Small non-coding RNAs in animal development

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Abstract | The modulation of gene expression by small non-coding RNAs is a recently discovered level of gene regulation in animals and plants. In particular, microRNAs (miRNAs) and Piwi-interacting RNAs (piRNAs) have been implicated in various aspects of animal development, such as neuronal, muscle and germline development. During the past year, an improved understanding of the biological functions of small non-coding RNAs has been fostered by the analysis of genetic deletions of individual miRNAs in mammals. These studies show that miRNAs are key regulators of animal development and are potential human disease loci.

Piwi-interacting RNAs
(piRNAs). Short RNA molecules (24–30 nt long) that are processed in a Dicer- and Drosha-independent manner. They associate with Piwi proteins and have a role in transposon silencing in flies. In mammals, they are restricted mostly to male germ cells.

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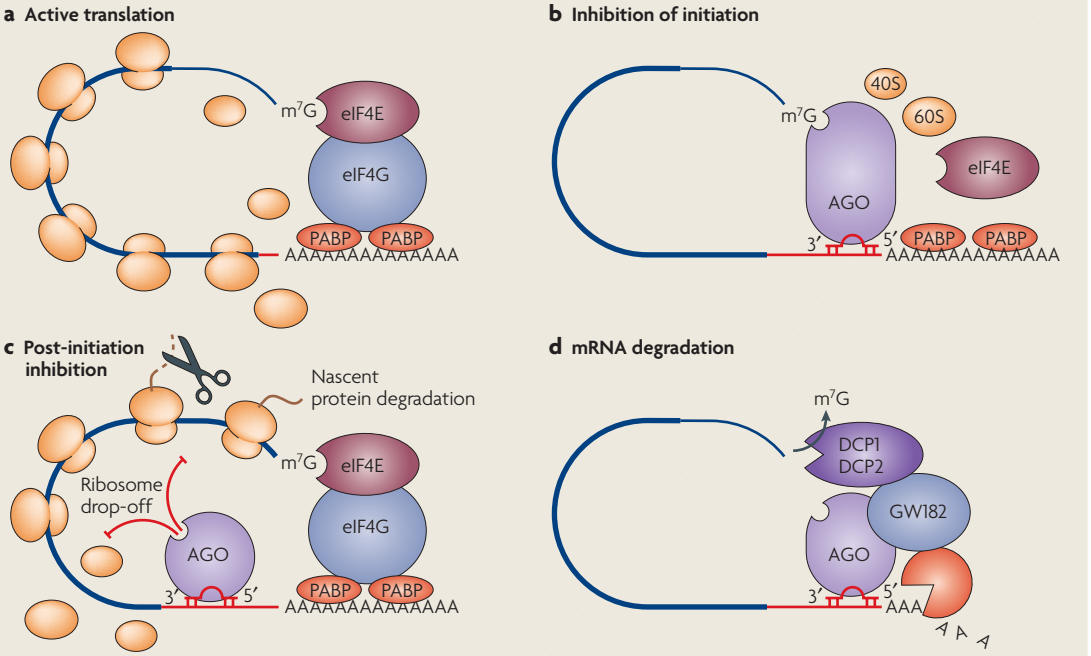
The traditional view of gene expression has relegated RNA to a somehow subsidiary role, reserving the main regulatory functions for proteins. Nonetheless, as early as 1961, Jacob and Monod proposed that RNAs could inhibit the expression of operons by base-pairing with the operator sequence[1]. The range of functions attributed to RNA has substantially expanded with the realization that RNA functions in the catalysis of crucial cellular processes, including pre-mRNA splicing and protein synthesis. In the past 15 years, the discovery of RNA-silencing phenomena that are mediated by an expanding assortment of small, non-coding RNAs has unveiled the ability of RNA to impact on an unanticipated variety of biological processes through the post-transcriptional modulation of gene expression. In particular, microRNAs (miRNAs) and Piwi-interacting RNAs (piRNAs) are implicated in various aspects of animal development (for example, neuronal, muscle and germline development). These recent advances place miRNAs and piRNAs firmly on the map of key developmental genes and point to their involvement in human diseases such as birth defects and cancer (reviewed in REF. 2).

From their humble discovery as regulators of developmental timing in *Caenorhabditis elegans*[3,4], miRNAs have emerged as important regulators of development in multiple plant and animal species. The first miRNAs described in animals, lin-4 and let-7, were identified by forward genetics as controllers of the timing of larval development in *C. elegans*: mutations of these genes resulted in the reiteration of larval cell fates and retarded the final differentiation of subsets of specialized cells[3–5]. Subsequent studies in invertebrates have shown the widespread involvement of miRNAs in various developmental processes. In *C. elegans*, miR-61 and miR-84 have been shown to modulate the expression of two orthologues of human oncogenes, *vav* and *ras*, in the context of development of the vulva[6,7]. Furthermore, lsy-6 and miR-273 are involved in a complex gene regulatory network that establishes the left–right asymmetry in the ASE chemosensory neurons (reviewed in REF. 8). In *Drosophila melanogaster*, the range of known functions of miRNAs is also wide[9,10]. The two miRNAs that are encoded by the *iab-4* locus (which are homologues of vertebrate miR-196) control expression of the *Ultrabithorax* gene and induce the homeotic transformation of halteres to wings when these miRNAs are ectopically expressed[11]. Bantam, miR-2, miR-6 and miR-14 regulate tissue growth through modulation of both apoptosis and cell proliferation[9,10,12–14]. Important signalling pathways, such as the Notch and epidermal growth factor pathways, are under the control of miRNAs[9,15,16], while the response to the steroid hormone ecdysone is modulated by miR-14 through regulated expression of the ecdysone receptor[17].

The genomic distribution of miRNAs in invertebrates, and to an even greater extent in vertebrates, is characterized by the presence of families of several identical or closely related mature miRNAs, which are sometimes encoded within the same genomic cluster (reviewed in REF. 18). A degree of functional redundancy among miRNAs is therefore to be expected, and studies of *C. elegans* strains that carry mutations of multiple miRNA-encoding loci suggest that this is indeed the case in certain instances[19,152]. However, several studies in the past year have shown that the deletion of single miRNAs can result in discernable phenotypes in mammals. Here, we review recent exciting discoveries that show that miRNAs regulate important developmental processes in vertebrates. We also include

Box 1 | **Mechanisms of miRNA-mediated gene regulation**



The mechanism of microRNA (miRNA)-mediated gene regulation, and how it affects active translation (see figure, part **a**), is a matter of controversy. In essence, the available data support two possible views: first, the translation of mRNAs is inhibited at the level of initiation, and the silenced mRNAs are occupied by few or no ribosomes[131]; second, the inhibition takes place at a step that is subsequent to initiation, and the silenced mRNAs sediment in the polyribosome fractions in a sucrose gradient. The former view has recently received support from several studies: Argonaute protein AGO2 has been shown to bind the m^7G cap of mRNAs through a domain that shares structural features with the translation initiation factor eIF4E, suggesting that when AGO2 is recruited to the 3′ UTR of a target mRNA by miRNAs, it hinders the m^7G cap recognition by the translation apparatus[132] (see figure, part **b**). Consistently, in an *in vitro* system, increased levels of the eIF4F complex (which includes the m^7G cap-binding eIF4E translation factor) reversed miRNA translational inhibition[133]. In further support of an effect on the translation initiation step, the assembly of the 48S complex (the translational complex that precedes the addition of the large ribosomal subunit to form the competent ribosome) was found to be inhibited by miR-2 *in vitro*, and eIF6, which inhibits joining of the 60S and 40S ribosomal subunits, was co-purified with the RNA-induced silencing complex (RISC)[134,135].

