REVIEW ARTICLE

Factors controlling the expression of mouse mammary tumour virus

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INTRODUCTION

Mouse mammary tumour virus (MMTV) is associated with mammary carcinomas in susceptible mouse strains (Nandi & McGrath, 1973; Bentvelzen, 1974). The virus is transmitted exogenously as an infectious agent through the milk of infected mice from mother to offspring (Bittner, 1936) or through the germ line as endogenous proviral loci (Varmus *et al.*, 1972; Cohen & Varmus, 1979, 1980; Groner & Hynes, 1980). Although the mammary gland is the major site of expression of the virus, certain other organs and cell types are now known to express MMTV but at lower levels (reviewed in Salmons & Günzburg, 1987).

It has long been known that the expression of this retrovirus is controlled by pregnancy hormones (reviewed by Ringold, 1983). The regulation of MMTV expression by glucocorticoids (McGrath, 1971; Dickson *et al.*, 1974; Fine *et al.*, 1974; Parks *et al.*, 1974; Ringold *et al.*, 1975) aroused much interest and consequently MMTV became one of the best studied hormone-regulated genes. However, despite the huge volume of literature concerning glucocorticoid regulation, it should be stressed that such hormones are not the only factors that control the expression of MMTV.

More recently, progress has been made in elucidating the regulatory mechanisms of some of the other factors involved in the control of MMTV expression. Of particular interest are negative regulatory elements (NREs), factors which specifically bind to NREs and the regulatory mechanisms involved in specifying lymphocyte specific expression. Such regulatory mechanisms are of interest with respect to the tissue specificity of expression shown by MMTV, the life cycle of the virus, its role in mammary tumorigenesis and its possible involvement in lymphomagenesis.

TISSUE SPECIFICITY AND TRANSGENIC MICE

Studies on the expression of endogenous (germ-line) transmitted MMTV proviruses in different organs of the mouse have revealed that few of these proviruses are transcriptionally active and that when active, the highest levels of expression occur in the mammary gland and, at much lower levels, in lymphoid cells (reviewed in Salmons & Günzburg, 1987). In view of the fact that MMTV-expressing cells do not necessarily have a phenotype and that most organs consist of many different cell types, only some of which may express MMTV, it is possible that these earlier studies failed to detect the expression of MMTV in some cell types. Support for the concept of more generalized MMTV

expression has been provided by more recent studies. Transgenic mice carrying constructs in which the MMTV LTR is linked to an easily assayable gene have revealed other sites of expression for MMTV (see also below) (Ross & Solter, 1985; Stewart *et al.*, 1988; Tremblay *et al.*, 1989). However, in such transgenic studies, sequences from the linked indicator gene probably also contribute to determining the site of expression (Günzburg *et al.*, 1991). A second potential caveat of such studies is that wild type MMTV expression levels and/or tissue specificity may well be determined additionally by MMTV sequences outside the LTR which are not included in the transgenic constructs (S. Wintersperger, B. Salmons and W. H. Günzburg, unpublished work).

However, using transgenic mice carrying an LTR–SV40 large T antigen construct (Choi *et al.*, 1987) expression of MMTV has been shown to occur in lungs, liver, salivary glands, seminal vesicles and/or prostate gland and testes. These organs were confirmed as sites for the expression of endogenous MMTV proviruses in a later study (Henrard & Ross, 1988) and corroborate earlier observations that the spleen and lymphoid cells express MMTV (Lopez *et al.*, 1985; Sarkar & Günzburg, 1986; Sharma *et al.*, 1988; King *et al.*, 1990). Nevertheless, it should be stressed that the expression levels in these tissues are considerably lower than those observed in the mammary gland during pregnancy and lactation.

Subsequently, Ross and co-workers demonstrated that the spectrum of expression of reporter transgenes under the transcriptional control of partially deleted MMTV-LTRs could be extended to include additionally brain, skeletal muscle and heart (Ross *et al.*, 1990). The LTR deletions define a region located between –655 and –201 in the U3 region as containing at least one negative regulatory element (NRE) (Fig. 1) to which an, as yet undefined, factor present in brain, skeletal muscle and heart presumably specifically binds and thereby inhibits expression from the MMTV promoter in these tissues. Clearly such factors could also be responsible for determining the other sites of expression of MMTV. The existence of NREs in the MMTV-LTR has also been documented *in vitro* in transfection studies by a number of groups and will be discussed below.

