“Myc’ed Messages”: Myc Induces Transcription of E2F1 while Inhibiting Its Translation via a microRNA Polycistron

Hilary A. Coller*, Joshua J. Forman, Aster Legesse-Miller

ABSTRACT

The recent revelation that there are small, noncoding RNAs that regulate the expression of many other genes has led to an exciting, emerging body of literature defining the biological role for these molecules within signaling networks. In a flurry of recent papers, a microRNA polycistron induced by the oncogenic transcription factor c-myc has been found to be involved in an unusually structured network of interactions. This network includes the seemingly paradoxical transcriptional induction and translational inhibition of the same molecule, the E2F1 transcription factor. This microRNA cluster has been implicated in inhibiting proliferation, as well as inhibiting apoptosis, and promoting angiogenesis. Consistent with its seemingly paradoxical functions, the region of the genome in which it is encoded is deleted in some tumors and overexpressed in others. We consider the possibility that members of this polycistronic microRNA cluster help cells to integrate signals from the environment and decide whether a signal should be interpreted as proliferative or apoptotic.

Introduction

microRNAs are 21–23-nucleotide noncoding RNAs processed from double-stranded hairpin precursors present in a wide range of organisms including worms, plants, flies, and mammals [1,2]. microRNAs are loaded into the RNA-induced silencing complex and subsequently hybridize to complementary sequences in target mRNAs. This results in inhibition of mRNA translation or reduced message stability [3,4]. Microarray analyses suggest that individual microRNAs can regulate hundreds of genes [5]. This finding has raised the interesting possibility that microRNAs can coordinate complex cellular responses. One emerging model of the role of microRNAs is to maintain the robustness of genetic networks by ensuring that genes that ought to be “off” are downregulated not only via decreased transcription but also by translational inhibition (Text Box 1) [6,7]. Recently, however, a microRNA cluster was found to be involved in a complex network structured like a feed forward loop (described further in Text Box 2). This network appears to play a central role in controlling proliferation, apoptosis and tumorigenesis.

The miR-17–92 Cluster

Many microRNAs are present within the genome not as an individual microRNA but rather as clusters of multiple microRNAs [8,9]. Usually these clusters contain two or three genes but larger clusters exist, including the miR-17–92 cluster, which contains seven mature microRNAs (miR-17–5p, miR-17–3p, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92–1). These microRNAs are organized in a polycistron; that is, they are all transcribed as a single pri-microRNA, and then subsequently processed to form the individual microRNAs. The entire miR-17–92 cluster is located within the third intron of an open reading frame termed the C13orf25 (Chromosome 13 open reading frame 25) gene located at 13q31–q32. The close proximity of the six clustered microRNAs to each other (all six encompass only ~800 base pairs of genomic DNA) makes it possible for all of the microRNAs to be transcribed and expressed similarly. In fact, a recent analysis of the expression of microRNAs in hematopoietic cell lines revealed similar expression patterns for all of the microRNAs in this cluster in the samples analyzed [8].

Myc and E2F Induce Expression of miR-17–92, and Individual microRNAs Inhibit E2F Translation

O'Donnell and colleagues demonstrated that activation of the oncogenic transcription factor c-myc induces the expression of microRNAs within the miR-17–92 cluster [10]. Chromatin immunoprecipitation confirmed that c-myc binds to its recognition sites, E-boxes, upstream of this cluster. Serum stimulation of fibroblasts induced the expression of c-myc and the miR-17–92 cluster with similar kinetics. Two of the microRNAs within the cluster, miR-17–5p and miR-20a, downregulated the protein—but not mRNA—abundance of their predicted target, the transcription factor E2F1. Transfection of antisense oligonucleotides that inhibit miR-17–5p or miR-20a resulted in increased E2F1 protein levels without affecting E2F1 transcript abundance. Consistent with this finding, c-myc induction led to a strong increase in E2F1 transcript levels, but only a modest increase in E2F1 protein...
Box 1. Genetic Buffering

