

Molecular imaging of transcriptional regulation during inflammation

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Abstract

Molecular imaging enables non-invasive visualization of the dynamics of molecular processes within living organisms *in vivo*. Different imaging modalities as MRI, SPECT, PET and optic imaging are used together with molecular probes specific for the biological process of interest. Molecular imaging of transcription factor activity is done in animal models and mostly in transgenic reporter mice, where the transgene essentially consists of a promoter that regulates a reporter gene. During inflammation, the transcription factor NF- κ B is widely involved in orchestration and regulation of the immune system and almost all imaging studies in this field has revolved around the role and regulation of NF- κ B. We here present a brief introduction to experimental use and design of transgenic reporter mice and a more extensive review of the various studies where molecular imaging of transcriptional regulation has been applied during inflammation.

Introduction

Historically conventional imaging techniques as radiography, computed tomography, ultrasonography and magnetic resonance imaging (MRI) were developed to visualize anatomical properties and changes for diagnostic purposes. Molecular imaging, which has emerged as a new discipline during the last decade, attempts to visualize functional properties. European Society for Molecular Imaging defines molecular imaging as the characterization of the dynamics of the molecular processes in the living organisms *in vivo*. The imaging modalities in molecular imaging are SPECT and PET that detect γ - and β - radiation; MRI that detect differences in relaxation time and optical imaging that mainly record luminescent and fluorescent light [1]. Essentially there are two types of molecular methods used in imaging: 1) administration of molecular probes that recognize and bind to a particular biochemical molecule or are activated by a specific process (e.g. enzymatic reaction); 2) reporter genes that are expressed in response to a gene regulatory event. To image activation of transcription factors, most commonly genetic constructs with a promoter coupled to reporter gene are used. This requires introduction of engineered genetic constructs in research animals as in transgenic

reporter mice, which has stably integrated the reporter construct in the genome.

Inflammation involves changes in hemodynamics, recruitment of leucocytes and platelets, and release of numerous signaling and effector molecules. All of this is adapted to type of tissue and stimuli (irritant, injury, infection); additionally it is timely regulated and adjusted to severity of insult. Ideally the inflammatory response is initiated on insult and terminated after homeostasis is reestablished. However, inflammation can become chronic, which is the case for diseases like rheumatoid arthritis and inflammatory bowel disease. The complexity of the inflammatory response requires that its many functional elements are controlled coordinately in some situations and independently in others. This regulation occurs through the specificity of recruited immune cells and their differentiation, signaling pathways and gene expression. Cellular protein composition is crucial for regulation at all levels, which gives transcriptional regulation a central role in orchestrating the inflammatory process. It is suggested that various sets of genes encode the different functional elements and that these genes are coordinately regulated by dedicated transcription factors [2]. For instance by using a systems biology approach in an LPS model a combination of three transcription factors (NF- κ B, ATF3, CEBP/ δ) was demonstrated to coordinate sustained expression of several inflammatory genes [3]. Of these, NF- κ B was regarded as the activator

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and thus illustrates how NF- κ B, which is required for most types of inflammatory responses, can engage in regulation of a specific set of inflammatory genes.

There are several hundred transcription factors involved in inflammation. In spite of this, imaging studies of NF- κ B has dominated research in this field. NF- κ B is attractive for inflammation studies due to the early activation, and the involvement in the large numbers of signaling pathways and the many genes related to immune functions that it controls [4,5]. The NF- κ B family of transcription factors is composed of five members (p50, p52, p65, c-Rel and RelB), which can form various hetero- and homodimers. In resting cells NF- κ B is retained in the cytosol bound to Inhibitors of NF- κ B (I κ Bs). Two distinct NF- κ B activation pathways have been described, the classical and the alternative pathway. In inflammation, the classical NF- κ B pathway is the more important of the two, and it is activated by a large number of stimuli, including proinflammatory cytokines, bacterial and viral products, and stress-inducing stimuli such as γ -radiation, ultraviolet light and reactive oxygen species. These stimuli induce the degradation of I κ B α and the nuclear translocation of mainly the p50/p65 heterodimer. In addition to being central for fighting infections and repair of tissue damage, a number of inflammatory diseases have been associated with elevated NF- κ B activity including rheumatoid arthritis, inflammatory bowel disease, asthma and cardiovascular disease [6-8]. Furthermore, results from animal models with genetic manipulations that either lead to increased or decreased NF- κ B activity demonstrates NF- κ B's significance in regulating inflammatory pathologies [4]. Based on such findings the development of NF- κ B modulators for treatment of inflammatory diseases has been given a lot of attention.

