Regulation of Microtubule Assembly and Stability by the Transactivator of Transcription Protein of Jembrana Disease Virus*\[5\]

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Microtubules are cytoskeletal polymers consisting of tubulin subunits that take part in diverse cell activities. Many viruses hijack cellular motor proteins to move on microtubules toward the cell interior during the entry process and toward the plasma membrane during the egress period. In addition, viruses often remodel microtubules to facilitate the generation of infectious progeny. In this study, we found that the transactivator of transcription protein of Jembrana disease virus (Jtat) bound tubulin and microtubules both in cells and in the purified system. Microtubule co-sedimentation and co-localization assays revealed a robust interaction of Jtat with microtubules. Tubulin turbidity assay further showed that Jtat promoted tubulin polymerization in vitro in a concentration-dependent manner. Moreover, Jtat promoted the partitioning of cellular tubulin toward the polymeric form, increased the level of tubulin acetylation, and significantly enhanced the cold stability of cellular microtubules. In addition, Jtat-mediated disruption of microtubule dynamics induced the release of Bim from microtubules, leading to profound apoptosis. These results not only identify Jtat as an important viral regulator of microtubule dynamics but also indicate that Jtat-induced apoptosis might contribute to Jembrana disease pathogenesis.

Microtubules, as one of the major components of the cytoskeleton, are long, cylindrical tubes that play important roles in a variety of cell activities such as the establishment and maintenance of cell shape and polarity, the beating of cilia and flagella, the migration of cells, and the transport of vesicles and organelles in the cytoplasm. In addition, microtubules are critical for cell division by orchestrating the alignment and segregation of chromosomes. Microtubules are intrinsically dynamic polymers composed of heterodimers of \(\alpha\) - and \(\beta\)-tubulin. The dynamic property is crucial for microtubules to carry out many of their cellular functions, such as the dramatic rearrangement of the microtubule network at the onset of mitosis and the reorientation of microtubules when cells undergo migration or morphological changes (1).

Microtubules also play an essential role in the infectious life cycle of viruses. As obligate cell parasites, viruses have evolved elegant mechanisms to explore host machinery to facilitate the generation of infectious progeny. They not only hijack cellular pathways to accomplish transcription, translation, and genome replication but also make full use of host cytoskeleton (2). Many viruses, such as human immunodeficiency virus (HIV),3 adeno-virus, and herpes simplex virus, are known to transport within host cells by using the microtubule cytoskeleton system (2).

However, viral association with cellular microtubules is not limited to proteins existing in viral capsids; viral regulatory proteins can also associate with microtubules and regulate their dynamic property. For example, the X protein of hepatitis B virus (HBV), HBx, which is critical for HBV replication, can regulate microtubule dynamics and facilitate the process of maturation and assembly of progeny particles during HBV replication (3).

Jembrana disease virus (JDV), which causes an acute infectious disease in cattle, belongs to the lentivirus family (4). Typical lentiviruses have a long incubation period followed by slowly developing and usually fatal diseases, but in experimentally JDV-inoculated cattle, the incubation period varies from 5 to 12 days before the outbreak of clinical symptoms that include fever, lymphadenopathy, and lymphopenia (5). Nevertheless, JDV is highly similar to other lentiviruses in protein structure, antigen reactivity, and genome sequence. Its genome contains the structural genes gag, pol, and env, the flanking long terminal repeats, and a number of accessory and regulatory genes (6). Jtat, the transactivator of transcription protein encoded by JDV, is highly homologous to the tat proteins encoded by other lentiviruses and has been implicated in JDV-mediated Jembrana disease pathogenesis (7). However, the molecular mechanisms underlying the above effect of Jtat remain largely unknown. In this study, our results reveal that Jtat binds both tubulin and microtubules, promotes tubulin polymerization, and increases

