

418 incompletely spliced mRNAs, respectively (12, 26, 30, 68), since the expression levels of  
419 these proteins were not significantly different between Tg and Wt cell lines. Additionally,  
420 these results indicate that induction of hCRM1 expression does not affect the expression of  
421 HTLV-1 regulatory proteins in virus-infected rat cells. We also observed variation in the  
422 levels of p19 production among the cell lines derived from hCRM1-Tg rats. Since the amount  
423 of p19 did not correlate with the expression levels of hCRM1, Tax, or Rex, the reason for the  
424 variation is not clear. Some other factors, including RanGTP and RanBP3, which play  
425 important roles in the nuclear export of CRM1-substrate complexes (14, 41, 47, 59), may  
426 affect the levels of p19 production in the rat cell lines. It is also possible that the integration  
427 sites of the provirus influence virus production. Further studies are required to identify the  
428 factors that result in varying p19 production among Tg-derived cell lines.

429 Variation was also observed in the expression of cell surface proteins among the established  
430 cell lines (Table 1). It is especially interesting that most of the wt-derived cells do not express  
431 CD3 or CD4, whereas the majority of the Tg-derived lines possess both of these molecules.  
432 Since others and we have established a number of CD4 positive cells from various strains of  
433 wt rats (31, 36), the present results may be due to the experimental disparity. However, it is  
434 possible that enhanced HTLV-1 production by the hCRM1 expressing cells and subsequent  
435 dissemination of the virus in the culture may influence the phenotypes of the transformed  
436 cells. Thus, additional studies are required to determine the significance and the cause of the  
437 difference.

438 The Tg rats showed minimal effects on HTLV-1 replication in vivo. Since dramatic  
439 enhancement of HTLV-1 production was observed in all hCRM1 expressing cells in vitro, it  
440 is possible that the number of HTLV-1-infected cells in vivo was too low to detect  
441 differences in virus production between Wt and Tg rats. From this point of view, alteration of  
442 experimental condition to improve the initial infection rate of HTLV-1 may lead to the  
443 enhanced viral replication in the Tg rats. Repression of viral protein expression in vivo may  
444 also reduce the effects of hCRM1, masking the enhanced viral replication in the Tg rats. Such  
445 responses have been well documented in HTLV-1 infected individuals (32, 33). It is also  
446 possible that HTLV-1 specific immune responses could affect the replication of HTLV-1 in

447 the Tg rats. Indeed. Our preliminary experiments indicated that induction of HTLV-1-specific  
448 CTL responses occurred as early as 1 week after virus infection. Alternatively, some other  
449 host factors may govern and modulate efficient HTLV-1 replication in vivo. Thus, further  
450 studies on both virological and immunological aspects are required to verify the importance  
451 of the Tg rats as an in vivo model of HTLV-1 infection.

452 The HTLV-1 Rex protein is able to functionally replace the Rev protein of HIV-1 (57).  
453 CRM1 is a nuclear export factor for HIV-1 Rev and a truncated Rev mutant with weakened  
454 binding affinity to CRM1 results in reduced levels of HIV-1 Gag production (20). These  
455 results raise the possibility that rat cells expressing hCRM1 protein can produce enhanced  
456 levels of HIV-1 structural proteins. Indeed, our preliminary results demonstrate that hCRM1  
457 promotes HIV-1 p24 Gag production in rat cells (unpublished observation). Thus, the  
458 hCRM1-Tg rats generated in this study may be also useful as a small animal model of HIV-1  
459 infection, when HIV-1 receptors are simultaneously expressed in these rats.

460 HIV latently infects reservoirs of resting T cells (7, 9, 10, 13, 61), which are thought to be in  
461 the G<sub>0</sub> state, and the virus is then reactivated during T cell activation. Alternatively, HIV has  
462 also been reported to propagate efficiently in non-replicating lymphatic T cells (18), which  
463 lack certain markers specific for activation. Since cytokines levels are high in lymphatic  
464 tissues, the progression of T cells from G<sub>0</sub> to G<sub>1</sub> may support HIV replication. Although  
465 release from cell cycle block has been extensively investigated at the transcriptional level, a  
466 recent study has shown that the synthesis of unspliced HIV Gag RNA increases rapidly during  
467 the HIV reactivation process, to a much greater extent than the synthesis of multiply spliced  
468 RNAs (7). Our results demonstrating a rapid increase in CRM1 expression during lymphocyte  
469 activation provide a clue to the underlying mechanism, the efficient action of the HIV Rev  
470 protein, which leads to robust synthesis of unspliced RNA. We suggest that HIV gene  
471 expression is regulated in lymphocytes at both the transcriptional and RNA export levels.

472 Independent of viral replication, the first phase of enhancement of CRM1 expression is also  
473 coincident with the induction of cytokines, such as IL-2 (4). CRM1 interacts with the ARE  
474 located in the 3' untranslated region of c-fos mRNA (via HuR and its ligands) and mediates  
475 export of this mRNA from the nucleus to the cytoplasm (6, 16). Therefore, CRM1 may

476 transport cytokine mRNAs belonging to the early response genes since many cytokine  
477 mRNAs harbor ARE sequences (24, 56). Collectively, these observations suggest that  
478 enhancement of mRNA export via the induction of CRM1 expression, in addition to  
479 regulation at the transcriptional and translational levels, may play an important role in  
480 coordinating gene expression during lymphocyte activation. The existence of a post-  
481 transcriptional mechanism leading to a rapid increase in CRM1 protein is consistent with this  
482 hypothesis.

