The *C. elegans* Ortholog of USP7 controls DAF-16 stability in Insulin/IGF-1-like signaling

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FOXO family transcription factors are downstream effectors of Insulin/IGF-1 signaling (IIS) and are regulated by posttranslational modification and coregulators, including components of the ubiquitin-proteasome system (UPS). Cofactors promoting DAF-16/FOXO protein stability and function in IIS have not been described yet. In a recent study, we have identified the deubiquitylating enzyme MATH-33, the ortholog of mammalian USP7/HAUSP, as an essential DAF-16 coregulator. We found that MATH-33 actively stabilizes DAF-16 protein levels when IIS is downregulated. Here we discuss how DAF-16/FOXO transcription factors are regulated by the UPS, in particular by the interplay of E3-ubiquitin ligases and deubiquitylating enzymes, which is critical for balancing DAF-16/FOXO activity and degradation. Recent findings raise the intriguing possibility that regulated oscillations in DAF-16/FOXO steady state levels play an integral role in mechanisms controlling healthspan and lifespan extension.

Keywords: aging, DAF-16, DUB, deubiquitinating enzyme, FOXO, HAUSP, Insulin/IGF-1 signaling, longevity, MATH-33, RLE-1, ubiquitin proteasome system, USP7

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Introduction

FOXO proteins are evolutionally conserved transcription factors involved in multiple biological processes including stress response, metabolism and lifespan regulation. The discovery of IIS-FOXO signaling in health and lifespan extension has greatly benefited from studies in invertebrates, in particular the nematode *Caenorhabditis elegans*.¹ Subsequently, a functional conservation of the

IIS-FOXO signaling axes has emerged as being critical for mammalian longevity determination.² More recently, genome-wide association studies (GWAS) have identified single nucleotide polymorphisms in the FOXO3a gene in humans, who lived to an extreme age³ supporting the evolutionary conservation of FOXO proteins in regulating the aging process.

The activity of FOXO proteins is primarily regulated by cytoplasmic to nuclear shuttling in response to upstream signaling networks. These phosphorylation dependent shuttling processes have been extensively characterized in previous studies.⁴ In addition, FOXO transcription factors and their *C. elegans* ortholog DAF-16 were found being tightly regulated at the level of protein turnover by the ubiquitin proteasome system (UPS).^{5,6} The UPS plays an essential role in protein degradation by tagging substrate proteins with ubiquitin chains, which are subsequently recognized by the proteasome. Ubiquitin residues are transferred and covalently attached to substrates by a sequential activation of 3 enzymes including an ubiquitin-activating enzyme (E1), an ubiquitin conjugating enzyme (E2) and an ubiquitin ligase (E3). The degradation of proteins tagged with polyubiquitin chains can be antagonized by deubiquitylases (DUBs), which are able to remove ubiquitin chains from substrate proteins rescuing them from degradation by the proteasome. Interestingly, several E3 ubiquitin ligases have been found to catalyze FOXO polyubiquitylation and proteasomal degradation in the context of growth factor signaling,^{6,7} however, deubiquitylases that actively promote FOXO protein stability downstream of IIS have not been discovered yet.

FOXO Proteins are Regulated by Mono- and Polyubiquitylation

Ubiquitylation is a reversible post-translational modification, in which ubiquitin is attached to lysine residues on substrate proteins or lysine residues of ubiquitin itself. Thus, ubiquitin can be attached to a substrate as a single ubiquitin molecule (monoubiquitin) or as a polyubiquitin chain. Polyubiquitin chains are assembled through isopeptide bond formation between the C-terminal Gly of ubiquitin and any one of 7 internal Lys residues of another ubiquitin molecule (i.e. Lys48 or Lys63). Lys48 and Lys63-linked chains have been extensively studied and linked to proteasomal degradation and signal transduction, respectively.

