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Review Article

The multifunctional role of intrinsic disorder in NF-κB signaling

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The nuclear factor-κB (NF-κB) transcription activation system involves disordered regions of both the NF-κB dimers and their inhibitors, the IκBs. The system is well-studied both at the cellular and biophysical levels affording a unique opportunity to compare and contrast the conclusions from both types of experiments. Through a combination of both experiments and theory, we have discovered that the RelA/p50 heterodimer and its inhibitor IκBα operate under kinetic control. Intrinsically disordered parts of both proteins are directly involved in temporal control and their folding and unfolding determines the rates of various processes. In this review, we show how the dynamic state of the intrinsically disordered sequences define the rates of intracellular processes.

Introduction to the NF-κB signaling system

The nuclear factor-κB (NF-κB) signaling system involves a family of NF-κB proteins and their inhibitors [1]. Although there are 15 possible homo- and heterodimers of NF-κB and at least five inhibitors (Figure 1A,B), the system is simpler than it looks because some dimers are preferred and these then each have a preferred inhibitor that binds with picomolar binding affinity. Thus, the RelA homodimer is bound most tightly by IκBβ (K_D 25 pM) but also by IκBα (K_D 47 pM), the RelA–p50 heterodimer is bound most tightly by IκBα (K_D 42 pM), and cRel heterodimers with either RelA or p50 bind IκBε with K_D s of 98 and 60 pM, respectively [2]. The very tight binding affinities of the inhibitors creates a situation where the NF-κB dimers are completely inhibited in resting cells and an active process must be initialized to remove the inhibitor (Figure 1C). For the rest of this perspective, we will focus on the case of the RelA–p50 heterodimer, which is the NF-κB responsible for rapid response to inflammatory signals, and for which the activation process is well understood.

How is NF-κB signaling kept in the 'off' state?

The binding of inflammatory signals to Tol-like receptors on the cell surface activates the IκB kinase (IKK) which phosphorylates IκBα at Ser 32 and 36 [3,4] in the disordered N-terminal sequence of IκBα. The doubly phosphorylated sequence then binds to the β-TrCP ubiquitin E3 ligase [5] which ubiquitylates the disordered IκBα sequence at Lys 21 and 22 targeting the protein for proteasomal degradation [6]. It is notable that in IKK knock-out cells, the half-life of the RelA–p50:IκBα complex is at least 48 h and phosphorylation does not change the binding affinity of IκBα for the RelA–p50 heterodimer. Therefore, proteasomal degradation is what causes the dissociation of the RelA–p50:IκBα complex. Thus, the only way that NF-κB can be dissociated from IκBα is by phosphorylation and ubiquitylation of IκBα and subsequent proteasomal degradation. In cells, IκBα is constantly being synthesized so as to capture any NF-κB that might not be inhibited keeping all of the NF-κB in the cytoplasm unable to activate genes. The newly synthesized IκBα that does not bind NF-κB is degraded keeping the overall concentration of free IκBα very low. This is the state of the cell prior to activation of the NF-κB signaling pathway.

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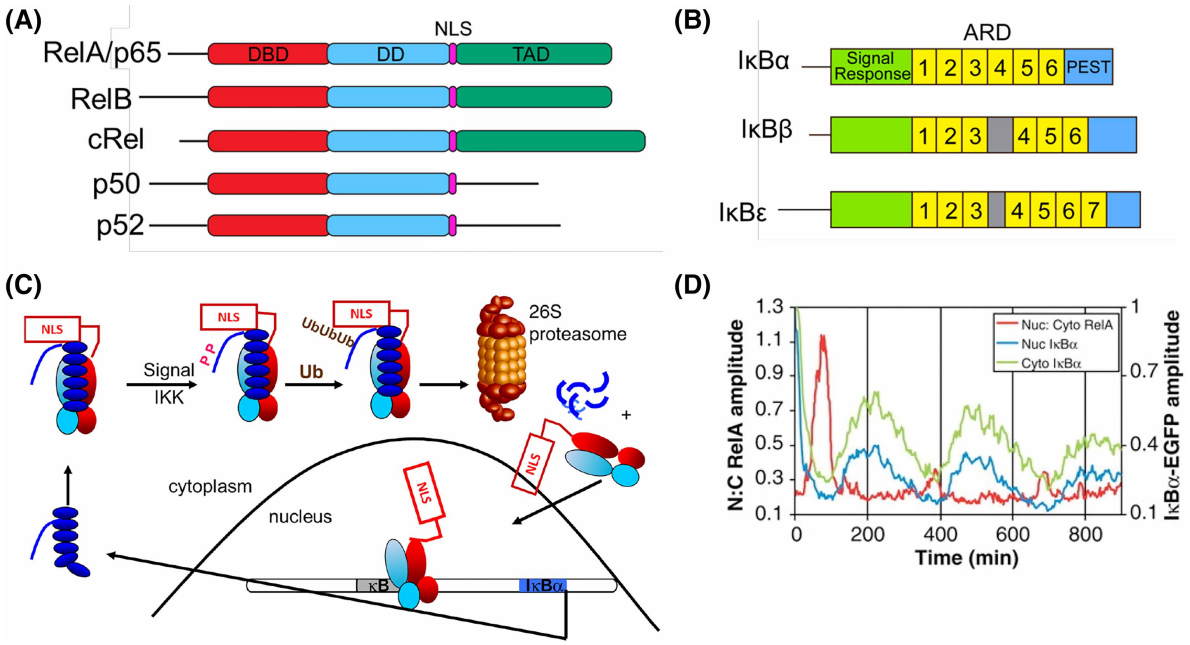