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3 The abbreviations used are: HIV, human immunodeficiency virus; JDV, Jembrana disease virus; Jtat, transactivator of transcription protein of JDV; DAPI, 4'-6-diamidino-2-phenylindole; PARP, poly(ADP-ribose)polymerase; GST, glutathione S-transferase; PBS, phosphate-buffered saline; MAP, microtubule association protein; MES, 4-morpholineethanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid.
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Jtat Interacts with Both Tubulin and Microtubules—Our two-dimensional polycrylamide gel electrophoresis initiative using Jtat-enriched cell extracts has implicated a potential interaction between Jtat and the microtubule subunit tubulin. To confirm the interaction between Jtat and tubulin, 293T cells were transfected with a plasmid expressing GST-tagged Jtat, with GST alone as a control. GST pulldown assay was then performed to examine the protein interactions. As shown in Fig. 1A, both α- and β-tubulin were detected in the pulldown preparation of GST-Jtat but not in that of GST, indicating a specific interaction between Jtat and tubulin. To examine whether the Jtat-tubulin interaction is direct, MAP (microtubule association protein)-free tubulin was incubated with bacterially purified GST-Jtat or GST immobilized on glutathione-Sepharose beads. GST pulldown assay revealed that purified GST-Jtat, but not GST, was able to interact with α- and β-tubulin (Fig. 1B), thus demonstrating a direct association between Jtat and tubulin.

We then performed microtubule co-sedimentation assay to examine whether Jtat interacts with microtubules. Purified reagents (Pierce Biotechnology). Equivalent amounts for each treatment group were loaded on the gel, and the level of tubulin was examined by Western blot analysis.

Western Blot Analysis—Proteins were resolved by polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were blocked in phosphate-buffered saline (PBS) containing 0.1% Tween 20 and 5% fat-free dry milk and incubated first with primary antibodies and then with horseradish peroxidase-conjugated secondary antibodies. Specific proteins were visualized with enhanced chemiluminescence detection reagent (Pierce Biotechnology). The intensity of protein bands was determined by the ImageJ software and corrected by subtracting the measured intensity with the background intensity.

Immunofluorescence Microscopy—Cells grown on glass coverslips were fixed with methanol for 5 min at −20 °C, washed with PBS, and blocked with 2% bovine serum albumin in PBS. Coverslips were incubated at 37 °C with the primary antibody for 1 h and then the fluorescein- or rhodamine-conjugated secondary antibody for 30 min, followed by staining with 0.5 μg/ml of DAPI for 5 min. Coverslips were mounted with 90% glycerol in PBS and examined with an Olympus fluorescent microscope.

Apoptosis Assays—To quantify the percentage of apoptosis, cells grown on coverslips were stained with DAPI and observed under the fluorescence microscope; apoptotic cells exhibited aberrant condensation of nuclear chromatin. To examine inter-nucleosomal DNA fragmentation, total cellular DNA was extracted and examined by electrophoresis on 2% agarose gel.

Flow Cytometry—Flow cytometric evaluation of cellular DNA content was performed as described previously (11, 12). Briefly, 2 10⁶ cells were collected, washed twice with ice-cold PBS, and fixed in 70% ethanol for 24 h. Cells were washed again with PBS and incubated with propidium iodide (20 μg/ml)/RNase A (20 μg/ml) in PBS for 30 min in the dark. Samples were analyzed on a BD FACSCalibur flow cytometer.

RESULTS

Jtat Interacts with Both Tubulin and Microtubules—Our two-dimensional polycrylamide gel electrophoresis initiative using Jtat-enriched cell extracts has implicated a potential interaction between Jtat and the microtubule subunit tubulin. To confirm the interaction between Jtat and tubulin, 293T cells were transfected with a plasmid expressing GST-tagged Jtat, with GST alone as a control. GST pulldown assay was then performed to examine the protein interactions. As shown in Fig. 1A, both α- and β-tubulin were detected in the pulldown preparation of GST-Jtat but not in that of GST, indicating a specific interaction between Jtat and tubulin. To examine whether the Jtat-tubulin interaction is direct, MAP (microtubule association protein)-free tubulin was incubated with bacterially purified GST-Jtat or GST immobilized on glutathione-Sepharose beads. GST pulldown assay revealed that purified GST-Jtat, but not GST, was able to interact with α- and β-tubulin (Fig. 1B), thus demonstrating a direct association between Jtat and tubulin.

