in the highly conserved 'YXDD' motif (Fig. 1*a*). The existence of alleles with an intact YXDD-motif, and therefore a presumably completely intact provirus, is not known. The HERV-K(HML-2.HOM)–encoded proteinase demonstrated self-cleavage and processing of the Gag protein[7] (Fig. 2). HERV-K10–encoded endonuclease has been reported to have enzymatic activity[8]. We have noted that the endonuclease ORF of HERV-K(HML-2.HOM) is nearly identical to the HERV-K10 endonuclease (data not shown), suggesting that it is likely to be active[9].

The 3′ portion of *pol*, the *env* gene and the 3′ LTR sequence share sequence identity with a described *env* mRNA (ref. 10), indicating that the formerly described *env* sequence originated from the HERV-K provirus and that the provirus retained transcriptional activity. Part of an *Alu* element (54 bp) was identified immediately downstream of the 3′ LTR, and therefore it is possible that the provirus integrated into an *Alu* element, with the remaining *Alu* sequence upstream of the 5′ LTR. FISH analysis using the *env* sequence of HERV-K(HML-2.HOM) localized the provirus to chromosome 7p22 (data not shown).

Using primers specific to the *env* sequence of HERV-K(HML-2.HOM) (nt 8,406–8,430) and 3′ flanking *Alu* sequence, we amplified a product of expected size (1.2 kb) in 54 human DNA samples from various ethnic groups (European, African and Asian). This indicates that at least one human chromosome 7 in each of these individuals harbours the proviral sequence. HERV-K(HML-2.HOM) therefore seems to be frequent, if not ubiquitous, in the human population. In contrast, no such PCR product was amplified from a chimpanzee DNA sample (data not shown), indicating that the provirus integrated into the human genome after the evolutionary split from chimpanzee.

HERV-K(HML-2.HOM) is the most intact human endogenous retrovirus yet identified. The explanation for why an intact HERV-K provirus, or any of its genes, should be preserved during evolution remains unclear. HERV-K(HML-2.HOM) may have arisen from retrotransposition, which in *Hominoidea* appears to have involved only the HERV-K(HML-2.HOM) proviruses harbouring a partially deleted *gag* gene[11]. The findings described here and in another study[12] suggest that the HERV-K(HML-2) provirus retained retrotranspositional activity until a relatively recent period in evolutionary time. Retrotransportation, however, would require a second, almost intact provirus, raising the question of the origin of the this latter provirus. This may have involved the germ cell integration of an exogenous HERV-K(HML-2) variant.

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300 million years of conserved synteny between chicken Z and human chromosome 9

Birds diverged from mammals 300–350 million years ago[1] (Mya). In mammals, the male is the heterogametic sex (XY male and XX female) and 'maleness' is under the control of a testis-determining factor, *SRY*, located on the Y chromosome. In contrast, sex determination in birds operates through a ZZ/ZW system in which the female is the heterogametic sex. It is not clear, however, whether this system is controlled by a dominant factor on the W chromosome or by Z-chromosome dosage[2,3].

Comparative gene mapping is an effective tool for the study of genome evolution in phylogenetically distant species that represent key stages in vertebrate evolution. The presence of orthologous genes on interspecific homologous chromosome segments (conservation of synteny) reflects the common phylogenetic origin of species and probably also the ancestral genomic organization[4,5]. Using both physical and genetic methods[6,7], we found that 11 of 18 genes of the chicken *Gallus gallus*, GGA Z chromosome have orthologues on human (HSA) chromosome 9, bands pter–q22 (Fig. 1). Although the overall homology between GGA Z and HSA 9 is extensive, the gene order has changed. For example, an inversion has moved *GGTB2–ACO1* to the Z-long arm or to the HSA 9-short arm. Additional translocations or insertion events during vertebrate evolution have moved smaller segments from the avian Z to other chromosomes. The distal long arm of HSA 9q32–qter shows conserved synteny with the chicken linkage group E41W17, which corresponds to a microchromosome. In contrast with Ohno's original proposition[8], the avian and mammalian sex chromosomes have evolved independently. Chicken Z and W share two genes and appear to be remnants of an ancestral pair of chromosomes[9]. Our results show that this ancestral autosome gave rise to most of avian Z and HSA 9. The delineated homology represents thus far the largest region of chromosomal synteny that has been conserved since separation of the avian and mammalian lineages over 300 Mya.

