The NFκB luciferase mouse: a new tool for real time measurement of NFκB activation in the whole animal

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Development of transgenic mice that express luciferase under the control of nuclear factor κB (NFκB) enables real time in vivo imaging of NFκB activity in intact animals

Carlsen H, Moskaug JØ, Fromm SH, *et al.* In vivo imaging of NF-kappa B activity. *J Immunol* 2002;**168**:1441–6.

A wide range of human disorders involves inappropriate regulation of NF-kappaB, including cancers and numerous inflammatory conditions. Toward our goal to define mechanisms through which NF-kappaB leads to the development of disease, we have developed transgenic mice that express luciferase under the control of NF-kappaB, enabling real-time in vivo imaging of NF-kappaB activity in intact animals. We show that in the absence of extrinsic stimulation, strong luminescence is evident in lymph nodes in the neck region, thymus, and Peyer's patches. Treating mice with TNF-alpha, IL-1alpha, or LPS increased the luminescence in a tissue-specific manner, with the strongest activity observed in skin, lungs, spleen, Peyer's patches, and the wall of the small intestine. Liver, kidney, heart, muscle, and adipose tissue displayed less intense activities. Also, exposure of skin to a low dose of UV radiation increased luminescence in the exposed areas. Furthermore, induction of chronic inflammation resembling rheumatoid arthritis produced strong NF-kappaB activity in the affected joints, as revealed by in vivo imaging. Thus, we have developed a versatile model for monitoring NF-kappaB activation in vivo.

Of the many different transcription factors found in mammalian cells by far the best known among scientists and clinicians alike is nuclear factor κB (NFκB). In addition to its pivotal role as a regulator of innate and adaptive immune responses, NFκB has also been linked as a contributory factor in many human disorders.[1] NFκB stimulates transcription of over 150 different genes encoding cytokines, adhesion molecules, immunoreceptors, acute phase proteins, enzymes, and regulators of the cell cycle and apoptosis.[2] Transcription of these genes is stimulated in response to binding of NFκB to a specific 10 base pair DNA sequence (GGGRNNYYCC, where R corresponds to a purine, Y represents a pyrimidine, and N can be any base) located in either the enhancer or upstream promoter regions of a gene. Binding of NFκB to these sequences is tightly regulated by the inhibitory function of the IκB-α protein which forms a complex with NFκB that masks functional domains of the transcription factor required for its nuclear localisation and DNA binding activities. The mechanism by which IκB-α bound NFκB is activated is now well understood (fig 1).[3] Following stimulation of the cell by an extracellular signal, such as the interaction of tumour necrosis factor α with its receptor, the recently discovered IκB kinase (IKK) complex is activated. The IKK complex then catalyses phosphorylation of IκB-α at two N terminal serine residues (ser 32 and 36) which then renders IκB-α a substrate for ubiquitination and finally for degradation by the 26S proteasome. These events are rapid, and within minutes of the initial stimulation event result in the nuclear translocation of active NFκB. A remarkable number of different inducers of the NFκB pathway have been described, including a plethora of cytokines and a surprising variety of different viral and bacterial gene products.[2] Given the ability of NFκB to be activated in response to this diverse range of extracellular stimuli and its subsequent transcriptional activation of a vast number of target genes, it is not surprising that the transcription factor has been implicated in so many disease processes. Gastrointestinal diseases in which NFκB has been linked as a contributor to pathogenesis include fibrogenesis, inflammatory bowel disease, acute pancreatitis, responses to infections by bacteria and viruses, and last but not least cancer.[4]

To date, most of what has been inferred about the potential role played by NFκB in gastrointestinal disease has been derived from a combination of in vitro and ex vivo studies measuring changes in NFκB nuclear localisation or DNA binding activity. The problem with these studies is that they are based on the often incorrect assumption that the transcriptional activity (or *trans*-activation) of NFκB can be directly correlated with its degree of nuclear location and DNA binding. Similarly, the suggestion that many commonly used anti-inflammatory drugs including aspirin, sulphasalazine, and glucocorticoids influence the activation of NFκB is largely based on this assumption. In fact, the degree of *trans*-activation by NFκB is also influenced by additional regulatory mechanisms including post-translational modifications (for example, phosphorylation) of NFκB and its interaction with transcriptional coactivators such as CBP/p300.[5] The paper by Carlsen *et al* describes the production of a new experimental tool that provides a significant advance in our ability to quantify changes in NFκB dependent transcription in real time, in a living animal and in a relatively non-invasive manner. What Carlson *et al* have produced is a transgenic mouse that carries in its genome a firefly luciferase gene under the control of three tandem NFκB DNA binding sites. Consequently, this mouse will express luciferase in any cell in which active NFκB is present; moreover, the level of luciferase expressed is proportional to the level of NFκB *trans*-activation in a cell or tissue. Measurement of luciferase expression is achieved by intravenous injection of the animal with a suitable substrate such as D-luciferin which in the presence of the enzyme leads to a bioluminescent reaction that can be detected in the whole animal using an image intensifier coupled to a CCD camera. As firefly luciferase has only a short biological half-life (two hours), the system is capable of measuring temporal fluctuations in NFκB activity which is a tremendous advance over other systems such as DNA binding assays that only provide a static snapshot of NFκB activity. But perhaps the most impressive advantage is that the system is sufficiently sensitive to image and quantify NFκB activity within the internal organs (including the small intestine and the liver) of the intact animal.