Molecular networks that can withstand chance perturbations and reproducibly produce the same phenotypic results have been favored over the course of evolution [7]. Genetic buffering refers to the stabilization of molecular networks, making them less sensitive to chance fluctuations in the levels of specific molecules. The best-understood example of a specific molecule with the capacity to buffer a network is the chaperone protein hsp90, which can serve as a capacitor for the build-up of genetic variation. In Drosophila, when the heat-shock protein hsp90 is mutated or impaired, phenotypes are observed in nearly every adult structure [61]. Thus, widespread variation affecting protein hsp90 is mutated or impaired, phenotypes are observed in nearly every adult structure [61]. Thus, widespread variation affecting protein hsp90 buffers the variation. When hsp90 is incapacitated, for instance, under conditions of high temperature, cryptic variants may be revealed. This process may promote evolutionary change by increasing phenotypic variance under stressful conditions. Chromatin regulators have also been suggested to play a role as genetic buffers. A systematic phenotypic variance under stressful conditions. Chromatin regulators revealed. This process may promote evolutionary change by increasing because hsp90 buffers the variation. When hsp90 is incapacitated, for instance, under conditions of high temperature, cryptic variants may be revealed. This process may promote evolutionary change by increasing.

miR-17–92 have been shown to reinforce the downregulation of transcripts in specific cell types or at times when the encoded proteins should not be present [6,7]. In several recent papers, microRNAs have been elegantly associated with regulatory loops that serve to reinforce lineage commitments, especially the irreversible commitment to a specific cell fate [63–65]. Under these conditions, a transient signal may result in one of two bistable states, characterized by either low microRNA levels and high target levels, or vice versa [64–66]. In addition to reinforcing low expression of genes that are intended to be “off,” robustness can also be improved by minimizing “noise” in protein expression levels similar to hsp90’s effects at the protein level. The microRNAs of the miR-17–92 complex have been proposed to help minimize noise in the levels of the E2F1 protein [7,23]. Because transcription is an inherently noisy process, frequent transcription coupled with infrequent translation results in lower intrinsic noise in protein levels compared with infrequent transcription [67–70]. Accordingly, in yeast, genes that are key regulators or essential have high rates of transcription and low rates of translation [71]. In this model, the miR-17–92 cluster could limit the extent of translation, thus allowing the cell to make many mRNA copies but have a low and carefully controlled amount of protein. This model could be tested by determining whether miR-17–92 actually affects inter-cell variability in E2F protein levels [72]. The amount of noise could be monitored with E2F1-YFP fusion proteins that include the relevant E2F 9-O-methyl oligoribonucleotides, or 2'-O-methyl oligoribonucleotides that target individual microRNAs within the cluster.

levels [10]. Since c-myc and E2F1 have been shown to activate each other’s transcription [11–13], this established an unusually structured network in which c-myc activates the transcription of E2F1 while simultaneously inhibiting its translation (Figure 1). The importance of the miR-17–5p/miR-20a binding sites for E2F1 expression were demonstrated with reporter assays [10,14]. The 3’ UTRs of E2F2 and E2F3 were also regulated via miR-17–5p/miR-20a binding sites, but the downregulation of E2F1 protein levels was stronger than for E2F2 or E2F3 [14].

To make matters even more complicated, the E2F transcription factors can also induce the microRNAs in the miR-17–92 cluster [14,15]. Overexpression of E2F1 or E2F3 results in increased miR-17–92 promoter activity [14,15]. Chromatin immunoprecipitation experiments by Woods and colleagues revealed stronger binding for E2F3 than E2F1 or E2F2 in three different regions of the miR-17–92 cluster, confirming a likely role for E2F3 in miR-17–92 expression. Because a control promoter at which all E2Fs are known to bind also resulted in stronger bands for E2F3 than for E2F1 or E2F2, this may reflect greater binding efficiency of the E2F3 antibody. Sylvestre and colleagues similarly concluded that immunoprecipitation followed by PCR resulted in bands of similar intensity for E2F1, E2F2, and E2F3 in one DNA region and a stronger band for E2F3 in a different region, which may also reflect higher binding affinity of E2F3 for this region, or higher binding affinity of the E2F3 antibody [14]. These findings suggest a complex network structure summarized in Figure 1.

Can miR-17–92 Prevent Runaway myc-E2F Activation?

