cross-linked pectin network. This network controls the porosity of the cell wall, as shown by the increased pore size of cell walls of boron-starved cell cultures. It will be interesting to see whether the altered pore size also affects the accessibility of polysaccharide-modifying enzymes to their substrates, which could be a mechanism by which reduced boron cross-linking affects growth of *mur1* plants. Does RGII cross-linking control normal plant growth? A reduction in pore size has been observed upon transition from the growth phase to the stationary phase in cell cultures. It is not known, however, whether RGII is involved in this change. What is the three-dimensional structure of this molecule? Insights come from molecular modeling, and attempts to crystallize this polysaccharide are under way. Maybe these approaches will explain its unusual stability. Finally, it will also be interesting to know whether this complex molecule carries out tasks other than those related to its ability to form dimers. Next time you drink a glass of red wine, rich in RGII, why not reflect on these intriguing questions.

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PERSPECTIVES: MOLECULAR BIOLOGY

Glimpses of a Tiny RNA World

Gary Ruvkun

Over the years, a steady stream of structural and regulatory RNAs have been identified. Three papers published in this issue on pages 853, 858, and 862 from the Tuschl, Bartel, and Ambros labs continue the tradition, but now prospecting for tiny RNAs of ~22 nucleotides (nt) (*1*–*3*). The chain of reasoning that simultaneously attracted these groups to 22 nt is convoluted but interesting.

The first 22-nt RNAs, *lin-4* and *let-7*, were identified by genetic analysis of *Caenorhabditis elegans* developmental timing (*4*, *5*). The expression of the *lin-4* RNA during the first larval stage and the *let-7* RNA during the fourth larval stage triggers the down-regulation of target mRNAs via 3′-untranslated region (UTR) elements that are complementary to each regulatory RNA to specify the temporal progression of cell fates (*6*, *7*). The *let-7* RNA, as well as its temporal regulation, are conserved across much of animal phylogeny (*8*). These 22-nt RNAs are called small temporal RNAs or stRNAs

Tiny RNAs also emerged from the biochemical analysis of RNA interference (RNAi) by experimentally induced double-stranded RNA (dsRNA): 21- to 25-nt small intermediate RNAs (siRNAs) are processed from dsRNA and act as templates for their own amplification and the degradation of target mRNAs during RNAi (*9*, *10*). *lin-4* and *let-7* are predicted to be processed from partially double-stranded precursors as well (*4*, *5*, *8*). The common size of ~22 nt for stRNAs and siRNAs suggested that they are generated and perhaps act by a common mechanism. In fact, the same Dicer ribonuclease (RNase) that is required to process dsRNA to siRNAs also processes the stRNAs from their precursors (*11*, *12*). The developmental defects caused by Dicer mutations in plants and animals may be due to defects in processing of other endogenous tiny regulatory RNAs (*11*–*13*).

The three teams use a range of biochemical techniques to clone 21- to 25-nt RNAs (*1*–*3*) from three different organisms, and thus reveal the richness of the tiny RNA world. They detect almost 100 new tiny RNAs—microRNAs or miRNAs. The Tuschl group identified 14 new miRNAs from the *Drosophila* embryo and 19 miRNAs from HeLa cells (*1*). The expression of all the new miRNAs was verified. The Bartel group identified 55 new miRNAs from mixed-stage *C. elegans* and verified the expression of 20 out of 22 miRNAs tested (*2*). Lee and Ambros cloned and verified the expression of 15 *C. elegans* miRNAs, 10 of which were also identified by the Bartel group (*3*). While the entry point of these studies was biochemical, complete genome sequences were key in the analyses. All three groups used the genome sequences of a variety of organisms to determine that these miRNAs are not breakdown products of mRNAs or structural RNAs, to infer precursors, to determine the genetic locations of the new genes, and to determine whether the miRNAs are conserved in evolution.

All of these miRNAs are predicted to be processed from multiply bulged and partially duplex precursors, like the stRNA precursors. Therefore, they are likely to be processed by Dicer, as demonstrated for two of the new miRNAs (*3*). More of the miRNAs are processed from the 3′ region of the precursor stem loop than from the 5′ region, from which stRNAs are processed. One precursor produces miRNAs from both stems (*2*). Thus, as in siRNA processing from dsRNA, Dicer probably processes both strands of these precursors, but in many cases only one strand may be stable. Some of the miRNAs are expressed only as longer precursors at some developmental stages (*2*, *3*), suggesting possible regulation of processing rather than transcription.

