A hypothetical MEK1-MIP1-SMEK multiprotein signaling complex may function in Dictyostelium and mammalian cells

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ABSTRACT In a previous study, we characterized Dictyostelium SUMO targeted ubiquitin ligase (StUbL) MIP1 that associates with protein kinase MEK1 and targets SUMOylated MEK1 to ubiquitination (Sobko et al., 2002). These modifications happen in response to activation of MEK1 by the chemoattractant cAMP. Second site genetic suppressor of mek1-null phenotype (SMEK) was also identified in Dictyostelium. MEK1 and SMEK belong to the same linear pathway, in which MEK1 negatively regulates SMEK, which then negatively regulates chemotaxis and aggregation. RNF4 is mammalian homologue of MIP: RNF4 interacts with hSMEK2, the human homologue of Dictyostelium SMEK. We propose the existence of an evolutionally conserved MEK1-SMEK signaling complex that upon MEK1 activation and SUMOylation, recruits ubiquitination ligase MIP1/RNF4, which, in turn, ubiquitinates SMEK and targets this protein for proteasomal degradation. This could be a mechanism for negative regulation of SMEK by MEK1 signaling.

KEY WORDS: RNF4, SUMO-targeted ubiquitin ligase, suppressor of MEK (SMEK), ubiquitination, cell migration

Cell motility and primitive mode of amoeboid migration are fundamental and ancient cellular behaviors that contribute to multicellular development, inflammation, immune responses, cancer metastasis and are conserved between mammals and non-mammalian model organisms, such as Dictyostelium discoideum (Stuelten et al., 2018). Dictyostelium presents a simple model to study directed cell migration (chemotaxis). The evolutionary conservation of canonical signaling pathway modules, accessible genetics (now including knock-outs, knock-ins, expression of engineered sequences, RNAi and CRISPR perturbations) (Sekine et al., 2018) and amenability to live imaging make Dictyostelium an important model to examine basic molecular mechanisms that govern chemotaxis. This organism permits direct observation of cells moving in complex native environment and allows large-scale genetic and pharmacological screening, as well as extensive biochemical and cell biology studies. Among genetically dissected pathways, MAP kinase pathways are central in control of aggregation and chemotaxis. The fundamental architecture of MAP kinase pathway is conserved between Dictyostelium and other eukaryotes, and this presents researchers with the opportunity to investigate conserved functions and common components, including protein kinases, kinase substrates, molecular scaffolds and regulatory proteins. Significant insights into functions of MAP kinase pathway were obtained using other genetically tractable model organisms, such as S. cerevisiae, C. elegans, and D. melanogaster. This cross-species data allows now to make comparisons between the phenotypes of these models, in which specific components of the pathway were perturbated (Shilo, 2014). It is also noteworthy, that ubiquitination machinery relevant for our discussion below has been extensively studied in Dictyostelium (Pergolizzi et al., 2019), and our study provides one important paradigm of how ubiquitination regulates protein kinase signaling and cellular functions (Sobko et al., 2002).

SUMO-targeted Ubiquitin Ligases (StUbLs) that recruit SUMOylated substrates to ubiquitination machinery have been characterized in fission and budding yeast, Drosophila and mammals (Sriramachandran and Dohmen, 2014), (Abed et al., 2018). In each system, specific individual substrates of StUbLs and their cellular functions were identified. In our previous study (Sobko et al., 2002), we characterized Dictyostelium StUbLMIP1 that associates with protein kinase MEK1 and targets SUMOylated MEK1 to ubiquitination. These modifications happen in response to activation of MEK1 by chemoattractant cAMP. Another study from Firtel lab characterized SMEK – second site genetic suppressor of mek1-null.
phenotype (Mendoza et al., 2005, Mendoza et al., 2007). Deletion of mek1 gene results in the phenotype, in which cells fail to aggregate properly and form very small aggregates, due to severe chemotaxis defect. Suppressor phenotype of SMEK implies that aggregation/chemotaxis defect of mek1 null cells is rescued upon deletion of smek gene in mek1- null cells. According to Mendoza et al., the analysis of smek phenotype shows, that not all effects of SMEK occur via MEK1 signaling. Nevertheless, at least in part, MEK1 sends the signal to SMEK, which, in turn, negatively affects chemotaxis and aggregation. Therefore, we propose the following scenario: MEK1 and SMEK belong to the same linear pathway, in which MEK1 negatively regulates SMEK, which then negatively regulates chemotaxis and aggregation.

Curiously, human SMEK1 and SMEK2 encode evolutionarily conserved isofoms of regulatory subunits of serine/threonine protein phosphatase 4, that among other functions, were implicated in control of cell migration (Martin-Granados et al., 2008, Gingras et al., 2005), (see also entries for SMEK1 and SMEK2 in GeneCards database for more information), (Rebhan et al., 1998).

