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Small But Mighty: The Emerging World of Microproteins

During initial efforts to annotate genomes, many potential coding sequences were identified that were assumed to be too short to encode functional proteins. To reduce the number of annotations and reduce the risk of false positives, an arbitrary cutoff was therefore applied, and sequences shorter than 100 amino acids (aa) were filtered out.[1] Later, it was discovered that many of these small open reading frames (smORFs) are translated to produce microproteins, or micropeptides, whose expression is apparently just as tightly controlled as for canonical proteins.[1,2]

Many microproteins are encoded in 5'-untranslated regions (5'-UTRs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), and a significant number of them are translated from non-AUG start codons.[3,4] These features traditionally made them difficult to identify, but the use of modern genomics technologies, such as ribosome profiling (Ribo-Seq) and sensitive proteomics workflows, has now firmly established that many smORFs are translated to produce detectable microproteins. Multiple studies have identified hundreds of microproteins in various cell types and tissues,[4–6] often including previously unannotated examples, but the function of most of them remains unknown. Nevertheless, the emerging evidence indicates key roles for microproteins in many cellular and physiological processes, and in this newsletter, we will review some of the most important findings so far.

Microprotein Regulation of the Cytoskeleton

The 82-aa microprotein Mozart 1 (MZT1) plays a central role in cytoskeletal organization as part of the γ-tubulin ring complex (γTuRC), an essential multiprotein assembly that acts as a master template for nucleation of new microtubules.[7] Binding of MZT1 to γ tubulin complex proteins (GCPs) controls recruitment of the γTuRC to microtubule-organizing centers (MTOCs) to spatially orchestrate microtubule nucleation.[8](Figure 1) Specifically, MZT1 binds to the N-terminal regions of GCPs—primarily GCP3 and GCP6—to form much of the luminal bridge of the γTuRC[9] and mediate its docking with MTOCs, via NEDD1.[10] Although MZT1 is not essential for either γTuRC assembly or microtubule nucleation, its absence strongly inhibits interaction of the γTuRC with NEDD1 and produces severe mitotic defects, identifying it as an essential targeting factor in microtubule organization.[10]

A number of microproteins have also been implicated in direct or indirect interactions with the actin cytoskeleton, and interestingly, these were mostly discovered due to their effects on cancer cells. The 51-aa "micropeptide inhibiting actin cytoskeleton" (MIAC) acts by regulating septin-2[11] and by suppressing EGFR signaling,[12] both of which are known to influence actin remodeling.[13,14] It appears to function as a tumor suppressor, since lower MIAC expression was correlated with poorer survival in cancer patients.[11,12]

Similarly, the 83 aa microprotein CASIMO1 (now annotated as SMIM22) was discovered in a breast cancer expression profiling screen, where it was particularly strongly overexpressed in ER+ tumors.[15] High expression was also identified in colon, ovarian, and lung cancer cell lines, and conversely to MIAC, knockdown of SMIM22 in MCF 7 breast cancer cells led to actin cytoskeleton dysregulation, impaired motility, and slower proliferation.

Short transmembrane mitochondrial protein 1 (STMP1) is also upregulated in multiple cancer types,[16] and high expression has been associated with poor prognosis. This 47-aa microprotein is a nuclear-encoded factor that localizes to the inner mitochondrial membrane, where it apparently interacts with complex IV of the electron transport chain. However, STMP1 also binds to myosin heavy chain 9 (MYH9) to promote mitochondrial fission and cell migration.[17] Mitochondrial fission specifically requires direct interaction of MYH9 with actin,[18] and the same protein has been implicated in cell migration through regulation of actin cytoskeleton organization.[19] Together, these actions may explain the pro-tumorigenic effects of *STMP1*,[16] and consistent with this, *STMP1* silencing prevented tumor metastasis in mouse xenografts.[17] Perhaps relatedly, an SNP that causes increased expression of *STMP1* is associated with elevated risk of Paget's disease,[20] a condition involving dysregulated bone remodeling.