483 In conclusion, we have established a novel Tg rat carrying the hCRM1 gene via examining  
484 its gene expression, and isolated several HTLV-1-infected T cell lines expressing hCRM1.  
485 Our results demonstrate that T cells from hCRM1 Tg rats produced enhanced levels of the  
486 HTLV-1 Gag protein compared to T cells from Wt control rats. These results indicate the  
487 essential role of hCRM1 in proper HTLV-1 replication and suggest the importance of this Tg  
488 rat in the development of HTLV-1 animal models. These animals may also contribute to the  
489 development of models for other human retroviruses, such as HIV-1.

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491

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#### REFERENCES

499

- 500 1. Adachi, Y., and M. Yanagida. 1989. Higher order chromosome structure is affected  
501 by cold-sensitive mutations in a *Schizosaccharomyces pombe* gene CRM1+ which  
502 encodes a 115-kD protein preferentially localized in the nucleus and its periphery. *J.*  
503 *Cell. Biol.* **108**:1195-1207.

- 504 2. **Akagi, T., I. Takeda, T. Oka, Y. Ohtsuki, S. Yano, and I. Miyoshi.** 1985.  
505 Experimental infection of rabbits with human T-cell leukemia virus type I. *Jpn. J.*  
506 *Cancer Res.* **76**:86-94.
- 507 3. **Alessi, D. R., A. Cuenda, P. Cohen, D. T. Dudley, and A. R. Saltiel.** 1995. PD  
508 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase  
509 kinase in vitro and in vivo. *J. Biol. Chem.* **270**:27489-27494.
- 510 4. **Ashwell, J. D., and R. D. Klusner.** 1990. Genetic and mutational analysis of the T-  
511 cell antigen receptor. *Annu. Rev. Immunol.* **8**:139-167.
- 512 5. **Bogerd, H. P., R. A. Fridell, R. E. Benson, J. Hua, and B. R. Cullen.** 1996. Protein  
513 sequence requirements for function of the human T-cell leukemia virus type 1 Rex  
514 nuclear export signal delineated by a novel in vivo randomization-selection assay.  
515 *Mol. Cell. Biol.* **16**:4207-4214.
- 516 6. **Brennan, C. M., I. E. Gailouzi, and J. A. Steitz.** 2000. Protein ligands to HuR  
517 modulate its interaction with target mRNAs in vivo. *J. Cell Biol.* **151**:1-14.
- 518 7. **Brooks, D. G., S. G. Kitchen, C. M. Kitchen, D. D. Scripture-Adams, and J. A.**  
519 **Zack.** 2001. Generation of HIV latency during thymopoiesis. *Nat. Med.* **7**:459-464.
- 520 8. **Callanan, M., N. Kudo, S. Gout, M. Brocard, M. Yoshida, S. Dimitrov, and S.**  
521 **Khochbin.** 2000. Developmentally regulated activity of CRM1/XPO1 during early  
522 *Xenopus* embryogenesis. *J. Cell Sci.* **113**:451-459.
- 523 9. **Chun, T. W., D. Engel, M. M. Berrey, T. Shea, L. Corey, and A. S. Fauci.** 1998.  
524 Early establishment of a pool of latently infected, resting CD4(+) T cells during  
525 primary HIV-1 infection. *Proc. Natl. Acad. Sci. USA* **95**:8869-8873.
- 526 10. **Chun, T. W., L. Stuyver, S. B. Mizell, L. A. Ehler, J. A. Mican, M. Baseler, A. L.**  
527 **Lloyd, M. A. Nowak, and A. S. Fauci.** 1997. Presence of an inducible HIV-1 latent  
528 reservoir during highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. USA*  
529 **94**:13193-13197.
- 530 11. **Daenke, S., S. Nightingale, J. K. Cruickshank, and C. R. Bangham.** 1990.  
531 Sequence variants of human T-cell lymphotropic virus type I from patients with

- 532 tropical spastic paraparesis and adult T-cell leukemia do not distinguish neurological  
533 from leukemic isolates. *J. Virol.* **64**:1278-1282.
- 534 12. **Fang, J., S. Kushida, R. Feng, M. Tanaka, T. Kawamura, H. Abe, N. Maeda, M.**  
535 **Onobori, M. Hori, K. Uchida, and M. Miwa.** 1998. Transmission of human T-cell  
536 leukemia virus type 1 to mice. *J. Virol.* **72**:3952-3957.
- 537 13. **Finzi, D., M. Hermankova, T. Pierson, L. M. Carruth, C. Buck, R. E. Chaisson,**  
538 **T. C. Quinn, K. Chadwick, J. Margolick, R. Brookmeyer, J. Gallant, M.**  
539 **Markowitz, D. D. Ho, D. D. Richman, and R. F. Siliciano.** 1997. Identification of a  
540 reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science*  
541 **278**:1295-1300.
- 542 14. **Fornerod, M., M. Ohno, M. Yoshida, and I. W. Mattaj.** 1997. CRM1 is an export  
543 receptor for leucine-rich nuclear export signals. *Cell* **90**:1051-1060.
- 544 15. **Fornerod, M., J. van Deursen, S. van Baal, A. Reynolds, D. Davis, K. G. Murti, J.**  
545 **Fransen, and G. Grosveld.** 1997. The human homologue of yeast CRM1 is in a  
546 dynamic subcomplex with CAN/Nup214 and a novel nuclear pore component Nup88.  
547 *EMBO J.* **16**:807-816.
- 548 16. **Gallouzi, I. E., and J. A. Steitz.** 2001. Delineation of mRNA export pathways by the  
549 use of cell-permeable peptides. *Science* **294**:1895-1901.
- 550 17. **Gessain, A., F. Barin, J. C. Vernant, O. Gout, L. Maurs, A. Calender, and G. de**  
551 **The.** 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical  
552 spastic paraparesis. *Lancet* **2**:407-410.
- 553 18. **Haase, A. T., K. Henry, M. Zupancic, G. Sedgewick, R. A. Faust, H. Melroe, W.**  
554 **Cavert, K. Gebhard, K. Staskus, Z. Q. Zhang, P. J. Dailey, H. H. Balfour, Jr., A.**  
555 **Erice, and A. S. Perelson.** 1996. Quantitative image analysis of HIV-1 infection in  
556 lymphoid tissue. *Science* **274**:985-989.
- 557 19. **Hakata, Y., T. Umemoto, S. Matsushita, and H. Shida.** 1998. Involvement of  
558 human CRM1 (exportin 1) in the export and multimerization of the Rex protein of  
559 human T-cell leukemia virus type 1. *J. Virol.* **72**:6602-7.