As imaging of transcription factor activity is basically only examined in transgenic reporter mice, we here briefly describe this technology. Furthermore, we review different studies related to transcription and inflammation, foremost connected to NF- κ B.

Design of transgenic reporter mice

Transgenic reporter mice have genomically inserted an engineered DNA construct (called transgene) essentially composed of a promoter and a reporter gene. Studies using reporter mice can determine activity of specific promoters and transcription factors that regulate them, which further can reflect physiological processes, disease progression and experimental manipulations. Reporter mice make possible non-invasive dynamic studies in living animals; meaning that a particular biological process can be monitored both over time and in all organs in the same animal.

Promoters used in transgenes are in principle designed by two approaches: natural promoters taken from a gene

of interest and artificial promoters where a set of selected cis-elements are combined. Often combinations of the two strategies are used. A promoter is composed of a core promoter containing DNA elements necessary for binding the polymerase and initiations of transcription, and a proximal promoter, placed upstream of the core promoter, containing regulatory cis-elements bound by transcription factors (Fig.1). In addition transgenes contain other critical elements including polyA sequences and translational start and stop codons. Furthermore, inclusion of an intron is sometimes used to increase expression efficiency [9].

Reporter genes are genetic markers that encode easily detectable proteins. The half-life of reporter proteins should be within hours when studying dynamic patterns to obtain close relationship with the biological process. Additionally, a short half-life will prevent accumulation of the reporter caused by potential background activity of the promoter. Extensive accumulation of the reporter can mask an induced expression. In basically all imaging studies of transgenic reporter mice, genes that encode bioluminescent or fluorescent proteins are used [10]. The bioluminescent reporter proteins are enzymes that catalyze a chemical reaction leading to light emission from an injected substrate. The most frequently used is firefly luciferase, but also renilla and click beetle luciferases are proven useful. Due to the scattering properties and absorption spectrum of animal tissue, reporter genes that emit light with the longest wavelength are favorable [11]. For fluorescent reporter genes this is particularly critical since autofluorescence of endogenous molecules is much lower at longer wavelengths. There are many fluorescent reporter genes available and new types are frequently introduced [12]. Recently the first infrared fluorescent protein was engineered [13]. The half-life of the fluorescent proteins are usually rather long, but the carboxy-terminal can be modified to reduce the stability of the protein [14,15]. Due to tissue autofluorescence, fluorescent imaging has much lower signal-to-noise ratio than bioluminescent imaging. However, fluorescent imaging, is superior for tomographic examination [11]. Optical imaging can be performed with a relatively rapid and easy procedure, which allows high through-put screening.

Co-regulated expression of different types of reporter genes is required to examine the same biological process with different imaging modalities. While bioluminescence is more sensitive in live animal imaging, fluorescent reporters are nearly always necessary for identification of single cells in tissue samples. In clinical imaging, expression of a therapeutic gene can be followed by co-regulated expression of a PET reporter gene [16]. To obtain co-regulated expression of genes various methods are used such as insertion of internal ribosome entry site (IRES) between the genes, repeated use of similar