Because c-myc and E2F1, E2F2, and E2F3 can activate one another’s transcription [11–13], one might imagine a cell in a runaway positive feedback loop in which c-myc induces the E2F transcription factors, which induce each other and, in turn, c-myc. This would result in excessively high levels of proliferative transcriptional regulators. High levels of c-myc have been clearly shown to have a tumor-promoting effect [16]. Just 2-fold differences in c-myc expression can affect cell size in flies or cell number in mice [17–20]. Indeed, dysregulated expression of c-myc is one of the most common abnormalities in human malignancy [21]. Thus, maintaining c-myc levels within a tight range can be considered critical for the prevention of cancer and accordingly, c-myc levels are tightly controlled at the level of transcription [16,22]. One hypothesized role for the miR-17–92 cluster is to further the goal of carefully minimizing noise in c-myc levels. By ensuring that E2F1 protein levels do not rise precipitously in response

Figure 1. The interactions among c-myc, the E2F1, E2F2, and E2F3 Transcription Factors and the microRNAs of the miR-17–92 Complex Are Shown

Black arrows indicate a transcriptional induction. Bidirectional arrows indicate mutual transcriptional induction. Darker arrows indicate stronger evidence of regulation while dashed arrows indicate that the evidence is less conclusive. Red lines indicate translational inhibition. Darker lines indicate stronger inhibition and dashed lines indicate weaker inhibition. Among members of the miR-17–92 cluster, only miR-17–5p and miR-20a have been shown to inhibit E2F translation.
to c-myc activation, miR-17–92 may act as a brake on this possible positive feedback loop, thus helping to ensure tightly controlled expression of c-myc and E2F1 proteins [10,23].

From this perspective, the miR-17–92 cluster might act as a tumor suppressor. Consistent with this hypothesis, loss of heterozygosity or deletion of the 13q31–32 region is observed in multiple types of tumors, including squamous cell carcinoma of the larynx [24], retinoblastoma [25], hepatocellular carcinoma [26], breast cancer [27], and nasopharyngeal carcinoma [28,29]. Copy number loss was observed frequently for the microRNAs of the miR-17–92 cluster in breast cancers (21.9%), ovarian cancers (16.5%), and melanomas (20.0%) [30]. In addition, miR-17–5p in particular is expressed at low levels in multiple breast tumor cell lines and has antiproliferative effects on breast cancer cells [31].

**Individual Members of the miR-17–92 Cluster Can Also Inhibit Apoptosis**

Paradoxically, while the miR-17–92 cluster has a putative role as an inhibitor of proliferation described above, the observed functional effect of overexpressing the microRNAs within the miR-17–92 cluster is not to inhibit proliferation but rather to induce proliferation and/or inhibit apoptosis. As an example of the role of microRNAs in the miR-17–92 cluster in inhibiting apoptosis, introduction of miR-17–92 in conjunction with c-myc overexpression resulted in B-cell lymphomas characterized by an absence of the high levels of apoptosis normally associated with c-myc-induced lymphomas [32]. In in vitro studies, Sylvestre and colleagues demonstrated that overexpression of miR-20a decreased doxorubicin-induced cell death in a prostate cancer cell line, while inhibition of miR-20a with antisense oligonucleotides increased cell death after doxorubicin [14]. Matsubara and colleagues discovered that antisense oligonucleotides directed against miR-17–5p, miR-20a, or both result in increased apoptosis as measured by TUNEL assays in cancer cell lines overexpressing the miR-17–92 cluster [33]. Little effect was observed when either miR-17–5p or miR-20a was introduced into cell lines that do not overexpress miR-17–92, or when antisense oligonucleotides against either miR-18a or miR-19a were introduced. These findings demonstrate that the microRNAs within this cluster have distinct functional effects, with at least miR-17–5p and miR-20a specifically inhibiting apoptosis.

Hayashita and colleagues also discovered that the microRNAs of the miR-17–92 cluster promote proliferation. Transfection of the entire miR-17–92 polycistron into lung cancer cells resulted in increased rates of cellular proliferation based on cell number. Introduction of the coding region of the CI3orf25 or the individual microRNAs miR-18, miR-19a, or miR-20a did not recapitulate the growth-promoting effects of the entire microRNA cluster [34]. Thus, one or more of the microRNAs in the cluster (but not miR-18, miR-19a, or miR-20a) may enhance proliferation via a mechanism distinct from the anti-apoptotic effect of miR-17–5p and miR-20a.

