The ups and downs of Tax and histones in adult T-cell leukemogenesis

Paul Laybourn

Department of Biochemistry
& Molecular Biology,
1870 Campus Delivery,
Colorado State University,
Fort Collins,
CO 80523-1870, USA
Tel.: +1 970 491 5100;
Fax: +1 970 491 0494;
paul.laybourn@colostate.edu

'HTLV-1 infection has been demonstrated to reduce core histone and linker histone protein levels by 20–40%, which would necessarily result in lower nucleosome density and produce similar effects as global loss of imprinting (DNA hypomethylation).'

Adult T-cell leukemia/lymphoma

Adult T-cell leukemia/lymphoma (ATLL) is a malignant cancer caused by human T-cell leukemia/lymphotropic virus Type 1 (HTLV-1) infection [1,2]. HTLV-1, more generally referred to as PTLV-1 (primate T-cell lymphotropic virus 1), is a member of the *Deltaretrovirus* genus (family *Retroviridae*) and infects 10–20 million people throughout the world [3]. The majority of those infected are concentrated in central Africa, Iran, Southeast Asia, southwestern Japan, the Caribbean region and western South America, but HTLV-1 is spreading into human populations of the USA and Western Europe. HTLV-1 is a blood-borne virus transmitted through sexual intercourse, breast-feeding, blood transfusion and needle reuse, the latter route accounting for most of the transmission in the USA and Western Europe [4]. ATLL develops in 2–7% of those infected, and develops primarily in those infected in infancy after a rather long latency (mean age of development: 45 years in Central and South America, 60 years in Japan) [5,6]. While the likelihood of infected individuals developing ATLL is relatively low, the average life expectancy of those progressing to ATLL is 6 months. An approximately equal proportion of HTLV-1-infected individuals develop the severe neurological disorder tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM), which has symptoms similar to amyotrophic lateral sclerosis (ALS) [4,7]. This editorial will focus on the molecular mechanisms of HTLV-1 leukemogenesis.

Human T-cell leukemia/lymphotropic virus Type 1

HTLV-1 is a complex retrovirus encoding six accessory proteins in addition to the *gag*, *pro*, *pol*

and *env* genes found in all retroviruses. This virus was initially isolated in 1980 and was the first human retrovirus identified [1,8–10]. HTLV-1 infects and transforms CD4⁺ T cells, although it can infect a broad range of cell types. In contrast to HIV-1, HTLV-1 infection of new human hosts requires transfer of whole infected cells, as few or no cell-free virions are found and infectivity of cell-free virions is very low [11–14]. Based on this virus' poor infectivity and the low degree of coding region sequence drift between virus isolates, host cell proliferation provides the primary means for HTLV-1 propagation in the provirus form [15,16]. Thus, this virus benefits significantly by T-cell transformation and immortalization. HTLV-1 is rather unique among oncogenic retroviruses in that it carries no oncogene captured from a host cell genome. In addition, host cell transformation does not correlate with integration of the provirus in or near particular cellular proto-oncogene loci [17]. Rather, transformation is mediated by the accessory gene products encoded within the so-called pX region located between the *env* gene and the 3' long terminal repeat (LTR) region.

HTLV-1 accessory gene products & their functions

The pX region of the HTLV-1 genome consists of four overlapping open reading frames (ORFI–IV) in transcripts initiated from the 5' LTR [5,18,19]. ORFI encodes p12^I, a protein that localizes to the endoplasmic reticulum and Golgi and causes increased cytoplasmic Ca²⁺ levels, likely functioning to promote cell survival and proliferation through intracellular signaling [20]. The p12^I protein also seems to reduce MHC expression, helping in the evasion of immunosurveillance [21]. ORFII encodes p13^{II} and p30^{II} proteins [22–25]. The former localizes to the mitochondrial inner membrane and affects K⁺ permeability and Ca²⁺ uptake, participating in elevation of cytoplasmic Ca²⁺ levels. The latter affects CBP/p300 functions, as well as Tax and Rex mRNA translation. ORFIII encodes p21^{III} and Rex. Rex binds the Rex response element (RxRE), a nucleotide sequence located in the 3' untranslated region (UTR) of the viral RNA, and functions to suppress splicing and promote nuclear export of the unspliced viral

RNAs [26–28]. The p21^{III} protein function is not known. ORFIV encodes the Tax protein, which functions to promote cell proliferation (passage through G1 into S phase), to interfere with cell-cycle checkpoints, to increase genomic instability, and to suppress apoptosis [5,29–31]. One viral gene, *hbx*, is transcribed from a 3′ LTR promoter back across the pX region and encodes both a protein and an RNA product [32–34]. The HBZ protein inhibits Tax transcriptional transactivation, whereas the HBZ RNA causes increased E2F1 transcription factor expression to promote cell proliferation [35,36].

