MicroRNA control of signal transduction

Masafumi Inui, Graziano Martello and Stefano Piccolo

Abstract | MicroRNAs (miRNAs) are integral elements in the post-transcriptional control of gene expression. After the identification of hundreds of miRNAs, the challenge is now to understand their specific biological function. Signalling pathways are ideal candidates for miRNA-mediated regulation owing to the sharp dose-sensitive nature of their effects. Indeed, emerging evidence suggests that miRNAs affect the responsiveness of cells to signalling molecules such as transforming growth factor-β, WNT, Notch and epidermal growth factor. As such, miRNAs serve as nodes of signalling networks that ensure homeostasis and regulate cancer, metastasis, fibrosis and stem cell biology.

Despite great advances, the miRNA world remains largely uncharted with regard to the physiological function of these molecules within cells and organisms, and uncovering the function of individual miRNAs is challenging. First, miRNAs are frequently present as families of redundant genes, which complicates genetic dissections. Second, each miRNA has numerous putative targets that have disparate functions, with no means to decide a priori which one is most meaningful and thus worthy of experimental validation. Third, the degree of target downregulation imposed by miRNAs often tends to be quantitatively modest; measured at the protein level, even an overexpressed miRNA typically downregulates most of its endogenous targets by less than 50% (REF 9). Therefore, most proteins should remain effective over this degree of inhibition, an argument supported by the paucity of haploinsufficient phenotypes that have been described so far. Conversely, few genes are known to have phenotypes when duplicated, perhaps mimicking the situation when miRNA-mediated regulation of a target is lost10. These considerations suggest that even though most genes are predicted to be miRNA targets, only a fraction of these interactions will prove instrumental for overt biological responses and phenotypes4,10–16. Remarkably, however, inhibition of miRNA biogenesis (for example, through the ablation of Dicer) clearly reveals that miRNAs are essential for a wide array of biological processes, including control of proliferative homeostasis, differentiation or embryonic stemness17–19.

As a resolution to this conundrum, rather than querying miRNA–target pairs to predict miRNA biological functions, the reverse question — asking which...
Box 1 | RNA biogenesis and mechanisms of action

MicroRNAs (miRNAs) are transcribed as primary transcripts (pri-miRNAs) by RNA polymerase II. Each pri-miRNA contains one or more hairpin structures that are recognized and processed by the microprocessor complex, which consists of the RNase III type endonuclease Drosha and its partner, DGCR8 (see the figure). The microprocessor complex generates a 70-nucleotide stem loop known as the precursor miRNA (pre-miRNA), which is actively exported to the cytoplasm by exportin 5.

In the cytoplasm, the pre-miRNA is recognized by Dicer, another RNase III type endonuclease, and TAR RNA-binding protein (TRBP; also known as TARBP2). Dicer cleaves this precursor, generating a 20-nucleotide mature miRNA duplex. Generally, only one strand is selected as the biologically active mature miRNA and the other strand is degraded. The mature miRNA is loaded into the RNA-induced silencing complex (RISC), which contains Argonaute (Ago) proteins and the single-stranded miRNA. Mature miRNA allows the RISC to recognize target mRNAs through partial sequence complementarity with its target. In particular, perfect base pairing between the seed sequence of the miRNA (from the second to the eighth nucleotide) and the seed match sequences in the mRNA 3′ UTR are crucial. The RISC can inhibit the expression of the target mRNA through two main mechanisms that have several variations: removal of the polyA tail (deadenylation) by fostering the activity of deadenylases (such as CCR4–NOT), followed by mRNA degradation; and blockade of translation at the initiation step or at the elongation step; for example, by inhibiting eukaryotic initiation factor 4E (EIF4E) or causing ribosome stalling RISC-bound mRNA can be localized to sub-cytoplasmatic compartments, known as P-bodies, where they are reversibly stored or degraded.

Figure is modified, with permission, from REF. 104 Nature Reviews Genetics © 2008 Macmillan Publishers Ltd. All rights reserved. m′G, 7-methylguanosine cap; ORF, open reading frame.

biological processes might be prime candidates for miRNA-mediated regulation — might be more productive. In this Review, we provide examples showing that signal transduction pathways are prime candidates for miRNA-mediated regulation in animal cells. Signalling complexes are indeed highly dynamic, ephemeral and non-stoichiometric molecular ensembles, which translate into well-established dose-dependent responses. As such, they are the ideal targets for the degree of quantitative fluctuations imposed by miRNAs. This might enable the multi-gene regulatory capacity of miRNAs to remodel the signalling landscape, facilitating or opposing the transmission of information to downstream effectors in an effective and timely manner26, TABLE 1 and Supplementary information S1 (table) provide a list of miRNAs targeting either positive or negative modulators of key signalling pathways. In the first part of this Review, we summarize some examples that relate the function of individual miRNAs to the regulation of cell signalling.

miRNAs may also help to explain a paradox in evolution. The core protein engines of developmental signalling networks are highly conserved devices, which can be traced back to the common ancestor of all Bilateria21–23. However, the development of increasingly complex body plans obviously required a great degree of plasticity in the use of those pathways, demanding the evolution of new layers of regulation. Just like transcription factor binding sites, 3′ UTR sequences are not constrained by coding needs and can potentially diverge rapidly to co-opt beneficial miRNA–target interactions and counter-select against deleterious pairs24,25. Nevertheless, although few new transcription factor families have arisen in animal evolution, continuous emergence of new miRNA families has paralleled the increased complexities in body plans and organs24,26,27. Thus, miRNAs may represent ductile and fast-evolving tools, which add sophisticated regulatory tiers to signalling pathways. We discuss the logic of these networks in the second part of this Review. In sum, crosstalk between growth factor signalling and miRNAs may substantially contribute to our current understanding of miRNA biology.
### Table 1 | microRNAs targeting signalling pathway components*