HORMONAL REGULATION

Over the last 10 years much effort has been directed towards an understanding of how gene expression is regulated by steroid hormones. MMTV has proved a very useful model system that has been extensively exploited in such studies, mainly using transfection techniques. The steroid hormone progesterone probably plays a major role *in vivo* where there is a huge increase in expression of MMTV in the mammary gland during pregnancy. However, other pregnancy hormones such as prolactin are also

Abbreviations used: MMTV, mouse mammary tumour virus; LTR, long terminal repeat; HRE, hormone responsive element; NRE, negative regulatory element; ORF, open reading frame; *MLs*, minor lymphocyte stimulating; CAT, chloramphenicol acetyltransferase; LPS, lipopolysaccharide.

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Fig. 1. Schematic representation of the location of regulatory elements in the MMTV-LTR

The LTR is divided into three regions, U3, R and U5. The site of transcription initiation (→ mRNA) is located at the 5' border of the R region. Regulatory elements (as indicated) are located within the U3 region. The numbers indicate location relative to the transcription initiation site (+1).

thought to play a role in this phenomenal increase in MMTV expression.

Steroid hormones

The sequences involved in mediating glucocorticoid stimulation of MMTV transcription are located in the MMTV promoter contained within the U3 region of the long terminal repeat (LTR) (Majors, 1990) (see Fig. 1). These sequences, located between nucleotides -202 and -50 relative to the transcription initiation site, were designated the hormone responsive element (HRE) (Hynes *et al.*, 1983; Buetti & Diggelmann, 1983; Majors & Varmus, 1983). The HRE contains four overlapping recognition sites (two proximal and two distal sites) to which both activated glucocorticoid and progesterin receptors bind (Chalepakidis *et al.*, 1988), thereby enhancing the transcription rate greatly (for steroid receptor reviews, see Gronemeyer, 1991; Muller & Renkawitz, 1991).

The same sequences have also been shown to mediate responsiveness to androgens, but not oestradiol (Darbre *et al.*, 1986; Parker *et al.*, 1987; Ham *et al.*, 1988; Cato *et al.*, 1987, 1988). Nevertheless, mutations in the HRE appear to affect differentially the response to these diverse steroid hormones (Gowland & Buetti, 1989), indicating that within the HRE there are distinct sequence differences required for its interaction with the different classes of steroid hormones.

Sequences located between the two distal binding sites of the HREs (-156 to -162) have been shown to regulate MMTV expression negatively (Langer & Ostrowski, 1988) (Fig. 1). This negative regulation is mediated *in trans* by a nuclear factor that was isolated from mammary epithelial cells. The biological significance of this negative regulatory mechanism is unclear, but it may be a determinant of tissue specificity, ensuring that hormone induction of MMTV expression in steroid-responsive organs other than the mammary gland does not occur. Such a

control mechanism is of importance in light of the fact that almost all cell types contain steroid receptors (Gustafsson *et al.*, 1987; Muller & Renkawitz, 1991).

Recent studies also implicate the involvement of chromatin in the glucocorticoid mediated regulation of MMTV transcription. Six nucleosomes (A-F) are positioned on the MMTV-LTR (Richard-Foy & Hager, 1987). Upon addition of glucocorticoid hormone, the activated receptor binds to the HRE and causes the displacement of nucleosome B. The displacement can be inhibited by sodium butyrate which, although it does not affect activated glucocorticoid receptor binding to the HRE, prevents promoter activation (Bresnick *et al.*, 1990; Pina *et al.*, 1990). Indeed, it appears that the displacement of nucleosome B is necessary for the transcription factor NF-1 to have access to its recognition site, thereby allowing the assembly of the transcription initiation complex (Archer *et al.*, 1991; Pina *et al.*, 1990).

Although the various factors involved in MMTV transcriptional regulation are discussed separately in this article, it is important to bear in mind that regulatory elements of genes interact with each other. This is illustrated by the recent study of Gouilleux *et al.* (1991) which shows that at least one positive regulatory element (-201 to -450, Fig. 1) co-operates with the HRE to enhance expression from the MMTV promoter.

Prolactin

The peptide hormone prolactin has been shown to enhance the effects of glucocorticoids on the expression of MMTV (Svec & Links, 1977). More recently it has been shown that prolactin at physiological concentrations may play a greater role in the control of MMTV expression *in vivo* than previously thought, particularly in parous mice (Munoz & Bolander, 1989). Although the prolactin receptor has been cloned (Boutin *et al.*, 1988), the mechanism by which prolactin regulates transcription of MMTV or indeed other mammary-specific genes has yet to be elucidated.

