SUMO Conjugation to Protein Phosphate 2A in the Regulation of Cell Signaling Through the Ras/MAPK Pathway

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SUMO

- Small Ubiquitin-Related Modifier (SUMO) is a protein modifier that alters the function of a target protein
- SUMO has been shown to be critical to the regulation of many cellular processes, such as signaling, transcription, the cell cycle, and many homeostatic functions[1]

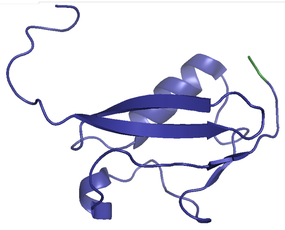

Figure 1. Crystal Structure of Drosophila SUMO. SUMO alters the function of a protein target by forming an isopeptide bond at SUMO's primed C-terminal highlighted in green with a lysine residue on a protein target

Ras/MAPK Pathway

- Ras/Mitogen Activated Protein Kinase(MAPK) pathway is conserved pathway from yeast to humans and is essential for cell proliferation
- Irregular Ras signaling is the reason for many developmental syndromes such as Noonan, Cardiofaciocutaneous, Legius, and Costello syndrome [2] [3]

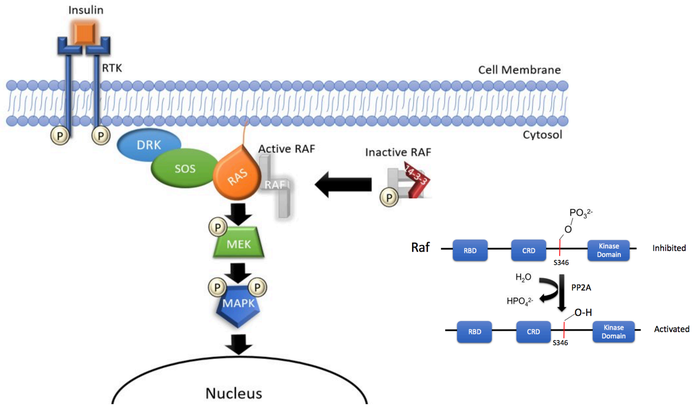

Figure 2. The Ras/MAPK signaling cascade. The Ras/MAPK pathway begins with the binding of an activating ligand to the RTK, leading to RTK autophosphorylation. Followed by the activation of DRK and Sos. Sos then activates Ras by catalyzing the exchange of GDP for GTP. In its GTP-bound form, Ras localizes to the membrane and recruits Raf which is activated by PP2A at serine 346. Raf then phosphorylates and activates MEK, which then di-phosphorylates MAPK. Activated MAPK then phosphorylates a number of transcription factors.

Proteomic evidence for SUMOylation in Ras Signaling

	Found in both Denaturing and Native Purification	Found only in Native Purification
Ras1 Signlaing	Leonardo, 14-3-3, PP2A Catalytic Subunit, PP2A Structural Subunit	PP2A Regulatory Subunit, Ras, ERK-A

Figure 3. Ras signaling proteins identified in the SUMO-ome. Nie et al., used Drosophila embryos expressing His_6-Flag SUMO established the first SUMO proteome study. Purification was done both in denaturing and native conditions, fractionated by molecular weight, trypsin digest, and analyzed via high pressure liquid chromatography followed by tandem mass spectrometry(ESI-QqTOF)[4].

SUMO Conjugation Increases Ras Signaling

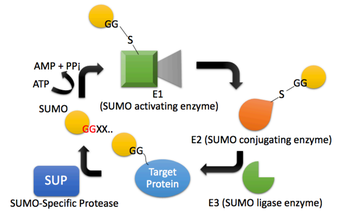

Figure 4. Mechanism of SUMO Conjugation. SUMO is primed at its C-terminal, resulting in an active SUMO conformation with di-glycine at the C-terminal. A series of SUMO enzymes catalyze the formation of an isopeptide bond to a lysine residue on a target protein. SUMO-Specific Proteases are responsible for reversing SUMO conjugation.

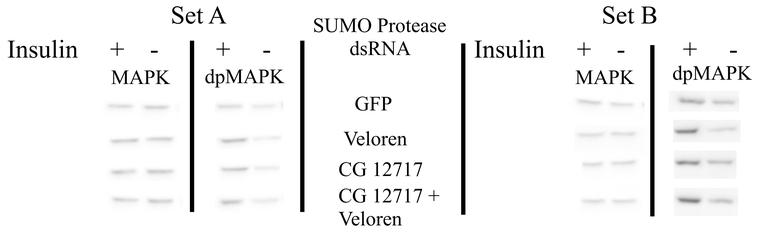

Figure 5. Knockdown of SUMO-Specific Protease increased Ras Signaling in Schneider 2 Cells. Schneider 2(S2) cells are a Drosophila melanogaster cell line. The reduction of SUMO-Specific Proteases (Veloren and CG12717) resulted in an increase of pathway sensitivity to insulin compared to the control(GFP). Knockdown of SUMO-Specific Proteases is reasoned to increase the global concentration of SUMOylated proteins.