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EXPERIMENTAL PROCEDURES

Materials—Propidium iodide, 4′,6-diamidino-2-phenylindole (DAPI), nucodazole, and antibodies against α-tubulin, acetylated α-tubulin, glutathione S-transferase (GST), and β-actin were purchased from Sigma-Aldrich. Antibodies against poly(ADP-ribose)polymerase (PARP), cleaved caspase-3, cleaved caspase-9, and Bim as well as Bim-specific small interfering RNA were used to express the

Cells, Plasmids, and Proteins—Cells were cultured in RPMI1640 medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO₂. Adenoviruses were prepared as described previously (8). The mammalian and bacterial expression plasmids for GST-tagged Jtat were constructed by insertion of Jtat cDNA in-frame into pEBG and pGEX6P1 vectors, respectively. The BL21(DE3) strain of Escherichia coli was used to express the GST-Jtat fusion protein or GST alone, and proteins were purified using glutathione-Sepharose 4B beads according to the manufacturer’s instruction (Promega). Tubulin (from bovine brain, >99% pure) was from Cytoskeleton Inc.

In Vitro Microtubule Co-sedimentation Assay—The in vitro microtubule co-sedimentation assay was performed as described (9), with minor modifications. In brief, bacterially purified GST-Jtat or GST was incubated at 30 °C for 30 min with paclitaxel-stabilized microtubules in PEMG buffer (100 mM PIPES, 1 mM EGTA, 1 mM MgSO₄, 1 mM GTP, pH 6.8). Microtubules were pelleted by centrifugation through a 60% glycerol/PEMG cushion at 40,000 × g for 20 min at 37 °C. The pellet and supernatant fractions were collected individually, and proteins present in each fraction were examined by Western blot analysis.

In Vitro Tubulin Polymerization Assay—Spectrophotometer cuvettes (0.4-cm path length) held a solution consisting of PEMG buffer with or without GST-Jtat. The cuvettes were kept at room temperature before the addition of 10 μM purified tubulin and shifted to 37 °C in a temperature-controlled Utrorspec 3000 spectrophotometer (Amersham Biosciences). Tubulin polymerization was monitored by measuring the changes in absorbance (350 nm) at 0.5-min intervals.

Preparation of Polymeric and Soluble Dimeric Tubulin—The polymeric (cytoskeletal) and soluble dimeric tubulin fractions were prepared as described previously (10). Briefly, cells were washed, and soluble proteins were then extracted under conditions that prevent microtubule depolymerization (0.1% Triton X-100, 0.1 M MES, pH 6.75, 1 mM MgSO₄, 2 mM EGTA, 4 mM KCl). The remaining polymeric (cytoskeletal) fraction was dissolved in 0.5% SDS in 25 mM Tris, pH 6.8. Total protein concentration was then determined in each fraction by BCA
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GST-Jtat or GST was incubated with preformed microtubules, and the mixture was pelleted by centrifugation. Proteins present in the pellet (P) and supernatant (S) fractions were examined by Western blotting. Jtat was found almost entirely in the pellet fraction, where microtubules resided (Fig. 1). In contrast, GST was present only in the supernatant fraction, which did not contain microtubules (Fig. 1D). These results thus revealed a robust interaction of Jtat with microtubules.

FIGURE 1. Jtat interacts with both tubulin and microtubules. A, Jtat interacts with tubulin in 293T cells. Cells were transfected with a plasmid expressing GST-Jtat or GST (control). GST pulldown and Western blotting were then performed to examine their interaction with tubulin. B, Jtat interacts with tubulin in vitro. MAP-free tubulin was incubated with bacterially purified GST-Jtat or GST (control) immobilized on glutathione-Sepharose beads. The presence of tubulin in the pulldown preparation was examined by Western blotting. The levels of GST-Jtat and GST used in the pulldown assay were detected by Coomassie blue staining. C, Jtat co-sediments with microtubules in vitro. Purified GST-Jtat was incubated with preformed microtubules, and the mixture was then pelleted by centrifugation. Proteins present in the pellet (P) and supernatant (S) fractions were examined by Western blotting. D, purified GST was incubated with preformed microtubules, and the rest of the experiments were performed as in panel C.

