Gene Regulation: A Tale of Germline mRNA Tails

Gene regulation often plays by different rules in the germline compared to the soma. In *Caenorhabditis elegans*, the spatial and temporal expression of germline genes is controlled post-transcriptionally via the 3′ UTR rather than transcriptionally via the promoter.

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Over the past several years, much of the research in gene regulation has focused on post-transcriptional mechanisms. With all the exciting discoveries in this field, however, it still remains to be seen how prominent the role of post-transcriptional regulation is in establishing and defining gene expression patterns, especially relative to transcriptional regulation. One tissue where post-transcriptional regulation has been long suspected to play a prominent role is the germline [1]. Many proteins that bind the 3′ and 5′ UTRs of mRNAs have diverse roles in the germline, such as maintaining germ-cell identity and ushering germ cells through the transitions from immature mitotic progenitors into meiosis and gametogenesis. For example, in the nematode *Caenorhabditis elegans*, concomitant loss of the RNA-binding proteins GLD-1 and MEX-3 causes germ cells to lose their identity and differentiate into a variety of somatic fates [2]. Additionally, two related RNA-binding proteins, FBF-1 and FBF-2, are required for the maintenance of germ cell progenitors and the switch from spermatogenesis to oogenesis in hermaphrodite worms [3,4]. However, only a small handful of mRNAs are known targets of RNA-binding proteins, and the corresponding consensus binding sequences are sufficiently unstructured to prevent reliable identification solely by computational methods. Thus, the generality of this mode of regulation, and its importance relative to transcriptional control, has not been well defined to date.

To address this question in a systematic way, members of the lab of Geraldine Seydoux writing in this issue of *Current Biology* [5] performed a series of transgenic reporter assays in the *C. elegans* germline, in which either promoters or 3′ UTRs were linked to a GFP reporter gene. The promoters and 3′ UTRs come from 30 genes whose protein products display 17 distinct spatial expression patterns. These patterns are specific for one or more germ cell types, including progenitor cells, meiotic cells, spermatocytes and oocytes. Strikingly, for 24 of the 30 different germline-expressed genes, the native 3′ UTRs were sufficient to cause GFP localization to closely mirror the endogenous protein expression pattern. Conversely, the promoters of these same genes did not confer any obvious cell-type specificity, but appeared generally permissive for low levels of expression in all germ cell types, beginning in larval progenitor cells.

These data suggest that transcription is initiated in immature germ cells and continues through all subsequent stages of germ cell development until the formation of mature sperm and oocytes, which are transcriptionally quiescent. From this ubiquitous expression, post-transcriptional regulation via the 3′ UTR then sculpts cell type-specific expression patterns by preventing or allowing protein expression at certain points during germ cell development. Which trans-acting factors are the sculptors? RNA-binding proteins, such as GLD-1 and MEX-3, appear to be at least partially responsible for defining cell-type specific expression patterns. Decreased levels of GLD-1 and MEX-3 caused the expression domain for many of these 3′ UTR reporters to expand from a more restricted pattern into a broader, less specific pattern [5]. Therefore, regulation via the 3′ UTR, possibly mediated by RNA-binding proteins, is the primary regulatory mechanism for many genes with diverse spatial and temporal protein expression patterns in the germline (Figure 1).

In multiple species, the embryonic germline employs chromatin- and transcription-based mechanisms to globally inhibit gene expression [6,7]. The prevailing hypothesis is that this transcriptional inhibition protects the germline from transcriptional programs that drive differentiation in somatic cells of the embryo [8]. By contrast, the general permissiveness of promoters in the larval and adult germline implies that the germline switches from this initial embryonic repressive state to a globally permissive state. This permissive state might inadvertently permit the expression of genes with roles in somatic differentiation. Post-transcriptional regulation could, therefore, be in place to inhibit such somatic transcripts from being translated. Consistent with this idea, *gld-1 mex-3* mutant germ cells undergo trans-differentiation into diverse somatic cell types such as neurons, muscle and intestine, implying that pre-existing transcripts present in the germline are inappropriately translated and promote somatic fates [2]. Thus, RNA binding proteins such as GLD-1 and MEX-3 might have dual roles in the germline, both to guard against inappropriate translation of somatic mRNAs, as well as to define the spatial patterns of germline mRNAs.

The apparent predominance of post-transcriptional regulation certainly does not preclude a contribution of transcriptional regulation toward defining some germline expression patterns. Regulation via the promoter might, for instance, provide an extra boost of expression at some point during germ cell development. In line with this idea, a subset of the genes that rely primarily on their 3′ UTRs also have E2F binding sites in their promoters and are responsive to the E2F transcription factor in germ cells in the mid-pachytene stage of meiosis [9]. Additionally, not all of the 30 genes tested in the transgenic reporter assay relied on the 3′ UTR [5]. Indeed, for five of the 30 genes, 3′ UTRs are dispensable and the promoters of these genes dictate the proper spatial and temporal restriction of gene expression.