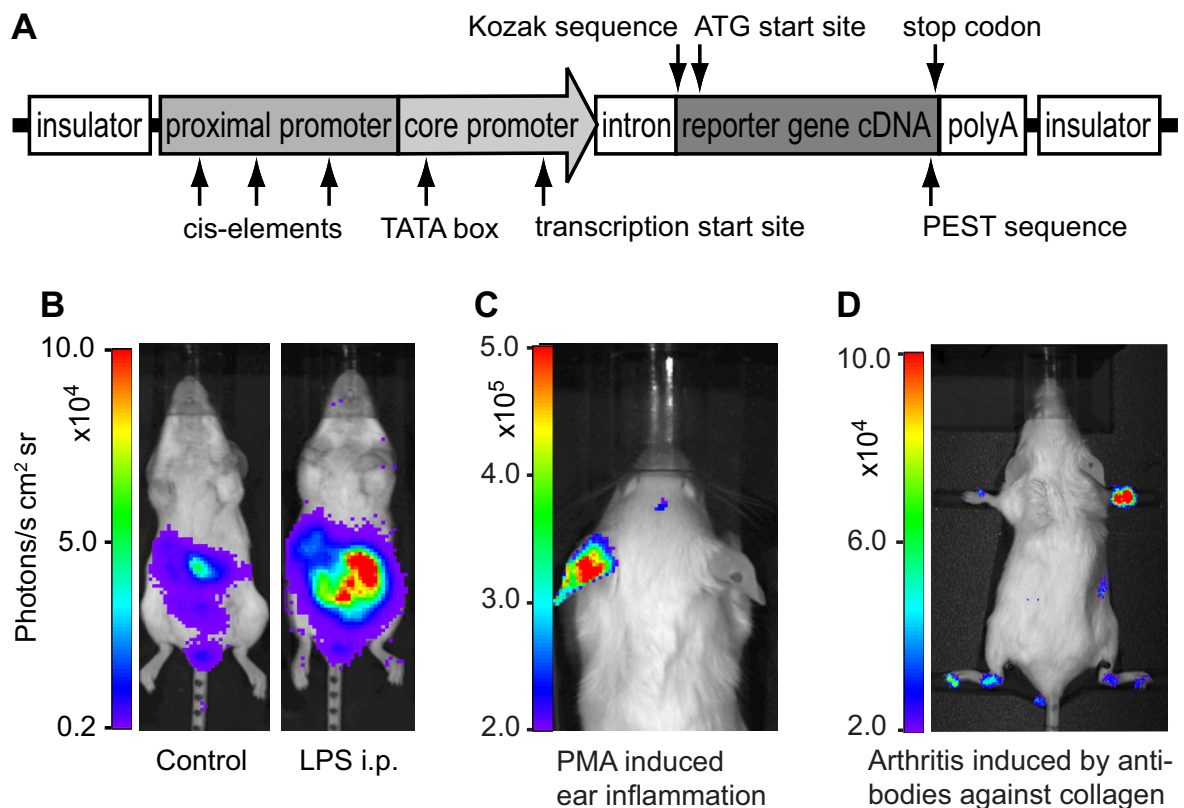


Figure 1 Transgenic reporter mouse. A) Schematic representation of a typical transgene, which is flanked by insulator sequences and including relevant elements for meaningful regulation of reporter gene expression. The core promoter often contains a TATA-box for binding of polymerase II and a transcriptional initiation site. The proximal promoter, which contains regulatory cis-elements, is usually localized upstream to the core promoter; however, enhancer elements can in principal be placed in other parts of the construct. An intron is often included to increase transcription efficiency. The polyA sequence is necessary to stabilize mRNA while the PEST sequence is introduced to exaggerate proteasome degradation and thus decrease the half-life of the protein. This is important to prevent accumulation of the reporter protein and to follow dynamic changes. Finally, the reporter gene needs the necessary elements for successful translation such as Kozak sequence and stop codon. B-D) Imaging of a transgenic reporter mouse after exposure to various inflammatory stimuli. This reporter mouse contains a transgene with NF- κ B sites that regulate expression of firefly luciferase generated.

promoters, polyproteins, fusion proteins and bidirectional promoters. IRES is commonly used, but the expression efficiency of the genes on each side of the IRES vary according to the type of transgene and tissue [17,18]. Multiple promoters are shown to cause mutual interference, which depends on environmental factors [19]. Polyproteins are proteins intermitted with a 2A-like peptide that is "cleaved" during translation [20]. Polyproteins as well as fusion proteins require molecular engineering, which introduce sequence changes that can affect the activity, stability and immunogenicity of the proteins. Bidirectional promoters are naturally occurring in the vertebrate genome, but have not been extensively tested in transgenic models [21].