To further investigate Jtat-microtubule interaction, CV-1 cells were transfected with a plasmid expressing GFP-tagged Jtat and stained with an antibody against α-tubulin. We found that GFP-Jtat partially co-localized with microtubules especially at cell periphery, and when microtubules were destroyed by nocodazole, such a co-localization pattern disappeared (Fig. 2). The co-localization of Jtat with microtubules was also observed by immunostaining cells with a Jtat antibody (data not shown).

The tubulin binding region of Jtat was characterized by transfection of cells with plasmids that express various truncated forms of Jtat tagged with GST (Fig. 3A). In vivo GST pulldown assay revealed that the amino acid sequences 1–51, 1–64, 38–64, and 38–97 were able to interact with tubulin but the sequences 52–97 and 65–97 were unable to interact (Fig. 3A and B), indicating the importance of the cysteine-rich domain in Jtat-tubulin interaction. Interestingly, the NH2-terminal amino acid sequence 1–37 was also able to interact with tubulin (Fig. 3A and B). Therefore, Jtat might possess at least two different regions that mediate its binding to tubulin.

Jtat Promotes Tubulin Polymerization in Vitro and in Cells—The specific and strong interaction of Jtat with tubulin and microtubules suggested that Jtat might play a role in regulating the dynamic property of microtubule assembly. To test this hypothesis, we first examined the effect of Jtat on the polymerization of tubulin subunits into microtubules in vitro by measuring changes in the turbidity produced upon tubulin polymerization in the presence or absence of Jtat. We found that Jtat promoted tubulin polymerization in a concentration-dependent manner (Fig. 4A).

To investigate whether Jtat could affect tubulin polymerization in cells, we prepared cell extracts that contain cytoskeletal (polymeric) and soluble (dimeric) tubulin, respectively, from Jurkat cells treated or untreated with Jtat. Our recent results have shown that exogenous Jtat can pass the plasma membrane and enter cells (13). Western blot analysis of the extracts of Jtat-treated cells revealed that Jtat dramatically promoted the partitioning of tubulin toward the polymeric form (Fig. 4B). Densitometric analysis of the Western blot bands showed that Jtat increased the percentage of cellular polymeric tubulin from 52 to 79% (Fig. 4C). Thus, Jtat was able to increase tubulin polymerization into microtubules in cells.

Jtat Enhances the Stability of Microtubules—We next studied the effect of Jtat on microtubule stability by examining the level of tubulin acetylation with a specific antibody against acetylated α-tubulin. Tubulin acetylation is a well established marker of microtubule stability; stable microtubules exhibit much...
higher extent of tubulin acetylation than dynamic, unstable microtubules (14). We found that the addition of Jtat significantly increased the level of tubulin acetylation in cells (Fig. 5, A and B). In addition, our result revealed that Jtat increased tubulin acetylation in a concentration-dependent manner (Fig. 5, C and D).

We tested the effect of Jtat on microtubule stability further by examining the cold stability of cellular microtubules in response to Jtat. HeLa cells are known to depolymerize microtubules when cooled to 4 °C (15). We thus incubated HeLa cells at 4 °C in the absence or presence of Jtat and then examined the morphology of cellular microtubules by immunofluorescence microscopy. We found that only a small subset (6.6%) of HeLa cells had clear microtubule fibers after incubation at 4 °C; however, the majority (91%) of Jtat-treated cells still had clear microtubules (Fig. 5E). Thus, we concluded that Jtat was able to enhance the stability of microtubules in addition to its increase of microtubule assembly.

