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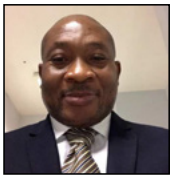
Phosphorylation-mediated regulation of ubiquitin recognition



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Abstract

Ubiquitin is an important cellular signaling protein in eukaryotes. Studies in recent years have shown that ubiquitin can itself be modified by phosphorylation which further modulates its regulatory functions, however, the full physiological (and pathological) significance of such additional modifications for instance, in PINK1-Parkin pathway during damaged mitochondrial clearance is unclear. Additionally, substrate-free or 'unanchored' polyubiquitin chains have also been identified to play essential functions such as in the NF-kB pathway, but whether these are modified by phosphorylation is unknown. The current investigation is based on the hypothesis that besides the characterised proteins parkin, NDP52 and OPTN, there are other undiscovered ubiquitin-binding proteins and ubiquitin-binding domains that can differentiate unmodified and phosphorylated ubiquitin. In addition, the project hypothesises that unanchored (substrate-free) polyubiquitin chains are regulated by phosphorylation. This investigation is therefore designed to catalogue mammalian effector proteins with selectivity for phospho-Ser65-ubiquitin and determine whether unanchored polyubiquitin chains are also regulated by phosphorylation. Affinity chromatography was employed with ubiquitin and phospho-Ser65-ubiquitin Sepharose to purify proteins from NSC-34 cells in a selected buffer and further explored in porcine brain cortex. Bound proteins were eluted from beads, resolved by SDS-PAGE and analysed by silver staining, western blotting and identified by Label-free LC-MS/MS-based protein sequencing. Endogenous unanchored polyubiquitin chains from HEK293T cells were purified by ZnF-UBP domain affinity chromatography. We provide evidence that the deubiquitinating enzymes USP5, USP3 and UCHL1 from NSC-34 cells preferentially bind to unmodified ubiquitin compared to phospho-Ser65- ubiquitin, whereas HDAC6 does not. Wild-type, phosphomimetics (S65D and S65E) and control (S65A) ubiquitin mutants were successfully generated as recombinant proteins in E. coli as an alternative to commercially available pSer65-ubiquitin and reduced binding of USP5 and UCHL1 confirmed. LC-MS/MS analyses suggested various potentially novel proteins which aredifferentially regulated by non-covalent attachment with ubiquitin and/or phospho-Ser65- (poly) ubiquitin. Also, increased levels of unanchored polyubiquitin chains were detected in MG- 132 treated HEK293T cells compared to control and these contained Lys48-linkages. We showed that purified unanchored polyubiquitin from CCCP treated HEK293T cells are Ser65 phosphorylated.

Biography

Julius Tieroyaare Dongdem obtained an M.Phil and Ph.D degrees in biochemistry, molecular pharmacology and molecular cell biology at the university of nottingham, UK. He had previously completed a B.Sc and mphil degrees in biochemistry at the university of Ghana, Legon, Ghana. He is a lecturer at the university for development studies, tamale, Ghana and has more than 10 articles to his credit in reputable Journals. He has also served in editorial board to more than seven journals.

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