Transgenic mice are most frequently generated by pronuclear injection of fertilized eggs [22]. With this method the transgene is incorporated more or less randomly and in unpredictable copies in the genome, which create two

problems. Firstly, genomic modification may unfavorably alter the phenotype of the animals because the transgene can be inserted in a location where it affects transcription. Secondly, the genomic DNA surrounding the inserted transgene can cause variable expression in different tissues through the influence of cis-elements and chromatin structure [23]. This is dependent on the location of insertion and a strategy to eliminate the problem is site specific insertion of the transgene. ROSA26 and Hprt are well described genomic loci where integration provides predictable and ubiquitous expression of inserted transgene [24,25]. This method is more laborious than pro-nuclear injection. The transgene is integrated by homologous recombination in embryonic stem cells, which are transferred to blastocysts for production of chimeric mice. A strategy to prevent influence by surrounding DNA of transgenes incorporated by pronuclear injection is the use of insulator elements. These are posi-

tioned at the ends of the transgene and have both enhancer-blocking properties preventing communications from cis-elements positioned outside the transgene, and barrier functions preventing the spread of heterochromatin [26]. As an alternative to generating transgenic reporter mice, which is time and work consuming, one can transfect liver cells by intravenous injection of a reporter construct and then study reporter activity in liver [27]. One drawback is that the expression of the reporter gene will be transient, making it difficult to perform longitudinal studies. Also viral vectors are used to visualize reporters *in vivo*, which have the potential for cell specific transfection [28]. To obtain the expected expression of reporter genes composition of the transgenic construct should be well planned with good understanding of assembled gene sequences. For instance, early versions of the firefly luciferase gene had numerous cis-elements, which took part in regulation of gene expression [29,30]. A list of unique transgenic reporter mice used to study gene regulation in inflammation is shown in table 1.

Applications of NF- κ B transgenic reporter models

The two first transgenic mice that convincingly showed that NF- κ B activity could be imaged *in vivo* either used the HIV long terminal repeat as promoter [31] or a synthetic promoter containing three NF- κ B binding sites from the immunoglobulin κ light chain promoter [32]. Both mice utilized the luciferase from firefly as reporter gene to mediate light emission. Activation of NF- κ B by classical stimuli (LPS, IL-1 β and TNF α) induced the expression of luciferase, which could be followed in the same animal over time and in multiple organs. Increase in NF- κ B activity during development of arthritis was visualized, as well as decrease by the anti-inflammatory agent Dexamethasone. Furthermore, NF- κ B dependent luciferase activity of individual organs was imaged after dissection from sacrificed animals and the signal strength in these *ex vivo* images was equal to the luciferase activity recorded in tissue homogenates. This confirms that the luminescent signal recorded in tissue reflects the actual level of reporter protein. These reporter mice have been used to examine the role of NF- κ B during inflammation, inflammatory mechanisms in general and to evaluate therapeutic strategies. A selection of these studies is reviewed in this chapter with a summary listed in table 2.

Imaging neural regulation of NF- κ B

Previous observations have shown that following acute brain injury, leukocytes are recruited particularly from the liver to the damaged brain. To test the hypothesis that NF- κ B has a critical role in this process, the dynamics of NF- κ B activity was imaged after induction of brain injury by intracerebral injection of IL-1 β . This led to an excep-

tionally rapid NF- κ B activation in the liver, suggestive of a signal transfer that involves the neural system. To determine the role of hepatic NF- κ B, it was selectively inhibited by intravenous adenoviral-mediated delivery of an I κ B α super-repressor. This treatment significantly reduced the number of neutrophils recruited to the brain [33].

The vagus nerve is shown to stimulate anti-inflammatory processes in the gut through cholinergic modulation of macrophages [34], and *in vivo* imaging of the gut region after blocking signaling from the vagus nerve by transection showed increased NF- κ B activity. Furthermore, in mice with experimentally induced colitis cutting the vagus nerve, and thus removing the cholinergic mediated anti-inflammatory signal, exaggerated the NF- κ B activity. This elevated NF- κ B activity coincided with disease severity and reduction in regulatory T-cells [35].

NF- κ B imaging in models of infectious diseases

Various constitutively active forms of I κ B kinases have been virally introduced in airway epithelium to test whether activation of NF- κ B pathways are sufficient to generate lung inflammation [36]. *In vivo* imaging was used to confirm up-regulation of NF- κ B activity in the lung, which also correlated well with disease parameters as cytokines, chemokines and recruitment of neutrophils. Imaging was also used to investigate to what extent duration of NF- κ B activation correlated with outcome of lung inflammation [37]. Models of acute or chronic lung infection were induced in reporter mice with single injection or continuous administration of LPS, respectively. NF- κ B activation was stronger and more sustained in mice with chronic disease, which progressed into more severe lung injury. Furthermore, an NF- κ B inhibitor (BMS-345541), which was delivered after onset of inflammation, reduced disease severity in parallel with reduction in NF- κ B activity. Although NF- κ B activity clearly correlated with disease outcome in the LPS model described above, two other studies shows that the host defense against *Pseudomonas* bacterial infection is impaired when NF- κ B activity is inhibited experimentally [38,39].