That the same microRNAs that inhibit E2F1 protein levels (miR-17–5p and miR-20a) also inhibit apoptosis suggests that E2F1 repression may be related to the inhibition of apoptosis conferred by the miR-17–92 cluster. Indeed, the anti-apoptotic activity of this cluster has been proposed to reflect differences in the physiological effects of the different E2F transcription factors. E2F1, as opposed to E2F2 or E2F3, has been particularly associated with an apoptotic response. For

---

**Box 2. Feed Forward Loops**

Genetic networks contain repeated regulatory motifs including feed forward loops. In biological systems, this motif has been defined as two transcription factors, one of which regulates the other, and both of which regulate a third gene (Figure 2). These transcription factor–based loops can be “coherent,” in which case the sign of the direct path to the target gene is the same as the sign of the indirect path to the reporter. Alternatively, they can be “incoherent,” in which case the direct and indirect paths have opposing effects on the target. For transcription factor–based loops, both coherent and, less frequently, incoherent feed forward loops have been identified in *S. cerevisiae* and *E. coli* [73]. In human and mouse, incoherent loops involving microRNAs and their targets have been inferred to be common based on microarray data [23]. Both coherent and incoherent feed forward loops involving two transcription factors have been theoretically modeled and experimentally tested [73,74]. The two components contributing to expression of the reporter may be organized in an “AND” conformation, such that both are required, or an “OR” conformation, so that either is sufficient for expression. An incoherent feed forward loop can cause a pulse of reporter activity under certain conditions. In this situation, the upregulation kinetics are expected to be faster than if the two factors were acting independently [73,75]. Indeed, in experimental systems, it has been shown that an incoherent feed forward loop can lead to accelerated turn-on dynamics, even if embedded within other loops [74,75].

We speculate that the structure of the feed forward loop involved in the c-myc–miR-17–92–E2F network may have an important impact on the kinetics of E2F1 activation. The network structure embedded within the c-myc–miR-17–92–E2F network may resemble a type 1 “incoherent” feed forward loop [73]. In the case of miR-17–92, microRNA-mediated inhibition of translation could allow a pulse of E2F activity. Once reaching a critical threshold, microRNAs could efficiently downregulate translation of the existing transcripts, thereby preventing a further rise in E2F1 abundance. This would allow a rapid shut-off of E2F1 activity that would be independent of the kinetics of E2F1 transcript degradation. Indeed, this mechanism might underlie the spike in E2F1 activity during cell-cycle entry [76]. Whether this network does in fact result in a rapid spike in E2F1 activity could be tested by a careful analysis of the time course of miR-17–92 and E2F1 induction in response to c-myc activation. A finding that E2F1 levels and activity are rapidly induced and then decline following c-myc induction would support this model as a possible advantage of the network structure discovered.

---

**Figure 2. Two Molecules (X and Y) Can Both Regulate Molecule Z Independently (A) or Can Form a Feed Forward Loop (B) in Which X Regulates Z Both Directly and Indirectly via Y**

Feed forward loops can be coherent as shown in (B), in which case the direction of the regulation on Z is the same in either the direct or indirect path, or incoherent, illustrated in (C), in which case the two pathways have opposing effects on the target gene.
instance, E2F1 is activated by the ATM/ATR DNA damage–
signaling pathway [35]. And, E2F1-responsive sites are present
in the promoters of caspases and other pro-apoptotic
molecules [36,37]. Although E2F2 and E2F3 are also somewhat
downregulated by the microRNAs in the cluster based on
reporter assays [14], the effect on E2F1 is much stronger.
Thus, the miR-17–92 cluster has been proposed to inhibit
apoptosis by decreasing E2F1 levels [14,15].

