
**100 YEARS AGO**

**It seems but the other day I saw London in a blaze of illumination to celebrate Her Majesty's happy accession to the throne. As in a few days the whole empire will be celebrating the Diamond Jubilee of our Queen, who will then have reigned over her multitudinous subjects for sixty years, what more suitable topic can I bring before you than that of diamonds! One often hears the question asked: "Why *Diamond* Jubilee?" I suppose it is a symbol intended to give a faint notion of the pure brilliancy and durability of the Queen's reign; and in thus associating Her Majesty with the precious diamond, to convey an idea of those noble qualities, public and private, which have earned for her the love, fealty, and reverence of her subjects. From the earliest times the diamond has occupied men's minds. ... The philosopher Steffans is accredited with the dictum that "Diamond is quartz which has arrived at self-consciousness!" and an eminent geologist has parodied this metaphysical definition, saying, "Quartz is diamond which has become insane!".**

**'Diamonds' – William Crookes, F.R.S.**

From *Nature* 5 August 1897.

**50 YEARS AGO**

**The "Oxford Dictionary" defines 'dialysate' as that portion of a mixture that remains after dialysis, and quotes Attfield's "Text-book of Chemistry" (1885). The quotation shows that the part that fails to pass through the membrane is referred to. From the point of view of the lexicographer, this is perfectly satisfactory; there seems to have been no earlier use of the word in print. ... Present-day usage does not agree with the "Oxford Dictionary". Nearly all the biochemists that I have consulted have understood by dialysate the more diffusible part of the system. At present, therefore, the word is ambiguous unless the context makes the meaning clear. Many authors and editors have not read Attfield, and some of those who have may question his authority in the matter. The popular meaning has probably been adopted because of the analogy with filtrate, distillate, sublimate, etc.; in each case the mobile part of the system is referred to. The continuing existence of this analogy will make it difficult to get universal acceptance of the dictionary meaning of dialysate.**

From *Nature* 9 August 1947.

of IκB now known, then stimulated an intense burst of research which to some extent clarified the signalling events. In response to extracellular stimuli, the IκBα molecule becomes phosphorylated by a serine-specific kinase at positions 32 and 36. This phosphorylation in turn induces polyubiquitination of the molecule, which targets it for rapid degradation (and not for dissociation from NF-κB, as originally postulated)[2].

During the same period, a search for the kinase or kinases responsible for the inducible phosphorylation of IκBα led to a flurry of papers describing the direct or indirect involvement of a number of known kinases in this phosphorylation event. A bona fide IκBα kinase would be expected to specifically phosphorylate serines 32 and 36 of IκBα and to be inducible by the stimuli known to activate NF-κB; unfortunately, none of the postulated kinases fulfilled all these requirements.

This is not the case for the kinase described by DiDonato and colleagues[3]: they have fractionated extracts from HeLa cells stimulated with TNF, and have examined the various fractions for their ability to specifically phosphorylate a fragment of the IκBα molecule containing serines 32 and 36. The authors observed that TNF stimulation of the cells resulted in the rapid activation of a kinase activity with the expected specificity, which eluted from a gel filtration column as a complex of relative molecular mass 900,000 (900K).

Last year, T. Maniatis's group[6] described the partial purification of a 700K complex from non-stimulated HeLa cells which contained a kinase activity able to specifically phosphorylate IκBα on the two critical serines. This kinase was unique in requiring ubiquitination in order to be activated. The same group have since demonstrated[7] that mitogen-activated protein kinase/ERK kinase kinase 1 (MEKK-1), a kinase involved in several signalling pathways and known to be activated by TNF, could directly activate the IκBα kinase activity of the 700K complex *in vitro*, and induce the site-specific phosphorylation of IκBα *in vivo*.

DiDonato *et al.* have purified and cloned the IκBα-specific kinase of their 900K complex, which turned out to be almost identical to a putative serine/threonine kinase of unknown function named CHUK[8]. The protein is composed of 744 amino acids, with a kinase domain in its amino-terminal region and several protein-interaction motifs, including a leucine zipper and a helix–turn–helix motif in its carboxy-terminal part. This kinase can specifically phosphorylate the two serines of IκBα and has been renamed IKKα.

IKKα can also phosphorylate the two critical serine residues of a second IκB species, IκBβ (ref. 9). Its activity is induced by TNF, IL-1 and to a lesser extent by PMA. Transfection experiments confirmed that IKKα overexpression can activate an NF-κB-dependent reporter gene, and that an antisense RNA can inhibit NF-κB activation by TNF, IL-1 or PMA. More interestingly, DiDonato *et al.* found that IKKα activity is sensitive to dephosphorylation by the protein phosphatase PP2A, consistent with the previous observation that NF-κB activity could be induced by okadaic acid, an inhibitor of PP2A, and suggesting that, *in vivo*, IKKα is negatively regulated by PP2A or a similar phosphatase. This in turn leads to the question of the identity of the kinase or kinases responsible for the phosphorylation and activation of IKKα.

So is that it? One obvious question concerns the identity of the other proteins in the 900K complex: although NF-κB/IκB is not present, this complex might contain some of the proteins involved in ubiquitination or in the process of degradation itself (or both). Alternatively, as has been seen in yeast[10], the entire kinase cascade leading to IκBα phosphorylation (or part of it) could be packed in a large complex including adaptor or scaffolding proteins, therefore ensuring speed and accuracy in the transmission of the signal (but maybe leaving less possibility for branching points).

Another question is whether IKKα is the unique integrator of the NF-κB response: in other words, do all the seemingly disparate stimuli known to activate NF-κB lead to the activation of IKKα, which in turn would phosphorylate the three known IκB species, IκBα, β and ϵ? If they do, then this kinase would be the perfect target for drugs aimed at controlling the inflammatory response. The report[11] that the mitogen-activated ribosomal S6 protein kinase, $p90^{rsk}$, can interact with IκBα and phosphorylate the protein upon serine 32 (but not serine 36), both *in vitro* and *in vivo* ($p90^{rsk}$ is induced by PMA, but not by TNF), indicates that the situation might be more complicated and that different stimuli might involve different kinases. Besides, the kinases in a certain number of signalling pathways known to date seem to exist as families, and it is quite possible that IKKα also belongs to such a group of related kinases, with possibly different specificities.

Finally, some discrepancies between the new work and that of the Maniatis group need to be resolved. The 700K complex which Maniatis and colleagues isolated from non-stimulated cells can be activated either by treatment with MEKK-1 or by ubiquitination; DiDonato *et al.* did not observe a requirement for ubiquitination and were unable to activate IKK *in vitro* with MEKK-1. Does this imply that the complexes purified by the two groups are different or differently regulated, while both are relevant to IκBα