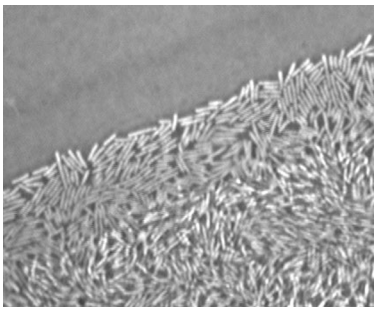

Figure 1. A video image taken in phase contrast of *E. coli* cells (wild-type strain AW405) swarming on 0.45% Eiken agar (in Eiken broth + 0.5% glucose) at room temperature.

A slight change in contrast extending into the cell-free region about 20 μm in front of the swarm indicates that the agar is conditioned in some manner. Cells near the edge of the swarm are in a monolayer in a quiescent band about 20 μm wide. Farther back, cells shuffle back and forth in coherent packs. Farther back still, cells are in multilayers, arranged in sub-domains that swirl, either clockwise or counterclockwise. For video clips showing swarming *Salmonella* (or *Serratia marcescens*, which swarms more vigorously) go to http://www.rowland.harvard.edu/labs/bacteria/index.html and click on Movies.

sigma factor σ^{28}, is suppressed by the anti-sigma factor FlgM. FlgM is pumped out of the cell by the flagellar transport apparatus once assembly of the basal part of the flagellum is complete [10]. This prevents the cell from wasting energy on flagellin synthesis when this protein cannot be put to use. When all goes well, filaments grow at their distal tips, with the flagellin subunits assembling beneath a terminal cap [11]. Wang *et al*. [9] found that when the plates are dry, flagellin assembly fails and FlgM is not excreted. This was shown directly by assaying for FlgM in the external medium. So FlgM builds up in the cytoplasm, and late-gene expression is suppressed. This build up was prevented by construction of a *cheY flgM* double mutant, which restored late-gene expression. But the flagella remained short, so the filament assembly defect is dominant. Evidently, when flagellin backs up in the filament, FlgM can no longer escape.

The real question, then, is why chemotaxis signaling mutants produce colonies that are dry. Is this just a matter of flagellar mechanics, or are the reasons more profound? How do cells make plates wet, anyhow? Wang *et al*. [9] speculate that flagellar filaments might stick to the swarm agar, and that the ability of the motor to change directions is important for them to unstick. Once unstuck, they stir, whipping lipopolysaccharide off the surface of neighboring cells. Lipopolysaccharide is known to have a surfactant/wetting function [12]. Cute. Perhaps it is time to learn what the flagella are actually doing.

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Germ Cells: Sex and Repression in Mice

The mouse *Blimp1* gene encodes a transcriptional repressor that is essential for B-cell development. Recent studies have shown that the Blimp1 protein also plays a critical role in the specification of mouse primordial germ cells.

Erez Raz

Primordial germ cells (PGCs), the progenitors of the gametes, sperm or egg, are typically segregated from all other cell lineages early in embryonic development. In mouse, and by extension other mammals, the PDFs are specified from a group of pluripotent cells in response to signalling events mediated by proteins of the bone morphogenetic protein (BMP) family [1,2]. These signals induce germ cell competence around embryonic day (E) 6.5, but it is only at E7.2 that a segregated population of PGCs is established. Defining the events that occur within this time frame and lead to the specification of the small PGC population (40–45 cells) is important for understanding of the molecular circuitry that control germ cell development, and likely also provide clues of general relevance to other, similar cell differentiation processes.

Detailed analysis of germ cell specification in mouse has been

carried out by monitoring the transcription profile of individual cells that reside in the region where the PGCs are induced [3]. This analysis provided a clear demonstration of the dynamic process by which the germ cell fate is acquired (Figure 1). Cells destined to give rise to PGCs exhibit high levels of the *fragilis* RNA, which encodes a member of the interferon-inducible transmembrane protein family, and then of the *stella* RNA, which encodes a novel protein that may function in chromatin remodelling or RNA processing. At the same time, however, strong repression of region-specific homeobox genes — *Hoxa1, Hoxb1, Lim1 and Evx1* — is observed in these cells.

