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man, confirming the mouse data [1].

The work with photolyase mice may therefore pave the way to our understanding of the actual causes of skin cancer in human populations. Combinations of photolyase mice lacking DNA repair — XP, CS or TTD mice — will soon be available and will help to unravel the complex, but exciting, open questions on the relationship of DNA damage and tumorigenesis. They will also help to answer the precise implications of UVB on sunlight effects, thus discriminating the genotoxicity of solar UVA irradiation, which is thought to act mainly through oxidative damage [20]. These studies will certainly be a careful alert to those people who still think of tanned skin as a healthy sign.

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MicroRNA Biogenesis: Drosha Can't Cut It without a Partner

The ribonuclease Drosha requires a dedicated double-stranded RNA binding protein to convert long, nuclear primary microRNA transcripts into shorter pre-microRNA stem–loops, the cytoplasmic precursors from which mature microRNAs are ultimately excised.

Yukihide Tomari and Phillip D. Zamore

MicroRNAs (miRNAs) are small RNA guides that repress the expression of their target genes. miRNAs generally have their own genes, distinct from the targets they regulate, but occasionally they cohabit with the introns or untranslated regions of genes that encode proteins. Human cells produce hundreds of distinct miRNAs, each of which is believed to act via the RNA silencing pathway to regulate mRNA stability or translation, or chromatin structure, much as small interfering RNAs do in the RNAi pathway. Regulation of gene expression by miRNAs has been proposed to be combinatorial, with different miRNAs acting together on a gene to tune its precise level of expression during development and in response to environmental stimuli. Ambros and colleagues [1] discovered the first miRNA in 1993, but miRNAs were not recognized as a new and extensive class of regulatory molecules until 2001. Hence there is great interest in how miRNAs are transcribed and processed to their mature form, how they function, and why they evolved.

miRNAs are transcribed by RNA polymerase II as primary miRNAs

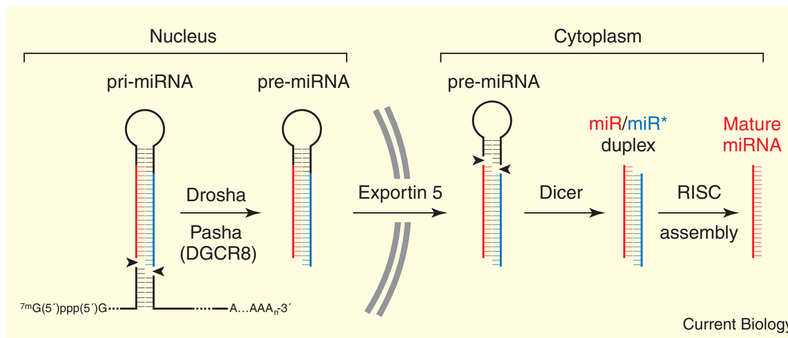

Figure 1. The miRNA biogenesis pathway in animals.

(pri-miRNAs) hundreds to thousands of nucleotides long [2–4]. The ribonuclease III (RNase III) enzyme Drosha cleaves the flanks of pri-miRNAs to liberate ~70 nucleotide stem–loop structures, called precursor miRNAs (pre-miRNAs). Pre-miRNAs contain the ~22 nucleotide mature miRNA in either the 5′ or 3′ half of their stem [5], and the pair of cuts made by Drosha establishes either the 5′ or the 3′ end of the mature miRNA [6]. Pre-miRNAs are moved from the nucleus to the cytoplasm by the protein Exportin 5, which recognizes the two- or three-nucleotide 3′ overhang left by Drosha at the base of the pre-miRNA stem, an end structure characteristic of RNase III cleavage [7–9]. In the cytoplasm, a second RNase III enzyme, Dicer, makes the pair of cuts that defines the other end of the miRNA, generating an siRNA-like duplex, the miR/miR* duplex. Assembly of the mature, single-stranded miRNA from the duplex into the RNA-induced silencing complex (RISC) completes miRNA biogenesis (Figure 1).