Possible Role for Apoptosis Inhibition by miR-17–92
in Spermatocytes

A physiological role for apoptosis inhibition mediated by
miR-17–92 has been suggested in spermatocytes [38]. In
individual pachytene spermatocytes within a normal testis,
high levels of E2F1 message but low levels of protein were
associated on a cell-by-cell basis with high miR-17–92
expression. This has been suggested to reflect the particular
needs of spermatocytes, cells in which meiotic recombination
induces extensive crossing over of sister chromatids and
multiple double-strand breaks, which would be expected to
result in apoptosis [38]. Inhibition of E2F1 by miR-17–92
could be important for preventing apoptosis in these cells
during meiotic recombination. These findings suggest that
the miR-17–92 cluster may be important for inhibiting
apoptosis under conditions in which it would be detrimental
to the organism. In addition, this same mechanism for
apoptosis inhibition may be associated with tumorigenesis, as
described further below.

c-myc Induction of miR-17–92 Induces Tumor
Angiogenesis

c-myc has been reported to promote neovascularization via
upregulation of pro-angiogenic VEGF [39] and
downregulation of anti-angiogenic thrombospondin-1 (tsp-1)
[40,41]. In addition, Dew and colleagues recently discovered
that the miR-17–92 microRNA cluster also plays a role in c-
myc–induced angiogenesis in a ras–myc tumorigenesis model.
When engrafted into mice, cells overexpressing ras form small
tumors while overexpression of both c-myc and ras results in
larger tumors with much more robust neovascularization [42].
In cell culture, miR-17–92 levels were elevated in the presence
of overexpressed c-myc, and levels of predicted targets tsp-1
and connective tissue growth factor (CTGF) declined.
Transfection of antisense oligoribonucleotides revealed that
miR-18 is especially important for CTGF regulation and miR-19
is important for tsp-1 regulation. Inhibition of miR-17,
miR-20a, or miR-92 had no effect on the levels of angiogenesis
target genes. Further, cells overexpressing both ras and miR-17–92
had lower levels of CTGF and formed tumors that were larger
and more vascular than tumors formed by cells expressing only ras.

miR-17–92 and c-myc Are Overexpressed in the
Same Tumors

While deletion of 13q31–32 has been observed in some
tumors as described above [24–29], paradoxically,
amplification at 13q31–32 is also frequently observed in
multiple tumor types, including liposarcoma [43], diffuse
large B-cell lymphomas [44], and colon carcinomas [45]. Fine
mapping of the 13q31–32 region in diffuse large B-cell
lymphomas revealed that C13orf25 is elevated in lymphoma
cell lines and patients [92,46]. Overexpression of miR-17–92
may be particularly tumor-promoting when c-myc is also
activated. Amplification of miR-17–92 in the B-cell lymphoma
tumor type is consistent with a role for miR-17–92 in
conjunction with c-myc, because human B-cell lymphomas are
often characterized by high c-myc expression [47]. Elevated
expression levels of miR-17–92 members has also been
associated with lung cell tumors, especially those with c-myc
amplification. miR-17–92 overexpression was detected at the
RNA level and in some cases at the DNA level as well,
suggesting amplification events [34]. In yet another study, a
human mantle cell lymphoma was shown to contain genomic
amplification of both c-myc and miR-17–92 [48].

One simple explanation for the concordance between high
levels of c-myc and miR-17–92 would be that c-myc directly
induces miR-17–92. But this is unlikely to represent the entire
story, as genomic amplification of the miR-17–92 cluster has
also been observed [34,48]. In addition, introduction of miR-
17–92 hastens lymphomagenesis in Eμ-myc overexpressing
mice [92]. As described above, lymphomas with high levels of
miR-17–92 exhibited less apoptosis than typically found in c-
myc–overexpressing tumors, consistent with the model that
miR-17–92 overexpression inhibits apoptosis. This finding
demonstrates that miR-17–92 overexpression confers a
further selective advantage in a high c-myc background.

miR-17–92 as an Integrator of Proliferative versus
Apoptotic Signals?

We conclude by hypothesizing that the unusual structure of
the c-myc–miR-17–92–E2F network may help the cell to
integrate external signals to make a cell fate decision, c-myc
activation can result in proliferation, and indeed, c-myc levels
are often markedly elevated in tumors. However, in different
cellular contexts, c-myc can be a potent inducer of apoptosis
[49–55]. In a mouse model with inducible c-myc, activation of
c-myc in pancreatic β cells induced uniform β cell
proliferation, but also overwhelming apoptosis, thus
counteracting the oncogenic potential of c-myc [56]. When c-
myc was activated in conjunction with overexpression of Bel-
xL, which suppresses c-myc–induced apoptosis, then c-myc
triggered rapid, uniform, and reversible progression to
tumorigenesis. c-myc/Bcl-xL-induced β cell tumors also
contained an extensive network of blood vessels that
regressed after c-myc was switched off.