Members of the RDE-1/Argonaute superfamily of proteins may also function in the maturation of miRNAs. The *C. elegans* Argonaute orthologs are required for the maturation and function of *let-7* and *lin-4* (*11*), and *C. elegans* RDE-1 and *Arabidopsis* Ago1 are necessary for RNAi (*14*, *15*). These proteins may form a complex with Dicer, as has been shown for *Drosophila* Argonaute2 (*16*). Genome sequences suggest that there are 24 *C. elegans* RDE-1/Argonaute genes, 7 in *Arabidopsis*, 4 in *Drosophila*, and 4 in humans. The distinct RDE-1/Argonautes may be specialized for processing subsets of miRNA genes. The developmental defects caused by mutations in *Drosophila* or *Arabidopsis* Argonaute genes may be due to defective processing of particular miRNAs (*17*, *18*).

Like *let-7*, a number of the miRNA genes are conserved in evolution. About 12% of the miRNAs are conserved between nematodes, flies, and mammals, but more than 90% of the *C. elegans* miRNAs are conserved in the 90% complete *Caenorhabditis briggsae* sequence (*2*). To detect these conserved segments in genome sequence comparisons, only one or two mismatches could be tolerated. But one of the new miRNAs, *mir-84*, is 5 nt diverged from *let-7*, temporally regulated like *let-7*, and conserved in flies and humans (*2*). Such a paralog could only be detected in the rarified sequence space of the miRNA sequence collection.

One of the more subtle results comes from what the papers did not find: There is almost no evidence of siRNAs diagnostic of RNA interference in normally growing animals (*2*). Thus, Dicer and its cofactors are normally used for miRNA production, and are only recruited for RNAi upon viral or other dsRNA induction.

Some of the miRNAs, like *lin-4* and *let-7*, are temporally regulated. A number of the *Drosophila* and *C. elegans* miRNAs are only expressed in germ line or early embryos, hotbeds of translational control. In addition, the analysis of miRNA expression in cell lines and tissues suggests cell type–specific expression (*1*, *3*). The regulated ex-

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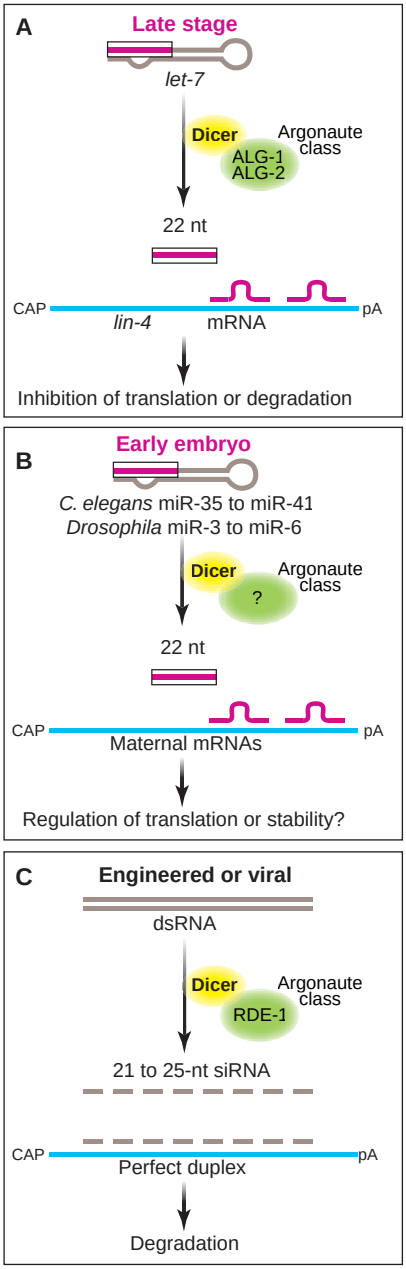
pression patterns of these miRNAs suggests functions in developmental control. However, some of the miRNAs are uniformly expressed, which could indicate more general roles in gene regulation, although in situ expression analysis has not been done to reveal possible cell-specific functions. One argument against an miRNA role in housekeeping gene regulation is that inhibition of *C. elegans* or *Arabidopsis* Dicer function, expected to generally decrease miRNA levels, causes developmental defects rather than cell-lethal defects (*11*, *13*).

Some of the miRNA genes are arranged in tandem clusters (*1*–*3*), as had been observed for the human *let-7* orthologs (*8*). The genes in the tandem clusters that have been studied are co-expressed, for example, in the germ line and early embryo of *C. elegans* and *Drosophila* (*1*, *2*). In fact, a set of seven highly related *C. elegans* miRNA genes that are expressed only in germ line and embryos are so tightly clustered within 1 kb that they are predicted to form a precursor from which all seven mature miRNAs might be processed (*2*).