FOXO proteins are subject to both mono- and polyubiquitylation.⁵ MDM-2 has been identified as an E3 ubiquitin ligase catalyzing FOXO monoubiquitylation under oxidative stress conditions.⁸ Monoubiquitylation on FOXO proteins causes an increase in FOXO transcriptional activity and enhances its association with chromatin.^{9,10} Exactly how FOXO monoubiquitylation results in increased transcriptional activation is not known, but it seems likely that monoubiquitin on FOXO is recognized by ubiquitin-binding proteins to promote FOXO target gene activation. In addition, it needs to be determined whether monoubiquitin adducts on FOXO associated with transcriptional activation could prime the synthesis of longer ubiquitin chains such as Lys-48-linked ubiquitin conjugates promoting proteasomal degradation. In this scenario an activated monoubiquitylated FOXO protein would be subject to subsequent degradation by priming polyubiquitin chain synthesis as a means of preventing hyperactivation of FOXO target genes.

Several E3 ubiquitin ligases have been found to polyubiquitylate FOXO proteins for the degradation by the proteasome. Interestingly, FOXO proteins need to be first phosphorylated by upstream kinases before they are targeted by E3 ubiquitin ligases including SCF-Skp2, CHIP and MDM2.^{11–13} For example, AKT phosphorylates FOXO1 at S256, which is required for FOXO1 association with Skp2, a subunit of the Skp1/Cul1/F-box

protein ubiquitin complex, leads to FOXO1 polyubiquitylation and degradation.¹² In another study, it was shown that FOXO3 phosphorylation by ERK at S294, S344 and S425 increases its binding to the E3 ubiquitin ligase MDM2, resulting in FOXO polyubiquitylation and proteasomal degradation.¹³ In these cases, canonical ubiquitylation on FOXOs via Lys-48-linked ubiquitin conjugates target FOXO proteins for degradation. Furthermore, an additional E3 ubiquitin ligase, atrogin-1/MAFbx, was found to ubiquitylate FOXO1 and FOXO3a in a non-canonical manner.¹⁴ Atrogin-1/MAFbx conjugates Lys-63-linked ubiquitin chains, which act as a non-proteolytic signal thereby enhancing FOXOs nuclear translocation and their transcriptional activity. For the studies described above, the ubiquitylated Lys residues on FOXO have not been identified. Altogether, the UPS regulates FOXO proteins in a complex manner, based on activating or degrading ubiquitin conjugates added by diverse E3-ubiquitin ligases in response to various upstream signaling networks.

As an additional layer of complexity, the function of E3-ubiquitin ligases can be opposed by deubiquitylases (DUBs). DUBs are able to remove mono- and polyubiquitin adducts from substrate proteins.¹⁵ USP7, a member of the ubiquitin-specific proteases (USPs) class of deubiquitylases, has been identified as a regulator for FOXO proteins that antagonizes monoubiquitylation on FOXO.^{9,10} USP7 is able to physically associate with FOXO proteins and de-conjugate monoubiquitin adducts counteracting FOXO activity, without affecting its protein stability. Interestingly a DUB controlling FOXO protein stability has not been found to date. This raises the question whether additional deubiquitylases antagonizing FOXO polyubiquitylation and degradation might be critical for balancing FOXO protein levels.

The Deubiquitylase MATH-33 Stabilizes DAF-16 Protein Levels in IIS

A previous study indicates that protein levels of the *C. elegans* FOXO ortholog

DAF-16 are regulated by the UPS.⁶ This work has demonstrated that the RING finger-containing E3-ubiquitin ligase RLE-1 (regulation of longevity by E3) is able to catalyze polyubiquitylation of DAF-16 and cause its proteasomal degradation. Conversely, *rle-1* deficiency results in an increase of DAF-16 protein stability, which, in turn, enhances DAF-16 dependent stress resistance and longevity, and delays DAF-16 dependent larval development of animals. Since RLE-1 triggers DAF-16 degradation by the UPS, RLE-1 functions as a negative regulator of DAF-16 protein stability and its physiological readouts. In a recent study, we took an unbiased approach toward the identification of novel proteins that play a role in stabilizing DAF-16. We isolated DAF-16 binding partners using Tandem Affinity Purification under conditions when DAF-16 was nuclear and transcriptionally active and discovered the deubiquitylase MATH-33 as a novel interacting factor. MATH-33 was required to stabilize DAF-16 protein steady-state levels when IIS was genetically downregulated and, thus, was positively regulating DAF-16 functions.