<table>
<thead>
<tr>
<th>Target</th>
<th>Effect†</th>
<th>miRNAs</th>
<th>Species</th>
<th>Validation assays used‡</th>
<th>Biological processes</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transforming growth factor-β</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>squint</td>
<td>+</td>
<td>miR-430</td>
<td><em>D. rerio</em></td>
<td>LOF on 3′ UTR GFP and ISH</td>
<td>Early embryogenesis</td>
<td>49</td>
</tr>
<tr>
<td>ACVR2A</td>
<td>+ and –</td>
<td>miR-15 and miR-16</td>
<td><em>H. sapiens</em> and <em>X. laevis</em></td>
<td>GOF and LOF on 3′ UTR luciferase reporter and WB</td>
<td>Early embryogenesis</td>
<td>48</td>
</tr>
<tr>
<td>Smad3</td>
<td>+</td>
<td>miR-23b</td>
<td><em>M. musculus</em></td>
<td>GOF on 3′ UTR luciferase reporter</td>
<td>Liver stem cell differentiation</td>
<td>50</td>
</tr>
<tr>
<td>Smad4</td>
<td>+</td>
<td>miR-23b</td>
<td><em>M. musculus</em></td>
<td>GOF on 3′ UTR luciferase reporter</td>
<td>Liver stem cell differentiation</td>
<td>50</td>
</tr>
<tr>
<td>Smad5</td>
<td>+</td>
<td>miR-23b</td>
<td><em>M. musculus</em></td>
<td>GOF on 3′ UTR luciferase reporter</td>
<td>Liver stem cell differentiation</td>
<td>50</td>
</tr>
<tr>
<td>lefty1, lefty2</td>
<td>–</td>
<td>miR-430</td>
<td><em>D. rerio</em></td>
<td>LOF on 3′ UTR GFP and ISH</td>
<td>Early embryogenesis</td>
<td>49</td>
</tr>
<tr>
<td><strong>WNT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tcf</td>
<td>+ and –</td>
<td>miR-8</td>
<td><em>D. melanogaster</em></td>
<td>GOF on 3′ UTR lacZ and WB</td>
<td>Eye formation</td>
<td>34</td>
</tr>
<tr>
<td>lef1</td>
<td>+</td>
<td>miR-203</td>
<td><em>D. rerio</em></td>
<td>LOF on WB</td>
<td>Fin regeneration</td>
<td>70</td>
</tr>
<tr>
<td>Wntless</td>
<td>+</td>
<td>miR-8</td>
<td><em>D. melanogaster</em></td>
<td>GOF on 3′ UTR lacZ and WB</td>
<td>Eye formation</td>
<td>34</td>
</tr>
<tr>
<td><strong>Hedgehog</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoothed</td>
<td>+</td>
<td>GOF and LOF on 3′ UTR luciferase reporter and WB</td>
<td><em>H. sapiens</em> and <em>M. musculus</em></td>
<td></td>
<td>Neural stem cell proliferation and medulloblastoma</td>
<td>35</td>
</tr>
<tr>
<td>GLI1</td>
<td>+ and –</td>
<td>miR-324-5p</td>
<td><em>H. sapiens</em></td>
<td>GOF and LOF on 3′ UTR luciferase reporter and WB</td>
<td>Neural stem cell proliferation and medulloblastoma</td>
<td>35</td>
</tr>
<tr>
<td><strong>Receptor tyrosine kinase and mitogen-activated protein kinase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRAS, KRAS</td>
<td>+</td>
<td>let-7</td>
<td><em>H. sapiens</em></td>
<td>GOF and LOF on 3′ UTR luciferase reporter and immunofluorescence</td>
<td>Cancer cell differentiation</td>
<td>54</td>
</tr>
<tr>
<td>ras</td>
<td>+</td>
<td>let-7</td>
<td><em>C. elegans</em></td>
<td>LOF on 3′ UTR lacZ</td>
<td>Vulva differentiation</td>
<td>54</td>
</tr>
<tr>
<td>Sprouty 1</td>
<td>–</td>
<td>miR-21</td>
<td><em>M. musculus</em></td>
<td>GOF on 3′ UTR luc reporter, WB, and LOF on WB</td>
<td>Cardiac fibrosis</td>
<td>64</td>
</tr>
<tr>
<td>spred1</td>
<td>–</td>
<td>miR-126</td>
<td><em>D. rerio</em></td>
<td>GOF and LOF on 3′ UTR luciferase reporter and WB</td>
<td>Angiogenesis</td>
<td>61</td>
</tr>
<tr>
<td><strong>Receptor tyrosine kinase and AKT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI3KR2</td>
<td>–</td>
<td>miR-126</td>
<td><em>M. musculus</em></td>
<td>GOF and LOF on 3′ UTR luciferase reporter and WB</td>
<td>Angiogenesis</td>
<td>62</td>
</tr>
<tr>
<td>pi3kr2</td>
<td>–</td>
<td>miR-126</td>
<td><em>D. rerio</em></td>
<td>GOF and LOF on 3′ UTR luciferase reporter and WB</td>
<td>Angiogenesis</td>
<td>61</td>
</tr>
<tr>
<td>PTEN</td>
<td>–</td>
<td>miR-21</td>
<td><em>H. sapiens</em></td>
<td>GOF and LOF on WB</td>
<td>Hepatocellular cancer</td>
<td>63</td>
</tr>
<tr>
<td>PTEN</td>
<td>–</td>
<td>miR-26a</td>
<td><em>H. sapiens</em></td>
<td>GOF on 3′ UTR luciferase reporter and WB</td>
<td>Glioma</td>
<td>66</td>
</tr>
<tr>
<td>PTEN</td>
<td>–</td>
<td>miR-216a and miR-217</td>
<td><em>H. sapiens</em></td>
<td>GOF and LOF on 3′ UTR luciferase reporter and WB</td>
<td>Glomerular mesangial cell survival and hypertrophy</td>
<td>67</td>
</tr>
<tr>
<td><strong>Notch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E(spl), Brd</td>
<td>+</td>
<td>GY-box, Brd-box and K-box miRNAs</td>
<td><em>D. melanogaster</em></td>
<td>GOF on 3′ UTR GFP</td>
<td>Wing formation</td>
<td>37</td>
</tr>
<tr>
<td><strong>Hippo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LATS</td>
<td>+</td>
<td>miR-372/373</td>
<td><em>H. sapiens</em></td>
<td>GOF on 3′ UTR luciferase reporter and WB</td>
<td>Testicular germ cell tumour</td>
<td>43</td>
</tr>
<tr>
<td>Expanded</td>
<td>+</td>
<td>miR-278</td>
<td><em>D. melanogaster</em></td>
<td>GOF on 3′ UTR luciferase reporter</td>
<td>Tissue growth</td>
<td>42,44</td>
</tr>
<tr>
<td><strong>p53</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>+</td>
<td>miR-125b</td>
<td><em>H. sapiens</em> and <em>D. rerio</em></td>
<td>GOF and LOF on 3′ UTR luciferase reporter and WB</td>
<td>Apoptosis in brain</td>
<td>41</td>
</tr>
<tr>
<td>TP63</td>
<td>+</td>
<td>miR-203</td>
<td><em>M. musculus</em></td>
<td>GOF on 3′ UTR luciferase reporter and WB</td>
<td>Keratinocyte differentiation</td>
<td>19</td>
</tr>
</tbody>
</table>