The effects of prolactin may also be indirect since it is known that the growth and differentiation status of mammary cells influences the expression of mammary-specific genes (Topper & Freeman, 1980).

DNA METHYLATION

Eukaryotic DNA contains 5-methylcytosine almost exclusively in the dinucleotide CpG (Razin & Riggs, 1980). The methylation status of cellular genes has been correlated with their expression status (Bird, 1984). The expression of MMTV proviruses is also correlated with hypomethylation. Generally, the provirus DNA methylation status is both locus- and tissue-specific and reflects the methylation status of the neighbouring host cell DNA (Günzburg & Groner, 1984; Günzburg *et al.*, 1984; Hu *et al.*, 1984; Fanning *et al.*, 1985). Endogenous MMTV proviruses are not usually expressed in mouse liver and these proviruses are highly methylated. However, the expression of such proviruses can be experimentally induced by treatment with 5-azacytidine or by partial hepatectomy, both of which result in a transient hypomethylation of the endogenous MMTV proviruses (Groner *et al.*, 1984; Ponta *et al.*, 1985). This suggests that methylation of proviral DNA may prevent its expression in hepatocytes. Conversely, the transition of mammary cells that express MMTV from a hormone-responsive to a hormone-unresponsive state is

accompanied by a loss of MMTV expression and an increase in methylation (Darbre *et al.*, 1983; Darbre & King, 1984). However, hypomethylation alone appears not to be sufficient for expression since at least one provirus (Mtv-20) is hypomethylated in a number of mouse organs and yet there is no evidence for the expression of this provirus (Günzburg & Groner, 1984). Taken together, these data implicate methylation as one, but not the only, level of control of expression. On the basis of results obtained from methylation studies of other cellular genes (Dyner, 1989), it seems probable that methylation at some critical, as yet undefined, methylation sites will influence the ability of transcription factors to bind their recognition sequences, thereby influencing gene expression of MMTV.

CELL-ENCODED TRANSCRIPTION FACTORS

Like most promoters that are recognized by RNA polymerase II, MMTV has a TATA motif to which the transcription factor TFIID binds (Davison *et al.*, 1983; Nakajima *et al.*, 1988) (Fig. 1). Mutations in the TATA sequence result in a 6–8-fold decrease in transcription (Toohey *et al.*, 1990). Sequences between the TATA motif and the initiation site of transcription do not appear to be important for regulation of transcription (Toohey *et al.*, 1990), except probably with respect to spacing.

A binding site for the transcription factor NF-1 has been

Fig. 2. Summary of the evidence that the ORF and gag regions of MMTV encode a negative-acting transcriptional regulator, *naf*

The packaging cell line carries a construct consisting of a Rous Sarcoma Virus (RSV)-LTR linked to the coding region (*gag*, *pol*, *env*) and 3' LTR of MMTV. A relatively high level of transcription of RSV-MMTV RNA ($\Rightarrow$ mRNA) can be detected in this cell line. Upon transfection of a *naf*-expressing MMTV retroviral vector carrying a neomycin-resistance gene (*neo*) driven from a herpes simplex virus (HSV) thymidine kinase promoter (*tk*), transcription from the RSV-MMTV is considerably reduced ($\rightarrow$ mRNA). Retroviral vectors that are unable to encode a functional *naf* (ORF deleted vector or *gag*-deleted vector) do not affect the level of RSV-MMTV RNA transcription ($\Rightarrow$ mRNA).

shown to be present in the MMTV-LTR (Nowock *et al.*, 1985; Buetti & Kuhnel, 1986; Cordingley *et al.*, 1987; Cordingley & Hager, 1988; Cato *et al.*, 1988) between -80 and -60 which partially overlaps with the most proximal glucocorticoid receptor binding site (Fig. 1). The cell-encoded NF-1 is involved in the transcriptional control of MMTV (Buetti & Kuhnel, 1986; Cordingley *et al.*, 1987; Cordingley & Hager, 1988; Kuo *et al.*, 1988) and is required for both basal and hormone-induced transcription (Toohey *et al.*, 1990). Interestingly, NF1 competes with steroid hormone receptors for binding to their respective recognition sequences within the MMTV-LTR and thus may regulate the maximal level of hormone inducible transcription.

The MMTV-LTR also contains two octamer-like motifs, located between -56 and -37 (Fig. 1), to which the transcription factor OTF-1 (also known as NFIII) binds (Meulia & Diggelmann, 1990; Bruggemeier *et al.*, 1991). The binding of OTF-1 to these sites is facilitated by glucocorticoid or progesterone receptor binding and is required for hormonal stimulation of transcription from the MMTV-LTR (Bruggemeier *et al.*, 1991). Additionally this factor may be involved in directing the expression of MMTV in B-lymphocytes (see below in 'Factors controlling lymphocyte-specific expression').

