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The Gene That Encodes the Herpes Simplex Virus Type 1 Latency-Associated Transcript Influences the Accumulation of Transcripts (Bcl-x_L and Bcl-x_S) That Encode Apoptotic Regulatory Proteins

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The herpes simplex virus type 1 latency-associated transcript (LAT) inhibits apoptosis. We demonstrate here that LAT influences the accumulation of the Bcl-x_L transcript versus the Bcl-x_S transcript in Neuro-2A cells. Bcl-x_L encodes an antiapoptotic protein, whereas Bcl-x_S encodes a proapoptotic protein. Promoting the accumulation of Bcl-x_L in neurons may inhibit apoptosis, thus enhancing the latency-reactivation cycle.

Herpes simplex virus type 1 (HSV-1) establishes latency in trigeminal ganglia (TG) following infection of the ocular, oral, or nasal cavity (6, 7). The latency-associated transcript (LAT) is the only viral transcript abundantly expressed in latently infected neurons (41). An unstable 8.3-kb LAT is spliced, yielding a stable 2-kb LAT intron (12, 28). Numerous researchers concluded that LAT promotes reactivation from latency by increasing the pool of latently infected neurons or directly stimulating the reactivation process (23, 46). The first 1.5 kb of the primary LAT is sufficient for spontaneous reactivation from latency in a rabbit ocular model of HSV-1 latency (40).

Plasmids expressing various LAT fragments enhance cell survival following an apoptotic insult (1, 17, 19, 38). The ability of these plasmids to promote cell survival correlates with the ability of viruses expressing the corresponding LAT fragments (LAT nucleotides 1 to 1499) to reactivate in the rabbit ocular model of HSV-1 latency (40). In the same rabbit ocular model of HSV-1 latency, a McKrae LAT null mutant (*d*LAT2903) had increased levels of apoptosis in rabbit TG (38). Furthermore, another LAT^- virus has increased neuronal apoptosis in acutely infected mice compared to a LAT^+ virus (1). Although LAT may have several functions, its antiapoptosis activity may be important for the latency-reactivation cycle.

Mammals have two major apoptotic pathways: the death receptor-mediated pathway (for example, Fas or tumor necrosis factor receptor) and the mitochondrial pathway (27, 42, 47). The death receptor-mediated pathway activates caspase 8, which induces a caspase cascade including caspase 3. Activation of the mitochondrial pathway results in the release of important proapoptotic molecules, including cytochrome *c* and Smac/Diablo (47). Released cytochrome *c* associates with Apaf-1, leading to caspase 9 activation and then caspase 3 activation. Bcl-2 family proteins regulate cytochrome *c* (13, 24, 25, 48) and Smac/Diablo (11, 45) release. One of the Bcl-2 family proteins, Bcl-x_L, can also inhibit caspase 8-dependent apoptosis by sequestering caspase 8 at the mitochondrial membrane and inhibiting cleavage of downstream targets (43). Since HSV-1 encodes several antiapoptotic genes (ICP27, U_S3, U_S5, gD, and LAT) (1, 4, 5, 8, 14, 19–22, 32, 33, 37, 38), regulating apoptosis is clearly important for the viral life cycle.

One of our goals is to understand the steps in the apoptotic pathway that LAT inhibits. Henderson et al. have previously demonstrated that LAT inhibits caspase 8- and caspase 9-induced apoptosis (17). Since LAT can inhibit apoptosis of a proapoptotic Bcl-2 family member, Bax (19), and Bcl-2 family members regulate caspase 8- and caspase 9-induced apoptosis, we hypothesized that LAT might alter the expression of Bcl-2 family members. To test this hypothesis, we examined RNA expression of Bcl-2 family members in productively infected cells and transiently transfected Neuro-2A cells. Neuro-2A cells were chosen for these studies because they are mouse neuroblastoma cells derived from the peripheral nervous system, and LAT inhibits apoptosis in these cells (17, 19, 38). Similar end point titers of infectious virus were present in Neuro-2A cells that were infected with strain *d*LAT2903, the wild-type (wt) McKrae strain, or the rescued *d*LAT2903 strain, which is consistent with previous studies using other cell lines (39). However, we have observed more apoptosis at 24 h after infection when Neuro-2A cells were infected with strain *d*LAT2903 versus the wt McKrae strain (data not shown).