In C.elegans, SMEK homologue was shown to function downstream FOXO transcription factor DAF-16 in canonical Insulin/IGF-1 signaling pathway, regulating longevity and stress responses (Wolff et al., 2006).

MIP1 is Dictyostelium RING Finger protein, which belongs to recently discovered evolutionarily conserved group of StUbL proteins, that contain also SUMO Interactive Motif (SIM) and drive SUMOylated proteins to ubiquitination and subsequent proteasomal degradation (Sobko et al., 2002, Sun et al., 2007, Geoffroy & Hay, 2009).

RNFL4 is mammalian homologue of MIP. It also contains SIM and RING Finger domains and it is most likely functions as StUbL. Recently, RNFL4-interacting proteins were systematically identified in high throughput proteomics/mass spectrometry study (Kumar et al., 2017). Intriguingly, the data of this study shows that RNFL4 interacts with human homologue of Dictyostelium SMEK – hSMEK2. This raises the possibility, that such complex is conserved in evolution, and exists in both human and Dictyostelium cells. If in human cells RNFL4 interacts with hSMEK2, then, MIP1 possibly interacts with SMEK in Dictyostelium. We propose that MEK1 and SMEK interact not only genetically, but also physically. Moreover, the mechanism of negative regulation of SMEK by MEK1 could be based on the existence of MEK1-SMEK complex that upon MEK1 activation and SUMOylation, recruits StUbL MIP1. It is possible, that MIP1 ubiquitinates SMEK and targets this protein for proteasomal degradation. This could be a basis for negative regulation of SMEK by MEK1 signaling.

Indeed, the data of high-throughput proteomics analysis of post-translational modifications (PTMs) indicates that SMEK homologues interact with each other (BioGRID database of protein-protein interactions), (Oughtred et al., 2019, Huttlin et al., 2017) and are ubiquitinated on multiple lysine residues that have been characterized (entries for SMEK1 and SMEK2 in PhosphoSitePlus database), (Hornbeck et al., 2015, Akimov et al., 2018, Povlsen et al., 2012, Wagner et al., 2011, Mertins et al., 2013, Udeshi et al., 2013).

Moreover, SMEK2 is also SUMOylated on one of the lysine residues, Lys726 (PhosphoSitePlus database), (Lumpkin et al., 2017), and both SMEK1 and SMEK2 possess putative uncharacterized SUMOylation sites, that fit the consensus SUMOylation motif (ψKXE/E, where ψ is a large hydrophobic amino acid, K is the target lysine, X is any amino acid and D/E is aspartate or glutamate), suggesting that MEK1-SMEK complex might be subject to SUMOylation that serves as a signal to bind StUbL MIP1/RNFL4, which likely targets the complex to subsequent ubiquitination and proteasomal degradation.

This data needs to be further validated under relevant physiological conditions and stimuli, such as exposure to the chemotactant. In our future studies, using Dictyostelium chemotaxis and aggregation, as experimental system, we would like to apply immune affinity purification and co-immunoprecipitation of tagged expressed proteins to prove that, MEK1, MIP and SMEK indeed form multi-protein complex. We would like to verify whether SMEK is SUMOylated and ubiquitinated upon chemoattractant stimulation. It will be curious to check the dynamic composition of SMEK-MIP1 complexes over the time course after chemoattractant stimulation. It will be

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**Fig. 1.** The MEK1-MIP1-SMEK signaling complex. (A) Regulation of chemotaxis by putative MEK1-MIP1-SMEK signaling complex. The known and the hypothetical connections (the latter in red text). (B) The known and hypothetical interactions within multiprotein signaling complexes.
TABLE 1
REPORTED COMPONENTS OF PUTATIVE MEK1-MIP1/RNF4-SMEK COMPLEX: KNOWN HOMOLOGUES IN OTHER ORGANISMS

| Gene/Protein | Homology, similarity of amino acid sequence to Dictyostelium counterpart | Reported protein-protein and genetic interactions | References |
|-------------|-------------------------------------------------|--------------------------------------------------|------------|
| Dicystostelium DdMEK1 | Homologue of MIP1 (Identical positions 222; similar positions 370) | MIP1 (yeast two-hybrid; co-IP) | Sobko et al, 2002 |
| Dicystostelium MIP1 | Homologue of SMEK (Identical positions 252; similar positions 314) | DdMEK1 (yeast two-hybrid; co-IP) | Sobko et al, 2002 |
| Dicystostelium SMEK | Homologue of SMEK (Identical positions 247; similar positions 319) | DdMEK1 (yeast two-hybrid; co-IP) | Sobko et al, 2002 |
| Human RNF4 | Homologue of MIP1 (Identical positions 47; similar positions 78) | hSMEK2 (IP-Mass spectrometry) | Mendoza et al, 2005, 2007 |
| Human MAP3K7 | MAP kinase kinase | RNF4 (reciprocal IP-Mass spectrometry) | Kumar et al, 2017 |
| C. elegans SMK-1 | Homologue of SMEK (Identical positions 222; similar positions 370) | Genetic interactions with FOXO transcription factor DAF-16 (Insulin/IGF-1 signaling pathway) | Wolff et al, 2006 |

Reported protein-protein and genetic interactions. Amino acid sequence identity and similarity were determined with UniProt (https://www.uniprot.org) Align tool.