Figure 1. Introduction to the NF-κB family of proteins and their inhibitors.

(**A**) The five NF-κB family members for homo- and heterodimers. Only RelA/p65, RelB and cRel have transcription activation domains (TADs). (**B**) The three inhibitors of the NF-κB family of proteins all have ankyrin repeat domains. (**C**) RelA–p50 is the most well-studied of the NF-κB family members. Its primary inhibitor is IκBα which keeps it in the cytoplasm until phosphorylation and ubiquitylation signals for IκBα degradation. Then the NF-κB nuclear localization signal is exposed facilitating nuclear import. Once in the nucleus, NF-κB turns on transcription of hundreds of genes including that of IκBα which then initiates a positive feedback loop. (**D**) IκBα is the inhibitor that is responsible for removing RelA–p50 from the nucleus which follows an oscillatory behavior due to remaining IKK kinase which can continue to signal for degradation of the newly synthesized IκBα in a second round of signaling.

How is NF-κB signaling turned on?

Our laboratory showed that a few critical interactions between IκBα and the NF-κB RelA–p50 heterodimer are all-important for maintaining the binding affinity between IκBα and the NF-κB. In fact, the large surface area of contact between the ankyrin repeat domain (ARD) of IκBα and the dimerization domains of RelA and p50 is misleading [7]. A key interaction that holds the interaction together is between Phe 309 in RelA and the 'top' of ankyrin repeat 1 in the IκBα ARD. Mutation of this residue causes a drastic loss of binding affinity of over 1000-fold [8]. Similarly, mutations within the PEST sequence following the sixth ankyrin repeat of IκBα strongly affect binding affinity [9]. NMR experiments revealed that the sequence of RelA surrounding Phe309 is disordered and remains somewhat dynamic even when it is bound to IκBα [10]. The sidechain of Phe 309 appears to fit into a pocket formed by Phe 77 and Leu 80 in the top of the IκBα somewhat like a button into a buttonhole [11]. Thus, one might imagine that when proteasomal degradation starts at the disordered N-terminal sequence of IκBα, this interaction might be disrupted causing dissociation of the IκBα and allowing interaction of the RelA nuclear localization signal with Importin-α.

NF-κB transcription activation does not require any new protein synthesis and as such is very rapid. Within 30 min of stimulation, over 100 000 molecules of NF-κB flood the nucleus and within 1 h, hundreds of genes are activated [12–14]. The amount of NF-κB in the nucleus follows an oscillatory behavior and this is because all of the NF-κB is kept in the cytoplasm inhibited by IκBα. Then once activation occurs, all of the NF-κB-bound IκBα is degraded. One of the genes that is most strongly activated by NF-κB is the gene for IκBα so that as soon as activation occurs, new IκBα is made. This newly synthesized IκBα then enters the nucleus and returns the NF-κB to the cytoplasm. However, if the kinase is still active, the process repeats, resulting in an oscillating concentration of nuclear NF-κB (Figure 1D).

The robustness of NF-κB activation depends on the absence of free IκBα in the cytoplasm that could re-capture some of the activated NF-κB. It turns out that this is accomplished by having an incredibly short intracellular half-life of free IκBα, less than 7 min [15]. Biophysical studies on free IκBα and sequence analysis of what constitutes a stable ankyrin repeat led to the creation of a more stable IκBα which is a double mutant (Y254L, T257A) with a half-life extended to over 12 min (Figure 2A). This 'prefolded' IκBα enabled us to discover much about how the protein structure of IκBα determines it free intracellular half-life. It turns out that the C-terminal ankyrin repeat of IκBα contains a bona fide degron. The existence of this degron was proven by attaching the sequence from the C-terminal ankyrin repeat of IκBα onto green fluorescent protein (GFP). Whereas GFP is highly stable in cells, the fusion of the IκBα sequence to its C-terminus reduced the intracellular half-life of GFP to the same as that for IκBα (Figure 2B). The degron in the C-terminal ankyrin repeat causes free IκBα to be degraded in a proteasome-dependent but ubiquitin independent manner [16]. It is still unclear whether there are accessory factors that bind this degron sequence and target it to the proteasome.