Now, four laboratories [10–13] have discovered that the double-stranded RNA-binding protein known as Pasha in flies, or its ortholog DGCR8 in *Caenorhabditis elegans* and mammals, acts together with Drosha to convert pri-miRNA to pre-miRNA. Pasha/DGCR8 is thought to bind directly to the central region and the RNase III domains (RIIIDs) of Drosha [12]. In fact, one route to identifying Pasha/DGCR8 was its published interaction in a genome-wide yeast two-hybrid screen of *Drosophila* proteins [10,13].

Drosha and Pasha/DGCR8 co-purify as a 500–650 kDa nuclear complex [10–12], the microProcessor, in which pri-miRNAs are envisioned to be converted to pre-miRNAs. The microProcessor may comprise more than one of each copy of Drosha and/or Pasha/DGCR8 or it may contain proteins in addition to Drosha and Pasha/DGCR8 [12]. Like Drosha, Pasha/DGCR8 is required *in vivo* to convert pri-miRNA to pre-miRNA. In flies, worms, and cultured mammalian cells, reducing the level of either Drosha or Pasha/DGCR8 by RNAi led to the accumulation of pri-miRNAs and a reduction in both pre-miRNAs and mature miRNAs [10–13], underscoring the remarkable conservation of the miRNA biogenesis and RNAi machinery among metazoans.

Reconstitution of pri-miRNA processing *in vitro* required only recombinant human Drosha and DGCR8; neither protein alone was active [11]. The finding that Drosha requires a double-stranded RNA-binding protein partner is striking, because *Drosophila* Dicer-2 forms a heterodimeric complex with the double-stranded RNA-binding protein R2D2, which is required for its function in RISC assembly [14], although Dicer alone suffices to convert long dsRNA into siRNAs [14,15] and pre-miRNA into miR/miR* duplexes [15].

Discovered by Zinder and colleagues in 1968, RNase III enzymes cut double-stranded RNA, using Mg^{2+} to facilitate catalysis. RNase III enzymes typically contain both RIIIDs and double-stranded RNA-binding domains. Class I RNase III proteins, found in bacteria and yeast, have a single RIIID. Drosha belongs to class II, and it contains two tandem RIIIDs. Dicer is a class III enzyme, with an amino-terminal helicase domain and a PAZ domain (thought to bind the single-stranded tails of siRNA duplexes) in addition to two tandem RIIIDs (Figure 2).

The crystal structure of the RIIID of the *Aquifex aeolicus* class I RNase III revealed it to be a homodimer and suggested that the catalytic centers lie at the dimer interface. Reasoning from the *Aquifex* crystal structure, Dicer was originally proposed to function as a dimer, with four RIIIDs breaking four phosphodiester bonds. Subsequently, Filipowicz and colleagues [15], in a tour-de-force structure–activity study, demonstrated that both *Escherichia coli* RNase III (class I) and human Dicer (class III) make a pair of cuts. They showed that Dicer's two RIIIDs form an intramolecular dimer that creates one pair of catalytic sites [15]. Kim and colleagues have now extended these studies to Drosha, a class II RNase III, demonstrating that it also contains a single processing center comprising two catalytic sites that each break one phosphodiester bond. The new data suggest that the two RIIIDs of Drosha also form an intramolecular dimer that defines the base of the pre-miRNA stem by cleaving the 5′ and 3′ sides of the pri-miRNA, leaving the approximately two nucleotide 3′ overhanging end required for recognition by Exportin 5.

Thus, all RNase III enzymes likely function as intermolecular (class I) or intramolecular (classes II and III) dimers of RIIIDs and break just two phosphodiester bonds at a time. So why do RNase III, Drosha and Dicer use such different substrate RNAs, and how do they yield products so different in length and structure? Their double-stranded RNA-binding protein partners may be part of the answer (Figure 2).

