Function and targets of the Urm1/Uba4 conjugation machinery in *Drosophila melanogaster*

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt förvar i hörsal E04, byggnad 6E, Norrlands Universitetsjukhus.

Fredagen den 26 Januari, kl. 13:00.

Avhandlingen kommer att förvaras på engelska.

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Posttranslational modification (PTM) of proteins is essential to maintain homeostasis and viability in all eukaryotic cells. Hence, besides the sequence and 3D folding of a polypeptide, modification by multiple types of PTMs, ranging from small molecular groups to entire protein modules, adds another layer of complexity to protein function and regulation. The ubiquitin-like modifiers (UBLs) are such a group of evolutionary conserved protein modifiers, which by covalently conjugating to target proteins can modulate the subcellular localization and activity of their targets. One example of such a UBL, is the Ubiquitin related modifier 1 (Urm1). Since its discovery in 2000, Urm1 has been depicted as a dual function protein, which besides acting as a PTM, in addition functions as a sulfur carrier during the thio-modification of a specific group of tRNAs. Due to this dual capacity, Urm1 is considered as the evolutionary ancestor of the entire UBL family. At present, it is well established that Urm1, with help of its dedicated E1 enzyme Uba4/MOCS3, conjugates to multiple target proteins (urmylation) and that Urm1 thus plays important roles in viability and the response against oxidative stress.

The aim of this thesis has been to, for the first time, investigate the role of Urm1 and Uba4 in a multicellular organism, utilising a multidisciplinary approach that integrates Drosophila genetics with classical biochemical assays and proteomics. In Paper I, we first characterized the Drosophila orthologues of Urm1 (CG33276) and Uba4 (CG13090), verified that they interact physically as well as genetically, and that they together can induce urmylation in the fly. By subsequently generating an Urm1 null Drosophila mutant (Urm1n123), we established that Urm1 is essential for viability and that flies lacking Urm1 are resistant to oxidative stress. Providing a molecular explanation for this phenotype, we demonstrated an involvement of Urm1 in the regulation of JNK signaling, including the transcription of the cytoprotective genes Jafrac1 and gstD1. Besides the resistance to oxidative stress, we have moreover (Manuscript IV) made an in-depth investigation of another phenotype displayed by Urm1 n123 mutants, an overgrowth of third instar larval neuromuscular junctions (NMJs), a phenotype which is shared also with mutants lacking Uba4 (Uba4n29).

To increase the understanding of Urm1 in the fly, we next employed a proteomics-based approach to identify candidate Urm1 target proteins (Paper II). Using this strategy, we identified 79 Urm1-interacting proteins during three different stages of fly development. Of these, six was biochemically confirmed to interact covalently with Urm1, whereas one was found to be associated with Urm1 by non-covalent means. In Manuscript III, we additionally identified the virally encoded oncogene Tax as a target of Urm1, both in Drosophila tissues and mammalian cell lines. In this study, we established a strong correlation between Tax urmylation and subcellular localization, and that Urm1 promoted a cytoplasmic accumulation and enhanced signalling activity of Tax, with implications for a potential role of Urm1 in Tax-induced oncogenesis.

Taken together, this thesis provides a basic understanding of the potential roles and targets of Urm1 in a multicellular organism. The four studies included cover different aspects of Urm1 function and clearly points towards a highly dynamic role of protein urmylation in fly development, as well as in adult life.

Keywords
Drosophila, Urm1, Uba4, MOCS3, Tax, HTLV, Posttranslational modification, ubiquitin-like modifiers, UBL, PTM, JNK, neuromuscular junctions, signaling