Nonetheless, mRNA that is inhibited by miRNAs has also been found to be associated with actively translating polysomes, suggesting that, at least in a subset of cases, miRNA does not inhibit the initiation of translation[136–139]. The post-initiation inhibition by miRNAs could result from rapid degradation of the protein product encoded by the targeted mRNA, or from a high rate of ribosome drop-off during elongation, resulting in incomplete protein products that would be rapidly degraded[138,139] (see figure, part **c**). Furthermore, the observation of translationally repressed mRNAs that co-sediment with polysomes can be explained by the formation of dense, translationally silent mRNPs ('pseudo-polysomes')[134].

A further element of uncertainty about the mechanism of action of miRNAs derives from observed variable levels of target mRNA degradation, and colocalization of the RISC component with mRNA degradation factors in the P bodies (reviewed in REF. 140; see figure, part **d**). Sequestration of mRNAs in the P bodies and degradation could be a step that follows blocking of translation, or a causative event in miRNA repression. GW182, a P-body component, has recently been shown to interact directly with AGO protein and to be recruited to the target mRNA in a let-7-dependent manner[141,142]. DCP1/2, decapping protein-1/2; eIF4G, eukaryotic initiation factor-4G; GW182, a conserved member of the GW182 protein family that is crucial for miRNA-mediated gene silencing; PABP, poly(A)-binding protein.

an overview of the roles of piRNAs, a recently discovered class of small non-coding RNA, in germline development. Given the breadth of the field, we focus our attention on the most recent literature (for excellent reviews based on the small RNA literature up to 2007, see REFS 20,21).

miRNA-mediated gene regulation

miRNAs are a class of short (19–25 nucleotide (nt)), single-stranded RNAs that are present in plants and animals[18]. Although they were previously thought to be exclusively present in multicellular organisms, miRNAs have recently been described in *Chlamydomonas reinhardtii*, a unicellular alga[22,23]. miRNAs can be encoded in independent transcription units, in polycistronic clusters or within the introns of protein-coding genes[18]. They are transcribed, mostly by RNA polymerase II, as capped and polyadenylated primary miRNAs (pri-miRNAs) that contain extended hairpin structures. Pri-miRNAs are cleaved in the nucleus by the RNase III enzyme Drosha, releasing the shorter (~65 nt long) precursor miRNA (pre-miRNA) hairpin structure (reviewed in REF. 24). Independently of Drosha, a subset of pre-miRNA hairpins can also be generated

RNase III
One of a highly conserved family of endoribonucleases that cleave double-stranded RNA and have an important role in the maturation of ribosomal RNA, among other processes.

Table 1 | **Mouse deletions of RNA-encoding and RNA-silencing genes**

Gene deleted	Phenotype	Refs
Mir-1-2	Cardiac morphogenetic defects, cardiac electrophysiological defects, fatal with variable penetrance.	52
Mir-208	Absence of cardiac hypertrophy in stress conditions, failure to upregulate βMHC in stress conditions.	82
Mir-155	Defective adaptive immunity, fibrosis and infiltration of the lung, defects in germinal centre reaction (decreased interleukin-2, interferon-γ), decreased production of immunoglobulin.	101, 102
Mir-150	Expanded lymphocyte B1 population, decreased lymphocyte B2, increased immunoglobulin production, increased c-Myb.	144
Miwi2	Male sterility, spermatogenesis arrest at early prophase meiosis I, complete loss of spermatogonia in adults.	122
Miwi	Male sterility: spermatogenesis arrest at early round spermatids stage.	120
Mili	Male sterility: spermatogenesis arrest at early prophase meiosis I.	121
Dicer-1	Lethality in early embryonic stages, depletion of multipotent stem cells, embryonic stem cells unable to differentiate, loss of epigenetic silencing of centromeric sequences.	44,45
Dicer-1 (tissue-restricted deletion)	Limb morphogenesis defects, lung development defects, incomplete embryonic myogenesis, loss of Purkinje cells in adult cerebellum, evagination of hair germs, epidermis hyperproliferation, impaired development of αβ-expressing thymocytes, reduced development of ventricular myocardium.	51, 145–149
Ago2	Lethality at mid-gestation, lethality in early embryonic stages.	150,151
Dgcr8	Lethality in early embryonic stages, embryonic stem cells unable to differentiate.	46

Ago2, Argonaute-2; βMHC, β-myosin heavy chain; c-Myb, transcription factor and proto-oncogene; *Dgcr8*, Drosha cofactor; *Mili*, Piwi-like homologue-2; *Miwi*, Piwi-like homologue-1; *Miwi2*, Piwi-like homologue-4.

from introns by the combined actions of the spliceosome and the lariat-debranching enzyme (LDBR)[25–27]. On export into the cytoplasm by exportin-5, the pre-miRNA is further processed by a second RNase III, Dicer, which excises a 19–25-nt double-stranded duplex. This short duplex is incorporated into the functional miRNA-induced silencing complex (miRISC), where the mature miRNA strand is preferentially retained. The miRISC contains miRNA, an Argonaute protein and other protein factors and is the effector complex of the miRNA pathway.

The miRISC is directed to mRNAs that are complementary to its miRNA component. miRISC inhibits the expression of mRNAs in one of two ways, depending on the degree of complementarity between miRNA and the target. If the complementarity is perfect, as is mostly the case in plants, the target mRNA is cleaved and degraded. By contrast, the complementarity between miRNAs and their targets in animals is frequently imperfect, and the mechanism leading to inhibition of mRNA expression is not well understood. Various mechanisms have been documented, including translational inhibition at the level of initiation and elongation, rapid degradation of the nascent peptide, mRNA sequestration into P bodies and mRNA degradation (reviewed in REF. 28). It is likely that features of the mRNA or of the proteins bound to it determine the method of suppression; however, the key features and methods of repression remain an intense focus of current research (BOX 1).

As noted above, animal miRNAs bind with imperfect complementarity to their targets, resulting in a variable degree of miRNA–target mismatches. As such, the search for targets of miRNAs is not straightforward in animals. Many studies have underscored the importance of high complementarity between residues 2–8 at the 5′ end of the miRNA with its target site, referred to as the seed region[29,30]. This model has recently been refined to account for the presence of secondary structures and other features of the 3′ untranslated region (UTR) sequence surrounding the target site, and for the ability of complementarity at the 3′ end of the cognate miRNA to compensate for imperfect seed matching[31–34].

The impact of miRNA-mediated biological regulation is estimated to be vast: hundreds of miRNAs have been cloned and thousands more have been predicted bioinformatically[35–38]. Furthermore, experiments *in vitro* have shown that overexpression of single miRNAs can result in decreased levels of >100 mRNAs, leading to the hypothesis that a large fraction of protein-coding genes are regulated by miRNAs[39]. Global analysis of mRNA levels relative to miRNAs shows low or undetectable levels of expression of predicted target mRNAs in tissues that express the targeting miRNA[40,41]. These observations have been interpreted as indicating that one role of miRNAs may be to function as developmental switches or, more subtly, to sharpen the borders of spatial or temporal gene-expression domains. Here they would ensure the silencing of unwanted messages resulting from leaky transcription or previous synthesis (reviewed in REFS 20,42), or allow the maintenance of target mRNA expression within an optimal range[43]. Therefore, it has been proposed that one function of miRNA-mediated control of gene expression in vertebrates may lie in conferring robustness to developmental programmes[42,20].