The mRNA that encodes the Bcl-x_L protein can be alternatively spliced within exon 2, yielding a proapoptotic protein (Bcl-x_S) (31). The Bcl-x primers used here amplified a 250-bp product from Bcl-x_L cDNA and a 64-bp product from the cDNA of the alternatively spliced Bcl-x_S product (Table 1), as previously reported (31). Reverse transcriptase PCR (RT-PCR) of total RNA prepared from Neuro-2A cells productively infected with LAT^- strain *d*LAT2903 for 24 h after infection produced a prominent 64-bp band corresponding to the Bcl-x_S RNA (Fig. 1A, lane dLAT). In Neuro-2A cells infected with the wt McKrae strain (Fig. 1A, lane WT) or strain *d*LAT2903-rescued virus (data not shown), the 64-bp RT-PCR product was not readily detected. In some cultures of mock-

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TABLE 1. Primers used to amplify cDNA[a]

Gene	Primer (type)[b]	PCR product(s) (bp)	Melting temp	No. of cycles	GeneBank accession no.
Bcl-2	GTCGCTACCGTCGTGACTT (F) CAGCCTCCGTTATCCTGGA (R)	267	60	35	NM 009741
Bcl-x	CCAGCTTCACATAACCCAG (F) ATCCACAAAAGTGTCCCAGC (R)	250, 64[c]	60	30–35	L35049
Casp 9	AAGACCATGGCTTTGAGGTG (F) AACAGCCAGGAATCTGCTTG (R)	333	60	35	AB01960
β-actin	GTGGGGCGCCCCAGGCACCA (F) C TCCTTAATGTCACGCACGATTTC (R)	400	53	20	
VHS	TGCTACATTCCCACGATCAA (F) AGGTCCTCGTCGTCTTCGTA (R)	346	60	30	AF007815
gC	AGGTCCTGACGAACATCACC (F) TAATACATTCCCTGGGTCGC (R)	208	60	30	JO2216
Bak	TTGCCCAGGACACAGAGGAGGT (F) GAATTGGCCCAACAGAACCACACC (R)	527	60	40	AF402617

[a] The melting temperatures of the respective primers and the numbers of cycles used for PCR are given for each primer pair.
[b] Forward (F) and reverse (R) primers were designed on the basis of the sequences of the respective genes. All primers are listed in a 5′ to 3′ direction.
[c] The Bcl-x primers can amplify a 250-bp cDNA product (Bcl-x_L) and a 64-bp cDNA product (Bcl-x_S).

infected cells, low levels of the Bcl-x_S splice product were detected, which is consistent with these cells undergoing apoptosis after growth in subcultures. When 30 to 35 cycles of amplification were performed, the Bcl-x_S-amplified product was consistently detected when Neuro-2A cells were infected with strain *d*LAT2903. However, the Bcl-x_S-amplified product was not readily detected when fewer than 25 cycles were used for amplification. The sequence of the 64-bp RT-PCR product amplified in Neuro-2A cells infected with strain *d*LAT2903 was identical to that of the Bcl-x_S spliced product.

Additional studies were performed to determine when the Bcl-x_S-amplified cDNA product was detectable in Neuro-2A cells that were infected with strain *d*LAT2903. In general, when Neuro-2A cultures were infected with *d*LAT2903, the Bcl-x_S spliced product was detectable between 6 and 8 h after infection (Fig. 1B). Cultures infected with the wt McKrae strain typically contained little or none of the Bcl-x_S-amplified product. In several experiments, Neuro-2A cultures infected with *d*LAT2903 contained reduced levels of the Bcl-x_L-amplified product (in conjunction with the increased levels of the Bcl-x_S-amplified product). This was particularly evident when reduced numbers of cycles were used for amplification of the cDNA (35 cycles [Fig. 1A] versus 31 cycles [Fig. 1B]). In cultures infected with *d*LAT2903, since Neuro-2A cells must be split every 2 to 3 days or they begin to detach from the dish and undergo an apoptotic death, the condition of the cell cultures can influence Bcl-x_L levels and the appearance of the Bcl-x_S product. In summary, the results of these studies suggested that LAT either inhibited splicing of the primary Bcl-x transcript into Bcl-x_S or increased the ratio of Bcl-x_L to Bcl-x_S by stabilizing Bcl-x_L RNA levels and/or destabilizing Bcl-x_S RNA levels.