Tax protein expression is necessary and sufficient for primary T-cell transformation and tumor formation in transgenic mice [37–42]. However, results from studies in animal model systems indicate that the other five accessory proteins are important for efficient viral infection *in vivo* [43–45]. Furthermore, Tax expression is lost in a significant proportion (>50%) of ATLL cell isolates, whereas HBZ expression has been observed in all ATLL cells analyzed thus far [5,19,46]. Cytotoxic T-cell elimination of HTLV-1 infected cells is primarily directed against Tax protein epitopes, thus selecting for infected cells that have lost Tax expression [47]. This expression pattern suggests that Tax function is required in the early stages of T-cell transformation, but is not required for maintaining the transformed state.

The long latency between HTLV-1 infection and ATLL development indicates that several additional changes must accumulate. The effects of Tax and the other accessory proteins on cellular homeostasis promote these changes. One could also speculate that some of these changes function to maintain the transformed state when Tax expression is reduced or eliminated late in ATLL development.

Effects of Tax protein on T cells

Tax is essential for provirus transcription and expression of the structural and accessory proteins. In addition, Tax suppresses virus infection-induced apoptosis by binding PI3 kinase and stimulating AKT (PKB) phosphorylation by PDK-1 [48,49]. Tax also promotes cell survival through binding IKK γ , leading to increased IKK $\alpha/\beta/\gamma$ complex phosphorylation [50,51]. This results in I κ B α destabilization and NF- κ B p100 processing to its p52 nuclear-localized, active form. Tax enhances CREB phosphorylation in T-cells, which, along with NF- κ B activation, has been shown to induce anti-apoptotic protein Bcl-X_L expression [52,53]. Tax functions to increase

the expression of IL-2 and IL-2R α , E2F, D2 and D3 cyclins, Cdk6, E cyclin and Cdk2 either directly or indirectly [54–58]. Tax also binds the cyclin D–Cdk4/6 complexes directly to activate them. Tax activates p21 (Waf1/CIP1) expression, which also binds and activates cyclin D2–Cdk4 complexes, and Tax binds and inactivates other cyclin-dependent kinase inhibitors (p15 [INK4b], p16 [INK4a] and p18 [INK4c]) [59–61]. The Tax protein stimulates DLL-1 phosphorylation, leading to constitutive Wnt pathway signaling [62,63]. Through these effects, Tax promotes cell-cycle progression, bypasses cell-cycle checkpoints and suppresses apoptosis.

Tax inhibits Tax1BP2 and RanBP1, leading to formation of multiple spindle pole bodies, and binds MAD1, interfering with normal spindle assembly checkpoint function [64,65]. Tax activates anaphase-promoting complex (APC), leading to early M-phase exit [66]. These effects result in aneuploidy in HTLV-1-infected T cells.

Tax reduces the expression of key genomic caretaker genes, including DNA polymerase- β , and increases the expression of proliferating cell nuclear antigen (PCNA), which function in base excision repair, nucleotide excision repair and mismatch repair systems [67,68]. Tax also interferes with G1 to S phase and G2 to M phase checkpoint controllers CHK1, CHK2 and p53 [69–73]. Tax inhibits telomerase (hTERT) expression and Ku80 protein function, leading to exposed chromosome ends and decreased double-strand DNA break repair [74–76]. These effects of Tax conspire to increase chromosome instability and mutation frequency, which accelerate the accumulation of cellular changes needed to produce a successful ATLL cell. At present, no proto-oncogenes or tumor suppressor genes have been identified that are consistently mutated in ATLL cells.

Epigenetic changes particular to ATLL cells have recently been investigated. Promoter DNA hypermethylation and repression of expression were seen at 54 tumor suppressor gene loci including p16 (Ink4a), Krüppel-like factor 4, early growth response 3, adenomatous polyposis coli, CD26 and PMS1; thus affecting regulation of cell-cycle progression, apoptosis and mismatch repair [77–80]. Hypomethylation of the proto-oncogene MEL1S resulting in overexpression was also observed, and is proposed to participate in suppression of the TGF- β signaling pathway, which is important for proper control of cell proliferation [81]. Due to the general experimental bias for identifying the appearance of methylation where there was none previously, few examples of

hypomethylation (also referred to as loss of imprinting [LOI]) are available for ATLL cells and for cancer cells in general [82–85]. However, examples are beginning to emerge and include aberrant chromosome rearrangements, biallelic (over) expression of cyclin D2 and the multidrug resistance gene (MDR1), and the reactivation of HPV oncogenes in cervical cancer cells [86–90].