To explore more deeply how the structure of IκBα exposes this degron, we carried out many studies on free IκBα and the YLTA stabilized mutant. We knew from NMR studies that this region of IκBα must adopt multiple structures because no resonances were observed for the fifth and sixth repeats of free IκBα even though they were clearly visible when IκBα was bound to NF-κB [17]. Using amide hydrogen/deuterium exchange, we showed that the fifth and sixth ankyrin repeats were highly exchanging indicative of the absence of stable structure [15]. Despite these two indicators of a lack of structure, no new helical structure is formed when IκBα binds to NF-κB indicating the presence of residual helical structure. To probe this region more thoroughly, we performed single molecule Forster resonance energy transfer (smFRET). We placed the two fluorophors that would undergo FRET on the AR2 and AR6 and observed the FRET from single molecules over time (Figure 3A). Based on our previous studies, we expected a stable, high-FRET signal for the IκBα bound to NF-κB and a lower FRET signal for the unbound IκBα. While we did observe a stable, high-FRET signal for the bound IκBα (Figure 3B), the signal for the unbound IκBα visited different states that ranged from high FRET to low FRET and we observed transitions in real time (Figure 3C,D) [18].

How is NF-κB signaling turned-off?

A fascinating part of the story is the mechanism by which IκBα enters the nucleus, binds the NF-κB and returns it to the cytoplasm as the inactive NF-κB:IκBα complex. It had been thought that IκBα enters the nucleus by passive diffusion, but very recently in-cell cross-linking led to the discovery that SQSTM-1/p62 is a chaperone that ensures rapid translocation of newly synthesized IκBα into the nucleus [19].

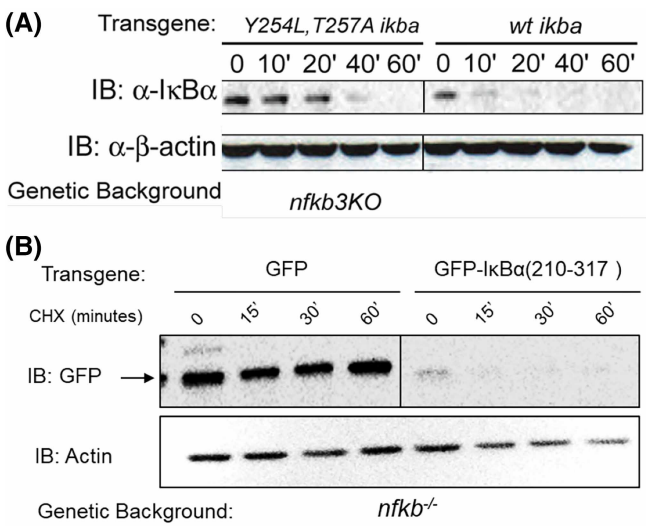

Figure 2. Evidence for a degron sequence in the C-terminal ankyrin repeats of IκBα.

(**A**) IκBα is degraded quickly in cells and stabilizing mutations increase its half-life [15]. (**B**) Green fluorescent protein (GFP) is very stable in cells, but appending the C-terminal residues 210–317 of IκBα to the C-terminus of GFP causes it to have the same dramatically faster degradation as IκBα [16].

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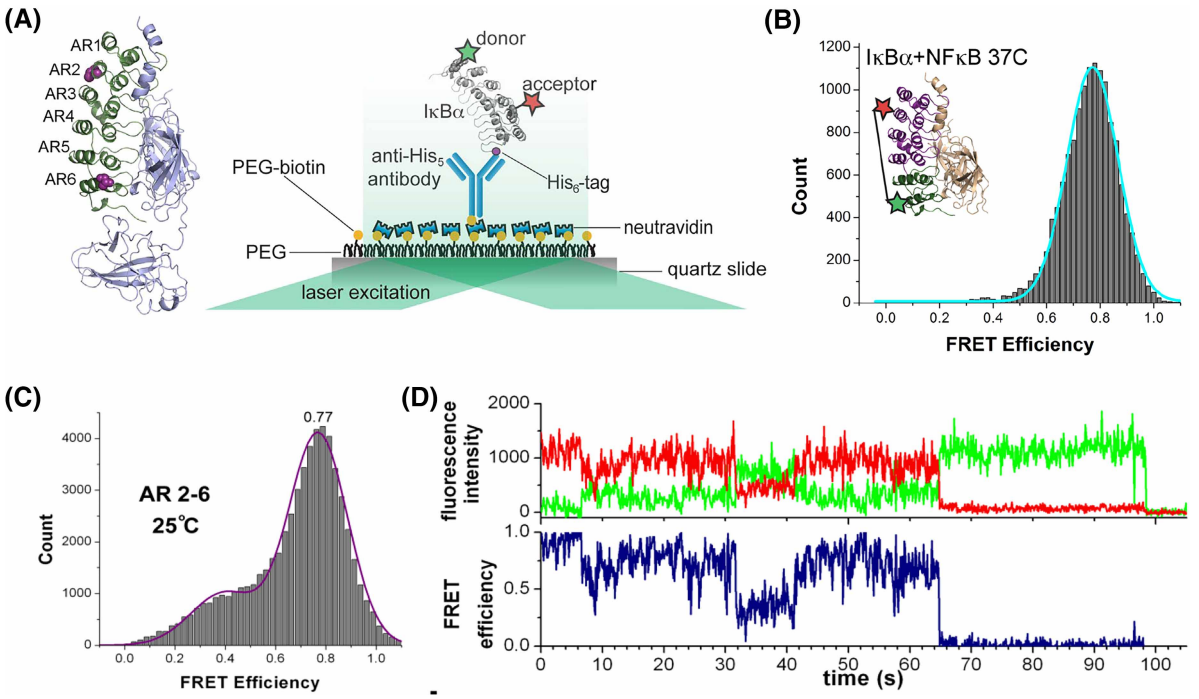

