The Homeobox Protein MSX2 Interacts with Tax Oncoproteins and Represses Their Transactivation Activity*†‡

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Bovine leukemia virus (BLV) tax is an essential gene involved in the transcriptional activation of viral expression. Tax is also believed to be implicated in leukemogenesis because of its ability to immortalize primary cells in vitro. To gain insight into the molecular pathways mediating the activities of this important gene, we identified cellular proteins interacting with Tax. By means of a two-hybrid approach, we show that Tax specifically interacts with MSX2, a general repressor of gene expression. GST pull-down experiments and co-immunoprecipitation assays further confirmed binding specificity. Furthermore, the N-terminal residues 1–79 of MSX2 are required for binding, whereas the C-terminal residues 201–267 of MSX2 do not play a critical role. Whereas the oncogenic potential of Tax in primary cells was only slightly affected by overexpression of MSX2, the other function of Tax, namely LTR-dependent transcriptional activation, was inhibited by MSX2 in human HeLa and bovine B-lymphoblastoid (BL3) cell lines. This MSX2 repression function can be counteracted by overexpression of transcription factors CREB2 and RAP74. The Tax/MSX2 interplay thus results in repression of viral transcriptional activation possibly acting as a regulatory feedback loop. Importantly, this viral gene silencing is not strictly associated with a concomitant loss of Tax oncogenicity as measured by its ability to immortalize primary cells. And interestingly, MSX2 also interacts with and inhibits the transactivation function of the related Tax1 protein encoded by the Human T-cell leukemia virus type 1 (HTLV-1).

Human T-cell leukemia virus type 1 (HTLV-1)§ and bovine leukemia virus (BLV) are members of the Deltaretrovirus genus in the Retroviridae family (1–4). In addition to the structural proteins Gag, Pol, and Env, these viruses also encode a series of regulatory proteins: Tax, Rex, p12/R3, and p13/G4. The Tax protein is a transcriptional activator, which increases the synthesis of viral proteins acting on a triplicate 21-bp element located in the 5’ long terminal repeat (LTR) (5–7). Tax does not interact directly with DNA but rather acts via cellular factors, such as members of the CREB/ATF family of basic leucine zipper proteins (8–11). The tax gene is believed to be essential because its presence is absolutely required for infectivity in vivo (12). Besides its role in the regulation of transcription, the Tax protein also exhibits an oncogenic potential (13). Tax behaves as an immortalizing oncogene because it is able to cooperate with the Ha-ras oncoprotein to fully transform primary rat embryo fibroblasts (14, 15). Both transactivation and immortalizing functions of Tax can be dissociated by mutations in specific regions of the protein. For example, mutation of the phosphorylation sites at serines 106 and 293 of BLV Tax abrogates immortalization potential in vitro but maintains transcriptional activity and viral oncogenicity in vivo (16, 17). Conversely, the transactivation of the LTR promoter is not required for Tax to transform primary cells in vitro (14). The Tax protein of HTLV-1 (Tax1) is known to activate several cellular genes including IL-2, IL-2Ra, IL-3, TNF-α, and GM-CSF (18–20). Tax1 is also involved in cell cycle regulation by direct activation of cyclin D3 and cyclin kinases cdk4 and cdk6 (21), or by inactivating the cyclin-dependent kinase inhibitor p16INK4a (22). In fact, protein-protein interactions with cellular factors are crucial for Tax1 to perturb the regulation of many cellular pathways (23). These HTLV-1 Tax binding factors include the human mitotic checkpoint protein HsMAD1 (24), MEKK1 (25), the IκB kinase (26), or the PCAF protein (27). In contrast, little is known about the cellular partners of the BLV Tax protein. In this study, we describe a functional interaction between the homeobox protein MSX2 and the BLV Tax oncoprotein.