Very recently, a new effect of Tax on T-cell homeostasis has been identified that constitutes a novel means for producing epigenetic changes [91]. HTLV-1 infection has been demonstrated to reduce core histone and linker histone protein levels by 20–40%, which would necessarily result in lower nucleosome density and produce similar effects as global LOI (DNA hypomethylation). In addition, decreased histone levels would produce nucleosome assembly defects during DNA replication and DNA double-strand break repair, contributing to increased chromosome instability [92,93]. Importantly, decreased histone levels could provide a means for HTLV-1 to counteract 5' LTR DNA methylation and transcriptional repression through chromatin structure, a common cellular antiretrovirus mechanism [94]. Initially, reduced histone levels may seem heretical. In actuality, only higher than normal histone levels have been shown to be severely problematic. In normal somatic cells, histone gene expression is closely coupled with DNA replication during the S phase [95,96]. Even a slight histone protein excess is highly deleterious to cells, and the accumulation of a small excess of histones has been shown to be highly cytotoxic and to severely compromise the transmission of chromosomal information [97–100]. By contrast, partial histone depletion has more subtle effects on cells [101].

'Upon development of immunodeficiency, selective pressure against Tax expression is lost. In T cells carrying a HTLV-1 provirus with methylated 5' LTR DNA, but otherwise wild-type sequences, viral gene expression could then return through the effects of decreased histone levels and increased Tax expression.'

Conclusions

Tax is necessary and sufficient for T-cell transformation. HBZ RNA and other accessory proteins appear to have supporting/enhancing

roles, particularly *in vivo*. The HBZ protein, p30^{II} and Rex actually function to reduce Tax expression and LTR promoter transactivation in the HTLV-1 early-phase to late-phase transition normal in retrovirus replication cycles. Furthermore, due to the selective pressure of immunosurveillance, Tax expression is often lost or suppressed in ATLL cells due to mutations in the Tax coding sequence, or through deletion or DNA methylation in the 5' LTR promoter. Thus, Tax could be considered to be essential early in ATLL cell development functioning to promote the accumulation of cellular changes required in addition to those induced by Tax, and to replace those induced by Tax directly when Tax expression is lost. Immunodeficiency often accompanies ATLL, and administration of immunosuppressive drugs has been found to accelerate ATLL development [102–104]. One could speculate that during the latency period, T cells carrying functional HTLV-1 proviruses are transcriptionally suppressed through a combination of promoter DNA methylation and HBZ, p30^{II} and Rex function. Upon development of immunodeficiency, selective pressure against Tax expression is lost. In T cells carrying a HTLV-1 provirus with methylated 5' LTR DNA, but otherwise wild-type sequences, viral gene expression could then return through the effects of decreased histone levels and increased Tax expression through a positive feedback mechanism.

Future perspective

The finding that Tax expression results in reduced histone levels raises two key questions that need to be investigated. First, what are the effects of reduced histone levels, when isolated from the many other effects of Tax, on HTLV-1 and cellular gene transcription and genomic stability? And second, through what mechanism does Tax reduce histone levels?

