Review

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RNA–RNA interactions in gene regulation: the coding and noncoding players

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The past few years have witnessed an exciting increase in the richness and complexity of RNA-mediated regulatory circuitries, including new types of RNA–RNA interaction that underlie key steps in gene expression control in an organized and probably hierarchic system to dictate final protein output. Both small (especially miRNAs) and long coding (lc) and noncoding (nc) RNAs contain structural domains that can sense and bind other RNAs via complementary base pairing. The versatility of the interaction confers multiple roles to RNA–RNA hybrids, from control of RNA biogenesis to competition for common targets. Here, we focus on the emerging evidence around RNA networks and their impact on gene expression regulation in light of recent breakthroughs around the crosstalk between coding RNAs and ncRNAs.

The rich landscape of the RNA world

There are more genes encoding regulatory RNAs than encoding proteins. This evidence, obtained in recent years from the sum of numerous post-genomic deep-sequencing studies, gives a good clue of the gigantic step we have taken from the years of the central dogma: one gene gives rise to one RNA to produce one protein. We now know that, besides its role as intermediary in protein synthesis, RNA is a functionally versatile molecule directly involved in the regulation of most cell processes, including epigenetic control, gene transcription, translation, RNA turnover, chromosomal organization, and genome defense, with key roles in cellular developmental and proliferation programs. In most instances, regulation is carried out by ncRNAs (for recent reviews, see [1–5]).

Almost without exception, RNA always works through interactions with proteins. Most ncRNA studies have described the functional effector unit as a ribonucleoprotein complex, in which specific interactions between RNA and RNA-binding protein partners constitute the essential functional unit. However, in a growing number of cases, evidence points to the existence of additional RNA–RNA interactions among different RNA species that add to the regulatory complexity mediated by ncRNA. For many years, the existence of direct RNA–RNA interactions had been associated with the functions of small nuclear RNAs (snRNAs) and nucleolar RNAs: within the core of the spliceosome, there exists a handful of essential RNA–RNA interactions between the U1, U2, U4, U5, and U6 snRNAs and the 5′, 3′ splice sites or branch point on the pre-mRNA. These interactions represent short [from just a few to approximately 15 nucleotides (nt) long] base-pairing regions, protein assisted, that act cooperatively and combinatorially to define *bona fide* splice sites that will be ultimately selected to engage in the catalytic reaction. RNA itself might be essential for the catalysis [6]. Similar RNA-guided reactions occur during the modifications of other RNAs, such as methylation or pseudouridylation of rRNAs, tRNAs, and snRNAs [7]. In trypanosomes, guide RNAs have essential roles in the proper editing of many mitochondrial mRNAs [8]. In prokaryotes, a defense system against invading RNAs involves the action of small RNAs derived from the clustered regularly interspaced short palindromic repeats (CRISPRs) loci, which act as guides for homology-dependent cleavage of target RNAs [9,10].

Here, we give an overview of the recent discoveries around RNA–RNA interactions and their impact on gene expression regulation, specially focusing on mammalian systems.

The discovery of miRNAs as components of a large RNA–RNA network

Two decades ago, a turning point was the finding of small RNA-mediated regulation in *Caenorhabditis elegans* with the discovery of miRNAs [11] and of the siRNA phenomenon in general [12,13]. miRNAs are a class of short (approximately 22 nt) ncRNAs that mediate post-transcriptional gene expression regulation, usually through imperfect base pairing to the 3′ untranslated regions (UTR) of target mRNAs (reviewed in [14]). miRNAs can regulate a high number of target mRNAs; for instance, a single miRNA can affect the expression of over 100 transcripts [15] and, conversely, a given mRNA can contain target sites for a large number of miRNAs, which suggests a complex regulatory network whose logic remains largely unexplored.

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Although generally functioning as repressors, there are some instances reported where binding of a miRNA to the target mRNA can result in upregulation of translation [16–18].

This is the conventional view of miRNA function, but, as discussed below, recent evidence points to additional modes of functioning for miRNAs that could add to the rich landscape of RNA-mediated regulation of cell programs. In many instances, these new functions coincide with an unexpected nuclear localization of the miRNA. Individual miRNAs had been detected in the nucleus at relatively high levels over a decade ago [19,20]. More recently, microarray analysis and deep-sequencing techniques revealed the presence of mature miRNA in the nucleus [21,22]. The import of mature miRNA to the nuclear compartment after Dicer-mediated maturation in the cytoplasm is still unclear (with the probable involvement of a hexanucleotide element at the 3′ end of mature miRNAs [23]), but it has been shown that nuclear miRNAs can inhibit or activate gene expression at the transcriptional level in the nucleus of human cells [24,25]. Importantly, the argonaute 2 (Ago2) protein, a key component of the RNA-induced silencing complex (RISC), is known to be imported into the nucleus from the cytoplasm [26], and mediate the cleavage of target RNAs in the nucleus [27–29].

