The cellular transcriptome reflects—in the first instance—the set of genes that are transcribed into their respective miRNAs. Yet, a deeper analysis reveals a more complex picture: a single gene may be transcribed into different, alternatively spliced mRNA isoforms. Moreover, alterations in the coding sequence yield different protein isoforms, while changes in the length of 5′- and 3′-untranslated regions (5′- and 3′-UTRs) impinge on the fate of the mRNA itself as they contain regulatory elements that determine its localization, translation efficiency and stability. The diversity of mRNA isoforms is dynamically regulated, both in time (during differentiation or in response to distinct stimuli) and space (from one cell type to another), thus generating a highly plastic mRNA landscape.

MicroRNAs (miRNAs) provide a sophisticated layer of gene regulation that coordinates a broad spectrum of biological processes, including cell growth, proliferation, and death. The interaction of miRNAs with their target mRNAs at binding sites localized within the 3′-UTRs causes degradation and/or translational repression of the message. Any change in the length of 3′-UTRs may alter the availability of miRNA-binding sites, thus affecting the activity of the miRNA.1,2 Hence, the functional outcome of miRNAs depends on the mRNA landscape present in the cell, represented by both the level of transcripts and their isoform repertoire. Given the spatiotemporal dynamicity of the transcriptome, miRNA-mediated regulation is also highly plastic, which, combined with the broad spectrum of targets regulated by a single miRNA, amplifies enormously the complexity of miRNA-mediated regulation (Fig. 1).

In a recently published study we addressed the plasticity of miRNA activity by investigating the role of the miR-17-92 cluster in B-cell lymphoma.3 miR-17-92 is transcribed as a polycistron that is processed into 7 mature miRNAs: miR-17 (miR-17-5p and miR-17-3p), miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a. Although the synergy between the truncated version of the cluster, miR-17-19b, and c-MYC (hereafter MYC) has been well documented during lymphomagenesis,4-6 little is known about the role of the cluster in lymphoma maintenance. We reasoned that in MYC-driven, fully established B-cell lymphomas, the contribution of both different transcriptional programs and changes in 3′-UTR lengths might trigger a distinct outcome of the cluster. In fact, the mRNA landscape is profoundly shaped during lymphoma progression by increasing MYC levels on one hand, and by pervasive 3′-UTR shortening, which has emerged as a widespread process during tumorigenesis,7,8 on the other hand.

We used a systems biology approach that integrates quotative transcriptomics and proteomics data with in silico prediction of miR-17-19b targets and in-depth 3′-UTR analysis to assess subtle changes in the transcriptome and proteome triggered by a mild induction of miR-17-19b. This combined strategy led us to unravel the effect of widespread 3′-UTR shortening in changing the set of miR-17-19b targets during lymphoma progression. In particular, we discovered that only 369 out of 1,000 predicted miR-17-19b targets are downregulated upon induction of the cluster. In addition, strikingly, one-third of the predicted and downregulated targets were actually expressed as miRNA isoforms with shorter 3′-UTRs, meaning that their downregulation was not caused by the induction of miR-17-19b as they lost the binding site for the cognate miRNAs. Hence, in-depth 3′-UTR analysis is crucial when trying to experimentally identify miRNA targets because in silico prediction disregards context-specific 3′-UTR isoforms. Based on this consideration, we defined as bona fide miR-17-19b targets only those genes that were significantly downregulated at the transcriptome or proteome level and expressed as a mRNA isoforms with 3′-UTRs containing the binding site for miR-17-19b. Interestingly, among the experimentally verified targets, we identified checkpoint kinase 2 (Chek2) as a novel miR-17-20 target. We further demonstrated that downregulation of Chek2 negatively regulates MYC translation in full-blown
lymphoma through modulation of the Elavl1 (better known as HuR)/RISC/MYC mRNA axis. Consequently, a decreased MYC level reduced lymphoma fitness both in vivo and in vitro.

Together, our data indicate that the activity of miR-17-92 is highly plastic and changes dynamically during tumor progression as a consequence of the plasticity of the transcriptome. We demonstrated that the set of real targets regulated by miR-17-92 in full-blown lymphoma is profoundly altered from that of early-stage tumor, unraveling the dual nature of the cluster: while miR-17-92 potentiates the pro-tumorigenic function of MYC during lymphomagenesis, it dampens MYC activity in full-blown lymphomas.

Our integrated analysis also demonstrates that limiting the activity of a miRNA to the downregulation of only one of its targets falls short in recapitulating the complexity of the regulatory circuitries sustained by this type of molecules. In-depth dissection of the molecular activity of single miRNA targets is certainly informative, but it represents only one piece of the composite puzzle describing the role of miRNAs in the cell. Only the combination of systems biology with in-depth analysis of the single targets provides the key to unlock miRNA-mediated regulatory networks in space and time.

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Figure 1. Plasticity of miRNA action during tumor progression. Dynamic changes in the mRNA landscape, which occur throughout tumor progression, elicit distinct miRNA functions between early- and late-stage tumors. Asterisk (*) indicates shortened 3’UTRs; symbols used for the miRNAs, RNA binding proteins, coding sequence, 3’ UTR, and poly(A) tail are explained within the figure; mRNAs expressed in both healthy cell/onset and in established tumor are in blue, whereas newly expressed RNAs present only in the established tumor are in red.