Rose, C. R. & Ransom, B. R. Regulation of intracellular sodium in cultured rat hippocampal neurones. *J. Physiol. (Lond.)* **499,** 573–587 (1997).
26. Lohof, A. M., Ip, N. Y. & Poo, M. M. Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. *Nature* **363,** 350–353 (1993).
27. Kahle, P., Barker, P. A., Shooter, E. M. & Hertel, C. p75 nerve growth factor receptor modulates p140trkA kinase activity, but not ligand internalization, in PC12 cells. *J. Neurosci. Res.* **38,** 599–606 (1994).
28. Blöchl, A. & Thoenen, H. Localization of cellular storage compartments and sites of constitutive and activity-dependent release of nerve growth factor (NGF) in primary cultures of hippocampal neurons. *Mol. Cell Neurosci.* **7,** 173–190 (1996).
29. Zhou, X. F. & Rush, R. A. Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. *Neuroscience* **74,** 945–953 (1996).
30. Goodman, L. J. *et al.* Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. *Mol. Cell Neurosci.* **7,** 222–238 (1996).

Acknowledgements

We thank R. Trautmann and E. Eilers for expert technical assistance and M. Ashdown for editorial assistance. This study was supported by a fellowship from the DFG to K.W.K. and by grants from the Deutsche Forschungsgemeinschaft and the Human Frontier Science Program to A.K.

Correspondence and requests for materials should be addressed to A.K. (akonnerth@med-rz.uni-sb.de).

Complete sequence and gene map of a human major histocompatibility complex

The MHC sequencing consortium*

Here we report the first complete sequence and gene map of a human major histocompatibility complex (MHC), a region on chromosome 6 which is essential to the immune system (reviewed in ref. 1). When it was discovered over 50 years ago the region was thought to specify histocompatibility genes, but their nature has been resolved only in the last two decades. Although many of the 224 identified gene loci (128 predicted to be expressed) are still of unknown function, we estimate that about 40% of the expressed genes have immune system function. Over 50% of the MHC has been sequenced twice, in different haplotypes, giving insight into the extraordinary polymorphism and evolution of this region. Several genes, particularly of the MHC class II and III regions, can be traced by sequence similarity and synteny to over 700 million years ago, clearly predating the emergence of the adaptive immune system some 400 million years ago. The sequence is expected to be invaluable for the identification of many common disease loci. In the past, the search for these loci has been hampered by the complexity of high gene density and linkage disequilibrium.

The impetus to obtain the complete sequence of the MHC was provided by the complex biology and genetics of the histocompatibility regions H-2 and HLA[1]. It is therefore no surprise that the MHC at 6p21.31 is among the first multi-megabase regions of the human genome to be completely sequenced. With over 200 identified loci, the MHC is the most gene-dense region of the human genome sequenced so far. It also encodes the most polymorphic human proteins, the class I and class II molecules, some of which have over 200 allelic variants. This extreme polymorphism is thought to be driven and maintained by the long-standing battle for supremacy between our immune system and infectious pathogens. Underlining its biomedical importance, the MHC is associated with more diseases than any other region of the human

* Corresponding authors representing the four sequencing centres: S. Beck (The Sanger Centre, Wellcome Trust Genome Campus, Hinxton CB10 1SA, UK); D. Geraghty (Fred Hutchinson Cancer Research Center, Seattle, Washington 98104, USA); H. Inoko (Tokai University School of Medicine, Department of Molecular Life Science, Bohseidai, Isehara, Kanagawa 259-11, Japan); and L. Rowen (University of Washington, Seattle, Washington 98195, USA). A full list of contributors appears at the end of the paper.

genome, including most, if not all, autoimmune conditions (for example, rheumatoid arthritis and diabetes[2]). Phenotypes with different aetiologies have also been linked to the region, ranging from cancer to sleeping and reading disorders.