- 560 20. **Hakata, Y., M. Yamada, N. MAbuchi, and H. Shida.** 2002. The carboxy-terminal  
561 region of the human immunodeficiency virus type 1 protein Rev has multiple roles in  
562 mediating CRM1-related Rev functions. *J. Virol.* **76**:8079-89.
- 563 21. **Hakata, Y., M. Yamada, and H. Shida.** 2003. A multifunctional domain in human  
564 CRM1 (exportin 1) mediates RanBP3 binding and multimerization of human T-cell  
565 leukemia virus type 1 Rex protein. *Mol. Cell. Biol.* **23**:8751-8761.
- 566 22. **Hakata, Y., M. Yamada, and H. Shida.** 2001. Rat CRM1 is responsible for the poor  
567 activity of human T-cell leukemia virus type 1 Rex protein in rat cells. *J. Virol.*  
568 **75**:11515-1525.
- 569 23. **Hall, W. W., C. R. Liu, O. Schneewind, H. Takahashi, M. H. Kaplan, G. Roupe,**  
570 **and A. Vahlne.** 1991. Deleted HTLV-I provirus in blood and cutaneous lesions of  
571 patients with mycosis fungoides. *Science* **253**:317-320.
- 572 24. **Hamilton, T. A., Y. Ohmori, and J. Tebo.** 2002. Regulation of chemokine  
573 expression by antiinflammatory cytokines. *Immunol. Res.* **25**:229-245.
- 574 25. **Hanabuchi, S., T. Ohashi, Y. Koya, H. Kato, A. Hasegawa, F. Takemura, T.**  
575 **Masuda, and M. Kannagi.** 2001. Regression of human T-cell leukemia virus type I  
576 (HTLV-I)-associated lymphomas in a rat model: peptide-induced T-cell immunity. *J.*  
577 *Natl. Cancer Inst.* **93**:1775-1783.
- 578 26. **Hidaka, M., J. Inoue, M. Yoshida, and M. Seiki.** 1988. Post-transcriptional  
579 regulator (rex) of HTLV-1 initiates expression of viral structural proteins but  
580 suppresses expression of regulatory proteins. *EMBO J.* **7**:519-523.
- 581 27. **Hinuma, Y., K. Nagata, M. Hanaoka, M. Nakai, T. Matsumoto, K. I. Kinoshita, S.**  
582 **Shirakawa, and I. Miyoshi.** 1981. Adult T-cell leukemia: antigen in an ATL cell line  
583 and detection of antibodies to the antigen in human sera. *Proc. Natl. Acad. Sci. USA*  
584 **78**:6476-6480.
- 585 28. **Holaska, J. M., and B. M. Paschal.** 1998. A cytosolic activity distinct from CRM1  
586 mediates nuclear export of protein kinase inhibitor in permeabilized cells. *Proc. Natl.*  
587 *Acad. Sci. USA* **95**:14739-14744.