The global functional role of miRNAs in development can be inferred from animals that lack Dicer1 or DGCR8, a cofactor that is required for Drosha function (TABLE 1). Deletion of *Dicer1* and *Dgcr8* results in early developmental arrest in mice, accompanied by defects in the proliferation of pluripotent stem cells[44–46]. Tissue-specific deletion of *Dicer1* in mice and ablation of *dicer* in zebrafish results in a seemingly unaffected overall patterning, while the establishment, maintenance and function of subsets of cells are impaired to variable degrees[47–53].

The role of individual miRNAs in the development of mammals has only recently begun to be assessed by genetic ablation. Next, we summarize recent studies that relate the functions of individual miRNAs to the regulation of early embryonic development or to the subsequent development and function of various tissues.

miRNAs in early embryonic development

Several recent studies have revealed a substantially conserved network of intercellular signalling mechanisms that specify the site of initiation of gastrulation in vertebrates and, therefore, the embryonic axis (reviewed in REF. 54).

miRNA-induced silencing complex
(miRISC). A multicomponent gene regulatory complex that is activated by a microRNA (miRNA) associated with an Argonaute protein and that regulates gene expression, mediated by the sequence complementarity between the miRNA and the target mRNA.

Argonaute protein
One of a family of evolutionarily conserved proteins that are characterized by the presence of two homology domains (PAZ and PIWI). Argonaute proteins are essential for diverse RNA-silencing pathways.

P bodies
Cytoplasmic foci that are thought to store and degrade translationally repressed RNA.

In the *Xenopus laevis* embryo, β-catenin accumulates in the future dorsal side in response to fertilization and synergistically interacts with Vg-1 to induce the Nieuwkoop centre, which induces the Spemann's organizer, which, in turn, initiates gastrulation and induces the ectoderm to become the neural plate and neural tube. The transforming growth factor-β (TGFβ) protein Nodal has a pivotal role in the induction and patterning of mesoderm: a dorsal-to-ventral gradient of Nodal activity is required for the formation of dorsal mesoderm and Spemann's organizer[55,56].

The mechanism that translates the early β-catenin dorsal–ventral gradient into the late blastula gradient of Nodal activity is not known. A recent study shows that, at least in *X. laevis*, miRNAs have a relevant role in this process[57]. Two miRNAs, miR-15 and miR-16, inhibited Nodal signalling by reducing the expression of one of its receptors, Acvr2a. Consistently, Spemann's organizer and head structures were reduced by overexpression of miR-15 and miR-16, but were increased by blockage of these miRNAs. Furthermore, miR-15 and miR-16 are epistatic to β-catenin, as blockage of miR-15 and miR-16 restored dorsal mesoderm induction in embryos in which Wnt/β-catenin signalling was suppressed. These data, and the observation of a ventral-to-dorsal gradient of miR-15 and miR-16 that is reciprocal to the β-catenin gradient, suggest that inhibition of miR-15 and miR-16 expression is a major mechanism through which the Wnt signalling pathway promotes Nodal signalling and dorsal mesoderm patterning[57].

Evidence of a role for miRNAs in the modulation of Nodal in the early embryo is seemingly at odds with the observed absence of gross defects of embryonic axis specification in zebrafish that lack Dicer[47]. A possible explanation for this discrepancy is provided by the absence of potential complementary sites for miR-15 and miR-16 in the 3′ UTR of zebrafish Acvr2a, whereas such sites are present in mammals, which require Dicer for early embryonic development[57,44]. However, other components of the Nodal signalling pathway in zebrafish are under the control of miRNA-mediated modulation. miR-430, a highly abundant miRNA that is required for the clearance of maternal mRNAs, has recently been shown to directly decrease the expression of squint (sqt), a member of the Nodal family[58]. Interestingly, lefty, an antagonist of Nodal, is also regulated by miR-430. The simultaneous relief from miR-430-mediated regulation of both squint and lefty resulted in either a modest effect or no effect on mesoderm induction, whereas other outputs of Nodal activity (such as the number of endoderm progenitors and specialized dorsal forerunner cells) were decreased.

Therefore, it was suggested that miR-430 fine-tunes the overall activity of the Nodal signalling pathway by balancing the relative levels of agonist and antagonist. Furthermore, miR-430 was shown to confer robustness by dampening the levels of signalling molecules because overexpression of squint or lefty did not produce an appreciable phenotype in the presence of miR-430, but resulted in disruption of development when miR-430 complementary sites were mutated[58].

miRNAs in neuronal development

It has long been suggested that the nervous system, with its astonishing variety of functionally specialized cellular subtypes and vast number of synaptic contacts, requires ways to expand the information content of a limited number of protein-coding genes more than any other tissue. A well-documented means of achieving this goal is alternative splicing (reviewed in REFS 59,60). Small non-coding RNAs offer another source of complexity. The ability of miRNAs to specify and maintain neuronal cell-type identity is strikingly demonstrated by the requirement for lsy-6 and miR-273 in the establishment of left–right asymmetry in the ASE neurons in *C. elegans* (reviewed in REF. 8). The role of miRNAs in late neuronal development, neuronal functions and synaptic plasticity have been exhaustively reviewed elsewhere[61]. In addition, recent evidence points to a role for miRNAs in neuronal cell differentiation. For instance, the neuronal-tissue-specific miR-124 helps to acquire and maintain the neuronal cellular identity by directly silencing a large number of target mRNAs, and through the repression of master regulators of gene expression.

miR-124 is expressed specifically and abundantly in the mouse brain and in P19 pluripotent cells on their differentiation to neuron-like cells[62,63]. Mis-expression of miR-124 in HeLa cells inhibited the expression of >100 genes that are normally expressed at low levels in neuronal tissue, suggesting that it may contribute to neuronal differentiation[39]. One target of miR-124 is polypyrimidine tract-binding protein (PTB, also called PTBP1 or hnRNP-I), a regulator of alternative splicing that inhibits the inclusion of alternative cassette exons[53,64]. During neuronal differentiation, the switch between the expression of PTB and nPTB (neuronal PTB, also called PTBP2 or brPTB), a highly homologous neuron-specific protein encoded by a separate gene, results in widespread changes in the splicing pattern of genes that are involved in crucial neuronal functions[65]. The mutually exclusive expression of the two PTB forms is directly enforced by PTB, which alters the splicing of nPTB by repressing the inclusion of an alternative exon, resulting in a message that carries a premature stop codon[65,53]. Therefore, miR-124 indirectly activates the expression of nPTB by inhibiting PTB[53] (FIG. 1a).

