Cell Death and Differentiation (2002) 9, 1167–1171

www.nature.com/cdd

News and Commentary

Retinoid-related orphan receptors (RORs): roles in cell survival, differentiation and disease

AM Jetten*[,1] and E Ueda[1]

[1] Cell Biology Section, Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of HealthResearch Triangle Park, North Carolina, NC 27709, USA

* Corresponding author: E-mail: jetten@niehs.nih.gov

Cell Death and Differentiation (2002) **9,** 1167–1171.
doi:10.1038/sj.cdd.4401085

Nuclear receptors constitute a superfamily of ligand-dependent transcription factors, that include receptors for steroid hormones, retinoids, thyroid hormone, and eicosanoid metabolites, and orphan receptors for which the ligand has not yet been identified. The retinoid-related orphan receptors alpha, beta and gamma (RORα, β, and γ also referred to as NR1F1 to -3, respectively) comprise a distinct subfamily of nuclear receptors.[1] RORs share a modular structure composed of an amino terminal domain, a DNA-binding domain, a hinge- and a ligand binding domain (LBD), that is typical of nuclear receptors. Through alternative splicing and promoter usage each ROR gene generates two or more isoforms (e.g. RORγ1 and γ2) that differ only in their amino terminus. The utilization of different promoters results in a cell type-specific expression of certain ROR isoforms. As a consequence these isoforms regulate different physiological processes and target genes. However, in cells where RORs are co-expressed, their functions may overlap. RORs regulate transcription by binding as a monomer to ROR-response elements (ROREs) consisting of the consensus sequence AGGTCA preceded by a 6 bp A/T rich region in the promoter regulatory region of target genes.[2,3] Despite intensive research, to date few ROR target genes have been positively identified (Table 1).

Transcriptional regulation by RORs is mediated via an interaction with co-repressor or co-activator complexes which through their histone deacetylase or acetylase activities, respectively, induce changes in chromatin structure and mediate the interaction of RORs with the basic transcriptional machinery.[1,4,5] RORs interact with co-repressors, including nuclear co-repressor (N-CoR), and co-activators, such as steroid receptor co-activator 1 (SRC-1), suggesting that they can function as repressors and activators of transcription.[1] Whether the activity of RORs is regulated by ligands or modulated by other signaling pathways is not yet clear. A recent study demonstrated that RORα- and γ-mediated transcriptional activation could be significantly enhanced by Ca^{2+}-dependent calmodulin kinase IV (CaMKIV).[6] These observations potentially link regulation of gene expression by RORs to a variety of signaling pathways that cause an increase in intracellular Ca^{2+}. Although the mechanism of this stimulation of transactivation is far from understood, it does not appear to involve direct phosphorylation of RORs by CaMKIV. Alternatively, the increase in ROR-mediated transcriptional activation by CaMKIV may be mediated by phosphorylation and activation of a specific co-activator, inactivation of a co-repressor or increased synthesis of an endogenous ligand.[1,6] Activation of the transcription factor MEF2 by CaMKIV has been reported to be linked to phosphorylation to histone deacetylases and their subsequent export to the cytoplasm.[7] Such a mechanism may also account for the CaMKIV-enhanced transcriptional activation by RORs.

