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Molecular characterization of full-length MLV-related endogenous retrovirus ChiRV1 from the chicken, *Gallus gallus*

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ABSTRACT

We report the first full-length sequence of an endogenous retrovirus from the genome of domestic chicken, that is not related to the Avian leukemia viruses (ALV). This retrovirus, designated ChiRV1, clusters with Murine leukemia virus (MLV)-related retroviruses and hence is the first complete gammaretrovirus from the genome of a bird. Nevertheless it is not related to exogenous MLV-related retroviruses infecting chicken. The provirus is 9133 bp long and contains 90%-identical LTRs as well as reading frames for the *gag*, *pol* and *env* genes, interrupted by in-frame stop codons. Expression analysis showed that ChiRV1 is a transcribed provirus. Screening of the chicken genome database revealed 100 ChiRV1-related sequences that are grouped into three classes based upon LTR alignment and subsequent phylogenetic analysis.

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Introduction

The genome of domestic chicken, *Gallus gallus*, is one of the intensively studied genomes. Numerous classes of repetitive elements, such as endogenous retroviruses, make up 9% of the chicken genome (ICGSC, 2004).

Retroviruses are currently classified into seven genera designated α-, β-, γ-, δ-, ε-retroviruses, spumaviruses and lentiviruses (van Regenmortel et al., 2000). Alpharetroviruses (also known as ALV genus) comprise the only genus confined to birds. Two groups of chicken endogenous retroviruses have been described previously: ev loci and EAV group with subgroups EAV-HP, EAV-0, E33/E51 (Borisenko and Rynditch, 2004). All of them are ALV-related and thus belong to alpharetroviruses. Recently it has become apparent that some gammaretroviruses, namely endogenous MLVs, are widespread within the genomes of all four classes of terrestrial vertebrates (Martin et al., 1999). Nevertheless these elements are only partially characterized, being mostly sequenced in the *pro–pol* region.

Here, we describe an endogenous chicken retrovirus ChiRV1. The phylogenetic analysis indicates that this element clusters with MLV-related retroviruses and therefore is the first full-length γ-retrovirus identified in the genome of domestic chicken.

Results and discussion

ChiRV1 genomic structure

The strategy for cloning of ChiRV1 was as follows. First, using degenerate PCR primers, we have cloned and sequenced the protease (*pro*) and reverse transcriptase (*pol*) genes of ChiRV1 from genomic DNA of domestic chicken (line CB). Then, in order to find a complete retroviral sequence, the sequences obtained were screened against a chicken genome database (http://www.genome.wustl.edu/genome.cgi?GENOME=Gallus%20gallus; released May 2006) with the chicken genome browser of the University of California Santa Cruz (http://genome.ucsc.edu/cgi-bin/hgGateway). Upon identification of putative LTRs, we designed PCR primers to amplify the full-length retrovirus from the same domestic chicken line CB. Finally, the full-length retrovirus thus obtained was again used to screen the chicken genome database as well as the GenBank/EMBL/DDJB databases.

The chicken genome database consists of sequences of red jungle fowl (*G. g. gallus*), the progenitor of domestic chicken (*G. g .domesticus*). Our analysis showed that the ChiRV1 provirus, that has been sequenced from the genome of domestic chicken and ChiRV1 from the chicken genome database presented a high level of identity (99.3%).

The ChiRV1 provirus was found to be 9133 bp in length, and has the usual genomic organization consisting of LTR-gag-pol-env-LTR (Fig. 1).

The 5′ and 3′ LTRs of ChiRV1 are 720 bp in length and contain putative polyadenylation signals AATAAA as well as the TATA-box. The 5′ and 3′ LTRs are about 90% identical. The primer binding site (PBS) of

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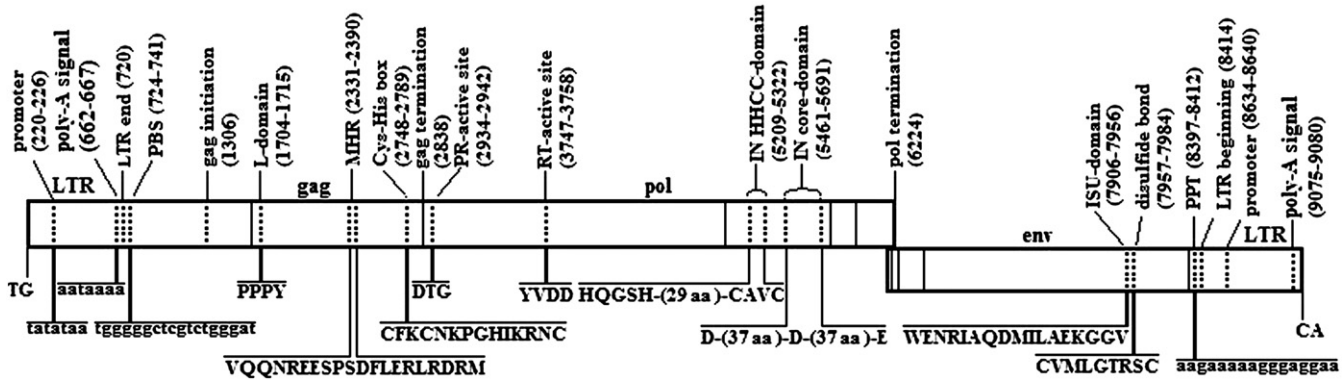

Fig. 1. Genetic map of ChiRV1 provirus. Structural features and conserved motifs are indicated with their positions (bp) in the ChiRV1 genome. Their borders are shown as dashed lines. Full lines indicate stop-codons (shown for coding sequences only) at the following positions: 1626, 1629, 2838 (*gag* stop), 5026, 5782, 5966, 6224 (*pol* stop), 6267, 6456, 8361 (*env* stop). See text for other explanations.