Financial & competing interests disclosure

Research described from the PJJ laboratory was supported by grants from the Colorado Cancer League and the American Heart Association awarded to PJJ. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Adult T-cell leukemia/lymphoma (ATLL) is a malignant cancer caused by human T-cell leukemia/lymphotropic virus Type 1 (HTLV-1) infection.
- Transformation is mediated by the accessory gene products encoded within the so-called pX region located between the *env* gene and the 3' long terminal repeat (LTR).
- Tax protein expression is necessary and sufficient for primary T-cell transformation. However, the other five accessory proteins are important for efficient viral infection *in vivo*.
- Tax promotes cell-cycle progression, bypasses cell-cycle checkpoints and apoptotic activation, promotes aneuploidy and increases chromosome instability and mutation frequency to accelerate the accumulation of cellular changes needed to produce a successful ATLL cell.
- Epigenetic changes (promoter DNA hypermethylation and repression of expression) particular to ATLL cells were seen at 54 tumor suppressor gene loci.
- HTLV-1 infection has been demonstrated to reduce core histone and linker histone protein levels by 20–40%.
- Tax could be considered to be essential early in ATLL cell development, functioning to promote the accumulation of cellular changes required in addition to those induced by Tax and as replacement for those induced by Tax directly when Tax expression is lost.
- In T cells carrying a HTLV-1 provirus with methylated 5' LTR DNA, but otherwise wild-type sequences, viral gene expression could return upon development of immunodeficiency through the effects of decreased histone levels and increased Tax expression through a positive feedback mechanism.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Gallo RC: The discovery of the first human retrovirus: HTLV-1 and HTLV-2. *Retrovirology* 2, 17 (2005).
2. Takatsuki K: Discovery of adult T-cell leukemia. *Retrovirology* 2, 16 (2005).
3. Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, Murphy EL: Global epidemiology of HTLV-I infection and associated diseases. *Oncogene* 24, 6058–6068 (2005).
4. Verdonck K, Gonzalez E, Van Dooren S, Vandamme AM, Vanham G, Gotuzzo E: Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect. Dis.* 7, 266–281 (2007).
5. Matsuoka M, Jeang KT: Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat. Rev. Cancer* 7, 270–280 (2007).
6. Yasunaga J, Matsuoka M: Human T-cell leukemia virus type I induces adult T-cell leukemia: from clinical aspects to molecular mechanisms. *Cancer Control* 14, 133–140 (2007).
7. Bangham CR, Osame M: Cellular immune response to HTLV-1. *Oncogene* 24, 6035–6046 (2005).
8. Gallo RC: History of the discoveries of the first human retroviruses: HTLV-1 and HTLV-2. *Oncogene* 24, 5926–5930 (2005).
9. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC: Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl Acad. Sci. USA* 77, 7415–7419 (1980).
10. Yoshida M: Discovery of HTLV-1, the first human retrovirus, its unique regulatory mechanisms, and insights into pathogenesis. *Oncogene* 24, 5931–5937 (2005).
11. Bangham CR: The immune control and cell-to-cell spread of human T-lymphotropic virus type 1. *J. Gen. Virol.* 84, 3177–3189 (2003).
12. Derse D, Hill SA, Lloyd PA, Chung H, Morse BA: Examining human T-lymphotropic virus type 1 infection and replication by cell-free infection with recombinant virus vectors. *J. Virol.* 75, 8461–8468 (2001).
13. Fan N, Gavalchin J, Paul B, Wells KH, Lane MJ, Poiesz BJ: Infection of peripheral blood mononuclear cells and cell lines by cell-free human T-cell lymphoma/leukemia virus type I. *J. Clin. Microbiol.* 30, 905–910 (1992).
14. Igakura T, Stinchcombe JC, Goon PK *et al.*: Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science* 299, 1713–1716 (2003).
15. Van Dooren S, Pybus OG, Salemi M *et al.*: The low evolutionary rate of human T-cell lymphotropic virus type-1 confirmed by analysis of vertical transmission chains. *Mol. Biol. Evol.* 21, 603–611 (2004).
16. Van Dooren S, Salemi M, Vandamme AM: Dating the origin of the African human T-cell lymphotropic virus type-I (HTLV-I) subtypes. *Mol. Biol. Evol.* 18, 661–671 (2001).
17. Seiki M, Eddy R, Shows TB, Yoshida M: Nonspecific integration of the HTLV provirus genome into adult T-cell leukaemia cells. *Nature* 309, 640–642 (1984).