Thus, transcription of specific germ cell markers, such as *stella*, and concomitant repression of genes characteristic of the neighbouring somatic cells may be characteristic of the path leading to mouse PGC specification. Interestingly, studies focusing on early germ cell development in *Drosophila* and *Caenorhabditis elegans* have also emphasized the role of transcription repression in establishing the germline lineage. In these organisms, in which the germ cell fate is dictated by maternally provided determinants, transcription is reduced in early germ cells [4–7]. The functional importance of the transcriptional repression in *C. elegans* has been demonstrated using mutants in which, in the absence of such a repression, the germline cells adopt somatic fates [5].

Counteracting somatic fates by repressing gene expression is thus an important step in establishing the germline lineage in different organisms, but the molecular basis for this phenomenon in mouse PGC development was until recently not known. Recent work from the Robertson [8] and Surani [9] labs has changed this situation by defining the role of B-lymphocyte-induced maturation protein-1 (Blimp1) in PGC specification.

Blimp1 is a transcriptional repressor with Krüppel-type zinc fingers which has previously been shown to play a critical role in the development of immunoglobulin secreting cells [10–12]. Specifically, forced expression of Blimp-1 is sufficient to drive B-cell differentiation into plasma cells and, conversely, inactivation of Blimp1 brings B-cell terminal differentiation to a halt. Importantly, Blimp1 promotes plasma cell differentiation by extinguishing the expression of genes required for earlier aspects of B-cell development, while allowing the expression of other genes whose function is important for plasma cell function, for example, genes involved in immunoglobulin secretion.

The expression pattern of *Blimp1* in the mouse embryo [13], coupled with a functional analysis of the gene in *Xenopus* [14] and zebrafish [15], suggested that the protein is required for development of various mouse cell types in addition to antibody producing cells. Indeed, *Blimp1* loss-of-function mouse mutants die with multiple defects, such as increased apoptosis, abnormal vasculature development and disrupted branchial arch morphology [8]. Importantly, *Blimp1* is also expressed in mouse PGCs during early stages of their development [8,9,13].

The unique expression pattern of *Blimp1* in the region where the founder PGCs reside has provided key clues concerning the critical role it plays in establishing the germline [9]. Notably, *Blimp1* is expressed already at E6.25 in a single cell layer of epiblast cells at one end of the short axis that will eventually occupy a proximal posterior position of the embryo. This expression is unlike that of *fragilis*, which at this stage is detected in the entire region of the proximal epiblast and is detected across several cell layers. This suggests that *Blimp1* expression may be regulated by other signalling factors in addition to BMPs. A day later (E7.25), 20–25 *Blimp1*-expressing cells are identified within the *fragilis* positive cell population and a few of these cells start expressing the definite PGC marker *stella*. Further studies demonstrated that *Blimp1*-expressing cells are restricted to the germline lineage, thus defining this gene as the earliest known marker for mouse PGCs [9].

To determine whether Blimp1 expression is in fact essential for early germ cell development, both groups generated null alleles of the gene and followed the fate of the PGCs [8,9]. Indeed, a dramatic reduction (more than 90%) in the number of PGCs was observed in mutant animals compared with wild-type counterparts. Significantly, analysis of heterozygous animals revealed a reduction in PGC number as well, indicating a strong sensitivity for Blimp1 level by the early germline. Notwithstanding the reduced PGC number in heterozygous embryos, these cells exhibited normal proliferation rate and migration.

These findings reflect a requirement for Blimp1 activity in determining the size of the founder cell population, but additional roles in other processes such as cell fate maintenance or survival have not been ruled out. These results are strikingly similar to those obtained with BMP4, the factor provided from the extraembyonic ectoderm to determine the initial size of the PGC population [2]. But in contrast to BMP4, the function of which is important also for the development of other derivatives of proximal epiblast cells, such as the allantois, Blimp1 function appears to be specifically required for PGC development.

What is the role of Blimp1 in early PGC development? A partial answer to this question came from analyzing the cellular phenotype of PGCs lacking Blimp1 [9]. In contrast to wild-type PGCs, which exhibit active migration just after their specification [16], the few *Blimp1* mutant PGC-like cells that did form remained together and did not leave their original cluster [9]. Therefore, one of the earliest and basic manifestations of the PGC fate, assuming motile behaviour, depends on the function of Blimp1.

