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Regulation of NF-κB: Arginine Methylation Takes the Stage

Han Wei[1], Rasika Mundade[1] and Tao Lu[1,2]*

1Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, USA
[2]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, USA

Abstract

Nuclear factor κB (NF-κB) is a family of transcription factors that play central roles in multiple biological functions, including immune and inflammatory responses and tumorigenesis. The regulation of the activity of NF-κB is very sophisticated. Post-translational modifications of NF-κB have long been known to play an essential role in the regulation of NF-κB activity. Since the p65 subunit (RelA) is the major subunit of the typical NF-κB heterodimer, more attention has been recently devoted to how p65 is regulated by the post-translational modifications. In this review, we provide an overview of the most recent developments in the regulation of p65 by post-translational modifications.

Keywords: Arginine methylation; Epigenetic regulation; NF-κB; Post-translational modifications

Overview of NF-κB signaling

Signaling through NF-κB plays an essential role in many disease conditions. Therefore, understanding elements that govern NF-κB has the potential to reveal targets for intervention to ameliorate a variety of diseases from cancer to inflammation. This review focuses on identifying new advances in our understanding of how NF-κB signaling is controlled.

The transcriptional factor NF-κB family includes p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2). All proteins of the NF-κB family share a Rel homology domain (RHD) in their N-terminus, which is required for their dimerization, nuclear targeting, binding to DNA, and interaction with its inhibitors, the IκB proteins (IκBs) [1]. A subgroup of NF-κB family members, namely the Rel proteins, including p65, RelB and c-Rel, also contain an additional carboxy-terminal transactivation domain (TAD). The prototype of NF-κB is the heterodimer of p65 and p50 subunits. In unstimulated cells, NF-κB exists as homo- or heterodimers by binding to IκBs [1]. There are seven IκB family members: IκBα, IκBβ, IκBγ, IκBε, BCL-3, and the precursor proteins p100 and p105, that bind to the RHD domains of NF-κB dimers [2]. Each IκB interacts with their specific NF-κB dimers, covers their nuclear localization signals, and thus keeps NF-κB in an inactive state in cytoplasm. NF-κB has both canonical and non-canonical activation pathways [3]. In the canonical NF-κB signaling pathway, extracellular signals such as proinflammatory cytokines activate IκB kinase (IKK), which phosphorylates IκBα, leading to its ubiquitinationand degradation by proteasomes [3]. The released NF-κB (mostly p65/p50 heterodimers) translocates into the nucleus, binds to DNA, and regulates the expression of multiple inflammatory and innate immune genes [3]. In the non-canonical pathway, phosphorylation of p100 on its C-terminal helps the processing of p100 to p52 [3]. The freed NF-κB (mostly p52/RelB heterodimers) translocates into the nucleus and regulates the expression of genes, many related to the development and maintenance of the immune system [3].

Post-Translational Modifications of NF-κB

The NF-κB pathway is regulated by multiple post-translational modifications including ubiquitination, phosphorylation, acetylation, sumoylation, and nitrosylationetc [4]. These regulatory modifications can vary with different NF-κB stimulators and even the same modifications may display quite different effects [4]. In this review, we will only focus on the development of the post-translational modifications regarding p65 since p65 is the major subunit of NF-κB.