- 588 29. **Hoshino, H., H. Tanaka, K. Shimotohno, M. Miwa, M. Nagai, M. Shimoyama,**  
589 **and T. Sugimura.** 1984. Immortalization of peripheral blood lymphocytes of cats by  
590 human T-cell leukemia virus. *Int. J. Cancer* **34**:513-517.
- 591 30. **Inoue, J., M. Yoshida, and M. Seiki.** 1987. Transcriptional (p40x) and post-  
592 transcriptional (p27x-III) regulators are required for the expression and replication of  
593 human T-cell leukemia virus type I genes. *Proc. Natl. Acad. Sci. USA* **84**:3653-3657.
- 594 31. **Ishiguro, N., Mabe, K. Seto, H. Sakurai, H. Ikeda, A. Wakisaka, T. Togashi, M.**  
595 **Tateno, and T. Yoshiki.** 1992. A rat model of human T lymphocyte virus type I  
596 (HTLV-I) infection. 1. Humoral antibody response, provirus integration, and HTLV-I-  
597 associated myelopathy/tropical spastic paraparesis-like myelopathy in seronegative  
598 HTLV-I carrier rats. *J. Exp. Med.* **176**:981-989.
- 599 32. **Kannagi, M., S. Matsushita, and S. Harada.** 1993. Expression of the target antigen  
600 for cytotoxic T lymphocytes on adult T- cell-leukemia cells. *Int. J. Cancer* **54**:582-588.
- 601 33. **Kannagi, M., K. Sugamura, K. Kinoshita, H. Uchino, and Y. Hinuma.** 1984.  
602 Specific cytolysis of fresh tumor cells by an autologous killer T cell line derived from  
603 an adult T cell leukemia/lymphoma patient. *J. Immunol.* **133**:1037-1041.
- 604 34. **Kim, F. J., A. A. Beeche, J. J. Hunter, D. J. Chin, and T. J. Hope.** 1996.  
605 Characterization of the nuclear export signal of human T-cell lymphotropic virus type  
606 1 Rex reveals that nuclear export is mediated by position-variable hydrophobic  
607 interactions. *Mol. Cell. Biol.* **16**:5147-5155.
- 608 35. **Kinoshita, T., A. Tsujimoto, and K. Shimotohno.** 1991. Sequence variations in  
609 LTR and env regions of HTLV-I do not discriminate between the virus from patients  
610 with HTLV-I-associated myelopathy and adult T-cell leukemia. *Int. J. Cancer* **47**:491-  
611 495.
- 612 36. **Koya, Y., T. Ohashi, H. Kato, S. Hanabuchi, T. Tsukahara, F. Takemura, K.**  
613 **Etoh, M. Matsuoka, M. Fujii, and M. Kannagi.** 1999. Establishment of a  
614 seronegative human T-cell leukemia virus type 1 (HTLV-1) carrier state in rats  
615 inoculated with a syngeneic HTLV-1-immortalized T-cell line preferentially  
616 expressing Tax. *J. Virol.* **73**:6436-6443.

- 617 37. **Kudo, N., S. Khochbin, K. Nishi, K. Kitano, M. Yanagida, M. Yoshida, and S.**  
618 **Horinouchi.** 1997. Molecular cloning and cell cycle-dependent expression of  
619 mammalian CRM1, a protein involved in nuclear export of proteins. *J. Biol. Chem.*  
620 *272:29742-29751.*
- 621 38. **Kurihara, K., N. Harashima, S. Hanabuchi, M. Masuda, A. Utsunomiya, R.**  
622 **Tanosaki, M. Tomonaga, T. Ohashi, A. Hasegawa, T. Masuda, J. Okamura. Y.**  
623 **Tanaka, and M. Kannagi.** 2005. Potential immunogenicity of adult T cell leukemia  
624 cells in vivo. *Int. J. Cancer* **114:257-267.**
- 625 39. **Kushida, S., H. Mizusawa, M. Matsumura, H. Tanaka, Y. Ami, M. Hori, K.**  
626 **Yagami, T. Kameyama, Y. Tanaka, A. Yoshida, H. Nyunoya, K. Shimotohno, Y.**  
627 **Iwasaki, K. Uchida, and M. Miwa.** 1994. High incidence of HAM/TSP-like  
628 symptoms in WKA rats after administration of human T-cell leukemia virus type 1-  
629 producing cells. *J. Virol.* **68:7221-7226.**
- 630 40. **LaGrenade, L., B. Hanchard, V. Fletcher, B. Cranston, and W. Blattner.** 1990.  
631 Infective dermatitis of Jamaican children: a marker for HTLV-I infection. *Lancet*  
632 **336:1345-1347.**
- 633 41. **Lindsay, M. E., J. M. Holaska, K. Welch, B. M. Paschal, and I. G. Macara.** 2001.  
634 Ran-binding protein 3 is a cofactor for CRM1-mediated nuclear protein export. *J. Cell*  
635 *Biol.* **153:1391-1402.**
- 636 42. **Mann, D. L., P. DeSantis, G. Mark, A. Pfeifer, M. Newman, N. Gibbs, M.**  
637 **Popovic, M. G. Sarngadharan, R. C. Gallo, J. Clark, W. Blattner.** 1987. HTLV-I--  
638 associated B-cell CLL: indirect role for retrovirus in leukemogenesis. *Science*  
639 **236:1103-1106.**
- 640 43. **Martinez-Martinez, S., P. Gomez del Arco, A. L. Armesilla, J. Aramburu, C. Luo,**  
641 **A. Rao, and J. M. Redondo.** 1997. Blockade of T-cell activation by dithiocarbamates  
642 involves novel mechanisms of inhibition of nuclear factor of activated T cells. *Mol.*  
643 *Cell. Biol.* **17:6437-6447.**
- 644 44. **Miyoshi, I., I. Kubonishi, S. Yoshimoto, T. Akagi, Y. Ohtsuki, Y. Shiraishi, K.**  
645 **Nagata, and Y. Hinuma.** 1981. Type C virus particles in a cord T-cell line derived by