The DAF-16 stabilizing function of MATH-33 was unexpected, as MATH-33 is the closest ortholog of mammalian USP7/HAUSP, a deubiquitylase, previously discovered as a negative regulator of FOXO activity.^{9,10} In mammals, USP7/HAUSP has been shown to deubiquitylate monoubiquitylated FOXO proteins under acute oxidative stress conditions and serum starvation, which negatively regulates FOXO function, but not its protein stability.^{9,10} Importantly, monoubiquitylation of FOXO proteins is associated with an increased FOXO transcriptional activity. Therefore, USP7/HAUSP acts as a negative regulator of FOXO activity in mammalian systems as it removes monoubiquitin residues on FOXO. In *C. elegans* we found that MATH-33 antagonizes RLE-1-mediated polyubiquitylation and degradation of DAF-16 when IIS is compromised (Fig. 1). The stabilization of DAF-16 by the deubiquitylase MATH-33 was essential for IIS-conferred DAF-16 physiological readouts in early development, stress response, lipid storage and lifespan extension.

MATH-33 was physically associated with DAF-16 in *C. elegans* and could also bind to non-ubiquitylated, recombinant DAF-16 *in vitro* as it has been described for the FOXO-USP7 interaction. These data suggest that the physical binding of MATH-33 is essential for its recognition of DAF-16 as a substrate rather than simply recognizing a specific ubiquitin chain type. By analogy, USP7, a member of the ubiquitin-specific protease (USP) category, likely belongs to a substrate-specific group of DUBs.¹⁵ USP7 removes ubiquitin chains from substrates in a relatively promiscuous manner with regard to the ubiquitin linkage type; however, it needs to recognize its substrates by physical association. The interaction domain for the MATH-33/USP7 – DAF-16/FOXO binding has not been mapped yet. Yeast 2-hybrid data indicate that the C-terminal part of USP7 might be critical for the association with the C-terminal region of FOXO4.⁹ The binding of MATH-33 to DAF-16 is enhanced when IIS is downregulated in *C. elegans* suggesting that DAF-16 nuclear shuttling is essential for the physical interaction with predominantly nuclear MATH-33.^{16,17} Moreover, MATH-33 nuclear localization is more pronounced when IIS is compromised indicating that the association of DAF-16 with MATH-33 could occur in the nucleus. However, according to the mammalian FOXO literature, FOXO proteins need to be cytoplasmic and phosphorylated to interact with E3-ubiquitin ligases, which mediate their polyubiquitylation and degradation,^{12,18,19} These data would suggest that the MATH-33 deubiquitylase could act on polyubiquitylated DAF-16 in the cytoplasm in *C. elegans* as well (Fig. 1). It is not known to date, if the E3-ubiquitin ligase RLE-1 recognizes phosphorylated DAF-16 in the cytoplasm or whether DAF-16 is non-phosphorylated and nuclear when it is polyubiquitylated by RLE-1. Further studies are needed to determine the subcellular localization in which RLE-1 and MATH-33 regulate the ubiquitylation state of DAF-16.

Interestingly, MATH-33 was not required to maintain DAF-16 steady state protein levels in wild type animals when IIS signals at normal physiological levels.¹⁶ This raises the question how

Figure 1. The USP7 ortholog MATH-33 promotes DAF-16 stability and functions in Insulin/IGF-1-like signaling (IIS). MATH-33 antagonizes RLE-1-mediated polyubiquitylation and proteasomal degradation of DAF-16 when IIS is downregulated. DAF-16 steady-state levels are critical for controlling DAF-16 functions in development, metabolism, stress response and longevity. It needs to be determined whether nuclear, transcriptionally active DAF-16 is monoubiquitylated like mammalian FOXO proteins. It is not known to date if DAF-16, polyubiquitylated by RLE-1, is phosphorylated and localized in the cytoplasm.