Consistent with the inhibition of PTB by miR-124, a strikingly complementary pattern of expression of PTB, nPTB and miR-124 was observed in mouse embryos. PTB was expressed in areas of the developing neuronal system where non-differentiated progenitor cells are present, whereas nPTB and miR-124 were expressed in differentiated neurons. Furthermore, the distribution of the exon-including isoforms of various genes regulated by PTB and/or nPTB precisely overlapped with miR-124 expression, which is consistent with the ability of miR-124 to antagonize PTB. Finally, the pattern of expression of splicing isoforms of PTB target genes was perturbed in mice carrying a telencephalon-restricted *Dicer*-null mutation, confirming that miRNAs are involved in the regulation of splicing[53]. The opposite switch between PTB isoforms, from nPTB to PTB, is also regulated by miRNAs; during muscle development, the muscle-specific miR-133 directly inhibits the expression of nPTB in myoblasts,

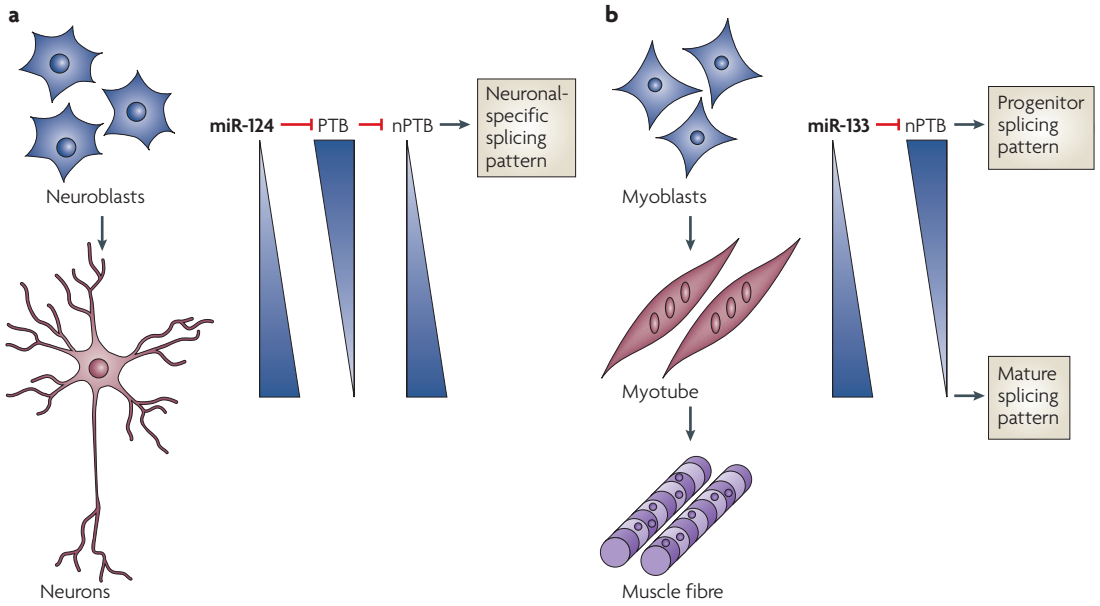


Figure 1 | **MicroRNAs in neuronal development.** Polypyrimidine tract-binding protein (PTB) and its neuron-specific homologue nPTB are regulators of gene expression at the interface between RNA silencing and splicing. **a** | In undifferentiated cells, the ubiquitous splicing regulator PTB represses the expression of nPTB by affecting the pattern of pre-mRNA splicing. As differentiation proceeds, miR-124 activates the expression of nPTB by inhibiting PTB. nPTB, in turn, shifts the alternative splicing of an array of genes to a neuron-specific pattern. Furthermore, miR-124 silences REST, a transcriptional inhibitor of neuron-specific genes that is expressed outside the neural system. **b** | As myogenesis progresses from the myoblast stage to the myotube stage, the level of the muscle-specific miR-133 increases. miR-133 inhibits the expression of nPTB, indirectly affecting the pattern of alternative splicing of several target genes.

resulting in changes in the splicing pattern of genes that are regulated by nPTB[66] (FIG. 1b). Intriguingly, these are the first described examples of an miRNA achieving a biological effect by indirectly modulating alternative splicing.

Besides modulating the PTB–nPTB switch, miR-124 affects another crucial regulator of neuron-specific gene expression, the RE1-silencing transcription factor (REST, also called NRSF). REST is a transcription factor that represses the extra-neuronal transcription of several genes, including miR-124 (REF. 67, reviewed in REF. 68). Reciprocally, miR-124 inhibits REST activity by targeting small C-terminal domain phosphatase-1 (SCP1), which is required for REST-mediated repression of neuronal genes[69]. These findings suggest the existence of a negative feedback loop: in non-neuronal cells and neuronal progenitors, the expression of neuronal-specific genes (including miR-124) is repressed by REST and SCP1; as cells progress towards neuronal differentiation and REST is transcriptionally inhibited, miR-124 ensures the fast cessation of the biological effects of REST by post-transcriptionally inhibiting the expression of its required cofactor SCP1 (REF. 69).

miRNAs in muscle development

The formation of mature muscle proceeds with the exit of myoblasts from the cell cycle, the expression of muscle-specific genes and the suppression of genes that are specific to other cell lineages and tissues. A role for miRNAs in this process was originally suggested by an enrichment of specific miRNAs in myocytes[63]. Blocking miRNA maturation specifically in the heart by deletion of Dicer led to heart failure at embryonic stages and poor development of the ventricular muscle[52]. The overall architecture of the heart chambers was grossly normal, as were molecular markers of early heart differentiation and patterning[52]. These broad observations led to the study of a particular miRNA, miR-1, for its role in controlling the development of skeletal and heart muscle.

miR-1 and the development of heart and muscle. miR-1 is highly expressed in skeletal and heart muscle across species from *D. melanogaster* to humans[63,70–72]. In mice and humans, miR-1 and its variant miR-206 are encoded by three separate loci (*MIR-1-1*, *MIR-1-2* and *MIR-206*); each of these loci co-expresses a closely linked gene, called *MIR-133* (REF. 73). Consistent with a crucial role for miR-1 in the proper establishment and maintenance of muscular and cardiac tissue, its expression is regulated by transcriptional master regulators of myogenesis: MEF (myocyte-specific enhancer-binding factor) and MYOD (myoblast determination protein-1) are required for somitic expression of *MIR-1-1* and *MIR-1-2*, respectively, whereas serum response factor (SRF) is required for cardiac expression of both[33].

In an *in vitro* model of myoblast differentiation, the expression of miR-1 was induced on growth in a differentiation medium, and coincided with the appearance of muscle-specific molecular markers[73]. Overexpression of miR-1 in myoblasts promoted differentiation while reducing cell proliferation[73]. A similar activity was observed in the developing heart, where miR-1 overexpression reduced cell proliferation, resulting in a thinner ventricular wall[33].

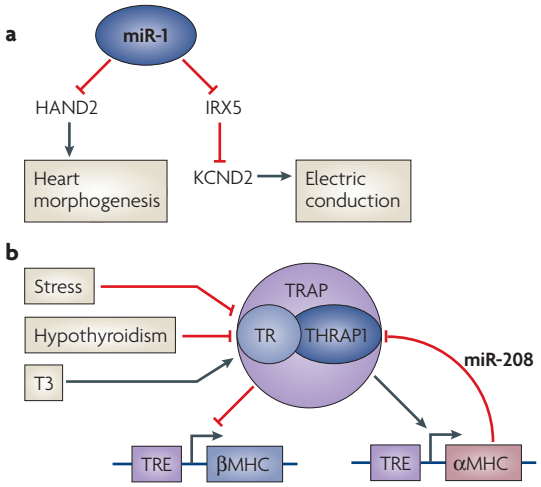


Figure 2 | **MicroRNAs in cardiac development. a** | miR-1 regulates cardiac morphogenesis by optimizing the level of the HAND2 transcription factor. Electric conduction is abnormal in mice that lack miR-1 as a consequence of de-inhibition of IRX5, a homeodomain-containing transcription factor that represses the expression of the KCND2 potassium channel. **b** | In normal conditions in wild-type animals, miR-208 maintains an optimal level of the thyroid hormone receptor (TR) cascade activity by acting on THRAP1 (thyroid hormone receptor-associated protein complex 240 kDa component) in a negative feedback loop. In transgenic mice that overexpress miR-208, inhibition of the TR pathways allows aberrant expression of β-myosin heavy chain (βMHC) in the adult. Similarly, in conditions of stress or hypothyroidism, decreased activity of the TR cascade leads to expression of βMHC and hypertrophy. In the absence of miR-208 in null mice, THRAP1 is de-repressed and baseline levels of TR activity are abnormally high and resistant to inhibition by stress signals. Therefore, *Mir-208*-null mice do not express elevated levels of βMHC or undergo cardiac hypertrophy in conditions of stress and hypothyroidism. T3, tri-iodothyronine; TRE, T3 response element.