Mastitis is defined as inflammation of the mammary gland mainly caused by microbial pathogens, such as bacteria. Mastitis is quite common in breast feeding women, and in the dairy industry intra-mammary infections are of great economical importance due to loss of milk production. The dynamics of NF- κ B activity was investigated in a mouse model of mastitis where *E.coli* was inoculated in the mammary glands of lactating mice [40]. NF- κ B was rapidly, but transiently activated, with a peak around 10 hours and termination after 24 hours. Interestingly, a systemic response was revealed as a mild increase in NF- κ B activity in the liver, which also was longer lasting. This systemic reaction was confirmed by increased circulating

Table 1: Overview of transgenic reporter mice available for studies of inflammation

Regulatory elements of transgenic mice #	Reporter gene	Method	Features of the transgenic mice
Three NF- κ B sites* separated by linker sequences (14 to 25 bp) [32,35,47,48,53,65-68]	Fluc (Firefly luciferase)	Pronuclear injection	<i>In vivo</i> imaging. Short half-life of reporter. Good induction. Used in numerous disease models. Distinct visualization of lymph nodes. Difficult to assess single cells
Three NF- κ B sites* separated by linker sequences (14 to 25 bp) Insulator sequences flank the transgene [40,49,51,54,69-76]	Fluc	Pronuclear injection	<i>In vivo</i> imaging. Short half-life of reporter. Insulators protect transgene against genomic interference. Used in many disease models. Difficult to assess single cells.
Six NF- κ B sites* separated by four bp. Bi-directional expression of two reporter genes [77]	Fluc dEGFP	Pronuclear injection	<i>In vivo</i> imaging and detection of dEGFP in single cells. Short half-life of both reporters. Used in a brain ischemia model. Weak dEGFP signal. Need antibodies for detection.
HIV-1 LTR with two NF- κ B sites* and three Sp1 sites [31,36,38,41,52,78-82]	Fluc	Pronuclear injection	<i>In vivo</i> imaging. Short half-life of reporter. Good induction. Used in various disease models mainly to study lung pathology. Difficult to assess single cells.
HIV-1 LTR with two NF- κ B sites* and three Sp1 sites [37]	EGFP/Fluc fusion protein	Pronuclear injection	<i>In vivo</i> imaging. EGFP signal detected in isolated macrophages. Short half-life of both reporters. Good induction. Need antibodies to detect EGFP in sections.
Two NF- κ B sites* [83-86]	Fluc	Pronuclear injection	Good induction. Successfully used to study T-cell regulation. No demonstration of <i>in vivo</i> imaging.
Five NF- κ B sites *[87]	Fluc	Pronuclear injection	<i>In vivo</i> imaging. Short half-life of reporter. Used in only one study.
Three NF- κ B sites* [88]	EGFP	Site specific in HPRT-locus	Signals detected from single cells and whole organs. Site specific integration prevents influence from regulatory elements outside the transgene. <i>In vivo</i> imaging not shown. Stable version of EGFP complicates assessment of dynamic NF- κ B regulation.
Twelve Smad 2/3 binding sites [55,89,90].	Fluc	Pronuclear injection	<i>In vivo</i> imaging. Used to study TGF β signaling and response to injury, particularly in brain. Difficult to assess single cells.
iNOS-promoter fragment (1.24 kb) [57]	Fluc	Pronuclear injection	<i>In vivo</i> imaging. Reflects iNOS mRNA in liver. Sensitive to pro- and anti-inflammatory agents. Used in only one study.