An AP-1-like transcription factor isolated from a mammary tumour cell line, which produces MMTV, has recently been shown to bind to a TRE-like sequence, TGAG/ATCA, located between -756 and -743 in the MMTV-LTR. This factor, NF-S, a *fos/jun*-related protein, has not been detected in normal mammary gland cells (Siddique & Sarkar, 1990), implying that the NF-S protein is present only in transformed mammary cells and may even be a consequence of MMTV expression.

REGULATORY ELEMENTS

A region around -1090 to -900 within the MMTV-LTR has been delimited in three independent studies as an enhancer (Mink *et al.*, 1990; Gouilleux *et al.*, 1991; Yanagawa *et al.*, 1991). It has been postulated that this enhancer may be involved in the activation of *int* proto-oncogenes (Mink *et al.*, 1990), one of the earlier events in mammary tumorigenesis (for a review of *int* genes see Nusse, 1991). In the study of Yanagawa and coworkers (1991), this enhancer was active in two human mammary tumour cell lines but not in HeLa cells and only weakly in Jurkat T cells. Further, a nuclear factor(s) present only in mammary extracts binds to this DNA fragment (Yanagawa *et al.*, 1991). Thus, this enhancer is a good candidate as a determinant of mammary specificity.

Three negative regulatory elements (NREs) have been defined within the MMTV-LTR that have been shown to repress transcription in transfection experiments (Morley *et al.*, 1987; Mink *et al.*, 1990; Gouilleux *et al.*, 1991) (Fig. 1). At least one of the elements (-428 to -364) functions in both orientations (Morley *et al.*, 1987; Mink *et al.*, 1990) and shows a cell type specificity with respect to its effects, suggesting that this element-factor interaction may also be involved in determining the tissue specificity of expression of MMTV (Mink *et al.*, 1990). The factors that bind to these regions and mediate the negative regulatory effect are as yet unknown.

VIRALLY ENCODED TRANSCRIPTION FACTORS

Retroviruses such as human immunodeficiency virus (HIV) (Cann & Karn, 1989), human T-cell lymphotropic virus (HTLV) (Hidaka *et al.*, 1988) and bovine leukaemia virus (BLV) (Derse, 1988; Willems *et al.*, 1989) encode regulatory factors, in addition to the structural proteins and enzymes (protease, integrase, reverse transcriptase) required for the life cycle (Majors, 1990).

These regulatory factors are presumed to be responsible for the establishment of the viral infection and the fine balance required for co-ordinated expression of the viral gene products, leading to efficient release of infectious virions.

MMTV has long been known to encode an enigmatic open reading frame (ORF) within the U3 region of the LTR (Donehower *et al.*, 1981; Kennedy *et al.*, 1982). This ORF is highly conserved among the various sequenced MMTV variants, arguing strongly for a biological function of the ORF (Dickson, 1987; Salmons & Günzburg, 1987). However, convincing expression of the ORF product *in vivo* has yet to be demonstrated.

A 1.7 kb mRNA, found in the mammary glands and tumours of BALB/c mice, has been described as a candidate ORF mRNA (van Ooyen *et al.*, 1983; Wheeler *et al.*, 1983). The putative ORF transcript consists of sequences located 5' to the *gag* region splice donor, linked to a splice acceptor site just outside of the 3' LTR. However this mRNA may be transcribed from an incomplete endogenous provirus known to reside at the *Mtv-6* locus in BALB/c mice (Cohen *et al.*, 1979) rather than representing an authentic transcript of a complete MMTV genome.

The LTR ORF potentially encodes a protein of up to 37 600 Da (Donehower *et al.*, 1981). A series of four methionine-rich candidate ORF proteins of 18, 21, 24 and 36 kDa have been detected using *in vitro* translation of RNAs from the 3' LTR (Dickson *et al.*, 1981; Sen *et al.*, 1981; Racevskis & Prakash, 1984). More recently, putative ORF products of 36 kDa and 45 kDa have been detected in a baculovirus expression vector system. The 45 kDa ORF gene product is a glycosylated form of the 36 kDa form (Brandt-Carlson & Butel, 1991). However, these proteins have yet to be convincingly detected *in vivo* even though antibodies, raised against potentially immunogenic synthetic peptides, recognize the *in vitro* translated products (Racevskis & Prakash, 1984; Brandt-Carlson & Butel, 1991).

