

# MicroRNAs in Development and Disease

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Micro ribonucleic acids (miRNAs) are small RNAs responsible for selective repression and degradation of mRNAs. Regulation by miRNAs during development facilitates the transition from one stage to the next and can control specialized characteristics of mature cells. When this regulation is disrupted, cancer can result through increases in proliferation and defects in differentiation.

## Introduction

Micro ribonucleic acids (miRNAs) are nonprotein-coding RNA molecules approximately 21–24 nucleotides in length that regulate messenger RNA (mRNA) translation and stability (Bartel, 2004; Bushati and Cohen, 2007). miRNAs may be transcribed from the genome as individual transcripts or as miRNA clusters. These primary miRNA transcripts are processed by the ribonuclease (RNase) III Droscha and its cofactor Pasha to form a hairpin of approximately 65 nucleotides known as the pre-miRNA. The hairpin is then exported to the cytoplasm and cleaved by Dicer to form the mature miRNA. Once processed, the miRNA is incorporated into the miRNA-induced silencing complex (miRISC), where the miRNA acts as a guide to target mRNAs for selective degradation or translational repression (Figure 1). Commonly, the 8 nucleotides at the 5' end of the miRNA, known as the 'seed', pair perfectly with the 3'UTR (untranslated region) of target mRNAs, followed by imprecise pairing at the 3' end (Ambros, 2004; Bartel, 2004; Bushati and Cohen, 2007). This imperfect binding causes translational repression, deadenylation and degradation of the mRNA. Exactly how miRNAs selectively target some 3'UTRs is still not well understood. The limited complementarity between miRNA–mRNA target pairs makes target prediction a continuing challenge in the field. Identification of targets will be crucial for determining the functional relevance of miRNAs in development and disease (Table 1). In this article, we will survey the role of miRNAs as developmental regulators as well as their potential to cause disease.

Development from a single cell into a multicellular organism is a complex process that requires spatial and

temporal regulation of gene expression. At the cellular level, this progression may be thought of as a stepwise process in which miRNAs have a potential role at each stage. Following fertilization, the totipotent cells of the early embryo can generate any cell in the adult organism. Early in development, cells are separated into either somatic cells or stem cells. These somatic cells then form particular cell lineages, for instance neuronal or muscle progenitor cells. Progenitors further develop into specialized cell types that work together to generate all tissues of the organism. The mature cells must be able to maintain their unique properties and regulate them as a part of the overall physiology of the organism (Figure 2). Progression through development requires the activation of new genes and the specific degradation of products from previous developmental stages, but exactly how this happens remains unclear. Evidence for the role of miRNAs indicates that these molecules play an important role in modulating the transition from one stage to the next as well as maintaining mature cells.

## Germline Stem Cell Maintenance and Differentiation

Stem cells have the ability to proliferate to generate more stem cells, while also being able to differentiate into specific cell types. Because of this potential, it is crucial that decisions involving maintenance and cell fate specification be highly regulated. Several lines of evidence indicate that miRNAs are involved in stem cell maintenance and differentiation. Differentiation of stem cells requires the down-regulation of factors responsible for maintaining the stem cell potential. In the early stages of development, cells operate by using maternally provided mRNA and proteins that may also reinforce preservation of the stem cell nature. However, during the maternal-to-zygotic transition, maternal products degrade and transcription of the zygotic genome begins. In zebrafish, miR-430 accelerates the degradation of maternally provided mRNA as embryonic cells progress towards specific cell lineages (Schier and Giraldez, 2006). This clearance may be ridding the cell of stem