In contrast to the results obtained with Bcl-x, the levels of rRNA, Bcl-2, caspase 9, and β-actin did not change dramatically after infection (Fig. 2). In general, these results suggested that when Neuro-2A cells were infected with the wt McKrae strain of HSV-1 or the strain McKrae-based LAT$^-$ mutant *d*LAT2903, the virus host shutoff (VHS) function was negligi-

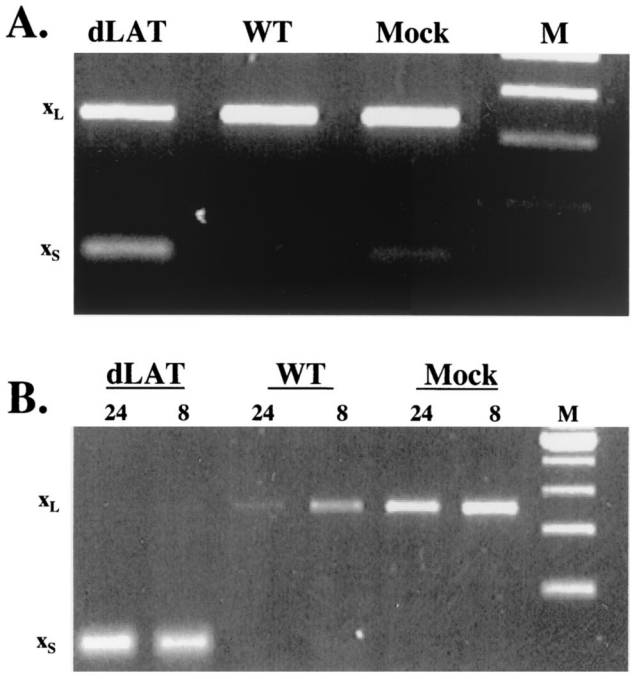

FIG. 1. Analysis of Bcl-x RNA expression in infected Neuro-2A cells. Using a multiplicity of infection of 4, Neuro-2A cells were infected with strain *d*LAT2903 (dLAT) or wt HSV-1 (McKrae strain) (WT). As controls, cultures were mock infected (Mock). At 24 h after infection (A) or at 8 and 24 h after infection (B), RNA was prepared using Trizol reagent and the RNA was subsequently treated with RNase-free DNase I for 30 min at room temperature. First-strand cDNA was synthesized using 2 μg of total RNA as the template, 0.5 μg of oligo(dT) 12-18 as a primer, and a SuperScript preamplification kit (Stratagene, La Jolla, Calif.). For each sample, 1/10 of the cDNA reaction mixture was used with the indicated primers (Table 1). Using 35 (A) or 31 (B) cycles, amplification was conducted as described in Table 1. The positions of the Bcl-x_L (x_L) and Bcl-x_S (x_S) bands are indicated. Omitting reverse transcriptase from the reaction eliminated amplification of the specific bands (data not shown). A 100-bp ladder was used as a marker (M) to estimate the size of the amplified cDNA products. These results are representative of at least five independent experiments.