Table 1: Overview of transgenic reporter mice available for studies of inflammation (Continued)

IκBα-promoter fragment (11.0 kb) [58]	Fluc	Pronuclear injection	<i>In vivo</i> imaging. Luciferase activity reflects IκBα mRNA in liver. Used in only one study.
SAA1-promoter fragment (7.7 kb) [59]	Fluc	Pronuclear injection	<i>In vivo</i> imaging. Luciferase activity reflects SAA1 mRNA in liver and protein in serum. Demonstrated in an acute arthritis model. Used in only one study.
GADD45β-promoter fragment (10.5 kb) [60]	Fluc	Pronuclear injection	<i>In vivo</i> imaging. Reflects GADD45β mRNA in multiple organs. Used to study effects of various stressful insults (inflammation, oxidative stress, toxins). Used in one study.
COX-2-promoter (endogenous) [91-93]	Fluc	Knock-in in the COX2 gene	<i>In vivo</i> imaging. Correlation between luciferase and COX-2 protein levels in multiple organs. Knock-in reflects endogenous promoter activity.

#Include most relevant references where the models have been used.

*Sequence of the NF-κB binding site: 5'-GGGACTTCC-3'. This sequence is found in numerous NF-κB regulated promoters including immunoglobulin κ light chain and HIV LTR, and it is used in all NF-κB reporter mice generated up to now.

levels of the acute phase protein serum amyloid A, tumour necrosis factor-α and interleukin-6. Interestingly, in a recent work it was shown that activation of NF-κB in mammary glands was sufficient to cause mastitis-like symptoms such as increased apoptosis and loss of milk production. Oppositely, specific inhibition of NF-κB in glands of mice with mastitis prevented milk loss [41]. These results indicate that NF-κB is a critical regulator of milk loss during infection, making NF-κB reporter mice useful to evaluate therapeutic strategies.

NF-κB imaging in autoimmune disease

Despite intense research efforts, the etiology of most autoimmune diseases remains obscure. It is previously shown that B-cells can present fragments of the variable region of their immunoglobulins, called idiotype (Id), on their MHC class II, which further can be recognized by T-helper cells. Such T-cells with Id-specific receptors have been described in a number of autoimmune diseases in humans [42-45]. In a mouse model where the collaboration between Id-presenting B-cell and Id-specific T cell are enhanced through genetic manipulation, a plethora of autoimmune diseases correlating with autoantibody production develops [46]. To determine the role of NF-κB during initiation and progression of autoimmune diseases, the mouse model was crossed with NF-κB reporter mice. Imaging revealed NF-κB activation before onset of clinical symptoms and it correlated with disease progression and autoantibody production. Activation was observed in secondary lymphoid organs, inflamed colon,

skin lesions, and arthritic joints. Moreover, *ex vivo* imaging of the small intestine demonstrated autoimmune disease, which had clinical parameters in agreement with celiaki [47]. Additionally, imaging of NF-κB activation has been used to quantify the effect of the IκB kinase 2 inhibitor ML120B in a model of rheumatoid arthritis. This was verified by reduced expression of NF-κB target genes [48]. These results suggest that *in vivo* imaging of NF-κB activation is a good marker for autoimmune disease in experimental mouse models.

Imaging of NF-κB together with new optical probes

Numerous molecular processes are involved in inflammation and probes that can detect some of these events have been developed. Imaging of NF-κB activation together with such probes has been performed to examine correlation of different but still connected processes. A near infrared fluorescent probe that emits light when cleaved by the protease activity of cathepsin B and K was utilized in a model of rheumatoid arthritis [48]. The intensity of the probe coincided with disease severity and NF-κB mediated luminescence intensity. Production of reactive oxygen species is a hallmark of inflammation. A bioluminescent probe (L-012) that reacts with some of these reactive oxygen species was shown to be activated in parallel with NF-κB in various inflammation models [49].

NF-κB imaging of dietary influence

The discovery that diet affects gene regulation has been crucial for the understanding of diet's role in health and