MATH-33 specifically regulates DAF-16 in the context of reduced growth factor signaling on a mechanistic layer. MATH-33 protein levels did not significantly change; however, we found that MATH-33 nuclear localization and colocalization with active nuclear DAF-16 was enhanced when IIS was diminished. This resulted in an increased physical association of MATH-33 with DAF-16 in the context of compromised IIS. Thus, it is possible that MATH-33 subcellular localization and physical binding to substrates are controlled by posttranslational modifications (PTMs), such as phosphorylation and ubiquitylation. MATH-33 subcellular localization and physical association with DAF-16 might be regulated on the posttranslational level by the Insulin/IGF-1 signaling cascade itself. PTMs including phosphorylation and ubiquitylation have been identified on mammalian USP7 using mass spectrometry,²⁰ however, their impact on USP7 substrate binding has not been addressed under reduced growth factor signaling conditions. In summary our data point to an IIS-specific regulation of DAF-16 stability by the deubiquitylase MATH-33, which correlates with an enhanced physical binding of MATH-33 to its substrate DAF-16 upon reduced growth factor signaling.

USP7 and MATH-33: Evolutionary Conserved or Species-Specific DUBs?

USP7 appears to be a relatively conserved deubiquitylase across species from man to yeast.²¹ The highest degree in sequence homology is observed in the catalytic domain; however, domains outside the catalytic domain are poorly conserved. For instance, human USP7 and *C. elegans* MATH-33 share 50% amino acid sequence identity in the catalytic domain, but reveal only 31% total sequence identity (Fig. 2). Such differences in regulatory domains outside the catalytic domain could result in distinct functions for USP7 orthologues between species. MATH-33 could have evolved a species-specific role in the regulation of DAF-16 polyubiquitylation and stability,¹⁶ in contrast to the role of USP7, which antagonizes FOXO transcriptional activity by removing monoubiquitin residues.^{9,10}

However, it is also possible that USP7 and MATH-33 act as dual-specificity DUBs. In this case the deubiquitylases would regulate FOXO/DAF-16 activity by antagonizing monoubiquitylation under one circumstance, but control FOXO/DAF-16 stability by reversing its polyubiquitylation under other circumstances. In this scenario the signaling intensities upstream of FOXO/DAF-16

Figure 2. Amino acid sequence alignment of human USP7 with *C. elegans* MATH-33. Isoform 1 of human USP7 (UniProt Q93009-1) was aligned with *C. elegans* MATH-33 (UniProt Q7JKC3). Percent sequence identity of proteins and individual domains are presented. TRAF, substrate-binding domain; C223, C202, active site cysteine residues in catalytic domain; HUBL, USP7/HAUSP ubiquitin-like domain.

could impact the extent of FOXO/DAF-16 nuclear localization and activity as well as their interaction with the cellular ubiquitylation machinery. In our study IIS was genetically reduced in *C. elegans* by using a temperature sensitive allele of the insulin/IGF-1 receptor ortholog *daf-2* [*daf-2(e1370)*], which compromised IIS efficiently and chronically. We induced DAF-16 activity by shifting temperature sensitive *daf-2* mutants to the semipermissive temperature (20°C) for 24 hours to several days or weeks depending on the experimental setting. Interestingly, the *daf-2(e1370)* mutant already reduces IIS at 15°C, which is considered as permissive temperature, but is actually semipermissive as well. Thus, the 15°C condition results in partial DAF-16 nuclear localization and lifespan extension. Overall, this would suggest that the *daf-2(e1370)* temperature sensitive mutant might cause chronically reduced IIS leading to a strong, permanent nuclear localization of DAF-16 at 20°C. This potentially could shift the ubiquitylation state of DAF-16 to polyubiquitylation mediated by the E3-ubiquitin ligase RLE-1 and its degradation by the proteasome. Under these conditions the activity of MATH-33 could predominantly remove polyubiquitin chains from DAF-16 and thus stabilize DAF-16. Conversely, the study of Van der Horst et al., described mammalian USP7 as a negative regulator for FOXO proteins by removing monoubiquitin and consequently reducing FOXO transcriptional activity without affecting its protein stability.⁹ In this report FOXO activation was induced transiently, through exposure of cultured cells to hydrogen peroxide for 5 to 120 minutes. Monoubiquitylation on FOXO was already observed after 5 minutes, and USP7 association with

