Two-Step Transformation of Rat 3Y1 Cells by the Adenovirus E1A and E1B Genes

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Abstract

The transformation of rodent cells by the adenovirus $E1A$ and $E1B$ genes was very efficient when these genes were physically linked. When they were cleaved, the transformation became very inefficient. To clarify this difference, the chimeric $E1B$ genes in which either the adenovirus enhancer or the human $\beta$-actin promoter was linked to the 5' side of the $E1B$ gene were introduced into rat 3Y1 cells. The saturation density of these cell lines (eB or APrB) was similar to that of parental 3Y1 cells. When eB or APrB cell lines were supertransfected with the $E1A$ gene, discrete dense foci were developed after 5–6 weeks, while the supertransfection of 3Y1 derivative cell lines, in which the enhancer-unlinked $E1B$ gene was introduced, did not develop any dense foci. Analysis of the $E1A$ and $E1B$ transcripts in these cell lines indicated that the $E1B$ gene is efficiently expressed in the presence of the $E1A$ gene products if the enhancer is linked to the $E1B$ gene and that an increased level of E1B proteins is required for an efficient expression of the $E1A$ gene. These results indicated that $E1A$ and $E1B$ genes in separate pieces of DNA are capable of cooperatively transforming 3Y1 cells if appropriate cis-acting elements are attached and high-level expressions are achieved.

Introduction

Transformation of rodent cells by the adenovirus requires both $E1A$ and $E1B$ genes (1–8). A close physical linkage of these genes, however, is required for
efficient transformation. When these genes were cleaved and the E1A and E1B genes on separate DNA molecules were cotransfected to rodent cells, the transformation became very inefficient (9,10). The reason is presently unknown. The inefficiency could have several causes. First, the 5' flanking region of the E1B gene, which overlaps with the 3' portion of the E1A coding region, may have some regulatory function for expression of the E1B gene. In fact, it has been reported that there are four protein binding sites in the XbaI-HpaI sequence at positions 1336–1569 (Fig. 1A) (11). Second, the enhancer located upstream of the