Advanced article

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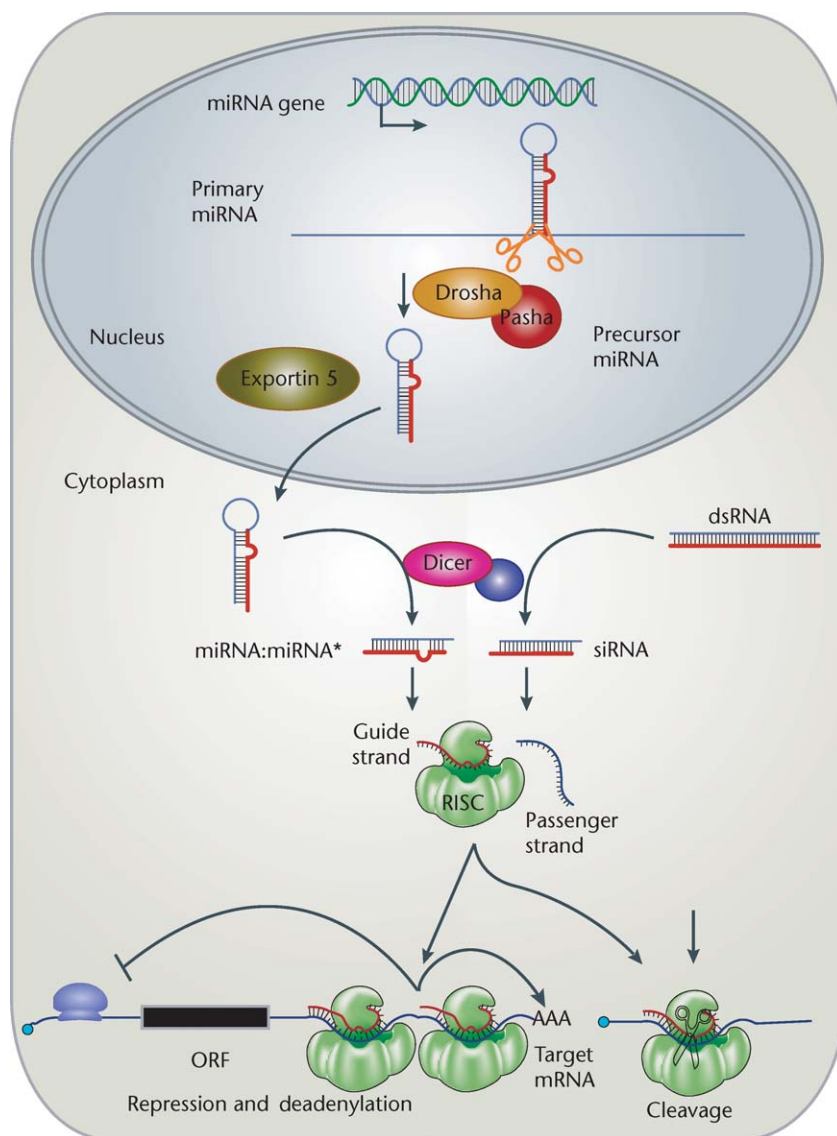
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**Figure 1** miRNA biogenesis and activity. miRNA precursors are processed by Drosha and Dicer to form mature miRNAs. After loading into miRNA-induced silencing complex (miRISC), the miRNA acts to guide the complex to target mRNAs. Double-stranded RNA can also be processed by a Dicer complex to generate small interfering RNAs (siRNAs) that are complementary to their target and mediate mRNA cleavage and degradation (Kosik, 2006; He and Hannon, 2004).

cell-associated factors as well as creating a clean slate for zygotic expression and further development.

As somatic cells differentiate, it is important that some cells, such as those in the germline, retain their stem cell identity. NANOS is a highly conserved protein that is important for germline development. *Nanos* mRNA is maternally provided and is selectively depleted from somatic cells. In zebrafish, *nanos* is repressed by miR-430 in somatic cells, but is selectively protected from repression in germ cells by the RNA-binding protein dead end (Mishima *et al.*, 2006; Kedde *et al.*, 2007). In this way, cell-specific expression of *nanos* is achieved, allowing germ cells to maintain their stem cell identity (Schier and Giraldez, 2006). As the differential expression of *nanos* seems to begin even before

miRNA targeting, this mechanism must be acting in parallel to others, and provides an additional level of regulation. It is also interesting that mRNAs may exhibit differential susceptibility to miRNA-mediated repression. This suggests that cell type-specific factors affect miRNA activity by protecting particular mRNAs to reinforce differences between somatic and germ stem cells.