ChiRV1 is complementary to $tRNA^{Pro}$. The canonical dinucleotides TG/CA terminate the ChiRV1 provirus.

Similarly to many endogenous retroviruses, ChiRV1 is not an intact retrovirus having many in-frame stop codons (Fig. 1). In addition to those indicated at Fig. 1, there are eight more stop codos. We eliminated them by adding 1-bp (positions 1552, 3732) and 2-bp (positions 3222 4476, 6459, 6513, 6548, 7833) insertions to the DNA sequence to maintain the open reading frame (these artificial insertions are not present in the ChiRV1 sequence submitted to GenBank). Despite ChiRV1 defectiveness, many protein motifs conserved among different retroviruses could be assigned to its deduced amino acid sequence.

ChiRV1 *gag* is 1.5 kb in length. The deduced amino acid sequence contains a late (L) domain (PPPY) required for efficient budding (Demirov and Freed, 2004) as well as a major homology region (MHR) within the capsid protein and one Cys-His box within the nucleocapsid (Vogt, 1997).

Pol was found to be 3.4 kb in length and its ORF is in frame with the *gag* ORF although the two genes are separated by a stop codon. *Pol* has the typical organization of Pro, RT/RnaseH, Int with a conserved protease-active center (DTG), a reverse transcriptase-active center (YVDD) and two integrase motifs: zinc-binding (HHCC) and putative core (DD35E) (Haren et al., 1999).

Env extends for 2.2 kb and contains an immunosuppressive domain (ISU), a Cys-domain and a transmembrane domain (Benit et al., 2001). The polypurine tract (PPT) of 16 nucleotides located immediately upstream of the 3′ LTR can be clearly seen as well. Compared to *pol*, the *env* gene seems to be in a different reading frame, although many stop codons at the *pol-env* border make it impossible to locate the exact beginning of *env*.

ChiRV1 expression

To determine ChiRV1 expression, we performed RT-PCR analysis with RNA prepared from embryo fibroblasts and the *v-src* induced tumor. An expected product of 833 bp (positions of primers used for RT-PCR: 2928 and 3761 in ChiRV1 genome) can be seen after hybridization with a probe amplified from chicken genomic DNA by the same primer pair (Fig. 2). In addition, Blast searches revealed many chicken ChiRV1-related sequences in the GenBank EST database that confirmed ChiRV1 transcription activity.

Evolutionary relationships of ChiRV1

To study the phylogenetic relationships of the novel chicken endogenous retrovirus, a phylogenetic tree was constructed (Fig. 3). We compared 142 amino acid residues of reverse transcriptase (from domain 1 to 5 as indicated by Xiong and Eickbush, 1990) from 33 retroelements representing all known retroviral groups. It is clear that there is strong bootstrap support for a clade containing ChiRV1 and HERV-I and hence for inclusion of ChiRV1 into the group of gammaretroviruses. Thus ChiRV1 is the first chicken provirus that is not related to ALV and is a member of the gammaretrovirus group.

Several features of the genomic organization of ChiRV1 and MLV are consistent with the close phylogenetic relationships that exist between these viruses. They encode *gag* and *pol* in the same ORF and therefore synthesis of the *gag–pol* polyprotein appears to be mediated via suppression of termination (Swanstrom and Willis, 1997). Both ChiRV1 and MLV have a $tRNA^{Pro}$ binding site, whereas the other chicken endogenous retroviruses known to date, namely ALV-related, have a $tRNA^{Trp}$ binding site. Some amino acid motifs of ChiRV1, e.g. immunosuppressive domain, Cys-domain and transmembrane domain (Benit et al., 2001), are more similar to motifs of MLV-related retroviruses, especially HERV-I, than to those of other retroviral groups.

Phylogenetic analysis also showed that ChiRV1 is not clustered with reticuloendotheliosis viruses (REVs) — REV-A, duck spleen necrosis virus (SNV) and chicken syncytial virus (CSV). The latter virus is the only MLV-related exogenous retrovirus infecting chickens. As suggested, REVs are of mammalian origin and their presence in the avian genome is the result of horizontal transmission (Martin et al., 1999). REVs are exogenous retroviruses and no endogenous REVs have been identified in birds so far. Moreover our analysis revealed no REV-related sequences in the chicken genome database using the available GenBank sequences of REVs.

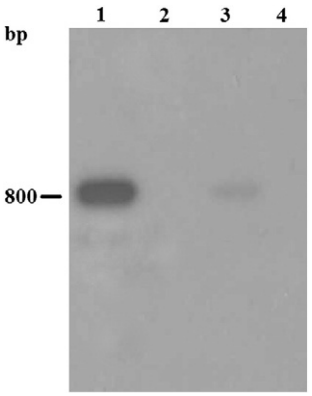

Fig. 2. Southern blot hybridization of RT-PCR products. 1 – RT-PCR on RNA from embryo fibroblasts, 2 – negative control (without reverse transcriptase) on RNA from embryo fibroblasts, 3 – RT-PCR on RNA from *v-src*-induced tumor, 4 – negative control on RNA from *v-src*-induced tumor.

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