The 3.6-megabase (Mb) virtual MHC sequence reported here is derived from a patchwork of different haplotypes and has been assembled from the work of four main groups[3] (see Acknowledgements). In addition to the gene map described below, further interesting attributes have started to emerge from preliminary analyses. Long before the term 'single nucleotide polymorphism' (SNP) was coined, variations in the coding and noncoding sequences were observed in the MHC of different individuals and were used to delineate ancestral haplotypes[4]. The available data already indicate that the extreme polymorphism characterizing the MHC is not homogeneous throughout the entire region. The variation in the noncoding sequences appears to peak around the most polymorphic gene loci and 'hitch-hiking', as the result of overdominant allele selection (heterozygote advantage), has been suggested to explain this phenomenon. Variation levels of 5–17% have been reported at some of these loci (HLA-DP, DQ, B and C), which are by far the highest levels found in the human genome so far[5–8]. A systematic analysis and verification of SNPs across the entire MHC is still in progress. Sequence comparisons revealed several MHC genes (TUBB, TNXB, PBX2, NOTCH4, RXRB and RPS18) to be syntenic in invertebrate genomes such as *Drosophila melanogaster* and *Caenorhabditis elegans*, indicating that the origin of the locus now known as MHC predates the emergence of the adaptive immune system[9].

Currently, the MHC is the second longest contiguous sequence in the human genome. The determination of very long sequences will allow the exploration of global chromosomal features such as isochores (long-range regions of homogenous G + C content), replicons and repeat dynamics in much greater detail than before. The low G + C isochore covering the classical class II region (see poster or Supplementary Information) is one of the best studied isochores in the human genome[10,11]. Its predicted boundaries correlate precisely with switching of replication timing from 'later' replication in the classical class II region to 'earlier' replication at the centromeric boundary (P. Johonnett and D. Sheer, personal communication) and at the telomeric boundary[12]. This may represent a link between isochores and the elusive replicon structure of the human genome[13,14].

Figure 1 and the poster accompanying this issue of *Nature* (also available as Supplementary Information) show the complete gene map of all 224 gene loci identified in this particular composite of MHC haplotypes. A list of the genes and their alternative designations is provided as Supplementary Information. Ninety-three of the 224 loci (41.5%) were discovered or located to the MHC as a direct result of the genomic sequence. Historically, the MHC has been divided into three regions: class II (centromeric), class III and class I (telomeric)[15]. Analyses of the immediate flanking regions reveal that the 'classical' class I and class II regions extend much further than previously thought[11,16]. These regions are here referred to as 'extended' class I and class II regions. A set of more than seven genes involved in inflammation, including three members of the tumour necrosis factor (TNF) superfamily, within the class III region is sometimes specified as the class IV region[17]. Various other genes associated with the immune system are distributed throughout the MHC. The total immune system constituent of the MHC is 39.8% of the expressed loci (see poster or Supplementary Information) including at least 10 novel genes identified from the genomic sequence (Fig. 1). The clustering of immune-related genes in the MHC region is so striking that it seems unlikely to be coincidental[18]. The classical class II region is particularly notable because almost all of the genes are of immune function, namely class II A and B genes, LMPs, TAPs and TAPBP in the extended class II region. This clustering of immunity genes in the MHC may reflect