Figure 3. Single molecule FRET studies of IκBα reveal nanospring behavior.

(**A**) Set-up of the smFRET experiment involved attaching the IκBα (labeled at AR2 and AR6) to the microscope slide by way of a His(6) tag that bound to an anti-His(6) antibody which was also biotinylated so it would bind to the nutravidin on the microscope slide. (**B**) IκBα bound to NF-κB shows a narrowly distributed high-FRET signal indicative of a completed folded ankyrin repeat domain. (**C**) Unbound IκBα showed a broader FRET signal with a population at lower FRET. (**D**) smFRET traces revealed that the lower FRET population was due to the nanospring behavior of IκBα which stochastically sampled states in which the AR2 and AR6 were further apart, but could then regain the high-FRET state.

Once IκBα is in the nucleus, it enhances the kinetics of NF-κB dissociation from the DNA. We first observed that IκBα accelerated the dissociation of NF-κB from DNA using surface plasmon resonance (SPR) experiments in 2009 [20]. In this first report, we were able to demonstrate that the dissociation rate of NF-κB from DNA was linearly dependent on the concentration of IκBα (Figure 4A). Both the N-terminal interaction between Phe 309 and IκBα as well as the disordered AR5–AR6 of IκBα were important for 'molecular stripping' as we later called this unique phenomenon. Several variants of IκBα that had a more well-folded AR5–AR6 were less able to facilitate dissociation. Later, we were able to directly observe the transient ternary complex between IκBα and the NF-κB–DNA complex (modeled in Figure 4B) both by NMR [21] and by stopped-flow fluorescence [22] (Figure 4C). Eventually, we were able to discover a mutant of IκBα that bound to NF-κB with nearly the same affinity as wildtype but that was much less able to strip NF-κB from the DNA. We hypothesized that the negatively charged PEST region of IκBα might repel the DNA and that this electrostatic repulsion might be involved in the facilitated dissociation. We therefore generated the '5x-PEST' mutant IκBα in which five negatively charged amino acids in the PEST region were neutralized (e.g. E → Q, D → N). The '5x-PEST' mutant formed a more stable ternary complex (NF-κB–DNA–IκBα). To test whether the facilitated dissociation was functionally important, we put the mutant IκBα into cells behind the native IκBα promoter and performed single cell studies of nuclear import/export of fluorescently labeled Rel A. These experiments showed that whereas in cells containing wildtype IκBα, Rel A was efficiently exported from the nucleus, in cells containing the '5x-PEST' mutant IκBα nuclear export of Rel A was much slower (Figure 4D). This was a really important result because it allowed us to define that the molecular mechanism and rate-determining process for nuclear export was, in fact, IκBα-mediated molecular stripping of NF-κB from the DNA [23]. Systems biology studies of the NF-κB signaling system demonstrated that without molecular stripping, NF-κB signaling could never be turned-off [24,25]. This result is due to a combination of factors; (1) NF-κB target sites (called κB sites) vary widely and (2) in the nucleus, ChIP studies show at least 40% of