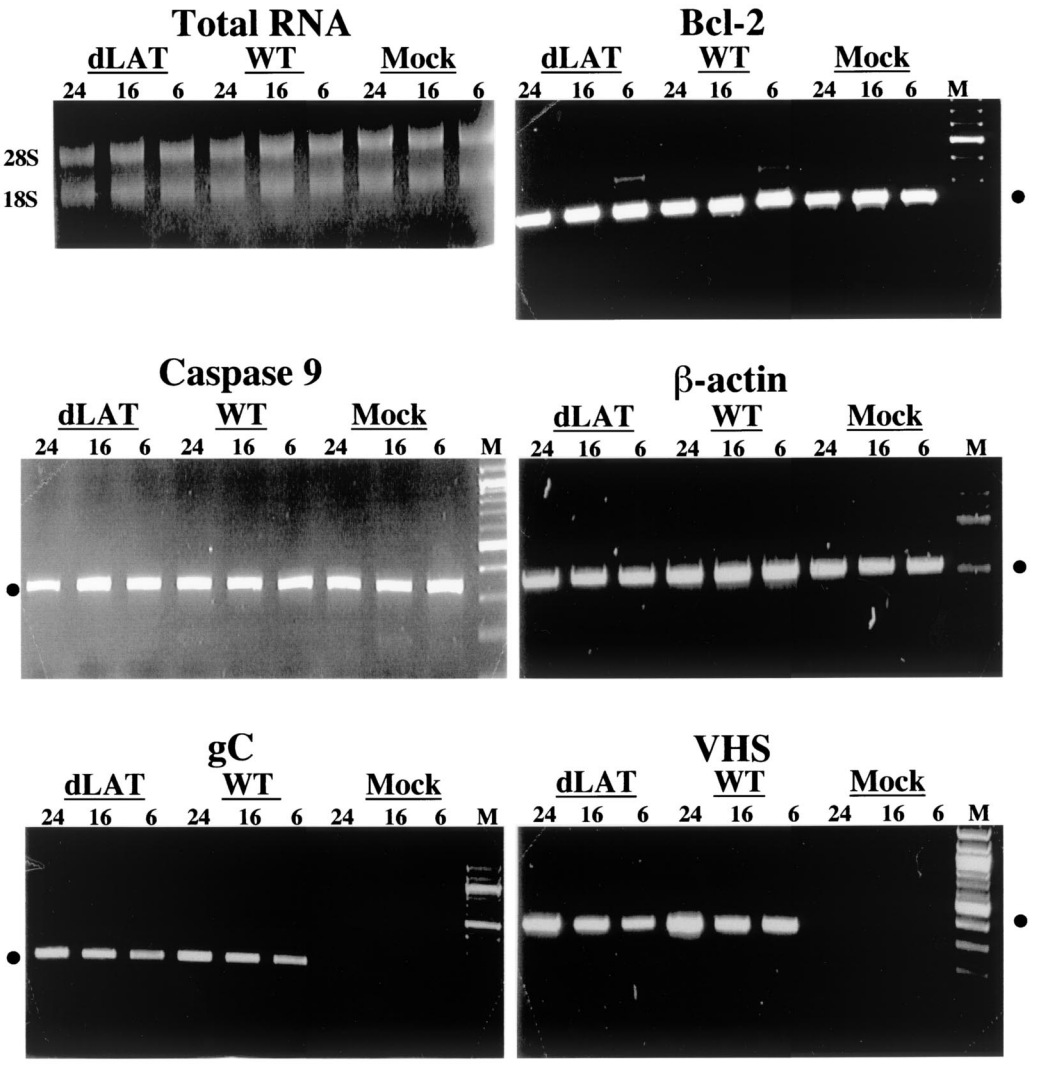

FIG. 2. Analysis of apoptosis regulatory genes in infected Neuro-2A cells. Using a multiplicity of infection of 4, Neuro-2A cells were infected with strain *d*LAT2903 (dLAT) or wt HSV-1 (McKrae strain) (WT). As controls, cultures were mock infected (Mock). At 6, 16, or 24 h after infection, RNA was prepared using Trizol reagent and the RNA was subsequently treated with RNase-free DNase I for 30 min at room temperature. Samples containing total RNA (1 μg) were electrophoresed on a 1.2% formaldehyde agarose gel, and the position of the rRNA was determined. First-strand cDNA was synthesized using 2 μg of total RNA as the template, 0.5 μg of oligo(dT)12-18 as a primer, and a SuperScript preamplification kit (Stratagene). For each sample, 1/10 of the cDNA reaction mixture was used with the indicated primers (Table 1). Amplification was conducted as described in Table 1. The closed circles denote the positions of amplified products. Omitting reverse transcriptase from the reaction eliminated amplification of the specific bands (data not shown). A 100-bp ladder was used as a marker (M) to estimate the size of the amplified cDNA products. These results are representative of at least four independent experiments.

ble. Since infection of sympathetic and sensory neurons with HSV has been reported to not elicit a potent VHS activity (34), this provided additional evidence that Neuro-2A cells have neuron-like properties. As expected, infection of nonneuronal cell types with the McKrae strain leads to reduction of the steady-state levels of β-actin RNA, indicating that the McKrae strain has a functional VHS activity (data not shown). Consistent with the finding that Neuro-2A cells infected with wt strain McKrae or strain *d*LAT2903 yield similar amounts of virus, expression of VHS RNA or glycoprotein C (gC) RNA was the same when Neuro-2A cells were infected.

Since HSV-1 encodes several antiapoptotic genes that are expressed during productive infection (1, 4, 5, 8, 14, 19–22, 32, 33, 37, 38), we tested whether, in the absence of other viral genes, LAT could inhibit accumulation of Bcl-x_S. Neuro-2A cells transfected with Bax (Fig. 3A, lane 3) and mock-transfected Neuro-2A cells (Fig. 3A, lane 4) both contained the 64-bp Bcl-x_S-amplified band. This was as expected, because Neuro-2A cells undergo apoptosis as a result of Bax expression (19) or when growth factors are depleted (17). In contrast, Neuro-2A cells transfected with LAT (Fig. 3A, lane 1) or the LAT plus Bax (lane 2) did not contain a prominent Bcl-x_S-amplified band, suggesting that in the absence of other viral genes, LAT inhibited accumulation of Bcl-x_S. Inclusion of reverse transcriptase in the cDNA reaction was required for detection of the 250-bp Bcl-x_L-amplified products and 64-bp Bcl-x_S-amplified products, confirming that cDNA, not contaminating DNA, was amplified (Fig. 3B). No dramatic changes