18. Kiyokawa T, Seiki M, Imagawa K, Shimizu F, Yoshida M: Identification of a protein (p40x) encoded by a unique sequence pX of human T-cell leukemia virus type I. *Gann* 75, 747–751 (1984).
19. Yasunaga J, Matsuoka M: Leukaemogenic mechanism of human T-cell leukaemia virus type I. *Rev. Med. Virol.* 17, 301–311 (2007).
20. Ding W, Albrecht B, Luo R *et al.*: Endoplasmic reticulum and *cis*-Golgi localization of human T-lymphotropic virus type 1 p12(I): association with calreticulin and calnexin. *J. Virol.* 75, 7672–7682 (2001).
21. Johnson JM, Nicot C, Fullen J *et al.*: Free major histocompatibility complex class I heavy chain is preferentially targeted for degradation by human T-cell leukemia/lymphotropic virus type 1 p12(I) protein. *J. Virol.* 75, 6086–6094 (2001).
22. Awasthi S, Sharma A, Wong K *et al.*: A human T-cell lymphotropic virus type 1 enhancer of Myc transforming potential stabilizes Myc-TIP60 transcriptional interactions. *Mol. Cell. Biol.* 25, 6178–6198 (2005).
23. D'Agostino DM, Ranzato L, Arrigoni G *et al.*: Mitochondrial alterations induced by the p13II protein of human T-cell leukemia virus type 1. Critical role of arginine residues. *J. Biol. Chem.* 277, 34424–34433 (2002).
24. Nicot C, Dunder M, Johnson JM *et al.*: HTLV-1-encoded p30II is a post-transcriptional negative regulator of viral replication. *Nat. Med.* 10, 197–201 (2004).
25. Zhang W, Nisbet JW, Bartoe JT, Ding W, Lairmore MD: Human T-lymphotropic virus type 1 p30(II) functions as a transcription factor and differentially modulates CREB-responsive promoters. *J. Virol.* 74, 11270–11277 (2000).
26. Inoue J, Seiki M, Yoshida M: The second pX product p27 chi-III of HTLV-1 is required for gag gene expression. *FEBS Lett.* 209, 187–190 (1986).
27. Inoue J, Yoshida M, Seiki M: Transcriptional (p40x) and post-transcriptional (p27x-III) regulators are required for the expression and replication of human T-cell leukemia virus type I genes. *Proc. Natl Acad. Sci. USA* 84, 3653–3657 (1987).
28. Younis I, Green PL: The human T-cell leukemia virus Rex protein. *Front. Biosci.* 10, 431–445 (2005).
29. Gatza ML, Watt JC, Marriott SJ: Cellular transformation by the HTLV-I Tax protein, a jack-of-all-trades. *Oncogene* 22, 5141–5149 (2003).
30. Marriott SJ, Semmes OJ: Impact of HTLV-I Tax on cell cycle progression and the cellular DNA damage repair response. *Oncogene* 24, 5986–5995 (2005).
31. Peloponese JM Jr, Kinjo T, Jeang KT: Human T-cell leukemia virus Type 1 Tax and cellular transformation. *Int. J. Hematol.* 86, 101–106 (2007).
32. Gaudray G, Gachon F, Basbous J, Biard-Piechaczyk M, Devaux C, Mesnard JM: The complementary strand of the human T-cell leukemia virus Type 1 RNA genome encodes a bZIP transcription factor that down-regulates viral transcription. *J. Virol.* 76, 12813–12822 (2002).
33. Larocca D, Chao LA, Seto MH, Brunck TK: Human T-cell leukemia virus minus strand transcription in infected T-cells. *Biochem. Biophys. Res. Commun.* 163, 1006–1013 (1989).
34. Lemasson I, Lewis MR, Polakowski N *et al.*: Human T-cell leukemia virus Type 1 (HTLV-1) bZIP protein interacts with the cellular transcription factor CREB to inhibit HTLV-1 transcription. *J. Virol.* 81, 1543–1553 (2007).
35. Basbous J, Arpin C, Gaudray G, Piechaczyk M, Devaux C, Mesnard JM: The HBZ factor of human T-cell leukemia virus Type I dimerizes with transcription factors JunB and c-Jun and modulates their transcriptional activity. *J. Biol. Chem.* 278, 43620–43627 (2003).