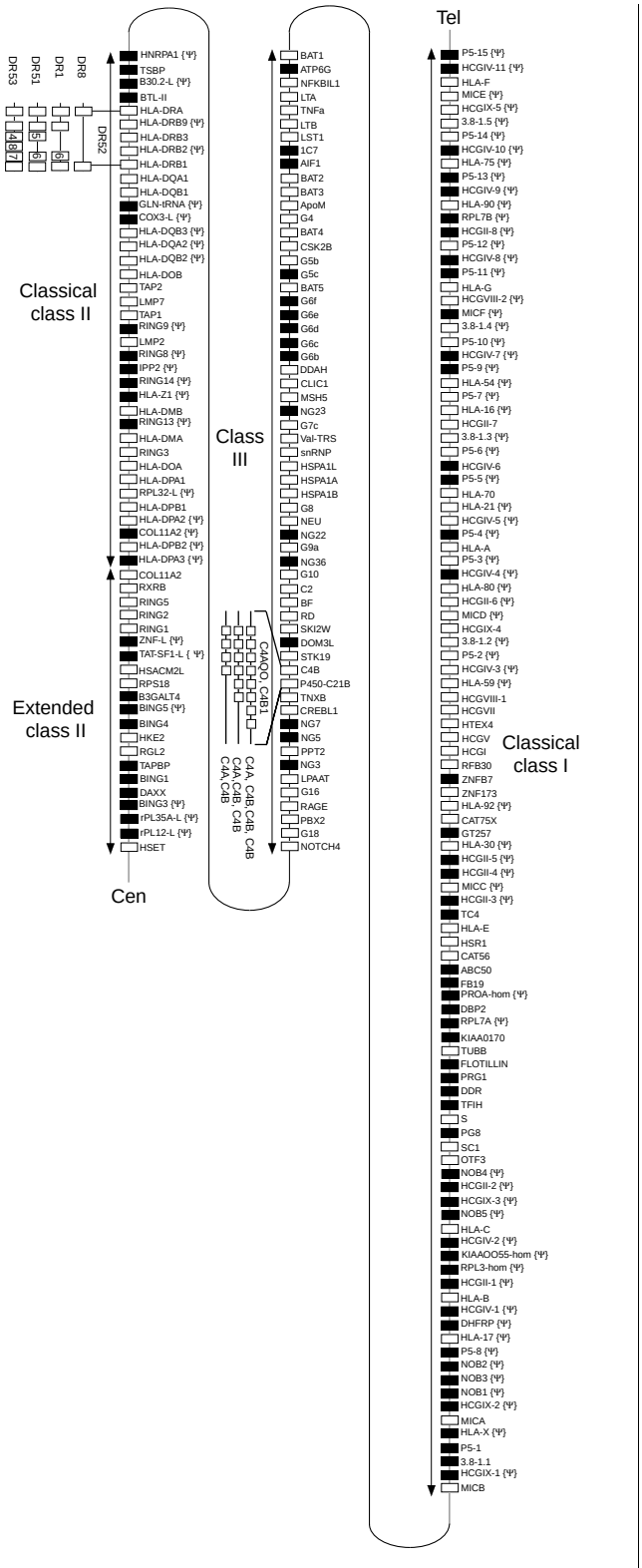

Figure 1 Complete gene map of the MHC reference sequence reported here. Genes are shown in order from telomere to centromere but not to scale. Gene loci that were discovered or located to the MHC as a direct result of the genomic sequence are indicated by filled boxes. As will be the case for the rest of the human genome, the MHC reference sequence is a composite of different haplotypes. However, in regions with known differences in gene content (C4 region in class III and DR region in the classical class II region) only single haplotypes were sequenced (C4AQ0, C4B1 in the class III region and DR52 in the class II region).

co-evolution of functions or co-expression of related transcripts. However, the proportion of immune system genes in the genome in general is not known at present and it may be equally high. The average gene density (including pseudogenes) over the entire 3.6 Mb of the MHC is 1 gene per 16 kilobases (kb), with distinct regional variations. Particularly interesting is the proportion of expressed genes in relation to pseudogenes. Except in certain haplotypes, where the C4 regions have duplicated (Fig. 1), there appear to be no or very few pseudogenes in the class III region. In contrast, the class I and class II regions are full of pseudogenes. Both class I and class II regions appear to have duplicated many times, generating novel gene family members which have then diverged into new functions. A possible explanation for maintaining such high levels of pseudogenes could be that they are involved in generating new alleles by gene conversion, a phenomenon that has been observed at other human immune loci[19].

The first complete MHC sequence provides an important new tool for studying the genetics, biology and evolution of human multigene families, populations and disease. In the long term, the biological importance of the MHC is likely to justify the re-sequencing and epigenetic analysis of several common haplotypes, which differ markedly in sequence and gene content. Efforts towards this goal are already in progress, as they will facilitate the precise identification of many disease loci. Typing of new micro-satellites derived from the genomic sequence has already allowed us to narrow down the candidate region for psoriasis vulgaris (an inflammatory skin disorder) to a critical segment including four genes—S, PG8, SC1 and OTF3 (H. Inoko, unpublished data). The discovery of regions paralogous to the MHC on chromosomes 1, 9 and 19 is indicative of ancient duplications and possibly the remnant of tetraploidization in the early vertebrate genome[20,21]. The sequences of these related regions and of other vertebrate MHCs are eagerly awaited[22]. □

Received 20 July; accepted 26 August 1999.