Jtat Induces Rapid and Profound Apoptotic Cell Death—An exquisite control of microtubule assembly and stability is known to be crucial for microtubule functions; aberration in microtubule assembly/stability by the alteration of MAPs or treatment with chemical agents can have severe consequences, such as cell death (1, 15–18). We thus asked whether the disruption of microtubule dynamics by Jtat could affect cell viability. Jurkat cells treated or untreated with Jtat were stained with the DNA dye DAPI; apoptotic nuclear morphology was found in cells as early as 4 h after Jtat treatment (Fig. 6A). Jtat-treated cells also displayed a DNA laddering pattern resulting from internucleosomal DNA fragmentation, and the appearance of DNA laddering was clearly dependent on the concentration of Jtat (Fig. 6B).

We then performed flow cytometry to further examine Jtat-induced apoptosis. Jurkat cells treated with Jtat were collected at different time points and stained with the DNA dye propidium iodide, and cellular DNA content was analyzed by a flow cytometer. The percentage of cells with less than 2N DNA content (sub-G₁ cell population) was quantified as a measure of apoptosis. Jtat was found to increase the percentage of sub-G₁ cells in a time-dependent manner (Fig. 6C). In addition, the percentage of sub-G₁ cells increased gradually with the increase of Jtat concentration (Fig. 6D).

Based on prominent DNA fragmentation and increased sub-G₁ population in Jtat-treated cells, our next aim was to examine the involvement of caspases that play a major role in the execution of apoptosis by cleaving a variety of substrates. The activation of caspase-3 and caspase-9, upon their cleavage by upstream proteases, is considered a hallmark of the apoptotic process (19). Therefore, we investigated the levels of cleaved active forms of caspase-3 and caspase-9 by Western blot analysis of cell extracts following Jtat treatment. We found that Jtat caused a concentration-dependent increase in the cleaved

![Figure 3](image3.png) Characterization of the tubulin binding region of Jtat. A, schematic representation of various truncated forms of Jtat that were tagged with GST and used to identify the tubulin binding region. The tubulin binding ability of each truncated form of Jtat is summarized on the right of the scheme. CRD, cysteine-rich domain; core, conserved core region; basic, region of basic amino acids. B, cells were transfected with a plasmid expressing GST or the indicated truncated form of Jtat tagged with GST. GST pulldown and Western blotting were then performed to examine their interaction with α-tubulin.

![Figure 4](image4.png) Jtat promotes tubulin polymerization in vitro and in cells. A, effects of 0, 1.25, 2.5, 5, and 10 μM Jtat on tubulin polymer formation were measured by light scattering, reflected as the absorbance at 350-nm wavelength. The data shown in this graph represent the means of three independent experiments. B, Western blot analysis of tubulin partitioning between polymer (P) and soluble dimer (S) in Jurkat cells untreated or treated with 1 μM Jtat for 4 h. C, experiments were performed as in panel B, and the percentage of polymeric tubulin in total tubulin (polymer + soluble dimer) was quantified by densitometric analysis of the Western blot bands. Error bars represent S.D. of date from three independent experiments.
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A. Con, Jtat

acetylated α-tubulin

α-tubulin

B. Tubulin Acetyl Level

0 0.25 0.5 0.75 1 1.25 μM

C. Western blot analysis of acetylated and total α-tubulin in Jurkat cells untreated or treated with 1 μM Jtat for 4 h.

D. Western blot analysis of acetylated α-tubulin.

E. Immunofluorescence microscopy of microtubules in HeLa cells after incubation at 4 °C for 2 h in the absence or presence of 0.5 μM Jtat.

FIGURE 6. Jtat induces apoptotic cell death. A. Jurkat cells were untreated or treated with 1 μM Jtat for 4 h and stained with DAPI. The nuclear morphology was then observed under a fluorescence microscope. B. DNA electrophoresis pattern in Jurkat cells treated with 0, 0.25, 0.5, or 0.75 μM Jtat for 4 h. C. Jurkat cells were treated with 1 μM Jtat for 0, 2, 4, 6, 8, or 12 h, and the percentage of cells with sub-G1 DNA content (sub-G1 population) was quantified as a measure of apoptosis. D. Jurkat cells were treated for 4 h with 0, 0.25, 0.5, 0.75, 1, or 1.25 μM Jtat, and the percentage of cells with sub-G1 DNA content was then quantified. E. Western blot analysis of cleaved caspase-3 and cleaved caspase-9 in Jurkat cells treated for 4 h with 0, 0.13, 0.25, 0.37, 0.5, or 0.63 μM Jtat. F. Western blot analysis of PARP in Jurkat cells untreated or treated with 1 μM Jtat for 4 h. G. Western blot analysis of PARP in Jurkat cells treated with 1 μM Jtat for 0, 0.5, 1, 1.5, 2, 3, 4, or 5 h. H. Western blot analysis of PARP in Jurkat cells treated for 4 h with 0, 0.25, 0.5, 0.75, 1, or 1.25 μM Jtat.