Recombinant human Drosha alone shows non-specific RNase activity, but the addition of

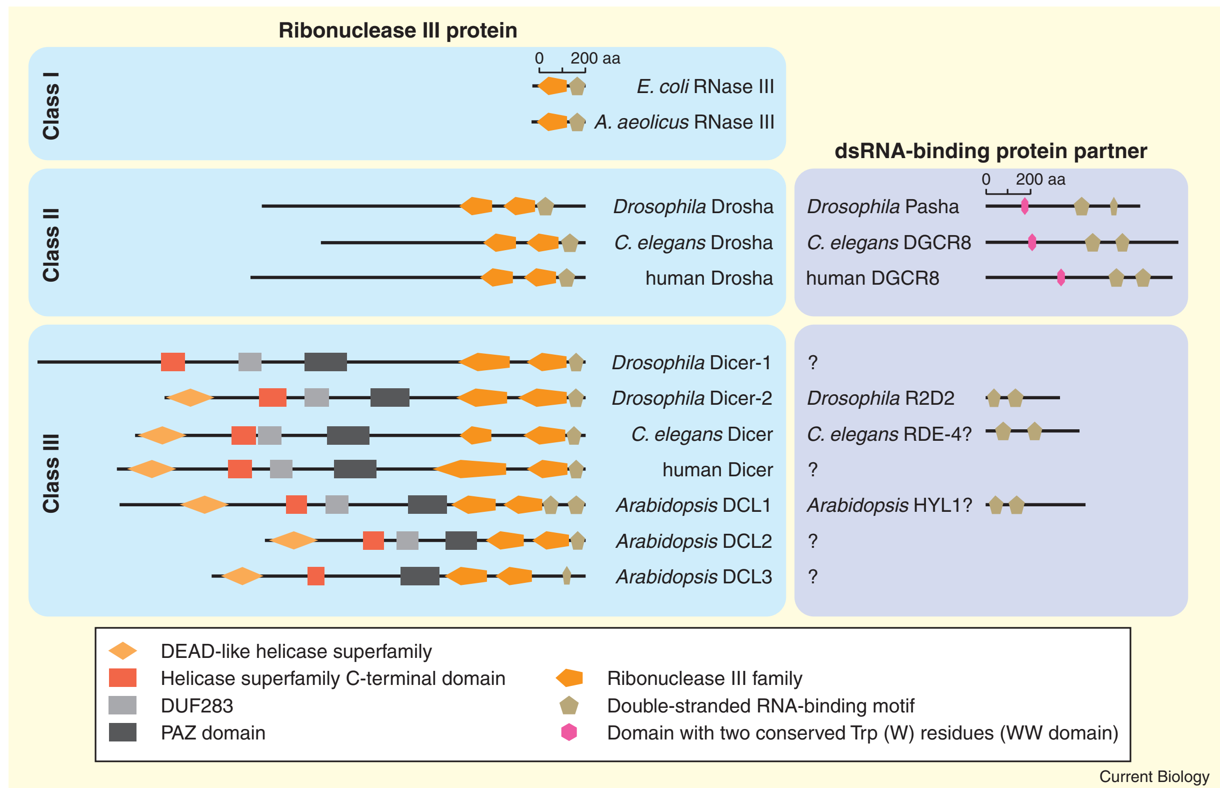

Figure 2. Ribonuclease III family proteins and their double-stranded RNA-binding protein partners.

Domain structures represent the combined predictions of Pfam (http://www.sanger.ac.uk/Software/Pfam/), InterPro (http://www.ebi.ac.uk/interpro/), PROSITE (http://www.expasy.org/prosite/) and SMART (http://smart.embl-heidelberg.de/).

DGCR8 renders it specific for pri-miRNA processing [11]. This implies that Pasha/DGCR8 is a specificity factor that organizes binding of Drosha on the pri-miRNA, much as R2D2 has been proposed to organize Dicer-2 binding to siRNA. How does the Drosha-Pasha/DGCR8 complex recognize pri-miRNA structure? *In vivo* and *in vitro*, processing pri-miRNA into pre-miRNA requires a terminal loop greater than 10 nucleotides [16]. The sites of Drosha cleavage appear to be measured from the loop. This distance corresponds to about 22 base pairs — two turns of the RNA helix [16]. Pasha/DGCR8 may bind the double-stranded stem near the loop, orienting Drosha to bind closer to the base. Thus Pasha binding may place the RIIIDs of Drosha ~22 base pairs from the loop, defining the ends of the pre-miRNA.