Germ stem cells are maintained in a niche, where signalling controls cell division to permit production of daughter cells that differentiate while maintaining the stem cell node. Loss of *dicer* in *Drosophila* germ stem cells reduces the number of egg chambers in the ovary, suggesting that the miRNA pathway is required for germline stem cell division in flies (Shcherbata *et al.*, 2006). In these mutants,

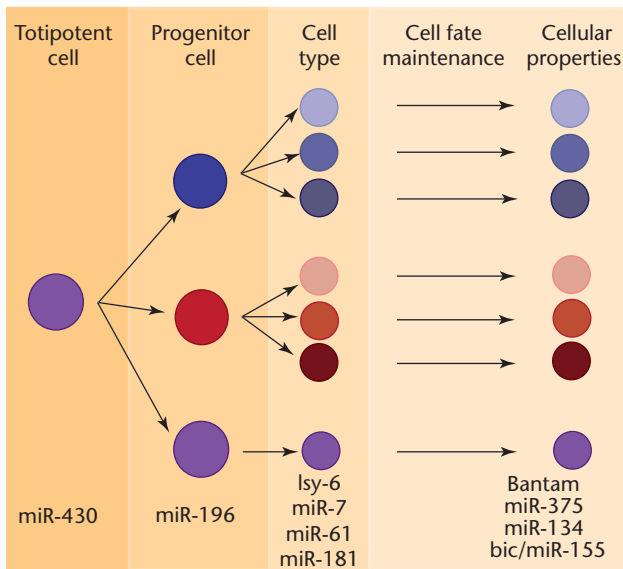
**Table 1** Many known miRNA targets play crucial roles in development and disease

Organism	miRNA	Targets	Function	References
<i>Caenorhabditis elegans</i>	let-7 family	lin-41	Developmental transition	Slack <i>et al.</i> (2000)
		hbl-1	Temporal patterning	Abrahante <i>et al.</i> (2003)
		daf-12	Temporal patterning, aging	Lin <i>et al.</i> (2007)
	lin-4	pha-4	Organogenesis	Grosshans <i>et al.</i> (2005)
		let-60	Proliferation	Johnson <i>et al.</i> (2005)
		lin-14	L1 larval transition	Lee <i>et al.</i> (1993); Wightman <i>et al.</i> (1993)
lsy-6	cog-1	Neuronal cell fate	Johnston and Hobert (2003)	
miR-61	vav-1	Vulval cell fate, Notch signalling	Yoo and Greenwald (2005)	
	miR-273	die-1	Neuronal cell fate	Chang <i>et al.</i> (2004)
<i>Drosophila</i>	bantam	hid	Tissue growth, apoptosis	Brennecke <i>et al.</i> (2003)
	miR-1	Delta	Muscle development and maintenance	Kwon <i>et al.</i> (2005)
	miR-7	Yan	Eye differentiation, EGFR signalling	Li and Carthew (2005)
		HLHm3	Neuronal differentiation and Notch signalling	Stark <i>et al.</i> (2003)
		m4		
	miR-8	Hairy	Neuronal maintenance	Karres <i>et al.</i> (2007)
miR-14	Atrophin	Tissue development, maintenance	Varghese and Cohen (2007)	
	miR-278	EcR	Insulin response, metabolism	Teleman and Cohen (2006)
Zebrafish and <i>Xenopus</i>	miR-1	HDAC4	Myogenesis	Chen <i>et al.</i> (2006)
	miR-10	HoxB1a	Neural development	Woltering and Durston (2008)
	miR-15	HoxB3a		
		Acvr2a	Embryonic development, nodal signalling	Martello <i>et al.</i> (2007)
	miR-16	Acvr2a	Embryonic development, nodal signalling	Martello <i>et al.</i> (2007)
	mir-133	SRF	Myoblast proliferation	Chen <i>et al.</i> (2006)
	miR-214	su(fu)	Muscle development, hedgehog signalling	Flynt <i>et al.</i> (2007)
	miR-200 family	zfhx1	Olfactory neuron differentiation	Choi <i>et al.</i> (2008)
	miR-430	lfng		
		GSTM1, SMARCA2	Unknown	Giraldez <i>et al.</i> (2006)
nanos, tdrd7		Germ cell development	Mishima <i>et al.</i> (2006)	
	Squint	Embryonic development and nodal signalling	Choi <i>et al.</i> (2007)	
	Lefty			
Mouse	miR-1	Irx5	Cardiac differentiation and maintenance	Zhao <i>et al.</i> (2007)
	miR-124	hand2	Proliferation	Zhao <i>et al.</i> (2005)
		PTBP1	Neuronal differentiation	Makeyev <i>et al.</i> (2007)
	miR-124a2	SCPI		Visvanathan <i>et al.</i> (2007)
	FoxA2	Pancreatic beta cell differentiation	Baroukh <i>et al.</i> (2007)	