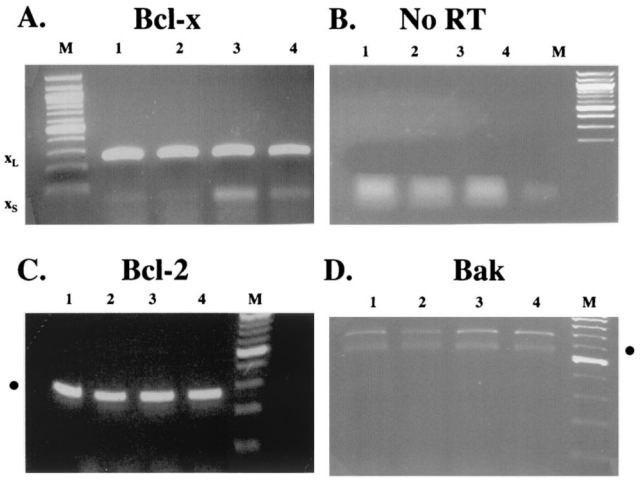

FIG. 3. LAT inhibits splicing of Bcl-x_S in transfected Neuro-2A cells. Neuro-2A cells were transfected with the plasmids described below. Lane 1, 4 μg of LAT3.3–1 μg of pcDNA3.1; lane 2, 4 μg of LAT3.3–1 μg of pCMVBax; lane 3, 4 μg of pcDNA3.1–1 μg of pCMVBax; lane 4, 5 μg of pcDNA3.1. LAT3.3 contains the LAT promoter plus the first 1.5 kb of LAT coding sequences (19). pCMVBax contains the full-length Bax cDNA downstream of the human cytomegalovirus promoter; the plasmid was obtained from Upstate Biotechnology (Lake Placid, N.Y.). At 48 h after transfection, using primers that amplify Bcl-x (A), Bcl-2 (C), or Bak (D), RNA was prepared and RT-PCR was performed as described for Fig. 1. (B) cDNA reactions that did not contain reverse transcriptase (No RT) were amplified using the Bcl-X primers. In the No RT reactions, the Bcl-x primers did amplify the expected products. The No RT reaction also yielded no bands when the Bcl-2 or Bak primers were used (data not shown).

were detected in the levels or mobility of the Bcl-2-amplified product regardless of the treatment (Fig. 3C). Studies were also performed to test whether LAT altered splicing of Bak, because Bak is a proapoptotic gene that belongs to the Bcl-2 family. Interestingly, Bak can be alternatively spliced in neurons to yield an antiapoptotic protein rather than the proapoptotic protein seen in nonneuronal cells (44). In contrast to Bcl-x, LAT did not have a dramatic effect on Bak splicing patterns in transiently transfected Neuro-2A cells (Fig. 3D) or in productively infected Neuro-2A cells (data not shown).

This study demonstrated that LAT inhibited accumulation of Bcl-x_S. This suggests that LAT promoted splicing of Bcl-x to Bcl-x_L rather than to Bcl-x_S or that LAT stabilized Bcl-x_L steady-state RNA levels while at the same time destabilizing Bcl-x_S RNA levels. Since the levels of Bcl-2, caspase 9, and β-actin mRNA were not reduced dramatically in infected Neuro-2A cells (Fig. 2), the reduction of Bcl-x_L levels in cells infected with strain *d*LAT2903 was not merely the result of viral host shutoff activity. These results are important, because the Bcl-x_L protein has antiapoptotic activity whereas the Bcl-x_S protein is proapoptotic. Thus, increasing the ratio of Bcl-x_L to Bcl-x_S transcripts, as LAT appeared to do in these studies, should enhance cell survival (9). The ability of the LAT to interfere with caspase 8- and caspase 9-induced apoptosis (17) may be linked to influencing splicing of Bcl-x to Bcl-x_L rather than Bcl-x_S, since Bcl-x_L inhibits both caspase 8 (43)- and caspase 9-induced apoptosis (18, 35). Bcl-x_L, but not Bcl-2, is highly expressed in the central and peripheral nervous systems of developing (26) and adult mice (15, 16, 30, 36), suggesting that Bcl-x_L is the most important Bcl-2 family protein expressed in TG.

