Laboratory of Viral and Cellular Genetics

Receptors for retroviruses, retroviral vectors, endogenous retroviruses, silencing of retroviruses, epigenetics

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Retroviruses multiply through a complex replication cycle in their host cells. They enter cells via specific receptors displayed at the cell surface, integrate into the host chromosomes, and use the cell transcription and proteosynthesis machineries to express retroviral structural or enzymatically active proteins. At multiple levels, cellular restriction factors regulate retroviral replication. Specific binding of retroviral envelope proteins to host cell receptors is the prerequisite for cell permissiveness to the infection. Retroviruses can broaden their host range by mutations of the env gene, and vice versa, host cells develop resistance to retroviruses by mutations of genes encoding the specific receptors. We have described such an interesting semi-resistant phenotype in chicken line P and in red jungle fowl and explained it by intronic mutations of the receptor Tva. Both mutations decrease the splicing efficiency of tva transcripts and diminish the display of receptor molecules. Another defence mechanism used by the host cells is the inactivation of integrated invaders at the level of transcription via DNA methylation and modifications of adjacent histones. This might be an obstacle in the case of retroviral vectors used for the gene transfer in gene therapy trials. We have demonstrated that vectors derived from avian sarcoma and leukosis viruses are efficiently silenced through DNA methylation and de novo DNA methyltransferase Dnmt3b plays a crucial role in this process. The epigenomics of retroviral integration sites revealed that only proviruses localized close to the transcription starts of targeted genes keep long-term transcriptional activity without provirus silencing and promoter methylation. The protective region around the transcription start site is marked by enrichment in H3K4 trimethylation. In gene bodies, out of H3K4me3 islands, the proviruses are silenced progressively with the distance from transcription start and in intergenic regions, the proviruses are efficiently silenced. Another example of epigenetic regulation is represented by endogenous retroviruses in the human genome. Fusogenic envelope glycoproteins encoded by two copies of HERVs, ERVWE1 and ERVFRDE1, are strictly placenta-specific, and their expression in other tissues must be prevented by DNA methylation and histone methylation. Another level of syncytin control, at least in syncytin-1, is splicing of retroviral RNA, which has been observed in trophoblastic cells and aberrantly in male germ line tumours. The possible role of hydroxymethyl cytosine in transcriptional regulation of both infectious and endogenous retroviruses is under study.

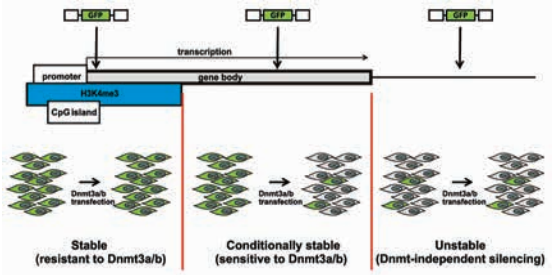

Fig. 1. Expression of proviruses integrated in different genomic localizations, an integrative model. Proviruses integrated close to the TSS within the H3K4 trimethylation region are stably expressed and insensitive to overexpression of de novo Dnmt (left). Proviruses integrated within the gene bodies outside of the H3K4me3 regions are silenced but their stable expression remains in the absence of Dnmt3a/Dnmt3b (conditionally stable expression; middle). Intergenic insertions result in rapid silencing of proviral expression, which is independent of de novo Dnmts (right). Provirus expression is indicated by a picture with green and gray cells.

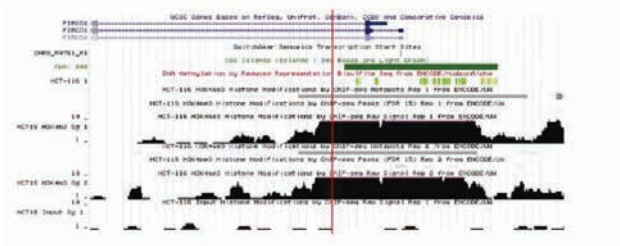

Fig. 2. Example of an integration site with stable provirus expression. The targeted gene (blue), integration site (vertical red line), CpG island (dark green), distribution of CpG dinucleotides (light green), and profile of H3K4 trimethylation (black) are shown.

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- GA CR, GA301/09/2031 – Fusogenic envelope glycoproteins of human endogenous retroviruses and their receptors, 2009-2011, J. Hejnar
- GA CR, GA301/09/P667 – Transcriptional silencing of retroviral vectors during their integration into the host genome, 2009-2011, F. Šenigl
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- GA CR, GAP502/11/2207 – Avian sarcoma and leukosis virus-based vectors and their potential for transgenesis in chicken, 2011-2013, J. Hejnar
- Ministry of Agriculture of the Czech Republic, QJ1210041 – New type of vaccine against chicken viral diseases, 2012-2016, J. Hejnar
- Ministry of Education, Youth and Sports of the Czech Republic, LK11215 – Regulation of active endogenous retroviruses in the mammalian genome, 2012-2016, D. Elleder
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