EXPERIMENTAL PROCEDURES

Plasmids—Plasmids pSGMSX2 and pEGFPMSX2 were constructed by subcloning the human MSX2-cDNA (kindly offered by Dr. T. Iimura, Tokyo Medical and Dental University, Japan) into pSG5 (Stratagene) and pEGFP-C1 (Clontech), respectively. Plasmids pCDNAMSX2-c-Myc, pCDNAMSX2AN-c-Myc, and pCDNAMSX2ΔC-c-Myc, which express respectively, C-terminal c-Myc-tagged full-length, amino acids 80–267 and amino acids 1–200 of the human MSX2 protein, were constructed by inserting the human MSX2 cDNA, or PCR-derived MSX2 truncation mutants into pcdNA3.1/myc-HisB (Invitrogen). Plasmid pCDNARAP74-flag was obtained by subcloning the human RAP74 cDNA (kindly offered by Dr. Z. Burton, Michigan State University) into pcdNA3.1Flag (Invitrogen). The pLTRLuc, pLTR1Luc, and pCMVLuc reporter constructs contain, respectively, the BLV LTR, the HTLV-1 LTR, and the cytomegalovirus (CMV) promoter upstream of the firefly luciferase gene. Vectors
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The PJ69-6a yeast strain was transformed with indicated plasmids as described under “Experimental Procedures.” Cell colony viability was evaluated: dead (−), efficiently growing (+), or forming very small colonies (*). The strengths of the interactions were next measured by titration of the β-galactosidase units (ONPG) assay. Values indicated are the means and S.D. of three independent experiments.

Table I: Tax interacts with MSX2 in yeast

| Vectors                          | Growth | β-Gal units |
|---------------------------------|--------|-------------|
| pBDTax + pADMSX2                | +      | 20 ± 5      |
| pBDTax(p106 + 293) + pADMSX2    | +      | 18 ± 4      |
| pBDTax                          | *      | 3 ± 2       |
| p53 + pADMSX2                   | −      | −           |
| pLaminC + pADMSX2               | −      | −           |
| p53 + pSV40                      | +      | 36 ± 8      |

RESULTS

Identification of MSX2 as a Ligand for Tax—Out of a yeast two-hybrid screen, we previously isolated several clones interacting with BLV Tax (29) among which three of them corresponded to the homedomain protein MSX2. To confirm the interactions of this primary screen, expression vectors for both partners were extracted and retransformed into yeast. The different clones were then tested for their ability to specifically activate a β-galactosidase reporter construct that can be induced only in the presence of both partners. As shown in Table I, yeast transformed with pBDTax and pADMSX2 were able to form colonies (+) and expressed the β-galactosidase enzyme (20 units). In contrast, yeast containing MSX2+ p53 or MSX2+ Lamin C failed to grow (Table I, −) whereas those expressing only the Tax protein formed small colonies
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Fig. 1. BLV Tax interacts with MSX2. A. MSX2, MSX2ΔN, MSX2ΔC, and MSX1 proteins were synthesized using rabbit reticulocyte lysates in the presence of 35S-labeled methionine and cysteine. Five microliters of the programmed lysates were then electrophoresed in a 12.5% SDS-PAGE gel, dried, and exposed to x-ray film. B, five microliters of lysates containing MSX2, MSX2ΔN, MSX2ΔC, or MSX1 were mixed with GST-Tax or GST protein bound to glutathione-Sepharose beads. The mixtures were incubated 3 h at 4°C, washed extensively, and electrophoresed onto a 12.5% SDS-PAGE gel. [35S]methionine-labeled MSX2 protein was then revealed by autoradiography. C, HeLa cells were co-transfected with plasmid pEGFPMSX2 expressing the GFPMSX2 protein and plasmids pSGTax for the BLV Tax protein. After 30 h, cells were lysed, and immunoprecipitation (IP) was then performed using rabbit anti-GFP antibody or, as control a rabbit antisera (Control IgG). After extensive washes, the immunoprecipitates were used in Western blotting experiment using a mouse anti-Tax antibody (5A5). As negative controls, the same experiments were performed in parallel using lysates from HeLa cells transfected with pEGFP and pSGTax, pEGFP, and pEGFPMSX2. The amount of transfected DNA was maintained constant with plasmid pSG5.

(Table I, *) with a background enzymatic activity of 3 units. It thus appears that Tax, when bound to DNA, can generate weak but significant basal transcriptional activation of the reporter gene. As a positive control, yeast transformed by p53 and pSV40 expressed high levels of β-galactosidase (36 units). We conclude that BLV Tax specifically binds to MSX2 in yeast.