As mentioned above, an important step in PGC specification is suppression of the ‘somatic program’. Is Blimp1 responsible for the repression of

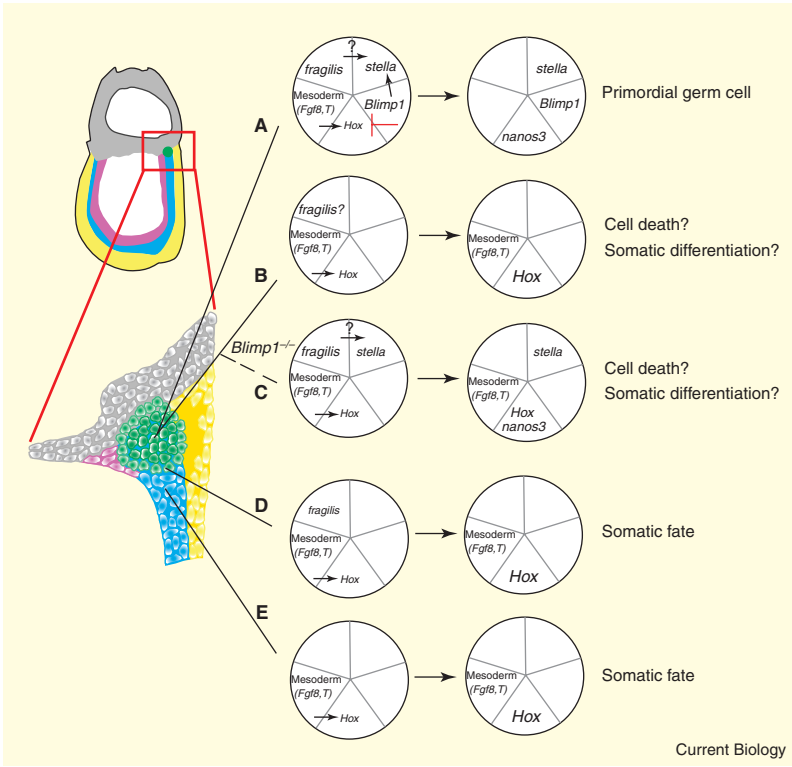

Figure 1. A model illustrating some of the events occurring in the niche where PGCs are specified.

BMP signals and presumably other signals from the extraembryonic ectoderm and primary endoderm cells predispose cells to give rise to common precursors of extra-embryonic mesoderm and PGCs. Germ cell specification correlates with the expression level of *fragilis* and depends on Blimp1 function. (A) Some of the cells expressing high levels of *fragilis* (dark green area) express *Blimp1* and subsequently *stella*. In these cells, Blimp1 inhibits the somatic program (for example, region-specific *Hox* gene expression) resulting in development into PGCs that later express PGC markers such as *nanos*3. (B) Most *Blimp1*-deficient cells do not express *stella* C. In rare cases, cells lacking *Blimp1* function that also express *stella* can be found. These cells fail to repress the somatic genes and do not develop into PGCs. (C and D) Cells that express low levels or no *fragilis* and do not express *Blimp1* develop into somatic cells. The arrows signify a requirement for the activity of a certain gene for the activation or suppression of the transcription of another gene rather than a direct interaction between the corresponding genes.

genes that should only be expressed in somatic cells? Ohinata *et al.* [9] obtained strong evidence in support of this possibility by analyzing the transcription of specific genes in PGCs lacking Blimp1 function. These rare mutant PGC-like cells, defined as *Blimp1* negative and *stella* positive cells, showed inconsistent gene expression patterns: most exhibited defects in repression of *Hoxa1* and *Hoxb1* expression; some lacked expression of PGC markers such as *Sox2* and *Nanos3*; and some coexpressed the *Hox* genes and the PGC markers (Figure 1C).

This work highlights the conservation of the principles governing germline development in different organisms. For example, whereas the molecular events directing cells to assume the germline fate differ between mouse and the invertebrate models such as *Drosophila* and *C. elegans* [17], the PGCs of these organisms exhibit early transcription repression albeit by employing different molecules to achieve it. Additionally, the finding that the development of both PGCs and immunoglobulin secreting plasma cells depend on the function of the same molecule is a demonstration of how cell differentiation modes are conserved between germline and somatic cells. Similarly, during their migration towards the gonad, PGCs respond to the same molecules used by somatic cells such as those of the haematopoietic system, as well as by migrating cells involved in pathological disorders, such as cancer cells [18–20]. Therefore, in addition to understanding the basis for germ cell specification, development and behaviour, an interesting focus on its own right, defining the principles and molecules governing these processes in this lineage is of general interest to biologists in a broad range of fields.