(Continued)

Table 1 Continued

Organism	miRNA	Targets	Function	References
	miR-133	PTB	Muscle differentiation, splicing	Boutz <i>et al.</i> (2007)
	miR-134	nanog, LRH1	Stem cell differentiation	Tay <i>et al.</i> (2008)
	miR-140	HDAC4	Bone development	Tuddenham <i>et al.</i> (2006)
	miR-181	HoxA11	Muscle differentiation	Naguibneva <i>et al.</i> (2006)
	miR-196	Hoxb8	Embryonic development	Yekta <i>et al.</i> (2004)
	miR-206	connexin43	Muscle development	Anderson <i>et al.</i> (2006)
	miR-208	THRAP1	Cardiac growth and function	van Rooij <i>et al.</i> (2007)
Rat	miR-1	KCNJ2 GJA1 (connexin 43)	Cardiac function	Yang <i>et al.</i> (2007)
	miR-134	limk	Neuronal cell properties	Schratt <i>et al.</i> (2006)
Human	let-7	Ras HMGA2 TRIM71	Proliferation Proliferation Embryonic development	Johnson <i>et al.</i> (2005) Park <i>et al.</i> (2007) Lin <i>et al.</i> (2007)
	miR-1	HSP60, HSP70	Apoptosis	Xu <i>et al.</i> (2007)
	miR-9	Onecut2	Insulin secretion	Plaisance <i>et al.</i> (2006)
	miR-10b	p21	Proliferation	Ivanovska <i>et al.</i> (2008)
	miR-15a	Bcl-2	Apoptosis	Cimmino <i>et al.</i> (2005)
	miR-16-1	Bcl-2	Apoptosis	Cimmino <i>et al.</i> (2005)
	miR-17-92	E2F2, E2F3 E2F1	Proliferation	Sylvestre <i>et al.</i> (2007) O'Donnell <i>et al.</i> (2005)
	miR-21	TPM1 PTEN PDCD4	Tumour suppressor Tumour suppressor Tumour suppressor	Zhu <i>et al.</i> (2007) Meng <i>et al.</i> (2007) Frankel <i>et al.</i> (2008)
	miR-23	Hes-1	Neuronal differentiation	Kawasaki and Taira (2003)
	miR-29	Mcl-1	Apoptosis	Mott <i>et al.</i> (2007)
	miR-122	Cat-1	Liver properties	Jopling <i>et al.</i> (2005)
	miR-124	laminin gamma1 integrin beta1	Neuronal differentiation	Cao <i>et al.</i> (2007)
	miR-125	lin-28	Neuronal differentiation	Wu and Belasco (2005)
	miR-133	Caspase 9	Apoptosis	Xu <i>et al.</i> (2007)
	miR-146	TRAF6, IRAK1	Immune system response (NF-KB, AP-1 signalling)	Taganov <i>et al.</i> (2006)
	miR-150	c-myb	Lymphocyte differentiation	Xiao <i>et al.</i> (2007)
	miR-155	TP53INP1 pu.1	Apoptosis Lymphocyte differentiation	Gironella <i>et al.</i> (2007) Vigorito <i>et al.</i> (2007)
		AT(1)R	Cardiovascular cell properties	Martin <i>et al.</i> (2006)
	miR-221	kit p27(kip1)	Promote erythropoiesis Proliferation	Felli <i>et al.</i> (2005) Galardi <i>et al.</i> (2007)
	miR-222	kit p27(kip1)	Promote erythropoiesis Proliferation	Felli <i>et al.</i> (2005) Galardi <i>et al.</i> (2007)
	miR-375	Mtpn	Insulin secretion	Poy <i>et al.</i> (2004)



**Figure 2** Stepwise development of a mature cell from a stem cell. Progression towards a mature cell involves a number of intermediate stages. The cells that make up the early embryo are capable of giving rise to all cells in the organism. These totipotent cells divide and differentiate into different lineages, such as muscle or neuron, which in turn further develop to form the functional cell types present in the adult organism. The unique cellular properties of these cells must then be maintained throughout its lifetime. miRNAs act at each level to ensure controlled expression of stage-specific factors and to facilitate transitions from one stage to the next.

upregulation of a cell cycle inhibitor slows the rate of transition through the cell cycle. The tight control of cell division by the miRNA pathway appears to be important for maintaining a pool of germ stem cells while permitting differentiation.