1. Klein, J. *Natural History of the Major Histocompatibility Complex* (Wiley, New York, 1986).
2. Tiwari, J. L. & Terasaki, P. I. *HLA and Disease Associations* (Springer, Berlin, 1985).
3. http://www.sanger.ac.uk/HGP/Chr6/MHC.shtml
4. Dawkins, R. *et al.* Genomics of the major histocompatibility complex: haplotypes, duplication, retroviruses and disease. *Immunol. Rev.* **167,** 275–304 (1999).
5. Limm, T. M., Ashdown, M. L., Naughton, M. J., McGinnis, M. D. & Simons M. D. HLA-DQA1 allele and suballele typing using non-coding sequence polymorphisms. *Hum. Immunol.* **38,** 57–68 (1993).
6. Horton, R. *et al.* Large-scale sequence comparisons reveal unusually high levels of variation in the HLA-DQB1 locus in the class II region of the human MHC. *J. Mol. Biol.* **282,** 71–97 (1998).
7. Guillaudeux, T., Janer, M., Wong, G. K., Spies, T. & Geraghty, D. E. The complete genomic sequence of 424,015 bp at the centromeric end of the HLA class I region: gene content and polymorphism. *Proc. Natl Acad. Sci. USA* **95,** 9494–9499 (1998).
8. Satta, Y., Kupfermann, H., Li, Y.-J. & Takahata, N. Molecular clock and recombination in primate MHC genes. *Immunol. Rev.* **167,** 367–379 (1999).
9. Trachtulec, Z. *et al.* Linkage of TATA-binding protein and proteasome subunit C5 genes in mice and humans reveals synteny conserved between mammals and invertebrates. *Genomics* **44,** 1–7 (1997).
10. Fukagawa, T. *et al.* A boundary of long-range G+C% mosaic domains in the human MHC locus: pseudoautosomal boundary-like sequence exists near the boundary. *Genomics* **25,** 184–191 (1995).
11. Stephens, R. *et al.* Gene organisation, sequence variation and isochore structure at the centromeric boundary of the human MHC. *J. Mol. Biol.* **291,** 789–799 (1999).
12. Tenzen, T. *et al.* Precise switching of DNA replication timing in the GC content transition area in the human MHC. *Mol. Cell. Biol.* **17,** 4043–4050 (1997).
13. Hamlin, J. L. & Dijkwel, P. A. On the nature of replication origins in higher eukaryotes. *Curr. Opin. Genet. Dev.* **5,** 153–161 (1995).
14. Delgado, S., Gomez, M., Bird, A. & Antequera, F. Initiation of DNA replication at CpG islands in mammalian chromosomes. *EMBO J.* **17,** 2426–2435 (1998).
15. Trowsdale, J. & Campbell, R. D. Map of the human major histocompatibility complex. *Immunol. Today* **18** (Suppl.) (1997).
16. Ruddy, D. A. *et al.* A 1.1-Mb transcript map of the hereditary hemochromatosis locus. *Genome Res.* **7,** 441–456 (1997).
17. Gruen, J. R. & Weissman, S. M. Evolving views of the major histocompatibility complex. *Blood* **90,** 4252–4256 (1997).
18. Bodmer, W. F. Evolutionary significance of the HLA system. *Nature* **237,** 139–145 (1997).
19. Haino, M. *et al.* Comparison and evolution of human immunoglobulin VH segments located in the 3′ 0.8-megabase region: Evidence for unidirectional transfer of segmental gene sequences. *J. Biol. Chem.* **269,** 2619–2626 (1994).
20. Sugaya, K. *et al.* Three genes in the human MHC class III region near the junction with the class II: Gene for receptor of advanced glycosylation end products, PBX homeobox genes and a Notch homolog, human counterpart of mouse mammary tumor gene int-3. *Genomics* **23,** 408–419 (1994).
21. Kasahara, M. The chromosomal duplication model of the major histocompatibility complex. *Immunol. Rev.* **167,** 17–32 (1999).
22. Kaufman, J. *et al.* The chicken B locus is a minimal essential major histocompatibility complex. *Nature* (this issue).

Supplementary Information is available at *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