To assess the specificity of the interaction between BLV Tax and MSX2 in vitro, we performed GST pull-down experiments. To this end, BLV Tax protein was expressed as a fusion protein with GST using the pGexTax vector. On the other hand, MSX2, truncated mutants MSX2ΔN (lacking residues 1–79 of MSX2) and MSX2ΔC (lacking residues 201–267 of MSX2) and MSX1 (which has 51% identity with MSX2 in their N-terminal regions and 62% identity in their C-terminal regions) were synthesized in rabbit reticulocyte lysates (Fig. 1A). The lysates containing the labeled MSX proteins were incubated with GST-Tax fusion protein bound to glutathione-Sepharose beads. The beads were then washed extensively in binding buffer, and the bound polypeptides subjected to SDS-PAGE analysis (Fig. 1B). MSX2, and its C-terminal-deleted mutant (MSX2ΔC) specifically bound to GST-Tax fusion protein but did not interact with GST alone. Under the same experimental conditions, MSX1 and MSX2ΔN mutant did not interact with GST-Tax (Fig. 1B). We conclude that BLV Tax binds to MSX2 in vitro, and that the N-terminal domain of MSX2 is required for the interaction.

To test whether BLV Tax and MSX2 interact in cell lysates, we first immunoprecipitated the MSX2 protein, and the presence of Tax in the complex was subsequently assessed by Western blot. For this purpose, two vectors pEGFPMSX2 (expressing a fusion protein between MSX2 and the green fluorescent protein) and pSGTax were transfected in HeLa cells and cultivated over 30 h. After cell lysis, proteins were immunoprecipitated using either rabbit anti-GFP or with a control rabbit antiserum (Fig. 1C). The immunoprecipitates were analyzed by SDS-PAGE, transferred onto a nylon membrane, and revealed with two Tax-specific monoclonal antibodies (5A5 and 6A7). A polypeptide of 34 kDa sharing antigenicity and co-migrating with the Tax protein (lane 1) was revealed in immunoprecipitates from lysates containing GFPMSX2 (lane 5) but not GFP alone (lane 4). As controls for specificity, Tax was not revealed when the cells were transfected with a control plasmid (lanes 2 and 3) or when the GFP antibody was omitted (lanes 7–10). It thus appears that Tax and MSX2 interact in HeLa cell lysates providing additional evidence for the specificity of their interaction.

Colocalization of Tax and MSX2—To assess the subcellular localization of Tax and MSX2 in mammalian cells we performed confocal microscopy. As demonstrated previously, MSX2 protein fused to GFP localizes mainly in the cell nucleus (29). To confirm and extend these observations, the MSX2 protein was tagged with the c-Myc epitope (in plasmid pcDNAMSX2-c-Myc). HeLa cells were transfected with this expression vector together with pSGTax coding for BLV Tax. Twenty-four hours post-transfection, cells were fixed, permeabilized, and incubated with antibodies against BLV Tax (5A5) and c-Myc (A-14). Finally, anti-Tax and anti-cMyc antibodies were revealed by Alexa-546- and FITC-coupled conjugates, respectively.

BLV Tax was localized in the nucleus (stained by TOPRO-3) as well as in the cytoplasm of the transfected cells, as revealed by the orange (Alexa 546) and red (TOPRO-3) fluorochrome profiles, two types of patterns were observed (Fig. 2, Tax). In contrast, MSX2-c-Myc protein was localized exclusively in the nucleus of HeLa cells (Fig. 2, MSX2). Tax and MSX2 co-localized in the nucleus, as revealed by the yellow color of the merged green and orange fluorochromes (Fig. 2, Tax+MSX2) and the perfect match of the profiles of the green and orange fluorochrome intensities (Supplemental Fig. 3). In addition, in Tax+MSX2 double positive cells, Tax was almost exclusively