36. Satou Y, Yasunaga J, Yoshida M, Matsuoka M: HTLV-I basic leucine zipper factor gene mRNA supports proliferation of adult T cell leukemia cells. *Proc. Natl Acad. Sci. USA* 103, 720–725 (2006).
37. Grassmann R, Berchtold S, Radant I *et al.*: Role of human T-cell leukemia virus Type 1 X region proteins in immortalization of primary human lymphocytes in culture. *J. Virol.* 66, 4570–4575 (1992).
38. Grassmann R, Dengler C, Muller-Fleckenstein I *et al.*: Transformation to continuous growth of primary human T lymphocytes by human T-cell leukemia virus type I X-region genes transduced by a *Herpesvirus saimiri* vector. *Proc. Natl Acad. Sci. USA* 86, 3351–3355 (1989).
39. Grossman WJ, Kimata JT, Wong FH, Zutter M, Ley TJ, Ratner L: Development of leukemia in mice transgenic for the Tax gene of human T-cell leukemia virus type I. *Proc. Natl Acad. Sci. USA* 92, 1057–1061 (1995).
40. Pozzatti R, Vogel J, Jay G: The human T-lymphotropic virus type I Tax gene can cooperate with the ras oncogene to induce neoplastic transformation of cells. *Mol. Cell Biol.* 10, 413–417 (1990).
41. Smith MR, Greene WC: Type I human T cell leukemia virus Tax protein transforms rat fibroblasts through the cyclic adenosine monophosphate response element binding protein/activating transcription factor pathway. *J. Clin. Invest.* 88, 1038–1042 (1991).
42. Tanaka A, Takahashi C, Yamaoka S, Nosaka T, Maki M, Hatanaka M: Oncogenic transformation by the Tax gene of human T-cell leukemia virus type I *in vitro*. *Proc. Natl Acad. Sci. USA* 87, 1071–1075 (1990).
43. Collins ND, Newbound GC, Albrecht B, Beard JL, Ratner L, Lairmore MD: Selective ablation of human T-cell lymphotropic virus Type 1 p12I reduces viral infectivity *in vivo*. *Blood* 91, 4701–4707 (1998).
44. Silverman LR, Phipps AJ, Montgomery A, Ratner L, Lairmore MD: Human T-cell lymphotropic virus Type 1 open reading frame II-encoded p30II is required for *in vivo* replication: evidence of *in vivo* reversion. *J. Virol.* 78, 3837–3845 (2004).
45. Younis I, Yamamoto B, Phipps A, Green PL: Human T-cell leukemia virus type 1 expressing nonoverlapping Tax and rex genes replicates and immortalizes primary human T lymphocytes but fails to replicate and persist *in vivo*. *J. Virol.* 79, 14473–14481 (2005).
- Provides a clear example of the power of infectious clones for dissecting the function and mechanism of HTLV-1 infection in ATLL development.
46. Ma T, Van Tine BA, Wei Y *et al.*: Cell cycle-regulated phosphorylation of p220(NPAT) by cyclin E/Cdk2 in Cajal bodies promotes histone gene transcription. *Genes Dev.* 14, 2298–2313 (2000).
47. Kannagi M, Ohashi T, Harashima N, Hanabuchi S, Hasegawa A: Immunological risks of adult T-cell leukemia at primary HTLV-I infection. *Trends Microbiol.* 12, 346–352 (2004).
48. Ikezoe T, Nishioka C, Bandobashi K *et al.*: Longitudinal inhibition of PI3K/Akt/mTOR signaling by LY294002 and rapamycin induces growth arrest of adult T-cell leukemia cells. *Leuk. Res.* 31, 673–682 (2007).
49. Peloponese JM Jr, Jeang KT: Role for Akt/protein kinase B and activator protein-1 in cellular proliferation induced by the human T-cell leukemia virus Type 1 Tax oncoprotein. *J. Biol. Chem.* 281, 8927–8938 (2006).
50. Iha H, Kibler KV, Yedavalli VR *et al.*: Segregation of NF- κ B activation through NEMO/IKK γ by Tax and TNF α :