Recent elegant work in mutant mice has demonstrated that miR-1 is not solely an early regulator of the proliferation-to-differentiation switch in muscle, but also has important roles in later cardiac functions[33,52]. Selective ablation of *Mir-1-2*, one of the two genes encoding miR-1 in the mouse genome, resulted in animals with enlarged hearts due to thickened walls. Cardiomyocytes in the *Mir-1-2* mutant mice failed to exit the cell cycle properly, resulting in hyperplasia. These defects, along with the failure of ventricular septation, led to the prenatal or early postnatal death of approximately half of the mutants. Surviving adult *Mir-1-2* mutant animals displayed a complex array of electrophysiological defects that resulted in sudden death, similar to clinical observations in humans with abnormal electrocardiograph traces[52].

miR-1 targets. Similar to all miRNAs, miR-1 could potentially regulate many genes, and putative targets have been identified through a combination of computational and experimental approaches[39]. During looping, a vital stage of heart morphogenesis, miR-1 controls the balance between proliferation and differentiation of myocardiocytes through translational inhibition of *HAND2*, which encodes a transcription factor[33] (FIG. 2a). Furthermore, several other genes that are involved in cell-cycle regulation or cardiac growth and differentiation have been suggested as possible targets of miR-1 (REF. 52).

The electrophysiological effects of miR-1 seem, at least in part, to be mediated by its control of the transcription factor IRX5, which in turn inhibits the expression of a gene that encodes a potassium channel, *KCND2* (REF. 52) (FIG. 2a). KCND2 has an important role in cardiac repolarization, which suggests a potential mechanism for the observed disturbances of cardiac conduction in *Mir-1-2* mutant mice. Interestingly, increased miR-1 levels are observed both in human patients with coronary artery disease and in animal models of heart ischaemia. Overexpression of miR-1 favoured the appearance of potentially fatal arrhythmias, whereas its blockage through chemically modified antisense oligonucleotides reduced their occurrence[74].

The importance of miR-1 function in skeletal muscle development is highlighted in the Texel sheep, a breed that has been selected over centuries for its increased muscularity. A recent study has identified a G→A transition in the 3′ UTR of a myostatin gene that negatively regulates muscle mass in these sheep[75]. This single nucleotide polymorphism optimizes a recognition site for miR-1, resulting in decreased levels of myostatin in Texel sheep and increased muscle growth[75].

Although it is clear that miR-1 activity is a vital component of muscle development and function, several aspects of miR-1-mediated regulation remain unclear. For example, the two copies of miR-1 in the mouse genome are expressed in a similar, although not identical, spatial and temporal pattern. However, they do not appear to act redundantly because ablation of *Mir-1-2* alone causes a profound phenotype[52]. Furthermore, both copies of *Mir-1* are transcribed as a primary transcript, which also contains *Mir-133*. miR-133 is detected at high levels specifically in the muscle and heart but, contrary to miR-1, overexpression of miR-133 causes increased proliferation and decreased myocyte differentiation[63,73]. The presumably coincident and simultaneous transcription of these two miRNAs with opposite effects on muscle maturation prompts the question of whether a post-transcriptional mechanism exists that balances their actions in different phases of development. In addition, targets of miR-1 and miR-133 might be differentially expressed at different phases of development. The functional interaction between miR-1 and miR-133 and the mechanism that balance their actions awaits further elucidation.

miRNAs involved in cardiac hypertrophy. The postnatal heart responds to various stress signals, such as hypertension or endocrine dysfunctions, with a hypertrophic (enlargement) response. This enlargement stems from an increase in cardiomyocyte volume, not proliferation. Although cardiomyocyte hypertrophy probably provides a functional advantage in its early phase, it is soon accompanied by deposition of fibrotic tissue and decreased contractility, and ultimately results in heart failure. The various signalling cascades that are implicated in hypertrophy

Hyperplasia
Enlargement of an organ resulting from an increased number of its cells.

activate a set of transcription factors that have early roles in cardiac development (reviewed in REF. 76). Several groups of researchers have identified a set of miRNAs that have abnormal levels of expression in mouse and rat hypertrophy models[77–81]. A subset of these miRNAs, when overexpressed, conferred the morphologic features that are typical of hypertrophy in primary cardiomyocytes. Furthermore, a single miRNA, miR-195, is sufficient to provoke heart dilative hypertrophy when it is overexpressed *in vivo* in transgenic mice. Interestingly, miR-195 expression is also elevated in failing human hearts[77].

Several miRNAs were also reduced in murine and human hypertrophic hearts, including miR-133 and miR-1 (REFS 79,80). These two miRNAs, which have a crucial role in heart development and function (see above), also affect cardiac hypertrophy[79,80]. Although miR-1 and miR-133 seem to act in opposition during skeletal muscle differentiation, they function cooperatively in the context of cardiac hypertrophy. Decreased levels of either miR-133 or miR-1 are sufficient to initiate a hypertrophic phenotype[73,79,80].

A hallmark of cardiac hypertrophy is the aberrant postnatal activation of fetal genes. For example, β-myosin heavy chain (βMHC) is aberrantly expressed during hypertrophy, at the expense of the adult form, αMHC. βMHC has lower ATPase activity than the adult form, and thus its expression results in contractile dysfunction in the adult heart. Intriguingly, the heart-specific miRNA miR-208 is encoded within an αMHC intron, which suggests the possibility of miRNA-mediated regulation. To explore this possibility *in vivo*, Van Rooij and collaborators deleted miR-208 by homologous recombination, without affecting the levels of expression of αMHC. The phenotype of untreated mutant animals was subtle, with decreased contractility and expression of fast skeletal muscle-specific genes in the heart. In experimental cardiac hypertrophy models, however, *Mir-208*-null animals failed to show heart hypertrophy and induction of βMHC, unlike wild-type animals. Reciprocally, transgenic overexpression of miR-208 was sufficient to induce robust expression of βMHC. These data hint at a role for miR-208 in setting the threshold of induction of βMHC in response to stress and hypothyroidism[82] (FIG. 2b).

Thyroid hormone receptor (TR), in combination with THRAP1 (thyroid hormone receptor-associated protein complex 240 kDa component), directly represses the expression of βMHC and promotes αMHC at the transcriptional level (reviewed in REFS 76,83). miR-208 maintains an optimal level of TR activity by negatively controlling the expression of THRAP1 in a negative feedback loop (FIG. 2b). In wild-type animals, this miR-208-mediated inhibition is not sufficient to allow expression of βMHC, but a threefold increase of miR-208 in transgenic mice resulted in robust induction of βMHC. In the absence of miR-208, the threshold of inhibition of the TR cascade is elevated beyond the ability of hyperthyroidism and stress stimuli to overcome it. Thus, a negative feedback loop has been revealed whereby the same locus that encodes αMHC also produces miR-208, which, by regulating the TR pathways, also modulates the expression of the two MHC genes and the contractility of the heart[82] (FIG. 2b).