## Cell Lineage Specification

After initial proliferation and patterning begins, it appears that miRNAs are not essential for somatic cells to differentiate into major cell lineages such as muscle, epithelia, neurons or blood. Studies in zebrafish indicate that the loss of all miRNAs does not impair the specification of cell lineages (Schier and Giraldez, 2006). In mice, conditional *dicer* knockouts in the limbs, skin and lung epithelia caused major defects in morphogenesis and cell death but all major cell lineages were still present (Andl *et al.*, 2006; Kloosterman and Plasterk, 2006). Thus, the regulation of gene expression at this stage appears to be largely transcriptional, while miRNAs may play a supplementary role to buffer against the inappropriate expression of gene products or maintain tissue homeostasis after differentiation.

## Cell Fate Determination

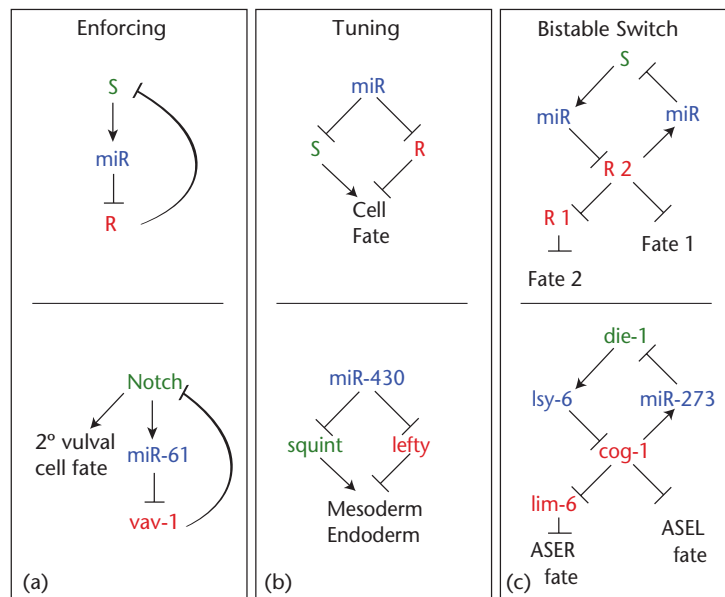
Once a major lineage is specified, progenitor cells differentiate into distinct cell types. Though it appears that

miRNAs play a role as modulators, a number of studies indicate a fundamental role as switches in terminal differentiation as well. In these instances, the miRNAs and their targets are part of a signalling cascade. This provides a way for cells to cause rapid inactivation of a developmentally important pathway and mediate large changes in gene expression by regulation of key transcription factors.

The first miRNAs identified have important functions as switches of gene expression. In *Caenorhabditis elegans*, the specification of many cell types is temporally regulated by the *lin-4* and *let-7* family miRNAs. These small RNAs were isolated from genetic screens for mutations altering developmental timing. *C. elegans* larvae pass through four developmental stages, L1–L4, before reaching adulthood. Loss of *lin-4*, the first miRNA identified, causes the L1 stage to be repeated later in development for specific cell types. *Let-7* is known to target transcription factors to mediate the transition from L4 to adult. Additionally, miRNAs in the *let-7* family are involved in the L2–L3 transition (Alvarez-Garcia and Miska, 2005). Although *let-7* has roles in other processes, as described later, it is not clear how heterochronic miRNAs might be acting in other organisms. One interesting possibility is that they are regulating transcription factors. In this way, by directly targeting a small set of transcripts, these miRNAs would in fact affect the expression of a much larger set of genes, thus having a broad impact on gene expression.

miRNAs can function in feedback loops in order to amplify or buffer specific signalling pathways. A positive feedback loop involving Notch signalling helps determine the fate of cells that form the vulva in *C. elegans* (Figure 3). In these cells, LIN-12/NOTCH activates miR-61, which targets and represses the NOTCH repressor, reinforcing expression of both NOTCH and the miRNA (Yoo and Greenwald, 2005; Kloosterman and Plasterk, 2006). Conversely, in the *Drosophila* eye, a feedback loop negatively regulates YAN, a factor involved in maintenance of the progenitor cell state. EGFR signalling upregulates expression of miR-7, which then represses YAN expression, allowing differentiation into photoreceptor cells (Li and Carthew, 2005; Kloosterman and Plasterk, 2006). Interestingly, YAN represses miR-7 expression. In *C. elegans* bilateral taste receptor neurons, asymmetric gene expression of chemosensory genes requires the presence of *lsy-6* miRNA in left neurons and miR-273 in right neurons. These examples illustrate that expression levels of both proteins and miRNAs are carefully balanced so that in response to a threshold level of signalling, the cell can respond quickly.