†ACVR2A, activin receptor type 2A; Brd, Branded; C. elegans, Caenorhabditis elegans; D. melanogaster, Drosophila melanogaster; D. rerio, Danio rerio; E(spl), enhancer of split; GFP, green fluorescent protein; GOF, gain of function; H. sapiens, Homo sapiens; ISH, in situ hybridization; KRAS, Kirsten rat sarcoma RAS; lef1, lymphoid enhancer-binding factor 1; LATS, large tumour suppressor; LOF, loss of function; miRNA, microRNA; M. musculus, Mus musculus; NRAS, neuroblastoma RAS; PI3KR2, PI3-kinase regulatory subunit-β; PTEN, phosphatase and tensin homologue; spred1, Sprouty-related, EVH1 domain-containing protein 1; Tcf, T cell factor; WB, western blot; X. laevis, Xenopus laevis. ‡This is a partial list referring to the examples cited in this Review. See Supplementary information S1 (table) for a more extensive description of miRNAs and corresponding signalling cascades. §Indicates whether the miRNA targets a positive (+) or negative (−) modulator of the signalling pathway. **GOF indicates miRNA overexpression, LOF indicates miRNA treatment with loss of function reagent.**
miRNA and signalling: common principles
During embryonic development, a handful of signalling pathways — transforming growth factor-β (TGFβ), WNT, Hedgehog, Notch, Hippo, and pathways driven by receptor tyrosine kinases (RTKs) such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) receptors — precisely coordinate tissue induction, patterning, growth and morphogenesis. The same pathways maintain tissue homeostasis in adults, and their perturbations account disproportionately for human diseases. The effectiveness of these signalling pathways relies on their capacity to tightly control the expression of target genes in time and space. Two common principles are adopted to achieve this result: context-dependent transcriptional activation and default repression.

miRNAs in default repression. Default repression ensures that target gene expression is turned on exclusively in the presence of signalling but kept actively repressed in its absence. This is primarily attained at the transcriptional level: typically, the same responsive element on a target gene promoter switches from mediating default repression to signal-dependent activation (Fig. 1b). But is transcriptional control sufficient to explain tight signalling regulation? It would seem unreasonable to assume that in vivo cells will be challenged only by unambiguous on or off situations. Much more frequently, cells will be challenged only by unambiguous on or off situations. Much more frequently, cells will have to distinguish between what is a real signal — one worthy of activating downstream targets — from inputs that are too weak or too transient. In this grey area, miRNAs could be crucial for signal interpretation: by dampening positive mediators of signalling cascades, miRNAs would raise the threshold for pathway activation, restricting it only to appropriate zones of competence (Fig. 1c).

WNT, Notch and Hedgehog are signalling pathways that are under strong default repression. The role of the transcription factor TCF in WNT signalling serves as a paradigm for this type of regulation. In the absence of WNT ligands, the cytosolic pool of β-catenin, which works in conjunction with TCF to activate specific genes, is phosphorylated and targeted for degradation. Following WNT signalling, a cascade is initiated that results in the stabilization and translocation of β-catenin to the nucleus, where it forms a transcriptional activating complex with TCF, which outcompetes co-repressors sitting on target genes. miR-8, the Drosophila melanogaster orthologue of the vertebrate miR-200 family, has been identified in a gain-of-function screen for negative regulators of...
Wingless signalling (WNT signalling in vertebrates)\(^4\). In *D. melanogaster* and mouse cells miR-8 and miR-200c, respectively, contribute to default repression by targeting both TCF and upstream positive modulators of the pathway, including Wntless (also known as evi), which is required for the secretion of WNT ligands\(^8\).

A similar example relates to Hedgehog signalling\(^2\). In mammals, this pathway controls the proliferation of cerebellar granule progenitor cells, and aberrant pathway activity causes medulloblastomas\(^29\). Using a miRNA high-throughput profile in human medulloblastomas, miR-324-5p was identified as a supressor of Hedgehog signalling, miR-324-5p targets the transcription factor GLI1, the mediator of Hedgehog signalling, and its loss upgrades pathway responsiveness, leading to tumour formation\(^35\).

Default repression by miRNAs does not necessarily have to target core pathway components; it may be equally effective when it intercepts their transcriptional targets [FIG 1c]. A classical example of default repression at the level of downstream targets is the miRNA-mediated regulation of the enhancer of split (*E(spl)*) and Bearded (*brd*) gene clusters, which are downstream effectors of Notch signalling in *D. melanogaster*. This is a highly redundant system, in which families of related miRNAs (miR-2, miR-4, miR-7, miR-11 and miR-79 ) promiscuously target a family of related mRNAs, preventing aberrant deployment of Notch-mediated developmental programmes\(^36,37\). Notably, the Notch targets in *E(spl)* and *brd* are among the few studied examples for which specific mutation of miRNA binding sites in a genomic transgene is sufficient to cause mutant phenotypes, indicating that miRNA regulation is essential for normal Notch signalling\(^38,39\).

miRNAs may themselves be mediators of default repression. For example, during DNA damage, a cascade of kinases activate the p53 tumour suppressor, leading to cell cycle arrest or apoptosis. p53 is normally a latent transcription factor that is inhibited by ubiquitin-mediated degradation\(^40\). The miRNA miR-125b is essential to complete p53 repression by targeting it, and loss of miR-125b causes p53-dependent apoptosis [FIG 1d]. Interestingly, miR-125b is itself part of the DNA damage network, as it is downregulated after genotoxic treatments\(^41\). Thus, by raising the threshold for p53 activation, miR-125b ensures a safe and robust DNA damage response.

