

but is organized into specific nucleo-protein complexes called mitochondrial nucleoids. In order to visualize mt-nucleoids in HepG2 and INS-1E cells, we have prepared several constructs coding for conjugates of a mitochondrial transcription factor A (TFAM) or a Twinkle helicase and various fluorescent proteins and photoconvertible fluorescent proteins, including GFP, DENDRA2, PS-CFP2 and Eos. Constructs for Viral Power lentiviral expression system (Invitrogen) were prepared as well as the corresponding lentiviral particles, that were subsequently used for cell transductions. In addition, immunocytology of mt-nucleoids was performed with the aid of anti-TFAM antibodies. Using confocal microscopy we have found co-localizations of each TFAM-conjugated fluorophores with TMRE or mitochondria-addressed DsRed fluorescent protein contouring mitochondrial reticulum network. Furthermore, an influence of oxidative stress, various metabolic states (glycolytic and obligatory oxidative phosphorylation) and several stress factors were tested in order to access changes in mt-nucleoids organization.

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### 18P.9 The large dynamin-like GTPase Mgm1 in mitochondrial fusion

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The mitochondrial network is constantly remodeled by fusion and fission events. To understand the molecular mechanisms underlying mitochondrial fusion is highly relevant in regard to its role in cell physiology and disease pathogenesis. It is particularly interesting, as the process of mitochondrial fusion is remarkable in several aspects compared to other characterized cellular membrane fusion events: (i) There are in total four membranes that need to be fused in a coordinated manner; and (ii) fusion of mitochondria is not mediated by SNARE proteins, but instead by large dynamin-like GTPases. In yeast, Mgm1 is the representative of this class in the inner mitochondrial membrane. It exists as two isoforms, one short (s-Mgm1) and one long (l-Mgm1). s-Mgm1 is created by a specific cleavage through the rhomboid protease Pcp1. Our results suggest that this specificity is not due to the cleaved hydrophobic region itself, but rather determined through an exosite. We hypothesize that the exceptional biogenesis pathway of Mgm1 plays a crucial role in the regulated proteolysis, involving a potential interplay between Pcp1 and the TIM23 translocase. As a balanced formation of both isoforms is crucial for mitochondrial fusion, we have analyzed the differential characteristics of the two isoforms. Both interact in homotypic as well as heterotypic manner, but are differentially distributed across the submitochondrial compartments. l-Mgm1 is preferentially found in cristae, while s-Mgm1 mainly resides in the inner boundary membrane. Surprisingly, it became apparent that a functional GTPase domain is only required in the short but not in the long isoform of Mgm1. These new findings let us propose that the two isoforms carry out distinct, presumably interrelated functions in mitochondrial fusion and cristae maintenance.

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