- 646 co-cultivating normal human cord leukocytes and human leukaemic T cells. *Nature*  
647 **294:770-771.**
- 648 45. Nakamura, H., M. Hayami, Y. Ohta, K. Ishikawa, H. Tsujimoto, T. Kiyokawa,  
649 M. Yoshida, A. Sasagawa, and S. Honjo. 1987. Protection of cynomolgus monkeys  
650 against infection by human T-cell leukemia virus type-I by immunization with viral  
651 env gene products produced in *Escherichia coli*. *Int. J. Cancer* **40:403-407.**
- 652 46. Nakamura, H., Y. Tanaka, A. Komuro-Tsujimoto, K. Ishikawa, K. Takadaya, H.  
653 Tozawa, H. Tsujimoto, S. Honjo, and M. Hayami. 1986. Experimental inoculation  
654 of monkeys with autologous lymphoid cell lines immortalized by and producing  
655 human T-cell leukemia virus type-I. *Int. J. Cancer* **38:867-875.**
- 656 47. Nemergut, M. E., M. E. Lindsay, A. M. Brownawell, and I. G. Macara. 2002.  
657 Ran-binding protein 3 links CRM1 to the Ran guanine nucleotide exchange factor. *J.*  
658 *Biol. Chem.* **277:17385-17388.**
- 659 48. Nishioka, K., I. Maruyama, K. Sato, I. Kitajima, Y. Nakajima, and M. Osame.  
660 1989. Chronic inflammatory arthropathy associated with HTLV-I. *Lancet* **1:441.**
- 661 49. Nishizuka, Y. 1984. The role of protein kinase C in cell surface signal transduction  
662 and tumour promotion. *Nature* **308:693-698.**
- 663 50. Nomura, M., T. Ohashi, K. Nishikawa, H. Nishitsuji, K. Kurihara, A. Hasegawa,  
664 R. A. Furuta, J. Fujisawa, Y. Tanaka, S. Hanabuchi, N. Harashima, T. Masuda,  
665 and M. Kannagi. 2004. Repression of tax expression is associated both with  
666 resistance of human T-cell leukemia virus type 1-infected T cells to killing by tax-  
667 specific cytotoxic T lymphocytes and with impaired tumorigenicity in a rat model. *J.*  
668 *Virol.* **78:3827-3836.**
- 669 51. Ohashi, T., S. Hanabuchi, H. Kato, Y. Koya, F. Takemura, K. Hirokawa, T.  
670 Yoshiki, Y. Tanaka, M. Fujii, and M. Kannagi. 1999. Induction of adult T-cell  
671 leukemia-like lymphoproliferative disease and its inhibition by adoptive  
672 immunotherapy in T-cell-deficient nude rats inoculated with syngeneic human T-cell  
673 leukemia virus type 1-immortalized cells. *J. Virol.* **73:6031-6040.**

- 674 52. **Ohashi, T., S. Hanabuchi, H. Kato, H. Tateno, F. Takemura, T. Tsukahara, Y.**  
675 **Koya, A. Hasegawa, T. Masuda, and M. Kannagi.** 2000. Prevention of adult T-cell  
676 leukemia-like lymphoproliferative disease in rats by adoptively transferred T cells  
677 from a donor immunized with human T-cell leukemia virus type 1 Tax-coding DNA  
678 vaccine. *J. Virol.* **74**:9610-9616.
- 679 53. **Oka, T., H. Sonobe, J. Iwata, I. Kubonishi, H. Satoh, M. Takata, Y. Tanaka, M.**  
680 **Tateno, H. Tozawa, S. Mori, T. Yoshiki and Y. Ohtsuki.** 1992. Phenotypic  
681 progression of a rat lymphoid cell line immortalized by human T-lymphotropic virus  
682 type I to induce lymphoma/leukemia-like disease in rats. *J. Virol.* **66**:6686-6694.
- 683 54. **Osame, M., K. Usuku, S. Izumo, N. Ijichi, H. Amitani, A. Igata, M. Matsumoto,**  
684 **and M. Tara.** 1986. HTLV-I associated myelopathy, a new clinical entity. *Lancet*  
685 **1**:1031-1032.
- 686 55. **Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C.**  
687 **Gallo.** 1980. Detection and isolation of type C retrovirus particles from fresh and  
688 cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad.*  
689 *Sci. USA* **77**:7415-7419.
- 690 56. **Raghavan, A., R. L. Robison, J. McNabb, C. R. Miller, D. A. Williams, and P. R.**  
691 **Bohjanen.** 2001. HuA and tristetraprolin are induced following T cell activation and  
692 display distinct but overlapping RNA binding specificities. *J. Biol. Chem.* **276**:47958-  
693 47965.
- 694 57. **Rimsky, L., J. Hauber, M. Dukovich, M. H. Malim, A. Langlois, B. R. Cullen,**  
695 **and W. C. Greene.** 1988. Functional replacement of the HIV-1 rev protein by the  
696 HTLV-1 rex protein. *Nature* **335**:738-740.
- 697 58. **Simpson, R. M., T. M. Zhao, B. S. Hubbard, S. Sawasdikosol, and T. J. Kindt.**  
698 1996. Experimental acute adult T cell leukemia-lymphoma is associated with thymic  
699 atrophy in human T cell leukemia virus type I infection. *Lab. Invest.* **74**:696-710.
- 700 59. **Stade, K., C. S. Ford, C. Guthrie, and K. Weis.** 1997. Exportin 1 (CRM1p) is an  
701 essential nuclear export factor. *Cell* **90**:1041-1050.

