Optimizing IRGM Expression for Structural Characterization

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Autophagy, a highly regulated process governing cellular component degradation and recycling, plays a pivotal role in maintaining cellular homeostasis and responding to various stresses [1], [2]. One of the basic objects of this intricate cellular performance resides the Immunity-Related GTPase M (IRGM), a member of the interferon-inducible GTPase family [3]. IRGM assumes multifunctionality in molecular and cellular biology, influencing innate immune responses and cellular equilibrium profoundly [4].

Our research endeavour sought to determine whether IRGM undergoes G domain dimerization, a critical event in stimulating GTPase activity and initiating plasma membrane fission [5]. However, elucidating the independent structural conformation of IRGM, with its small size (~23 kDa), under native conditions remains an insurmountable challenge for cryo-electron microscopy (cryo-EM). In connection with this, we decided to crystallize the protein in parallel, as well as to study potential complexes with binding proteins, such as Beclin-1 and Endophilin B1. These complexes unveil multiple potential binding sites. Primarily, the coiled-coil domains emerge as prime candidates for interaction, driving autophagy regulation—responsible for cellular component degradation and recycling.

In connection with these tasks, we needed to establish stable expression of the protein, and subsequently co-expression with partner proteins. We had at our disposal previously expressed SF9 cell pellets obtained by transfection with baculovirus. The isolated protein was sufficient for the first crystallization tests. Since our studies require stable overexpression of the protein, we attempted to express the protein in faster ways: using E. coli constructs, as well as expression in SF9 using transient transfection. When trying to express it in E. coli, the protein was predominantly in the insoluble fraction, and we also identified a problem with protein folding. At the same time, after a series of tests and optimization of expression in SF9, we obtained the protein in sufficient quantities.

The obtained results of protein expression in SF9 cells allow us to continue working on the study of IRGM and its potential partners and we plan to start a series of tests on the formation of complexes. This endeavour promises to illuminate the mechanisms governing autophagic regulation.

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