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the control of several promoters including the osteocalcin promoter (34, 35) and the SV40 promoter (36). Because MSX2 binds to Tax, we examined whether MSX2 could interfere with transcriptional activation of the viral LTR promoter. Therefore, we co-transfected HeLa cells with expression vectors for Tax (pSGTax) and MSX2 (pSGMSX2) together with the pLTRLuc reporter construct. This latter plasmid contains the BLV promoter cloned upstream of the firefly luciferase gene. Twenty-four hours post-transfection, the cells were harvested, and the luciferase activities were measured in the lysates. It appeared that, luciferase activity was gradually decreased by MSX2 in a dose-dependent manner (Fig. 3A). As illustrated for BLV Tax by a Western blot in B, inhibition of transcription was not because of a decrease in the levels of Tax. We conclude that MSX2 very efficiently represses Tax transcription of the LTR promoter. As expected, MSX2 also inhibited, but to a lesser extent, the cytomegalovirus promoter (Fig. 3C, CMV-Luc) further confirming the role of MSX2 as a general inhibitor of transcription. Because BLV infects B-lymphocytes, we performed similar transfection experiments using the bovine B-cell lymphoblastoid cell line (BL3). It appeared that MSX2 also inhibited Tax-dependent transcription in BL3 cells (Fig. 3D). Together, these data demonstrate that Tax-dependent LTR transcription activity is also inhibited by the homeodomain protein MSX2, similar to many cellular and viral promoters. Neither MSX1 nor the MSX2N mutant unable to bind Tax (Fig. 1B) inhibited the Tax transcription activity, confirming the specificity of the MSX2 repression of Tax-dependent LTR transcription (Fig. 3F).

Transcription Factors CREB2 and RAP74 Interfere with MSX2-dependent Repression of Tax Transactivation—To activate viral transcription, the Tax protein requires a 21-bp sequence (Tax-responsive element), which is repeated three times within the LTR (7, 37). These repeats share a motif resembling the cyclic AMP (cAMP)-responsive element (CRE) core sequence (TGACGTCA) (38). Tax does not bind directly to these motifs but interacts with CREB/ATF cellular transcription factors (8, 9). Among these, CREB2 induces LTR-dependent activation in the presence of PKA and activates Tax-dependent transcription (28).

To determine the role of MSX2 in this process, we co-transfected the pLTRLuc reporter vector together with expression vectors for Tax, CREB2, PKA, and MSX2 as indicated on Fig. 4. As expected, MSX2 represses Tax transactivation (Fig. 4A, compare lanes 2 and 6). Co-transfection of bovine CREB2 partially reverted MSX2 inhibition of Tax-transactivation (Fig. 4A, compare lanes 2, 3, 6, and 7). In the presence of bovine CREB2 and PKA, MSX2 completely lost its ability to inhibit Tax transactivation (Fig. 4A, compare lanes 4 and 8). Taken together, these data demonstrate that overexpression and activation of CREB2 by PKA reverts MSX2 repressor function.

It has been shown that MSX2 suppresses transcription through interaction with RAP74, the large subunit of TFIIIF, and

| DNA | Cell culture | Nude mice |
|-----|--------------|------------|
| Myc | 50 foci (≥7) | 6/6 (5000–7000 mm³) |
| pSG5 | 0 | 6/6 (<150 mm³) |
| Tax | 22 foci (≥5) | 6/6 (900–3000 mm³) |
| MSX2 | 0 | 6/6 (<300 mm³) |
| Tax + MSX2 | 25 foci (≥4) | 6/6 (400–900 mm³) |

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MSX2 does not interfere with the Tax immortalizing function in vitro

Primary REF cells were transfected with expressing vectors for indicated proteins and, as negative control, the empty vector pSG5. One-half of the cells were stably selected and transformed foci counted. The other half were injected into nude mice and tumor volumes calculated as described under “Experimental Procedures.” The number of nude mice and the range of their tumor volumes are indicated.

MSX2 does not interfere with the Tax immortalizing function in vitro

In contrast, MSX2 by itself is not tumorigenic in LSM510 coupled with an Axiovert 200 microscope). The orange arrows indicate differential localization of Tax in MSX2 + Tax double positive cells whereas the yellow arrows depict Tax single positive cells.

FIG. 2.

Co-localization between BLV Tax and MSX2 in HeLa cells.