We speculate that the miR-17–92 cluster may also have an
important role in the balance between a proliferative versus
apoptotic response to c-myc induction. Under normal
proliferative conditions, induction of miR-17–92 in response
to c-myc activation could serve as a brake on excessive
proliferation. miR-17–92–mediated E2F1 inhibition would
attenuate the hypothetical positive feedback loop of c-myc–
E2F activity. The absence of the miR-17–92–mediated
dampening of proliferative signals could be tumorigenic, and
this might explain the deletions in the 13q31–32 region in
multiple types of tumors [24,25,28]. miR-17–92 induction
would also limit apoptosis under normal proliferative
conditions.

Under other conditions, for instance, in stressful situations,
we hypothesize that c-myc activation might occur in the
absence of miR-17–92 induction. Our model would predict
that miR-17–92 would not be induced, or would be induced less robustly, under conditions in which c-myc activation results in apoptosis. The molecular mechanisms that control miR-17–92 in response to c-myc induction are thus of particular interest. One possible model would be that both c-myc and E2F are required to induce expression of miR-17–92, and that the presence of both transcription factors signals to the cell that c-myc activation should be interpreted as a proliferative rather than apoptotic signal. In this model, E2F transcription factors would act as sensors for whether conditions are suitable for proliferation [14,15]. Monitoring both miR-17–92 expression and the occupancy of the miR-17–92 promoter by c-myc and E2Fs in response to proliferative and apoptotic signals would likely shed light on whether the expression of this cluster reflects an “AND” signaling switch.

We further hypothesize that miR-17–92 has a distinct effect in a c-myc-overexpressing environment. c-myc activation induces proliferation that is simultaneously held in check by apoptosis. If a cell has constitutive c-myc overexpression, then this would override the anti-proliferative effects of the miR-17–92 cluster. If the same cell also acquires constitutively high levels of miR-17–92, it will lose the apoptotic response that keeps its proliferative capacity in check, and may proceed to proliferate and form a tumor. Thus, overexpression of both c-myc and miR-17–92 would be expected to create a tumor-promoting environment. Indeed, overexpression of both c-myc and miR-17–92 has been observed in the same tumors [34,48], as described above. Under these conditions, the proangiogenic effects of c-myc and miR-17–92 may also cooperate with the anti-apoptotic effects of the miR-17–92 cluster to simultaneously create multiple conditions conducive for a tumor growth.

**Future Research**

In summary, a series of elegant recent papers has illuminated a fascinating and unexpected network of interactions involving the c-myc and E2F transcription factors, and the members of a microRNA cluster. This network may be organized in the format of an incoherent feed forward loop, in that c-myc induces E2F1 transcription while repressing E2F1 translation. The microRNAs within this cluster may act as a brake on proliferation, inhibiting apoptosis and promoting angiogenesis. We look forward to further research that will clarify the roles of c-myc, different E2F transcription factors, and other regulators in controlling expression of the miR-17–92 cluster. In particular, experiments addressing whether c-myc and the E2Fs act synergistically or independently to control miR-17–92 expression would help to define its potential role as a signal integrator. It will also be interesting to determine whether miR-17–92 plays a role in tumorigenesis mediated by other genetic mechanisms. For instance, in chronic myeloid leukemias, expression of the miR-17–92 cluster was downregulated by RNAi directed against the pro-oncogenic fusion protein bcr-abl [57]. Other microRNAs have been implicated in tumorigenesis, either as oncogenes or tumor suppressors [58–60]. The mechanisms by which these microRNAs affect tumorigenesis, in particular, whether they affect the same or different molecules and employ the same or different molecular circuitry, would shed light on the key elements in the transition to tumorigenesis. Recent analysis of gene expression patterns between microRNAs and their targets suggest that networks of this type, in which the expression of the microRNA and its targets are positively correlated, are common in human and mouse, especially in neural tissues [23]. This makes it of particular importance to discover the potential advantages conferred by this seemingly paradoxical network structure.