**miRNAs in default activation.** Although miRNAs repress gene expression, their function is not just repressive. Indeed, their effect on the output of signalling cascades is strictly dependent on pathway topology. For example, in the Hippo tumour suppressor pathway, which controls tissue growth in *D. melanogaster* and mammals by regulating cell proliferation and apoptosis, active signalling actually leads to the inactivation of the two downstream transcription factors of the pathway, the Yes-associated protein (YAP) and Tazfazin (TAZ) proto-oncogenes. Specifically, signalling causes YAP and TAZ phosphorylation, which inhibits their nuclear activities by localizing them to the cytoplasm\(^22\). YAP and TAZ phosphorylation is mediated by the large tumour suppressor (LATS) kinase and its upstream regulator, Expanded. These Hippo pathway components are targeted by two miRNAs: miR-372 and miR-373 in mammals (LATS) and miR-278 in *D. melanogaster* (Expanded)\(^42-44\). This results in the absence of YAP and TAZ phosphorylation, nuclear retention and consequently transcriptional activation of YAP and TAZ target genes. Thus, in the case of Hippo, suppression of signalling mediators leads to the transcription of YAP and TAZ target genes, explaining the oncogenic potential of these miRNAs [FIG 1e]. Interestingly, a key transcriptional target of the Hippo pathway in *D. melanogaster* is the miRNA bantam, which serves as an essential mediator of organisational growth and patterning\(^45,46\).

**miRNAs and context-dependent signalling**

The arrival of an extracellular signal does not typically deliver specific instructions. Instead, it is the cell that interprets the signal according to its history and actual environment, sorting out the repertoire of target genes that better suits its needs. The unique miRNA milieu of each cell type is ideally suited to serve in such context-dependent gene expression [FIG 1a]. This would allow great plasticity in the system, and explains the constant recycling of these signalling cascades in different cell types and stages in development and evolution.

A key challenge in biology is explaining how naive cells acquire distinct fates in response to a limited number of signalling cues. An elegant solution to this riddle relies on the cell’s ability to perceive extracellular signals quantitatively (that is, by their intensity and duration) and to couple these ‘readings’ with the activation of distinct gene expression programmes\(^47\). Such dose dependency seems particularly amenable to miRNA regulation. In this section, we provide examples relating to miRNAs as generators of graded responses or as signalling amplifiers.

**miRNAs sharpen morphogen gradients in TGFβ signalling.** The effects of signalling by TGFβ superfamily ligands, such as TGFβ and bone morphogenetic protein (BMP), are widespread during morphogenesis and adult tissue homeostasis. This is highlighted by a range of hypomorphic and haploinsufficient phenotypes in mutants for TGFβ pathway components observed in fly, worm and vertebrate model systems\(^42\). This also suggests that these components must be somehow limiting in vivo and thus could be ideal targets of miRNA regulation.

Nodal is a TGFβ superfamily ligand that serves as a potent morphogen during induction of the germ layers and specification of the body axes. Importantly, in early vertebrate embryos, Nodal activity is asymmetric: in *Xenopus laevis*, the highest Nodal activity is required for the formation of the Spemann’s organizer, which defines the embryo’s most dorsal and anterior structures\(^43\) [FIG 2a]. Recent work highlighted the role of two miRNAs, miR-15 and miR-16, in this asymmetry. miR-15 and miR-16 are enriched on the ventral side of the embryo, where they attenuate Nodal signalling by targeting the Nodal receptor activin receptor type 2A (ACVR2A)\(^48\) [FIG 2b,c]. miR-15 and miR-16 also provide a first example of integration between distinct signalling pathways.
Smad proteins are transcription factors that transduce TGFβ signals downstream of their receptors, and they can also be targeted by miRNAs. Indeed, within the developing liver, the miR-23b cluster has been shown to target three Smads (SMAD3, SMAD4 and SMAD5), thereby inhibiting the anti-proliferative response mediated by TGFβ and fostering hepatocyte proliferation\textsuperscript{36}. The fact that a single miRNA cluster targets several Smads concomitantly offers an interesting example of how, despite having a weak effect on their own, the simultaneous attack of miRNAs on a common set of regulatory proteins can amplify their effect.

\textbf{Attenuation of RAS signalling by let-7.} Beyond spatial regulation, miRNAs can modify tissue responsiveness over time. let-7 was the first nematode miRNA for which clear homologues could be identified in diverse metazoan lineages\textsuperscript{\textsuperscript{31}}. In nematodes, let-7 functions as regulator of the transition from undifferentiated and proliferative stem cells of the late larva to quiescent, differentiated cells of the adult\textsuperscript{31}. This is strikingly reminiscent of the function of mammalian let-7, which is expressed at low levels by progenitor cells, but at high levels by their differentiated progeny\textsuperscript{31}. In cancer cells, loss of let-7 seems to be associated with a reverse embryogenesis programme; that is, with the reactivation of genes that positively regulate proliferation and stem cell self-renewal in youth, but the activity of which typically declines with age\textsuperscript{31}. One of the evolutionary conserved targets of let-7 is RAS, and this regulation controls breast cancer cell self-renewal and, likely, the response to chemotherapy (FIG. 2d). Clearly, this explains only part of the complexity of let-7 biology: in the breast cancer example, epithelial de-differentiation occurs through let-7-mediated downregulation of a different target, high mobility group box A2 (HMGA2; also known as HMGIC)\textsuperscript{34,35}. By taming proto-oncogenic signalling, let-7 may oppose cancer, but it may also contribute to the decline of normal stem cell function and tissue renewal associated with ageing\textsuperscript{36}. Recent advances on the mechanisms of let-7 biogenesis by LIN-28, a protein that promotes pluripotency (see below), suggest exciting possibilities for the targeted manipulation of let-7 expression in different contexts.

