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Mouse mammary tumor virus: A model system for regulation of gene expression

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Zusammenfassung

Mouse mammary tumor virus (MMTV) kommt prizipiell in zwei Formen vor. Erstens als integrierte virale DNA (endogen vererbt), die in allen Zellen der Maus enthalten ist und zweitens als infektiöse Form, bei der sich die DNA nur im Kern von Brustdrüsenzellen integriert. Die erste Form verhält sich wie ein stummes Gen während die zweite Form aktiv ist, durch Glukocorticoide stimuliert wird und zum Mamma-Karzinom führt. Wir haben beide Typen von viralen Genen molekular geklont und durch Transfektion in verschiedene Zellen in Gewebekultur eingeführt. Wir konnten zeigen, dass sowohl die endogene DNA, wie die infektiöse DNA in transfektierten Zellen aktiv ist und dass die Expression beider Gene durch Glukocorticoide stimuliert wird. Wir konnten die DNA Sequenzen, die für die Hormonstimulierung nötig sind, in einem kleinen Fragment der viralen DNA lokalisieren. Bei der Sequenzanalyse dieses DNA-Stückes haben wir ein neues virales Gen entdeckt, das die Information für ein Protein von ca. 40000 Molekulargewicht enthält. Mit Hilfe eines Antikörpers suchen wir in verschiedenen Brustdrüsenzellen und- Tumoren nach diesem Protein, dessen Funktion noch nicht bekannt ist.

Introduction

The biology of the mouse mammary tumor virus (MMTV) has several features that make it particularly interesting. Its target cell specificity results in the appearance of mammary carcinomas in mouse strains with high mammary tumor incidence. There exist numerous strains of MMTV, which are transmitted either horizontally as infectious agents in the milk, or vertically, in form of integrated viral DNA (provirus) in the germ line. Infection of mice with milk-borne (exogenous) virus generally leads to mammary tumors early in life. Among the genetically transmitted (endogenous) variants, some have been associated with slowly growing mammary tumors late in life. Others seem to have no effect on tumor incidence (for review see Bentvelzen and Hilgers, 1980). Whether variations in the viral sequences, different genomic locations or differences in the target cells are responsible for the different patterns of expression was one of the questions asked in our studies.

Another peculiarity of MMTV is its hormonal regulation (reviewed by Varmus *et al.*, 1979). Both virus-induced mammary tumors and viral gene expression in tissue culture cells are regulated by glucocorticoid hormones. The increase in viral gene expression seems to be due to a direct action of the receptor-hormone complex. The experiments summarized below aimed at the localization of the DNA sequences responsible for the glucocorticoid response.

The mechanism by which MMTV causes mammary tumors is still unclear. The lack of a simple tissue culture transformation assay made it extremely difficult to tackle this problem. As large amounts of molecularly cloned MMTV DNA are now available for transfection studies new approaches to this problem can be envisaged.

Comparison of the biological activity of exogenous and endogenous MMTV DNA in transfected mouse and mink cells

Recently we molecularly cloned the unintegrated DNA of GR-MMTV (Buetti *et al.*, 1981) and an integrated endogenous provirus from AJ mice (Diggelmann *et al.*, 1982). Both types of proviruses have been transfected into mouse L-cells and their expres-

sion has been studied. The cloned exogenous provirus is efficiently expressed in transfected cells, as it is *in vivo* in infected mammary gland cells and in mammary tumors. Viral gene expression is also strongly stimulated by glucocorticoid hormones as it is *in vivo*, demonstrating that viral sequences are sufficient for hormone susceptibility (Buetti and Diggelmann, 1981). Endogenous proviruses which are not expressed *in vivo* and which do not respond to glucocorticoid stimulation (Dudley *et al.*, 1978) are capable of being normally expressed after cloning in bacteria and transfection into L-cells. These experiments (Hynes *et al.*, 1981; Diggelmann *et al.*, 1982) suggest that endogenous proviruses are potentially functional genes which are subject to a negative control in normal mouse cells. One of the important differences of endogenous proviruses *in vivo* and after transfection is their degree of methylaction. The L-cell endogenous, silent proviruses are highly methylated (Diggelmann *et al.*, 1982; Hynes *et al.*, 1981b). The transfection process in itself does not activate these proviruses and their methylation pattern does not change. By its replication in E. Coli the cloned MMTV DNA loses its specific methylation pattern and it remains undermethylated after transfection into mouse L-cells for as long as it has been examined (over one year). It is reasonable to assume that undermethylation is at least one condition for expression of MMTV DNA. These results ae summarized in table 1.