**Acknowledgments**

We also thank Leonid Kruglyak (Princeton University) and Chi Van Dang (Johns Hopkins University) for their helpful input. HAC is the Milton E. Cassel scholar of the Rita Allen Foundation. HAC and ALM are supported by National Institute of General Medical Sciences Center of Excellence grant P50 GM071508.

**Funding.** The authors received no specific funding for this article.

**Competing interests.** The authors have declared that no competing interests exist.

**References**

1. Bartel DP (2004) MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116: 281–297.
2. Carrington JC, Ambros V (2003) Role of microRNAs in plant and animal development. Science 301: 576–579.
3. Wightman R, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 75: 855–862.
4. Olsen PH, Ambros V (1999) The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis prior to the initiation of translation. Dev Biol 216: 671–680.
5. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, et al. (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433: 769–773.
6. Stark A, Brennecke J, Bushati N, Russell RB, Cohen SM (2005) Animal MicroRNAs confer robustness to gene expression and have a significant impact on 3’UTR evolution. Cell 123: 1135–1146.
7. Hornestein E, Shomron N (2006) Canalization of development by microRNAs. Nat Genet 38: S20–S24.
8. Yu J, Wang F, Yang GH, Wang FL, Ma YN, et al. (2006) Human microRNA clusters: Genomic organization and expression profile in leukemia cell lines. Biochem Biophys Res Commun 340: 59–68.
9. Altuvia Y, Landgraf P, Lithwick G, Elefant N, Pfeffer S, et al. (2005) Clustering and conservation patterns of human microRNAs. Nucleic Acids Res 33: 2697–2706.
10. O’Donnell KA, Wentzel EA, Zeller KL, Dang CV, Mendell JT (2005) c-Myc-regulated microRNAs modulate E2F1 expression. Nature 435: 839–843.
11. Leone G, DeGregori J, Sears R, Jakoi L, Nevins JR (1997) Myc and Ras collaborate in inducing accumulation of active cyclin E/Cdk2 and E2F. Nature 387: 422–429.
12. Fernandez PC, Frank SR, Wang L, Schroeder M, Liu S, et al. (2005) Genomic targets of the human c-Myc protein. Genes Dev 17: 1115–1129.
13. Matsumura I, Tanaka H, Kanakura Y (2005) E2F1 and c-Myc in cell growth and death. Cell Cycle 2: 333–338.
14. Sylvestre Y, De Guire V, Querido F, Mukhopadhyay UK, Bourdeau V, et al. (2006) An E2F/miR-20a autoregulatory feedback loop. J Biol Chem 282: 2155–2143.
15. Woods K, Thomson JM, Hammond SM (2006) Direct regulation of an oncogenic microRNA cluster by E2F transcription factors. J Biol Chem 282: 2130–2134.
16. Chung HJ, Levens D (2005) c-myc expression: Keep the noise down! Mol Cells 20: 157–166.
17. Gallant P (2005) Myc, cell competition, and compensatory proliferation. Cancer Res 65: 6485–6487.
18. Johnston LA, Gallant P (2002) Control of growth and organ size in *Drosophila*. Bioessays 24: 54–64.
19. de la Cova C, Abril M, Bellosa P, Gallant P, Johnston LA (2004) *Drosophila* myc regulates organ size by inducing cell competition. Cell 117: 107–116.
20. Moreno E, Basler K (2004) dMyc transforms cells into super competitors. Cell 117: 117–129.
21. Cole MD, McMahon SB (1999) The Myc oncoprotein: A critical evaluation of transactivation and target gene regulation. Oncogene 18: 2916–2924.
22. Weber A, Liu J, Collins I, Levens D (2005) TFIIH operates through an heterochronic gene lin-14 by blocking LIN-14 protein synthesis prior to the initiation of translation. Dev Biol 216: 671–680.
23. Tsang J, Zhu J, van Oudenaarden A (2007) MicroRNA-mediated feedback and feedforward loops are recurrent network motifs in mammals. Mol Cell 26: 753–767.
24. Stembalska A, Blin N, Ramsey D, Sasiadek JM (2006) Three distinct regions of deletion on 13q in squamous cell carcinoma of the larynx. Oncol Rep 16: 417–421.

25. Zhang XL, Fu WL, Zhao HX, Zhou LX, Huang JF, et al. (2005) Molecular studies of loss of heterozygosity in Chinese sporadic retinoblastoma patients. Clin Chim Acta 358: 75–80.

