別紙４

報告番号	※　甲　第　　号

主　論　文　の　要　旨

論文題目　Histidine phosphorylation-mediated signal transduction regulates axon regeneration in *C. elegans*.
(ヒスチジンリン酸化を介したシグナル伝達は線虫の神経軸索再生を制御する)

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論　文　内　容　の　要　旨

Protein phosphorylation is one of the most important post-translational modifications that regulate and diversify protein functions. In animals, the phosphorylation of serine, threonine, and tyrosine are well characterized, but relatively little is known about the phosphorylation of histidine (pHis). Previous studies with cultured mammalian cells have shown that His-phosphorylation is regulated by the His-kinase NDPK and the pHis-phosphatase PHPT1. However, their physiological functions *in vivo* remain largely unknown.

To understand the *in vivo* role of His-phosphorylation in animals, I used the nematode *Caenorhabditis elegans* (C*. elegans*) as a model organism. *C. elegans* has one NDPK orthologue, NDK-1, and one PHPT1 orthologue, PHIP-1. Since NDK-1 has a housekeeping function independent of protein histidine phosphorylation, and its loss kills animals, I first focused on PHIP-1 and explored a phenotype in *phip-1* mutants. As a result, I found that PHIP-1 is required for axon regeneration, an evolutionarily conserved neuronal response in which neurons regenerate damaged axons for functional recovery. I also found that overexpression of NDK-1 inhibits axon regeneration. These results suggest that

His-phosphorylation has an inhibitory role in axon regeneration.

To elucidate the molecular mechanism by which His-phosphorylation inhibits axon regeneration, I searched for PHIP-1-binding proteins by yeast two-hybrid screen. I identified two genes, *gpb-1* and *unc-51*, which encode the heterotrimeric G protein β subunit (Gβ) and the homolog of human ULK kinase. Since Gβ is a known substrate of His-phosphorylation, I focused on GPB-1. From genetic and biochemical analyses, I found that NDK-1 inhibits axon regeneration by phosphorylating GPB-1 Gβ at His-266, while PHIP-1 promotes axon regeneration by counteracting this phosphorylation. Thus, GPB-1 His-266 phosphorylation inhibits axon regeneration.

Previous studies have shown that Gβ His-phosphorylation activates the heterotrimeric G protein α subunit (Gα) in a receptor-independent manner. Also, the *C. elegans* Goα protein, GOA-1, is known to inhibit axon regeneration. Therefore, I examined the genetic interactions between GPB-1 His-phosphorylation and GOA-1. As a result, I found that GPB-1 His-phosphorylation inhibits axon regeneration by activating GOA-1. Taken together, these results suggest that in wild-type animals, PHIP-1 promotes axon regeneration by dephosphorylating GPB-1 and inactivating GOA-1.

Next, I examined how PHIP-1 is activated during axon regeneration. To this end, I focused on another PHIP-1-binding protein, UNC-51. Since UNC-51 is a protein kinase, I tested whether UNC-51 phosphorylates PHIP-1. I found that UNC-51 phosphorylates PHIP-1 at serine 112 (S112). Furthermore, using a phospho-deficient PHIP-1(S112A) and a phospho-mimetic PHIP-1(S112E) mutant, I found that phosphorylation of PHIP-1 S112 is important for its catalytic activity and axon regeneration. These results suggest that UNC-51 activates PHIP-1 through S112 phosphorylation.

Based on the results above, I propose the following model. Axon injury activates UNC-51, which in turn phosphorylates and activates PHIP-1. Next, PHIP-1 dephosphorylates GPB-1, thereby inactivating GOA-1 signaling and promoting axon regeneration. Therefore, this study provides one example of how reversible His-phosphorylation regulates biological functions in living animals.