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MicroRNAs: Loquacious Speaks out

In *Drosophila*, Dicer-2 requires the double-stranded RNA binding protein R2D2, to mediate the assembly of short interfering RNAs into the RNA-induced silencing complex. New data show that Dicer-1 also requires a double-stranded RNA binding protein called Loquacious for efficient microRNA-mediated gene silencing.

Philipp J. F. Leuschner, Gregor Obernosterer and Javier Martinez

A major breakthrough in molecular biology was the finding that eukaryotic cells harness mechanisms of RNA interference (RNAi) to regulate the expression of endogenous genes [1,2]. Just as in the 'canonical' RNAi pathway, short single-stranded RNA molecules called microRNAs (miRNAs) serve as sequence specific guides to target silencing complexes containing an Argonaute (Ago) protein to cognate sequences — typically in the 3′-untranslated region of target messenger RNAs [3].

miRNAs originate from long primary transcripts (pri-miRNAs) [4], which are processed in the nucleus by the RNase III-like enzyme Drosha and its cofactor Pasha/DCGR8 into ~65 nt, hairpin shaped precursors, or pre-miRNAs [5–8]. Pre-miRNAs are exported to the cytoplasm, where another RNase III-like enzyme, Dicer, liberates a ~22 nt long miRNA duplex from the hairpin. One strand of the duplex is integrated into an active RNA-induced silencing complex (RISC) [9].

While human Dicer is able to process long double-stranded RNA (dsRNA) as well as pre-miRNAs, the two pathways are separated in *Drosophila* (Figure 1). Here, Dicer-2 does not play a role in miRNA biogenesis, but is required to cleave long dsRNAs into short interfering RNAs (siRNAs), which are then assembled into siRISCs (Figure 1A). Dicer-1 processes pre-miRNAs and loads the resulting miRNAs into miRISC containing Ago-1 (Figure 1B). However, Dicer-1 also seems to be required downstream of siRNA-production in siRISC assembly [10–12]. It has been shown that the activities of Drosha and Dicer-2 absolutely depend on the auxiliary dsRNA binding domain (dsRBD) proteins, Pasha and R2D2, respectively [5–8,13]. Now Saito *et al*. [14] and Förstemann *et al.* [15] provide a missing piece in the RNAi versus miRNA puzzle: they conclusively demonstrate that Dicer-1 also requires a dsRBD protein to efficiently process pre-miRNAs into miRNA duplexes. Saito *et al*. [14] relied on an RNAi-based functional screen for *Drosophila* dsRBD proteins that affect miRNA biogenesis, while Förstemann *et al*. [15] searched a database for conserved dsRBDs containing proteins. Both laboratories identified the same candidate — a paralogue of *Drosophila* R2D2, featuring two canonical and one non-canonical dsRBDs. This candidate was baptized *loquacious* (*loqs*), as endogenous RNA-mediated silencing is lost in mutant flies.

Both groups show that reduced levels of Loqs result in the accumulation of endogenous pre-miRNAs. The same phenotype is observed when cells are depleted of Dicer-1, but not Dicer-2 or R2D2. The physical association of Loqs and Dicer-1 was confirmed by reciprocal co-immunoprecipitation, and did not depend on a pre-miRNA substrate. Saito *et al.* [14] demonstrate that both proteins also co-immunoprecipitate with Ago1, providing further evidence that miRNA processing may be directly linked to the assembly of miRISC. If assayed for processing activity *in vitro*, immunoprecipitates of Dicer-1 or Loqs readily generated mature miRNAs from synthetic precursors and were also found to associate with pre- and mature miRNAs *in vivo.*

So what is the actual function of Loqs in this complex? It is certainly not simply the stabilization of Dicer-1, because Dicer-1 protein levels did not decrease significantly in the absence of Loqs. According to Saito *et al.* [14], Loqs confers substrate specificity for pre-miRNAs to Dicer-1. Surprisingly, Dicer-1 processes long dsRNA as well as pre-miRNA substrates, if Loqs is removed from the complex. Re-addition of Loqs inhibited dsRNA processing and enhanced pre-miRNA processing. But how is this achieved? Three splice variants of *loqs* are known, of which only two isoforms interact with Dicer-1. Interestingly, the third isoform lacks the non-canonical dsRBD, suggesting that this domain may be essential for association with Dicer-1. Loqs could enhance the binding