HeLa cells were transfected with pSGTax (coding for BLV Tax), and pcDNAMSX2-c-Myc (expressing MSX2-tagged by c-Myc), as indicated. Twenty-four hours after transfection, cells were fixed, permeabilized, and labeled with Tax and c-Myc-specific antibodies and Alexa-546 or FITC-conjugated secondary antiserum. Finally, labeled cells were visualized, and labeled with Tax and c-Myc-specific antibodies and Alexa-546 and pcDNAMSX2-c-Myc (expressing MSX2-tagged by c-Myc), as indicated. HeLa cells were transfected with pSGTax (coding for BLV Tax), and pcDNAMCX2-c-Myc (expressing MSX2-tagged by c-Myc), as indicated. HeLa cells were transfected with pSGTax (coding for BLV Tax), and pcDNAMCX2-c-Myc (expressing MSX2-tagged by c-Myc), as indicated. HeLa cells were transfected with pSGTax (coding for BLV Tax), and pcDNAMCX2-c-Myc (expressing MSX2-tagged by c-Myc), as indicated.
that RAP74 is able to counteract the MSX2-repression of the osteocalcin promoter (33). To determine the role of RAP74 in MSX2 repression of the LTR promoter activation, we co-transfected pLTRLuc reporter vector together with expression vectors for Tax, MSX2, and increasing amounts of RAP74. As shown in Fig. 4B, RAP74 was able to reverse, in a dose-dependent manner, the MSX2 repression of LTR transactivation (Fig. 4B, compare lanes 7–10). To analyze the effects of RAP74 and Tax on MSX2 repression, we co-transfected the LTR reporter vector with expression vectors for RAP74, MSX2, and increasing amounts of Tax. We then calculated the percentage of MSX2 inhibition of LTR activity by comparing luciferase data from cell samples overexpressing MSX2 to those from cells lacking MSX2. As shown in Fig. 4C, MSX2 repression of LTR promoter activity gradually decreased in the presence of increasing amounts of Tax (Fig. 4C, compare lanes 1–5) and, as expected, the presence of RAP74 also interfered with MSX2 inhibition of the LTR activation (Fig. 4C, lanes 6–10). Taken together, these data demonstrate that overexpression of transcription factors CREB2 or RAP74 and the presence of high doses of Tax can independently interfere with MSX2 repressor function.

MSX2 Also Interacts with HTLV-1 Tax and Represses Its Transactivation Activity—To test whether MSX2 also interacts with HTLV-1 Tax, both proteins were synthesized separately in rabbit reticulocyte lysates using plasmids pSGTAX1 and pDNA/MX2-c-Myc in the presence of 35S-labeled methionine and cysteine (Fig. 5A). Equal volumes (10 μl) of each lysate were incubated over 4 h, and the Tax1 protein immunoprecipitated using either the Tax1 antibody or a control antiserum. The immunoprecipitates were analyzed by SDS-PAGE and revealed by Western blotting with anti-c-Myc antibody. As shown in Fig. 5B, c-Myc-tagged MSX2 protein specifically co-immunoprecipitated with Tax1.

To examine whether MSX2 could also interfere with transcriptional activation of the HTLV-1 LTR promoter, we co-transfected HeLa or Jurkat cells with expression vectors for Tax1 (pCMVTax) and MSX2 (pSGMSX2) together with a HTLV-1 LTR luciferase reporter construct. Twenty-four hours post-transfection, the cells were harvested, and the luciferase activities were measured in the lysates. It appeared that MSX2 represses HTLV Tax1 transactivation in a dose-dependent manner (Fig. 5C).

