CRISPR is actually applicable more broadly than RNAi since it can be used to disrupt nonprotein coding regions of the genome. Also, some labs have modified the Cas9 protein to eliminate the nuclease activity and introduce other functions so that CRISP methods can also provide to shut off or enhance transcription and provide a platform for gain-of-function screens (GoF) (Cheng et al. 2013). Gilbert and Horlbeck et al. reported 2014 the development of two related tools called CRISPRi and CRISPRa to repress or induce, respectively, individual transcripts with minimal off-target effects, which provide complementary biological insights through both loss- and gain-of-function genetic screens (Gilbert Luke et al. 2014).

There are of course also for CRISPR/Cas9-based gene-editing potential off-targeting effects to be anticipated. Because of the experience with RNAi-based screening, researchers try to address these with highest priority (Doench et al. 2014; Sanjana et al. 2014; Hendel et al. 2015). One factor is the exposure of the genome to the nuclease. Although cells transfected with the Cas9 nuclease without co-expressing single sgRNAs have not been found to have an increased mutagenesis rate at any of the interrogated sites (Hendel et al. 2015), studies have found that changing exposure time to the nuclease in the presence of sgRNAs can improve the "specificity ratio," defined as the ratio of on-target activity to off-target activity (Hendel et al. 2015). Also a fusion of a catalytically inactive Cas9 to the FokI nuclease (Cas9-FosI or fCas9) has been found to improve the specificity of the nuclease tremendously (Guilinger et al. 2014). Because the sequence of a genome is not random, another potential off-target causing factor is if there are homologous sites to the intended target site found in the genome and how many (Hendel et al. 2015). This has been one reason to further improve the design of the already existing sgRNA libraries (Doench et al. 2014; Sanjana et al. 2014; Hendel et al. 2015)

As for every novel technology, there is still a lot to be learned and tested, but clearly CRISPR provides already a core technology on which to build new exciting tools to explore the function not only by interrupting protein coding genes but also by specifically modifying them.

6 Conclusion

In conclusion, RNAi and recently also CRISPR-based high-throughput functional screening has clearly had an impact on our biological understanding of diseases as well as for the discovery of targets most notably perhaps for cancer treatment. With the growing knowledge about how to address the intrinsic flaws of the RNAi screening technologies, the confidence in the validity of RNAi screening results and the enthusiasm about the technology will increase again (Fennell et al. 2014). Specifically, investing in thorough assay development including the choice of reagent and assay design, subsequent sophisticated data analysis and data integration, and very careful follow-up experimental validation, large-scale RNAi screens will be successful at uncovering new genes, signaling pathways, and gene networks involved in disease mechanisms and will continue to be a valuable experimental