- implications for stimulus-specific interruption of oncogenic signaling. *Oncogene* 22, 8912–8923 (2003).
51. Xiao G, Cvijic ME, Fong A *et al.*: Retroviral oncoprotein Tax induces processing of NF- κ B2/p100 in T cells: evidence for the involvement of IKK α . *EMBO J.* 20, 6805–6815 (2001).
52. Kim YM, Ramirez JA, Mick JE, Giebler HA, Yan JP, Nyborg JK: Molecular characterization of the Tax-containing HTLV-1 enhancer complex reveals a prominent role for CREB phosphorylation in Tax transactivation. *J. Biol. Chem.* 282, 18750–18757 (2007).
- **Describes a thorough investigation demonstrating the criticality of CREB phosphorylation in Tax recruitment and transcriptional activation and the previously unknown ability of Tax to stimulate CREB phosphorylation in T cells.**
53. Mori N, Fujii M, Cheng G *et al.*: Human T-cell leukemia virus Type I Tax protein induces the expression of anti-apoptotic gene *Bcl-xL* in human T-cells through nuclear factor- κ B and c-AMP responsive element binding protein pathways. *Virus Genes* 22, 279–287 (2001).
54. Akagi T, Ono H, Shimotohno K: Expression of cell-cycle regulatory genes in HTLV-I infected T-cell lines: possible involvement of Tax1 in the altered expression of cyclin D2, p18Ink4 and p21Waf1/Cip1/Sdi1. *Oncogene* 12, 1645–1652 (1996).
55. Fraedrich K, Muller B, Grassmann R: The HTLV-1 Tax protein binding domain of cyclin-dependent kinase 4 (CDK4) includes the regulatory PSTAIRE helix. *Retrovirology* 2, 54 (2005).
56. Haller K, Wu Y, Derow E, Schmitt I, Jeang KT, Grassmann R: Physical interaction of human T-cell leukemia virus Type 1 Tax with cyclin-dependent kinase 4 stimulates the phosphorylation of retinoblastoma protein. *Mol. Cell. Biol.* 22, 3327–3338 (2002).
57. Iwanaga R, Ohtani K, Hayashi T, Nakamura M: Molecular mechanism of cell cycle progression induced by the oncogene product Tax of human T-cell leukemia virus Type I. *Oncogene* 20, 2055–2067 (2001).
58. Santiago F, Clark E, Chong S *et al.*: Transcriptional up-regulation of the cyclin D2 gene and acquisition of new cyclin-dependent kinase partners in human T-cell leukemia virus Type 1-infected cells. *J. Virol.* 73, 9917–9927 (1999).
59. Kehn K, Deng L, De La Fuente C *et al.*: The role of cyclin D2 and p21/waf1 in human T-cell leukemia virus Type 1 infected cells. *Retrovirology* 1, 6 (2004).
60. Low KG, Dorner LF, Fernando DB, Grossman J, Jeang KT, Comb MJ: Human T-cell leukemia virus Type 1 Tax releases cell cycle arrest induced by p16INK4a. *J. Virol.* 71, 1956–1962 (1997).
61. Suzuki T, Kitao S, Matsushime H, Yoshida M: HTLV-1 Tax protein interacts with cyclin-dependent kinase inhibitor p16INK4A and counteracts its inhibitory activity towards CDK4. *EMBO J.* 15, 1607–1614 (1996).
62. Hirata A, Higuchi M, Niinuma A *et al.*: PDZ domain-binding motif of human T-cell leukemia virus Type 1 Tax oncoprotein augments the transforming activity in a rat fibroblast cell line. *Virology* 318, 327–336 (2004).
63. Ishioka K, Higuchi M, Takahashi M *et al.*: Inactivation of tumor suppressor Dlg1 augments transformation of a T-cell line induced by human T-cell leukemia virus Type 1 Tax protein. *Retrovirology* 3, 71 (2006).
64. Ching YP, Chan SF, Jeang KT, Jin DY: The retroviral oncoprotein Tax targets the coiled-coil centrosomal protein TAX1BP2 to induce centrosome overduplication. *Nat. Cell Biol.* 8, 717–724 (2006).
65. Peloponese JM Jr, Haller K, Miyazato A, Jeang KT: Abnormal centrosome amplification in cells through the targeting of Ran-binding protein-1 by the human T cell leukemia virus Type-1 Tax oncoprotein. *Proc. Natl Acad. Sci. USA* 102, 18974–18979 (2005).
66. Liu B, Hong S, Tang Z, Yu H, Giam CZ: HTLV-I Tax directly binds the Cdc20-associated anaphase-promoting complex and activates it ahead of schedule. *Proc. Natl Acad. Sci. USA* 102, 63–68 (2005).
67. Jeang KT, Widen SG, Semmes OJ, Wilson SH: HTLV-I *trans*-activator protein, Tax, is a *trans*-repressor of the human β -polymerase gene. *Science* 247, 1082–1084 (1990).
68. Lemoine FJ, Kao SY, Marriott SJ: Suppression of DNA repair by HTLV Type 1 Tax correlates with Tax *trans*-activation of proliferating cell nuclear antigen gene expression. *AIDS Res. Hum. Retroviruses* 16, 1623–1627 (2000).
69. Ariumi Y, Kaida A, Lin JY *et al.*: HTLV-1 Tax oncoprotein represses the p53-mediated *trans*-activation function through coactivator CBP sequestration. *Oncogene* 19, 1491–1499 (2000).
70. Haoudi A, Semmes OJ: The HTLV-1 Tax oncoprotein attenuates DNA damage induced G1 arrest and enhances apoptosis in p53 null cells. *Virology* 305, 229–239 (2003).
71. Park HU, Jeong JH, Chung JH, Brady JN: Human T-cell leukemia virus Type 1 Tax interacts with Chk1 and attenuates DNA-damage induced G2 arrest mediated by Chk1. *Oncogene* 23, 4966–4974 (2004).
72. Pise-Masison CA, Mahieux R, Jiang H *et al.*: Inactivation of p53 by human T-cell lymphotropic virus Type 1 Tax requires activation of the NF- κ B pathway and is dependent on p53 phosphorylation. *Mol. Cell. Biol.* 20, 3377–3386 (2000).
73. Tabakin-Fix Y, Azran I, Schavinsky-Khrapunsky Y, Levy O, Aboud M: Functional inactivation of p53 by human T-cell leukemia virus Type 1 Tax protein: mechanisms and clinical implications. *Carcinogenesis* 27, 673–681 (2006).
74. Gabet AS, Mortreux F, Charneau P *et al.*: Inactivation of hTERT transcription by Tax. *Oncogene* 22, 3734–3741 (2003).
75. Majone F, Jeang KT: Clastogenic effect of the human T-cell leukemia virus Type 1 Tax oncoprotein correlates with unstabilized DNA breaks. *J. Biol. Chem.* 275, 32906–32910 (2000).
76. Majone F, Luisetto R, Zamboni D, Iwanaga Y, Jeang KT: Ku protein as a potential human T-cell leukemia virus Type 1 (HTLV-1) Tax target in clastogenic chromosomal instability of mammalian cells. *Retrovirology* 2, 45 (2005).
77. Morimoto H, Tsukada J, Kominato Y, Tanaka Y: Reduced expression of human mismatch repair genes in adult T-cell leukemia. *Am. J. Hematol.* 78, 100–107 (2005).
78. Tsuji T, Sugahara K, Tsuruda K *et al.*: Clinical and oncologic implications in epigenetic down-regulation of CD26/dipeptidyl peptidase IV in adult T-cell leukemia cells. *Int. J. Hematol.* 80, 254–260 (2004).
79. Yang Y, Takeuchi S, Tsukasaki K *et al.*: Methylation analysis of the adenomatous polyposis coli (*APC*) gene in adult T-cell leukemia/lymphoma. *Leuk. Res.* 29, 47–51 (2005).
80. Yasunaga J, Taniguchi Y, Nosaka K *et al.*: Identification of aberrantly methylated genes in association with adult T-cell leukemia. *Cancer Res.* 64, 6002–6009 (2004).
81. Yoshida M, Nosaka K, Yasunaga J, Nishikata I, Morishita K, Matsuoka M: Aberrant expression of the *MELIS* gene