Because BLV and HTLV-1 infect different cell types (i.e., respectively, B- and T-lymphocytes), we next compared the endogenous levels in a bovine B-lymphoblastoid cell line (BL3) and in Jurkat T-cells. In both cell lines, the endogenous MSX2 levels were similar (lanes 1 and 3 of Fig. 6). In addition, the amount of MSX2-cMyc generated by plasmid transfection compared with the endogenous levels is about 2-fold higher in BL3 cells and 3-fold higher in Jurkat T-cells (lanes 2 and 4, Fig. 6). We conclude that the homeobox protein MSX2 targets both BLV and HTLV-1 Tax oncoproteins and reduces their transactivation activities.
DISCUSSION

BLV and HTLV-1 are closely related retroviruses sharing similar genomic organizations and affecting cells from the hematopoietic system. However, these two viruses infect different cell types (CD4 and CD8 T-lymphocytes for HTLV-1 and B cells for BLV) and are not subject to mutual cross-transmissions. The induced diseases are also different: ATL (adult T-cell leukemia) or TSP (tropical spastic paraparesis) for HTLV-1 and EBL (enzootic bovine leukemia) for BLV. However, for both viruses, the tax gene is thought to be a major actor in pathogenesis, and the aim of this report is to unravel the metabolic pathways involved in this process.

By means of the two-hybrid system, we identified MSX2 as a cellular partner specifically interacting with BLV Tax. Several
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FIG. 5. MSX2 interacts with HTLV-1 Tax and represses its transactivation activity. A, MSX2 tagged by c-Myc and Tax1 proteins were separately synthesized using rabbit reticulocyte lysates in the presence of 35S-labeled methionine and cysteine. Five microliters of the programmed lysates were then electrophoresed in a 12.5% SDS-PAGE gel, dried, and exposed to an x-ray film. The empty vector pcDNA3.1 was used as a negative control. B, 10 μl of each lysate containing in vitro translated Tax1 and MSX2-c-Myc proteins were mixed in 500 μl of NET buffer and incubated for 4 h at 4 °C. The Tax1 protein was then immunoprecipitated using specific antibody (αTax1) or a control antisera (Control IgG). The immunoprecipitates were analyzed by SDS-PAGE, transferred onto a nylon membrane and revealed with anti-c-Myc antibody. C, 1 μg of reporter plasmid HTLV1LTR-Luc, and, as indicated, different amounts of expressing vectors for MSX2, and Tax1 were transfected into HeLa or Jurkat cells by the GeneJammer or TransIT-Jurkat procedures, respectively. Luciferase activities were determined 24 h post-transfection. The data are the mean of relative luciferase activities of three independent experiments normalized to protein concentrations. Error bars represent S.D.

FIG. 6. Expression of MSX2 in BL3 and Jurkat cell lines. BL3 and Jurkat cell lysates were separated by SDS-PAGE and subjected to Western blot analysis using anti-Msx2 antibody (lanes 1 and 3, respectively). Lysates from BL3 and Jurkat cell lines transfected with 1 μg of pcDNA-MSX2-c-Myc were analyzed in parallel on lanes 2 and 4, respectively. Arrows indicate transfected (Msx2-cMyc) and endogenous Msx2 proteins. The protein band intensities were measured using Scion Image tools (www.scioncorp.com).

lines of evidence support the specificity of interaction between Tax and MSX2. First, when the expression vectors for both partners were isolated and re-introduced into yeast, the resulting cells yielded high levels of β-galactosidase activity. It should be mentioned here that a weak signal was measured in yeast containing only the bait vector (Table I, pBDTax), further supporting the existence of an activation domain within Tax, as described previously (39). Other evidence for specificity for BLV Tax and MSX2 interaction have been obtained by two other independent techniques: GST pull-down and co-immunoprecipitations experiments (Fig. 1). Furthermore, we showed that the N-terminal residues 1–79 of MSX2 are required for binding, whereas the C-terminal residues 201–267 of MSX2 do not play a critical role in the interaction. The MSX gene family comprises three members (MSX1, MSX2, and MSX3). MSX1 and MSX2 are often co-expressed in tissues and share 98% of primary structure identity in their homeodomain, 62% identity in their C-terminal regions, and 51% identity in their N-terminal regions. We found that MSX1 did not bind Tax, further confirming the specificity of Tax/MSX2 interaction. Similar approaches extended these observations to HTLV Tax (Fig. 5). In vivo, BLV and HTLV infect different cell types (B and T lymphocytes, respectively). In this report, we showed that MSX2 affects both Tax proteins in the same way and that the MSX2 endogenous expression levels are comparable in B and T cell lines (Fig. 6). These data suggest that mechanisms involving similar cellular pathways are shared by the BLV and HTLV viruses.