Phosphorylation of p65

Phosphorylation of p65 was nicely summarized by Perkins [4]. Since then, only one more new serine (S) site, S547, has been identified [5]. Here, we only name a few examples. For instance, three phosphorylated serine sites have been found in the RHD domain (amino acid 1-301) of p65, including S205, 276, and 281 [6-8]. Phosphorylation of S276 is catalyzed by either protein kinase K (PKA) [7] or mitogen- and stress-activated protein kinase 1 (MSK1) [8] in different cell systems, which in turn leads to the enhanced NF-κB transcriptional activity. Another study demonstrated that in response to lipopolysaccharide (LPS), S205 and 278 can be phosphorylated by unknown serine/threonine-directed protein kinases, which further increase NF-κB transcriptional activity [6]. In addition to the RHD domain, four other serines were found to be phosphorylated in the TAD domain (amino acid 428-551): S468, 529, 536, and 547 [5,9-13]. Most phosphorylations on serine serve as an NF-κB activating signal. For example, upon TNFα treatment, S529 is phosphorylated by casein kinase II (CKII) which leads to the increased transactivation potential of NF-κB [12]; whereas S536 phosphorylation by the IKKβ increased p65 transcriptional activity in monocytes/macrophages [13]. S468 was found to be phosphorylated by IKKε [9], IKKβ [10], or GSK3β [11] in different cell systems. In response to T-cell co-stimulation, IKKε mediates inducible phosphorylation of S468, which enhance p65 transcriptional activity without influencing DNA binding ability [9]. However, S468 can also inhibit p65 transactivation. For example, phosphorylation of S468 by GSK-3β negatively regulates NF-κB activity in unstimulated HeLa cells [11]. Also phosphorylation of S468 catalyzed by IKKβ deceases NF-κB-dependent transcription in response to TNF-α and IL-1β treatment [10]. By using p65S468A-reconstituted p65−/− mouse embryonic fibroblasts (MEFs), Schwabe

***Corresponding author:** Tao Lu, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, USA, Tel: 317-278-0520; E-mail: lut@iupui.edu

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and Sakurai [10] found that S468A mutant significantly increases NF-κB activity after TNF-α and IL-1β treatment in comparison to wtp65-reconstituted MEFs [10]. Other examples also suggest that phosphorylation on p65 is not always seen as an activation signal. A new study shows that phosphorylation of S547 by Ataxia telangiectasia mutated (ATM) represses NF-κB-dependent transcription in HEK-293 cells in response to DNA double-strand breaks (DSB) [5]. Besides the 7 serine sites located on either the RHD or TAD domains described above, S311, a site located in the linker region (amino acid 302-427) between the RHD and TAD domains, is phosphorylated by the atypical protein kinase C ζ (PKCζ) [14]. This important phosphorylation is required for CBP-enhanced NF-κB transcriptional activity in embryo fibroblasts (EFs) in response to TNFα stimulation [14].

In addition to serine phosphorylation, another major amino acid player in phosphorylation is threonine. To date, three threonines have been reported to be phosphorylated with different stimulations, including T254 [15] in the RHD domain, and T435 [16], 505 [17] in the TAD domain of p65. For instance, in response to cisplatin, T505 is phosphorylated by the checkpoint kinase Chk1 [17] leading to the proapoptotic effect of p65 and negatively regulating autophagy, cellular proliferation, and migration [18].

Therefore, the above examples further illustrate the significant importance of phosphorylation in the regulation of the p65 subunit of NF-κB.

Acetylation of p65

As illustrated by Perkins [4], five acetylated lysine (K) sites have been identified on p65. They are K122, 123, 218, and 221, all located in the RHD domain, and K310, which is located in the linker region of p65. From 2006 to present, no additional acetylation site has been identified on p65. Chen et al. showed that in response to TNFα, K310 is acetylated by p300/CBP [19]. This modification is required for the full transcriptional activity of p65 [20]. In another report, K218, 221 are also seen to be acetylated by p300/CBP [20]. Acetylation of K221 enhances p65 DNA binding, and in conjunction with acetylation of K218, blocks the interaction of p65 with newly synthesized IκBα, thereby, activating NF-κB. Acetylation at K122 and 123 by both p300 and p300/CBP-associated factor (PCAF) decreases the DNA binding ability, and helps the removal of NF-κB and subsequent export to the cytoplasm by IκBα [21].

Other Modifications

In addition to the above modifications, it has also been seen that suppressor of cytokine signaling 1 (SOCS1) catalyzes the ubiquitination on amino acids 220–335 of p65 which results in the p65 degradation and negative regulation of NF-κB inducible genes expression [22]. Nitration on tyrosine Y66 and Y152 of p65 triggered by nitric oxide treatment inhibits NF-κB activity [23]. More details regarding other types of modifications of p65 can be found in the thorough review by Perkins [4].

