

backward in a computer simulation, Charnoz *et al.* (1) demonstrate that the spiral arose as a localized cloud, about 300 km in radial extent, in early 2004. In other words, the spiral began with an event. But whereas the clumps imaged by Voyager dissipated on time scales of months or less, this pattern has persisted for at least 1.5 years and is likely to continue for years more. (The spiral strands will continue to wind ever tighter until, eventually, they blend into a more uniform skirt around the F ring.)

The discovery of spiral structure reopens the debate over external versus internal collisions. Charnoz *et al.* (1) emphasize the role of S/2004 S6, although they acknowledge that its mass is far too small to create such an immense pattern. They suggest instead that scattering of ring particles off the surface of S/2004 S6 may be the cause of the spiral. As for interactions of the ring with other moons, Prometheus is large enough, but we know that it has not collided with the F ring in the recent past.

So what is left as an explanation? When particles collide, the energy of impact is distributed among particles typically comprising 10 to 100 times as much mass as that of the impactor. As a result, any impactor that produced the spiral probably came from a distance far greater than 300 km. So an alternative to the model of Charnoz *et al.* (1)

is that we are seeing the outcome of a rare, very large impact into the F ring. By this argument, S/2004 S6 may still play a role but, as a consequence, not a cause—it may be a particularly large shard left over from the impact, which now finds itself on a very different orbit from the rest of the ring.

Further monitoring by Cassini should enable us to distinguish between these models. If S/2004 S6 has a continuing role, then we would expect a new spiral to form when its orbital orientation again intersects the ring's core. If Cassini is still operational in 2009, then we might observe the formation of something similar when Prometheus begins to skim the inner edge of the F ring. On the other hand, meteoroid impacts would be expected to occur randomly, with a broad distribution of sizes from the numerous small, clump-forming events to the very rare, large, spiral-forming events.

Perhaps the most fundamental questions concern how the F ring came to exist and why it is so strange. A few factors are important. First, the ring orbits at the edge of the Roche limit, the boundary that separates rings from moons. Inside the Roche limit, Saturn's tidal forces overcome self-gravity, preventing moons from accreting. In the F ring, some reaccretion is possible, so ring bodies are continuously breaking up and joining back together. Second, it is faint

and narrow, so that small injections of new dust are quite noticeable; in the A ring, the equivalent of a spiral-forming event might pass unnoticed. Third, the ring and its nearby "shepherds" all follow highly eccentric orbits, which means that the ring is perturbed quite radically as the moons approach and recede on each orbit. (Here "shepherd" is probably a misnomer; elsewhere in the solar system, nearby moons act to confine rings, whereas the perturbations by Prometheus and Pandora are quite disruptive.) The ring also presumably contains a large mass of its own; how else could it maintain a fixed eccentricity against the tendency of ring particles to precess at different speeds? The picture that emerges is that of a ring that arose from the disruption of a small moon—perhaps the size of Prometheus—that lives in an environment too severely perturbed to ever settle down into a uniform, circular ring. If history is any guide, the F ring harbors a few more surprises that are awaiting Cassini's instruments and science teams.

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DEVELOPMENTAL BIOLOGY

Encountering MicroRNAs in Cell Fate Signaling

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Organisms must tightly regulate where and when each of their genes is expressed, lest their development goes awry with potentially lethal consequences. The mechanisms that control these important time and place decisions have been of great investigative interest. Hence, it

came as a huge surprise that a major level of gene regulation

was completely unknown until the recent discovery of a class of small regulatory RNA molecules known as microRNAs (1, 2). Ever since, we have been racing to understand microRNA function during the development of multicellular animals and

plants. On page 1330 in this issue, Yoo and Greenwald (3) describe a direct connection between the miR-61 microRNA and LIN-12/Notch, the cell surface receptor that controls a fundamental and highly conserved signaling pathway. The link marks an important advance in our understanding of the role of microRNAs in developmental processes.