Table: MEK1-MIP1-SMEK signaling complex

It will also be important to validate RNF4 – hSMEK2 interactions and existence of the complex in human cells (using suitable cell lines with high expression levels of these proteins). One may hypothesize that RNF4-SMEK complex also interacts with the protein kinase of one of mammalian MAP kinase pathways. Activation of this kinase would be expected to trigger SUMOylation, ubiquitination and proteasomal degradation of SMEK and possibly other components of the complex. For example, we would need to establish whether putative RNF4-hSMEK2 complex also contains mitogen-activated protein kinase kinase kinase, MAP3K7, which was found reciprocally as either “bait” or “pray” in mass spectrometry/proteomics study of RNF4-interacting proteins (BioGRID entries for RNF4 and MAP3K7), (Tan et al., 2015). MAP3K7 is known to be ubiquitinated in response to cytokine activation and signaling. Does MAP3K7 ubiquitination require RNF4, as a component of StUbL? Is MAP3K7 also SUMOylated? Is SUMOylation a prerequisite for ubiquitination? All these questions await further experimentation. Ultimately, we would like to apply systems biology analysis of protein-protein interactions and molecular pathways to identify other putative components of these conserved multi-protein signaling complexes and explore their functions.

The availability of data from global studies of protein-protein interactions and PTMs of protein kinases and other signaling proteins now makes it possible to predict and validate functional connections between the kinases, their putative substrates, modulators and other proteins (Sobko, 2006).

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Conflict of interest
The author has no a conflict of interest.

Data references
GeneCards Encyclopedia (www.genecards.org):
REBHAN, M., CHALIFA-CASPI, V., PRILUSKY, J., AND LANCET, D. (1998)

References
ABED M, BITMAN-LOTAN E, ORIANA. (2018) The Biology of SUMO-Targeted Ubiquitin Ligases in Drosophila Development, Immunity, and Cancer. J Dev Biol 6: 2.
AKIMOV V, BARRIO-HERNANDEZ I, HANSEN SVF, HALLENBORG P, PEDERSEN AK, BEKKER-JENSEN DB, PUGLIA M, CHRISTENSEN SDK, VANSELOW JT, NIELSEN MM, KRATCHMAROVA I, KELSTRUP CD, OLSEN JV, BLAGEBOV. (2018) UbiSite approach for comprehensive mapping of lysine and N-terminal ubiquitination sites. Nat Struct Mol Biol 25: 631-640.
HUTTLIN EL, BRUCKNER RJ, PAULO JA, CANNON JR, TING L, BALTIER K, COLBY G, GEBREAB F, GYIGI MP, PARZEN H, SZPYT J, TAM S, ZARRAGA G, PONTOANO-VAITES L, SWARUP S, WHITE AE, SCHWEPPE DK, RAD R, ERICKSON BK, OBAR RA, GURUHARSHA KG, LI K, ARTAVANIS-TSAKONAS S, GYIGI SP, HARPER JW. (2017) Architecture of the human interactome defines protein communities and disease networks. Nature 545: 505-509.
Geoffroy MC, Hay RT. (2009). An additional role for SUMO in ubiquitin-mediated proteolysis. Nat Rev Mol Cell Biol 10: 564-568.

Reported protein-protein and genetic interactions. Amino acid sequence identity and similarity were determined with UniProt (https://www.uniprot.org) Align tool.
GINGRAS AC, CABALLERO M, ZARKE M, SANZECA A, HAZBUN TR, FIELDS S, SONENBERG N, HAFEN E, RAUGHT B, AEBERSOLD R (2005) A novel, evolutionarily conserved protein phosphatase complex involved in cisplatin sensitivity. Mol Cell Proteomics 4: 1725-1740.

HORNBECK PV, ZHANG B, MURRAY B, KORNHAUSER JM, LATHAM V, SKRZYPEK E. (2015) PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. Nucleic Acids Res 43: D512-520.

KUMAR R, GONZÁLEZ-PRIETO R, XIAO Z, VERLAAN-DE VRIES M, VERTEGAAL ACO. (2017) The STUbI RNF4 regulates protein group SUMOylation by targeting the SUMO conjugation machinery. Nat Commun 8: 1809.