\textbf{miRNAs as signalling amplifiers.} Once a miRNA targets an inhibitor of a signalling cascade, it serves as a positive regulator by either amplifying signal strength or duration, or empowering cell responsiveness to otherwise sub-threshold stimuli. The RAS–RAF–mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)–AKT cascades are two pleiotropic pathways that branch from activated RTKs\textsuperscript{31}. The characteristic trait of these pathways is potent signal amplification; this is achieved by numerous kinases acting sequentially and activating one another, as well as by second messengers, such as phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)\textsubscript{P})\textsuperscript{31,35}. The intrinsic risk of this design is mounting a potential chain reaction that leads to senescence or cancer initiation. Negative regulators are in place to prevent this risk; these include phosphatase and tensin homologue (PTEN; which reverses PI3K-mediated

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{\textbf{MicroRNAs and signalling gradients.} a) The conventional view of a morphogenetic signalling gradient, as exemplified by mesoderm induction by Nodal in amphibian embryos. A dorso–ventral gradient of Nodal ligand expression is translated into graded SMAD2 signalling (indicated by arrows), thus inducing mesodermal tissues of different dorso–ventral identities. The highest dose of signalling induces the Spemann’s organizer (ORG), the most dorsal structure of the embryo. b,c | MicroRNAs (miRNAs) can modify the interpretation of signalling gradients. b | In Xenopus laevis embryos, miR-15 and miR-16 are enriched on the ventral side, where they inhibit the Nodal receptor activin receptor type IIα (ACVR2A) and consequently SMAD2 signalling. c | The asymmetric expression of miR-15 and miR-16 is regulated by dorsally enriched β-catenin signalling, which reflects the dorsal-to-ventral activity of the Nodal receptor ACVR2A (magenta triangle, lower panel). d | miRNAs can modify tissue responsiveness over time. let-7 expression increases during differentiation, resulting in progressive inhibition of RAS signalling. This enables self-renewal in progenitor cells but restrains it while cells differentiate. e | Signalling downstream of receptor tyrosine kinases (RTKs) involves two main branches, namely the phosphoinositide 3-kinase (PI3K)–AKT and the RAF–mitogen-activated protein kinase (MAPK) cascades. Some miRNAs, such as miR-21 and miR-126, target inhibitors of both branches, leading to a general upregulation of RTK signalling. miR-26a specifically upregulates the PI3K–AKT branch by inhibiting phosphatase and tensin homologue (PTEN). PIK3R2, PI3-kinase regulatory subunit-β; SPRY, sprouty-related genes (Sprouty or SPRED1).}
\end{figure}
miRNAs in signalling crosstalk

The regulatory capacity of miRNAs may be exploited to connect distinct signalling pathways (Fig. 3a). We mentioned above the example of miR-15 and miR-16, linking WNT and TGFβ signalling. Furthermore, recent evidence suggests that there are several miRNAs that can act as mediators of signalling crosstalk. One particular example is how TGFβ signals through AKT during renal fibrosis (Fig. 3b); this link is assured by a cascade of miRNAs. Specifically, TGFβ turns on the transcription of miR-192, which inhibits the expression of zinc finger E-box-binding homeobox 2 (ZEB2). Because ZEB2 is a transcriptional repressor of miR-216a and miR-217, its inhibition allows the derepression of miR-216a and miR-217, leading to AKT activation. The ensuing AKT activation causes glomerular mesangial cell survival and hypertrophy in diabetic mouse models. Thus, antagonizing either miR-192 or miR-216a and miR-217 could offer new therapeutic potentials for diabetic nephropathy.

Similarly to embryogenesis, adult tissue regeneration and wound repair also require the strict, coordinated and dynamic control of various signalling pathways (Fig. 3b). Not surprisingly, miRNAs are emerging as key micro-managers of some of these processes. For example, the skin constantly rejuvenates during homeostasis through the self-renewing capacity of the innermost basal layer. As cells move suprabasally, they embark on a terminal differentiation programme. Integral to this switch is the downregulation of the p53-related factor p63, which is essential for epithelial stem cell maintenance. miR-203 sharpens the border between basal progenitors and their differentiating progeny because it targets residual p63 expression in suprabasal layers. Skin appendages, such as hair follicles or fins, can also regenerate through WNT stimulation. Remarkably, another conserved target of miR-203 is lymphoid enhancer-binding factor 1 (LEF1), a b-catenin DNA-binding partner. Although the role of miR-203 in hair follicle regeneration has not been investigated, miR-203 inhibits Lef1-mediated fin regeneration in zebrafish. It is therefore tempting to speculate that inhibition of miR-203 may sustain the regenerative capacities of the whole epidermis.

miRNAs confer signalling robustness

Robustness is the capacity of biological systems to generate an invariable phenotype, even when facing genetic or environmental perturbations, and miRNAs have been proposed to contribute to robustness by several mechanisms.

Gliomagenesis

Emergence of a tumour that arises from glial cells in the brain or spinal cord.

Glomerular mesangial cell

A component of the renal glomerulus that can contract and relax and secrete inflammatory cytokines.

Basal layer

The deepest layer of pluristratified epithelia, which are attached to the basement membrane and enriched in stem or transient amplifying progenitor cells.

Suprabasal

The layer of cells in mammalian epithelia that are located just above the deepest (or basal) layer, typically generated by the asymmetric cell division of basally located stem cells.

phosphorylation of PtdIns(3,4,5)P3 (REF 58), PI3-kinase regulatory subunit-β (PIK3R2; also known as p85β) (which inhibits PI3K through many mechanisms) and Sprouty-related, ETV1 domain-containing protein 1 (SPRED1) or Sprouty (two related antagonists of RAS-mediated RAF activation). Several miRNAs have been shown to target both positive and negative regulators of these cascades (TABLE 1; Supplementary information S1 (table)). Here, we highlight two miRNAs, miR-21 and miR-126, as examples of miRNAs that amplify signals through the coordinated and coherent regulation of multiple targets.

miR-126 is the most highly enriched miRNA in endothelial cells, where it sustains VEGF signalling by targeting SPRED1 and PIK3R2 mRNAs (Fig. 2e). By inhibiting the production of natural repressors of VEGF signalling, miR-126 promotes angiogenesis and vascular integrity, suggesting that it may serve as an effective target for anti-angiogenic therapies.

miR-21 targets both PTEN and Sprouty. Thus, miR-21 serves as a general enhancer of RTK signalling, perhaps explaining its frequent upregulation in various human tumours. Interestingly, increased levels of miR-21 not only characterize cancer cells but are also present in other pathological growths. In cardiac fibrosis, miR-21 is highly expressed in proliferating cardiac fibroblasts. Fibrosis has been traditionally considered to be a secondary consequence of failing cardiomyocytes. Strikingly, however, RTK signalling attenuation in cardiac fibroblasts following silencing of miR-21 led to significant attenuation of heart disease, suggesting that fibroblasts may have a more direct and causal role in cardiac hypertrophy and dysfunction than previously thought (Fig. 2e).

