Review

SUMO and NF-κB ties

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Abstract. Members of the NF-κB family of transcription factors play critical roles in regulating immunity and cell survival and contribute to cancer progression and chemoresistance. Over the past 20 years, much has been learned about the remarkable complexity in regulation of NF-κB signaling. In particular, recent studies have added to our current understanding of the roles of a multitude of post-translational modifications in this signaling system: these include phosphorylation, acetylation, nitrosylation, ubiquitination, neddylation and sumoylation. This review will highlight our current knowledge of the roles of sumoylation in regulating NF-κB signaling and functions and will address future perspectives.

Keywords. SUMO, NF-kappaB, posttranslational, transcription, Ubc9, PIAS, NEMO, IkappaBalpha.

Overview of the NF-κB signaling system

It has been more than 20 years since the initial report of the discovery of the transcription factor nuclear factor kappa B (NF-κB) [1]. NF-κB refers to a collection of protein dimers, each composed of members of the NF-κB/Rel family of proteins. In the mammalian system, there are five members: p65/RelA, RelB, c-Rel, p105/p50 and p100/p52. Conserved members of this family can also be found in other eukaryotic systems, including Drosophila (Dorsal, Dif and Relish), cnidarians (Nv-NF-κB) and others [2]. In mammalian cells, the most ubiquitous NF-κB dimer consists of a p65/p50 heterodimer [3] that is found in the cytoplasm bound to members of a family of inhibitors known as inhibitor of NF-κB (IκB). The binding of IκB family members, such as IκBα and IκBβ, can prevent nuclear accumulation of NF-κB, in part via masking the nuclear localization sequence (NLS) of NF-κB [4, 5]. IκBα can also promote nuclear export of bound NF-κB, further ensuring cytoplasmic localization of inactive NF-κB dimers [6–10].

Activation of NF-κB involves its release from IκB, followed by nuclear translocation and a multitude of posttranslational modifications that enable its transcriptional activation function. In this review, the term “signaling” (or “signal transduction”) is used specifically to refer to molecular events leading up to, but not including, those directly associated with NF-κB-dependent transcription in the nucleus. The “canonical” NF-κB signaling includes the release of active NF-κB in the cytoplasm by the activation of the cytoplasmic kinase complex, known as the IκB kinase (IKK). IKK consists of three major proteins: two kinases, IKKα/IKK1 and IKKβ/IKK2, and a regulatory subunit, IKKγ/NEMO (NF-κB essential modulator). A large body of literature describes the complex mechanisms that are employed to cause IKK activation in response to a wide array of structurally and functionally unrelated extracellular and intracellular signals [11]. In most cases, activated
IKK promotes phosphorylation of IkBα and its subsequent degradation by the ubiquitin-dependent 26S proteasome system, thereby exposing the NLS of NF-κB, leading to its translocation into the nucleus. During its liberation from IkBα and subsequent to nuclear translocation, NF-κB subunits can undergo several posttranslational modifications to acquire maximal capacity to regulate the transcription of many different target genes involved in inflammatory responses. B and T cell receptor activation and various stress responses. The specific outcomes of NF-κB-dependent transcription are dependent on the cell types and the signals leading to NF-κB activation [12]. In addition to this highly studied NF-κB activation mechanism, another well-established “noncanonical” pathway has also emerged, which involves proteolytic processing of p100/NF-κB2 to selectively activate p52/RelB heterodimers. This pathway is solely dependent on IKKα, without the need for IKKβ and NEMO, and regulates secondary lymphoid organogenesis and B cell development, among other biological functions [13]. Readers are encouraged to consult more comprehensive and excellent reviews of the NF-κB family members, distinct activation mechanisms and their role in transcription [2, 3, 12].

It has been demonstrated time and again that differential posttranslational modifications aid in determining the specificity of signal transduction pathways both activating and sometimes inhibiting NF-κB induced by diverse agents. Modifications such as phosphorylation, ubiquitination and acetylation and, more recently, nitrosylation, neddylation and sumoylation have been demonstrated to play critical roles in regulating NF-κB signaling and transcriptional function [14]. One area of NF-κB biology that has not been extensively reviewed in the literature is the role of protein modifications by SUMO (small ubiquitin-like modifier). Thus, the focus of this review will be geared towards current knowledge regarding the roles of sumoylation of different protein targets in NF-κB signaling and transcriptional pathways. We will first briefly describe the process of sumoylation and then delve into different situations in which sumoylation and SUMO regulatory proteins have been implicated in the regulation of NF-κB. Finally, we will highlight some of the unanswered issues that may be further addressed in future research.

Overview of protein sumoylation

Sumoylation is the process of posttranslational covalent modification of target proteins by a relatively small peptide (~20 kDa) called SUMO. SUMO was cloned over 10 years ago in *Saccharomyces cerevisiae* and called *SMT3* (suppressor of mif two 3) [15]. In humans there are currently four known SUMO isoforms (SUMO-1, SUMO-2, SUMO-3 and SUMO-4), of which only SUMO-1, -2, and -3 can be conjugated to target substrate proteins [16]. SUMO is first translated in an unconjugatable precursor state and needs to be C-terminally processed by SUMO proteases to expose glycine-glycine (“di-glycine”) residues critical for the conjugation reaction (see below). SUMO-4 contains a proline residue N-terminally adjacent to the di-glycine moiety, which seems to prevent its maturation into a functional conjugatable entity [17].

Protein modification by SUMO occurs on specific lysine residues of target proteins and takes place via a series of enzymatic steps. A heterodimeric SUMO-activating enzyme (E1), SAE1/SAE2 in human (also known as Aos1/Uba2 in *S. cerevisiae*), forms a high-energy thioester bond with the active site cysteine residue C173 in SAE2 and the C-terminal glycine residue of SUMO in a manner that is ATP-dependent. The SUMO moiety is then transferred to the catalytic cysteine residue (C93) of Ubc9 (ubiquitin-conjugating enzyme 9), the SUMO-conjugating enzyme (E2). The E2 can then directly interact with a substrate by recognition of a consensus sequence, yKxE/D (“ψ” representing a hydrophobic amino acid residue and “x” representing any amino acid residue), and transfer SUMO to the epsilon amino group of the lysine residue embedded within the consensus site to form a covalent isopeptide bond; this is referred to as sumoylation [18]. In yeast and mammals, there is only one known SUMO E1 and one known SUMO E2. Both SUMO E1 and E2 are predominantly found in the nuclear compartment, although the E2 has also been shown to be localized to the cytoplasmic filament of the nuclear pore complex (NPC) [19]. Sumoylation affects many protein substrates and is involved in many important physiological and pathological processes [16, 20]. In fact, deletion of *Ubc9* has been shown to be lethal in both yeast and mice [21, 22].

Similar to the ubiquitination system [23], sumoylation can utilize a group of enzymes known as SUMO ligases (E3), which can promote the protein sumoylation reaction both *in vitro* and *in vivo*. There are multiple SUMO E3s identified, and the list is still growing. Like E1 and E2, the cellular location of E3s appears to be concentrated in the nucleus or on the nuclear membrane [24]. The largest group of SUMO E3s belongs to the protein inhibitors of activated STAT (PIAS) family, which was originally identified to be inhibitors of the STAT (signal transducer and activator of transcription) family of transcription factors [25, 26]. Characteristic of the PIAS family is their SP-RING (Siz/PIAS-really interesting new gene) domain, which is critical to promote protein