ciency. (1). A muscle-specific eukaryotic gene expression plasmid, pSV40E/MCK-HIF1a, was constructed by integrating SV40-enhancer with MCK promoter to regulate HIF-1a gene expression. (2) *In vitro* and *in vivo* studies both indicated that, compared with the natural MCK promoter, the SV40E/MCK hybrid promoter significantly increased HIF-1a gene expression, while retaining a good muscle-cell specificity. Although less efficient than the nonspecific CMV promoter, the hybrid promoter provided more stable gene expression and represented a good compromise between transcriptional activity and muscle specificity. (3) *In vitro* biological effects of increasing HIF-1a gene expression were analyzed in myoblasts to evaluate the function of the muscle-specific gene expression system. Real-time PCR showed up-regulation of several critical angiogenic genes expression, such as VEGF, ANGPT-1, MMP-2 and SDF-1, which were previously demonstrated to facilitate new blood vessel formation and/or maturation. Transwell cell migration assay revealed that pSV40E/MCK-HIF1a transfected L6 cells could recruit progenitor cells derived from bone marrow and muscle tissue. These observations suggested the muscle-specific gene expression system may be useful for stimulating new blood vessel growth and maturation in ischemic limbs while restricting the therapeutic effect to muscle tissue. (4) When reporter gene was transferred into mice limb skeletal muscles, using various nonionic natural polymers, including hyaluronic acid, alginic acid and dextran, the formulated plasmid/polyer resulted in different levels of reporter gene expression, depending upon the type and concentration of the polymers. Some of them showed better performance than naked DNA and these results indicated that the pSV40E/MCK-HIF1a combined with a suitable nonionic polymer may provide a safe and efficient gene therapy system for treatment of limb ischemia.

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Amphipathic CPPs upregulate Ca in cells' cytosol and induce lysosomal exocytosis

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Cell penetrating peptides (CPP) promote the uptake of different bioactive cargo molecules that makes the harnessing of CPPs a promising strategy for drug design and delivery. The translocation mechanism of CPPs into cells, however, has still remained elusive. Direct passage of peptides across the plasma membrane might interfere with its integrity and introduce disturbances. In our study we assessed how cells compensate the disturbances and which processes are induced in response to CPP uptake. Applying fluorometry, flow cytometry and fluorescence microscopy we demonstrate that the uptake of various CPPs enhances the calcium levels in Jurkat and HeLa cells' cytoplasm. The elevated cytoplasmic free calcium concentration evokes downstream effects of membrane repair response and lysosomal exocytosis. Our results indicate that ten of the most commonly used CPPs can be divided into three groups based on their interaction with plasma membrane, the induction of calcium influx, and downstream responses: (1) primary amphipathic CPPs (e.g. MAP, TP) that modulate plasma membrane integrity inducing influx of calcium ions into cells and activate membrane repair and lysosomal exocytosis starting, from low concentrations; (2) arginine-rich, secondary amphipathic, CPPs (e.g. Penetratin, pVEC) that induce changes in the intracellular calcium concentration or subsequent responses at relatively high concentrations and (3) non-amphipathic CPPs (e.g. Tat, Arg9) that do not evoke changes in the intracellular calcium concentration or subsequent responses even at high concentrations. Triggering of the plasma membrane repair response may help cells to recover by replacing the misorganized or membrane active CPPs containing plasma membrane regions, whereas non-amphipathic CPPs could infiltrate without subsequent cellular responses.

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Cellular delivery of oligonucleotides by PepFect

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PepFect (PF) series of peptide based transfection reagents have been developed for the delivery of oligonucleotides (ON) and plasmids into cells. Some PFs are also capable of nuclear delivery of oligonucleotides, for example phosphorothioate 2′-O-methyl RNA oligomers translocate into nucleus and rescue the luciferase expression in the splicing redirection assay after coupling to PF. The optimal ratio of ON with PF for obtaining the functional complexes has been described earlier, but it is not known how such particles interact with the cell surface, enter cells, and reach nucleus. In order to characterize the oligonucleotide delivery by PFs, we labelled 2′-OMe ON with 1.4 nm Nanogold (NG) particles. The membrane interaction, uptake, and intracellular traffic of ON–NG after complexing with PFs were mapped by transmission electron microscopy to unravel their internalization mechanism. PFs pack the Nanogold-labelled ON into small (~200 nm) particles in solution. Smaller particles of ON–NG–PF complexes associate later to form bigger assemblies at the surface of HeLa cells and are taken up by cells in vesicles. The size, electron density and regularity of ON–NG–PF containing structures vary largely depending on the PepFect and its concentration. In cells the majority of the complexes locate in the endosomal/lysosomal vesicles after four hours of incubation. However, the vesicles often have a discontinuous membrane and the Nanogold-labelled oligonucleotides can be found in the cytosol. In addition, with the help of some PFs, the oligonucleotides also reach the cell nucleus. Our results demonstrate that non-covalent complexes of Nanogold-labelled oligonucleotides with PepFects form particles that concentrate at the cell surface and enter cells by endocytotic mechanism. The finding that oligonucleotides have reached nucleus suggests that ON–PF complexes could induce the destabilization of endosomal mem-