In mammals, sex is determined by the male-dominant factor on the Y chromosome, *SRY* (ref. 10). Sex-reversal syndromes in humans, however, indicate the presence of other downstream sex-determining genes. Monosomy for the distal short arm of HSA 9 has been associated with failure of testicular development and XY sex reversal, which is most likely due to haploinsufficiency of a dosage-sensitive gene[11,12]. Recently, the human DM-domain gene expressed in the testis,

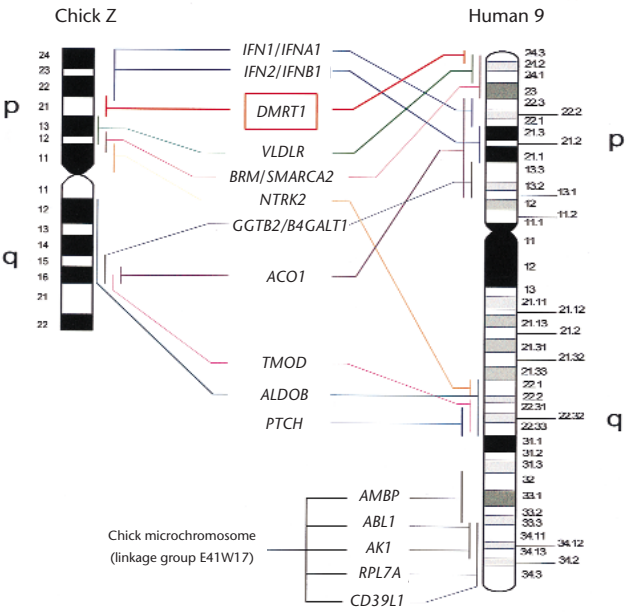

Fig. 1 Comparative location of orthologous genes on chicken Z and human chromosome 9. The G-banded idiogram of chicken (GGA) Z chromosome (left) opposite an idiogram of G-banded human (HSA) chromosome 9 (right). Comparatively mapped genes are indicated between the idiograms. The distal long arm of HSA 9q32–qter appears to be homologous to chicken linkage group E41W17 on a microchromosome. Human mapping information was obtained from OMIM.

DMRT1, which shares significant structural homology with male sexual regulatory genes from *Caenorhabditis elegans* (*mab-3*) and *Drosophila melanogaster* (*dsx*), has been identified in the critical region[13]. We reasoned that an orthologous gene on GGA Z might be involved in avian testis development.

A human *DMRT1* EST (AA412330) was used to isolate a 1.5-kb chicken cDNA (TUPSp573J1773Q3, RZPD library 573). This cDNA was used as a probe to isolate a cosmid (MPMGc125B0641Q5, RZPD library 125) that was then FISH mapped to GGA Zp21 (Fig. 2). The amino acid translation of the cDNA sequence (GenBank accession number AF123456) revealed a DM domain showing 86% similarity to human *DMRT1*, indicating that it is a true homologue of *DMRT1*. Hybridization of chicken genomic and cosmid DNA with *DMRT1* cDNA under low stringency conditions indicated the presence of a single DM-domain gene in chicken.

As *SRY* is not sex-specific in birds and reptiles, it cannot be considered an ancestral vertebrate sex-determining gene. Because of its chromosomal location and the fact that its male regulatory function is highly conserved across evolution, *DMRT1* is, so far, the only candidate testis-determining gene in birds. Two other Z-linked genes, *ZOV3* and *VLDLR*, have been implicated in the function of the chicken ovary[14,15]. It may be that two copies of *DMRT1* are required for testis formation, whereas a single copy along with the W chromosome leads to female sexual differentiation. This is consistent with the view that, although sex determination has undergone considerable evolutionary changes, regulatory genes such as *DMRT1* are highly conserved in their function[3].

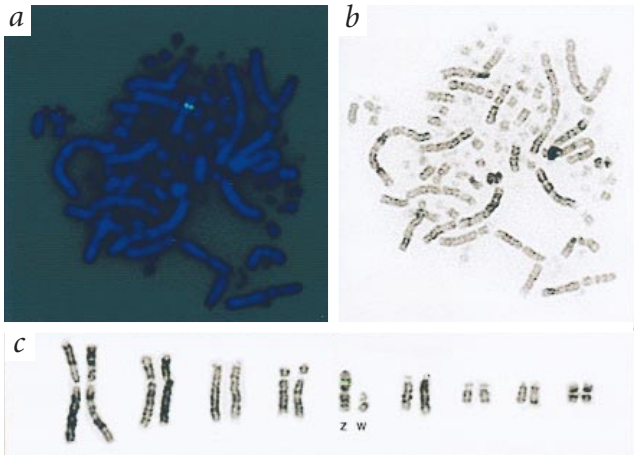

Fig. 2 FISH mapping of *DMRT1* on chicken Z chromosome. ***a***, Hybridization of *DMRT1* cosmid to a female chicken metaphase spread. The biotinylated DNA probe is detected by FITC-avidin (green fluorescence). Chromosomes are counterstained with DAPI. ***b***, DAPI banding of the same metaphase spread, converted by Oncor Image software into G-like bands. ***c***, G-banded karyotype of chicken macrochromosomes hybridized with *DMRT1* (green spot on Z chromosome).

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