transmembrane span topology with cytoplasmically oriented N- and C-terminal domains. Quality control of membrane proteins is crucial for membrane maintenance. We examined Acr3 degradation and showed that Acr3 transporter undergoes endocytosis and vacuolar degradation through MVB pathway. Degradation of Acr3 does not require a functional proteasome. Acr3 degradation is Rsp5 ubiquitin ligase and Doa4 deubiquitinase dependent. Based on the 10-transmembrane model of Acr3 we selected 11 cytoplasmically oriented lysine residues potentially subjected to a posttranslational modification by ubiquitination and replaced each residue with arginine. Single K to R substitution did not increase Acr3 stability, however, triple mutants accumulated in endosome and vacuolar membranes. We also observed that ubiquitination is indispensable for proper vacuolar degradation of Acr3 through MVB pathway.

Autophagy

P.4.3.B-001
Macrophage migration inhibitory factor-induced autophagy is involved in dengue virus replication

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Dengue virus (DENV) infection is the most common mosquito-borne viral infection, which is common in tropical and subtropical countries. DENV infection can cause mild dengue fever and life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Cytokine storm plays a vital pathogenic role in DHF/DSS. However, currently there is no effective antiviral drugs available. Previous studies have shown that DENV infection can induce autophagy of infected cells which facilitate the replication of virus. In addition, the amount of a pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF) in dengue patients' sera is correlated with the severity of the disease. Since MIF is able to induce autophagy formation of cells, we propose and test the hypothesis that MIF-induced autophagy is involved in DENV replication. We found that MIF secretion was increased in DENV-infected human hepatoma cell line (HuH-7) in a time dependent manner. Utilizing shRNA to knock down endogenous MIF, we found that autophagy, virus replication and viral titer were all inhibited. Moreover, after treating with MIF inhibitors, ISO-1 and p425, both autophagy and DENV infection were inhibited. In conclusion, we demonstrated in this study that DENV infection induces MIF release, thus facilitating DENV replication via autophagy formation. Further study by targeting MIF may prevent not only inflammation but also inhibit DENV replication, which may develop into a new strategy to treat DENV infection.

P.4.3.B-002
Cardiomyocyte BECN1-dependent autophagy in acute overload of the left ventricle

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Hemodynamic overload of the left ventricle of the heart is accompanied by activation of some mechanisms responsible for regulated cell death including apoptosis. In the recent years there has been an increasing interest to cardiomyocyte autophagy, which may be a phenomenon involved in programmed (or regulated) death in some cases, but might also have reparative potential in other circumstances providing cell survival at the expense of its internal resource mobilization. In this work BECN1-dependent cardiomyocyte autophagy was evaluated by the content of Beclin 1 (BECN1) in cardiomyocytes without morphological evidence of plasma membrane damage in rabbit left ventricular myocardium on 1, 3 and 5 days of acute hemodynamic overload caused by narrowing of the ascending aorta by 1/3 of its initial diameter. Activity of cardiomyocyte autophagy was assessed on the basis of BECN1 content estimation immunochemically with the use of primary goat polyclonal antibodies (SantaCruzBiotechnology, Inc., USA). It was shown that the content of BECN1 was rather low in the control group (2.77 vol.%) and significantly decreased (0.54 vol.%) on day 1 after the onset of cardiac overload compared with the controls. On day 3 there was a further decrease in BECN1 in cardiomyocytes (0.20 vol.%). On day 5 this index negligibly increased (0.38 vol.%), but still remained significantly lower in comparison with controls. It was also found that intensity of positive stain was much higher in the myocardial sites with denser vascular tree. It might be suggested that cardiomyocyte autophagy is a mechanism of cell resource mobilization in the intact myocardium rather than regulated cell death. Its inhibition under acute cardiac overload is most probably due to a severe energy deficit.

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P.4.3.B-003
Activity and distribution of senescence-associated β-galactosidase in aging oocytes and eggs

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Senescence in somatic cells is commonly characterized by enhanced activity of senescence-associated β-galactosidase (SA-β-gal), reflecting alteration of autophagocytosis. However, little is known about the autophagy-associated changes of SA-β-gal activity in aging gamete cells. To address this issue, in the present work, we measured intracellular activity and distribution of SA-β-gal in aging oocytes and eggs of the African clawed frog *Xenopus laevis*. Database mining revealed the presence of three homologous β-galactosidase isoforms in the annotated *Xenopus* genome. Their transcripts were abundant in the ovarian tissue and eggs. The protein products were predicted to contain an N-terminal signal peptide sequence, suggesting enzyme translocation to the cellular organelle fraction. Biochemical analysis of SA-β-gal activity confirmed its localization mainly in a particulate fraction of oocytes and eggs, with the optimum of catalytic activity at pH 4.0–5.0. These data indicate that SA-β-gal is a largely lysosomal enzyme in *Xenopus* oocytes and eggs, so the changes in its activity may reflect autophagy dynamics. Further analysis revealed gradual increase of SA-β-gal activity in *Xenopus* oocytes and eggs aged *in vitro* over 72 h. The activity increase observed in the eggs was significantly higher than that in the oocytes, suggesting faster aging of eggs vs. oocytes. We further used the novel cell-permeable fluorescent substrate of SA-β-gal, SPiDER-β-Gal, to visualize the lysosomal compartment in *Xenopus* oocytes and eggs. Strong fluorescent signal was observed in a fraction of dense cytoplasmic granules of the average size 8.9 ± 3.6 μm. It colocalized with a subpopulation of yolk platelets, specialized late endosomes that accumulate and store processed vitellogenin in frog oocytes, eggs and early embryos. Altogether, our results demonstrate that detecting SA-β-gal