Acknowledgements

Sequencing of the class I region in the US was funded by a Department of Energy grant to M. Olson and by US NIH grants to D.G. Sequencing of the class I region in Japan was funded by the Japanese Science and Technology Corporation (JST). Sequencing of the class II region at the Sanger Centre was funded by the Wellcome Trust. Early stages of the class II sequence were funded by the Imperial Cancer Research Fund and the EU BioMed1 programme. Sequencing of the class III region at the University of Washington Multi-megabase Sequencing Center was funded by the US NIH and Department of Energy. We thank all members past and present of the University of Washington Genome Center (http://www.genome.washington.edu/uwgc/), the Genome Sequencing Group at Tokai University (http://www.alis.tokyo.jst.go.jp/HGS/top.html), the Chromosome 6 Project Group at the Sanger Centre (http://www.sanger.ac.uk/HGP/Chr6) and the University of Washington Multimegabase Sequencing Center (http:/chroma.mbt.washington.edu/msg_www/) for their contributions. R.D.C. was funded by the UK Medical Research Council and J.T. was funded by the Wellcome Trust. The work was divided as follows: The Sanger Centre, Hinxton, UK: HSET to TSBP, 1.2 Mb; Tokai University, Isehara, Japan: HSPA1B to P5-15, 2.2 Mb; Fred Hutchinson Cancer Research Center and the University of Washington Genome Center (UWGC), Seattle, USA: NFKBIL1 to HLA-F and beyond, 2.2 Mb; University of Washington Multimegabase Sequencing Center (UWMSC), Seattle, USA: NOTCH4 to LTA, 0.76 Mb. From these sequences a 3,673,800-base-long consensus was assembled covering P5-15 to HSET (http://www.sanger.ac.uk/HGP/Chr6/MHC_990719.fasta).

Correspondence and requests for materials should be addressed to D. Geraghty (e-mail: geraghty@fhcrc.org) or H. Inoko (e-mail: hinoko@is.icc.u-tokai.ac.jp) for the class I region, S. Beck (e-mail: beck@sanger.ac.uk) or J. Trowsdale (e-mail: jt233@mole.bio.cam.ac.uk) for the class II region and D. Campbell (e-mail: rcampbel@hgmp.mrc.ac.uk) or L. Rowen (e-mail: leerowen@u.washington.edu) for the class III region.

Contributors in alphabetical order (numbers in parentheses indicate addresses):
B. Aguado (5), S. Bahram (7), S. Beck (1), R. D. Campbell (5), S. A. Forbes (6), D. Geraghty (2), T. Guillaudeux (2), L. Hood (4), R. Horton (1), H. Inoko (3), M. Janer (2), C. Jasoni (2), A. Madan (4), S. Milne (1), M. Neville (5), A. Oka (3), S. Qin (4), G. Ribas-Despuig (5), J. Rogers (1), L. Rowen (4), T. Shiina (3), T. Spies (2), G. Tamiya (3), H. Tashiro (8), J. Trowsdale (6), Q. Vu (2), L. Williams (2), M. Yamazaki (8)
(1) The Sanger Centre, Wellcome Trust Genome Campus, Hinxton CB10 1SA, UK
(2) Fred Hutchinson Cancer Research Center, Seattle, Washington 98104, USA
(3) Tokai University School of Medicine, Department of Molecular Life Science, Bohseidai, Isehara, Kanagawa 259-11, Japan
(4) University of Washington, Seattle, Washington 98195, USA
(5) HGMP Resource Centre, Wellcome Trust Genome Campus, Hinxton CB10 1SB, UK
(6) Cambridge University, Department of Pathology, Division of Immunology, Tennis Court Road, Cambridge CB2 1QP, UK
(7) Centre de Recherche d'Immunologie et d'Hematologie, 4 Rue Kirschleger, 67085 Strasbourg, France
(8) Bioscience Research Laboratory, Fujiya Co., Ltd., 228 Soya, Hadano, Kanagawa 257, Japan

The chicken B locus is a minimal essential major histocompatibility complex

Jim Kaufman*, Sarah Milne†, Thomas W. F. Göbel‡, Brian A. Walker*, Jansen P. Jacob*, Charles Auffray§, Rima Zoorob§ & Stephan Beck†

** Institute for Animal Health, Compton RG20 7NN, UK*
† The Sanger Centre, Wellcome Trust Genome Campus, Hinxton CB10 1SA, UK
‡ Institute for Animal Physiology, Munich 80539, Germany
§ CNRS, Genetique Moleculaire et Biologie du Developpement, UPR420, Villejuif Cedex, France

Here we report the sequence of the region that determines rapid allograft rejection in chickens, the chicken major histocompatibility complex (MHC). This 92-kilobase region of the B locus[1–4] contains only 19 genes, making the chicken MHC roughly 20-fold smaller than the human MHC[5]. Virtually all the genes have counterparts in the human MHC, defining a minimal essential set of MHC genes conserved over 200 million years of divergence between birds and mammals. They are organized differently, with