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Influence of Mouse-Strain-Specific Factors on Position-Dependent Transgene DNA Methylation Patterns

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In previous work from this laboratory, an inverse dependence was established for the adenovirus type 2 E2A late promoter between sequence-specific DNA methylation and promoter activity [1-5; for reviews see ref. 6, 7]. The effect of DNA methylation on promoter activity was also assessed in the transgenic mice, which were obtained from microinjections of unmethylated or in vitro *Hpa*II-premethylated pAd2E2AL-CAT DNA [1] into F_2 zygotes from B6D2F_1 (C57BL/6×DBA/2) hybrid mice. In CAT assays carried out on organ extracts from the pAd2E2AL-CAT mice, the inverse relationship was confirmed [2].

We studied the stability of the pAd2E2AL-CAT DNA methylation patterns in up to eight mouse generations and assessed the influence of the strain-specific genetic background. Three pAd2E2AL-CAT mouse lines were crossed with inbred DBA/2, C57BL/6 or B6D2F_1 mice. Parent-of-origin effects were controlled by exclusive hemizygous transgene transmission either via females or males. The founder animal of line 7-1 carried two groups of transgenes (A and B) on separate chromosomes. The transgene methylation patterns of the 7-1B transgenes and those of the lines 5-8 and 8-1 were stably transmitted.

Southern blot hybridization experiments [8, 9] revealed that the 7-1A transgene methylation pattern was a cellular mosaic. In mixed-genetic-background offspring from 7-1A animals, 10% carried transgenes with *Hpa*II-DNA methylation levels that were reduced from 40 to 10-15%. This finding suggested that in this background the factors that supported high methylation levels were dominant. When inbred DBA/2 mice were the mates, 40% of the siblings carried demethylated transgenes, whereas this ratio amounted to only 10% in C57BL/6 offspring (comparable to B6D2F_1 crossings). Transgene methylation patterns were not detectably influenced by the parent-of-origin.

Four DBA/2 offspring generations were analyzed beyond a 7-1A animal that carried demethylated transgenes. All offspring maintained the demethylated status and in some animals the transgenes had become even more demethylated. However, in this almost pure genetic background, a low level of transgene methylation remained, which was still

cellular mosaic. Hence, the detected cellular mosaicism was presumably not the consequence of a mixed genetic background. Rather, the strain-dependent cellular mosaic methylation patterns of the 7-1A transgenes might be the consequence of a chromosomal position effect, since the 7-1B transgenes in the same founder animal and the transgenes in the other mouse lines did not show such patterns.

Transgene methylation patterns were identical in the DNAs from all organ systems that were analyzed, except the testis, where the transgene DNA appeared to be almost completely demethylated, and epididymal sperm DNA, where the transgenes were de novo methylated. These different methylation levels might be indicative of methylation reprogramming events that take place in the germ line. However, the sperm transgene methylation patterns did not serve as a template for the (demethylated) patterns found in the 7-1A offspring. A more detailed account of this work will be published elsewhere [9].

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