- 702 60. **Stein, G. M., U. Pfeller, M. Schietzel, and A. Bussing.** 2000. Expression of  
703 interleukin-4 in apoptotic cells: stimulation of the type-2 cytokine by different toxins  
704 in human peripheral blood mononuclear and tumor cells. *Cytometry* **41**:261-270.
- 705 61. **Stevenson, M., T. L. Stanwick, M. P. Dempsey, and C. A. Lamonica.** 1990. HIV-1  
706 replication is controlled at the level of T cell activation and proviral integration.  
707 *EMBO J* **9**:1551-1560.
- 708 62. **Taguchi, H., T. Sawada, A. Fukushima, J. Iwata, Y. Ohtsuki, H. Ueno, and I.**  
709 **Miyoshi.** 1993. Bilateral uveitis in a rabbit experimentally infected with human T-  
710 lymphotropic virus type I. *Lab. Invest.* **69**:336-339.
- 711 63. **Tanaka, Y., A. Yoshida, H. Tozawa, H. Shida, H. Nyunoya, and K. Shinotohno.**  
712 1991. Production of a recombinant human T-cell leukemia virus type-I trans-activator  
713 (tax1) antigen and its utilization for generation of monoclonal antibodies against  
714 various epitopes on the tax1 antigen. *Int. J. Cancer* **48**:623-630.
- 715 64. **Tateno, M., N. Kondo, T. Itoh, T. Chubachi, T. Togashi, and T. Yoshiki.** 1984.  
716 Rat lymphoid cell lines with human T cell leukemia virus production. I. Biological  
717 and serological characterization. *J. Exp. Med.* **159**:1105-1116.
- 718 65. **Wang, G., R. Miskimins, and W. K. Miskimins.** 2000. Mimosine arrests cells in G1  
719 by enhancing the levels of p27 (Kip1). *Exp. Cell Res.* **254**:64-71.
- 720 66. **Werlen, G., E. Jacinto, Y. Xia, and M. Karin.** 1998. Calcineurin preferentially  
721 synergizes with PKC-theta to activate JNK and IL-2 promoter in T lymphocytes.  
722 *EMBO J.* **17**:3101-3111.
- 723 67. **Yoshida, M., M. Osame, K. Usuku, M. Matsumoto, and A. Igata.** 1987. Viruses  
724 detected in HTLV-I-associated myelopathy and adult T-cell leukaemia are identical on  
725 DNA blotting. *Lancet* **1**:1085-1086.
- 726 68. **Yoshida, M., T. Suzuki, J. Fujisawa, and H. Hirai.** 1995. HTLV-1 oncoprotein tax  
727 and cellular transcription factors. *Curr. Top. Microbiol. Immunol.* **193**:79-89.
- 728 69. **Zhang, X., Y. Hakata, Y. Tanaka, and H. Shida.** 2006. CRM1, an RNA transporter,  
729 is a major species-specific restriction factor of human T cell leukemia virus type 1  
730 (HTLV-1) in rat cells. *Microbes Infect.* **8**:851-859.



732 **FIGURE LEGENDS**

733

734 **Figure 1. Expression of CRM1 during activation of PBMCs.** (A) PBMCs isolated from donor  
735 1 and donor 2 were activated with ionophore, PMA, and IL-2 and analyzed by Western  
736 blotting. (B) PBMCs isolated from donor 1 (□) and donor 3 (◇) were activated with  
737 ionophore, PMA, and IL-2 and analyzed by quantitative RT-PCR. Each value is the average  
738 of duplicate measurements. (C) PBMCs isolated from donor 2 were activated with various  
739 combinations of ionophore, PMA, and IL-2 and analyzed by Western blotting. (D) PBMCs  
740 isolated from donor 2 were activated in the presence of various inhibitors and analyzed by  
741 Western blotting.

742

743 **Figure 2. Time course of CRM1 induction during activation of CD4<sup>+</sup> T cells.** (A) CD4<sup>+</sup> T  
744 cells isolated from donor 1 and donor 4 were activated with ionophore, PMA, and IL-2 and  
745 analyzed by Western blotting. (B) Time course of CRM1 mRNA induction during activation  
746 of CD4<sup>+</sup> T cells. CD4<sup>+</sup> T cells isolated from donor 1 (□) and donor 4 (◇) were activated  
747 with ionophore, PMA, and IL-2 and analyzed by quantitative RT-PCR. Each value is the  
748 average of duplicate measurements.

749

750 **Figure 3. Tissue distribution of human and rat CRM1 in hCRM1 Tg rats.** (A) Immunoblot  
751 assays showing the relative levels of h and rCRM1 in rat tissues. Each protein level was  
752 determined on immunoblots containing 10 µg of total protein per lane. An FCMT18 cell  
753 extract was used as a positive control. (B) Relative levels of h/rCRM1 expression among  
754 organs are shown. Protein expression was quantified by ImageGauge software and relative  
755 values are normalized to the amount of actin.

756

757 **Figure 4. Expression of HTLV-1 Gag and hCRM1 in the cell lines immortalized with HTLV-**  
758 **1.** (A) Detection of the hCRM1 transgene in the cell lines by PCR. DNA extracted from each  
759 cell line (100 ng) was subjected to PCR with the primers for hCRM1 and for G3PDH as an

760 internal control. (B) Protein expression of hCRM1 was detected by immunoblotting. Samples  
 761 (10 µg of total protein per each lane) were subjected to SDS-PAGE. HeLa cell extract was  
 762 used as a positive control. (C) HTLV-1 Gag protein in the supernatant of 2 day and 4 day  
 763 cultures was quantified by HTLV-1 p19 ELISA. The results are shown as the mean of three  
 764 independent experiments. (D) Based on the data of (C), the average p19 Gag production of Tg  
 765 and Wt cell lines was calculated. (E) The amount of intracellular Gag in each cell line was  
 766 analyzed by flow cytometry. Open histograms indicate the cells stained with anti p19/p55  
 767 Gag MAbs. Solid histograms represent the cells stained with control mouse IgG. (F) Growth  
 768 rate of Wt or Tg cell lines was measured. In parallel with the experiments described in Figure  
 769 4(C), the growth rate was monitored by the cell counting Kit-8 (Dojinndo Laboratories). The  
 770 relative cell numbers of 2 or 4 day vs. 0 day cultures are shown.