Table 1

	Endogenous provirus		Exogenous provirus	
	in vivo	after cloning and transfection	in vivo	after cloning and transfection
Integration of DNA	+	+	+	+
Viral RNA synthesis	—	+	+	+
Viral protein syntheis	—	+	+	+
Viral particles	—	not done	+	+
Glucocorticoid stimulation of transcription	—	+	+	+
Methylation of MMTV DNA	high	low	low	low
Oncogenicity of provirus	low	no results yet	high	no results yet

To exclude the possibility that the observed expression of viral products in transfected mouse cells resulted from recombination of the transfecting DNA with cellular sequences, or by activation of cellular MMTV copies, it was necessary to demonstrate the biological activity of these MMTV clones in cells which do not contain MMTV-related sequences. We therefore transfected the same DNA's into mink lung epithelial cells (CC164). Transformation by SV40 DNA was used as a selective marker to isolated transfected cells containing MMTV DNA. The results demonstrated that MMTV DNA of endogenous and exogenous origin was expressed in mink cells and viral gene expression was hormonally stimulated in both cases (Owen and Diggelmann, 1982). These experiments further confirm that the cloned endogenous and exogenous MMTV DNA's contain the complete viral information and that the sequences responding to glucocorticoid hormones are carried on the viral genome.

To localize the hormone responding sequences within the viral DNA we performed transfection studies with subgenomic DNA fragments. Figure 1A shows a schematic map of MMTV DNA and the fragments used for these experiments. The 1.45 kb Pst I fragment containing almost the complete region of the long terminal repeat (LTR) plus a few unique DNA sequences has been transfected into Ltk^- cells and we were able to demonstrate hormone stimulated synthesis of viral RNA (fig. 1B spots 4 and 5) (Fasel *et al.*, 1982) in these cells. This suggests that the hormone responsive sequence is located within the 1.45 kb Pst I fragment.

We also transfected Ltk^- cells with the 2.6 kb Sst I fragment which contains 103 bases upstream from the initiation site of viral RNA synthesis and reaches into the pol gene (see fig. 1A). One transfected cell clone obtained in this experiment synthesized considerable amounts of viral RNA, but dexamethasone had no effect on transcription (fig. 1B, spots 6, 7, 8). This result suggests that the hormone responsive sequences are located more than 70 nucleotides upstream from the

