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Toward Whole-Transcriptome Editing with CRISPR-Cas9

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Targeted regulation of gene expression holds huge promise for biomedical research. In a series of recent publications (Gilbert et al., 2014; Konermann et al., 2015; Zalatan et al., 2015), sophisticated, multiplex-compatible transcriptional activator systems based on the CRISPR-Cas9 technology and genome-scale libraries advance the field toward whole-transcriptome control.

Spatial and temporal control of gene expression is the fundamental basis in the generation of complex organisms that consist of hundreds of cell types engaging multiple biological responses and activities. In addition to coding genes, a large and still-growing number of non-coding RNA species, such as miRNAs, lncRNAs, enhancer RNAs, or circular RNAs, have been identified, and their precise functions in development and disease remain largely unknown. It is thus not astonishing that for decades scientists have been pursuing the aim to shape gene expression at will, and at best on genome scale.

For years cDNA or shRNA libraries have been the tools of choice for targeted perturbations of the transcriptome. However, multiple isoforms of a gene, action at specific subcellular localization, or clear *cis*-regulatory functions could not be recapitulated by these technologies, emphasizing the need for a new generation of tools. Genome editing, with its ability to site-specifically target DNA and remove or introduce a sequence into the genome, appeared promising to overcome limitations of the former approaches but was hampered by a lack of versatility and difficulties and costs of assembly on a large scale. Discovery and application of the CRISPR-Cas9 system (clustered regularly interspaced short palindromic repeats [CRISPR] and CRISPR-associated 9 [Cas9]) has recently opened new avenues for transformative innovations in genome editing without a number of the limitations seen with zinc finger nucleases (ZFNs) or transcriptional activator-like effector nucleases (TALENs) (Jinek et al., 2012). Genome-scale libraries for loss-of-function (LOF) screenings that leverage the DNA cleavage function of the CRISPR-Cas9 system have been established and proven their superiority compared to shRNA-based approaches (Shalem et al., 2014). First steps toward gain-of-function (GOF) with the CRISPR-Cas9 system as an RNA-guided transcription factor (CRISPRa) were performed (Gilbert et al., 2013; Figure 1A), but no major advances or applications have been described, likely due to the rather low transcriptional activation achieved in these approaches.

In two recent publications, Gilbert et al. (Gilbert et al., 2014) and Konermann et al. (Konermann et al., 2015) describe two novel CRISPRa systems that now offer the potency to perform informative genome-scale GOF perturbations and screening (Figures 1B–1E). Former approaches relied on direct fusion of an activator domain to an enzymatically inactivated Cas9 (dCas9). Instead, both systems are based on binding of multiple secondary molecules that activate transcription, either to the dCas9 (Gilbert et al., 2014; Figure 1B) or to the short guide RNA (Konermann et al., 2015; Figure 1C).

Gilbert and colleagues used a dCas9 fusion protein with a 10-fold repeat of an antibody epitope while co-expressing a single-chain fragment variable (scFv) fused to GFP and a 4-fold repeat of the VP16 (VP64) transcriptional activator, as introduced by Tanenbaum et al. (Tanenbaum et al., 2014) in the same issue of *Cell*. This accumulation of up to 40 VP16 domains at a single location was able to induce an up to 40-fold increase in gene expression. Based on tilling experiments and phenotypic readout of ricin resistance, the authors demonstrated that a 350 bp window upstream of the transcriptional start site (TSS) could be targeted to achieve phenotypic changes of the cell with no obvious off-target activity or toxicity detected in their screening using almost 200,000 sgRNAs.