FOXO was increased after 15 minutes suggesting a fast response of USP7 for removing monoubiquitin adducts from FOXO under transient activation conditions. In another study, FOXO1 activity was induced in hepatocytes by serum starvation and forskolin, a cAMP activator used to mimic the conditions of fasting.¹⁰ FOXO1 was activated relatively transiently, from 6 hours to overnight, to study the effect of USP7 on FOXO1 monoubiquitylation. Under these conditions USP7 antagonized monoubiquitylation on FOXO1, its transcriptional activity and ability to bind to promoter regions. Altogether, these data suggest that the nature of signaling networks upstream of FOXOs such as stress or Insulin/IGF-1 pathways, and differences in signaling intensities and their duration could impact how the deubiquitylase activities of MATH-33/USP7 affect DAF-16/FOXO. It is possible that MATH-33 and USP7 have dual specificities toward monoubiquitin and polyubiquitin chains linked to DAF-16/FOXO. Upstream signaling events could affect transcription factor activity by reversing monoubiquitylation when DAF-16/FOXO is transiently activated and protein stability by antagonizing polyubiquitylation under DAF-16/FOXO hyper-stimulation.

Concluding Remarks

DAF-16/FOXO transcription factors are critical regulators of the aging process and age-related diseases such as cancer and diabetes. Recent studies revealed that FOXO function is tightly regulated by the UPS. In particular, mammalian FOXO activity is balanced by the interplay of E3-ubiquitin ligases and the deubiquitylase USP7/

HAUSP. In contrast, we found that the *C. elegans* ortholog of USP7, MATH-33, is essential to promote DAF-16 stability and its functions by antagonizing the activity of the E3-ubiquitin ligase RLE-1 when IIS is compromised and lifespan is extended. Since USP7 is a relatively well-conserved deubiquitylase from yeast to man, it will be imperative to dissect whether USP7 acts as a regulator for FOXO stability in the context of IIS in mammals. Knowledge gained through these studies will have important implications for our understanding of the aging process in humans as well as for age-related diseases including diabetes and cancer.

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No potential conflicts of interest were disclosed.

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References

1. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 1993; 366:461-4; PMID:8247153; <http://dx.doi.org/10.1038/366461a0>
2. Kenyon CJ. The genetics of ageing. *Nature* 2010; 464:504-12; PMID:20336132; <http://dx.doi.org/10.1038/nature08980>
3. Wheeler HE, Kim SK. Genetics and genomics of human ageing. *Philos Trans R Soc Lond B Biol Sci* 2011; 366:43-50; PMID:21115529; <http://dx.doi.org/10.1098/rstb.2010.0259>
4. Calnan DR, Brunet A. The FoxO code. *Oncogene* 2008; 27:2276-88; PMID:18391970; <http://dx.doi.org/10.1038/onc.2008.21>
5. Huang H, Tindall DJ. Regulation of FOXO protein stability via ubiquitination and proteasome degradation. *Biochim Biophys Acta* 2011; 1813:1961-4; PMID:21238503; <http://dx.doi.org/10.1016/j.bbamcr.2011.01.007>
6. Li W, Gao B, Lee SM, Bennett K, Fang D. RLE-1, an E3 ubiquitin ligase, regulates *C. elegans* aging by catalyzing DAF-16 polyubiquitination. *Dev Cell* 2007; 12:235-46; PMID:17276341; <http://dx.doi.org/10.1016/j.devcel.2006.12.002>
7. Zhao Y, Wang Y, Zhu WG. Applications of post-translational modifications of FoxO family proteins in biological

