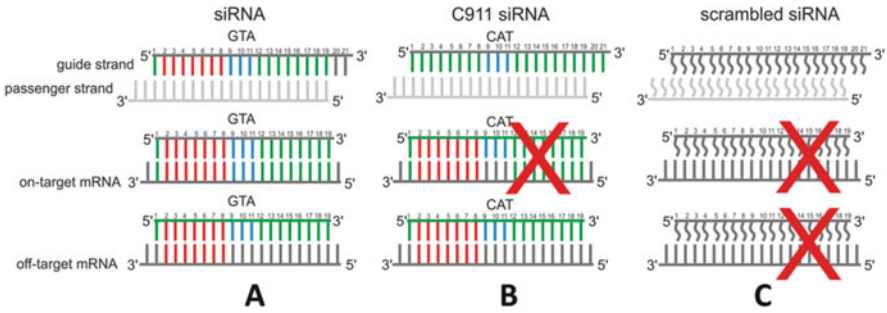

Fig. 6 On- and off-target effects of siRNAs and their controls. An siRNA (**a**, *left panel*) consisting of two complementary 19-mers of RNA (with two-base overhangs) is divided here conceptually into the 5′ end of the antisense strand (*red*), the middle of the siRNA (*blue*), and the 3′ end of the antisense strand (*green*). An on-target siRNA is reverse complement of the target mRNA sequence intended to be downregulated (**a**, *middle panel*), but this siRNA can also match to the seed sequence of a 3′ UTR of other mRNAs and can downregulate as in their off-target (**a**, *bottom*). Because it lost full complementarity in the middle three bases (*blue*), the C911 mismatch control (**b**) does downregulate the targeted mRNA, but because the antisense and sense strand seed sequence is still intact, it maintains the off-target effects of the original siRNA. The comparison of effects elicited by the original siRNA and the C911 mismatch control allows to distinguish phenotypes that are due to downregulation of the intended target rather than off-target effects. A scrambled siRNA (**c**) eliminates the match to the target mRNA and thus will not downregulate it but also eliminates the off-target effects due to matches to the seed sequence (however, it often creates new off-target effects against the new seed sequence) (Buehler et al. 2012a, b)

reagents will contain different seed sequences. For siRNA screens, seven or more independent reagents per gene might be assayed; for pooled shRNA screens, some investigators screen libraries using more than 15 constructs per gene (Bassik et al. 2009).

Taking these possible pitfalls of RNAi screening in consideration when designing and analyzing the screening project and planning follow-up strategies will be absolutely crucial for the success of an RNAi-based screen for novel targets. Screens against pathways or cellular processes that involve large numbers of different components (cell proliferation, cell death, cell signaling) may be especially prone to a high rate of false-positive results (Sigoillot and King 2011). Therefore, choosing more complex readouts or performing complementary screens might reduce the false-positive hit rate. The emphasis however lies on the thorough verification of screening results.

5 CRISPR/Cas9 Target Screening

A new technique has been developed as an alternative approach for RNAi-based LoF genetic screens. Identified as a prokaryotic viral defense response that utilizes clustered regularly interspaced short palindromic repeats (CRISPR) to target DNA, CRISPR/Cas9 has been adapted to be used to create “gene knockouts” by disrupting