Although LAT contains small open reading frames, a recent study concluded that LAT protein expression is not detected in infected cells or infected mice (29). The small open reading frames within the first 1.5 kb of LAT, a region of the LAT that promotes spontaneous reactivation, do not show a high degree of amino acid similarity between HSV-1 strains (10), suggesting that LAT RNA sequences, rather than a LAT protein, influences Bcl-x_S accumulation. The ability of LAT to interact with splicing factors (2) may influence splicing of certain cellular transcripts, including Bcl-x_L. Although caspase 9 (3) and Bak (44) are alternatively spliced, LAT expression did not dramatically alter the levels of these spliced products. Consequently, we conclude that LAT has the potential to influence expression of certain spliced cellular transcripts.

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REFERENCES

1. **Ahmed, M., M. Lock, C. G. Miller, and N. W. Fraser.** 2002. Regions of the herpes simplex virus type 1 latency-associated transcript that protect cells from apoptosis in vitro and protect neuronal cells in vivo. J. Virol. **76:**717–729.
2. **Ahmed, M., and N. Fraser.** 2001. Herpes simplex virus type 1 2-kilobase latency-associated transcript intron associates with ribosomal proteins and splicing factors. J. Virol. **75:**12070–12080.
3. **Angelastro, J. M., N. Y. Moon, D. X. Liu, A. S. Yang, L. A. Greene, and T. F. Franke.** 2001. Characterization of a novel isoform of caspase-9 that inhibits apoptosis. J. Biol. Chem. **276:**12190–12200.
4. **Asano, S., T. Honda, F. Goshima, D. Watanabe, Y. Miyake, Y. Sugiura, and Y. Nishiyama.** 1999. US3 protein kinase of herpes simplex virus type 2 plays a role in protecting corneal epithelial cells from apoptosis in infected mice. J. Gen. Virol. **80:**51–56.
5. **Aubert, M., and J. A. Blaho.** 1999. The herpes simplex virus type 1 regulatory protein ICP27 is required for the prevention of apoptosis in infected human cells. J. Virol. **73:**2803–2813.
6. **Baringer, J. R., and P. Swoveland.** 1973. Recovery of herpes-simplex virus from human trigeminal ganglions. N. Engl. J. Med. **288:**648–650.
7. **Bastian, F. O., A. S. Rabson, C. L. Yee, and T. S. Tralka.** 1972. *Herpesvirus hominis*: isolation from human trigeminal ganglion. Science **178:**306–307.
8. **Blaho, J. A., and M. Aubert.** 2001. Modulation of apoptosis during herpes simplex virus infection in human cells. Microbes Infect. **3:**1–8.
9. **Boise, L. H., M. Gonzalez-Garcia, C. E. Postema, L. Ding, T. Lindsten, L. A. Turka, X. Mao, G. Nunez, and C. B. Thompson.** 1993. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell **74:**597–608.
10. **Drolet, B. S., G. C. Perng, J. Cohen, S. M. Slanina, A. Yukht, A. B. Nesburn, and S. L. Wechsler.** 1998. The region of the herpes simplex virus type 1 LAT gene involved in spontaneous reactivation does not encode a functional protein. Virology **242:**221–232.
11. **Du, C., M. Fang, Y. Li, L. Li, and X. Wang.** 2000. Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. Cell **102:**33–42.
12. **Farrell, M. J., A. T. Dobson, and L. T. Feldman.** 1991. Herpes simplex virus latency-associated transcript is a stable intron. Proc. Natl. Acad. Sci. USA **88:**790–794.
13. **Finucane, D. M., E. Bossy-Wetzel, N. J. Waterhouse, T. G. Cotter, and D. R. Green.** 1999. Bax-induced caspase activation and apoptosis via cytochrome *c* release from mitochondria is inhibitable by Bcl-xL. J. Biol. Chem. **274:**2225–2233.
14. **Galvan, V., R. Brandimarti, and B. Roizman.** 1999. Herpes simplex virus 1 blocks caspase-3-independent and caspase-dependent pathways to cell death. J. Virol. **73:**3219–3226.
15. **Gonzalez-Garcia, M., I. Garcia, L. Ding, S. O'Shea, L. H. Boise, C. B. Thompson, and G. Nunez.** 1995. bcl-x is expressed in embryonic and postnatal neural tissues and functions to prevent neuronal cell death. Proc. Natl. Acad. Sci. USA **92:**4304–4308.
16. **Gonzalez-Garcia, M., R. Perez-Ballestero, L. Ding, L. Duan, L. H. Boise, C. B. Thompson, and G. Nunez.** 1994. bcl-XL is the major bcl-x mRNA form