Deep sequencing techniques
Sequencing to high coverage, where coverage (or depth) corresponds to the average number of times that a nucleotide is sequenced.

miRNAs in lymphocyte development

The haematopoietic system offers an ideal model for studies that correlate gene regulation with cell lineage specification owing to the ease of isolation and expansion *in vitro* of precursor and intermediate staged cells. In addition, there is a wealth of molecular markers that are specific for various phases of differentiation. Here, we focus our attention on recent studies that demonstrate the role of miRNAs in lymphocyte maturation. Studies that examine miRNA function in myeloid lineage development and macrophage function have been reviewed elsewhere[84,85].

miR-181. The miRNA content of the haematopoietic system has recently been surveyed using DNA microarray and deep sequencing techniques[86–88]. Characterization of the composition of the miRNA repertoire of cells at various stages of T-lymphocyte maturation showed that only a handful of miRNAs displayed significantly altered expression levels across T-lymphocyte development[88]. Nonetheless, dynamic changes in the miRNA abundance during T-lymphocyte maturation define a miRNA 'signature' that is specific for each stage[88]. In particular, miR-181 is elevated at the double positive (DP) stage, when thymocytes expressing both CD4 and CD8 undergo positive and negative selection, suggesting a role for miR-181 in this process.

miR-181 appears to increase the sensitivity of DP cells to stimulation of the T-cell receptor (TCR). TCR signalling within thymocytes must be strictly regulated because it is responsible for selecting cells that will only strongly interact with non-self ligands. During development in the thymus, the TCR on DP cells must bind to the major histocompatibility complex (MHC)–peptide complex with low affinity in order to be selected for further development in lymphocytes. The negative selection of strong binders at this stage is required to eliminate cells that could induce autoimmunity. However, once the naïve lymphocytes exit the thymus, the TCR must bind MHC–peptide complexes with high affinity for activation into a mature lymphocyte. The modulation of T-cell responsiveness is therefore crucial for the cellular outcomes at different phases of differentiation. T lymphocytes that overexpress miR-181 display a stronger activation of the TCR signalling cascade in response to low-affinity MHC–peptide complexes compared with untreated cells[89]. Blocking miR-181 in DP cells suppresses both positive and negative selection. Because wild-type DP thymocytes have a tenfold higher miR-181 abundance than their mature counterparts, it appears that miR-181 is responsible for the intrinsic modulation of cellular sensitivity to TCR activation. miR-181 sets a lower threshold of TCR cascade activation in DP cells than in mature lymphocytes by repressing the expression of several phosphatases, resulting in increased levels of activation of two TCR signalling molecules, LCK (lymphocyte cell-specific protein-tyrosine kinase) and ERK (extracellular signal-regulated kinase)[89] (FIG. 3a).

miR-155. The *BIC* gene (B-cell integration cluster) was originally identified as a site of frequent integration of avian leukosis virus, leading to B-cell lymphoma induction[90]. *BIC* encodes a ~1,700-nt polyadenylated

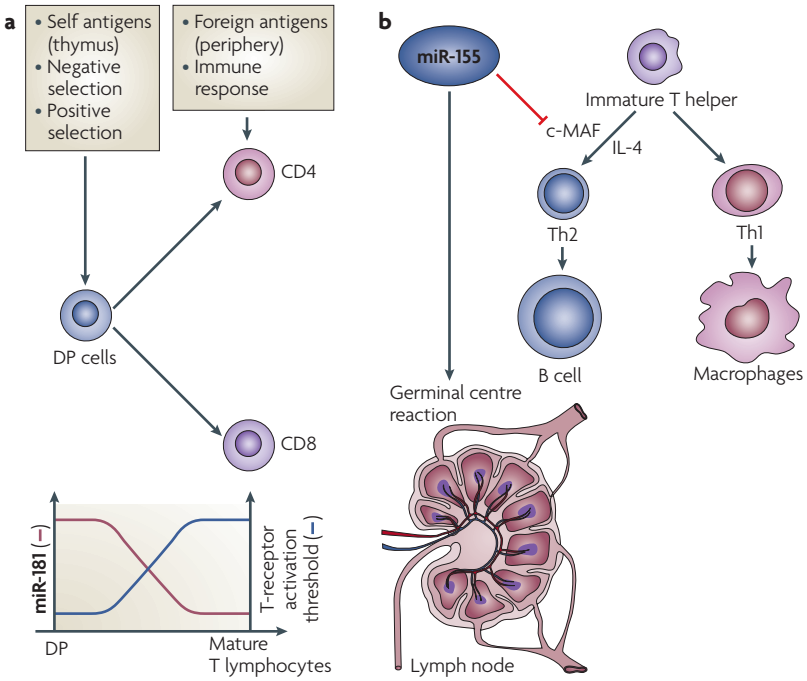


Figure 3 | **Role of miR-181 and miR-155 in lymphocyte development. a** | Higher levels of miR-181 in double positive lymphocytes (DP cells) compared with mature T cells is accompanied by the higher sensitivity of the T-cell receptor to stimulation by MHC–peptide complexes. As miR-181 levels decrease during maturation, the activation threshold of T-cell receptors increases as a result of increased levels of several phosphatases modulated by miR-181 (see graph). **b** | *Mir-155*-null mice are characterized by complex defects in homeostasis of the immune system and globally impaired immune responses. Among the defects that were characterized in detail, the loss of miR-155-mediated inhibition of the transcription factor c-MAF led to increased production of interleukin-4 (IL-4) and T helper-2 (Th2) cells. The germinal centre reaction was disrupted, resulting in impaired T cell-dependent antibody responses (see main text).

and spliced transcript that lacks a recognizable protein-coding sequence. The gene is poorly conserved except for a ~100-bp region[91]. Interest in *BIC* has recently been reignited by the finding that the conserved region encodes miR-155 (REF. 92). BIC/miR-155 expression is increased in activated B and T cells, macrophages and dendritic cells[93,94]. Elevated levels of miR-155 have also been found in Burkitt lymphoma, Hodgkin lymphoma, other B-cell lymphomas, breast carcinoma and lung carcinoma[95–100]. Furthermore, high levels of miR-155 correlated with a poor prognosis in lung cancer[100].

More recently, two groups have used genetic deletion of the *Mir-155* gene in mice to investigate its function *in vivo*[101,102]. The absence of miR-155 results in a complex alteration of the immune response, as determined by tests for B-cell, T-cell and dendritic-cell function and by a failure to achieve protective immunity against a bacterial pathogen[102]. Furthermore, *Mir-155*-null mutants showed a lung histopathology that was reminiscent of human autoimmune diseases, with diffuse fibrosis, increased collagen deposition and immune cells in the bronchioli[102]. In the context of generalized altered homeostasis of the immune system, specific abnormalities of the lymphocytes were identified. First, *Mir-155*-null mutants displayed an altered equilibrium between the two classes of helper T lymphocytes, Th1 and Th2. This balance appears to be shifted in favour of Th2 in *Mir-155*-knockout mice. This result is, at least in part, explained by loss of miR-155-mediated inhibition of c-MAF, a transcription factor that promotes the expression of interleukin-4 (IL-4), one of the major outputs of Th2 cells (FIG. 3b). Second, in *Mir-155*-null mice, B lymphocytes were decreased in the germinal centres, which are areas within lymph nodes where B lymphocytes divide, differentiate to plasma cells and start immunoglobulin production[101] (FIG. 3b). The phenotype of *Mir-155*-null mice demonstrates a complex role for miR-155 in various aspects of the adaptive immune response. Further analysis of these mutants will probably also reveal roles for miR-155 in innate immunity because miR-155 expression in macrophages has recently been described[94].

piRNAs and germline development

A large part of eukaryotic genomes is occupied by transposons and retrotransposons — repetitive sequences that have duplicated themselves many times and can move into new locations. Similar to retroviruses, retrotransposons propagate themselves by transcription into RNA from their location in the genome, followed by reverse transcription back to DNA and integration into a new genomic location. Activation of transposable elements in the germline leads to the transmission of an increased copy number to the next generation. As such, transposons are mainly active in the germline. Because active transposable elements lead to genomic instability, the impairment of genes that are responsible for transposon control often results in sterility and other abnormalities of the germline. A role for small interfering RNA (siRNA) in the control of retrotransposon mobility has previously been demonstrated in *C. elegans*[103,104]. Recent studies are beginning to elucidate how mechanisms that are based on RNA silencing have specialized to curb the activity of selfish genetic elements, specifically in the germline, by deploying a novel set of short non-coding RNAs.