Recent studies of ROR-deficient mice have implicated RORs in the regulation of a number of biological processes and revealed potential roles for these proteins in several pathologies (Table 1).[1] ROR$\alpha^{-/-}$ mice and *staggerer* (*sg*/*sg*) mice, a natural mutant strain disrupted in RORα expression, display an ataxic phenotype caused by severe cerebellar neurodegeneration.[8–10] The latter involves a defective regulation of the differentiation and/or maintenance (possibly increased apoptosis) of cerebellar Purkinje cells. Interestingly, the cerebellar anomalies observed in hypothyroid animals are very similar to those displayed by RORα-deficient mice and involve extensive apoptosis in cerebellar granule cells.[11] Whether the cerebellar neurodegeneration in RORα-deficient mice are mediated through a similar mechanism needs further study. RORα-deficient mice also exhibit abnormalities in bone formation and maintenance of bone tissue indicating that RORα functions as a positive regulator of osteogenesis.[12] These studies suggest that changes in RORα signaling could play a role in osteoporosis. In addition, ROR$\alpha^{-/-}$ mice display an increased susceptibility to arteriosclerosis and show alterations in several immune responses.[13,14] Overexpression of RORα has been reported to suppress TNFα-induced expression of COX-2, IL-6 and IL-8 in smooth muscle cells.[15] This suppression appears to be linked to the transcriptional activation of the *IκBα* gene by RORα through an RORE present in the NF-κB promoter. This would subsequently lead to an increase in IκBα and inhibition of the NF-κB signaling pathway. These observations suggest that RORα may function as a negative regulator of inflammatory responses *in vivo*.

Compared to RORα, which is widely expressed, the expression of RORβ is much more restricted.[1] RORβ is expressed in regions of the central nervous system involved in processing sensory information, in the retina, and pineal gland. ROR$\beta^{-/-}$ mice manifest a duck-like gait and become blind due to a degenerative loss of the retina.[16] Whether this degeneration is mediated through apoptosis has yet to be determined. The abnormalities in circadian rhythm displayed by ROR$\beta^{-/-}$ mice and the differential regulation of RORβ expression in the pineal gland as a function of circadian rhythm suggest a role for RORβ in the control of circadian rhythm.[16,17]

Table 1 Characteristics of members of the ROR subfamily

Receptor type[a]	Human chromosomal localization	Tissue/cell type specific expression	Biological functions	Phenotype of ROR-deficient mice	Putative target genes[b]
$ROR\alpha_{1-4}$	15q21–22	Various tissues, including brain (cerebellum, thalamus), kidney, lung, testis, hair follicle	Purkinje cell development, bone metabolism, immune response	Cerebellar neurodegeneration, deficits in muscular coordination, changes in immune responses, hypoalphalipoproteinemia, osteopenia, and increased susceptibility to arteriosclerosis	Apolipoprotein A–I, lipoxygenase 5, Purkinje cell protein-2, $I\kappa B\alpha$
$ROR\beta_{1,2}$	9q22	Retina, brain (thalamus, supraoptic nerve, suprachiasmatic nucleus, pineal gland), epididymus	Circadian rhythm	Blind (degeneration of retina), changes in circadian behavior, duck-like gait	
$ROR\gamma_{1,2}$	1q21	Various tissues, including liver (hepatocyte), kidney, heart, brown fat, thymus (DP thymocytes), lung (alveolar Type I cells), lymph node progenitor cells ($CD3^-CD4^+CD45^+IL\text{-}7R\alpha^+$)	Lymph node organogenesis, thymopoiesis	Lack of lymph nodes, increased apoptosis in DP thymocytes, changes in DN to DP transition, increased susceptibility to thymic lymphomas	T early alpha, Bcl-x_L

[a]Subscript indicates ROR isoforms identified in mouse. [b]No proven target gene has yet been identified. Genes indicated may be regulated either directly or indirectly by RORs

Recently, studies of mice deficient in the expression of RORγ unveiled several important physiological functions for this orphan receptor.[1,18,19] ROR$\gamma^{-/-}$ mice lack all lymph nodes and Peyer's patches suggesting that RORγ is essential for the development of secondary lymphoid organs. RORγ is highly expressed in $CD3^-CD4^+CD45^+IL\text{-}7R\alpha^+$ cells, early progenitors of lymphoid organ development. The lack of lymph node organogenesis in ROR$\gamma^{-/-}$ mice is likely related to the loss of this population of precursor cells. Although the molecular mechanism by which RORγ regulates lymph node development has yet to be elucidated, RORγ appears to be required for differentiation or survival of these progenitor cells.