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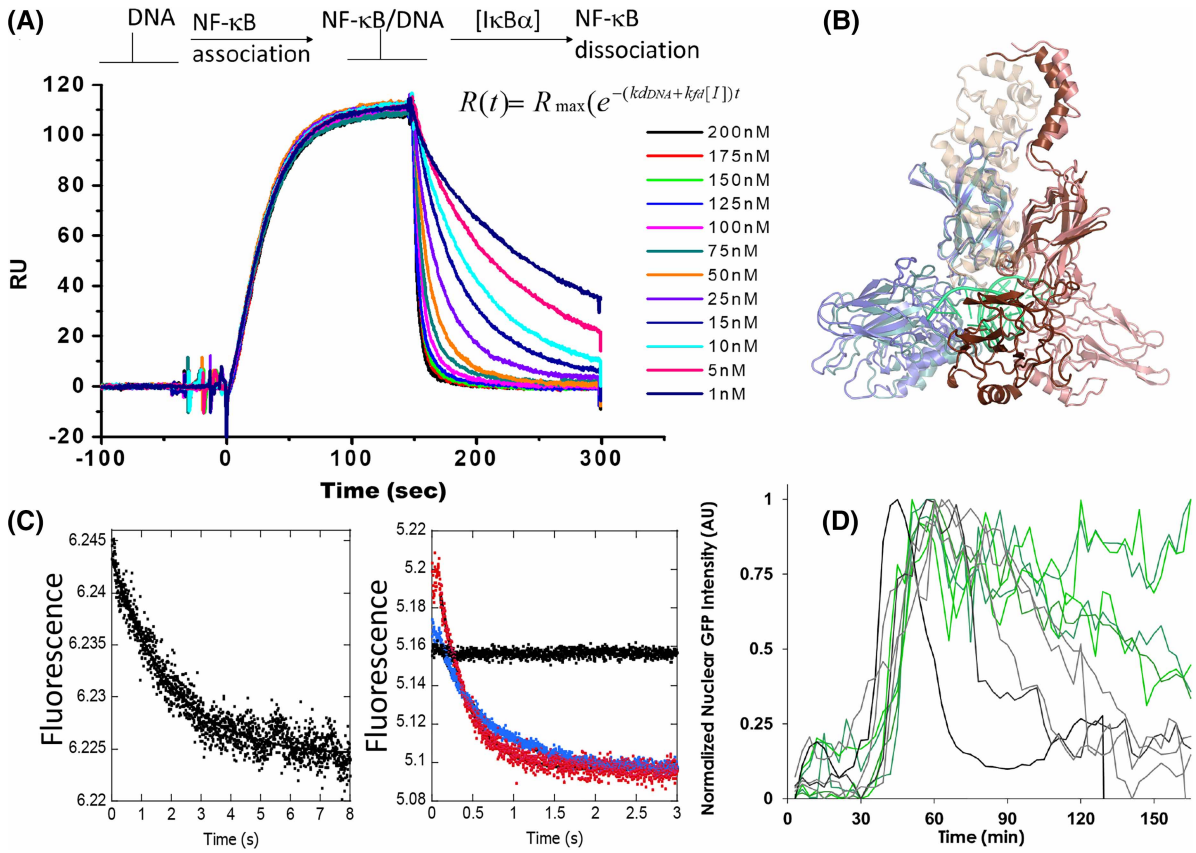

Figure 4. Evidence for IκBα-mediated facilitated dissociation of NF-κB from the DNA (aka molecular stripping).
(**A**) The first evidence for molecular stripping came from SPR experiments in which varying concentrations of IκBα were flowed into a channel containing a pseudo-flowing equilibrium of NF-κB–DNA complexes. NF-κB would dissociate from the DNA at increasing rates that depended on the IκBα concentration [20]. (**B**) Model of the ternary complex between NF-κB, DNA and IκBα showing that the DNA-binding domains must rearrange upon IκBα binding [29]. (**C**) Stopped-flow fluorescence of DNA bound to fluorescently labeled DNA (left panel) shows that the dissociation of the NF-κB–DNA complex dissociates at a rate of ~0.5 s^{-1} but upon addition of IκBα, dissociation happens much more rapidly and is concentration dependent (right panel). In addition, evidence for formation of a transient ternary complex can be seen because the fluorescence increases prior to decreasing also in a concentration-dependent manner [22]. (**D**) Single cell traces of fluorescently tagged RelA show that NF-κB enters the nucleus rapidly and is also rapidly removed by IκBα (black and gray traces). Introduction of the '5x-PEST' mutant form of IκBα behind the native IκBα promoter results in the same rapid nuclear entry of NF-κB but a much slower removal of NF-κB from the nucleus (green traces) [23].

the NF-κB is bound to sites that do not resemble κB sites. Therefore, the total number of 'specific' sites to which NF-κB could bind is very large. Furthermore, we recently discovered that for full-length NF-κB (which includes the transcription activation domain (TAD) of Rel A) the difference between the binding affinity for a bona fide κB site and for non-specific sequences is not as large as was originally gleaned from studies of NF-κB truncated before the TAD [26].

Finally, one might wonder how IκBα is able to engage NF-κB when it is bound to DNA. All of the crystal structures of NF-κB homo- and heterodimers bound to DNA show the DNA engulfed in a cavity formed by both of the N-terminal DNA-binding domains and the bottoms of the dimerization domains (Figure 5A). To understand whether the NF-κB–DNA structures were more dynamic than they seemed from the crystal structures, we again set-out to perform smFRET experiments, this time with the fluorophores on each of the DNA-binding domains (Figure 5B,D). Remarkably, both the DNA-free and DNA-bound NF-κB molecules showed a wide range of FRET signals without adopting any defined states such as open or closed (Figure 5C,E) [27]. Rather, the two N-terminal domains spent long periods of time at many different FRET states.