From the biochemical activities of *let-7, lin-4*, and the siRNAs, the new miRNAs are expected to regulate the translation or stability of other mRNAs (see the figure). The targets of the *let-7* and *lin-4* miRNAs emerged from genetic analysis of suppressors of the *let-7* or *lin-4* heterochronic mutant phenotypes (*6*, *7*). These target mRNAs bear regions of complementarity to the *let-7* and *lin-4* RNAs, but with bulges and loops that make their informatic detection in total genome sequences difficult. The significance of the complementary sites has been proven by mutation that renders the site unresponsive to the regulatory RNA (*6*, *7*). A variety of mRNAs bearing regulatory 3′ UTRs have been identified in the germ line and early embryo of *Drosophila* and *C. elegans*. A search of these 3′ UTRs for regions complementary to the embryonically expressed miRNAs could reveal potential targets and genetic pathways. Consistent with such a role, inhibition of the *C. elegans* Dicer gene *dcr-1* causes sterility and embryonic lethality (*11*).

miRNAs could act in other pathways, such as in the translational control of mRNAs tethered in dendritic regions of neurons, which has been proposed to mediate synaptic plasticity (*19*). The 3′-UTR sequences that mediate dendritic translational control of calcium-calmodulin–dependent kinase II have been determined (*20*), and it would be interesting to see if there are miRNAs that are complementary to these cis-acting sequences. Finally, because siRNAs in plants mysteriously regulate target gene transcription as well as mRNA stability (*9*), it is possible that the new miRNAs regulate target gene transcription as well.

The most definitive test of the function of the new miRNAs is to isolate mutations in these genes, and there may be genetic loci that have not been molecularly analyzed at the genetic location of these miRNAs in *C. elegans* or *Drosophila*. Gene knockout, or perhaps RNAi, increased gene dosage, or misexpression strategies, also could be used to establish the function of these miRNAs.



Slicing and dicing miRNAs. (**A**) The two founder miRNAs from *C. elegans, lin-4* and *let-7*, are processed from partially duplexed precursors by the Dicer/Argonaute complex, and then down-regulate the expression of protein-coding mRNAs by base-pairing to partially complementary elements on those mRNAs. The new miRNAs are expected to function similarly (**B**). For example, the *Drosophila* and *C. elegans* miRNAs that are expressed during embryonic stages may regulate the translation of maternal mRNAs that pattern early development. (**C**) The small interfering RNAs (siRNAs) that are intermediates in RNAi are also 21 to 25-nt long, processed by a Dicer/RDE-1 complex, and target mRNAs. The molecular components of RNAi may have been recruited for viral and transposon surveillance from an original role in miRNA regulation, or vice versa.

Because genes that encode gene-regulatory proteins constitute a large fraction of *C. elegans* and *Drosophila* genetically identified loci, why have more of the miRNAs not been revealed by genetic analysis? One view is that they constitute a small genetic target, although multiple *lin-4* and *let-7* mutant alleles emerged from relatively limited genetic screens. Alternatively, given that less than 5% of the many *C. elegans* KH or RRM domain RNA-binding protein genes have been genetically identified, the miRNA genes may be emerging from genetics at about the expected rate. And if these miRNA genes regulate the expression of fewer target mRNAs than other RNA-regulatory proteins, or if multiple miRNAs regulate adjacent sites on common target mRNAs, mutations in them may cause more subtle phenotypes. Or perhaps researchers have simply been focusing on the canon of protein-coding genes.

Why use a tiny RNA to regulate the expression of target genes? First, the expression of a miRNA of 70 nt occurs much more rapidly than that of a typical protein-coding primary transcript of 1 to 1000 kb and is not further delayed by translation. A transcriptional cascade of miRNA genes—for example, during the short cell cycles of *Drosophila* or *C. elegans* early embryogenesis—could trigger developmental transitions by translational control of preexisting maternal mRNAs through translational control. The expression of miRNAs after a synaptic transmission may regulate the translation of mRNAs tethered in a dendrite before the decay of a phosphorylation event that may mark a recently fired synapse. Second, the 22-nt siRNAs are remarkably potent at mRNA inactivation. There is good evidence that they can act systemically in plants and nematodes, with probable amplification (*9*). Given that the miRNAs use the same processing and presentation machinery as the siRNAs, the miRNAs also may be amplified from their precursors, and may spread throughout an organism from a single source of expression.

How complete are these miRNA surveys? They are just the first steps and not proposed to be saturating. But many of the miRNAs identified by the Ambros and Bartel group coincide, and *let-7* constituted about 30% of the 100 miRNAs isolated from HeLa cells (*1*). While these biochemical procedures are skewed toward abundant miRNAs, the multiple isolations of particular miRNAs circumscribe the abundant miRNA world. On the other hand, many miRNAs were isolated just once, suggesting

more miRNAs expressed at lower levels. Many of the miRNAs were isolated from mixed-stage RNA preparations (*2*, *3*). Biochemical collection of miRNAs from selected cell types or finely staged preparations may reveal rare miRNAs that act in particular cells or at particular times. But the comprehensive detection of miRNAs expressed in few cell types or under particular conditions may demand informatic approaches based on the now-extensive training set of miRNAs revealed in these papers.

