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Regulation of Intracellular Delivery by Nanocarrier Design

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Extended Abstractr

Targeting strategies to consistently and efficiently deliver nanocarriers and other nanostructures to the nucleus have enormous potential in gene transfer technologies as well as multiple other applications in biological research and therapeutics. Most delivery approaches assume that nanocarriers must escape endomembrane vesicles such as endosomes as a prerequisite for nuclear entry. Hence, the addition of endosomal buffering or membranolytic moieties is a common design approach, yet recent evidence indicates that endosomal escape is often uncorrelated with improved nuclear delivery, and membranolytic structures are often cytotoxic. Meanwhile, once in the cytoplasm, nanocarriers are either assumed to enter the nucleus during cellular mitosis (in dividing cells), or programmed, through the addition of nuclear localization sequences (NLSs), to enter the nucleus through the nuclear pores (in non-dividing cells). The efficacy of both endosomal escape and nuclear entry strategies is cell-specific and also depends upon detailed features in the nanocarrier. Hence, we sought to develop improved nuclear targeting approaches and unravel key delivery mechanisms by studying structure-function behavior of nature-inspired (histone-mimetic) gene carriers (polyplexes) in several types of cells relevant to regenerative medicine and cancer.

Previously, we showed that histone-targeted polyplexes trafficked to the nucleus through a route involving caveolae as well as the Golgi and endoplasmic reticulum (ER), using pathways similar to several pathogens.(Reilly et al. 2012) We hypothesized that the efficacy of these polyplexes was due to both an increased utilization of native vesicular trafficking as well as regulation by histone effectors. Accordingly, we used confocal microscopy, cellular inhibition experiments, and subcellular fractionation to determine that a key effect of histone-targeting was to route polyplexes away from clathrin-mediated recycling pathways by harnessing endomembrane transfer routes regulated by histone methyltransferases. Based on these studies, an unprecedented finding was that polyplexes accumulated in Rab6-labeled Golgi/ER vesicles and ultimately shuttled directly into the nucleus during ER-mediated nuclear envelope reassembly. These novel findings highlight alternative mechanisms to subvert endolysosomal trafficking and harness the ER to enhance gene transfer.

References

Reilly M.J., Larsen J.D., et al. (2012). Polyplexes traffic through caveolae to the Golgi and endoplasmic reticulum en route to the nucleus. Mol Pharm, 9, 1280-1290.