The finding that an additional helical turn at the base of the stem, beyond the end of the pre-miRNA is also required in the pri-miRNA for its processing [16] suggests that the double-stranded RNA-binding domain of Drosha binds the portion of the double-stranded stem contained in the pri-miRNA but not the pre-miRNA. Of course, the positions of Pasha/DGCR8 and the Drosha double-stranded RNA-binding domain could be reversed, with Pasha/DGCR8 positioned at the base of the stem and the Drosha double-stranded RNA-binding domain near the loop. Currently, we cannot distinguish between the two models.

In plants, Dicer-like-1 (DCL1) is thought to catalyze both pri-miRNA and pre-miRNA processing [17,18]. DCL1 has two double-stranded RNA-binding domains, which are required for accurate pri-miRNA processing, yet the R2D2- or Pasha/DGCR8-like double-stranded RNA-binding protein HYL1 is required for miRNA biogenesis [19,20]. Tantalizingly, HYL1, a nuclear protein, is a component of a protein complex of unknown composition, but whose reported molecular weight is roughly the size of HYL1 plus a DCL1 protein [20]. Might HYL1 play the role of Pasha/DGCR8 for DCL1 in plants?

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β-Catenin: A Pivot between Cell Adhesion and Wnt Signalling

Mutual adhesion of animal cells is intimately linked to Wnt signaling through a shared component: β-catenin, or Armadillo in *Drosophila.* Recent work indicates how β-catenin shifts from cell adhesion to Wnt signaling, a switch associated with epithelial–mesenchymal transitions and cancer.

Mariann Bienz

The canonical Wnt signaling pathway is highly conserved throughout the animal kingdom [1] and controls numerous developmental processes in animals as distant as flies, frogs and hydra. These include early embryonic patterning, epithelial–mesenchymal interactions and maintenance of stem cell compartments. A key effector of the Wnt pathway is β-catenin, the *Drosophila* version of which is Armadillo. Inappropriate activation of β-catenin in the intestinal epithelium, and in other tissues, often leads to cancer [2].

The β-catenin protein was initially discovered for its role in cell adhesion [3]. As a component of adherens junctions, it promotes cell adhesion by binding to the intracellular domain of the transmembrane protein cadherin, a Ca^{2+}-dependent homotypic adhesion molecule, and linking cadherin to the actin cytoskeleton through the adaptor protein α-catenin (Figure 1). This adhesion function is based on a subcellular pool of β-catenin that is membrane-associated and stable.

In contrast, the signaling function of β-catenin is conferred by a soluble cytoplasmic pool that is highly unstable in the absence of a Wnt signal, as a result of multiple phosphorylations in the protein's amino terminus (Figure 2) that earmark it for proteasome-mediated degradation. This earmark depends on the combined actions of the Adenomatous polyposis coli (APC) tumor suppressor, the Axin scaffolding protein and two serine/threonine protein kinases: glycogen synthase kinase 3β (GSK3) and its priming kinase, casein kinase 1. Upon Wnt signaling, GSK3 is inhibited; as a consequence, unphosphorylated ('activated') β-catenin accumulates, and promotes the transcription of Wnt target genes by binding to TCF transcription factors in the nucleus (Figure 1) [2]. These transcriptional changes are the key read-outs of canonical Wnt signaling, and are the basis for Wnt-induced changes in normal and malignant development [4,5].

Armadillo/β-catenin is thus a truly dual-function protein, encoded by a single gene in most animals and in humans. This sharing of a critical component between two fundamental processes — cell adhesion and cell signaling — may reflect a need for coordinate control between them. Indeed, cell signaling is coupled to a loosening of adhesion between epithelial cells during epithelial–mesenchymal transitions and other developmental processes [6]. The same intrinsic link is also manifest during cancer whose progression typically depends on inappropriate cell signaling and loss of cadherin-mediated adhesion [7].

The pivot between these two processes appears to reside in β-catenin, which potentially couples loss of cell adhesion to increased Wnt signaling if diverted from the plasma membrane to the nucleus. Indeed, although the two β-catenin pools are normally well buffered and functionally separated from each other, experimental manipulations of the levels of one pool can affect the function of the other under some circumstances (reviewed in [8]). The question arises whether there are control switches that flip the