26. Lin YW, Sheu JC, Liu LY, Chen CH, Lee HS, et al. (1999) Loss of heterozygosity at chromosome 13q in hepatocellular carcinoma. Identification of a putative independent region. Eur J Cancer 35: 1730–1734.

27. Eriksson G, Johannesburg G, Ingvarson S, Jordansdottr IB, Jonsson JG, et al. (1998) Mapping loss of heterozygosity at Chromosome 13q: Loss at 13q12–q13 is associated with breast tumour progression and poor prognosis. Eur J Cancer 34: 2076–2081.

28. Lo KW, Teo PM, Hui AB, To KF, Tsang YS, et al. (2000) High resolution allelotype of microdissected primary nasopharyngeal carcinoma. Cancer Res 60: 3548–3553.

29. Shao J, Li Y, Wu Q, Liang X, Yu X, et al. (2002) High frequency loss of heterozygosity on the long arms of Chromosomes 13 and 14 in nasopharyngeal carcinoma in Southern China. Chin Med J (Engl) 115: 571–575.

30. Zheng L, Huang J, Yang N, Greshock J, Megraw MS, et al. (2006) microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci U S A 103: 9136–9141.

31. Hossain A, Kuo MT, Samders GF (2006) Mir-17–5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. Cell Biol 26: 819–821.

32. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, et al. (2005) A microRNA polycistron as a potential human oncogene. Nature 438: 828–833.

33. Matsubara H, Takeuchi T, Nishimura N, Furukawa Y, Satoh M, Endo H, et al. (2002) Apaf-1 is a mediator of E2F-1-induced apoptosis. J Biol Chem 277: 39760–39767.

34. Lin WC, Lin FT, Nevins JR (2001) Selective induction of E2F1 in response to DNA damage mediated by ATM-dependent phosphorylation. Genes Dev 15: 1835–1844.

35. Lin VC, Lin FT, Neijs IM (2001) Selective induction of EF1 in response to DNA damage mediated by ATM-dependent phosphorylation. Genes Dev 15: 1835–1844.

36. Nahle Z, Polakov J, Davuluri R, McCurrach ME, Jacobson MD, et al. (2002) Direct coupling of the cell cycle and cell death machinery by E2F. Nat Cell Biol 4: 859–864.

37. Fuhraka Y, Nishimura N, Furukawa Y, Satoh M, Endo H, et al. (2002) Apaf-1 is a mediator of E2F-1-induced apoptosis. J Biol Chem 277: 39760–39767.

38. Novotny GW, Sonne SB, Nielsen JE, Jonstrup SP, Hansen MA, et al. (2007) Transcriptional repression of EF1 mRNA in carcinoma in situ and normal testis correlates with expression of the mir-17–92 cluster. Cell Death Differ 14: 879–882.

39. Knies-Bamforth UE, Fox SB, Poulsom R, Evan GI, Harris AL (2004) c-Myc interacts with hypoxia to induce angiogenesis in vivo by a vascular endothelial growth factor-dependent mechanism. Cancer Res 64: 5653–5657.

40. Tikhonenko AT, Black DJ, Limil ML (1996) Viral Myc oncproteins in infected fibroblasts down-modulate thrombospondin-1, a possible tumor endothelial growth factor-dependent mechanism. Cancer Res 56: 801–806.

41. Venturini L, Battmer K, Castoldi M, Schultheis B, Hochhaus A, et al. (2007) Expression of the mir-17–92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. Blood 109: 4399–4405.

42. Zindy F, Eschen GM, Randle DH, Kamijo T, Cleveland JL, et al. (1998) Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. Genes Dev 12: 2421–2433.

43. Mangan S, Alon U (2003) Structure and function of the feed-forward loop network motif. Proc Natl Acad Sci U S A 100: 11980–11985.

44. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, et al. (2002) Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 99: 15244–15249.

45. Pelengaris S, Khan M, Evan GI (2002) Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. Cell 109: 321–334.

46. Venturini L, Battmer K, Castoldi M, Schultheis B, Hochhaus A, et al. (2007) Expression of the mir-17–92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. Blood 109: 4399–4405.