sequences 1–97 (full-length), 1–37, 1–51, 1–64, 38–64, and 38–97, which interacted with microtubules, were able to induce apoptosis, whereas the sequences 52–97 and 65–97, which did not interact with microtubules, were unable to cause apoptosis (Fig. 7A).

We next sought to gain more mechanistic insights into the relationship between Jtat-mediated disruption of microtubule dynamics and induction of apoptosis. Bim, a pro-apoptotic protein of the Bcl-2 family, is known to associate with microtubules and postulated as a sensor of microtubule integrity; microtubule-interfering agents have been reported to cause the release of Bim from microtubules, which then promotes apoptosis presumably by translocation to mitochondria (20, 21). It is possible that Jtat might adopt a similar strategy to transduce signals from the alteration of microtubule dynamics to apoptosis. To test this possibility, we examined Bim distribution in microtubule pellet and supernatant fractions in Jurkat cells. We found that Bim was entirely present in the microtubule pellet fraction in the absence of Jtat and a portion of Bim was detected in the supernatant fraction upon Jtat treatment (Fig. 7B), indicating Jtat induced the release of Bim from microtubules. Moreover, we found that Jtat-induced apoptosis was remarkably enhanced by Bim adenoviruses (Fig. 7C) and inhibited by Bim-specific small interfering RNA (Fig. 7D).
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FIGURE 7. Jtat-induced apoptosis is mediated by Bim. A, 293T cells were transfected with a plasmid expressing GST or the indicated truncated form of Jtat tagged with GST, and the percentage of cells with sub-G1 content was determined by flow cytometry. The expression of GST and GST-Jtat fusion proteins was examined by Western blot analysis. B, Western blot analysis of Bim distribution in microtubule pellet (P) and supernatant (S) fractions in Jurkat cells untreated or treated with 1 μM Jtat for 2 h. C, Jurkat cells infected with Bim adenoviruses were treated with 0, 1, or 2 μM Jtat for 4 h, and the percentage of sub-G1 cells was quantified by flow cytometry. D, Jurkat cells transfected with Bim-specific small interfering RNA were untreated or treated with 2 μM Jtat for 4 h, and the percentage of sub-G1 population was then quantified. E, a schematic model showing the roles of Jtat in the infectious life cycle of JDV, regulation of microtubule dynamics, and induction of apoptosis.

suggested a critical role for Bim in linking Jtat-induced disruption of microtubule dynamics to apoptosis.

DISCUSSION

Viruses are obligatory cellular parasites with a life cycle critically dependent on host cells and often employ host signaling pathways and machinery to accomplish their infectious life. Many viruses are known to interact with host microtubules or microtubule-associated proteins, and the interactions have proven to be critical at multiple stages throughout viral life cycle. Incoming viruses use motor proteins to travel along microtubules from the plasma membrane to the nuclear or perinuclear replication site, whereas progeny viruses depend on microtubules and motors to move from the assembly site to the cell periphery (2, 22). In addition, viruses can regulate microtubule dynamics through various mechanisms to exert their cellular effects (22). A better understanding of the virus-microtubule interaction will provide insights into both viral pathogenesis and microtubule biology.

