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The activation of cellular genes in transformed cells

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We have used differential cDNA cloning techniques to isolate a number of genes that are activated as a result of transformation by the DNA tumour virus Simian virus 40. From the nucleotide sequences of the cDNA clones we have been able to identify some of these genes. One of them derives from the major histocompatibility complex and contains a repetitive element that identifies a large number of RNAs present in pluripotential embryonic cells.

Introduction

The transformation of established lines of cultured cells *in vitro* requires the action of only one gene product, be it of viral or cellular origin (Cooper 1982; Tooze 1981; Weiss *et al.* 1982). However, the resultant transformed cell lines differ from their normal parents in a large number of biological and biochemical properties. It is unlikely that these changes all result from direct actions of the transforming protein and it therefore seems necessary that one of the functions of such proteins is to reprogramme the cell's pattern of gene expression.

We have sought to identify cellular genes that are activated as a result of transformation by the DNA tumour virus Simian virus 40 (SV40). There were a number of reasons for choosing SV40 as the model system for this kind of experiment. Transformation by SV40 depends on the gene encoding large T-antigen (Martin 1981) which is a nuclear DNA-binding protein known to be able to regulate transcription (Rigby & Lane 1983). Large T-antigen represses the transcription of its own mRNA (Hansen *et al.* 1981; Rio & Tjian 1983) and activates transcription of the late region of the viral genome (Keller & Alwine 1984). Furthermore, functional large T-antigen is required for the induction of cellular enzyme synthesis which is the first detectable response to SV40 infection (Postel & Levine 1976) and an activity of large T-antigen is involved in the transcriptional reactivation of the silent rDNA complement in human–mouse somatic cell hybrids (Soprano *et al.* 1979). Moreover, Williams *et al.* (1977) used the technique of cDNA–mRNA hybridization in solution to show that there are differences between the cytoplasmic mRNA populations of an SV40-transformed human cell line and its normal parent. It therefore seemed sensible to use SV40-transformed cells to search for cellular genes that are activated by the viral transforming protein.

We have developed a differential cDNA cloning protocol which has allowed us to isolate a number of genes activated as a result of transformation by SV40 (Scott *et al.* 1983*a*, *b*). We shall here consider the identity of several of these genes and discuss possible roles for a repetitive element contained within one of them.

Results

Transcription units activated in SV40*-transformed cells*

We used preparative hybridization, both to immobilized cDNA and in solution, to prepare a cDNA probe enriched in sequences expressed at a higher level in the SV40-transformed cell line SV3T3 Cl38 than in the parental Balb/c 3T3 A31 line. This probe was used to screen a cDNA library prepared from SV3T3 Cl38 mRNA and we thus isolated 42 cDNA clones that were divided into Sets on the basis of restriction endonuclease mapping and cross-hybridization experiments (Rigby *et al.* 1984; Scott *et al.* 1983*b*; unpublished results). Clones within a Set derive from the same or closely related mRNAs; these results are summarized in table 1.

Table 1. cDNA clones corresponding to genes activated in transformed cells

designation	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
prototype	pAG64	pAG59	pAG82	pAG88	pAG10	pAG23
insert length/kilobases	1.55	1.8	1.58	0.70	1.6	0.7
pAG clones related by cross-hybridization	15, 22, 38, 64, 71, 85, 86, 104	37, 57, 58, 59, 69	1, 31, 75, 82	13, 47, 48, 74, 77, 88, 97, 98, 105, 109	10	23
number of clones in Set	8	5	4	10	1	1
identity	Class I MHC antigen (1.6 kilobase RNA) unknown (0.7 kilobase and 0.6 kilobase RNAs)	endogenous retrovirus	mitochondrial	mitochondrial	mitochondrial	unknown

We have determined the nucleotide sequences of a number of these cDNA clones and have, by comparing these sequences with the databanks, been able to identify some of the activated transcription units. Set 1 cDNA clones hybridize to three RNAs, of 1.6, 0.7 and 0.6 kilobases, and contain a dispersed repetitive element (Scott *et al.* 1983*b*). These three RNAs are detected because each contains the repetitive element, which is a member of the B2 family (Krayev *et al.* 1982). The 1.6 kilobase Set 1 mRNA encodes a Class I antigen of the major histocompatibility complex and is found at high levels in a wide variety of transformed mouse cell lines (Brickell *et al.* 1983).