- functions. *J Mol Cell Biol* 2011; 3:276-82; PMID:21669942; <http://dx.doi.org/10.1093/jmcb/mjr013>
8. Brenkman AB, de Keizer PL, van den Broek NJ, Jochimsen AG, Burgering BM. Mdm2 induces monoubiquitination of FOXO4. *PloS One* 2008; 3:e2819; <http://dx.doi.org/10.1371/journal.pone.0002819>
9. van der Horst A, de Vries-Smits AM, Brenkman AB, van Triest MH, van den Broek N, Colland F, Maurice MM, Burgering BM. FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. *Nat Cell Biol* 2006; 8:1064-73; PMID:16964248; <http://dx.doi.org/10.1038/ncb1469>
10. Hall JA, Tabata M, Rodgers JT, Puigserver P. USP7 attenuates hepatic gluconeogenesis through modulation of FoxO1 gene promoter occupancy. *Mol Endocrinol* 2014; 28:912-24; PMID:24694308; <http://dx.doi.org/10.1210/me.2013-1420>
11. Li F, Xie P, Fan Y, Zhang H, Zheng L, Gu D, Patterson C, Li H. C terminus of Hsc70-interacting protein promotes smooth muscle cell proliferation and survival through ubiquitin-mediated degradation of FoxO1. *J Biol Chem* 2009; 284:20090-8; <http://dx.doi.org/10.1074/jbc.M109.017046>
12. Huang H, Regan KM, Wang F, Wang D, Smith DI, van Deursen JM, Tindall DJ. Skp2 inhibits FOXO1 in tumor suppression through ubiquitin-mediated degradation. *Proc Natl Acad Sci USA* 2005; 102:1649-54; PMID:15668399; <http://dx.doi.org/10.1073/pnas.0406789102>
13. Yang JY, Zong CS, Xia W, Yamaguchi H, Ding Q, Xie X, Lang JY, Lai CC, Chang CJ, Huang WC, et al. ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. *Nat Cell Biol* 2008; 10:138-48; PMID:18204439; <http://dx.doi.org/10.1038/ncb1676>
14. Li HH, Willis MS, Lockyer P, Miller N, McDonough H, Glass DJ, Patterson C. Atrogin-1 inhibits Akt-dependent cardiac hypertrophy in mice via ubiquitin-dependent coactivation of Forkhead proteins. *J Clin Invest* 2007; 117:3211-23; PMID:17965779; <http://dx.doi.org/10.1172/JCI31757>
15. Komander D, Clague MJ, Urbe S. Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* 2009; 10:550-63; PMID:19626045; <http://dx.doi.org/10.1038/nrm2731>
16. Heimbucher T, Liu Z, Bossard C, McCloskey R, Carrano AC, Riedel CG, Tanasa B, Klammt C, Fonslow BR, Riera CE, et al. The Deubiquitylase MATH-33 Controls DAF-16 Stability and Function in Metabolism and Longevity. *Cell Metab* 2015; 22:151-63; PMID:26154057; <http://dx.doi.org/10.1016/j.cmet.2015.06.002>
17. McCloskey RJ, Kemphues KJ. Deubiquitylation Machinery Is Required for Embryonic Polarity in *Caenorhabditis elegans*. *PLoS Genetics* 2012; 8:e1003092; PMID:23209443; <http://dx.doi.org/10.1371/journal.pgen.1003092>
18. Matsuzaki H, Daitoku H, Hatta M, Tanaka K, Fukamizu A. Insulin-induced phosphorylation of FKHR (Foxo1) targets to proteasomal degradation. *Proc Natl Acad Sci USA* 2003; 100:11285-90; PMID:13679577; <http://dx.doi.org/10.1073/pnas.1934283100>
19. Fu W, Ma Q, Chen L, Li P, Zhang M, Ramamoorthy S, Nawaz Z, Shimajima T, Wang H, Yang Y, et al. MDM2 acts downstream of p53 as an E3 ligase to promote FOXO ubiquitination and degradation. *J Biol Chem* 2009; 284:13987-4000; PMID:19321440; <http://dx.doi.org/10.1074/jbc.M901758200>
20. Fernandez-Montalvan A, Bouwmeester T, Joberty G, Mader R, Mahnke M, Pierrat B, Schlappe JM, Wornpenberg S, Gerhartz B. Biochemical characterization of USP7 reveals post-translational modification sites and structural requirements for substrate processing and subcellular localization. *FEBS J* 2007; 274:4256-70; PMID:17651432; <http://dx.doi.org/10.1111/j.1742-4658.2007.05952.x>
21. Holowaty MN, Sheng Y, Nguyen T, Arrowsmith C, Frappier L. Protein interaction domains of the ubiquitin-specific protease, USP7/HAUSP. *J Biol Chem* 2003; 278:47753-61; PMID:14506283; <http://dx.doi.org/10.1074/jbc.M307200200>