disease. It has been demonstrated that dietary components can be both pro-inflammatory and anti-inflammatory. Intake of high fat diet can for instance lead to low grade inflammation, which again is linked to metabolic syndrome and type 2 diabetes [50]. In two related studies, NF- κ B reporter mice were fed high fat diet for several weeks and both studies found a modest but significant increase in NF- κ B dependent bioluminescence, indicative of low grade inflammation [51,52]. Interestingly, in one of these studies the NF- κ B target gene I κ B ϵ was chronically elevated, whereas genetic manipulations to inhibit this gene protected against type 2 diabetes [52]. Diet can also contribute to a reduction in inflammation through regulation of NF- κ B. Previous studies showed that vitamin A deficiency was linked to increased infection and inflammation. In an attempt to clarify the relationship of vitamin A and NF- κ B activity, vitamin A deficient diet were fed to NF- κ B reporter mice to deplete the vitamin A stores. Vitamin A deficient mice had an overall increased NF- κ B induction of 2.2 fold. Conversely, when mice on normal diet were given a single oral dose of vitamin A in the form of retinoic acid, NF- κ B activity was rapidly and transiently decreased [53]. This inhibition was also observed in LPS treated mice [54]. Thus, the use of NF- κ B reporting mice may prove to be a powerful tool to evaluate anti-inflammatory effects of other dietary factors.

Imaging TGF β signaling

Besides NF- κ B reporter mice, very few other transgenic reporter models exists for reporting specific transcription factors related to inflammation. One exception is the luciferase reporter mice for the transcription factors Smad 2 and 3 [55]. Activation of these transcription factors is the canonical signaling pathway for transforming growth factor β (TGF β). TGF β plays a wide role in the immune system, and affects all populations of leukocytes in a stimulatory or inhibitory manner [56]. The Smad reporter mouse was used to assess global regulation of Smad 2 and 3 activities after LPS stimulation. LPS rapidly induced luciferase expression in liver and brain. Moreover, the signal was much more prolonged in the brain demonstrating differences in organ regulation by TGF β signaling.

Imaging promoter regulation relevant for inflammation

In addition to imaging inflammatory regulation of single transcription factors, several reporter mice have been developed for studies of natural promoters involved in inflammation. Such reporter mice are valuable both for assessing the transcriptional regulation during inflammation and to evaluate the relative contribution of distinct

inflammatory genes. Zhang et al., have developed four different transgenic luciferase reporter mice with promoters from important response genes in inflammation: Serum Amyloid A (SAA), inducible Nitric Oxide Synthetase (iNOS), I κ B and Growth arrest and DNA-damage-inducible β (GADD45 β). *In vivo* imaging following induction of inflammation by various stimuli showed robust activation in all the reporter mice. In addition they showed, by exploiting specific inhibitors and activators, that NF- κ B is central to the regulation of all the promoters. However, the influence of NF- κ B differed between the four. The SAA and GADD45 β promoter were primarily regulated by NF- κ B, whereas the I κ B promoter was in addition influenced by the p38 signaling pathway and the iNOS promoter also needed interferon regulated factor for maximal activation [57-60].

Imaging non-conventional transgenic mice

While basically all studies of molecular imaging of transcriptional regulation in inflammations is done in mice produced by pronuclear injection there are some studies with other methods. For instance, transfection and expression of reporter genes can specifically be obtained in liver following intravenous injection of naked DNA. Such an approach has been used to image luciferase activity under the control of NF- κ B [61]. In these mice, administration of thioacetamide or LPS showed strong induction of liver bioluminescence and the signal was reduced by catalase. Using the same type of method a fusion protein between firefly luciferase and I κ B α was expressed in liver cells. Degradation of I κ B is prerequisite for activation of NF- κ B. Since degradation of I κ B also leads to degradation of firefly luciferase, disappearance of light emission will reflect NF- κ B activation [62]. This approach enables a close to real-time imaging of the NF- κ B signaling pathway. Another approach is to utilize adenoviral transfection. Brain nuclei have been transfected with reporter constructs for NF- κ B and AP1 and imaged *in vivo*. In this study, luciferase activity could be quantified and followed over several weeks after LPS stimulation [63].

Conclusion and future perspective

Molecular imaging of transcription factors is a young research field, but has demonstrated the usefulness of visualizing regulation of transcription factors *in vivo*. We have basically only reviewed studies on NF- κ B regulation in inflammation, which have dominated this research area. The various studies illustrate the importance and advantage both to track activity in individual mice over time, to quantify the relative changes in activity and to visualize the spatial patterns of activation.