In some cases, the decision being made is more complex than when to begin differentiation. For instance, a variety of mature cells are derived from haematopoietic precursors. miR-181 is found in progenitors and is strongly expressed in B cells (Chen *et al.*, 2004; Ambros, 2004). Overexpression of miR-181 causes a greater proportion of progenitor cells to form B cells, though the mechanism behind this process is not yet known. Thus, the abundance of an miRNA in a particular cell type can reinforce the decision to form that cell type, potentially through the



**Figure 3** miRNAs in signalling cascades. miRNAs in signalling cascades can affect activity of the pathway in a number of ways. (a) An initial signal (S) may reinforce its own activity by activating an miRNA that downregulates a repressor (R) of the pathway. An example of this positive feedback loop is Notch signalling in *C. elegans* vulval precursor cells, as shown in the lower panel of (a). (b) Signalling cascades must be strictly regulated. An miRNA may finely tune expression of downstream signalling molecules through inhibition of a repressor of miRNA expression. For instance, regulation by miR-430 of *squint* and *lefty*, genes that are involved in TGF $\beta$  signaling, dampens and balances the input to the pathway to regulate the extent of mesoderm and endoderm differentiation. (c) Expression of several miRNAs within a pathway can act as a bistable switch to mediate the decision between cell fates. In the case of *C. elegans* chemosensory neurons, *die-1* signalling induces *lsy-6* expression, which then represses *cog-1*, allowing the 'left' cell fate (ASEL) to be established. Alternatively, expression of miR-273 in neurons inhibits *die-1* signalling so that *lim-6* is repressed and the 'right' cell fate (ASER) occurs.

repression of transcripts specific to other types of cells in the lineage.

## miRNA-mediated Control of Cellular Properties

Expression of miRNAs continues after cellular identities have been established, suggesting that miRNAs have roles in the regulation of special properties and maintenance of mature cells. miR-1 is expressed in the developing muscle of the fly, but it also appears to be essential for the maintenance of these cells (Kloosterman and Plasterk, 2006). Fly larvae lacking miR-1 establish muscle early in development but die during growth (Sokol and Ambros, 2005). It has been speculated that another role of miRNAs may be to prevent expression of transcripts that do not belong in that cell type. Studies in cell culture have shown that when miR-1 is overexpressed in HeLa cells, many of its targets are nonmuscle transcripts (Lim *et al.*, 2005; Wiemer, 2007). Conversely, genes coexpressed with the miRNA tend to lack miRNA target sites in their 3' UTRs (Stark *et al.*, 2005; Farh *et al.*, 2005). This suggests that one of the functions of miRNAs could be to prevent accidental expression of 'noisy' genes that are not supposed to be present in that cell type. It would be interesting to further study this phenomenon *in vivo* and to determine if other tissue-specific miRNAs might function similarly to stabilize the

type-specific properties of a given cell after they are established during development.

miRNAs are also potential regulators of the properties of highly specialized cells. These properties may include cell shape, size, adhesion and secretion. In the pancreatic islet cells, insulin is released from vesicles in response to glucose in the blood stream. miR-375 suppresses glucose-induced insulin secretion by targeting proteins involved in exocytosis (Poy *et al.*, 2004; Kloosterman and Plasterk, 2006). In the nervous system, dendritic spines form the receiving end of neuronal synapses. The volume of the dendritic spine has been tied to the signalling potential at the synapse. Neuron-specific miR-134 regulates dendritic spine volume by repressing a regulator of actin filament formation (Schratt *et al.*, 2006; Kloosterman and Plasterk, 2006). These examples further support the idea of miRNAs as a mechanism for modulating cellular properties postdifferentiation to allow the balance between normal physiological functioning and adaptation to changing conditions.