SUMO regulation occurs parallel to Raf

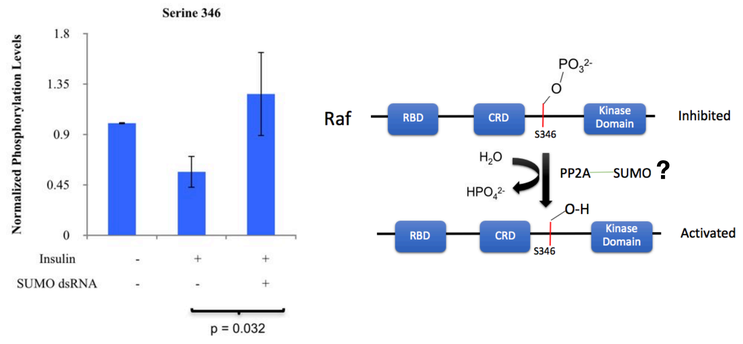

Figure 6. Knockdown of SUMO Increased Phosphorylation of Raf in Schneider 2 Cells. Raf in Ras signaling is found to be inactive when phosphorylated. Phosphorylation of Raf at serine 346 is increased when SUMO is knockdown and in the presence of insulin, suggesting that SUMO positively regulates Ras signaling parallel to Raf. We speculated SUMOylation to occur at PP2A.

PP2A Knockdown Decreased Ras Signaling

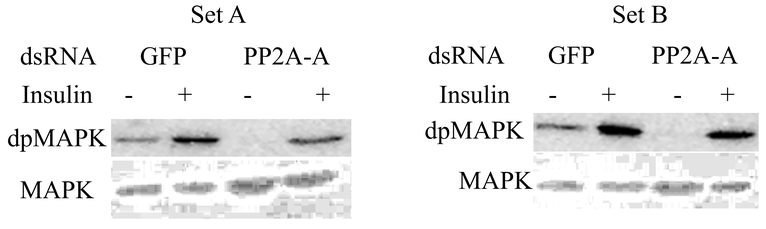

Figure 7. Knockdown of PP2A-A Decreased Ras Signaling in Schneider 2 Cells. PP2A is one of the known regulators of Raf in the Ras/MAPK pathway. Here we set to understand the importance of the structural subunit of PP2A(PP2A-A) in Ras signaling. Knocking down PP2A-A resulted in a decrease of pathway sensitivity to insulin suggesting that PP2A-A is necessary for proper pathway activity.

Proposed Ras/MAPK Pathway

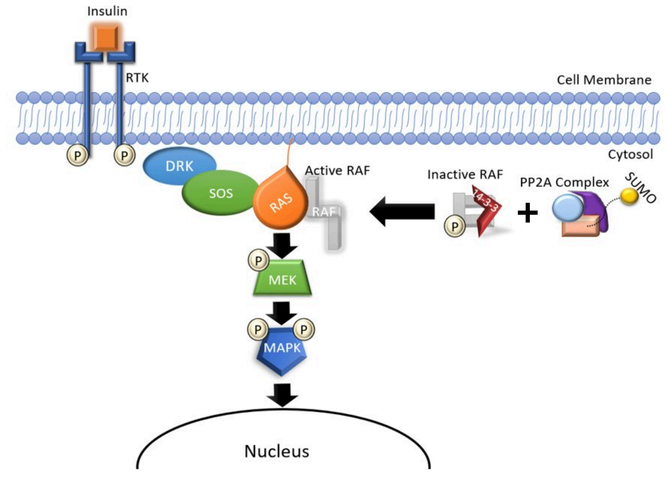

Figure 8. A proposed model for Ras/MAPK signaling cascade. The proposed model shows that the SUMOylation of the PP2A complex is necessary for the de-phosphorylation of Raf at serine 346, thereby activating Raf and resulting in proper Ras signaling.

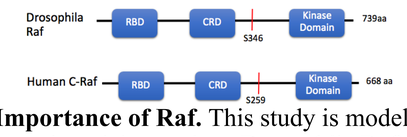

Figure 9. Importance of Raf. This study is modeled in Drosophila which serves as a good model organism. Drosophila Raf is homologous to human C-Raf and serine 346 in Drosophila is equivalent to serine 259 in humans.

Current Project

Proteomic approach for Identifying Site-Specific SUMOylation in Drosophila melanogaster

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1. SUMOylated Protein:
His x 6-Flag-N-Term … KHTPLRKLMN AYCDRAGLSM QVVRFRFDGQ PINENDTPTS LEMEEGDTIE VYQQQTGG~K(Protein)

2. Immobilized Metal Ion Affinity Chromatography(IMAC) under denaturing conditions
His-His-His-His-His-His-Flag-N-Term … … … QVVRFRFDGQ PINENDTPTS LEMEEGDTIE VYQQQTGG~K(Protein)
|    Nickle Column    |

3. Fractionation of SUMOylated Proteins via Molecular Weight
His x 6-Flag-N-Term - … … AYCDRAGLSM QVVRFRFDGQ PINENDTPTS LEMEEGD-TIE VYQQQTGG~K(Protein)

4. Protease Digestion of Isolated SUMOylated Proteins
His x 6-Flag-N-Term - … … AYCDRAGLSM QVVRFRFDGQ PINENDTPTS LEMEEGD~TIE VYQQQTGG~K(Protein)
                                                                    Glu-C

5. LC-MS/MS:
SUMOylated Peptides: VYQQQTGG~K(Peptide)
Signature Molecular Formula:C35H54N10O13  Signature Mass: 822.387722 m/z
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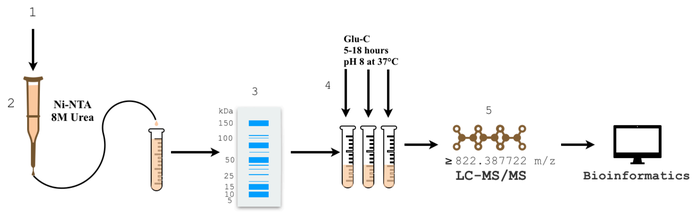

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Acknowledgments

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