Recently, during the construction of a retroviral vector system based upon MMTV, it was shown that the ORF product has an associated negative transcriptional regulatory activity that functions *in trans*. The MMTV vector system consists of a vector construct (Günzburg & Salmons, 1986) and a packaging cell line which expresses high levels of MMTV transcripts and structural proteins (Salmons *et al.*, 1989) (Fig. 2). This packaging cell line contains a construct, RSV-MMTV, which serves as an assay gene. Upon introduction of the vector provirus into the packaging cell line, the expression of MMTV transcripts and structural proteins from the assay construct is reduced. Run-on analysis revealed this to be due to a decreased rate of transcription (Salmons *et al.*, 1990), suggesting that the vector construct encodes a factor that, *in trans*, down-regulates MMTV expression. Using mutation analysis, two regions of the vector provirus were shown to be required for the down regulation: the ORF contained within the 3' LTR and a region in the *gag* gene (Salmons *et al.*, 1990) (Fig. 2). The involvement of *gag* sequences offers an explanation as to why previous attempts to express the ORF alone by coupling the MMTV-LTR to a strong promoter have failed to reveal its biological activity. Consequently, we have designated the ORF product *naf* (negative acting factor) (Salmons *et al.*, 1990). In this light it is of interest that the amount of putative ORF mRNA has been shown to be inversely correlated to the levels of *gag*, *pol* and *env* proteins in a number of preneoplastic outgrowths and derived tumours (Knepper *et al.*, 1989), supporting a role *in vivo* for *naf*.

The involvement of the ORF product in transcriptional regulation of MMTV has also been reported by van Klaveren & Bentvelzen (1988). However, in apparent contrast to the role of ORF as a negative-acting transcriptional factor, these authors provide evidence that the ORF product is a *trans*-activator of the

MMTV-LTR, analogous to the *tat* product of HIV. In this system, the MMTV-LTR was coupled to the chloramphenicol acetyltransferase (CAT) gene. This indicator construct was then transfected into non-infected or MMTV-infected rat mammary tumour cell lines. Interestingly, CAT activity is lower in the MMTV infected cells as compared to the non-infected cells, implying that the MMTV provirus in the infected cells is making a *naf*-like factor. When the LTR-CAT construct is co-transfected with constructs expected to express ORF into mouse fibroblast NIH3T3 cells, CAT activity is somewhat increased. Two 'minimal' constructs are able to *trans*-activate the LTR-CAT construct, both having the complete MMTV-LTR in common and no other MMTV sequences (van Klaveren & Bentvelzen, 1988). The cloning of an ORF/*naf* cDNA and the expression of this cDNA in cells should resolve the apparent differences in functionality of the ORF/*naf*.

A transcriptional activator activity has been detected in cells transfected with an MMTV provirus carrying a mutated ORF (W. H. Günzburg, F. Heinemann, G. Brem, V. Erfle & B. Salmons, unpublished work). This is of particular interest because one interpretation of these results would be that the ORF/*naf* product has regulatory domains that determine whether it functions as a positive or negatively acting transcription factor, a possibility which may also reconcile the apparently conflicting reports of ORF/*naf* as a transcriptional repressor and as an activator. Alternatively, MMTV may encode a second, transcriptional *trans*-activator, the coding sequences of which are as yet unknown.

Taken together, the experimental evidence available to date suggests that MMTV encodes one or more autoregulatory transcription factors and is therefore functionally more similar to lentiviruses than C-type retroviruses such as murine leukaemia virus. Such regulatory factors may, in analogy in their role in the lentiviruses, play an important role in the establishment of infection, tissue specificity of expression and/or in MMTV-mediated mammary tumorigenesis.

FACTORS CONTROLLING LYMPHOCYTE-SPECIFIC EXPRESSION

It has long been known that MMTV can be expressed in lymphoid cells, but because of the relatively high level of expression in the mammary gland and the association of MMTV with mammary tumorigenesis, this area of research has not received as much attention as it deserved. The realization that MMTV is also associated with lymphomagenesis together with the more recent finding that an MMTV-encoded product directly influences the immune system has provided the impetus for a more extensive investigation into the molecular mechanisms controlling MMTV expression in lymphoid cells.

