Subcellular Processes in Plants and Animal Cells

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KillerRed expressing Tol2 transposon enhancer trap transgenics for in vivo studies of protein transport during zebrafish development.

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KillerRed (KR) protein is a genetically-encoded photosensitizer that fluoresces red upon illumination with green light. Membrane-tagged KillerRed (mem-KR) contains the membrane localization signal of neuromodulin. The Tol2-based transgenic approach was used to generate several stable transgenic lines expressing mem-KR in tissue-specific manner. For example, SqKR1 embryos mem-KR is found at the membrane and/or Golgi apparatus domains of cells in the most superficial cell layer of embryonic and larval zebrafish - enveloping layer (EVL). Interestingly, intracellular distribution of mem-KR in the dorsal and ventral EVL cells differs. In the ventral EVL mem-KR was found primarily in large aggregates representing Golgi, whereas in the dorsal EVL mem-KR localisation varies between Golgi and plasma membrane. Currently, we use various inhibitors of signalling pathways to identify a pathway responsible for developmental regulation of membrane protein transport.

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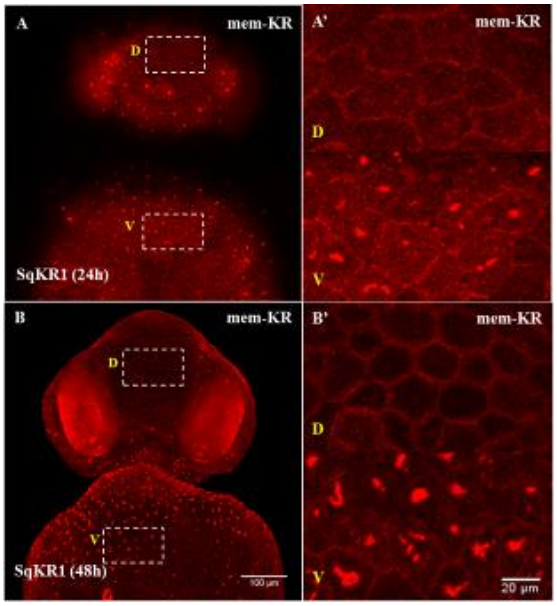

Figure 1. Protein distribution pattern of mem-KR is different in dorsally situated and ventrally situated EVL cells of SqKR1 embryos. (A, B and C) Confocal stack image of SqKR1 embryos at 24, 48 and 72 hpf show differential protein distribution pattern of mem-KR in dorsal versus ventral EVL cells. (A', B' and C') Magnified view of dorsal versus ventral EVL cells at 24, 48, and 72 hpf show that mem-KR in dorsally situated EVL cells are mainly localized to the plasma membrane while mem-KR in ventrally situated EVL cells are localized to the plasma membrane but also found in large aggregates.

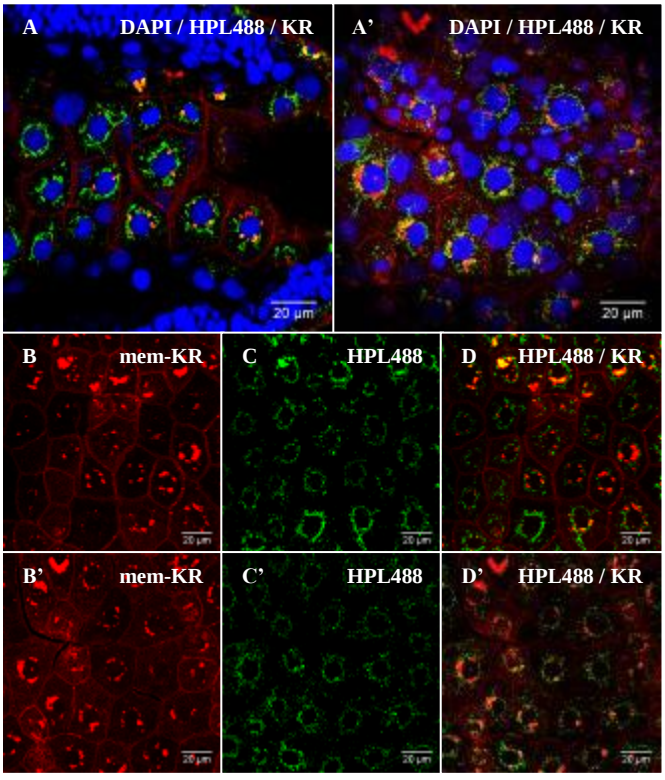

Figure 2. Aggregates of mem-KR proteins co-localize with a Golgi apparatus marker. SqKR1 embryos were fixed at 30hpf and stained for nucleus with DAPI, stained for Golgi apparatus with HPL488 and stained for mem-KR with Alexa Fluor 555 conjugated antibody against anti-KillerRed antibody. (A – D) Confocal stack image of dorsally situated EVL cells of stained 30hpf SqKR1 embryos show that mem-KR is found mainly at the plasma membrane while small aggregates co-localize with the Golgi apparatus, which is peri-nuclear. (A' – D') Confocal stack image of ventrally situated EVL cells of stained 30hpf SqKR1 embryos show that a vast amount of mem-KR are present as large aggregates that co-localize with the Golgi apparatus.