Another anomaly observed in ROR$\gamma^{-/-}$ mice is associated with thymopoiesis. The thymus of 6–8-week-old ROR$\gamma^{-/-}$ mice contains a greatly reduced number of thymocytes compared to thymus from wild-type mice.[18,19] Homeostasis in the thymus is maintained by a delicate balance between proliferation, differentiation, selection, and apoptosis.[20] Loss of RORγ expression enhances apoptosis as well as proliferation of distinct thymocyte populations. These observations indicate that loss of RORγ disturbs homeostasis in the thymus. In addition to increased apoptosis *in vivo*, the rate of apoptosis of cultured ROR$\gamma^{-/-}$ thymocytes is greatly accelerated.[18,19] Normal thymocytes undergo 'spontaneous' apoptosis over a period of 3–4 days of culture while accelerated apoptosis in ROR$\gamma^{-/-}$ thymocytes occurs within several hours.

Thymopoiesis is a tightly regulated developmental program controlled by multiple signaling pathways involving cell:cell interactions, various cytokines and different transcription factors.[20–23] The major stages in thymopoiesis are defined by the expression of the cell membrane co-receptors CD8 and CD4. At the first stage, $CD4^-CD8^-$ (double negative, DN) thymocytes undergo a series of distinct changes, including TCRβ rearrangement and extensive proliferation, before they differentiate into $CD4^+CD8^+$ (double positive, DP) cells. After TCRα-recombination and expression, DP thymocytes containing TCRs with intermediate affinity for self-peptide-major histocompatibility complexes (MHC) are selected and continue maturation (positive selection) into single positive (SP) $CD4^+CD8^-$ (T helper) or $CD4^-CD8^+$ cells. During this selection process more than 95% of the immature DP thymocytes, cells with low or high affinity for self-antigen-MHC complexes, are eliminated by apoptosis through processes referred to as death by neglect or negative selection, respectively. The precise mechanisms of these two processes of apoptosis are still poorly understood.[24,25] In ROR$\gamma^{-/-}$ mice the number of both DP and SP cells are greatly diminished due to increased apoptosis in DP cells.[18,19] This enhanced apoptosis together with the fact that the expression of RORγ is largely restricted to DP thymocytes suggest that this population is a target for RORγ regulation. The enhanced cell death in DP thymocytes does not require TCR expression and is not mediated through the Fas-activated signaling pathway since DP thymocytes from ROR$\gamma^{-/-}$ mice that were made defective in TCRα expression or Fas ligand (FasL) function did not differ in their phenotype from those of ROR$\gamma^{-/-}$ mice.[19] Moreover, expression of FasL was not altered in ROR$\gamma^{-/-}$ thymocytes. These observations suggest that this increase in apoptosis does not occur through negative selection but appears to mimic death by neglect. Recent studies have shown that in addition to enhancing the survival of DP thymocytes, RORγ promotes the maturation of DN cells, and in particular that of the immature CD8 single positive (ISP) subpopulation,[26] into DP thymocytes.[27]