In fact, the number of genes in the tiny RNA world may turn out to be very large, numbering in the hundreds or even thousands in each genome. Tiny RNA genes may be the biological equivalent of dark matter—all around us but almost escaping detection, until first revealed by *C. elegans* genetics and then more comprehensively charted by these papers. The next step is to figure out whether these regulatory RNAs use principles of amplification and systemic spread that have selected for their conservation as well as their ramification into so many apparently new sequences.

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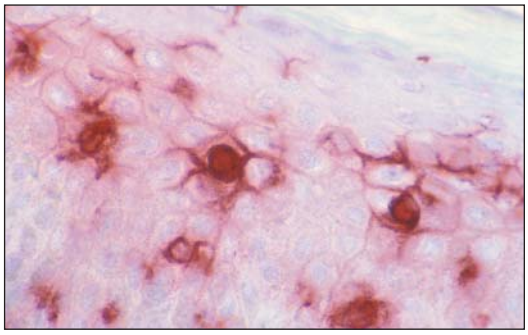
PERSPECTIVES: IMMUNOLOGY

Chip Shots—Will Functional Genomics Get Functional?

Robert L. Modlin and Barry R. Bloom

Of every 10 people infected with the tubercle bacillus, only one will develop tuberculosis; of a hundred individuals infected with the malaria parasite, only 0.5% will die. When people are exposed to the same point source of infection, some become ill, for example from enteritis caused by *Escherichia coli* in contaminated food, whereas others do not; some may die from influenza whereas others suffer only mild symptoms. How can we understand the basis for these variations in susceptibility to infection? In the genomic age, the easy answer could be that pathogens switch on different genes or differentially alter expression of the same genes in susceptible versus resistant individuals. But surely variations in disease susceptibility cannot be explained so readily. Indeed, given the presumed strong selective pressure of infection on the human genome, it is striking how few genes exclusively expressed in the immune system are strongly associated with disease susceptibility, and how small the effects of known polymorphisms (sequence differences) in individual genes appear to be (*1*). On page 870 of this issue, Huang *et al*. (*2*) compare the gene expression profiles of human dendritic cells challenged with three different pathogens (a bacterium, a yeast, and a virus). In particular, these investigators wanted to know whether antigen-presenting dendritic cells could discriminate the three microbial challenges and whether discrimination reflected the program of genes activated uniquely by the interaction of a specific pathogen (or its products) with the dendritic cells. As they report, gene chip array analysis revealed that the patterns of dendritic cell gene expression varied according to the pathogen encountered, with some genes being uniquely activated.



Dendritic cells on the frontline. Photomicrograph of skin dendritic cells (Langerhans cells) from a patient with leprosy. Dendritic cells, components of the innate immune response, recognize and respond to microbial pathogens by activating T cells (adaptive immunity) and by releasing inflammatory mediators that mobilize neutrophils and macrophages (innate immunity). Patterns of dendritic cell gene expression differ according to the microbial pathogen encountered (*2*).

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Dendritic cells, members of the body's innate immune system (see the figure), are a good choice of immune cell for this type of study. They are important for initiating both adaptive immunity (activating T and B cells expressing receptors that recognize microbial antigens) and innate immunity (activating phagocytic cells such as neutrophils and macrophages). With their gene chip arrays, Huang *et al*. (*2*) discovered a hierarchy of gene expression in human dendritic cells. The outright winner, the *E. coli* bacterium, activated 685 genes; next came the influenza virus with 531 genes activated; and last came yeast (*Candida albicans*), which activated the fewest genes (289). Of the 6800 genes on the chip array, about 2000 were expressed in resting dendritic cells, and 1330 genes showed alterations in expression after infection. Based on database annotations, the expressed genes were categorized into the following families: innate or adaptive immunity, immune receptors, immune transcription, glycolysis and energy, apoptosis, growth factors, tissue remodeling, cell stress, and immune inhibitors. Despite the fact that the three microbes are completely different organisms, a large number (166) of core genes were activated by all three pathogens, and a smaller number of overlapping genes were activated by two of the three pathogens. Some genes were activated early, some 8 hours after infection, and others 12 hours after infection. The innate immune response appears to be programmed to respond to widely different pathogen challenges with a common core pattern of gene activation. More interesting still, pathogen-specific responses were also observed—118 and 58 genes were activated only by *E. coli* or influenza virus, respectively. Curiously, *Candida* did not induce any pathogen-specific genes. This could imply that the core genes induced by all three pathogens would be sufficient to combat yeast infection. Alternatively, other genes not represented on the chip array, or immune cells in addition to dendritic cells, may be required to deal effectively with this pathogen.

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