Exogenous infectious virus

Exogenous MMTV is delivered to offspring suckling mice in the milk of the infected mother at a time when the mammary gland is not well developed (Dickson & Peters, 1983). At a later time the virus infects mammary epithelial cells. Thus there must be a second cell type or organ which harbours the virus and enables it to be transported from the gut to the mammary gland at the correct time. One proposed mechanism is that the virus comes into contact with lymphocytes which then become infected, serving as viral replication intermediates. The infected lymphocytes eventually reach the mammary gland where infection of the final target cells occurs. This is supported by the observation that exogenous MMTV is capable of infecting lymphocytes (Liegler & Blair, 1986). Although both B and T cells are potential carriers of MMTV, it appears that T-cells are

essential for the transmission of the virus to the mammary gland (Tsubura *et al.*, 1988). However it should be noted that the production and/or processing of *gag* and *env* proteins is impaired in lymphoid cells (Nusse *et al.*, 1979; Vaidya *et al.*, 1980) and hence the virus may be transmitted by cell-to-cell contact, rather than by infection.

Endogenous germ line transmitted proviruses

Endogenous MMTV proviruses are also expressed in lymphoid cells (Bentvelzen & Brinkhof, 1977). Spleen-specific expression of the *Mtv*-6 associated provirus has been documented using recombinant inbred mouse strains (Sarkar & Günzburg, 1986). More recently, the expression of genomic length MMTV transcripts in BALB/c splenic lymphocytes was reported (Pauley & Lopez, 1991). King and co-workers have cloned a cDNA for an MMTV mRNA expressed in B lymphocytes. The transcripts arise from the endogenous *Mtv*-9-associated provirus and sequencing of the U3 region reveals that it is intact (King *et al.*, 1990). This is in sharp contrast to MMTV proviruses cloned from T-cell lymphomas which always carry a deletion in the U3 region of the LTR (see below). Expression of MMTV in B lymphocytes occurs in the absence of hormonal stimulation, indicating that either B-cells express a factor that promotes MMTV expression or that factors that normally silence MMTV expression are not active in B-cells (King *et al.*, 1990). In this light it is interesting to note that the MMTV LTR contains an octamer binding motif, OTF-1 (see 'Cell-encoded transcription factors' and Fig. 1), which is a binding sequence for factors involved in B-cell specific gene expression (e.g. Ig heavy chain) suggesting a mechanism of hormone-independent expression.

The expression of the *Mtv*-9 provirus in B-cells is stimulated in the presence of the synthetic glucocorticoid dexamethasone. However, the B lymphocyte mitogen lipopolysaccharide (LPS) also stimulates expression from the *Mtv*-9 provirus in these cells by a mechanism that is independent of and distinct to that of glucocorticoids (King & Corley, 1990). LPS probably plays an important role in regulating MMTV expression in lymphocytes since high levels of MMTV expression co-segregate with the *lps* locus on mouse chromosome 4 (Traina-Dorge *et al.*, 1985). Moreover, the injection of LPS directly into the mammary glands of LPS-responder mice has been shown to enhance the expression of MMTV (Carr *et al.*, 1985). Recently, Lund & Corley (1991) have presented evidence for the existence of certain factors that are responsible for MMTV expression in B-cells. These appear to be novel factors, and not the regulatory factors NF-KB and OTF-2 that are highly expressed in many pre-B and B-cells. Additionally, MMTV expression is enhanced during the differentiation of pre-B cells into mature antibody secreting B-cells. Although this increased MMTV expression is correlated with enhanced Ig gene transcription, these two events appear to be regulated by independent factors. One currently favoured hypothesis (Lund & Corley, 1991; Corley & Lund, 1991) is that the as-yet-unidentified MMTV-enhancing factor present in mature B-cells may be indirectly induced as a result of the expression of MMTV-encoded superantigens (see under 'MMTV and superantigens').

MMTV and lymphomagenesis

In certain inbred strains of mice T-cell lymphomagenesis is directly correlated with the presence and expression of newly acquired multiple copies of MMTV (Ball & McCarter, 1979; Michalides *et al.*, 1982; Ball *et al.*, 1983; Dudley & Risser, 1984; Ball & Dekaban, 1987). The cloning and subsequent sequencing of such newly integrated proviruses revealed a consistent deletion and substitution of sequences within the U3 region of the LTR (Michalides *et al.*, 1985; Dudley *et al.*, 1986; Michalides &