Given their diverse cellular localization, miRNAs in particular and ncRNAs in general can guide and direct changes at several different steps in gene expression. In the next section, we review key aspects of mRNA and ncRNA biogenesis that are subject to regulation by RNA–RNA interactions.

Regulation of splicing, translation, and miRNA biogenesis through direct RNA–RNA interaction

RNAs that control splicing

Both small ncRNA and ncRNA (lncRNA) can control splicing through direct base pairing. Kornblihtt and colleagues have shown that siRNAs complementary to intronic or exonic sequences close to an alternative exon can regulate the splicing of that exon by inducing facultative heterochromatin formation [30,31]. In the case of the zinc finger E-box binding homeobox 2 (*Zeb2*) gene (a transcriptional repressor of E-cadherin), a natural antisense transcript that overlaps the 5′ splice site of an intron harboring an internal ribosome entry site (IRES) necessary for the expression of *Zeb2* prevents its splicing [32]. This example illustrates how lncRNA can regulate alternative splicing patterns of other target RNAs through direct base pairing. Related to this point, a genome-wide survey of sense–antisense gene pairs in lymphoblastoid cell lines indicated that areas of overlap were more prone to alternative splicing events [33].

RNAs that control translation

The group of Khavari and coworkers discovered that terminal differentiation-induced ncRNA (*TINCR*), a lncRNA controlling epidermal differentiation, binds to mRNAs of many skin development genes and regulates their expression by stabilizing its bound mRNAs [34]. Binding occurs through a 25-nt motif that is strongly enriched in interacting mRNAs.

RNAs that control the biogenesis of miRNAs

miRNA biogenesis comprises several highly regulated steps [35]. A plethora of proteins have been identified as regulators of miRNA abundance through either direct binding to the miRNA precursors or interaction with the processing machineries. Recently, a direct role for RNA species in regulating miRNA biogenesis has been uncovered. In mouse, *miR-709*, which is predominantly nuclear, binds to a 19-nt recognition element on *pri-miR-15a / 16-1*, thereby blocking the processing by Drosha and, thus, reducing the amount of *pre-miR15a / 16-1* produced [36]. The recognition element is located 0.8 kb downstream of the *miR-15a / 16-1* gene loci and, thus, it is unlikely that the mechanism involved is a direct result of *miR-709* interference with Microprocessor machinery; further mechanistic details will be necessary to understand the regulation involved. *miR-15a / 16-1* targets the antiapoptotic protein B cell lymphoma 2 'Bcl-2' and, therefore, regulation by *miR-709* impacts the control of cell apoptosis. Similarly, in *C. elegans*, the work of Zisoulis and coworkers has shown that *let-7* miRNA regulates its own expression by binding to a complementary site near the 3′ end of its primary transcript (500 nt downstream of the mature *let-7*) and promoting Ago-mediated processing. This creates a direct autoregulatory loop in which mature *let-7* is able to upregulate its own biogenesis [37]. The authors extend their work to show that, in HeLa cells, approximately 50% of all mature *let-7* is in the nucleus (Figure 1A).

Other ncRNAs may regulate miRNA biogenesis: a transcript derived from an ultraconserved region can also act as a regulator of *miR-195* biogenesis through direct binding to the pri-miRNA stem-loop structure [38]. Binding to this region prevents efficient recognition of the structure by DiGeorge syndrome chromosomal region 8 (DGCR8)/DROSHA and diminishes the amount of processed miRNA. As a consequence, there is a negative impact on the abundance of active *miR-195*. The short stretch of base pairing between the two RNAs suggests that some unknown protein factor is involved in stabilization of the interaction (Figure 1B).

Another recent study in human cells characterizes how a lncRNA, the mitochondrial dynamic-related lncRNA (*MDRL*), regulates Drosha processing of the *miR-484* primary transcript to pre-miRNA through binding to another miRNA, *miR-361* [39]. The work shows that mature *miR-361* is able to bind to a region downstream of the *pri-miR-484* stem-loop in the nucleus, reducing DROSHA cropping and, therefore, the levels of processed *miR-484*. Thus, *MDRL* would act as a functional reservoir (or 'sponge', see below) for *miR-361* and reduce the amount of *miR-361* able to interact with *pri-miR-484*. Given that *miR-484* inhibits mitochondrial fission and apoptosis in cardiomyocytes, the interplay between these three ncRNAs is especially relevant in the context of myocardial infarction (Figure 1C). Nevertheless, the detailed mechanism by which *miR-361* reduces Drosha cleavage is unclear, as is the effect of *MDRL–miR-361* interaction on the levels of both RNAs.

Given evidence along these lines, it is possible that nuclear localization of mature miRNAs serves to both sequester them from the cytoplasm (thus fine-tuning their