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Inhibition of Simian Virus 40 T-Antigen Expression by Cellular Differentiation

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Murine 3T3T stem cells transfected with pSV3neo DNA were employed to study the effects of somatic cell differentiation on simian virus 40 (SV40) T-antigen expression. This experimental approach was used because the 3T3T cell line is a well-characterized in vitro adipocyte differentiation system and the pSV3neo plasmid contains the early region of the SV40 genome and a selective marker, G418 resistance. Cell clones containing stably integrated pSV3neo which expressed T antigen were isolated in G418-containing medium. Most of these cell clones differentiated poorly. However, several clones retained the ability to efficiently differentiate into adipocytes, and with these cell clones, it was established that adipocyte differentiation markedly repressed T-antigen expression. The differentiation-specific repression of T-antigen expression did not result from a loss of proliferative potential associated with terminal differentiation, because it was observed in adipocytes that could be restimulated to proliferate. In such cells, restimulation of cell growth induced reactivation of T-antigen expression. Repression of T-antigen expression was also demonstrated during differentiation of SV40 T-antigen-immortalized human keratinocytes. These results establish that the process of cellular differentiation can repress T-antigen expression in at least two distinct biological systems.

During the process of differentiation, numerous changes in cellular gene expression occur. Since viral genes are dependent on certain host factors for their expression, differentiation can also affect the expression of viral genes. For example, in undifferentiated embryonal carcinoma cells (14) and pluripotent mouse embryonic cells (44), the genes of several oncogenic viruses are expressed poorly or not at all. In contrast, proficient transcription occurs if the viral DNA is introduced into these cells after differentiation. This change in the ability of viral genes to be expressed is thought to involve the loss of diffusible repressor molecules during differentiation which were present previously in the undifferentiated cells. These factors do not appear to be specific because a wide variety of tumor viruses fail to replicate in undifferentiated embryonal carcinoma cells (26, 35, 37), and promoter or enhancer regions of simian virus 40 (SV40) (30), polyomavirus (13, 31), murine leukemia virus (10), and adenovirus type 5 E1A (45) can each compete for these putative negative regulatory factors (1). However, the biological events that occur when pluripotent embryonal cells differentiate are thought not to be representative of regulatory processes involved in control of somatic cell differentiation. This conclusion is supported by observations that there is active transcription of the SV40 early region and other viral genes in undifferentiated somatic cells in culture (19, 24). In fact, the expression of some viral genes actually inhibits somatic differentiation in certain cells (4, 38).

To determine if somatic cell differentiation can modulate T-antigen expression, 3T3T cells were transfected with pSV3neo DNA and selected for resistance to the antibiotic G418. Although 3T3T cells normally differentiate very efficiently into adipocytes, most pSV3neo-transfected cells differentiated very poorly. Nevertheless, some cell clones were isolated which differentiated well. One clone, CSV3-1, could in fact differentiate into adipocytes at a frequency equal to that of the parental 3T3T cell line. In CSV3-1 and 12 other pSV3neo-transfected clonal cell lines, T-antigen expression was found to be markedly repressed during adipocyte differentiation. The repression of T antigen was reversible when CSV3-1 adipocytes were stimulated to grow and was therefore not a result of terminal differentiation. In addition, the repression of T antigen was evident during the differentiation of SV40 T-antigen-immortalized human keratinocytes.

MATERIALS AND METHODS

Cell culture. 3T3T murine mesenchymal stem cells were derived from BALB/c 3T3 clone A31 cells (7) and were cultured as previously described (16, 25, 42). Briefly, 3T3T cells and pSV3neo-transfected derivative cell clones (see below) were grown in Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS). Cultures were maintained at subconfluent cell densities and were mycoplasma-free. The HE-SV cell line, an SV40 T-antigen-immortalized human keratinocyte cell line, was kindly provided by P. M. Howley (2). HE-SV cells were cultured in serum-free MCDB 153 medium containing various cofactors and growth factors (41).

DNA transfection and isolation of cell clones expressing T antigen. Exponentially growing 3T3T cell cultures were transfected with pSV3neo (33) at 5 μg of DNA per 2.5×10^5 cells, using a standard calcium phosphate technique (9). Cells with stable chromosomal integration of this plasmid were selected by culturing in medium containing 500 μg of G418 per ml for ~4 weeks (5, 6). G418-resistant cells were placed in differentiation-inducing conditions described below, and after 7 days, single adipocytes and single undifferentiated cells were isolated from the same dishes and cloned by using the microchip method described previously (34). Clonal lines were developed from these individual cells by inducing cell proliferation with DMEM containing 30% FBS and 50 μg of insulin per ml.

The HE-SV cell line was derived previously from human keratinocytes after transfection with plasmid DNA containing the SV40 early region by Banks-Schlegel and Howley (2).

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