Table 1. MMTV proviruses and associated *Mls* superantigens

Endogenous MMTV locus*	<i>mls</i> antigen locus	V β deleted	Reference(s)
Mtv-1†	Mls-2 ^a	3	Frankel <i>et al.</i> (1991), Choi <i>et al.</i> (1991)
Mtv-2†		14	Acha-Orbea <i>et al.</i> (1991)
Mtv-6	Mls-3 ^a	3	Frankel <i>et al.</i> (1991), Woodland <i>et al.</i> (1991b)
Mtv-7	Mls-1 ^a	6,7,8,1,9	Frankel <i>et al.</i> (1991)
Mtv-8	Dvb11-1	11,17	Dyson <i>et al.</i> (1991), Woodland <i>et al.</i> (1991b)
Mtv-9	Dvb11-2/Etc-1	5,1,5,2,11,17	Dyson <i>et al.</i> (1991), Woodland <i>et al.</i> (1991a)
Mtv-11	Dvb11-3	11	Dyson <i>et al.</i> (1991)
Mtv-13	Mls-2 ^a	3	Frankel <i>et al.</i> (1991)

* For review of the chromosomal location and properties of endogenous MMTVs, see Salmons & Günzburg (1987).

† These proviruses give rise to infectious virus.

Wagenaar, 1986; Lee *et al.*, 1987; Ball *et al.*, 1988) (Fig. 1). This consistent finding suggests that elements within the U3 region of LTR may be responsible for determining target cell specificity. This notion has received support from a number of groups (Hsu *et al.*, 1988; Theunissen *et al.*, 1989; Yanagawa *et al.*, 1990) who show that the rearranged LTR, but not the normal MMTV-LTR, directs transcription from an indicator CAT gene in three different T-cell lines. Further, Yanagawa and co-workers showed that these rearranged LTRs had lost a negative regulatory element (Yanagawa *et al.*, 1990) and contained a sequence that is highly homologous to the A enhancer of polyoma virus (Veldman *et al.*, 1985; Kryszke *et al.*, 1987). Although transgenic mice carrying constructs in which indicator genes are expressed from such a deleted LTR do not show expression in T-cells, this has been interpreted to indicate that other events in addition to U3 deletion and rearrangement are required to generate a functional T-cell promoter (Ross *et al.*, 1990).

The U3 deletions, which are present in both LTRs, also disrupt the ORF (Fig. 1) and consequently would be expected to give rise to a truncated *naf* product. It is tempting to speculate that the truncation may abolish the *naf*-mediated negative regulation, thus allowing these proviruses to be over-expressed in lymphoid cells.

MMTV and superantigens

MMTV exists as a multicopy endogenous proviral gene at distinct loci located on different chromosomes in the germ line of mice (Salmons & Günzburg, 1987). Specific endogenous MMTV loci segregate with the deletion of certain specific classes of T-cells during the establishment of tolerance (Table 1) (Dyson *et al.*, 1991; Frankel *et al.*, 1991; Marrack *et al.*, 1991; Woodland *et al.*, 1991a). Previously such deletions of classes of T-cells had only been observed in response to bacterial antigens, called superantigens or minor lymphocyte stimulating (*Mls*) antigens. These superantigens bind to specific classes of the V- β chain, a component of the T-cell receptor, in conjunction with class II MHC molecules. The binding event initially causes the T-cells to proliferate and eventually results in the deletion of these cells. The loss of particular classes of T-cells causes tolerance which greatly influences immune responses (for reviews see Janeway, 1991; Palmer, 1991).

The previously identified *Mls* antigen loci cosegregate with various endogenous MMTV proviruses (Table 1), leading to the speculation that the endogenous MMTV genomes might encode at least some of the previously described *Mls* antigens. Excitingly, this has recently been verified by introducing MMTV constructs either into lymphocytes in cell culture or into transgenic mice which do not express a given *Mls* antigen. The genetically modified lymphocytes express the newly introduced *Mls* antigen

which stimulates the specific class of T-cells to which it is reactive (Choi *et al.*, 1991) in an *in vitro* assay. In the transgenic mouse study, the relevant T-lymphocyte subclass is deleted in response to the newly introduced superantigen (Acha Orbea *et al.*, 1991), albeit at a slower rate than has been observed for normal endogenous *Mls* antigens.

The biological relevance of expression of superantigens remains to be shown. It is possible that exogenous virus infection causes a gradual immunosuppression by deleting T-cells, in a manner akin to that described for the generation of peripheral tolerance. However, recent data from Wei and colleagues argues against this hypothesis. In their study, preneoplastic or neoplastic syngeneic mammary cells that express a newly acquired exogenous (infectious) MMTV provirus were introduced into the mammary fat pads of BALB/c mice. This resulted in a reduction in T-cells carrying the V β 2 chain, as expected, but after surgical removal of the tumour, no immunosuppression was detected (Wei *et al.*, 1991).

