Condensation in the endocytic protein network

Mamta

mamta.mamta@unige.ch

Clathrin-mediated endocytosis (CME) is spatially and temporally regulated by a choreographed interaction of over 50 proteins though the precise mechanisms of this intricate network remains poorly understood. In *Saccharomyces cerevisiae*, the early phase of CME is variable in duration (1-3 min). During this time, the early proteins cluster at the plasma membrane and define the endocytic site. Upon the recruitment of the late coat proteins and the actin regulators, the endocytic pathway becomes regular (30-35 sec).The mechanism for the transition from variable early to regular late phase is still an open question in the field. We aim to understand the role of weak multivalent interactions formed by the clathrin adaptors and endocytic scaffolds in regulation of spatiotemporal dynamics of endocytosis.

Recently, one of the early arriving yeast endocytic protein i.e. Ede1 has been shown to form liquid condensates upon over-expression. The ability of Ede1 to form condensates is proposed to promote the initiation of endocytosis. To explore the possibility of other proteins forming condensates, we performed an over-expression screen of multiple endocytic proteins. While most of the proteins were dispersed in cytoplasm in higher concentration, one of the late coat proteins, Pan1, formed condensates. Pan1 is an EH domain protein which shares structural similarities with Ede1. Pan1 condensates exhibited liquid like properties such as fast exchange of molecules, and sensitivity to temperature changes or hexanediol treatment. Electron microscopy revealed Pan1 condensates as a ribosome exclusion zones devoid of membranes. The Pan1 condensates specifically recruited late endocytic proteins like Abp1, Sla1 which might reflect Pan1's function at endocytic sites. We hypothesize that the condensation properties of Pan1 give flexibility to the endocytic machinery to allow dynamic rearrangements of the proteins for successful endocytic events. A set of deletion mutants revealed that multiple parts of Pan1 can contribute to its condensation property.

Ede1 and Pan1 can form weak multivalent interactions with the conserved NPF domains across clathrin adaptor proteins. These weak multivalent interactions might be driving phase separation of Ede1 and Pan1 at the endocytic sites. This interaction is thought to be regulating the transition from early to late phase. Upon precise disruption of these multivalent interaction by mutating 16 NPFs of the four clathrin adaptors, contrary to the prevailing view, we found that the late phase along with the actin polymerisation remains unperturbed. We aim to assess the possibility of EH domains regulating other aspects of endocytosis beyond forming weak multivalent interactions with the NPFs.