Methylation of NF-κB

As shown in Figure 1, beyond the more traditional modifications that are mentioned above, in the past few years, six methylated K sites have been identified on p65. They are: K37, 218, 221, 310, 314 and 315 [24-28]. In an attempt to identify the novel regulators of NF-κB by using a novel genetic approach, Lu et al. [25,29-31] discovered that the nuclear receptor binding SET domain protein 1 (NSD1) and the demethylase F-box leucine-rich protein 11 (FBXL11) regulate NF-κB through the reversible methylation of K218 and 221 of p65. Zhang et al. [26] reported that the plant homeodomain finger protein 20 (PHF20, also called glioma-expressed antigen 20 promotes NF-κB transcriptional activity by interacting with methylated p65 at K218 and 221. The interaction between PHF20 and methylated p65 blocks the recruitment of phosphatase PP2A to maintain the p65 phosphorylation status. Ea and Baltimore [24] reported that, upon activation of NF-κB by TNFα, Set9 monomethylates p65 at K37, and this epigenetic modification regulates the promoter binding of p65 to a subgroup of genes, such as IκBα, IP-10, and TNFα. The induction of IP-10 and TNFα was greatly reduced in $p65^{-/-}$MEF cells that express K37Q mutant as compared to those that are reconstituted with wild type p65 [24]. Yang et al. reported that p65 is monomethylated by Set9 at K314 and 315 [28], leading to the decreased NF-κB activity and its target gene expression. Levy et al. identified that SETD6 monomethylates p65 on K310, leading to the induction of a repressed state at NF-κB target genes through the binding of G9a-like protein in U2OS cells [27]. Why does p65 need the methylation on multiple K sites? Recently, we studied

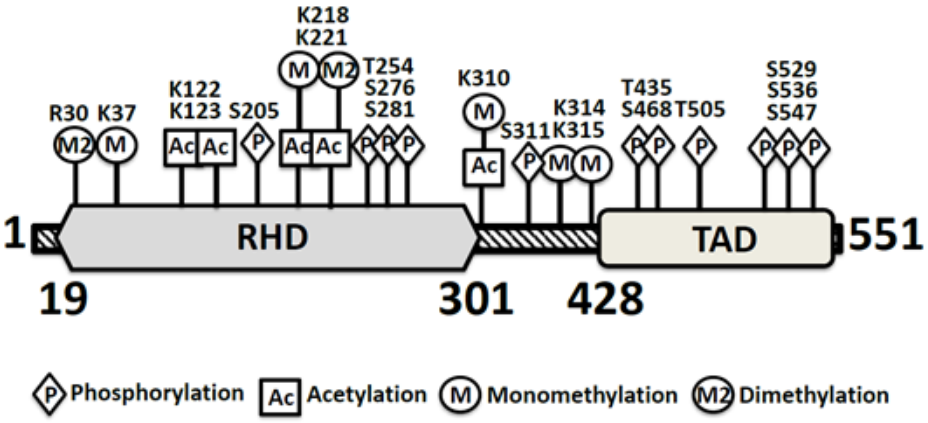

Figure 1: Post-translational modifications of p65 [4].

Figure shows schematic diagram of the principal structural motifs of p65: Rel Homology domain (RHD, amino acid 19-301), Transactivation domain (TAD, amino acid 428-551) and the linker region (amino acid 302-427). The mapped sites are the post-translation modifications in p65; only phosphorylation, acetylation and methylation (Mono- or Di-methylation) sites are shown here.