We have determined a part of the nucleotide sequence of pAG59, the prototype clone of Set 2 (unpublished results); this is related to the sequence of Moloney murine leukaemia virus (Mo-MuLV). pAG59 can not derive from Mo-MuLV itself as this is an exogenous virus, but presumably derives from one of the many endogenous retroviral sequences present in the mouse genome. Given the retroviral origin of pAG59 it is clear that the 3.2 kilobase transcript to which this cDNA clone hybridizes is derived from the viral *env* gene which encodes the viral surface glycoprotein gp70. In support of this we have shown, using appropriate monoclonal antibodies, that SV3T3 Cl26 and SV3T3 Cl38, the SV40-transformed cell lines that express the 3.2 kilobase mRNA (Scott *et al.* 1983*b*), contain a gp70 polypeptide (unpublished results).

The cDNA clones of Sets 3, 4 and 5 are homologous to the mitochondrial genome and we

are currently analysing the precise coding potentials of the novel mitochondrial mRNAs which we detect in transformed cells (unpublished results). We have determined the nucleotide sequence of pAG23, the cDNA clone of Set 6, which shows no homology to any sequence within the currently available databases (unpublished results).

A repetitive element found in many embryonic mRNAs

An understanding of the role of the activated genes in transformation might be aided by knowledge of when and where these genes are expressed during the normal life cycle of the mouse. With the exception of the presence of the 1.6 kilobase Set 1 mRNA in thymus (Brickell *et al.* 1983) we have been unable to detect the Set 1 and Set 2 mRNAs in normal, adult tissues. We therefore examined embryos and were surprised to observe that RNA isolated from 8.5 d and 9.5 d embryos hybridized to Set 1 cDNA probes more strongly than mRNA from SV3T3 Cl38 (Murphy *et al.* 1983). We have shown that this strong hybridization is caused by the presence within the embryo of many mRNAs that contain the B2 repetitive element found within the Set 1 cDNA clones. These Set 1-related mRNAs are quantitatively regulated during embryonic development and during the *in vitro* differentiation of EC and EK cells (Murphy *et al.* 1983). Our data indicate that the expression of these mRNAs is a characteristic of pluripotential stem cells and that the mRNAs decrease in abundance in differentiated cells.

Discussion

We have been particularly intrigued by the observation that the three Set 1 RNAs found at elevated levels in a wide variety of transformed and tumour cell lines (Brickell *et al.* 1983; Rigby *et al.* 1984; Scott *et al.* 1983*b*) share a B2 repetitive element with a large number of mRNAs found in pluripotential embryonic stem cells (Murphy *et al.* 1983). Britten and Davidson and their colleagues have previously presented a considerable body of data on the presence of repetitive sequences in mRNAs expressed during sea urchin development (Davidson & Posakony 1982; Davidson *et al.* 1982; Posakony *et al.* 1983) and have also considered the possibility that such elements might be important in the control of gene expression. The B2 repeat family was originally isolated by virtue of its homology to nuclear 'fold-back' RNA (Kramerov *et al.* 1979) and the consensus B2 sequence contains homology to the consensus RNA polymerase III promoter (Krayev *et al.* 1982).

We have not yet isolated cDNA clones corresponding to the small Set 1 RNAs and therefore can not comment on their structure. However, we do note that Sutcliffe *et al.* (1982) have isolated a repetitive element, which they call an identifier and which is found within many brain-specific transcription units (Milner *et al.* 1984). This identifier repeat also contains a consensus RNA polymerase III promoter sequence and will act as a polymerase III promoter in *in vitro* systems (Sutcliffe *et al.* 1984). The identifier sequence hybridizes to small RNAs, of 160 and 110 nucleotides, which are specific to neural cells and appear to be transcripts of the repetitive element plus some flanking sequences. It is therefore possible that the small Set 1 RNAs are not messages but are polymerase III transcripts of B2 elements and flanking sequences. We are presently testing the *in vitro* promoter activity of various cloned B2 elements.

We have no direct evidence as to whether the B2 repetitive elements are involved in the control of gene expression. We are currently exploring this possibility by constructing chimaeric genes that contain the element, and transfecting them into a variety of cell types, particularly

pluripotential embryonal carcinoma cells for which we have developed an efficient gene transfer system (Gorman *et al.*, this symposium).

Whatever the function, if any, of the repetitive element we can use it as a probe to isolate cDNA clones corresponding to mRNAs present in pluripotential embryonic stem cells and thus study the structure and expression of genes regulated during early embryonic development. Such experiments are in progress.

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