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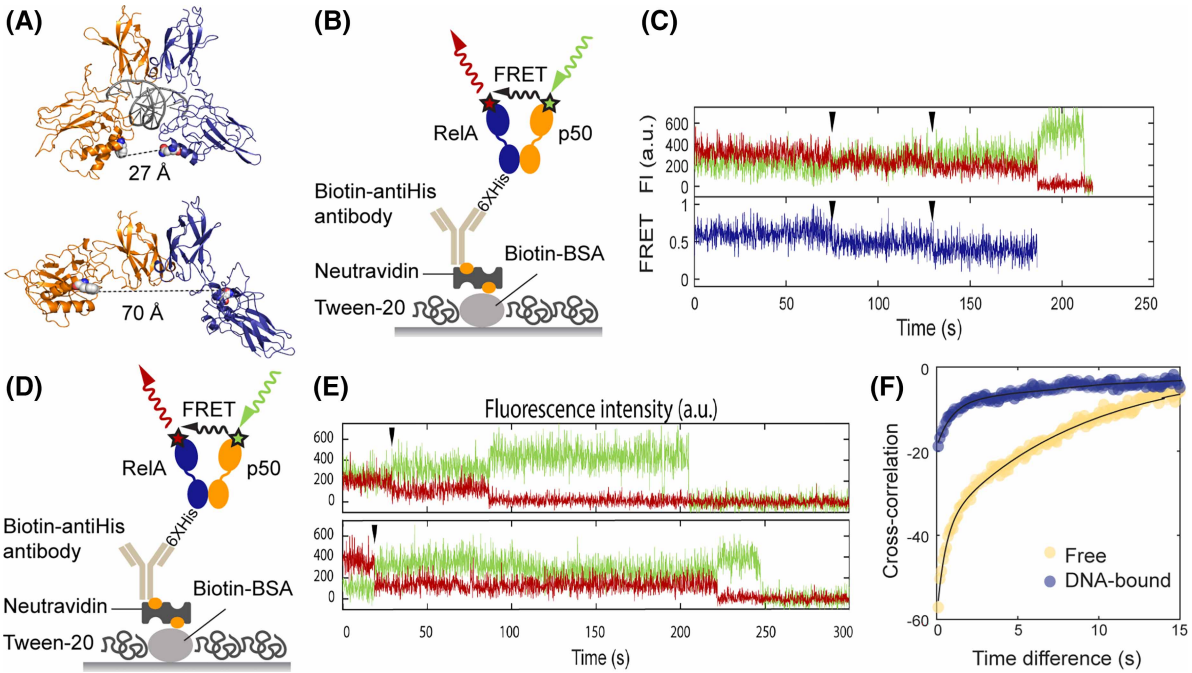

Figure 5. Single molecule FRET experiments to probe the dynamics of the N-terminal DNA-binding domains of NF-κB.
(**A**) Many crystal structures of DNA-bound NF-κB show that the DNA binds in a cleft formed between the N-terminal DNA-binding domains and the base of the dimerization domains as if the NF-κB is hugging the DNA. Several molecular dynamics simulations suggest that if the DNA is not present, the DNA-binding domains become further apart. (**B**) The smFRET set-up for studying the motions of the NF-κB DNA-binding domains includes specific fluorescent labeling of each DNA-binding domain and immobilization of the NF-κB on the microscope slide using a His(6) tag as already described. (**C**) A representative smFRET trace showing the NF-κB domains adopting different FRET states and slowly transitioning between them. (**D**) The smFRET set-up for studying the motions of the NF-κB DNA-binding domains includes specific fluorescent labeling of each DNA-binding domain and immobilization of κB site DNA on the microscope slide to which the labeled NF-κB binds. (**E**) A representative smFRET trace showing the DNA-bound NF-κB domains adopting different FRET states and slowly transitioning between them. (**F**) Cross-correlation analysis reveals a different time constant of motion for the free vs. the DNA-bound NF-κB.

Interconversions between the FRET states were slow and stochastic (Figure 5C,E). Cross-correlation analysis revealed that the DNA-bound domains more readily interconverted than they did when not bound to DNA (Figure 5F). These results indicated that the DNA-binding domains did not both have to be fully engaged in DNA binding for the molecule to stay bound to the DNA. The many DNA-bound states that were not fully engaged likely explain the ease with which IκBα can engage the NF-κB and facilitate its dissociation from the DNA.

Future directions

The 'elephant in the room' yet to be explored is the RelA TAD. Whereas the p53 TAD interacts with the DNA-binding domains of p53 and heightens their ability to discriminate between specific and non-specific DNA-binding sites [28], the opposite seems to be true for the RelA TAD [26]. The RelA TAD is large, 230 amino acids, and is predicted to be entirely disordered except for two small helical regions that bind the transcriptional co-activator with PDZ-binding zinc finger (TAZ) domain and the kinase-inducible domain interacting (KIX) domain in the transcriptional co-activator, CREB-binding protein (CBP). Small angle x-ray scattering analyses of the TAD indicate that it is a compact globule and that it likely does not fold back to interact with the NF-κB Rel Homology Domain (RHD) [26]. Does binding of CBP alter the conformational ensemble of the RelA TAD? Does the RelA TAD ever influence DNA binding? These questions await future studies of the NF-κB signaling system.