LUMPKIN RJ, GU H, ZHU Y, LEONARD M, AHMAD AS, CLAUSER KR, MEYER JG, BENNETT EJ, KOMIVES EA. (2017) Site-specific identification and quantitation of endogenous SUMO modifications under native conditions. Nat Commun 8: 1171.

MARTIN-GRANADOS C, PHILP A, OXENHAM SK, PRESCOTT AR, COHEN PT. (2008) Depletion of protein phosphatase 4 in human cells reveals essential roles in centrosome maturation, cell migration and the regulation of Rho GTPases. Int J Biochem Cell Biol 40: 2315-2332.

MENDOZA MC, DU F, IRANFAR N, TANG N, MAH LOOMIS WF, FIRTEL RA. (2005) Loss of SMEK, a novel, conserved protein, suppresses MEK1 null cell polarity, chemotaxis, and gene expression defects. Mol Cell Biol 25: 7839-7853.

MENDOZA MC, BOOTH EO, SHAULSKY G, FIRTEL RA. (2007) MEK1 and protein phosphatase 4 coordinate Dictyostelium development and chemotaxis. Mol Cell Biol 27: 3817-3827.

MERTINS P, QIAO JW, PATEL J, UDESHI ND, CLAUSER KR, MANI DR, BURGESS MW, GILLETTE MA, JAFFE JD, CARR SA. (2013) Integrated proteomic analysis of post translational modifications by serial enrichment. Nat Methods 10: 634-637.

OUGHTRED R, STARCK C, BREITKREUTZ BJ, RUST J, BOUCHER L, CHANG C, KOLAS N, O’DONNELL E, LEUNG G, MCADAM R, ZHANG F, DOLMA S, WILEMS A, COULOMBE-HUNTINGTON J, CHATR-ARYAMONTRI A, DOLINSKI K, TYERS M. (2019) The BioGRID interaction database: 2019 update. Nucleic Acids Res 47(D1):D529-D541.

PERGOLIZZI B, BOZZARO S, BRACCIO E. (2019) Dictyostelium as model for studying ubiquitination and deubiquitination. Int J Dev Biol 63: 529-539.

POVLSEN LK, BELIP, WAGNER SA, POULSEN SL, SYLVESTersen KB, POULSEN JW, NIELSEN ML, BEKKER-JENSEN S, MAILAND N, CHOUDHARY C. (2012) Systems-wide analysis of ubiquitylation dynamics reveals a key role for PAF15 ubiquitination in DNA-damage bypass. Nat Cell Biol 14: 1089-1098.

REBHAN M, CHALIFA-CASPI V, PRILUSKY J, LANCET D. (1998) GeneCards: a novel functional genomics compendium with automated data mining and query reformulation support. Bioinformatics 14: 656-664.

SEKINE R, KAWATA T, MURAMOTO T. (2018) CRISPR/Cas9 mediated targeting of multiple genes in Dictyostelium. Sci Rep 8: 8471.

SOBKO A, MA H, FIRTEL RA. (2002) Regulated SUMOylation and ubiquitination of DdMEK1 is required for proper chemotaxis. Dev Cell 2: 745-756.

SOBKO A. (2006) Systems biology of AGC kinases in fungi. Sci STKE 352:re9.

SRIRAMACHANDRAN AM, DOHMEN RJ. (2014) SUMO-targeted ubiquitin ligases. Biochim Biophys Acta 1843: 75-85.

SHILO BZ. (2014) The regulation and functions of MAPK pathways in Drosophila. Methods 68: 151-159.

STUETLENCH, PARENT CA, MONTELL DJ. (2018) Cell motility in cancer invasion and metastasis: insights from simple model organisms. Nat Rev Cancer 18: 296-312.

TAN B, MU R, CHANG Y, WANG YB, WU M, TU HQ, ZHANG YC, GUO SS, QIN XH, LI T, LI WH, ZHANG XM, LI AL, LI HY. (2015) RNF4 negatively regulates NF-κB signaling by down-regulating TAB2. FEBS Lett 589: 2850-2880.

UDESHI ND, SVINKINA T, MERTINS P, KUHN E, MANI DR, QIAO JW, CARR SA. (2013) Refined preparation and use of anti-diglycine remnant (K-ε-GG) antibody enables routine quantification of 10,000s of ubiquitination sites in single proteomics experiments. Mol Cell Proteomics 12: 825-831.

WAGNER SA, BELIP, WEINERT BT, NIELSEN ML, COX J, MANI N, MANN M, CHOUDHARY C. (2011) Proteome-wide, quantitative survey of in vivo ubiquitination sites reveals widespread regulatory roles. Mol Cell Proteomics 10: M111.013284.
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