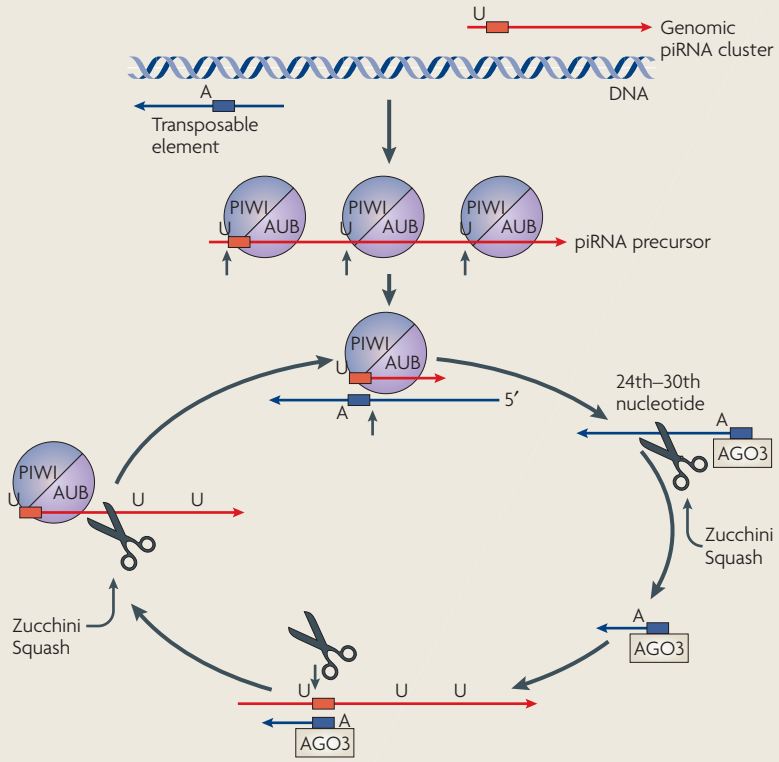
Repeat-associated RNA and germline development in flies. Forward-genetic studies provided early indications of an involvement of proteins, which were later recognized as members of the Argonaute family, in germline development[105]. The *piwi* gene (P-element induced wimpy testis) was first identified as a mutation that impairs asymmetric division in germ-line stem cells (GSCs), resulting in severe defects in spermatogenesis and female sterility[105,106]. Also, Aubergine (encoded by *aub*) was originally identified as a mutation that leads to sterility[107]. Aubergine is required for the formation of pole cells, from which primordial germ cells originate[108]. Piwi, Aubergine and Argonaute-3 (encoded by *Ago3*) are germline-specific Argonaute proteins in *D. melanogaster*. The other two members of the Argonaute family in flies, AGO1 and AGO2, which are expressed more abundantly in the soma, are involved in miRNA- and siRNA-mediated RNA-silencing pathways, respectively, which strongly suggests an involvement of small non-coding RNA-mediated pathways in the *aub* and *piwi* phenotypes. Recent studies show that this is indeed the case, and led to the discovery of an entirely new family of RNAs.

Small interfering RNA
(siRNA). Short double-stranded RNA molecules (~21–23 nt) that guide the cleavage and degradation of RNA that is complementary to one of its strands.

Germ-line stem cells
(GSCs). Cells that have the ability to self-renew and to generate differentiated cells that are restricted to the germ cell lineage.

Primordial germ cells
Embryonic cells that give rise to the germ cell lineage.

Box 2 | **Generation of piRNAs**



The peculiar features of the Piwi-interacting RNAs (piRNAs) that are connected with individual members of the Piwi family of Argonaute proteins have led to a model for the generation and amplification of piRNAs (see figure). In *Drosophila melanogaster*, most of the piRNAs that co-purify with PIWI (P-element induced wimpy testis) and AUB (Aubergine) proteins are in the antisense orientation to functional transposons, whereas the piRNAs that co-purify with AGO3 are mostly in the sense orientation; therefore, extensive complementarity exists between the AGO3-associated and AUB/PIWI-interacting pools of piRNAs[111,112]. Interestingly, the 5′ ends of complementary piRNAs that are associated with AGO3 and AUB/PIWI are separated by ten nucleotides. Consistent with the complementarity of the first ten nucleotides, most piRNAs that are associated with AUB and PIWI carry a uridine residue at their 5′ end, whereas there is a strong bias for adenosine residues at the tenth position in piRNAs from the pool that co-purifies with AGO3. These features imply that piRNAs are amplified and maintained by a mechanism that is different from the biogenesis of small interfering RNAs and microRNAs, which relies on Dicer activity.

According to the proposed model, antisense primary piRNAs, which are generated from fragments of transposons in the genome, associate with AUB and PIWI proteins and target complementary active transposons. AUB and PIWI cut the target transposon at the residue that is complementary to the tenth nucleotide of the piRNA, generating the 5′ end of a secondary piRNA. Subsequent processing — possibly involving the putative nucleases Zucchini and Squash that cleave it 24–29 nucleotides downstream[143] — generates secondary piRNAs in the sense orientation, which are bound by AGO3. The AGO3–piRNA complex can then generate new antisense piRNAs from primary transcripts that are encoded from the piRNA-generating clusters[111,112]. Thus, sites of integration of defective transposons (such as the *flamenco* locus), from which primary antisense piRNAs are generated, might serve as a genetic memory of previous invasions by parasitic genetic elements[111].

AGO3, AUB and PIWI bind a family of thousands of small non-coding RNAs, called piRNAs[109,110]. Approximately 80% of the piRNAs in *D. melanogaster* are repeat-associated small interfering RNAs (rasiRNAs), a family of RNAs with a sequence that corresponds to or is complementary to transposable elements[110–113]. piRNAs are single stranded and slightly longer than the other known small non-coding RNAs (24–29 nucleotides in *D. melanogaster*), with a phosphorylated 5′ end and a 2′-*O*-methyl (2′-*O*-me) modification at their 3′ ends, similar to siRNAs (but unlike miRNAs)[113–115]. Flies that lack a functional *pimet* gene (the homologue of *Arabidopsis thaliana* HEN1), which encodes the enzyme responsible for the 2′-*O*-methylation of piRNAs, are viable and fertile but show defects in the ability of piRNAs to repress retrotransposons[115]. In *D. melanogaster*, piRNA sequences are clustered in discrete sites that are located in areas of pericentromeric and subtelomeric heterochromatin, which are enriched in repetitive sequences that derive from transposable elements[111]. Although the biogenesis of piRNAs has not yet been entirely characterized, it requires Piwi proteins but, unlike miRNAs and siRNAs, is not affected by deletion of Dicer[109] (BOX 2). The role of piRNAs in the repression of transposable genetic elements was demonstrated by the simultaneous increase of transposons and disappearance of piRNAs in *piwi* and *aub* mutants[109]. Furthermore, previously characterized master regulators of transposon activity, such as Flamenco, coincide exactly with piRNA clusters[111].