MSX2 is a general negative regulator of gene expression known to interact with some components of the basal transcription machinery (33). Direct interaction with DNA is apparently not required for MSX2 suppressor function. MSX2 rather inhibits transcription via protein-protein interactions with components of the basal transcription machinery such as TFIIF (RAP74 and RAP30) (33). MSX2 belongs to a family of homeobox proteins, which have been implicated in epithelial-mesenchymal interactions during embryogenesis in various body tissues and their expression is often associated with high cellular growth potential (41–45). The MSX2 homeodomain protein has also been involved in the transcriptional mechanisms that regulate osteoblast proliferation, differentiation, and gene expression. MSX2 suppresses the osteocalcin promoter and induces craniosynostosis syndrome, characterized by precocious differentiation of calvarial osteoprogenitor (33, 46, 47). Most interestingly, overexpression of MSX2 was found in a large proportion of carcinoma cell lines and in several tumors (47). In addition, overexpression of antisense MSX2 cDNA interferes with cell transformation induced by the v-Ki-ras oncogene, suggesting its possible role in carcinogenesis (32).

Here, we have shown that the interaction between Tax and MSX2 results in an inhibition of LTR-directed gene expression in fibroblasts and lymphoid cell lines. Comparatively, transcription directed by the LTR was apparently more affected than that of other promoters like CMV (Fig. 3), SV40, and TRE (data not shown), suggesting a possible specificity of inhibition. Under these conditions, viral expression would thus be silenced without affecting the transcription of other cellular genes. We speculate about a possible mechanism allowing silencing of viral gene expression during activation and replication of the
host cell. Interestingly, MSX2 repression could be overcome by overexpression of CREB2 and the catalytic subunit of protein kinase A (Fig. 4A). In this context, several models are conceivable: (i) the CREB/PAK pathway activates a gene that inhibits MSX2, (ii) the CREB/Tax complex displaces MSX2 from the pretranslational complex, (iii) CREB and MSX2 have similar or overlapping recognition sites on Tax resulting in mutual exclusion of both proteins. In any case, we have shown that activation via the CREB/PAK pathway reverses MSX2 repression of Tax transactivation. It has been shown that RAP74 binds directly to the core suppressor domain of MSX2 and reverses inhibition of the osteocalcin promoter activity (33). Here, we extended these observations to the BLV LTR promoter activation by providing evidences that RAP74 can also interfere with MSX2-repression of BLV LTR-directed transactivation (Fig. 4, B and C). However, RAP74 and Tax require two different regions of MSX2 for binding (residues 132–148 and 1–79, respectively) and Tax does not influence RAP74/MSX2 interaction (Supplemental Fig. 1). Another major point of this report concerns the immortalizing potential of BLV Tax. We have indeed shown that MSX2 does not abolish the ability of BLV Tax to transform REF cells (Table II). It thus appears that MSX2 represses Tax-dependent transactivation but still permits cell transformation indicating that both functions of Tax are differently modulated by MSX2. It should be mentioned here that separable domains within the BLV Tax protein mediate transactivation and transformation (16). Indeed, mutations in the zinc finger structure abrogate the capacity of Tax to activate viral expression without concomitant loss of immortalization (14). On the other hand, phosphoserines 106 and 293 of BLV Tax are required for in vitro transformation but not for transactivation (16). Dissociation of these two activities of Tax might be a major process which allows cell immortalization in the absence of viral structural gene expression. The Tax/MSX2 interplay may therefore play an important role in viral silencing and persistence as well as oncogenesis in vivo.

Acknowledgments—We thank E. Wattel (Centre Léon Bérard, Lyon, France), Z. Burton (Michigan State University), F. Bex (Free University of Brussels, Belgium), T. Iimura (Tokyo Medical and Dental University, Japan), D. Portetelle (Faculty of Agronomy, Gembloux, Belgium), C. Abate-Shen (University of Medicine and Dentistry of New Jersey (UMDNJ)-Robert Wood Johnson Medical School, NJ), and K. Yoshikawa (Institute for Protein Research, Osaka University, Japan) for generously providing reagents.

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