Leveraging the same principle of multiple effector molecule recruitment to a single dCas9 binding site, Konermann and colleagues developed an independent CRISPRa system (Figure 1C). The coat protein of the MS2 phage (MCP) binds as a dimer to a small RNA stem-loop (MS2-SL) structure. This interaction has already been used in cell biology, and as a proof of concept, Mali and colleagues (Mali et al., 2013) already attached the MS2-SL to an sgRNA combined with a MCP-VP64 fusion to activate transcription. Despite the use of two MS2-SLs attracting four MCP-VP64, the efficacy was rather low. Konermann et al. instead based their novel design principles on their Cas9-sgRNA-DNA crystallization studies (Nishimasu et al., 2014) showing exposition of the sgRNA loops from the Cas9 protein. Based on this observation, they did not simply

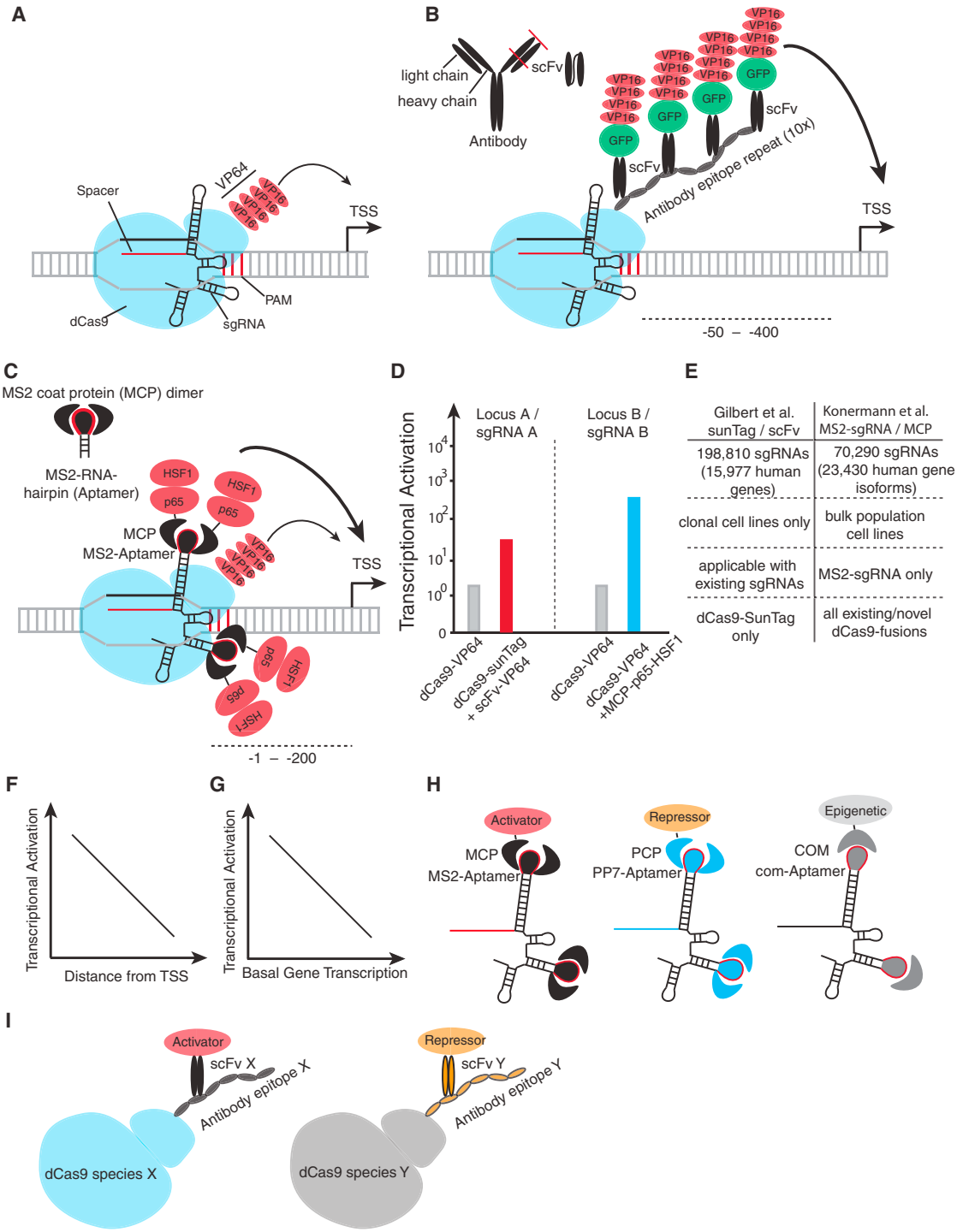

Figure 1. Second-Generation CRISPRa Systems

(A–C) Schematic representation of first-generation and second-generation CRISPRa systems. First-generation CRISPRa with dCas9-VP64 (4× VP16) fusion is shown in (A). Second-generation SunTag-dCas9 with 10× antibody epitope fusion bound by scFv-sfGFP(GFP)-VP64 fusion proteins is shown in (B). Reported window of activity relative to the TSS is indicated (−50 to −400 nucleotides from TSS). In (C), a second-generation dCas9-VP64 fusion with MS2-aptamer modified sgRNA bound by MCP-p65-HSF1 fusion proteins is shown. Reported window of activity relative to the TSS is indicated (−1 to −200 nucleotides from TSS).
(D) Approximate reported activation of the second-generation systems compared to first generation. Activation was measured at different loci with different sgRNAs excluding direct comparison of the SunTag- and MS2-based systems.
(E) Reported sgRNA libraries and applicability of the SunTag- and MS2-based systems.
(F) Inverse correlation of binding distance to TSS and transcriptional activation reported by Konermann et al.

(legend continued on next page)

attach the MS2-SLs to the sgRNA but prolonged the exposed sgRNA loops with the MS2-SLs. This rational design yielded a more than 100-fold stronger activation of gene expression. To further optimize the CRISPRa system, the authors again applied rational design, noting that endogenous gene expression is activated by synergizing transcription factors. In line with this observation, they could show that combined fusion of different transcriptional activator domains (i.e., p65, HSF1/MyoD1) to the MCP could further enhance the activation potential