Delivery of siRNA and ribozyme for gene therapy by 35-nm nanoparticles of phi29 motor pRNA

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siRNA and ribozymes have shown great promise for the treatment of genetic diseases, cancers, and viral infections. However, their therapeutic value has been hindered by the lack of an efficient and safe *in vivo* delivery system to target specific cells. This work is to investigate the use of self-assembling motor pRNA of bacterial virus phi29 as a novel targeting and delivery vector for therapeutic RNAs. The natural function of pRNA is to form a hexameric ring as part of a motor complex which gears phi29 DNA packaging. This hexamer formation is enabled by the "hand in hand" interaction of pRNA loops. By making complementary mutations in these interlocking loops, we designed pRNAs that self-assemble *in vitro* in a programmed way to form dimers or trimers with nearly 100% efficiency. Apart from the loop region, pRNA contains another independent, self-folding domain, which is essential for DNA packaging but not for dimer, trimer or hexamer formation. We designed several modifications including extensions, truncations and circular permutations of this domain to engineer pRNAs which carry additional functionalities. One such functionality was a CD4 receptor-binding RNA aptamer. The aptamer mediated specific delivery of siRNA or ribozyme to a CD4-overexpressing thymic T cell line via receptor-mediated endocytosis. The therapeutic potential of the pRNA nanocomplex was investigated by targeting the anti-apoptosis factor survivin in breast cancer cells and leukemia model lymphocytes. Both for reporter genes and anti-apoptosis factors, the knock-down was stronger for pRNA-escorted siRNA compared to siRNA alone. This gene silencing effect was specific and exclusive of the 5'-phosphate caused interferon effect, since our RNAs were transcribed with a special transcription initiator. We studied specific targeting and delivery by forming programmed dimeric or trimeric pRNA nanocomplexes. Incubation of a CD4-overexpressing thymic T cell line with the pRNA dimer containing CD4-binding aptamer and siRNA to survivin resulted in binding and entry of the pRNA dimer into these cells, and subsequently induced significant apoptosis of only the targeted CD4-overexpressing cells.

The significance of this work is to show a viable approach towards using powerful ribozyme or siRNA-mediated gene knock-down for therapy. The pRNA nanocomplex vector system is ideal for delivering therapeutic RNAs, because the programmable self-assembly allows for simultaneous multiple delivery, and the size of the nanocomplexes in the 30 to 40 nanometer range avoids the problem of short half-life of small molecules *in vivo* due to short retention time, and the problem of undeliverability due to a larger than 100 nM size.

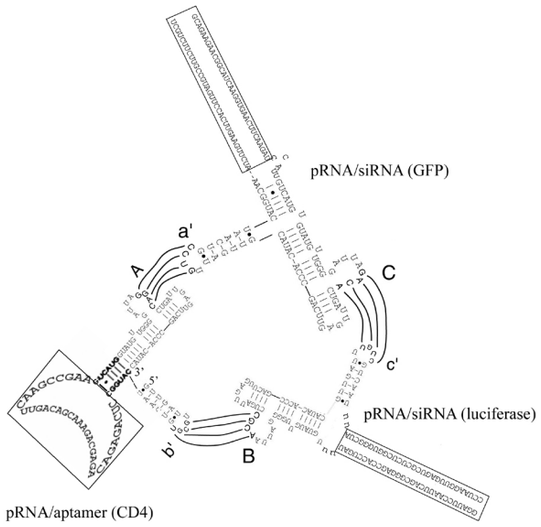

Fig. 1: A pRNA trimer linking a targeting moiety (CD4-aptamer) to two siRNAs targeting the reporter genes for GFP and luciferase. The pRNA serves as interconnecting link, stabilizer and siRNA-enhancer.

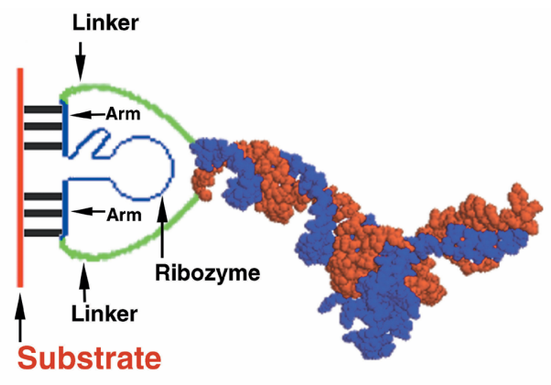

Fig. 2: A pRNA monomer harboring a ribozyme designed to cleave a specific RNA sequence.