It has also been suggested that the deletion of particular subclasses of T-cells may protect mice from further infection with exogenous virus by eliminating target cells. However such a mechanism would only protect against exogenous infection with the same MMTV variant that caused the T-cells deletion (Table 1). It also assumes that T-cells are the only target for the establishment of MMTV infection.

Alternatively and more attractively, the *Mls* antigens may have evolved as a mechanism to stimulate T and B cells transiently so that virus replication can occur. After gut-mediated infection of lymphoid cells, stimulation of T-cells carrying the relevant V β class by MMTV expression of the *Mls* antigen presented presumably by infected immature B-cells would lead to a clonal expansion of B lymphoid cells, thereby amplifying the number of infected cells and increasing the likelihood of mammary gland infection (Acha Orbea & Palmer, 1991; Corley & Lund, 1991). It has also been suggested that B-cells may directly be stimulated and undergo clonal expansion in response to superantigens (Pascual & Capra, 1991).

The constructs employed for the superantigen studies (Acha Orbea *et al.*, 1991; Choi *et al.*, 1991) were designed to express the ORF from the MMTV-LTR. This would imply that the MMTV ORF product is a superantigen and at first sight appears to be at variance with the proposed function of the ORF as a transcription factor (van Klaveren & Bentvelzen, 1988; Salmons *et al.*, 1990) (see under 'Virally encoded transcription factors'). It is interesting to note that sequence analysis of the part of the *naf* coding region defined in the *gag* gene suggests that it may represent a transmembrane domain (W. H. Günzburg & B. Salmons, unpublished work). If the product of the ORF gene were a transmembrane protein, expression of the C-terminal of the

protein may be sufficient for the superantigen effects but the presence of the *N*-terminal, cytoplasmic domain may be required for the transcriptional regulation function. Clearly, the cloning of the complete ORF/*naf* cDNA together with convincing expression data is required to clarify the apparent multiple biological functions assigned to the ORF region.

CONCLUSIONS AND FUTURE PROSPECTS

The expression of MMTV underlies multiple complex mechanisms that we are only just beginning to understand. Some of these mechanisms will be shared with other pregnancy- and lactation-induced mammary-specific genes. Mammary cell growth, interaction and differentiation play major roles in the regulation of such genes. However, significant progress is being made with respect to how some of the factors that are involved in modulating the expression of MMTV function.

One class of factors involved in controlling the expression of MMTV are cellular transcription regulators that can be positively acting (e.g. glucocorticoids) or negatively acting (of which there seems to be at least three). The availability of combinations of these factors in particular tissues and cell types and their concerted action determines the expression pattern of the virus in a manner analogous to that which has been proposed for cellular genes (Renkawitz, 1990). Negatively acting transcription factors are particularly important with respect to determining tissue specificity. At least some of these factors may prove to be key players in the tissue specific regulation of the expression of a number of cellular genes also.

Additionally, MMTV encodes autoregulatory factors which have obvious analogies with factors already described for some of the better understood retroviruses, particularly those of the lentivirus family. These factors act either by directly influencing expression from the MMTV-LTR or indirectly by subtly influencing the growth control of the MMTV-infected cell. The expression of *MIs* antigens represents a second mechanism by which the fate of MMTV-infected cells is manipulated by virally encoded products.

These factors of both viral and cellular origin await further investigation with the promise of revealing new insights into gene regulatory mechanisms, tissue specificity and ultimately retrovirally mediated pathogenesis.

NOTE ADDED IN PROOF

The negative regulatory element previously defined by Morley *et al.* (1987) (−455 to −364; NRE d and e, Fig. 1) has been further characterized and shown to function synergistically with the NRE located within the HRE (−162 to −156; NRE f, Fig. 1) to repress MMTV transcription (Lee *et al.*, 1991). Evidence has also recently been presented that suggests that histone H1 normally binds to block the binding sites for NF-1 and TFIID. Glucocorticoid receptor binding to nucleosome B causes displacement of H1, thus permitting access of NF-1 and TFIID to their respective recognition sequences (Bresnick *et al.*, 1992).

We would like to acknowledge Professors Volker Erfle and Gottfried Brem for their constant encouragement and support. We would also like to thank the members of our laboratory (Frank Heinemann, Andreas Kolb, Inge Janka, Robert Saller, Stefan Wintersperger and Karen Edelmann) for their never-ending challenging questions and suggestions which has generated such a stimulating scientific environment.

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