JDV is a bovine lentivirus that causes an acute and severe disease in infected animals after a short incubation period (4). The genome of all lentiviruses contains accessory and regulatory genes as well as structural genes. In HIV, at least six accessory and regulatory genes, tat, rev, nef, vif, vpr, and vpu, have been identified and functionally implicated in viral replication and pathogenesis, among which tat is most extensively studied and best understood and is an essential determinant in HIV pathogenesis (23–25). Jtat, a member of the tat protein family, is the transactivator of transcription protein of JDV and plays an important role in JDV replication and pathogenesis (26). A comparison of the amino acid sequences of Jtat and HIV tat shows a high homology in the cysteine-rich domain and the core activation domain (7). Similar to HIV tat, Jtat may affect various cell activities, thereby modulating cellular signals and gene activation in both the virus-infected cells and the neighboring uninfected cells (27). Elucidating novel functions of Jtat is of fundamental importance in our understanding of JDV virology and pathogenesis.

In this study, by GST pulldown, microtubule co-sedimentation, and microtubule co-localization assays, we have identified an interaction between Jtat and microtubules both in vitro and in cells. The microtubule binding domains of Jtat appear novel, because they do not show obvious similarity in either amino acid sequence or predicted structure with known MAPs, including MAP1, MAP2, MAP4, and Tau.

Our studies show that Jtat promotes microtubule polymerization and increases their stability. An accurate modulation of microtubule assembly and stability is known to be crucial for microtubule function and cell viability; alteration of microtubule assembly/stability by abnormal expression of MAPs or by drug treatment can result in serious phenotypes such as cell death (1, 15–18, 28). We have also found that overexpression of MAPs such as MAP2c and Parkin in cells or treatment of cells with microtubule drugs such as Taxol can trigger apoptosis (data not shown). In this scenario, it is not difficult to understand that the alteration of microtubule dynamics by Jtat induces rapid and profound apoptosis. However, it is noteworthy that in our experiments overexpression of Tau in cells does not cause obvious apoptosis (data not shown), reflecting the diversity and complexity of microtubule-binding proteins.

Although it is still elusive today as to how the alteration of microtubule dynamics by many MAPs and microtubule drugs leads to apoptosis, our results demonstrate that Bim is released from microtubules upon Jtat treatment and is required for Jtat-induced apoptosis, establishing a mechanistic link between the disruption of microtubule dynamics and induction of apoptosis. The development of various diseases upon viral infection is known to involve a complex interaction between viruses and host immune responses, as well as an interplay between viral and cellular regulatory factors (29, 30). HIV tat-induced apoptosis has been implicated in the depletion of CD4+ T lymphocytes during the outbreak of the acquired immunodeficiency syndrome (31). Given the high similarity between Jtat and HIV
tat in amino acid sequence and transcription activating functions, it is conceivable that Jtat-induced apoptosis may contribute to the pathogenesis of Jembrana disease. Based on the known background of JDV and Jtat and the advance of this study, we have proposed the following model to describe the roles of Jtat in the infectious life cycle of JDV, regulation of microtubule dynamics, and induction of apoptosis (Fig. 7E). In this model, Jtat is expressed upon JDV infection and transactivates the transcription of genes critical for JDV replication. Jtat can be released from JDV-infected cells and enter the neighboring cells. In cells, Jtat interacts with microtubules and interferes with microtubule dynamics, which in turn causes the release of Bim from microtubules and activates caspases. As a result, apoptosis is triggered, presumably in lymphocytes, ultimately leading to immune system defects and Jembrana disease.