771

772 Figure 5. Viral states in HTLV-1-transformed T cells derived from Tg and wild type rats. (A)  
 773 Proviral load of each cell line was measured by quantitative real time PCR. The copy number  
 774 of HTLV-1 provirus was normalized by dividing with the G3PDH copy number in the same  
 775 sample. (B) Production of intracellular Tax in each cell line was analyzed by flow cytometry.  
 776 Solid histograms indicate the cells stained with anti-Tax MAb. Open histograms represent the  
 777 cells stained with control mouse IgG. (C) Rex expression of each cell line was detected by  
 778 immunoblotting. Ten µg of total protein per each lane was subjected to SDS-PAGE. Lower  
 779 bands in FCMS1 and TCMS18 samples represent p21 Rex.

780

781 Figure 6. Dissemination of HTLV-1 in hCRM1 Tg rats. (A) Mean plasma p19 concentration  
 782 in Wt (n=9; ●) or hCRM1 (n=7; ■) Tg rats after intraperitoneal inoculation of mitomycin  
 783 C-treated MT2 cells ( $1 \times 10^7$  per animal). (B) Mean plasma p19 concentration in Wt (n=16;  
 784 □) or hCRM1-Tg (n=17; ■) rats 1 week after intraperitoneal inoculation of MT2 cells  
 785 ( $5 \times 10^6$  per animal). (C) Detection of HTLV-1 provirus in thymus derived from rats used in  
 786 (B). Presence of HTLV-1 provirus was analyzed by nested PCR. Results are mean percentage  
 787 of HTLV-1 provirus positive rats in 5 independent experiments. (D and E) HTLV-1 proviral  
 788 loads of rats used in (B). HTLV-1 proviral loads in peripheral blood cells (D) or thymus (E)

789 were quantified by real-time PCR. The relative copy numbers of HTLV-1 provirus per  $2 \times 10^7$   
790 copies of G3PDH are shown. Results are expressed as mean + standard deviation. The  
791 statistical significance of differences was determined with the Student's t-test, using  
792 Microsoft Excel 2004 for Mac software, as indicated in Figure 6B-E.

793 Table 1. Constructed cell lines and surface markers

794

795

796	Cell lines	hCRM1	Surface markers							
			CD3	CD4	CD5	CD8	CD25	MHC1	MHC2	
797	FWT1	-	+	+	+	-	+	+	+	
798	FWS1-11	-	-	-	+	-	+	+	+	
799	FWS1-27	-	-	-	+	-	+	+	+	
800	FWS1-31	-	-	-	+	(+)	+	+	-	
801	FWS1-34	-	-	-	+	-	+	+	-	
802	FWT11	-	+	-	+	-	+	+	+	
803	FCMS1	+	+	+	+	-	+	+	(+)	
804	FCMT1	+	+	+	+	-	+	+	+	
805	FCMT18	+	+	+	-	-	+	+	-	
806	FCMS18	+	+	+	(+)	-	+	+	(+)	
807	FCMT27	+	+	+	(+)	-	+	+	(+)	
808	FCCT13-1	+	-	-	(+)	-	+	+	+	
809	FCCT13-2	+	+	-	(+)	(+)	+	+	+	
810	FCCS13-1	+	-	-	-	-	+	+	+	
811	FCCS13-2	+	-	(+)	(+)	-	+	+	-	
812	+:positive (+):weakly positive -negative									



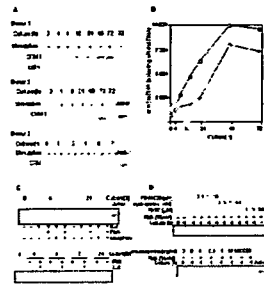


Figure 1

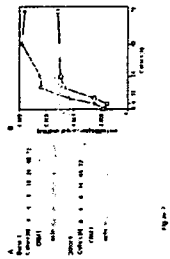


Figure 2

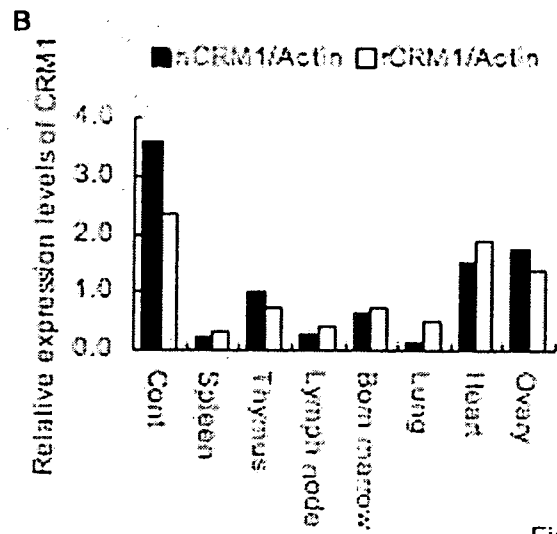
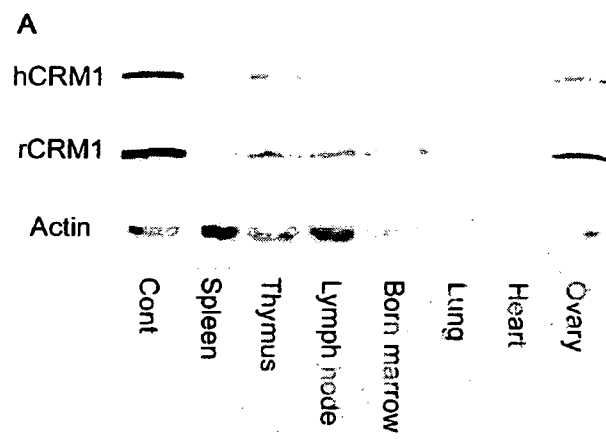