Disruption of the rasiRNA pathway also led to defects in specification of the embryonic axis. Mutations in *aub* and two other genes involved in rasiRNA pathways — the putative helicases Armitage (*armi*) and Spindle-E (*spn-E*) — resulted in premature expression of the posterior determinant Oskar and defects in the polarization of the microtubule cytoskeleton[116]. However, loss-of-function mutations of genes encoding ATR and CHK2 kinases, which function in DNA-damage signalling, suppressed the embryonic axis defects of *armi* and *aub,* but not the defects in transposon suppression[117,118]. Furthermore, *armi* and *aub* mutants accumulated DNA breaks, as shown by increased accumulation of foci of the histone γ-H2Av[117]. These findings indicated that the rasiRNA pathway does not directly control axis specification in *D. melanogaster* embryos, which was perturbed in *armi* and *aub* mutants as a result of increased DNA damage and activation of ATR and CHK2, possibly caused by increased transposon mobilization[117].

piRNAs in vertebrate germline development. The piRNA pathway in vertebrates shows similarities to the rasiRNA pathway in *D. melanogaster*, as well as some intriguing differences. Similar to flies, mice have three genes that belong to the Piwi family, called *Mili*, *Miwi* and *Miwi2*, which are expressed exclusively in the testis at different stages of development. The zebrafish *piwi* orthologue *ziwi* is also expressed in the ovaries[119]. In the mouse, *Mili* is expressed in mitotic spermatogonia and disappears towards the end of prophase of the first meiosis, whereas *Miwi* appears during prophase of the first meiosis. As in *D. melanogaster*, null deletion of these genes caused profound defects in gametogenesis, albeit only in males. In *Miwi*- and *Mili*-null mutants, spermatogenesis was arrested during meiosis, with defects appearing earlier in *Mili* mutants, consistent with its early expression[120,121]. Meiotic defects were observed in *Miwi2* mutants, along

rasiRNA
Repeat-associated small interfering RNA that is derived from highly repetitive genomic loci. rasiRNA is involved in the establishment and maintenance of heterochromatin and transposon control.

Seminiferous tubules
Structures in the testis where spermatocytes mature.

with the disappearance of spermatogonia in adult animals, resulting in completely empty seminiferous tubules[122].

Similar to *D. melanogaster*, the estimated 2×10^5 potential vertebrate piRNAs are characteristically longer than miRNAs and siRNAs, and are encoded by a small number of genomic clusters[119,123–128]. Within each cluster, all piRNAs are encoded from the same strand, suggesting the possibility of a primary transcript that encompasses the cluster, but with no predicted hairpin structures, unlike miRNA primary transcripts. Furthermore, although the genomic location of the clusters is conserved between mammals, the sequences of the single piRNAs are not. Interestingly, important differences exist between the piRNAs that are expressed in early spermatogenesis (when Mili is expressed) and the piRNAs that are expressed after the first meiosis[129]. Mili-associated piRNAs contain many sequences that match transposable elements and these piRNAs are encoded by genomic clusters that are rich in nested, often defective, transposon sequences, similar to the master regulators of transposon activity in *D. melanogaster*[129]. Unexpectedly, only ~17% of the clusters encoding piRNAs that are expressed late in spermatogenesis are located within repeated sequences, which is less than the percentage expected by chance, given that ~40% of the mouse genome is made up of such sequences[123,124].

Consistent with a conserved role in controlling the mobilization of transposable elements, both *Mili*- and *Miwi2*-null mouse mutants show increased levels of active transposons. In contrast to the situation in *D. melanogaster*, the increased mobilization of transposable elements in *Mili* and *Miwi2* mutants was accompanied by decreased DNA methylation of the mobilized elements, suggesting the possibility of a functional relationship between Piwi and DNA methylation[122,129]. Furthermore, analysis of early piRNAs complementary to transposable elements shows an enrichment of stretches of precisely ten complementary nucleotides starting from the 5′ end; reciprocally, a significant enrichment of adenosine residues was detected in position 10, matching the uridine residue at the 5′ end of most piRNAs. These features are similar to *D. melanogaster* piRNAs and allow the hypothesis about the feed-forward loop of transposon degradation and piRNA amplification to be extended to vertebrates[129] (BOX 2).

Thus, shared features of piRNAs in *D. melanogaster* and vertebrates suggest a common mechanism of action that involves the control of transposable elements. Nonetheless, most piRNAs in mouse spermatocytes do not match transposable element sequences and their sequences are not conserved. It is therefore plausible that many piRNAs in the mouse act through entirely different mechanisms or regulate different biological functions altogether.

Concluding remarks

The targeted deletion of genes in mice has provided an invaluable strategy to understand the functional role of protein-coding genes. Null mutations in invertebrate miRNA genes resulted, in some cases, in dramatic developmental phenotypes, while in other cases, only the simultaneous deletion of more than one functionally related miRNA resulted in appreciable phenotypes[5,19,152]. Given the large number of duplications of miRNA-encoding sequences in vertebrate genomes, and the presence of large families of miRNAs that are similar in their sequence and pattern of expression, functional redundancy is to be expected to an even higher degree in mammals. By contrast, some miRNAs are likely to affect the expression of a large number of functionally related protein-encoding genes, and their absence is expected to result in profound phenotypes. The recent descriptions of mice that carry deletions of single miRNAs provide great insight into the roles of these molecules *in vivo*.

In the future, genetic deletion of single or multiple miRNA or piRNA loci is likely to become an essential aspect of the functional analysis of small non-coding RNAs, as it has been in the past two decades for protein-coding RNAs. As demonstrated by the example of miR-208 in mice, or of miR-14 in *D. melanogaster,* miRNA deletion can result in increased vulnerability to stress conditions, which might be difficult to assess under standard laboratory conditions[14,82] (reviewed in REF. 130). One specific challenge in assessing the role of miRNAs that are involved in the maintenance of homeostasis in the face of external stimuli will be to devise experimental assays that mimic aspects of the complexity of life in a natural environment.

Furthermore, the diverse phenotypes that are associated with genetic deletions of miRNAs generally derive from increased expression of their target genes. Another challenge for research in this field in the next few years will be the reliable identification of the *in vivo* mRNA targets of miRNAs. Although computational methods of miRNA target prediction have rapidly improved, experimental methods for the reliable identification of regulated mRNAs could greatly foster our understanding of miRNA function *in vivo.* In addition, these studies are likely to focus attention on small RNA genes as important loci in various aspects of human disease, including birth defects, cardiac arrhythmia, organ failure and the different forms of neoplasia.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BIC | *HAND2* | *KCND2* | *Mili* | *Miwi* | *Miwi2*
FlyBase: http://www.flybase.org
Ago3 | *aub* | *piwi*
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Burkitt lymphoma | Hodgkin lymphoma
UniProtKB: http://beta.uniprot.org/uniprot
αMHC | βMHC | DGCR8 | Dicer | Drosha | IRX5 | Nodal | nPTB | MEF | MYOD | PTB | REST | THRAP1

FURTHER INFORMATION

Frank J. Slack's homepage: http://www.yale.edu/slack

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