Assignment¹ of the chicken orthologue of the human protooncogene EVI1 (Ectotropic viral integration site 1) to chromosome GGA 9qter. Conserved and compared synteny between avian, mouse and human

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¹ To our knowledge this is the first time this gene has been mapped in chicken.

Rationale and significance

The protooncogene *Evi1* was initially identified in the mouse (Mucenski et al., 1988a). It encodes a protein that has multiple 28 amino acid repeats containing the consensus sequence found in the zinc finger domains of many transcriptional regulatory proteins (Morishita et al., 1988). Activation of its expression is frequently found in murine myeloid leukemias due to murine ecotropic retrovirus integration in its 5' region. EVI1 is not normally expressed in hematopoietic cells. In humans, the gene can be activated in myeloid leukemias and myelodysplastic diseases by chromosome rearrangements implicating human chromosome HSA3q26 like inv(3)(q21q26) or t(3;3)(q21q26) (Ogawa et al., 1996; Suzukawa et al., 1997; van Waalwijk et al., 2002). Overexpression of EVI1 has also been detected in patients with "normal" karyotypes suggesting that this inappropriate activation occurs through other mechanisms (Morishita et al., 1990).

A chicken EVI1 cDNA clone was identified by random sequencing of a cDNA chicken spleen library and used as probe to screen a chicken genomic BAC library (Zoorob et al., 1996). Two BAC clones were detected, isolated and checked for the presence of the EVI1 gene by Southern blot experiments. We report here the cytogenetic localization of the chicken orthologue of the human protooncogene EVI1 by fluorescent in situ hybridization (FISH).

Materials and methods

The DNA of BAC P1(211)D8 and P1(50)G1 were respectively labeled with digoxigenin dUTP and biotin dUTP by nick translation and used as a probe for fluorescent in situ hybridization (FISH) on chicken chromosomes. FISH experiments were realized as previously described (Coullin et al., 2002), on the metaphases of chicken cells (*Gallus gallus*) obtained from embryonic cell cultures. The digoxigenin was detected using an anti-digoxigenin-rhodamin-labeled antibody and DAPI was used as counterstaining. Pictures were acquired on a Zeiss epifluorescent microscope using a tri-CCD camera and Vysis computer software (Smart Capture 2). Chromosome assignment was made by reference to the DAPI banded ideogram of the chicken chromosomes GGA1-8;ZW (Ladjali-Mohammed et al., 1999) and G banding report for GGA1-10;ZW (Ladjali et al., 1995).

Probe names: P1(211)D8 and P1(50)G1

Insert sizes: 120 kb

Proof of authenticity: Sequencing and Southern blot

Probe type: BAC clones

Vector: pbeloBACII

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Fig. 1. Chromosomal localization in GGA9q14 of the protooncogene EVI1 to chicken (*Gallus gallus*) metaphases by fluorescent in situ hybridization (FISH). BAC P1(211)D8 and BAC P1(50)G1 were respectively labelled with digoxigenin and biotin and revealed in red and green using anti-Dig-Rhodamin antibody and Avidin-FITC. Banding was obtained by reverse DAPI. (a) BAC P1(211)D8 (red) on a metaphase classified according to Ladjali et al. (1995) and Ladjali-Mohammed et al. (1999). The two chromo-

somes 9 (*) are respectively evidenced with the FISH signal and with the banding alone, in the right upper corner of the figure. (b) BAC P1 (50) G1 (green) and reverse DAPI banding; (c) Double FISH of BAC p1(211)d8 (red) and (P1(50) g1 (green) on chromosomes GGA 9 extracted from metaphase (b) (arrow) and from another experiment. (d) Proposed ideogram from reverse DAPI banding of GGA 9 and regional assignment of EVI1.

Results and conclusions

Mapping data:

FISH experiments and results are illustrated in Fig. 1.

Most precise location: chicken chromosome GGA9qter (9q14) *

Number of cells examined: 50

Number of cells with specific signals: BAC P1 (211) D8
1(0), 2(7), 3(12), 4(29), 7(1)***, 8(1)** chromatids per cell
BAC P1 (50)G1
1(0), 2(1), 3(3), 4(45), 7(0)***, 8(1)** chromatids per cell

The murine and human EVI1 loci were respectively mapped on chromosome MMU3 (Mucenski et al., 1988b), and HSA3q26 (Morishita et al., 1990; Levy et al., 1993). We localized the chicken locus on GGA9 (9q14) where the assignment of *TFRC*, *EIF4A2*, *NCL*, *PAX3*, *SKIL*, and *MCW0134* was previously reported (Schmid et al., 2000). These 6 markers (and EVI1) were found together as a conserved syntenic group on human HSA3q (Schmid et al., 2000). However only the chicken distal markers *SKIL* and *MCW0134* (and *EVI1*) were located

on mouse chromosome MMU3. *TFRC* and *EIF4A2* segregated on MMU16 and *NCL* and *PAX3* segregated on MMU16 and MMU1, respectively (Schmid et al., 2000).

The order of genes on the chicken map is still subject to change. Furthermore, based on detailed analyses on other chicken chromosomes it is known that many rearrangements may have occurred within syntenic regions (Crooijmans et al., 2001). However, with these restrictions, the – Evi1 region – illustrates one of the frequent examples where the synteny is better conserved between chicken and man than between man and mouse.

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* A GTG banded ideogram was proposed for GGA9.

** A percentage of tetraploid cells, present in the embryonic chicken fibroblast culture, was taken into account.

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