- identified in association with hypomethylation in adult T-cell leukemia cells. *Blood* 103, 2753–2760 (2004).
82. Ducasse M, Brown MA: Epigenetic aberrations and cancer. *Mol. Cancer* 5, 60 (2006).
 83. Feinberg AP, Ohlsson R, Henikoff S: The epigenetic progenitor origin of human cancer. *Nat. Rev. Genet.* 7, 21–33 (2006).
 - **This review brings together the mounting evidence supporting a view that epigenetic changes play at least an equal role in the progression from normal cells to cancer cells.**
 84. Feinberg AP, Tycko B: The history of cancer epigenetics. *Nat. Rev. Cancer* 4, 143–153 (2004).
 85. Ting AH, McGarvey KM, Baylin SB: The cancer epigenome – components and functional correlates. *Genes Dev.* 20, 3215–3231 (2006).
 86. Badal V, Chuang LS, Tan EH *et al.*: CpG methylation of human papillomavirus Type 16 DNA in cervical cancer cell lines and in clinical specimens: genomic hypomethylation correlates with carcinogenic progression. *J. Virol.* 77, 6227–6234 (2003).
 87. Eden A, Gaudet F, Waghmare A, Jaenisch R: Chromosomal instability and tumors promoted by DNA hypomethylation. *Science* 300, 455 (2003).
 88. Nakayama M, Wada M, Harada T *et al.*: Hypomethylation status of CpG sites at the promoter region and overexpression of the human *MDR1* gene in acute myeloid leukemias. *Blood* 92, 4296–4307 (1998).
 89. Qu GZ, Grundy PE, Narayan A, Ehrlich M: Frequent hypomethylation in Wilms tumors of pericentromeric DNA in chromosomes 1 and 16. *Cancer Genet. Cytogenet.* 109, 34–39 (1999).
 90. Suter CM, Martin DI, Ward RL: Hypomethylation of L1 retrotransposons in colorectal cancer and adjacent normal tissue. *Int. J. Colorectal Dis.* 19, 95–101 (2004).
 91. Bogenberger JM, Laybourn PJ: Human T lymphotropic virus type 1 protein Tax reduces histone levels. *Retrovirology* 5, 9 (2008).
 - **In this study, a new flow cytometry-based assay is used to demonstrate that HTLV-1 Tax protein functions to perturb T-cell homeostasis, at least in part, by decreasing the chromosomal nucleosome density.**
 92. Lewis LK, Karthikeyan G, Cassiano J, Resnick MA: Reduction of nucleosome assembly during new DNA synthesis impairs both major pathways of double-strand break repair. *Nucleic Acids Res.* 33, 4928–4939 (2005).
 93. Ye X, Franco AA, Santos H, Nelson DM, Kaufman PD, Adams PD: Defective S phase chromatin assembly causes DNA damage, activation of the S phase checkpoint, and S phase arrest. *Mol. Cell* 11, 341–351 (2003).
 94. Taniguchi Y, Nosaka K, Yasunaga J *et al.*: Silencing of human T-cell leukemia virus type I gene transcription by epigenetic mechanisms. *Retrovirology* 2, 64 (2005).
 - **Describes a key study investigating the mechanisms of reversible silencing of HTLV-1 provirus transcription in viral latency.**
 95. Marzluff WF, Duronio RJ: Histone mRNA expression: multiple levels of cell cycle regulation and important developmental consequences. *Curr. Opin. Cell Biol.* 14, 692–699 (2002).
 96. Zhao J: Coordination of DNA synthesis and histone gene expression during normal cell cycle progression and after DNA damage. *Cell Cycle* 3, 695–697 (2004).
 97. Gunjan A, Verreault A: A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in *S. cerevisiae*. *Cell* 115, 537–549 (2003).
 98. Gunjan A, Paik J, Verreault A: Regulation of histone synthesis and nucleosome assembly. *Biochimie* 87, 625–635 (2005).
 99. Jaeger S, Barends S, Giege R, Eriani G, Martin F: Expression of metazoan replication-dependent histone genes. *Biochimie* 87, 827–834 (2005).
 100. Marzluff WF: Metazoan replication-dependent histone mRNAs: a distinct set of RNA polymerase II transcripts. *Curr. Opin. Cell Biol.* 17, 274–280 (2005).
 101. Prado F, Aguilera A: Partial depletion of histone H4 increases homologous recombination-mediated genetic instability. *Mol. Cell Biol.* 25, 1526–1536 (2005).
 102. Kawano N, Shimoda K, Ishikawa F *et al.*: Adult T-cell leukemia development from a human T-cell leukemia virus Type I carrier after a living-donor liver transplantation. *Transplantation* 82, 840–843 (2006).
 103. Roudier M, Lamaury I, Strobel M: Human T cell leukemia/lymphoma virus Type I (HTLV-I) and *Pneumocystis carinii* associated with T cell proliferation and haemophagocytic syndrome. *Leukemia* 11, 453–454 (1997).
 104. Suzuki S, Uozumi K, Maeda M *et al.*: Adult T-cell leukemia in a liver transplant recipient that did not progress after onset of graft rejection. *Int. J. Hematol.* 83, 429–432 (2006).

Affiliation

- **Paul Laybourn**
Department of Biochemistry & Molecular Biology, 1870 Campus Delivery, Colorado State University, Fort Collins, CO 80523-1870, USA
Tel.: +1 970 491 5100;
Fax: +1 970 491 0494;
paul.laybourn@colostate.edu