expressed during murine development and its product localizes to mitochondria. Development **120:**3033–3042.
17. **Henderson, G., W. Peng, L. Jin, G.-C. Perng, A. B. Nesburn, S. L. Wechsler, and C. Jones.** 2002. Regulation of caspase 8- and caspase 9-induced apoptosis by the herpes simplex virus latency-associated transcript. J. Neurovirol. **8**(Suppl. 2)**:**103–111.
18. **Hu, Y., M. A. Benedict, D. Wu, N. Inohara, and G. Nunez.** 1998. Bcl-XL interacts with Apaf-1 and inhibits Apaf-1-dependent caspase-9 activation. Proc. Natl. Acad. Sci. USA **95:**4386–4391.
19. **Inman, M., G. C. Perng, G. Henderson, H. Ghiasi, A. B. Nesburn, S. L. Wechsler, and C. Jones.** 2001. Region of herpes simplex virus type 1 latency-associated transcript sufficient for wild-type spontaneous reactivation promotes cell survival in tissue culture. J. Virol. **75:**3636–3646.
20. **Jerome, K. R., Z. Chen, R. Lang, M. R. Torres, J. Hofmeister, S. Smith, R. Fox, C. J. Froelich, and L. Corey.** 2001. HSV and glycoprotein J inhibit caspase activation and apoptosis induced by granzyme B or Fas. J. Immunol. **167:**3928–3935.
21. **Jerome, K. R., R. Fox, Z. Chen, A. E. Sears, H. Lee, and L. Corey.** 1999. Herpes simplex virus inhibits apoptosis through the action of two genes, Us5 and Us3. J. Virol. **73:**8950–8957.
22. **Jerome, K. R., J. F. Tait, D. M. Koelle, and L. Corey.** 1998. Herpes simplex virus type 1 renders infected cells resistant to cytotoxic T-lymphocyte-induced apoptosis. J. Virol. **72:**436–441.
23. **Jones, C.** 1998. Alphaherpesvirus latency: its role in disease and survival of the virus in nature. Adv. Virus Res. **51:**81–133.
24. **Jurgensmeier, J. M., Z. Xie, Q. Deveraux, L. Ellerby, D. Bredesen, and J. C. Reed.** 1998. Bax directly induces release of cytochrome *c* from isolated mitochondria. Proc. Natl. Acad. Sci. USA **95:**4997–5002.
25. **Kluck, R. M., E. Bossy-Wetzel, D. R. Green, and D. D. Newmeyer.** 1997. The release of cytochrome *c* from mitochondria: a primary site for Bcl-2 regulation of apoptosis Science **275:**1132–1136.
26. **Krajewska, M., J. K. Mai, J. M. Zapata, K. W. Ashwell, S. L. Schendel, J. C. Reed, and S. Krajewski.** 2002. Dynamics of expression of apoptosis-regulatory proteins Bid, Bcl-2, Bcl-X, Bax and Bak during development of murine nervous system. Cell Death Differ. **9:**145–157.
27. **Kruegger, A., S. Baumann, P. H. Krammer, and S. Kirchhoff.** 2001. FLICE-inhibitory proteins: regulators of death receptor-mediated apoptosis. Mol. Cell. Biol. **21:**8247–8254.
28. **Krummenacher, C., J. M. Zabolotny, and N. W. Fraser.** 1997. Selection of a nonconsensus branch point is influenced by an RNA stem-loop structure and is important to confer stability to the herpes simplex virus 2-kilobase latency-associated transcript. J. Virol. **71:**5849–5860.
29. **Lock, M., C. Miller, and N. W. Fraser.** 2001. Analysis of protein expression from within the region encoding the 2.0-kilobase latency-associated transcript of herpes simplex virus type 1. J. Virol. **75:**3413–3426.
30. **Merry, D. E., D. J. Veis, W. F. Hickey, and S. J. Korsmeyer.** 1994. bcl-2 protein expression is widespread in the developing nervous system and retained in the adult PNS. Development **120:**301–311.
31. **Minn, A. J., L. H. Boise, and C. B. Thompson.** 1996. Bcl-x(S) antagonizes the protective effects of Bcl-x(L). J. Biol. Chem. **271:**6306–6312.
32. **Munger, J., A. V. Chee, and B. Roizman.** 2001. The U_S3 protein kinase blocks apoptosis induced by the *d*120 mutant of herpes simplex virus 1 at a premitochondrial stage. J. Virol. **75:**5491–5497.
