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A Nuclear Factor in B Cells and Beyond

Michael J. May1

The mid-1980s was a very exciting time for researchers exploring the regulation of eukaryotic gene transcription. When this month’s Pillars of Immunology paper was published in 1986 (1), it was clear that cis-acting motifs remote from the RNA start site were critical for the transcription of an expanding list of viral, prokaryotic, and eukaryotic genes. These DNA elements, named enhancers, were described by Serfling, Jasin, and Schaffner as “modular arrangement(s) of short sequence motifs each with a specific function in conferring inducibility, tissue specificity, or a general enhancement of transcription” (2). Despite this understanding, exactly how these elements exerted their “enhancer effects” to regulate eukaryotic gene transcription was not known.

The prevailing hypothesis was based upon pioneering work in the late 1970s and early 1980s dissecting the mechanisms of viral gene expression (2, 3). The model that emerged was that viral enhancers bound sequence-specific trans-acting proteins and that these factors directly or indirectly regulated Pol II activity (3). A notable example was Sp1 that was identified as a trans-acting regulator of SV40 early gene transcription (4, 5). Supporting this as the mechanism of enhancer activity in mammalian genes, several DNase footprinting and competition studies pointed to the existence of similar enhancer-specific trans-activating factors (2, 3). Furthermore, motifs including the Sp1-binding site were identified as constitutive elements contributing to the transcription of some eukaryotic genes (2, 3). This model was perceptively accurate, but in 1986, definitive evidence of an enhancer-associated trans-acting factor regulating mammalian gene expression had not yet appeared.

Armed with this model, Ranjan Sen and David Baltimore set out to identify nuclear factors required for Ig gene expression. Several laboratories had identified regulatory elements within the Ig H and L chain genes including downstream enhancers located within introns in both the \( \mu \) H and \( \kappa \) L chain loci (1, 2, 6). Given the cell specificity of Ig transcription for B cells, it is not surprising that the hypothesis Sen and Baltimore were pursuing was that a factor binding to these elements would be tissue specific. However, what they reported in their seminal publication (1) rapidly transcended B cells and became one of the most intensely studied signaling paradigms of the last two decades (7–14). The transcription factor they identified was NF-kB.

Sen and Baltimore’s study was facilitated by their prudent use of the new technique of EMSA (15–17). EMSAs exploit the fact that a protein-bound radiolabeled DNA probe is retarded in polyacrylamide gels, and their utility was elegantly demonstrated in an earlier study describing a NF bound to octamer motifs (ATTTGCAT) within the Ig H chain enhancer (18). This factor (named IgNF-A and later identified as Oct-1) was also found in HeLa cells, and its consensus binding sequence was functional in the promoters of several vertebrate small nuclear RNA (snRNA) genes (18). IgNF-A was not, therefore, a strictly B cell-specific factor, and this was precisely what Sen and Baltimore sought to find.

The generation of EMSA reagents today is very simple. However, in 1986, Ranjan Sen had to subclone large DNA fragments encompassing the regulatory regions, then excise, purify, dephosphorylate, and \(^{32}\)P-label a panel of smaller fragments (1). Sen generated probes covering the Ig H chain enhancer (\( \mu \)E), the \( \kappa \) L chain promoter (kpr) and the \( \kappa \) L chain intronic enhancer (\( \kappa \)E) and incubated these with nuclear extracts from B cell lines representing various stages of differentiation. Multiple factors bound to each regulatory region and further analysis revealed that most were not B cell specific (Fig. 4 of Ref. 1). One exception was a factor bound to the third fragment (\( \kappa \)3) of \( \kappa \)E that was not competed away by cold probes from any other region but was competed for by the SV40 enhancer (Fig. 5 of Ref. 1). Sequence comparison and methylation interference analysis revealed the nucleotide sequence (GGGGACTTTCC) that bound this factor in both \( \kappa \)E and SV40 (Fig. 6 of Ref. 1). This was named the \( \kappa \)B site for the B cell-specific protein-binding site, and the factor that bound to it was named nuclear factor B (NF-kB). Intriguingly, NF-kB was only detected in mature B and myeloma cell lines and was not constitutively present in pre-B cells (Fig. 7 of Ref. 1). Sen and Baltimore therefore concluded that NF-kB was not only B cell-specific but was stage specific within the B lymphoid lineage (1).