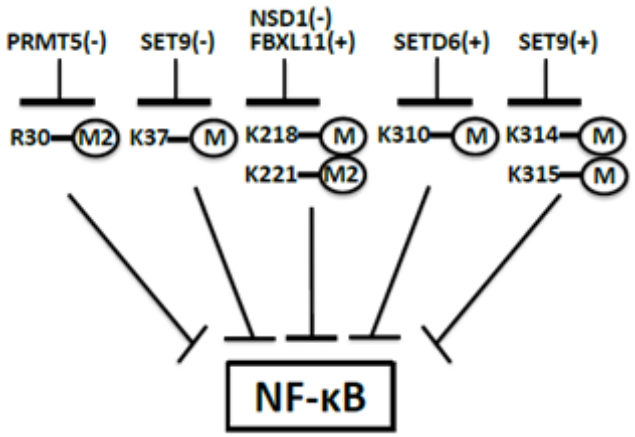

Figure 2: Predicted potential druggable targets through regulation of p65 methylation.We predict that NF-κB can be inhibited either by blocking the activities of the histone modifying enzymes, such as PRMT5, SET9 [24], NSD1, and SETD6, or by targeted activation of histone modifying enzymes FBXL11, or SETD6, or SET9 (reported in different cell system, [28]). Therefore, these enzymes could be potential druggable targets for controlling the NF-κB activity in diseases.

the effect of methylation on K37 and K218/221 sites of p65 [32], and we found that mutation on K218/221 greatly reduced the expression of ~50% of NF-κB inducible genes, whereas the K37Q mutation reduced the expression of only ~25% of NF-κB inducible genes. Chromatin Immunoprecipitation sequencing (CHIP-Seq) analysis showed that the mutant of K218/221Q greatly reduces the affinity of p65 for many promoters, and that the K37Q mutation does not. Structural modeling shows that the newly introduced methyl groups of K218/221 interact directly with DNA to increase the affinity of p65 for specific κB sites. Thus, the K218/221 and K37 mutants have dramatically different effects because methylations of these residues affect different genes by distinct mechanisms [32]. Furthermore, it is worth noting that it is a normal phenomenon to see that the same site of lysine can be either methylated or acetylated on the same protein, such as K218, 221[20,25,32] and K310 [19,27] on p65. Besides NF-κB, there are numerous examples of the same amino acid being altered by different modifications. For instance, tumor suppressor p53 has been found to be methylated and acetylated on the same lysine sites including K370, 372 and 382, which leads to quite different biological functions [33]. Furthermore, histone subunits, such as histone H2BK5, H3K9, 23, 27, 36 and H4K12 and 20 are all found to be modified by either methylation or acetylation on the same lysine with quite different biological consequences [34]. This phenomenon further highlights the complexity of the post-translational modifications of proteins.

Most recently, our lab discovered that the arginine (R) 30 of p65 (Figure 1) can be dimethylated by the protein arginine methyltransferase 5 (PRMT5), leading to activation of NF-κB [35]. Microarray analysis revealed that ~85% of the NF-κB–inducible genes that are down regulated by the R30A mutation are similarly down regulated by knocking down PRMT5. This extremely novel finding suggests that, like tumor suppressor p53 [36], methylation of arginine of p65 also plays an essential role in the regulation of NF-κB. We predicted the crystal structure of p65 showing that methylated R30 can mediate Van der Waals contacts therefore increasing the ability of p65 to bind to DNA and consequently, affect gene expression [35]. PRMT5 is a member of the PRMTs superfamily. Since PRMT5 is frequently seen overexpressed—often to a striking degree—in many types of cancer, such as colon, ovary, kidney, lung, bladder, liver, pancreas, breast, prostate, cervix, and skin, we suggest that high levels of this enzyme may promote tumorigenesis, at least in part by facilitating NF-κB-induced gene expression [36].

Perspective

As we described above, post-translational modifications of NF-κB are complex and diverse. These modifications determine the activity of NF-κB and regulate its gene expression. Among all these known modifications, acetylation and methylation are the most attractive ones, as both are catalyzed by the histone modifying enzymes, a class of enzymes proven to be more promising druggable targets than others. Some inhibitors for histone deacetylase (HDAC) have already been developed and approved by US Food and Drug Administration (FDA), but with limited treatment outcome. Therefore, there is urgent need for identifying more promising druggable targets than HDAC. Since methylation is the newest type of modification found on NF-κB, this makes histone methyltransferases or demethylases the hottest drug targets today [37].

Based on the information regarding novel methylation sites (Figure 1) on NF-κB and their catalyzing enzymes, we predict that in different cell systems, histone methylasesNSD1, Set9 and PRMT5 could become promising druggable targets for small inhibitors that will decrease the activity of NF-κB (Figure 2). On the other hand, targeted activation of Set6D, FBXL11 or Set9 (in different cell system, [28]) by small molecules may negatively regulate NF-κB activity (Figure 2).

