Engineered riboswitches – an alternative means to control gene expression

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Numerous synthetic RNA-based control devices, so called engineered riboswitches, have been developed in the last years. We have engineered riboswitches by insertion of *in vitro* selected, small molecule binding aptamers into untranslated regions of mRNAs, exploiting the fact that upon ligand binding the RNA structure interferes either with translation initiation or pre-mRNA splicing in yeast. An advantage of these regulators is that they can be designed in principle to any non-toxic, cell-permeable ligand of choice. In addition, the direct RNA-ligand interaction renders auxiliary protein factors unnecessary.

While many RNA aptamers have been identified that bind to a plethora of small molecules, only very few are capable of acting as riboswitch. Using a screening approach we identified a set of aptamers which confer neomycin-dependent regulation, however, to a various degree. In a combination of genetic, biochemical and structural studies we addressed the molecular basis for these differences. We demonstrated that a destabilized and open ground state accompanied by extensive structural changes upon ligand binding is necessary for regulation while inactive aptamers are already prestructured without the ligand. We identified a switching element responsible for the destabilization of the ligand free form without compromising the bound form. We will exploit this knowledge for the further development of RNA-based devises for the conditional control of gene expression.