NF-κB has entered the nucleus, as has been proposed on the basis of genetic and biochemical analysis[11,12].

We also examined the claim that Akt activates IKK through phosphorylation of IKKα at T23. The results of Ozes *et al.* were obtained *in vitro*[5], but we examined whether endogenous IKKα or IKKβ are phosphorylated on threonine residues in TNFα-treated cells. In both non-stimulated or TNFα-stimulated cells, IKKα and IKKβ were phosphorylated only at serine residues and no threonine phosphorylation could be detected (data not shown). In addition, overexpression of constitutively active Akt in HEK293 cells did not result in stimulation of IKKα-associated IκB kinase activity (data not shown).

It should also be noted that IKK can be fully activated by TNFα or interleukin-1 in IKKα-deficient cells[7,8]. Thus, IKKα phosphorylation by Akt or any other protein kinase is not essential for IKK activation. Although PI(3)K activation can potentiate NF-κB activity in the nucleus by IKK-independent mechanisms[11,12], we find no evidence for the involvement of the PI(3)K–Akt signalling pathway in IKK activation, which is essential for sending NF-κB to the nucleus[1].

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Ozes et al. *reply* — Delhase *et al.* take issue with our claim[1] that Akt induces activation of NF-κB by phosphorylating IKKα, contending that IKKα plays no role in the activation by TNF of NF-κB, and consequently that Akt could not affect NF-κB through IKKα. They point out that Hu *et al.*[2] have shown that cells deficient in IKKα have normal TNF-induced NF-κB activity, but this has been refuted by Li *et al.*[3], who reported significant reduction of TNF-induced NF-κB in IKKα-deficient cells. Indeed, the observations of Hu *et al.*[2] show that degradation of IκBα is diminished in cells from IKKα-deficient mice and are therefore not consistent with the conclusion that IKKα plays no role in TNF induction of NF-κB. Furthermore, deficiency of IKKβ only partially impairs TNF-induced NF-κB activation[4,5], which reserves a role for IKKα in this pathway. Others[5–7] have shown that activation of the IKK complex is dependent on the kinase activity of IKKα to activate IKKβ. Thus, strong evidence supports a role for IKKα in TNF induction of NF-κB.

Delhase *et al.* only tested the role of Akt on NF-κB activation in HeLa cells in which they did not observe activation of Akt by TNF. As the involvement of inflammatory stimuli, including TNF, TRAF-6, IL-1 and LPS[1,8–12] in PI(3)K/Akt activation is well documented, Delhase *et al.* should have investigated the Akt/NF-κB connection in some of these systems.

The properties of HeLa cells are known to vary greatly among different laboratories, and the inability of Delhase *et al.* to detect Akt activation in a particular line of HeLa cells is not only inconsistent with our observations[1] but also with others[13,14]. In view of our results, it is interesting that NF-κB reporter gene activity in HeLa cells can be induced by constitutively active Akt and that this is inhibited by dominant-negative IKKβ (ref. 15). We have done experiments in seven cell types and found that the amount of Akt, the extent to which it is activated by TNF, and its effects on IKKα are cell-type specific.

PI(3)K/Akt signalling has been implicated in NF-κB activation induced by inflammatory[1,8,11], mitogenic[16] and oncogenic stimuli[9,17], as well as by T-cell activation[17] and signalling through G-protein receptors[15]. Significant mechanistic differences exist as to how Akt is incorporated into NF-κB signalling, with the most unsettled issue being the role of the IκB kinase in NF-κB activation. Several groups[1,9,16] have reported direct physical and functional interactions between Akt and IKK, and as the only established function of IKK is to phosphorylate IκB, this suggests that IκB is a target for Akt[1,9,16,18].

Others have found no evidence for the involvement of Akt in IκB degradation, but rather propose an IκB-independent mechanism in which Akt affects the transcriptional activity of NK-κB (refs 8,11,17). However, even those in favour of an IκB-independent mechanism acknowledge the requirement for IKK activation for Akt effects on NF-κB transactivation[17]. This raises the question of why, if IKK activity is required for regulation of the transactivation function of NF-κB, activated IKK has stopped acting on IκB, its preferred substrate.

These disparate observations point to deficiencies in our understanding of NF-κB activation, and also suggest that the Akt/NF-κB connection is cell-type- and stimulus-specific. Consistent with this idea, constitutively active Akt alone induces NF-κB reporter gene activity in some cells[1,11,15,17], whereas in others, signals from other pathways are required for Akt to manifest its effect[8,18]. Likewise, activation of NF-κB by TNF is mediated by a PI(3)K/Akt pathway in some cell types[1,8] but not others[12,16]. We believe that the attempt of Delhase *et al.* to define a complex and incompletely understood signalling system based on experiments with a single cell type is an example of misplaced reductionism.

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Correction

Energy for microbial life on Europa

C. Chyba

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In this communication, I estimated the biomass that could be supported by mixing the ice irradiation products HCHO and H_2O_2 into Europa's ocean, finding values that ranged from ~10^7 g to ~10^{10} g. However, a calculational error in the higher estimate has recently come to my attention (E. J. Gaidos, K. N. Nealson and J. L. Kirschvink, personal communication). The correct result is ~10^8 g, making detectability of potential Europan life under this model considerably more difficult. I present a revised calculation and discuss related practical issues in the search for life on Europa at http://www.seti.org/pdf/chyba_lander.pdf.

Erratum

Do cockroaches 'know' about fluid dynamics?

D. Rinberg, H. Davidowitz

Nature **405,** 756 (2000)

The units of the r.m.s. of the square of the wind velocity shown in the *x*-axis label of Fig. 1a should be $cm^2\ s^{-2}$, and not $m^2\ s^{-2}$ as published.