Analysis of Bcl-2 family members revealed that the expression of Bcl-x_L mRNA and protein was almost totally repressed in ROR$\gamma^{-/-}$ thymocytes (Figure 1).[18,19] Little change was observed in the expression of proapoptotic Bax and Bak mRNAs. However, Bax protein which in normal thymocytes is largely localized to the cytoplasm, is translocated to the mitochondria in ROR$\gamma^{-/-}$ thymocytes. Since Bax can form an oligomer with Bcl-x_L, the repression of Bcl-x_L may be directly responsible for the translocation of Bax. Thymocytes from ROR$\gamma^{-/-}$ mice expressing Bcl-x_L under the control of the proximal promoter of the *lck* gene, which directs expression to DP thymocytes, exhibit a lifespan and rate of apoptosis similar to those of wild-type thymocytes. These findings suggest that suppression of Bcl-x_L is an essential and early event in the enhanced apoptosis in ROR$\gamma^{-/-}$ thymocytes. This conclusion is in agreement with studies showing that loss of Bcl-x_L comprises the survival of DP thymocytes in Bcl-$x_L^{-/-}$ mice.[28] Interestingly, during thymopoiesis the expression of Bcl-x_L, as that of RORγ2, is restricted to DP thymocytes.[28,29] The fact that Bcl-x_L expression is repressed in ROR$\gamma^{-/-}$ thymocytes, coupled with the observation that RORγ and Bcl-x_L are co-expressed in wild-type DP thymocytes suggests that RORγ positively controls *Bcl-x_L* transcription. However, little is known about the transcriptional regulation of the *Bcl-x_L* gene in thymocytes and the mechanism by which RORγ controls *Bcl-x_L* expression. RORγ may regulate *Bcl-x_L* expression directly by interacting with an RORE in the promoter regulatory region of *Bcl-x_L*. Although no RORE-like sequence was identified in the 700 bp promoter flanking region of *Bcl-x_L*, ROREs important for RORγ-mediated regulation could be located further up- or downstream of this region. Alternatively, RORγ may regulate *Bcl-x_L* expression indirectly by regulating the expression of other transcription factors or signaling pathways. Rel/NF-κB transcription factors have been implicated in the up-regulation of *Bcl-x_L* expression and shown to promote survival of lymphocytes.[30,31] It has to be established whether changes in the Rel/NF-κB signaling pathway are involved in the regulation of *Bcl-x_L* and survival of DP thymocytes in ROR$\gamma^{-/-}$ mice.

The activity of cyclin-dependent kinase 2 (CDK2) was found to be dramatically enhanced in thymocytes from RORγ-deficient mice.[19] Roscovitine, an inhibitor of CDK2 activity, greatly inhibits accelerated apoptosis in RORγ-deficient thymocytes suggesting that CDK2 plays an active role in this induction of apoptosis. Although roscovitine inhibits apoptosis, it does not totally prevent apoptosis suggesting that alternative apoptotic pathways may operate in ROR$\gamma^{-/-}$ thymocytes. The observed reduction in the level of the CDK-inhibitor $p27^{Kip1}$ in ROR$\gamma^{-/-}$ thymocytes appears to be at least partly related to the increase in CDK2 activity (Figure 1). Exogenous expression of Bcl-x_L blocks accelerated apoptosis in ROR$\gamma^{-/-}$ thymocytes and prevents the increase in CDK2 activity and the reduction in $p27^{Kip1}$ suggesting that Bcl-x_L acts upstream of these proteins.[19] A similar relationship between Bcl-2/Bcl-x_L expression, CDK2 activity and the level of $p27^{Kip1}$ protein has been observed in thymocytes during dexamethasone-mediated apoptosis.[32,33] The regulation of CDK2 activity by Bcl-x_L and Bcl-2 in thymocytes appears to be linked at least in part to a delay in the degradation of $p27^{Kip1}$ by the