33. **Munger, J., and B. Roizman.** 2001. The US3 protein kinase of herpes simplex virus 1 mediates the posttranslational modification of BAD and prevents BAD-induced programmed cell death in the absence of other viral proteins. Proc. Natl. Acad. Sci. USA **98:**10410–10415.
34. **Nichol, P. F., J. Y. Chang, E. M. Johnson, Jr., and P. D. Olivio.** 2000. Infection of sympathetic and sensory neurones with herpes simplex virus does not elicit a shut-off of cellular protein synthesis: implications for viral latency and herpes vectors. Neurobiol. Dis. **1:**83–94.
35. **Pan, G., K. O'Rourke, and V. M. Dixit.** 1998. Caspase-9, Bcl-XL, and Apaf-1 form a ternary complex. J. Biol. Chem. **273:**5841–5845.
36. **Parsadanian, A. S., Y. Cheng, C. R. Keller-Peck, D. M. Holtzman, and W. D. Snider.** 1998. Bcl-xL is an antiapoptotic regulator for postnatal CNS neurons. J. Neurosci. **18:**1009–1019.
37. **Perng, G.-C., B. Maguen, L. Jin, K. R. Mott, N. Osorio, S. M. Slanina, A. Yukht, H. Ghiasi, A. B. Nesburn, M. Inman, G. Henderson, C. Jones, and S. L. Wechsler.** 2002. A gene capable of blocking apoptosis can substitute for the herpes simplex virus type 1 latency-associated transcript gene and restore wild-type reactivation levels. J. Virol. **76:**1224–1235.
38. **Perng, G.-C., C. Jones, J. Ciacci-Zanella, M. Stone, G. Henderson, A. Yukht, S. M. Slanina, F. M. Hoffman, H. Ghiasi, A. B. Nesburn, and S. Wechsler.** 2000. Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript (LAT). Science **287:**1500–1503.
39. **Perng, G. C., E. C. Dunkel, P. A. Geary, S. M. Slanina, H. Ghiasi, R. Kaiwar, A. B. Nesburn, and S. L. Wechsler.** 1994. The latency-associated transcript gene of herpes simplex virus type 1 (HSV-1) is required for efficient in vivo spontaneous reactivation of HSV-1 from latency. J. Virol. **68:**8045–8055.
40. **Perng, G. C., H. Ghiasi, S. M. Slanina, A. B. Nesburn, and S. L. Wechsler.** 1996. The spontaneous reactivation function of the herpes simplex virus type 1 LAT gene resides completely within the first 1.5 kilobases of the 8.3-kilobase primary transcript. J. Virol. **70:**976–984.
41. **Rock, D. L., A. B. Nesburn, H. Ghiasi, J. Ong, T. L. Lewis, J. R. Lokensgard, and S. L. Wechsler.** 1987. Detection of latency-related viral RNAs in trigeminal ganglia of rabbits latently infected with herpes simplex virus type 1. J. Virol. **61:**3820–3826.
42. **Schmitz, I., S. Kirchhoff, and P. H. Krammer.** 2000. Regulation of death receptor-mediated apoptosis pathways. Int. J. Biochem. Cell Biol. **32:**1123–1136.
43. **Stegh, A. H., C. Bryan, J. Volkland, A. Algeciras-Schimnich, N. Ke, J. C. Reed, and M. E. Peter.** 2002. Inactivation of caspase-8 on mitochondria of Bcl-XL-expressing MCF7-Fas cells: role for the bifunctional apoptosis regulator protein. J. Biol. Chem. **277:**4351–4360.
44. **Sun, Y.-F., L.-Y. Yu, M. Saarma, T. Timmusk, and A. Arumae.** 2001. Neuron-specific Bcl-2 homology 3 dominant-only splice variant of Bak is anti-apoptotic in neurons, but pro-apoptotic in non-neuronal cells. J. Biol. Chem. **276:**16240–16247.
45. **Verhagen, A. M., P. G. Ekert, M. Pakusch, J. Silke, L. M. Connolly, G. E. Reid, R. L. Moritz, R. J. Simpson, and D. L. Vaux.** 2000. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell **102:**43–53.
46. **Wagner, E. K., and D. C. Bloom.** 1997. Experimental investigation of herpes simplex virus latency. Clin. Microbiol. Rev. **10:**419–443.
47. **Wang, X.** 2001. The expanding role of mitochondria in apoptosis. Genes Dev. **15:**2922–2933.
48. **Yang, J., X. Liu, K. Bhalla, C. N. Kim, A. M. Ibrado, J. Cai, T. I. Peng, D. P. Jones, and X. Wang.** 1997. Prevention of apoptosis by Bcl-2: release of cytochrome *c* from mitochondria blocked. Science **275:**1129–1132.