This picture of NF-kB did not last long. In December 1986, Sen and Baltimore published a second paper showing that incubation with LPS induced NF-kB activity in the nuclei of pre-B cells (19), and similar results were reported almost concurrently by Michael Atchison and Robert Perry (20). Furthermore, NF-kB was detected in PMA-treated Jurkat and HeLa cells demonstrating for the first time the non-cell-type-specific nature of inducible NF-kB (19). Sen and Baltimore also noted that induction of NF-kB occurred in the presence of protein synthesis inhibitors, and they concluded that NF-kB “may be activated by posttranslational modification of precursor factors” (19). In other words, inactive NF-kB pre-exists in resting cells and stimulation causes something to happen that activates it.

What followed can only be described as an information explosion. NF-kB proteins were purified to homogeneity and found to be relatives of the avian v-rel oncogene and Drosophila dorsal gene products (13, 21). In all, five members of the mammalian NF-kB family were identified and shown to be

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1 Address correspondence and reprint requests to Dr. Michael J. May, Department of Animal Biology, University of Pennsylvania School of Veterinary Medicine, 3800 Spruce Street, Philadelphia, PA 19104. E-mail address: maym@vet.upenn.edu
ubiquitously expressed transcriptional regulators (14). Intense effort focused on the mechanisms of induced activation identified the inhibitory family of IkB proteins that retain NF-κB in the cytoplasm of resting cells (12, 14, 21). Further work revealed that stimulus-induced phosphorylation, ubiquitination, and degradation of IkB by the proteasome releases NF-κB to the nucleus. This mechanism explained the “posttranslational modification” observations of Sen and Baltimore (19) and provided early evidence for regulated protein ubiquitination playing a role in a signaling cascade (10). During the mid- to late-1990s, many key players in the NF-κB activation pathway were identified including the elusive IkB kinase (IKK). This turned out to reside in a high m.w. multisubunit complex that has itself been the target of intense scrutiny (22).

The late 1980s also heralded an era of frenetic identification of inducible genes containing κB sites (8). These now number in the hundreds, and a comprehensive list can be found at www.nf-kb.org. Among the first κB-regulated genes reported were those encoding proinflammatory and immunoregulatory proteins (8) providing early glimpses of the critical role of inducible NF-κB in inflammation and immunity. This was accompanied by an expanding list of NF-κB-inducing stimuli that included engagement of the T and B cell receptors and members of the TLR family. Not surprisingly, NF-κB emerged as an essential regulator of both innate and adaptive immune responses (7, 12, 14, 22). Furthermore, in vivo genetic manipulation of the NF-κB, IkB, and IKK proteins largely resulted in immune-defective phenotypes underscoring the importance of the NF-κB system in maintaining a functional immune system (12, 14, 23). The significance of NF-κB to a far wider range of biomedical fields also became apparent with a prime example being the revelation of its role as a survival factor during oncogenesis (11). The NF-κB signaling pathway has therefore become a major target for the development of drugs aimed at a spectrum of pathologies, and many patents have been issued for NF-κB-blocking strategies (9).

Neither Sen nor Baltimore could have predicted that the B cell-specific factor they sought to identify would turn into this remarkable paradigm of inducible membrane to nuclear signaling. A PubMed search performed today lists over 25,000 “NF-κB” publications demonstrating the far-reaching impact of their 20-year-old study. Their paper unquestionably resides among the most important and influential publications considered to be Pillars of Immunology.

References