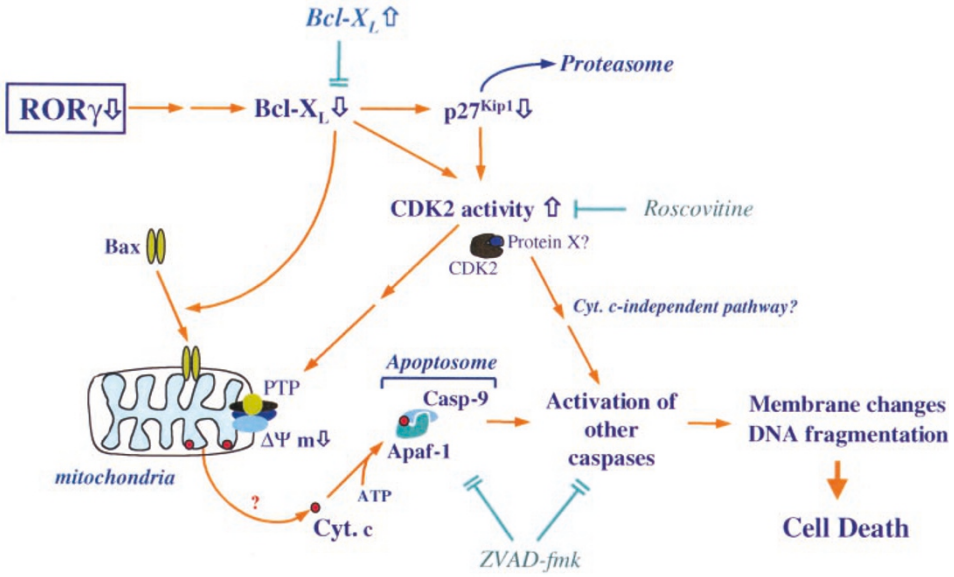

Figure 1 RORγ regulates the survival of DP thymocytes. Loss of RORγ expression results in repression of Bcl-x_L expression which in turn leads to degradation of $p27^{Kip1}$ protein by the proteasome and increased CDK2 activity. The repression of Bcl-x_L further induces the translocation of Bax to the mitochondria, dissipation of $\Delta\Psi_m$ and subsequently the activation of caspases and cell death. CDK2 activity plays an active role in accelerated apoptosis since the CDK2 inhibitor roscovitine inhibits the collapse of $\Delta\Psi_m$ and steps further downstream in apoptosis. The apoptosis promoting function of CDK2 may be related to changes in the specificity of its kinase activity due to either its association with a protein other than cyclin A or E (a yet unidentified protein X) or to alterations in its subcellular localization. Ectopic expression of Bcl-x_L restores survival of DP thymocytes. Whether accelerated apoptosis in ROR$\gamma^{-/-}$ thymocytes involves release of cytochrome *c* or is mediated by a cytochrome *c*-independent pathway has yet to be established. The pancaspase inhibitor ZVAD-fmk has little effect on the dissipation of $\Delta\Psi_m$ but inhibits steps further downstream in the execution phase of apoptosis

proteasome.[34] Although increased CDK2 activity plays an important role in the induction of apoptosis in thymocytes, how it affects apoptosis is not understood. The apoptosis-promoting role of CDK2 contrasts with the well-established function that nuclear CDK2-cyclin A or -cyclin E complexes have in the regulation of cell cycle progression. Interestingly, in its apoptosis-promoting role in dexamethasone-induced apoptosis, CDK2 was not found in complex with cyclin A or E[34] and it has been suggested that CDK2 may be associated with another protein that may change the target specificity of the CDK2 kinase. Alternatively, the apoptotic fate of the cell may depend on the subcellular localization of CDK2 as has recently been demonstrated in ultraviolet irradiation-induced apoptosis in mesangial cells.[35] The apoptosis enhancing activity of CDK2 may involve phosphorylation of (a) specific target protein(s) that promote(s) cell death. Since roscovitine inhibits the loss of mitochondrial membrane potential ($\Delta\Psi_m$), elevation of CDK2 activity may influence the function of proteins involved in controlling $\Delta\Psi_m$ (Figure 1).

As mentioned above, ROR$\gamma^{-/-}$ DP thymocytes undergo accelerated apoptosis when placed in culture.[18,19] This apoptosis is associated with loss of $\Delta\Psi_m$, an increase in caspase activity and annexin V binding, and ultimately internucleosomal degradation. Release of cytochrome *c* from mitochondria into the cytoplasm is often observed during early stages of apoptosis. Cytochrome *c* has been demonstrated to form a complex with APAF-1 and pro-caspase 9, referred to as apoptosome, resulting in the activation of caspase 9.[36] However, preliminary results appear to suggest that apoptosis in ROR$\gamma^{-/-}$ thymocytes may not involve mitochondrial release of cytochrome *c*. Future studies have to determine the role of cytochrome *c*-dependent and -independent mechanisms play in this apoptosis. The broad-spectrum caspase inhibitor ZVAD-FMK inhibited but did not block apoptosis and had little effect on the collapse of $\Delta\Psi_m$ suggesting that caspase activation may not be involved in early steps of accelerated apoptosis in ROR$\gamma^{-/-}$ thymocytes. The relationship between the repression of Bcl-x_L, Bax translocation, increased CDK2 activity, collapse of $\Delta\Psi_m$, and apoptosis is still controversial and not yet completely understood.[37–41] Although the molecular mechanism of accelerated apoptosis in cultured ROR$\gamma^{-/-}$ thymocytes is not yet precisely known, clearly the down-regulation of Bcl-x_L and increased CDK2 activity are critical events. However, other signals, e.g. reactive oxygen species or loss of cell–cell interactions, may act synergistically in accelerating apoptosis in cultured ROR$\gamma^{-/-}$ thymocytes.