Mature microRNAs are small RNAs, about 22 nucleotides in length, that are encoded in the genomes of every multicellular organism examined so far. MicroRNAs block gene expression by binding to complementary sequences in the 3' untranslated region (3' UTR) of messenger RNAs and directing either degradation of the messenger RNAs or inhibition of their translation (2). MicroRNAs were first discovered in the microscopic roundworm *Caenorhabditis elegans* as important regulators of developmental timing. They have since been impli-

cated in other aspects of development in plants (4) and animals, including vertebrates and invertebrates (1, 5). However, little is known about the precise role that microRNAs play in many important developmental decisions. For example, communication between cells via molecular signals is a universal mechanism to coordinate cell fate decisions during animal development. What role do microRNAs play, if any, in signaling pathways? Using *C. elegans* vulval development as a model system, Yoo and Greenwald provide an answer.

The vulva is a specialized adult structure that provides a connection from the uterus of the worm to the external environment (6) (see the figure). In wild-type larvae, three vulval precursor cells, called P5.p, P6.p, and P7.p, are specified to eventually form the adult vulva. Each of these cells adopts one of two vulval cell fates—primary (1°) or secondary (2°)—in the precise spatial pattern 2°-1°-2° (see the figure). LIN-12 (the worm homolog of Notch) specifies the 2° fate in P5.p and P7.p, whereas the epidermal growth factor signaling pathway specifies the 1° fate in P6.p. Cross-talk between the two pathways ensures the spatial precision of these cell fate decisions (6). To understand how LIN-12 signaling specifies the 2° fate, one must identify the target

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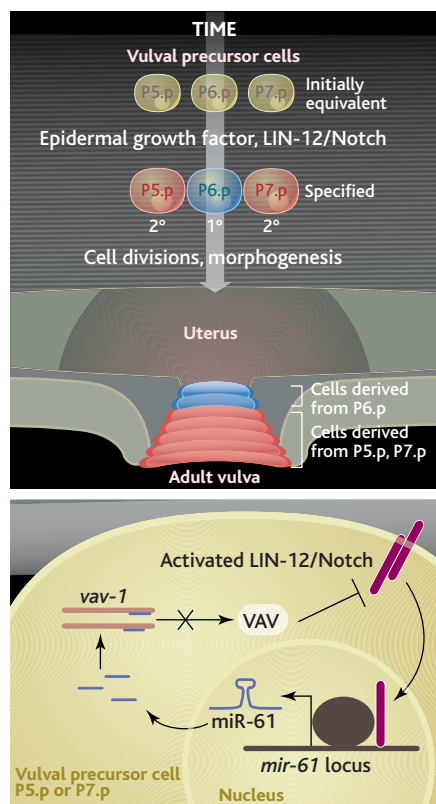
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genes whose transcription is turned on in response to LIN-12 activation. The LIN-12 target genes identified previously seemed to function by keeping epidermal growth factor signaling turned off in P5.p and P7.p (7, 8). Yoo and Greenwald now identify a target gene that promotes the 2° fate apparently independently of epidermal growth factor signaling. Interestingly, this gene encodes a microRNA, miR-61 (3).

Using computational methods, Yoo and Greenwald identified *mir-61* as a potential transcriptional target of LIN-12 signaling. Specifically, the *mir-61* locus was found to contain DNA sequences typical of those to which LIN-12/Notch receptors and their cofactors bind to regulate transcription of a target gene. The authors verified that *mir-61* is a true LIN-12 target gene in P5.p and P7.p by using a fluorescent reporter protein whose expression was driven by the *mir-61* regulatory region, including the predicted target sites. The reporter protein was visible in P5.p and P7.p, but was no longer expressed in those cells when the predicted target sites were mutated. When miR-61 was expressed in P6.p, where it is not normally expressed, the microRNA was sufficient to cause P6.p to adopt 2°, rather than 1°, cell fate characteristics (3). These results show that expression of a microRNA can be directly activated by a signaling pathway and that this can influence cell fate decisions (see the figure).