Perspectives

- The NF-κB signaling system turns on hundreds of genes and is involved in many diseases. We show the system is tightly controlled by interconverting weakly folded regions of each protein.
- The intrinsically disordered C-terminal ankyrin repeats of IκBα control its degradation rate as well as its ability to facilitate dissociation of NF-κB from the DNA. The weakly folded linkers between the DNA-binding domains and the dimerization domains of NF-κB allow IκBα access to dissociate it from DNA.
- The intrinsically disordered TAD of NF-κB has multiple functions, many of which are yet to be explored.

Competing Interests

The author declares that there are no competing interests associated with this manuscript.

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Abbreviations

ARD, ankyrin repeat domain; GFP, green fluorescent protein; IKK, IκB kinase; NF-κB, nuclear factor-κB; smFRET, single molecule Forster resonance energy transfer; TAD, transcription activation domain.

References

1 Baeuerle, P.A. and Baltimore, D. (1988) I kappa B: a specific inhibitor of the NF-kappa B transcription factor. *Science* **242**, 540–546 https://doi.org/10.1126/science.3140380
2 Ramsey, K., Chen, W., Marion, J., Bergqvist, S. and Komives, E. (2019) Exclusivity and compensation in NFκB Dimer distributions and IκB inhibition. *Biochemistry* **58**, 2555–2563 https://doi.org/10.1021/acs.biochem.9b00008
3 Ghosh, S. and Baltimore, D. (1990) Activation in vitro of NF-kappa B by phosphorylation of its inhibitor I kappa B. *Nature* **344**, 678–682 https://doi.org/10.1038/344678a0
4 Traenckner, E., Pahl, H., Henkel, T., Schmidt, K., Wilk, S. and Baeuerle, P. (1995) Phosphorylation of human I kappa B-alpha on serines 32 and 36 controls I kappa B-alpha proteolysis and NF-kappa B activation in response to diverse stimuli. *EMBO J.* **14**, 2876–2883 https://doi.org/10.1002/j.1460-2075.1995.tb07287.x
5 Yaron, A., Hatzubai, A., Davis, M., Lavon, I., Amit, S., Manning, A. et al. (1998) Identification of the receptor component of the IkappaBalpha-ubiquitin ligase. *Nature* **396**, 590–594 https://doi.org/10.1038/25159
6 Kroll, M., Margottin, F., Kohl, A., Renard, P., Durand, H., Concordet, J. et al. (1996) Inducible degradation of IκBα by the proteasome requires interaction with the F-box protein h-βTrCP. *J. Biol. Chem.* **271**, 376–379 https://doi.org/10.1074/jbc.271.1.376
7 Huxford, T., Huang, D.-B., Malek, S. and Ghosh, G. (1998) The crystal structure of the IκBα/NF-κB complex reveals mechanisms of NF-κB inactivation. *Cell* **95**, 759–770 https://doi.org/10.1016/S0092-8674(00)81699-2
8 Bergqvist, S., Croy, C., Kjaergaard, M., Huxford, T., Ghosh, G. and Komives, E. (2006) Thermodynamics reveal that helix four in the NLS of NF-kappaB p65 anchors IkappaBalpha, forming a very stable complex. *J. Mol. Biol.* **360**, 421–434 https://doi.org/10.1016/j.jmb.2006.05.014
9 Bergqvist, S., Ghosh, G. and Komives, E. (2008) The IkappaBalpha/NF-kappaB complex has two hot spots, one at either end of the interface. *Protein Sci.* **17**, 2051–2058 https://doi.org/10.1110/ps.037481.108
10 Cervantes, C., Bergqvist, S., Kjaergaard, M., Kroon, G., Sue, S., Dyson, H. et al. (2011) The RelA nuclear localization signal folds upon binding to IκBα. *J. Mol. Biol.* **405**, 754–764 https://doi.org/10.1016/j.jmb.2010.10.055
11 Jacobs, M.D. and Harrison, S.C. (1998) Structure of an IκBα/NF-κB complex. *Cell* **95**, 749–758 https://doi.org/10.1016/S0092-8674(00)81698-0
12 Hoffmann, A., Levchenko, A., Scott, M.L. and Baltimore, D. (2002) The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. *Science* **298**, 1241–1245 https://doi.org/10.1126/science.1071914
13 Tong, A., Liu, X., Thomas, B., Lissner, M., Baker, M., Senagolage, M. et al. (2016) A stringent systems approach uncovers gene-specific mechanisms regulating inflammation. *Cell* **165**, 165–179 https://doi.org/10.1016/j.cell.2016.01.020
14 Brignall, R., Moody, A., Mathew, S. and Gaudet, S. (2019) Considering abundance, affinity, and binding site availability in the NF-κB target selection puzzle. *Front. Immunol.* **10**, 609 https://doi.org/10.3389/fimmu.2019.00609