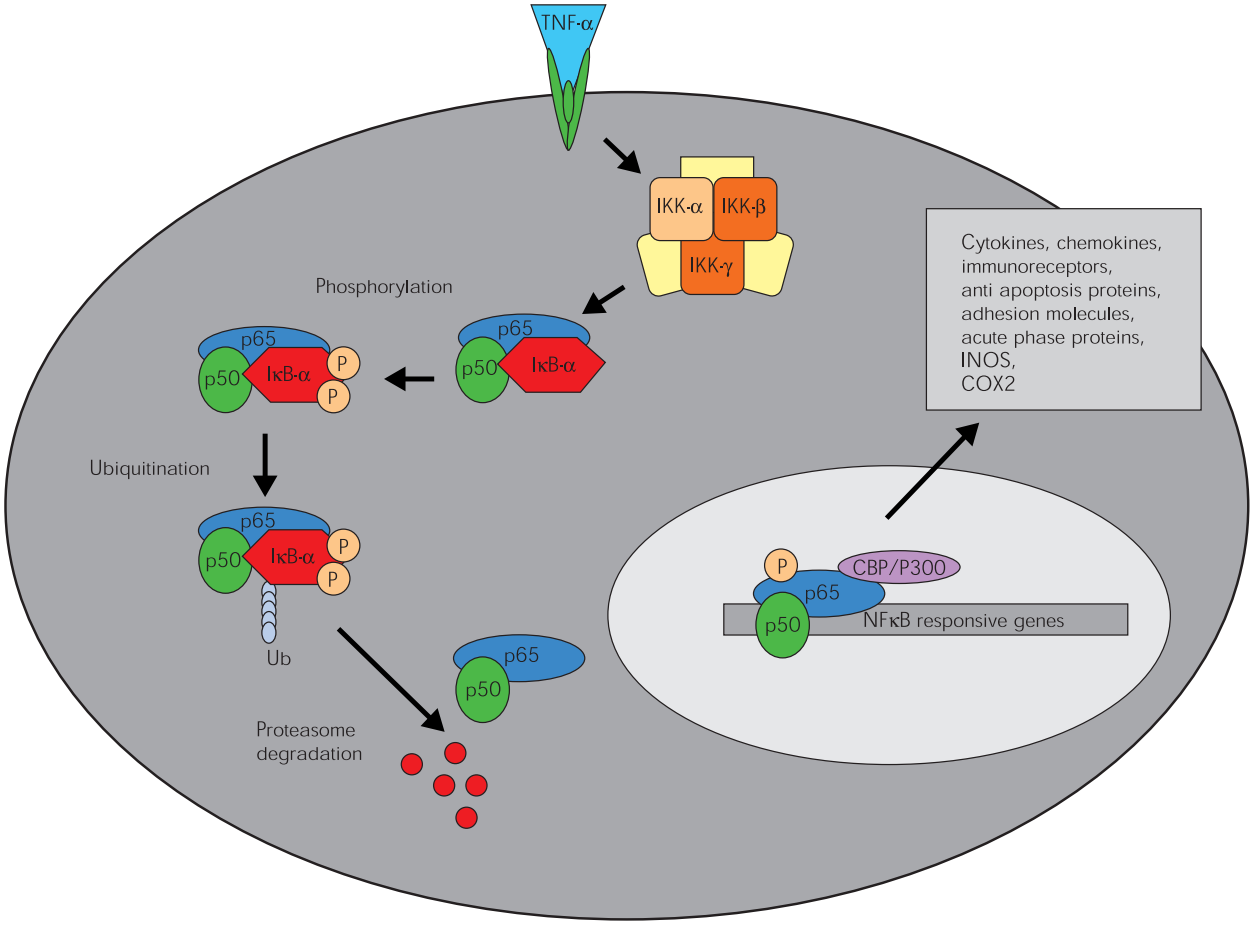

Figure 1 Events leading to activation of nuclear factor κB (NFκB) dependent gene transcription. Following the interaction of a cell surface receptor with its ligand (for example, tumour necrosis factor α (TNF-α)), the IκB kinase (IKK) complex is activated resulting in phosphorylation (P), ubiquitination (Ub), and proteasome mediated degradation of IκB-α. Free NFκB (p65:p50) dimers then translocate to the nucleus where additional phosphorylation events on p65 and recruitment of the coactivator and histone acetyltransferase CBP/p300 leads to transcriptional activation of up to 150 different genes. INOS, inducible nitric oxide synthase; COX2, cyclooxygenase 2.

How does the availability of the NFκB luciferase mouse impact on gastroenterology? The answer is that for the first time we can now properly monitor the influence of exogenous agents and pathological conditions on NFκB activity in the gut and liver. This includes the potential to screen for new drugs that target aberrant NFκB activity. It should also be possible to accurately determine the effective dose and administration regimen for a given anti-NFκB drug in an organ specific manner. Rather than simply determining the delivery and uptake of these drugs into the gut and liver, the NFκB luciferase mouse will now tell us the biological efficacy of the drug within the tissue and over what timeframe the drug can be expected to remain effective. As well as impacting on clinical research the NFκB reporter mouse has tremendous potential in basic research, especially when combined with experimental in vivo models of disease. One of the major problems associated with studying NFκB is its amazing sensitivity to changes in the cellular environment; the very process of isolating tissues and organs from an animal may itself alter NFκB activity. To date it has therefore been virtually impossible to determine changes in the transcriptional activity of NFκB during a disease process. Carrying out in vivo models of gastrointestinal disease in the NFκB luciferase mouse will enable us to accurately quantify the degree to which NFκB dependent transcription is increased throughout the entire course of a disease. Such studies will significantly improve our understanding of the role played by NFκB in gastrointestinal diseases.

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