Table 2: Overview of imaging studies related to transcriptional regulation in inflammation

Type of study	Results
Imaging neural regulation of NF-κB	Hepatic NF-κB is crucial for recruitment of neutrophils to the injured brain [33]. Vagus nerve signaling regulates NF-κB activity [35].
Imaging of infection models	<i>In vivo</i> imaging of NF-κB in lung [31]. NF-κB is sufficient to cause lung inflammation [36]. Duration of NF-κB activity is determining for lung injury [37]. Bacterial lung infection induces NF-κB. Lack of oxidative burst and targeted inhibition of NF-κB worsens <i>Pseudomonas</i> infection [38,39]. NF-κB is induced in infected mammary glands [40]. NF-κB is central for regulating milk production of mammary glands [41].
Imaging autoimmune disease	<i>In vivo</i> imaging of NF-κB during arthritis [32]. Tracking NF-κB in a transgenic model with various autoimmune diseases [47]. Evaluation of NF-κB inhibitor in arthritis. Combined imaging of NF-κB activity and protease specific near infrared probe [48]. Evaluation of probe for reactive oxygen species and NF-κB activity in arthritis [49].
Imaging of dietary influence	NF-κB activity during high fat feeding and obesity [51]. NF-κB and its role in energy balance of obese mice [52]. Vitamin A regulates NF-κB activity [54].
Imaging host immune reaction	Interaction between host and biomaterial induces NF-κB [87]
Imaging TGFβ signaling	Imaging Smad2/3-dependent TGF-beta signaling reveals prominent tissue-specific responses to inflammatory stimulus and injury [55]. Orally administered TGF-beta is biologically active in the intestinal mucosa and enhances oral tolerance [89]. Imaging of Smad signaling shows correlation with excitotoxic neurodegeneration [90].
Imaging regulation through natural promoters of inflammatory genes	iNOS-promoter activity used to evaluate effect of anti-inflammatory compounds [57]. Regulation of IκBα expression involves both the NF-κB and MAP kinase signaling pathways [58]. Serum amyloid A is induced by inflammatory stimuli. NF-κB is an important regulator [59]. GADD45β-promoter regulation by NF-κB and not MAPK pathway in acute inflammation [60]. Imaging Cox-2 gene expression in living animals with a luciferase knock-in reporter gene [93].
Imaging inflammation in non-conventional transgenic mice	NF-κB activation during liver inflammation in mice and prevention by catalase delivery [61]. Real-time imaging of ligand-induced IKK activation in liver [62]. Viral delivery of reporter constructs to discrete brain region used to monitor longitudinal NF-κB and AP1 activity [63].

The present reporter models of NF-κB have provided valuable information in a variety of experiments, but still have potential to elucidate a number of unresolved questions related to inflammation. However, NF-κB, whose binding sites are represented in more than 200 genes, is a rather ubiquitous marker of inflammation and it is also present in genes not directly involved in inflammation. Therefore, development of promoters that are more specifically regulated is attractive in order to investigate distinct functional elements of inflammation. Such promoters must be under regulation of defined sets of transcription factors. An important improvement in this

regard will be the ability to more precisely image gene regulation in individual organs, and functional events such as activation of specific cell types and productions of distinct cytokines.

Due to the rapid evolution of imaging technology combined with advances in molecular biology more quantitative and localized characterization of the reporter signal will represent an important and valuable improvement. The studies presented in this review have utilized conventional two dimensional imaging of reporter gene activity, which indeed has created valuable information from distinct organs such as liver, lungs, brain and intes-

tine; however, due to light scattering and poor tissues penetration, it is difficult to localize and quantify signals from deeper lying tissues. Information on inflammation in specific organs is vital for the understanding of biological mechanisms and to validate more precisely the effect of a treatment regime. New developments of brighter versions of optical reporter proteins as well as more red shifted fluorescent proteins is therefore crucial for obtaining tissue specific imaging. The combination of anatomical images (Computer tomography and MRI) with molecular imaging is forthcoming [64] and will clearly be utilized more extensively in anatomical characterizations. Molecular imaging of transcription factor activity in inflammation will definitively also in the future be an essential tool to provide knowledge in basic biological mechanisms in preclinical studies.

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Competing interests

HC owns stocks in the company Cgene AS, who holds the commercial rights of certain NF- κ B-luciferase reporter mice. AK has been partly employed by Cgene AS.

Authors' contributions

AK and HC authored the manuscript. Both authors read and approved the final manuscript.

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