15 Truhlar, S., Mathes, E., Cervantes, C., Ghosh, G. and Komives, E. (2008) Pre-folding IkappaBalpha alters control of NF-kappaB signaling. *J. Mol. Biol.* **380**, 67–82 https://doi.org/10.1016/j.jmb.2008.02.053

16 Fortmann, K., Lewis, R., Ngo, K., Fagerlund, R. and Hoffmann, A. (2015) A regulated, ubiquitin-independent degron in IκBα. *J. Mol. Biol.* **427**, 2748–2756 https://doi.org/10.1016/j.jmb.2015.07.008

17 Sue, S., Cervantes, C., Komives, E. and Dyson, H. (2008) Transfer of flexibility between ankyrin repeats in IkappaB* upon formation of the NF-kappaB complex. *J. Mol. Biol.* **380**, 917–931 https://doi.org/10.1016/j.jmb.2008.05.048

18 Lamboy, J., Kim, H., Lee, K., Ha, T. and Komives, E. (2011) Visualization of the nanospring dynamics of the IkappaBalpha ankyrin repeat domain in real time. *Proc. Natl Acad. Sci. U.S.A.* **108**, 10178–10183 https://doi.org/10.1073/pnas.1102226108

19 Liu, Y., Trnka, M., He, L., Burlingame, A. and Correia, M. (2023) In-cell chemical crosslinking identifies hotspots for SQSTM-1/p62-IκBα interaction that underscore a critical role of p62 in limiting NF-κB activation through IκBα stabilization. *Mol. Cell. Proteom.* **22**, 100495 https://doi.org/10.1016/j.mcpro.2023.100495

20 Bergqvist, S., Alverdi, V., Mengel, B., Hoffmann, A., Ghosh, G. and Komives, E.A. (2009) Kinetic enhancement of NF-kappaBxDNA dissociation by IkappaBalpha. *Proc. Natl Acad. Sci. U.S.A.* **106**, 19328–19333 https://doi.org/10.1073/pnas.0908797106

21 Sue, S.C., Alverdi, V., Komives, E.A. and Dyson, H.J. (2011) Detection of a ternary complex of NF-kappaB and IkappaBalpha with DNA provides insights into how IkappaBalpha removes NF-kappaB from transcription sites. *Proc. Natl Acad. Sci. U.S.A.* **108**, 1367–1372 https://doi.org/10.1073/pnas.1014323108

22 Alverdi, V., Hetrick, B., Joseph, S. and Komives, E.A. (2014) Direct observation of a transient ternary complex during IkappaBalpha-mediated dissociation of NF-kappaB from DNA. *Proc. Natl Acad. Sci. U.S.A.* **111**, 225–230 https://doi.org/10.1073/pnas.1318115111

23 Dembinski, H.E., Wismer, K., Vargas, J.D., Suryawanshi, G.W., Kern, N., Kroon, G. et al. (2017) Functional importance of stripping in NFkappaB signaling revealed by a stripping-impaired IkappaBalpha mutant. *Proc. Natl Acad. Sci. U.S.A.* **114**, 1916–1921 https://doi.org/10.1073/pnas.1610192114

24 Wang, Z., Potoyan, D. and Wolynes, P. (2016) Molecular stripping, targets and decoys as modulators of oscillations in the NF-κB/IκBα/DNA genetic network. *J. R. Soc. Interface* **13**, 20160606 https://doi.org/10.1098/rsif.2016.0606

25 Potoyan, D. and Wolynes, P. (2017) Stochastic dynamics of genetic broadcasting networks. *Phys. Rev. E* **96**, 052305 https://doi.org/10.1103/PhysRevE.96.052305

26 Baughman, H., Narang, D., Chen, W., Villagrán Suárez, A., Lee, J., Bachochin, M. et al. (2022) An intrinsically disordered transcription activation domain increases the DNA binding affinity and reduces the specificity of NFκB p50/RelA. *J. Biol. Chem.* **298**, 102349 https://doi.org/10.1016/j.jbc.2022.102349

27 Chen, W., Lu, W., Wolynes, P. and Komives, E. (2021) Single-molecule conformational dynamics of a transcription factor reveals a continuum of binding modes controlling association and dissociation. *Nucleic Acids Res.* **49**, 11211–11223 https://doi.org/10.1093/nar/gkab874

28 Krois, A., Dyson, H. and Wright, P. (2018) Long-range regulation of p53 DNA binding by its intrinsically disordered N-terminal transactivation domain. *Proc. Natl Acad. Sci. U.S.A.* **115**, E11302–E11310 https://doi.org/10.1073/pnas.1814051115

29 Potoyan, D.A., Zheng, W., Komives, E.A. and Wolynes, P.G. (2016) Molecular stripping in the NF-kappaB/IkappaB/DNA genetic regulatory network. *Proc. Natl Acad. Sci. U.S.A.* **113**, 110–115 https://doi.org/10.1073/pnas.1520483112