## miRNAs and Cancer

Proliferation and apoptosis are cellular processes that must be tightly regulated to maintain tissue homeostasis. When improperly regulated, these processes often result in development of cancer. The cell cycle is strictly controlled to ensure that cells only divide at the appropriate time. Several examples indicate that miRNAs play an important role in

cell cycle regulation. *MYC* is a well-characterized oncogene, meaning that its overexpression predisposes cells for becoming cancerous. Its role as a regulator of both cell cycle and apoptosis makes its overexpression an important step in the formation of many cancers. The miR-17 cluster of miRNAs is upregulated when *MYC* is overexpressed. It is possible that members of this miRNA cluster downregulate tumour suppressor genes, such that upon increased expression, the tumour suppressor levels are no longer adequate. The transcription factor E2F1, a regulator of cell cycle progression, has been identified as a likely target of miR-17-5p and miR-20a, two members of this cluster. Interestingly, *MYC* is known to induce E2F1 expression (Esquela-Kerscher and Slack, 2006). This suggests further complexity in the regulation of E2F1 abundance, with *MYC* inducing both E2F1 and miR-17 cluster transcription, followed by the fine-tuning of E2F1 levels by the miRNAs. Altered expression levels of either of these mediators of regulation could result in changes in cell cycle progression.

miRNAs also negatively regulate proliferation. RAS family proteins are small guanidine triphosphatases (GTPases) responsible for triggering signal cascades involved in a number of cellular processes including proliferation, differentiation and cancer. Let-7 in humans represses a number of genes involved in cell proliferation, including *RAS*. An examination of lung cancer samples showed that in comparison to normal tissue, cancerous cells expressed lower levels of let-7 and higher levels of RAS (Esquela-Kerscher and Slack, 2006). Let-7 also represses the chromatin-associated protein *HMGA2* (Mayr *et al.*, 2007). This protein alters chromatin structure to permit proliferation and is found in undifferentiated cells as well as in a number of types of cancerous cells. Because let-7 seems to act as a tumour suppressor by keeping proliferation in check, mutations altering either the expression of let-7 or its targeting ability could act as predisposing factors for cancer. Transient expression of let-7 in a cell culture system inhibits cell proliferation. This suggests that if let-7 could be delivered to cancer cells, it may be able to slow tumour growth. Additionally, low levels of let-7 in cancer cells are associated with a poor prognosis (Esquela-Kerscher and Slack, 2006), suggesting that let-7 expression could become a useful diagnostic biomarker in the future.

Cell number is also controlled by cell death. Again, some miRNAs have been identified as inducers of cell death, whereas others suppress it. A mutation in *bantam* was identified in flies that had larger than normal body size. This miRNA inhibits apoptosis while increasing proliferation (Alvarez-Garcia and Miska, 2005). A mammalian homologue of *bantam* has not been found, though it is possible that similarity could be independent of sequence, and other miRNAs may have evolved in vertebrates to have a similar function. miR-15a and miR-16-1 act as tumour suppressors and hinder cancer formation by targeting the antiapoptotic protein BCL-2 and facilitating apoptosis. (Wiemer, 2007). Their downregulation or misexpression is thought to contribute to leukaemias and lymphomas. Additionally, lower levels of BCL-2 are

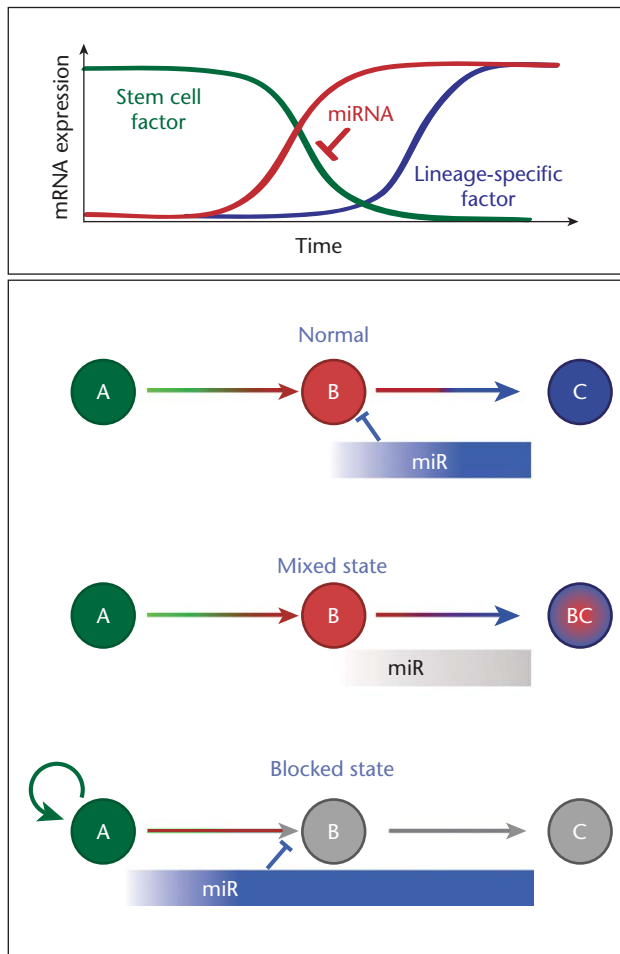
caused by blocking miR-21, resulting in increased apoptosis (Wiemer, 2007). miR-21 is upregulated in glioblastomas, a common and aggressive form of brain cancer. Yet, the connection between miR-21 and BCL-2 remains unclear, as a mechanism for increased miRNA expression causing increased protein levels has not yet been identified. Identification of miR-21 targets may reveal a repressor of BCL-2 and provide new insights into the mechanisms governing tumour progression.