It is worth of noting that the identification of PRMT5 as the enzyme that can methylate R30 of p65 has been particularly important in shedding light on this previously under studied area due to the fact this is the first and only R residue that has been reported to be methylated on NF-κB. Like lysine methylation of p65, it would not be surprising if we were to see more methylated arginine sites of p65 subunit of NF-κB identified in the years to come. Tangled with other post-translational modifications of p65, known or to be discovered, as well as the modifications of other subunits of NF-κB, the fine-tuned regulation of NF-κB is a fertile field for the inspired researcher to explore.

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References

1. Hayden MS, Ghosh S (2004) Signaling to NF-kappaB. Genes Dev 18: 2195-2224.
2. Gilmore TD (2006) Introduction to NF-kappaB: players, pathways, perspectives. Oncogene 25: 6680-6684.
3. Bonizzi G, Karin M (2004) The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25: 280-288.
4. Perkins ND (2006) Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene 25: 6717-6730.
5. Sabatel H, Di Valentin E, Gloire G, Dequiedt F, Piette J, et al. (2012) Phosphorylation of p65(RelA) on Ser(547) by ATM represses NF-Î°B-dependent transcription of specific genes after genotoxic stress. PLoS One 7: e38246.
6. Anrather J, Racchumi G, Iadecola C (2005) cis-acting, element-specific transcriptional activity of differentially phosphorylated nuclear factor-kappa B. J Biol Chem 280: 244-252.
7. Zhong H, Voll RE, Ghosh S (1998) Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Mol Cell 1: 661-671.

8. Reber L, Vermeulen L, Haegeman G, Frossard N (2009) Ser276 phosphorylation of NF-kB p65 by MSK1 controls SCF expression in inflammation. PLoS One 4: e4393.

9. Mattioli I, Geng H, Sebald A, Hodel M, Bucher C, et al. (2006) Inducible phosphorylation of NF-kappa B p65 at serine 468 by T cell costimulation is mediated by IKK epsilon. J Biol Chem 281: 6175-6183.

10. Schwabe RF, Sakurai H (2005) IKKbeta phosphorylates p65 at S468 in transactivaton domain 2. FASEB J 19: 1758-1760.

11. Buss H, Dörrie A, Schmitz ML, Frank R, Livingstone M, et al. (2004) Phosphorylation of serine 468 by GSK-3beta negatively regulates basal p65 NF-kappaB activity. J Biol Chem 279: 49571-49574.

12. Wang D, Westerheide SD, Hanson JL, Baldwin AS Jr (2000) Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. J Biol Chem 275: 32592-32597.

13. Yang F, Tang E, Guan K, Wang CY (2003) IKK beta plays an essential role in the phosphorylation of RelA/p65 on serine 536 induced by lipopolysaccharide. J Immunol 170: 5630-5635.

14. Duran A, Diaz-Meco MT, Moscat J (2003) Essential role of RelA Ser311 phosphorylation by zetaPKC in NF-kappaB transcriptional activation. EMBO J 22: 3910-3918.

15. Ryo A, Suizu F, Yoshida Y, Perrem K, Liou YC, et al. (2003) Regulation of NF-kappaB signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. Mol Cell 12: 1413-1426.

16. Yeh PY, Yeh KH, Chuang SE, Song YC, Cheng AL (2004) Suppression of MEK/ERK signaling pathway enhances cisplatin-induced NF-kappaB activation by protein phosphatase 4-mediated NF-kappaB p65 Thrdephosphorylation. J Biol Chem 279: 26143-26148.

17. Rocha S, Garrett MD, Campbell KJ, Schumm K, Perkins ND (2005) Regulation of NF-kappaB and p53 through activation of ATR and Chk1 by the ARF tumour suppressor. EMBO J 24: 1157-1169.