In the above examples, a miRNA simultaneously targets distinct RTK signalling branches, thus acting as a general amplifier of the signal response. In other cases, miRNAs can impart specificity to the signalling flow by channelling it towards specific branches. miR-26a, for example, targets only PTEN, thus specifically amplifying AKT-driven gliomagenesis following PDGF stimulation (Fig. 2e).

miRNAs in signalling crosstalk

The regulatory capacity of miRNAs may be exploited to connect distinct signalling pathways (Fig. 3). We mentioned above the example of miR-15 and miR-16, linking WNT and TGFβ signalling. Furthermore, recent evidence suggests that there are several miRNAs that can act as mediators of signalling crosstalk. One particular example is how TGFβ signals through AKT during renal fibrosis (Fig. 3a); this link is assured by a cascade of miRNAs. Specifically, TGFβ turns on the transcription of miR-192, which inhibits the expression of zinc finger E-box-binding homeobox 2 (ZEB2). Because ZEB2 is a transcriptional repressor of miR-216a and miR-217, its inhibition allows the derepression of miR-216a and miR-217, leading to AKT activation. The ensuing AKT activation causes glomerular mesangial cell survival and hypertrophy in diabetic mouse models. Thus, antagonizing either miR-192 or miR-216a and miR-217 could offer new therapeutic potentials for diabetic nephropathy.

Similarly to embryogenesis, adult tissue regeneration and wound repair also require the strict, coordinated and dynamic control of various signalling pathways (Fig. 3b). Not surprisingly, miRNAs are emerging as key micro-managers of some of these processes. For example, the skin constantly rejuvenates during homeostasis through the self-renewing capacity of the innermost basal layer. As cells move suprabasally, they embark on a terminal differentiation programme. Integral to this switch is the downregulation of the p53-related factor p63, which is essential for epithelial stem cell maintenance. miR-203 sharpens the border between basal progenitors and their differentiating progeny because it targets residual p63 expression in suprabasal layers. Skin appendages, such as hair follicles or fins, can also regenerate through WNT stimulation. Remarkably, another conserved target of miR-203 is lymphoid enhancer-binding factor 1 (LEF1), a b-catenin DNA-binding partner. Although the role of miR-203 in hair follicle regeneration has not been investigated, miR-203 inhibits Lef1-mediated fin regeneration in zebrafish. It is therefore tempting to speculate that inhibition of miR-203 may sustain the regenerative capacities of the whole epidermis.
miRNAs as signalling balancers and buffers. In a simple scenario, miRNAs can target both an activator and an inhibitor of the same pathway. A case in point is the regulation of the Nodal pathway by miR-430 in zebrafish, which targets both the Nodal homologue Squint and its inhibitor, Lefty. In this scenario, after lowering miR-430 levels, any gain of Nodal is balanced by a corresponding reduction in Lefty, which remains highly expressed and helps to maintain the Nodal pathway in a poised state. In this context, the miRNA-sensing cell can adopt two alternative fates: state A is the default fate, in which miRNA expression dominates and the expression of the TF is turned off. In state B, an extrinsic cue (signal) stabilizes the TF, promoting cell differentiation and leading to repression of the miRNA to stabilize the fate choice (upper panel). Reciprocal inhibition between the transcription factors zinc finger E-box-binding homeobox 1 (ZEB1) and ZEB2 and the miR-200 family regulates the switch between epithelial and mesenchymal states. Transforming growth factor-β (TGFβ) signalling induces epithelial to mesenchymal transition (EMT) by stabilizing the expression of ZEB1 and ZEB2 at the expense of miR-200. MET, mesenchymal to epithelial transition.

miRNAs in signalling networks. Signalling pathways are highly interconnected, and the flow of information they carry is controlled by many feedback loops. This renders their appearance and functionality more similar to a network rather than to a linear cascade. Theoretical models predict that miRNAs are crucial elements of these loops. First, miRNAs can act as reinforcers and backups of tissue-specific transcription programmes. This defines a coherent feedback loop in which the miRNA and its target are oppositely regulated by the same signal. This suggests that a miRNA participates in signalling networks to stabilize fine tissue patterning by repressing its target mRNA in cells where it should not be expressed (that is, ‘leaky’ miRNAs). Pioneering work in the embryo of D. melanogaster provided some vivid examples of this regulation, with a miRNA and its target being detected in mutually exclusive domains of the embryo. However, the concept of coherent regulation can equally apply to less extreme situations; that is, those in which the miRNA and its target are also co-expressed. Thus, irrespective of the relative ratio between a miRNA and its target, in coherent regulation the miRNA acts in concert with patterning signals for better control over gene expression.

Although the logic of coherent loops is intuitive, this hardly exhausts the regulatory potential of miRNAs. Not only do evolutionary conserved miRNA–target pairs seem to be co-expressed, but genome-wide computational and transcriptomic analyses showed that the expression of miRNAs is more positively than negatively correlated with that of their targets. This extends the functional importance of miRNAs to incoherent network topologies, in which the miRNAs and their target are co-activated (or co-repressed) by the same signalling cues. There are two main advantages of such a design. First, it prevents undesired pathway activation from stochastic signalling noise, as only bona fide stimuli can surpass inhibition by the co-expressed miRNA. Second, it may also act homeostatically to maintain steady-state levels of the target protein from unwanted signalling fluctuations, as the miRNA would tune the translation of its target in a direction opposite to that of the signal. Of note, this ensures uniform responsiveness in equivalent groups of cells within a range of signal distribution. For example, pluripotent embryonic stem (ES) cells must tightly control Nodal activity because this pathway defines opposing self-renewal and differentiation fates in a narrow window of concentrations and close temporal succession (M.I. and S.P., unpublished observations). Precise control over Nodal availability is mediated by an incoherent regulatory circuit, in which the pluripotency factors, such as OCT4, turn on the Nodal antagonist Lefty, which remains constantly in check by the concomitant OCT4-mediated activation of the miR-290–miR-295 cluster. It is important to note that the dynamic kinetic properties of miRNAs are ideally suited to serve in loops that confer robustness, as miRNA processing is faster than protein translation, allowing miRNAs to affect gene expression with a shorter delay than transcriptional repressors. Thus, miRNAs can confer cells exquisite temporal and quantitative precision over cell signalling.
This also has implications in cancer, in which numerous oncogenic mutations hit several signal transduction elements. If miRNAs buffer the fluctuations of signal transduction elements, then loss of miRNAs should exacerbate the aberrant activity of signalling molecules. Strikingly, expression profiling experiments show a global downregulation of miRNAs in tumour samples compared with normal tissues. Similarly, genetic inhibition of miRNA biogenesis greatly accelerates Ras-induced tumorigenesis. As cancer has been effectively portrayed as a Darwinian system that is based on the competition between distinct cellular variants, one interesting possibility is that escaping miRNA control may allow the exploitation of more aggressive signalling variants, thereby accelerating cancer progression.