Fig.3



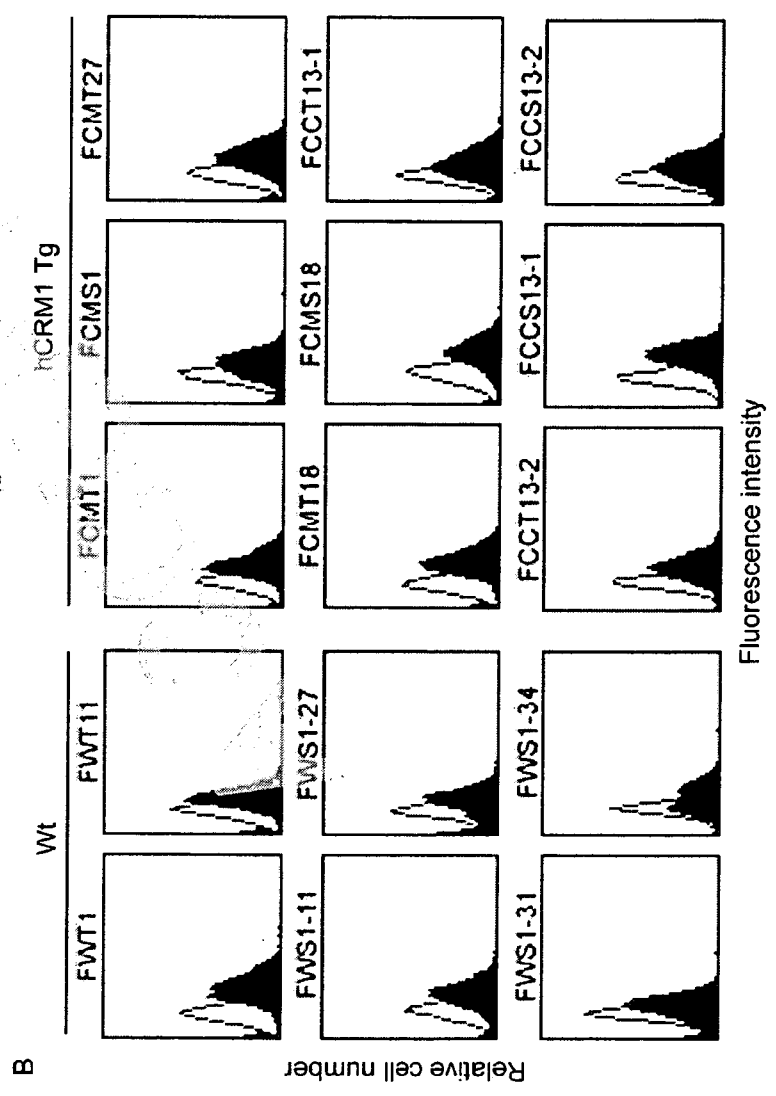
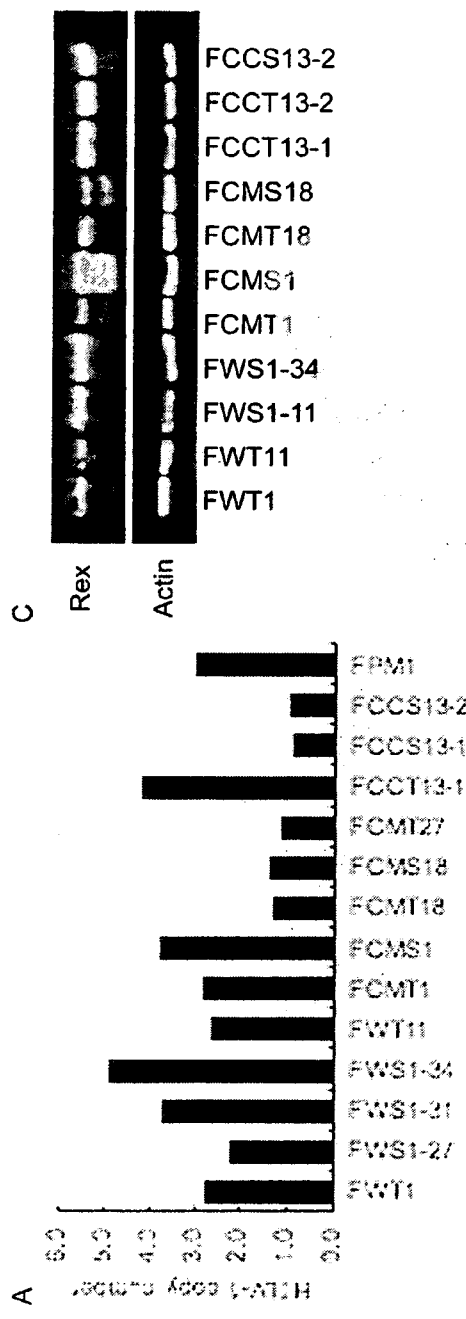


Fig.5