18. Msaki A, Sánchez AM, Koh LF, Barré B, Rocha S, et al. (2011) The role of RelA (p65) threonine 505 phosphorylation in the regulation of cell growth, survival, and migration. Mol Biol Cell 22: 3032-3040.

19. Chen LF, Williams SA, Mu Y, Nakano H, Duerr JM, et al. (2005) NF-kappaB RelA phosphorylation regulates RelA acetylation. Mol Cell Biol 25: 7966-7975.

20. Chen LF, Mu Y, Greene WC (2002) Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. EMBO J 21: 6539-6548.

21. Kiernan R, Brès V, Ng RW, Coudart MP, El Messaoudi S, et al. (2003) Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. J Biol Chem 278: 2758-2766.

22. StrebovskyJ, Walker P, Lang R, Dalpke AH (2011) Suppressor of cytokine signaling 1 (SOCS1) limits NFkappaB signaling by decreasing p65 stability within the cell nucleus. FASEB J 25:863-874.

23. Park SW, Huq MD, Hu X, Wei LN (2005) Tyrosine nitration on p65: a novel mechanism to rapidly inactivate nuclear factor-kappaB. Mol Cell Proteomics 4: 300-309.

24. Ea CK, Baltimore D (2009) Regulation of NF-kappaB activity through lysine monomethylation of p65. Proc Natl Acad Sci U S A 106: 18972-18977.

25. Lu T, Jackson MW, Singhi AD, Kandel ES, Yang M, et al. (2009) Validation-based insertional mutagenesis identifies lysine demethylase FBXL11 as a negative regulator of NFkappaB. Proc Natl Acad Sci U S A 106: 16339-16344.

26. Zhang T, Park KA, Li Y, Byun HS, Jeon J, et al. (2013) PHF20 regulates NF-ÎºB signalling by disrupting recruitment of PP2A to p65. Nat Commun 4: 2062.

27. Levy D, Kuo AJ, Chang Y, Schaefer U, Kitson C, et al. (2011) Lysine methylation of the NF-ÎºB subunit RelA by SETD6 couples activity of the histone methyltransferase GLP at chromatin to tonic repression of NF-ÎºB signaling. Nat Immunol 12: 29-36.

28. Yang XD, Huang B, Li M, Lamb A, Kelleher NL, et al. (2009) Negative regulation of NF-kappaB action by Set9-mediated lysine methylation of the RelA subunit. EMBO J 28: 1055-1066.

29. Lu T, Stark GR (2010) Use of forward genetics to discover novel regulators of NF-kappaB. Cold Spring Harb Perspect Biol 2: a001966.

30. Stark GR, Wang Y, Lu T (2011) Lysine methylation of promoter-bound transcription factors and relevance to cancer. Cell Res 21: 375-380.

31. Lu T, Jackson MW, Wang B, Yang M, Chance MR, et al. (2010) Regulation of NF-kappaB by NSD1/FBXL11-dependent reversible lysine methylation of p65. Proc Natl Acad Sci U S A 107: 46-51.

32. Lu T, Yang M, Huang DB, Wei H, Ozer GH, et al. (2013) Role of lysine methylation of NF-ÎºB in differential gene regulation. Proc Natl Acad Sci U S A 110: 13510-13515.

33. Sims RJ 3rd, Reinberg D (2008) Is there a code embedded in proteins that is based on post-translational modifications? Nat Rev Mol Cell Biol 9: 815-820.

34. Latham JA, Dent SY (2007) Cross-regulation of histone modifications. Nat Struct Mol Biol 14: 1017-1024.

35. Wei H, Wang B, Miyagi M, She Y, Gopalan B, et al. (2013) PRMT5 dimethylates R30 of the p65 subunit to activate NF-ÎºB. Proc Natl Acad Sci U S A 110: 13516-13521.

36. Wei H1, Mundade R1, Lange KC2, Lu T3 (2014) Protein arginine methylation of non-histone proteins and its role in diseases. Cell Cycle 13: 32-41.

37. Arrowsmith CH, Bountra C, Fish PV, Lee K, Schapira M (2012) Epigenetic protein families: a new frontier for drug discovery. Nat Rev Drug Discov 11: 384-400.