Mitochondria Also Produce Microproteins

Mitochondrial DNA (mtDNA) harbors hundreds of potential microprotein-encoding smORFs, although only a handful of these have been confirmed to date.[21] The first example was humanin, a 24-aa microprotein expressed from the 16S rRNA gene (*MT-RNR2*) in

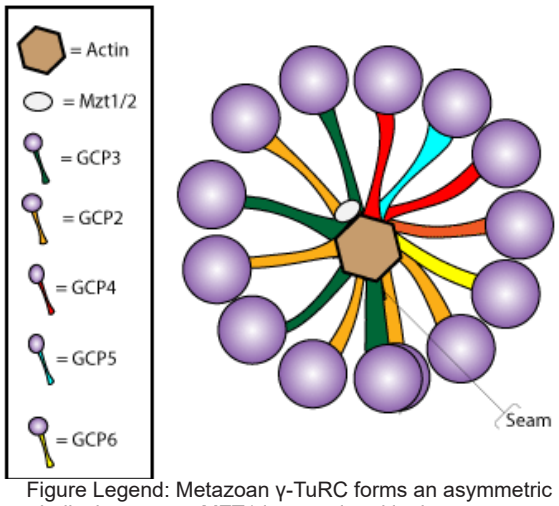

Figure Legend: Metazoan γ-TuRC forms an asymmetric helical structure, MZT1 is associated in the structure.

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Microprotein Associated PRODUCTS

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mtDNA.[22] It was initially identified in a cytoprotection assay using a cDNA library from the brain of an Alzheimer's disease patient, but has since been described as a protective factor in multiple cell types.[21]

Relatedly, the SHMOOSE microprotein was recently discovered by mapping an Alzheimer's disease-associated SNP to a previously uncharacterized smORF overlapping the *MT-ND5* gene in mtDNA.[23] This small 58-aa protein stays localized in mitochondria, where it modulates gene expression and increases mitochondrial oxygen consumption. The pathogenic variant was found to increase Alzheimer's disease risk by up to 50%, and introduces an amino acid substitution (D47N) that abolishes the cytoprotective effect of the wild type protein against amyloid-β-induced toxicity.

Also encoded in mtDNA, the MOTS-c microprotein is a small, 16-aa peptide that regulates insulin sensitivity and is primarily expressed in skeletal muscle.[24] It is strongly induced by exercise,[25] hinting that it may contribute to the metabolic benefits associated with regular physical activity. Transcription of MOTS-c appears to be regulated independently of the 12S rRNA gene (*MT-RNR1*) where its smORF is found, and its levels in muscle and plasma also vary differentially with age.[26] An SNP that causes a missense mutation (K14Q) in MOTS-c produces a hypomorphic form of the protein associated with reduced insulin sensitivity and a higher risk of type 2 diabetes, but interestingly, only in men.[27] It is also downregulated in type 1 diabetes, where it has been proposed as a therapeutic target due to its influence on T cell metabolism and its protective effects in a mouse model of the disease.[28]

Conclusions & Outlook

Research on microproteins is still a relatively young field. Even though the evidence suggests that hundreds to thousands of them are actively expressed, they remain a comparatively understudied protein class, and only a few of them have been investigated in detail. However, what we do know tells us that microproteins are just as important as canonical proteins in terms of their biological functions and significance, meaning that we can expect this area to remain an exciting topic for some time. In an important recent development, microproteins were identified as a cargo in extracellular vesicles (EVs),[29] suggesting that they might also mediate cell-to-cell and systemic communication in addition to their intracellular functions. Importantly, the microprotein profile of EVs differed between glioma patients and healthy controls, suggesting that certain microproteins could be useful in the future as disease biomarkers or novel therapeutic targets.

Cytoskeleton Proteins

Product	Amount	Cat #
Microtubule associated protein rich fraction Source : porcine brain	1 x 100 µg 5 x 100 µg	MAPF-A MAPF-C
Tubulin protein (>99% pure) Source : porcine brain	1 x 1 mg 5 x 1 mg	T240-A T240-B
Actin Protein (>99% Pure): Source: Rabbit Skeletal Muscle	4x250 µg 2x1 mg 5x1 mg 10x1 mg 20x1 mg	AKL99-A AKL99-B AKL99-C AKL99-D AKL99-E

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MemGlow 590 - Fluorogenic Plasma Membrane probe (A MEMBRIGHT Family Probe)	Ex 580 / Em 620	MG03
MemGlow 640 - Fluorogenic Plasma Membrane probe (A MEMBRIGHT Family Probe)	Ex 650 / Em 673	MG04
MemGlow 700 - Fluorogenic Plasma Membrane probe (A MEMBRIGHT Family Probe)	Ex 650 / Em 720	MG04

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