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Short communication

Loop formation by the transgene *WAP:6xHishGH* in transgenic rabbit fibroblasts, revealed by fluorescence *in situ* hybridization to nuclear halos

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Abstract. Using fluorescence *in situ* hybridization (FISH) to somatic nuclear halos from transgenic rabbits *WAP:6xHishGH*, we present evidence for stability of transgenesis at the chromatin level. FISH performed on fibroblasts from a homozygous individual showed 2 independent loops from both chromosomes of pair 7. On a heterozygous individual, FISH detected a single loop. According to the concept of chromatin loops and their influence on gene expression, this shows that the human growth hormone transgene, which was actively expressed in mammary gland under the influence of the tissue-specific promoter, was inactive in examined skin fibroblasts.

Key words: FISH, growth hormone, loop domain, rabbit, transgenesis.

The basic concept of chromatin loops emphasizes that the chain of nucleosomes packed into a fibril of 30 nm in diameter is organized into loop domains attached to the nuclear scaffold or matrix (Iarovaia et al. 2004). The loop domain is the basic structural and functional unit of eukaryotic chromatin. The size of loops ranges from 5 to 200 kb (Nickerson 2001). The structure of chromatin loops is associated with fundamental processes like DNA replication and gene expression (Heng et al. 2001). There are only limited data concerning the structure of transgenes at the chromatin level integrated into the genome. It is interesting to indicate how foreign DNA is integrated with the host genome and how this new sequence is maintained in the nucleus.

The aim of this study was to show the higher level of the transgene *WAP:6xHishGH* packaging in homozygous and heterozygous transgenic rabbits by using high-resolution fluorescence *in situ* hybridization (FISH) to somatic nuclear halos.

The preparation of nuclear halos was based on biochemical fractionation to remove histones (Gerdes et al. 1994; Kurz et al. 1996) with minor modifications. Ear fibroblasts from 2 rabbits (1 homozygous and 1 heterozygous) were grown on glass slides. Slides were washed in PBS, then incubated in permeabilization buffer (100 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 10 mM Tris-HCl, 0.5% Triton X-100, pH 7.4) for 15 min at 4°C, and again washed in PBS. Subsequently, extraction with a buffer (2 M NaCl, 10 mM Tris-HCL, 10 mM EDTA, pH 7.4) was performed for 5 min at 4°C to remove histones and other soluble proteins, leaving the nucleus surrounded by DNA loops. After extraction, the slides were

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washed in PBS and an ethanol series (10, 30, 50, 95%). Prior to FISH, the slides were baked at 70°C for 2 h. The plasmid *WAP:6xHishGH* (Lipiński et al. 2003) used as a probe for FISH was labeled with biotin-16-dUTP in a random-priming reaction. After denaturation of the probe and DNA on slides, hybridization was conducted overnight at 37°C. Standard posthybridization washes were performed and the signal was detected by using a combined layer of antibodies: FITC-avidin, goat anti-avidin and again FITC-avidin. The slides were stained with DABCO/DAPI and analyzed by using a fluorescence microscope.

FISH detection of the loop domain formed by the transgene *WAP:6xHishGH* is presented in Figure 1. Nuclear halos from the heterozygous transgenic rabbit showed a single loop domain (Figure 1a), but those from the homozygous individual showed 2 loops, each from chromosomes of pair 7 (Figure 1b). Approximately 30 cells for each animal were investigated. In 80% of cells, specific signals were observed, corresponding to the homozygous or heterozygous status. The length of the signals from loops varied between nuclei due to preparation of nuclear halos. The cells lacking signals resulted probably from the fine structure of loops on slides and 2-day washes during FISH preparation.

Limited information is available about the packaging of the genome in the nucleus and about the distribution of genomic sequences in interphase nuclei and its effect on the control of gene expression (Chevret et al. 2000) despite the extensive studies of chromatin structure and organization conducted since the early 1980s. Ciejek et al. (1983), using Southern blot, showed that actively expressed genes were bound to the nuclear matrix, while untranscribed sequences were localized in the loop structures. It was the most important discovery concerning chromatin structure. Gerdes et al. (1994) confirmed this observation by using FISH on nuclear halo preparations. Visual evidence that packaging of DNA is correlated with gene activity was presented. After FISH analysis the signals from inactive genes were positioned on the extended nuclear halo, while signals from active genes remained as condensed spots associated with the residual nuclei. The dystrophin gene fragment that is inactive in lymphocytes was studied by FISH and yielded a long signal on the long loop from residual nuclei. The same situation is presented in our study of transgenic fibroblasts. The transgene *WAP:6xHishGH*, efficiently expressed in the mammary gland due to the presence of the WAP promoter in the gene construct (up to 100 μg mL^{-1}), in fibroblasts was inactive and generated a large loop domain from interphase nuclei.

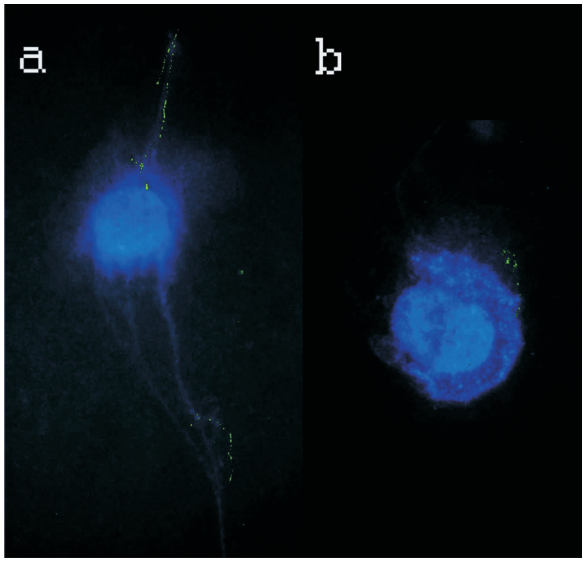

Figure 1. Loop formation by the transgene *WAP:6xHishGH* detected by FISH: (a) nuclear halo from a homozygous transgenic rabbit cell, demonstrating 2 loops; (b) nuclear halo from a heterozygous transgenic rabbit cell, showing a single loop domain formed by the transgene.

There is a possibility that the gene can create few loops. The largest known dystrophin gene in a single transcription unit may be organized into several loops and, what is important, the attachment sites do not affect transcription (Iarovaia et al. 2004). One gene can create different loops in different tissues. Nadel et al. (1995) demonstrated different organization of the 5S rDNA gene cluster in somatic cells and spermatozoa. In somatic (liver and brain) nuclei, the 5S rDNA gene cluster formed one large loop with little condensed FISH signals within the nuclear matrix, but in sperm nuclei the 5S rDNA gene cluster was organized into 3 small loops. The 5S rDNA gene cluster is a segment of DNA that is repeated from 100 to 1000 times, depending on species (Gerdes et al. 1994; Nadel et al. 1995). We predict that the *WAP:6xHishGH* transgene was integrated into the rabbit genome in many copies and considering the 5S rDNA gene cluster it could be interesting to compare the organization of this transgene in different tissues.

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