Post-translational control of Nur77

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Abstract

Nur77 is a nuclear orphan receptor that has been implicated in both cell survival and apoptosis. With the exception of T-cells, translocation of Nur77 to the cytoplasm promotes cell death, while its retention in the nucleus promotes survival and proliferation. Nur77 appears to be a true orphan receptor, indicating that its activity must be controlled by ligand-independent mechanisms. Here, we discuss the role of phosphorylation in the regulation of Nur77.

Nur77, Nur1 and Nor1 have shown that the putative binding site is independent of exogenously added ligand. Consistent with this, the crystal structures of the ligand-binding domains of Nur77 and Nor1 have shown that the putative binding site is in a closed conformation that would be inaccessible to a ligand [11]. As NR4A proteins can act independently of ligand, this raised the question of how their activity is regulated in vivo. Two of the most likely methods for this are the regulation of protein expression and post-translational modifications such as phosphorylation. Nur77, Nur1 and Nor1 are all encoded by immediate early genes whose transcription is induced in response to a variety of signals including mitogens and cellular stress [1,2]. In many circumstances, this requires the transcription factors CREB (cAMP-response-element-binding protein) or ATF1 (activating transcription factor 1) that can also be activated by mitogens and cellular stress after phosphorylation by the kinases MSK1 (mitogen- and stress-activated kinase 1) and MSK2 [12]. In addition, both mRNA and protein for Nur77, Nur1 and Nor1 appear to be unstable in vivo and their induction is transient following stimulation [12]. Thus regulation of protein levels could clearly play a role in regulating the activity of the NR4A proteins.

Another mechanism by which the activity of NR4A proteins may be regulated is phosphorylation. The phosphorylation of Nur77 was first demonstrated in PC12 cells where NGF stimulation was found to result in a band shift for Nur77 on SDS/polyacrylamide gels [13]. Treatment of the samples with a phosphatase was shown to reverse this band shift, confirming that it was due to Nur77 phosphorylation. Subsequent studies have confirmed this and further shown that, in PC12 cells, KCl and EGF treatment resulted in different degrees of band shift, indicating that Nur77 was phosphorylated on multiple sites [14]. Mapping all these phosphorylation sites has proved more challenging. One site that has been identified, Ser354 (amino acid numbers refer to the mouse Nur77 sequence; GenBank™ accession number
NP_034574), lies in the C-terminal region of the DNA-binding domain [15]. This was first identified as an in vitro site by peptide mapping and mutational analysis and was subsequently confirmed as being phosphorylated in cells. Ser354 was initially suggested to affect DNA binding, as phosphorylation of this site in vitro reduced the ability of Nur77 to bind to NBRE oligonucleotides in an EMSA [15]. This effect is consistent with the crystal structure of the Nur77 DNA-binding domain complexed to DNA [16]. In this structure, Ser354 lies close to the DNA backbone, and modelling of a phosphate at this position suggested that it would inhibit DNA binding. Consistent with this, NGF or EGF stimulation of PC12 cells was found to promote phosphorylation of Ser354 in Nur77, and correlated with a reduced activation of Nur77-dependent luciferase reporters [15]. In contrast, KCl stimulation did not affect Nur77-dependent luciferase reporter genes, and was unable to induce Ser354 phosphorylation [17]. It is not however clear whether Ser354 phosphorylation alone is sufficient to inhibit the transcriptional activity of Nur77. Mutation of Ser354 to an acidic residue in vitro a Nur77 fragment strongly induces Ser354 phosphorylation, was not sufficient to block the transcription of Nur77-dependent reporter genes in HEK-293 (human embryonic kidney) cells [18].

The identity of the kinase that phosphorylates Ser354 in Nur77 has also been controversial. Several kinases, including PKA (protein kinase A), PKC, RSK (p90 ribosomal S6 kinase) and PKB, have been reported to phosphorylate Nur77 or a Nur77 fragment in vitro [17,15,19,20]. More recently it has been shown that full-length Nur77 is not an effective in vitro substrate for either PKA or PKB when the rate of phosphorylation was compared with that for a known physiological substrate [18]. Several kinases have also been suggested to phosphorylate Ser354 in cells, particularly RSK and PKB. Two studies on PKB suggested a role for this kinase in the phosphorylation of Nur77; however, much of this work relied on overexpression of PKB, which can lead to breakdown of the specificity of signalling [21,20]. Recently, we have shown that stimuli that strongly activate PKB, such as insulin-like growth factor, are unable to induce Nur77 Ser354 phosphorylation [18]. In contrast, stimuli that activate RSK, such as PMA, do induce Ser354 phosphorylation, and this is blocked by inhibitors of both RSK and the ERK1/2 (extracellular-signal-regulated kinase 1/2) cascade (the upstream activators of RSK). Even for stimuli that activate both RSK and PKB, such as EGF, Nur77 Ser354 phosphorylation was completely blocked by inhibitors of either RSK or the ERK1/2 cascade [18]. Furthermore, in PDK1 L155E knock-in embryonic stem cells, which activate PKB normally but cannot activate RSK, PKA was unable to induce Ser354 phosphorylation. The Ser354 phosphorylation site is conserved in Nurr1 and Nor1. Phosphorylation at this equivalent site by RSK, but not PKB, has been demonstrated in HEK-293 cells [18].

The identity of other phosphorylation sites in Nur77 is still not clear. Thr145 has been identified as an in vitro site for ERK2, but the phosphorylation of this site has not been demonstrated in cells [22]. Ser422 has been identified as a site phosphorylated in cells by an NGF-inducible kinase [23], and the most likely candidate for this in vitro is ERK1/2. Phosphorylation of the N-terminal kinase domain by JNK (c-Jun N-terminal kinase) has also been reported, but the sites have not been identified [24]. Phosphorylation by JNK in this study was suggested to promote the nuclear export of Nur77 and lead to increased apoptosis [24].

In addition to phosphorylation, other mechanisms may also regulate Nur77. Nur77 has been reported to interact with several proteins including retinoid X receptor, Hif1α (hypoxia-inducible factor 1α), NF-κB (nuclear factor κB) and TRAP220 (thyroid hormone receptor-associated protein 220). Interestingly, Nurr1 has recently been reported to interact with the SUMO (small ubiquitin-related modifier) E3 ligase PIA54 [protein inhibitor of activated STAT (signal transducer and activator of transcription) 4], and expression of PIA54 represses Nurr1 transcriptional activity. Nurr1 does contain two conserved sumoylation motifs; however, mutation of either one of these sites was insufficient to block the repression by PIA54 [25]. Further work will be required to characterize the importance of these findings for the physiological roles of the NR4A proteins.

References

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