in comparison to the accumulation of more VP64 domains. Tilling of several promoter regions demonstrated robust gene activation within 200 bp from the TSS with a significant drop at further distance (Figure 1F). Of note, Konermann et al. measured gene expression of the tilled gene promoters instead of using a phenotypic readout as done by Gilbert et al., who noted that linear correlation between gene expression and phenotype was not granted. To further characterize the CRISPRa system, Konermann et al. addressed the marked variance of gene activation seen between different genes and could show inverse correlation of the basal transcription of a gene with its activatibility (Figure 1G). This finding has important implications for future studies in non-homogeneous cell systems, like primary cell isolates or differentiation of pluripotent stem cells, since activation of gene expression may vary even within one culture, or over time. Despite the potential caveats, Konermann et al. could show that their CRISPRa system yielded high-confidence results in a drug resistance screening and was capable of highly multiplexed gene activation, thus presenting an important resource for future studies.

Taken together, both studies are a significant advance for cell biology and biomedical research, now fully enabling gene activation at endogenous loci, including non-coding transcripts. In combination with classical CRISPR-Cas gene disruption or targeted modification, novel disease models can be generated and subsequently screened for therapeutic targets. Further development of these systems incorporating alternative RNA-aptamer-binding molecules (Zalatan et al., 2015) for multiplexed gene activation or repression, epigenetic modification (Hilton et al., 2015; Figure 1H), optical or chemical activation control, and potential incorporation of orthogonal Cas9 proteins (Fonfara et al., 2014; Figure 1I) will allow very elaborative studies of cellular functions and drug development in the future.

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(G) Correlation of basal gene transcription and transcriptional activation reported by Konermann et al.
(H) Potential multiplexing of different RNA-aptamer/aptamer-binding proteins as proposed by Zalatan et al. transferred to the sgRNA2.0 design by Konermann et al. Conceivable use of activators, repressors, or epigenetic modifiers at different loci is represented.
(I) Potential multiplexing of orthogonal Cas9 proteins and different scFv/scFv-epitope combinations as in (H).