miRNA and targets: reciprocal regulation

Another interesting type of positive feedback module is defined by a miRNA that is negatively regulated by its own target. This double negative configuration is similar to bistable electrical circuits — or toggle switches — and can be used to convert a transient signal into a longer-lasting cellular response: once one of two alternative states is established, the signalling cue that induced the transition is no longer necessary and the status is maintained by itself. Recent studies have reported several examples of miRNAs in toggle switches (Fig. 4c).

miR-200 and EMT: cell memory and epithelial plasticity

Epithelial to mesenchymal transition (EMT) is a complex gene expression programme that is characterised by loss of cell adhesion through repression of E-cadherin and activation of genes associated with motility, invasion and stensness. As such, EMT is activated during embryonic development and adult tissue remodelling. However, in epithelium-derived tumours, EMT is usurped to foster metastasis and gain of cancer stem cell phenotypes. The miR-200 family of miRNAs plays a major part in specifying the epithelial phenotype by preventing the expression of the transcriptional repressors of E-cadherin, ZEB1 and ZEB2 (Ref. 84). In turn, the miR-200 primary transcript is repressed by ZEB1 and ZEB2 (Ref. 85), establishing a double-negative feedback loop between ZEB1 and ZEB2 and the miR-200 family. TGFβ signalling, a potent inducer of EMT, activates ZEB1 and ZEB2 (Ref. 85), which then repress miR-200 expression. Once miR-200 levels fall below a threshold, the reprogramming from an epithelial to a mesenchymal phenotype is locked in place (Fig. 4c). The embedded stability of this double-negative feedback loop represents a new form of epigenetic memory. For example, this may explain why, at least in vitro, the stem cell characteristics endowed by a transient EMT stimulation continue to be manifested in the distant descendants of a cell long after the EMT-inducing stimulus has been removed.

miR-145, pluripotency and differentiation in ES cells

miR-145 has a crucial role in the transition between stemness and differentiation through a reciprocal negative feedback loop that includes pluripotency factors. miR-145 is expressed at low levels in pluripotent human ES cells because its promoter is directly repressed by OCT4; in turn, miR-145 targets the 3’ UTR of OCT4 and those of the other pluripotency-associated genes, such as SOX2 and Krueppel-like factor 4 (KLFR4). BMP signalling promotes the differentiation of human ES cells by inhibiting OCT4 and by sustaining the expression of miR-145, thus ensuring a firm inhibition of pluripotency genes.

miR-7 in signalling networks

An intrinsic risk of double-negative feedback loops is that stochastic fluctuations can fuel loop acceleration, rapidly flipping between alternative cell decisions. However, robustness can be imbued in the system by regulating the miRNA toggle switch downstream of a coherent feed-forward loop. This type of network has been characterized in D. melanogaster eye development. In this model, miR-7 is downstream of EGF receptor signalling, a pathway that is essential for the differentiation of progenitor cells into photoreceptors. As shown in Fig. 5, miR-7 and its target Yan (also known as pokkuri) are locked in a reciprocal negative feedback loop that keeps Yan expressed in progenitor cells and miR-7 activated as cells begin differentiating into photoreceptors. EGF signalling breaks tissue homeostasis by inciting a coherent feed-forward loop: it upregulates miR-7 and promotes transient Yan degradation, relieving miR-7 from repression. This two-tiered network embodies most of the sophisticated functions of miRNAs in signalling: stable cellular responses through the reciprocal inhibitory loop and noise buffering effects, as signalling must accumulate miR-7 above a threshold to induce stable changes in gene expression. Interestingly, robustness in gene expression attained through the coherent loop downstream of EGF could be visualized only after challenging miR-7-deficient flies with environmental perturbations, such as heat shock.

Signalling regulation of miRNA processing

A major gap in our knowledge on miRNAs relates to the mechanisms of their expression. Clearly, the identification of the genetic and epigenetic elements responsible for this event is essential to dissect the role of miRNAs in signalling networks. Most miRNA genes are located at intergenic regions, suggesting that they are derived from independent transcriptional units that are regulated by RNA polymerase II or, to a lesser extent, RNA polymerase III. Other miRNAs (about 25–30%) are embedded within the introns of known coding genes and might be regulated by the promoter of their host gene. Intriguingly, chromatin immunoprecipitation studies have shown that the body of mature miRNA genomic coding regions is unusually occupied by nucleosomes. The functional importance of this repressive epigenetic mark is unclear, but it may serve as a code to assemble factors involved in processing the miRNA precursors, as suggested by the fact that cleavage of primary miRNAs occurs co-transcriptionally. Indeed, miRNA maturation, rather than expression, might be the key regulatory step in miRNA generation: during mouse embryogenesis many miRNA precursors are present at high levels but remain unprocessed, but it is still unknown how
processing by Drosha and Dicer is actually regulated. A similar scenario is observed in cancer, in which most miRNAs are effectively downregulated irrespective of their genomic organization.

Some of the connections between signalling networks and miRNA biogenesis are starting to be revealed at the molecular level. The best-understood example for the regulation of miRNA biogenesis is let-7 miRNA. The levels of mature let-7 increase during differentiation, but this is not caused by an increase in its transcription rate. Instead, LIN-28 negatively regulates the processing of let-7 by recognizing the terminal loop of the let-7 pri-miRNA and blocking its cleavage by Drosha and Dicer, respectively. Following the binding to pre-let-7, LIN-28 induces 3’ terminal uridylation and subsequent degradation of let-7 by recruiting a terminal ribonucleotransferase or poly(U) polymerase, such as terminal uridylyltransferase 4 (REF 92). It is unknown whether other RNA-binding proteins use a mechanism that is similar to that used by LIN-28 to regulate processing of specific miRNAs. Nevertheless, the evolutionary conservation of the terminal loop in many miRNAs at least suggests a widespread regulatory role of this sequence.