## Conclusion

miRNAs facilitate developmental transitions both by repressing factors important for the previous developmental stage and by degrading factors that inhibit the new stage. At the cellular level, development occurs as cells progress from stem cell to lineage-specific progenitor to specific cell types. miRNAs may play important roles in this process through two mechanisms. miRNA-mediated repression may act as a master switch that affects a number of downstream changes. Alternatively, miRNAs may act to tune the expression levels of key developmental regulators. There are examples for each of these types of control, demonstrating that miRNAs can act in either way, depending on the biological context in which they are expressed. When placed within a signalling pathway, an miRNA can act as a switch. However, miRNAs can also be placed in an auxiliary position in which their job is to dispose of unnecessary transcripts or prevent fluctuations in expression. Current evidence indicates that switch-mediated effects occur more commonly in cell type determination, while other stages employ miRNAs mainly as modulators.

The role of miRNAs in development also makes them excellent candidates for the mediation of disease states. Misexpression of miRNAs that mediate transitions has the potential to act in tumourigenesis. For instance, recent evidence indicates that some cancers have a pool of stem cells that proliferate and are responsible for tumour growth. If an miRNA that degrades a differentiation factor is prematurely expressed, it could prevent the cell from progressing beyond the stem cell stage, a feature observed in many cancers. Alternatively, the failure of an miRNA to degrade its targets at the proper time could lead to a hybrid of a progenitor and mature cell (Figure 4). This incomplete differentiation could have profound effects on the properties of the mature cell.

The maintenance of normal cellular properties involves pathways that control stem cell maintenance, cell number and differentiation, the same characteristics that go awry in cancerous cells. Therefore, many of the miRNAs that modulate these properties have the potential to also function in cancer development. For instance, it is possible that the role of the miRNA pathway in germline stem cell division could explain the unrestricted proliferation and tumour formation if certain elements of the pathway are overexpressed. As the roles of miRNAs in controlling these disease-related processes become clearer, miRNAs are



**Figure 4** The importance of miRNAs in normal development. miRNAs aid the transition from one developmental stage to the following by repressing transcripts important to the earlier stage. As shown in this model (top), mRNAs for stem cell-specific factors (green) are rapidly degraded upon miRNA expression (red). This clearance creates a clean slate for expression of lineage-specific factors (blue). As mediators of transitions, both miRNA expression level and timing must be carefully regulated. In this model of a differentiating cell, a cell progresses from state A to B to C. In a normal state, the B–C transition occurs as an miRNA is activated and represses B state-specific transcripts to sharpen and accelerate progression to the mature C state (top). If the miRNA is not expressed, B transcripts will accumulate, resulting in a mixed B/C cell state (middle). This cell, with properties of both the progenitor and mature cell, will likely be defective in performing its role in the adult organism. In a blocked state, premature miRNA expression results in downregulation of transcripts important for establishing the B state, and the cell remains in the A state (bottom). By inhibiting differentiation, the stem cell state is maintained. This model supports a common theory in cancer biology in which proliferation of the tumour is supported by a core of cancer stem cells (Choi *et al.*, 2007).

likely to become an important element of preventative or therapeutic technologies in human disease.

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