Yoo and Greenwald found that the worm gene *vav-1*, homologous to the vertebrate oncogene *Vav*, contains sequences in its 3' UTR that are complementary to miR-61, and predicted that miR-61 could promote the 2° fate by repressing the production of VAV-1 protein (3). They confirmed this with two elegant experiments, again using fluorescent reporter proteins. The authors first showed that fusing the 3' UTR of *vav-1* to the messenger RNA encoding the reporter protein is sufficient to down-regulate expression of the reporter when miR-61 was present in the same cell. They then showed that the miR-61 binding sites in the *vav-1* 3' UTR are critical for down-regulating *vav-1* expression in P5.p and P7.p. When *vav-1* regulatory sequences were used to express the reporter, fluorescence was observed in vulval precursor cells but not in P5.p and P7.p, where endogenous miR-61 is expressed. However, when the miR-61 binding sites in the 3' UTR were mutated, the fluorescent protein became visible in P5.p and P7.p.

Finally, when Yoo and Greenwald lowered *vav-1* activity, LIN-12 was better able to specify the 2° fate, indicating that *vav-1* antagonizes *lin-12* activity in vulval precursor cells (3). Importantly, *vav-1* does not accomplish this by promoting the 1° fate, because in this experiment, epidermal growth factor signaling was removed.



A microRNA, miR-61, regulates a major developmental signaling pathway. (Top)

Simplified schematic view of *C. elegans* vulval development. Vulval precursor cell specification during larval development leads to the formation of an adult vulva. (Bottom) Signaling feedback loop proposed by Yoo *et al.* (3). The LIN-12/Notch receptor is activated in the vulval precursor cells P5.p and P7.p, resulting in the translocation of the receptor intracellular domain to the nucleus. There, it binds to cofactors and activates *mir-61* expression. miR-61 blocks expression of its target, messenger RNA encoding VAV-1, to promote 2° fate.

Together these observations suggest a feedback loop in P5.p and P7.p in which LIN-12 signaling directly activates expression of miR-61, thereby preventing the expression of VAV-1 protein, which could otherwise interfere with *lin-12* activity (see the figure). The precise molecular mechanism whereby *vav-1* opposes *lin-12* activity should prove to be interesting.

Yoo and Greenwald therefore show a new role for a microRNA in an important signaling pathway—in this case, as a direct target of LIN-12/Notch and as a determinant of the 2° cell fate. This is one of the very first descriptions of microRNA involvement in a signaling pathway. The only other study implicating microRNAs in the LIN-12/Notch pathway was carried out by Lai *et al.*, although in fruit flies (9). Lai *et al.* found that many protein-encoding genes whose transcription is up-regulated by Notch signaling are also posttranscriptionally

repressed by certain ubiquitously expressed microRNAs, and that this repression is important to prevent overexpression of the Notch targets. Lai *et al.* suggested a model whereby these microRNAs help dampen or “tune” expression of these target genes in Notch-responsive cells.

The evidence provided by Yoo and Greenwald points to a more specific role for *mir-61* in influencing a cell fate decision compared with the proposed “tuning” role of microRNAs in fly Notch signaling. This highlights a major question in the micro RNA field: How is it that microRNAs seem to be so remarkably versatile? For example, why do some microRNAs seem to function as developmental switches, controlling cell fate decisions by turning off a few key target genes, whereas other microRNAs seem to function more globally to modulate many target genes in many cells, apparently to ensure the precision of gene expression patterns?

In theory there are several factors that may contribute to these diverse roles. First may be the specificity of the expression of the microRNA; thus, a microRNA that is expressed in response to a particular signal may have a more specific function than a microRNA that is more ubiquitously expressed. Second, the number of target messenger RNAs that a microRNA finds is also likely to be important. A microRNA with very few critical targets, such as the canonical worm microRNA *lin-4* (1), is likely to act in a more specific manner than a microRNA with many targets. Finally, it is worth noting that a single microRNA may in fact function as a developmental switch in some circumstances and in “tuning” expression in other circumstances. For example, *mir-61* appears to be a LIN-12 target in some cells, but not others (3), so the role *mir-61* plays in these various cells may be quite different. Careful analysis of loss-of-function microRNA mutants, including *mir-61*, will be important to address this question. Such analysis may be complex because of redundancy between microRNAs that target the same messenger RNA (10).

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