Although ROR$\gamma^{-/-}$ mice are initially healthy, they are highly susceptible to early onset of thymic lymphoma.[42] At 4 months of age more than 50% of the mice have died as a result of T cell lymphoma formation. These tumor cells frequently metastasize to liver and spleen and occasionally to kidney. No other tumor types have been detected in ROR$\gamma^{-/-}$ mice. Although analysis of the immunophenotype of the lymphoblastic cells showed some heterogeneity in CD4/CD8 subpopulations among the different ROR$\gamma^{-/-}$ lymphomas often an increase in the number of (immature?) SP CD8$^+$ cells was observed. All T cell lymphomas stain highly for PCNA and TUNEL indicating a high rate of both proliferation and apoptosis within the tumors. ROR$\gamma^{-/-}$ lymphoma cells also undergo accelerated apoptosis when placed in culture. This apoptosis is, in contrast to that of ROR$\gamma^{-/-}$ thymocytes, resistant to inhibition by roscovitine suggesting differences in the regulation of apoptosis between lymphoblastic cells and ROR$\gamma^{-/-}$ thymocytes. Lymphoma formation does not appear to involve alterations in p53 expression. Although the mechanism that leads to lymphoma development has yet to be determined, it likely relates to the changes in control proliferation and survival of thymocytes in ROR$\gamma^{-/-}$ mice. Increased proliferation of ROR$\gamma^{-/-}$ thymocytes may promote the probability of genetic alterations in tumor suppressor or proto-oncogenes and as a consequence lead to development of lymphoma.[42] Future identification of target genes may help in elucidating the molecular mechanisms by which RORγ controls thymic homeostasis and lymphoma development.

Recently, the crystal structure of the LBD of RORβ in complex with stearate, a fortuitous ligand that co-purifies with the protein, and a NR-box peptide of the coactivator SRC-1 has been determined.[43] These studies revealed that RORβ and γ contain a relatively large ligand binding pocket of 766[3] and 705,[3] respectively, while that of RORα is smaller (568).[3] Stearate occupies only a small fraction of the ligand binding pocket and does not induce RORβ-mediated transactivation suggesting that it does not function as a true ligand. Based on their studies, Stehlin *et al*.[43] concluded that the activity of RORs is regulated by a ligand-mediated mechanism. However, based on homology modeling of RORα, Harris *et al*.[44] concluded that the RORα is a constitutively active receptor. Although the question of whether the activity of RORs is regulated by endogenous ligands is still controversial, insight into the structure of the binding pockets of RORs may enhance the design of synthetic agonists and antagonists that regulate the activity of RORs. Such synthetic compounds will provide powerful tools in the study of ROR signaling pathways and in the identification of ROR target genes. Although the precise roles for RORs in human disease still have to be determined, recent studies have revealed potential roles for RORs in arteriosclerosis, osteoporosis, cancer, and anomalies in circadian behavior.[12–16,42] Discovery of ROR ligands may lead to the development of novel therapeutic strategies for these diseases.