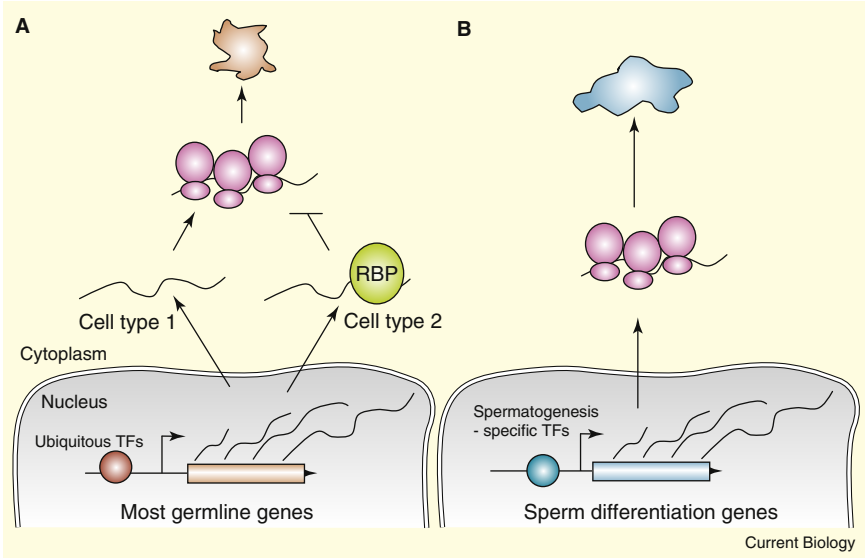

Figure 1. Two modes of germline gene regulation.

(A) Most genes expressed in the germline are broadly transcribed, and then depending on the presence of RNA-binding proteins (and likely small RNAs), the ability of the mRNA to be translated is permitted or inhibited in distinct germ cell types. (B) By contrast, genes expressed during spermatogenesis are regulated transcriptionally, likely by spermatogenesis-specific transcription factors (TFs). Once transcribed, the mRNA is generally translated without major post-transcriptional regulation.

Strikingly, all five genes are expressed primarily or specifically during spermatogenesis, suggesting that gene expression in this particular cell type is preferentially controlled transcriptionally, rather than post-transcriptionally (Figure 1). Thus, transcriptional control can dictate a specific expression pattern for at least one type of germ cell, although it is not the primary mode controlling spatial gene regulation in the *C. elegans* germline.

Why are genes expressed during spermatogenesis preferentially regulated transcriptionally, when the dominant mode of gene regulation in the rest of the germline appears to be post-transcriptional? During sperm differentiation, most cytoplasmic components — including mRNAs, RNA-binding proteins and ribosomes — are discarded into a 'residual body' [10] in order to minimize the size of the sperm. Because these cytoplasmic components are lost, it makes sense that spermatocytes rely instead on transcriptional regulation in the nucleus. Additionally, sperm and oocytes make unequal contributions to the embryo upon fertilization. Mature sperm retain minimal cytoplasm and, therefore, deliver relatively few mRNAs to the embryo. By contrast, oocytes provide an abundance of maternal mRNAs to the embryo. Because the embryo is initially transcriptionally quiescent, these mRNAs need to be regulated post-transcriptionally. The embryonic germline remains transcriptionally silent even after somatic cells have initiated transcription, relying even more heavily on post-transcriptional mechanisms of regulation. Thus, with the exception of spermatogenesis, post-transcriptional regulation modulates gene expression at all stages of germ cell development.

The post-transcriptional regulatory network in the germline is probably at least as complex as that governing transcription in other tissues. Many RNA-binding proteins beyond GLD-1, MEX-3, and the FBFs are preferentially expressed in the *C. elegans* germline [11], and a large fraction of germline mRNAs are likely to be influenced by the action of these diverse RNA-binding proteins. The next step is to clarify the underlying structure and logic of the network. Transgenic assays similar to that employed in the Seydoux lab [5] should permit systematic investigation of the requirement of specific sequences in the 3′ UTRs. Biochemical analysis will determine which RNA-binding proteins affect mRNA stability, localization and availability to the translational machinery. A potential role for miRNAs or other small RNA species in controlling gene expression via the 3′ UTRs of *C. elegans* germline mRNAs is currently unknown, but will be a fascinating possibility to explore. And finally, determining whether at least some somatic tissues utilize post-transcriptional regulation as prominently as the germline will be an important endeavor for the future.

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DOI: 10.1016/j.cub.2008.08.001