During DNA damage, p53 binds to the Drosha complex and promotes the post-transcriptional maturation of many miRNAs to pre-miRNA93. Among the miRNAs that depend on p53 are several putative tumour suppressors, so this could be a new mechanism by which DNA damage induces cell cycle arrest. Mutations in p53 are frequent in cancer; these disable DNA recognition but lead to the gain of metastatic properties in response to signalling cues such as those transmitted by TGFβ94. Intriguingly, mutant p53 also interferes with the formation of the Drosha processing complex, attenuating miRNA biogenesis and thus probably contributing to tumour progression95.

Another example of post-transcriptional regulation of miRNA relates to TGFβ and BMP signalling and the Smad-dependent processing of pri-miR-21 (REF 95). Smads promote a rapid increase in the expression of mature miR-21 by associating with the Drosha complex. As a consequence, miR-21 mediates the TGFβ-induced differentiation of vascular smooth muscle cells into contractile cells96. Because both p53 and Smads interact with p68 DEAD box RNA helicase (DDX5) of the Drosha complex, a housekeeping factor that is required for processing numerous miRNAs, it is unclear how transcription factors (such as Smads and p53) could control the biogenesis of a limited, and yet diverse set of miRNAs. It is possible that specificity emerges from the recognition of specific Drosha–pri-miRNA complexes; for example, it will be important to determine whether p53 or Smads directly recognize consensus sites in the RNA duplex that are similar to their cognate DNA responsive element.

Conclusions and future challenges

With the identification of a vast number of miRNAs, each one carrying a long list of putative targets, the challenge is now to understand their biological function. This challenge is further complicated by the apparent subtlety of the effects of the miRNA–target interaction on gene activity. However, in reviewing the emerging role of miRNAs in signal transduction, it becomes apparent how the highly dose-sensitive nature of developmental signalling pathways renders them prime candidates for miRNA regulation.

A future challenge will be to identify systematically all the miRNAs affecting, and regulated by, cell signalling. Although we are far from this goal, the experimental tools are definitively in place, including the capacity to screen for miRNAs that contribute to discrete signalling events using unambiguous and pathway-specific readouts in cultured cells or other model systems95,96. Importantly, we can expect that the use of new loss-of-function reagents, such as antagonomiRs and locked nucleic acids (LNA), will greatly accelerate the discovery of endogenous miRNA functions97,98.

As developmental signalling pathways are disproportionately relevant in human diseases in general, and in cancer in particular, relevant hints to decipher miRNA function will emerge from the identification of miRNAs that are consistently dysregulated in various types of tumour. This should pinpoint the miRNAs that are selected for their oncogenic function or the miRNAs that are downstream effectors of aberrantly dysregulated pathways of human cancers99. In any case, merging activity-based or expression-based screens with new RNA-based therapeutics may offer opportunities for a signalling therapy for cancer. Instead of focusing on protein-coded oncogenes, which are difficult to target therapeutically, one could focus on their target miRNAs. If these are causal for the malignant phenotype, then anti-miRNA therapeutics could represent readily available smart drugs that can potentially inhibit tumour growth or the metastatic burden of a given tumour.

Another clear indication from current studies is that miRNAs participate in signalling networks, both as backups of transcriptional control and as feed-forward or feedback devices that confer robustness to the output of cell signalling. Thus, the effect of a miRNA can
be the result of the net effect of opposing regulations or of the activities against mutually inhibiting factors. Therefore, new miRNA functions may be revealed as we refine our understanding of the networks in which they operate. As outlined in this Review, miRNAs and their targets can be wired with upstream signalling cues in such a way that perturbations in network components can be buffered or tolerated by the system. This fascinating link with biological robustness also carries with it numerous questions. If the main function of miRNAs is to serve robustness, then one might expect that the network, reciprocally, may absorb miRNA perturbations without overt or immediate consequences. In so doing, however, the network may lose its robustness and become more sensitive to genetic or environmental fluctuations. Now the challenge will be to experimentally identify these robustness loops. For example, simple inactivation of a miRNA may not suffice, but dual inhibition together with another transducer may cause the collapse of the entire signalling network, with dramatic phenotypic effects. Alternatively, miRNA functions may not be revealed under uniform laboratory conditions but may need more sophisticated experimental assays that take into account the cell’s own complexity and the cell’s interactions within tissues or organisms[13,110].

Little is known about the subcellular localization, turnover and dynamics of many of the macromolecular complexes carrying out miRNA functions; so, an emerging issue is how miRNA processing and activity cross-talk with signalling at the cell biology level. For example, a link has been established recently between RNA silencing and endosomal trafficking, with RISC assembly and turnover occurring at multivesicular bodies. This is intriguing because signalling and endocytic pathways are intimately intertwined: by regulating endosomal trafficking, signalling cues may tune RISC function[101,102]. Conversely, miRNAs might be secreted by exosomes for non-cell autonomous regulatory purposes[103].

Finally, facing the potential complexity of the miRNA-signalling network relationship, it is difficult to escape the prediction that any reductionist approach will greatly benefit from the guidance of quantitative mathematical modelling. The involvement of miRNAs in feed-forward and feedback motifs makes miRNAs ideal reagents in the hands of systems biologists to offer insights into the physical properties of signalling pathways, something that could not be reached by intuition alone. Curiously, aspects of miRNA function can already be perceived in virtue of their analogy with human engineering devices, as in the case of electronic switches. In turn, it may not be long before we might wish to look at the cellular miRNA and signalling framework to borrow new operational principles for the management of complex systems. This understanding may have applications in so far enigmatic, and necessarily holistic, aspects of tissue biology, such as regeneration, self-assembly and homeostasis and, perhaps, even on computing and engineering in general.


This paper suggests that some conserved miRNAs, such as miR-7, can enter into new genetic relationships to build developmental programmes against variation and impart robustness to diverse regulatory signalling networks.

This review discusses how miRNAs act as players in gene networks to confer robustness to developmental processes.
Wakioka, T. ′
This paper reveals a requirement for a miRNA in differentiation by targeting Smads. This paper reveals a previously unrecognized function of p55 in miRNA processing, which may underlie key aspects of cancer biology. Adorno, M. et al. Multifaceted miR‑23b complex opposes p53 to promote Tgfβ‑induced metastasis. Cell 137, 87–98 (2009).

| REFERENCES |}

NATURE REVIEWS | MOLECULAR CELL BIOLOGY | VOLUME 11 | APRIL 2010 | 263 | © 2010 Macmillan Publishers Limited. All rights reserved