1. Jetten AM *et al*. (2001) Prog. Nucl. Acid Res. 69: 205–247
2. Giguere V *et al*. (1994) Genes Dev. 8: 538–553
3. Yan ZH *et al*. (1997) J. Biol. Chem. 272: 10565–10572
4. Glass CK and Rosenfeld MG (2000) Genes Dev. 14: 121–141
5. McKenna NJ *et al*. (1999) J. Steroid Biochem. Mol. Biol. 69: 3–12
6. Kane CD and Means AR (2000) EMBO J. 19: 691–701
7. McKinsey TA *et al*. (2002) Trends Biochem. Sci. 27: 40–47
8. Dussault I *et al*. (1998) Mech. Dev. 70: 147–153
9. Hamilton BA *et al*. (1996) Nature 379: 736–739
10. Steinmayr M *et al*. (1998) Proc. Natl. Acad. Sci. USA 95: 3960–3965
11. Xiao Q and Nikodem VM (1998) Front Biosci. 3: A52–A57
12. Meyer T *et al*. (2000) Proc. Natl. Acad. Sci. USA 97: 9197–9202
13. Kopmels B *et al*. (1992) J. Neurochem. 58: 192–199
14. Mamontova A *et al*. (1998) Circulation 98: 2738–2743

15. Delerive P *et al.* (2001) EMBO Rep. 2: 42 – 48
16. Andre E *et al.* (1998) EMBO J. 17: 3867 – 3877
17. Schaeren-Wiemers N *et al.* (1997) Eur. J. Neurosci. 9: 2687 – 2701
18. Kurebayashi S *et al.* (2000) Proc. Natl. Acad. Sci. USA 97: 10132 – 10137
19. Sun Z *et al.* (2000) Science 288: 2369 – 2373
20. Sebzda E *et al.* (1999) Annu. Rev. Immunol. 17: 829 – 874
21. Fehling HJ and von Boehmer H (1997) Curr. Opin. Immun. 9: 263 – 275
22. Ellmeier W *et al.* (1999) Annu. Rev. Immunol. 17: 523 – 554
23. Killeen N *et al.* (1998) Curr. Opin. Immunol. 10: 360 – 367
24. Zhang J *et al.* (2000) J. Immunol. 165: 2970 – 2974
25. Bouillet P *et al.* (2002) Nature 415: 922 – 926
26. Miyazaki T (1997) J. Exp. Med. 186: 877 – 885
27. Guo J *et al.* (2002) Nat. Immunol. 3: 469 – 476
28. Ma A *et al.* (1995) Proc. Natl. Acad. Sci. USA 92: 4763 – 4767
29. He YW *et al.* (1998). Immunity 9: 797 – 806
30. Hettmann T *et al.* (1999) J. Exp. Med. 189: 145 – 158
31. Chen C *et al.* (2000) Mol. Cell. Biol. 20: 2687 – 2695
32. Hakem A *et al.* (1999) J. Exp. Med. 189: 957 – 968
33. Distelhorst CW (2002) Cell Death Differ. 9: 6 – 19
34. Gil-Gomez G *et al.* (1998) EMBO J. 17: 7209 – 7218
35. Hiromura K *et al.* (2002) Oncogene 21: 1750 – 1758
36. Zou H *et al.* (1999) J. Biol. Chem. 274: 11549 – 11556
37. Vander Heiden MG *et al.* (1997) Cell 91: 627 – 637
38. Zamzami N and Kroemer G (2001) Nat. Rev. Mol. Cell. Biol. 2: 67 – 71
39. Zimmermann KC *et al.* (2001) Pharmacol. Ther. 92: 57 – 70
40. De Giorgi F *et al.* (2002) FASEB J. 25: in press
41. Ott M *et al.* (2002) Proc. Natl. Acad. Sci. USA 99: 1259 – 1263
42. Ueda E *et al.* (2002) Cancer Res. 62: 901 – 909
43. Stehlin C *et al.* (2001) EMBO J. 20: 5822 – 5831
44. Harris JM *et al.* (2002) Mol. Endocrinol. 16: 998 – 1012