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REFERENCES
1. Desai, A., and Mitchison, T. J. (1997) *Annu. Rev. Cell Dev. Biol.* **13**, 83–117
2. Smith, G. A., and Enquist, L. W. (2002) *Annu. Rev. Cell Dev. Biol.* **18**, 135–161
3. Kim, S., Kim, H. Y., Lee, S., Kim, S. W., Sohn, S., Kim, K., and Cho, H. (2007) *J. Virol.* **81**, 1714–1726
4. Wilcox, G. E., Chadwick, B. J., and Kertayadnya, G. (1995) *Vet. Microbiol.* **46**, 249–255
5. Soesanto, M., Soeharsono, S., Budiantono, A., Sulistyana, K., Tenaya, M., and Wilcox, G. E. (1990) *J. Comp. Pathol.* **103**, 61–71
6. Chadwick, B. J., Coelen, R. J., Sammels, L. M., Kertayadnya, G., and Wilcox, G. E. (1995) *J. Gen. Virol.* **76**, Pt. 1, 189–192
7. Chen, H., Wilcox, G., Kertayadnya, G., and Wood, C. (1999) *J. Virol.* **73**, 658–666
8. Zhou, J., Liu, M., Aneja, R., Chandra, R., Lage, H., and Joshi, H. C. (2006) *Cancer Res.* **66**, 445–452
9. Yang, J. T., Laymon, R. A., and Goldstein, L. S. (1989) *Cell* **56**, 879–889
10. Zhou, J., Panda, D., Landen, J. W., Wilson, L., and Joshi, H. C. (2002) *J. Biol. Chem.* **277**, 17200–17208
11. Aneja, R., Zhou, J., Vangapandu, S. N., Zhou, B., Chandra, R., and Joshi, H. C. (2006) *Blood* **107**, 2486–2492
12. Liu, M., Aneja, R., Liu, C., Sun, L., Gao, J., Wang, H., Dong, J. T., Sarli, V., Giannis, A., Joshi, H. C., and Zhou, J. (2006) *J. Biol. Chem.* **281**, 18090–18097
13. Deng, G., Qiao, W., Su, Y., Sha, R., Geng, Y., and Chen, Q. (2006) *Virus Res.* **121**, 122–133
14. Westermann, S., and Weber, K. (2003) *Nat. Rev. Mol. Cell. Biol.* **4**, 938–947
15. Lieuvin, A., Labbe, J. C., Doree, M., and Job, D. (1994) *J. Cell Biol.* **124**, 985–996
16. Amos, L. A., and Schleiper, D. (2005) *Adv. Protein Chem.* **71**, 257–298
17. Downing, K. H. (2000) *Annu. Rev. Cell Dev. Biol.* **16**, 89–111
18. Jordan, M. A., and Wilson, L. (2004) *Nat. Rev. Cancer* **4**, 253–265
19. Riedl, S. J., and Shi, Y. (2004) *Nat. Rev. Mol. Cell. Biol.* **5**, 897–907
20. Puthalakath, H., Huang, D. C., O’Reilly, L. A., King, S. M., and Strasser, A. (1999) *Mol. Cell* **3**, 287–296
21. Marani, M., Tenev, T., Hancock, D., Downward, J., and Lemoine, N. R. (2002) *Mol. Cell Biol.* **22**, 3577–3589
22. Matarrese, P., and Malorni, W. (2005) *Cell Death Diff.* **12**, Suppl. 1, 932–941
23. Weiss, R. A. (1993) *Science* **260**, 1273–1279
24. Weisfab, M. O., Frank, R., Ochsenauber, C., Stricker, K., Dhein, J., Walczak, H., Debatin, K. M., and Krammer, P. H. (1995) *Nature* **375**, 497–500
25. Zagury, J. F., Sill, A., Blattner, W., Lachgar, A., Le Buanc, H., Richardson, M., Rappaport, J., Hendel, H., Bizzini, A., Gringeri, A., Carcagno, M., Criscuolo, M., Burny, A., Gallo, R. C., and Zagury, D. (1998) *J. Hum. Virol.* **1**, 282–292
26. Chen, H., He, J., Fong, S., Wilcox, G., and Wood, C. (2000) *J. Virol.* **74**, 2703–2713
27. Gibellini, D., Vitone, F., Schiavone, P., and Re, M. C. (2005) *New Microbiol.* **28**, 95–109
28. Hirokawa, N. (1994) *Curr. Opin. Cell Biol.* **6**, 74–81
29. Fultz, P. N. (1991) *J. Virol.* **65**, 4902–4909
30. Novembre, F. J., Johnson, P. R., Lewis, M. G., Anderson, D. C., Klumpp, S., McClure, H. M., and Hirsch, V. M. (1993) *J. Virol.* **67**, 2466–2474
31. Roshal, M., Zhu, Y., and Pannelles, V. (2001) *Apoptosis* **6**, 103–116