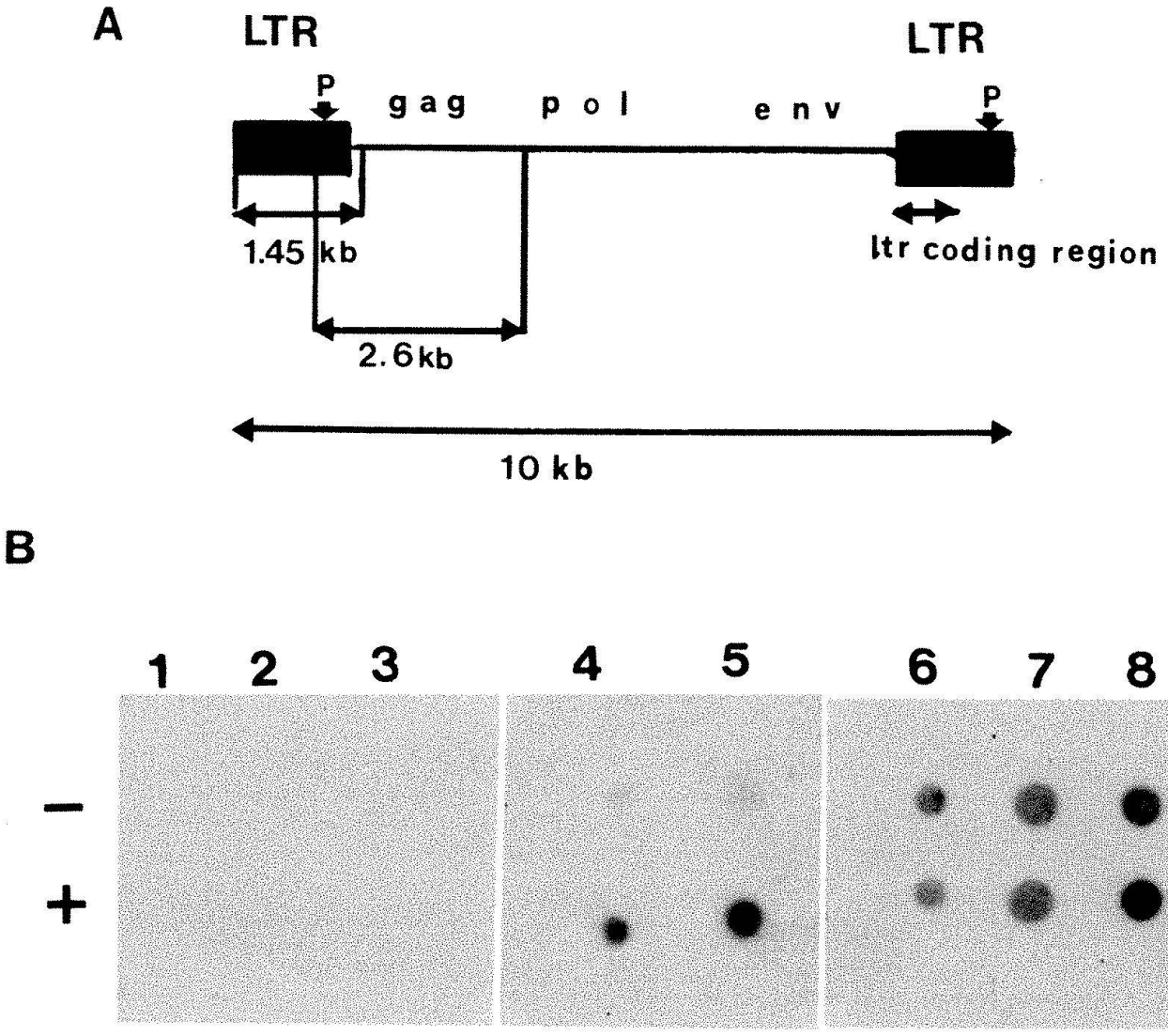

Fig. 1. A. Schematic representation of an MMTV genome. P = site of promotor for viral transcription. gag = gag-gene (major internal structural proteins). pol = polymerase gene (reverse transcriptase). env = envelope gene (glycoproteins). LTR = long terminal repeat. 1.45 kb fragment (Pst I restriction enzyme fragment). 2.6 kb fragment (Sst I restriction enzyme fragment).

B. Analysis of virus specific RNA synthesized in Ltk^- control cells (1, 2, 3) and Ltk^+ cells transfected with the 1.45 kb fragment (4, 5) or the 2.6 kb fragment (6, 7, 8). − total RNA from cells kept without hormone. + total RNA from cells kept overnight in 10^{-6} M dexamethasone 1, 4, 6 10 ug RNA spotted, 2, 5, 7 20 ug RNA spotted, 3, 8 30 ug RNA spotted. Filters were hybridized with ^{32}...-labelled MMTV DNA.

viral promotor (P in fig. 1A). Further transfection experiments using DNA fragments with deletions in the important region of -70 to -1100 upstream of the viral promotor are in progress.

Does MMTV have a transforming gene or does it cause mammary gland cell transformation by an alternative mechanism?

Retroviruses can be divided into two classes with respect to their mechanism of cell transformation. The first group (e.g. the classical Rous Sarcoma Virus) contains a transforming gene (viral *onc* gene). This gene is derived from a cellular gene (cellular onc gene) present in all animal cells. It is thought that these genes were picked up by passage of retroviruses in their host cells and by reverse transcription of the cellular onc gene mRNA (for review see Bishop, 1982). Viruses containing such an onc gene transform cells rapidly and cause tumors with high frequency and early in the life of the host. Viruses which do not contain an onc gene are capable of transforming cells by activation of a cellular onc gene (Neel *et al.*, 1981).

Viruses transforming cells by this mechanism cause tumors much more rarely and late in the life of the animal.

When we sequenced the long terminal repeat region of MMTV DNA we found a coding region of 960 basepairs potentially giving rise to a protein of 320 amino acids (36 K) (Fasel et al., 1982). Such a protein could be involved in mammary gland cell transformation. On the basis of the proposed amino acid sequence B. Gutte (University of Zürich) synthesized a peptide of 23 amino acids. This oligopeptide has been injected into rabbits in order to produce antibodies. We are now searching for the corresponding protein in normal and lactating mammary gland cells, in mammary tumors and in cells transfected with cloned MMTV DNA. In preliminary experiments using these antibodies we found a 50 K protein which is present at highest levels in lactating mammary gland cells, detectable in mammary tumor cells and transfected cells but not in normal mammary gland cells. This protein is larger than predicted by the sequencing data. This could be due to modifications of the protein; e.g. other transforming proteins are phosphoproteins. Experiments to characterize this 50 K protein in more detail are under way. Its function is for the moment still unknown. Much more work has still to be done to understand the mode of tumor formation by MMTV.

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