Translational repression: **A duet of Nanos and Pumilio**

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Recent studies have shed new light on translational repression by Nanos and Pumilio proteins. The ancestral function of this repression mechanism appears to be in early germline development; later, species-specific applications in embryonic patterning and spermatogenesis–oogenesis switching evolved.

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Studies of how gene expression is regulated during development frequently focus on control at the level of transcription, but regulation at the level of mRNA translation plays an important part in determining when and where certain specific proteins are produced. A major aspect of translational regulation is the directed repression of target mRNAs — a mechanism that is heavily used during early embryonic and germline development in diverse organisms.

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nanos and *pumilio* genes. Recent studies have shed new light on the biochemical mechanism by which Nanos and Pumilio interact to control repression, and also the way that this repression mechanism has been put to different uses during metazoan evolution.

The *nanos* and *pumilio* genes were first discovered as key components of a common pathway that acts in the posterior patterning of the *Drosophila* embryo. The Nanos and Pumilio proteins achieve their patterning function by suppressing the translation of the maternally supplied *hunchback* transcript progressively towards the posterior of the embryo, thereby creating a concentration gradient of the Hunchback transcription factor across the embryo with its highest level at the anterior pole. This Hunchback protein gradient, in turn, choreographs a cascade of downstream target genes that lead to the formation of the segmental pattern of the embryo.

In addition to embryonic patterning, both *nanos* and *pumilio* function in various aspects of *Drosophila* germline development. In *Drosophila*, as in many other organisms, embryonic primordial germ cells are characterized by their transcriptional quiescence, mitotic arrest and extensive migration to the somatic gonadal sites. These features ensure the proper development of the germline. In the past four years, several studies have shown the essential role of *nanos* as a cell autonomous factor in the migration and transcriptional quiescence of embryonic primordial germ cells, as well as in the maintenance of germline stem cells during oogenesis in adult *Drosophila* [1,2]. Similarly, *pumilio* is also required for germline stem cell maintenance during oogenesis [2,3]. These findings suggest that the Nanos/Pumilio-mediated translational repression mechanism plays an important role in multiple processes during germline development.

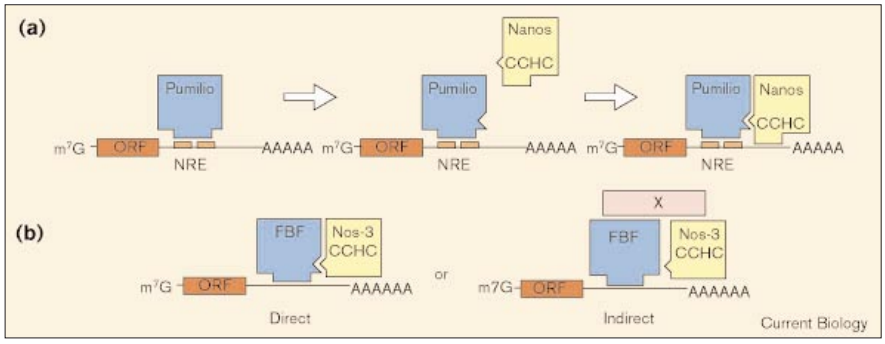
Two recent studies [4,5] have further underscored the importance of the Nanos/Pumilio-mediated mechanism in primordial germ-cell development. Deshpande *et al.* [4] tracked back the action of *nanos* in primordial germ cell migration and transcriptional quiescence to the syncytial blastoderm stage — the initial period of germ-cell formation. At this stage, germ cells in embryos lacking maternal Nanos already start to express ectopically the sex determination gene *Sex-lethal* (*Sxl*) and somatic segmentation genes *fushi tarazu* (*ftz*) and *even-skipped* (*eve*). This derepression of gene expression appears to be at the transcriptional level. As Nanos is only known to repress translation, *Sxl*, *ftz* and *eve* are unlikely direct targets of Nanos. Instead, their transcrip-

the Nanos repression activity. Moreover, at the syncytial blastoderm stage, *nanos*⁻ germ cells fail to suppress cyclin B expression and slow down the cell cycle, suggesting that Nanos is also involved in mitotic quiescence as early as the initial stage of germ-cell development.

Does *pumilio* have a parallel function with *nanos* in *Drosophila* primordial germ cell development? A recent study by Asaoka-Taguchi *et al.* [5] has shown that indeed it does. They found, firstly, that *pumilio*⁻ primordial germ cells are similarly defective in migration into the embryonic gonads. Secondly, the *pumilio* deficiency disrupts transcriptional quiescence. And finally, the *pumilio*⁻ germ cells fail to arrest mitosis at the G2/M stage, but precociously express cyclin B. All these defects parallel those of *nanos*⁻ germ cells, suggesting that *pumilio* works together with *nanos* to regulate embryonic primordial germ-cell development. In addition, *pumilio* is required for postembryonic primordial germ-cell development, gonadogenesis and oogenesis, as we have recently shown [6]. Given the known parallel functions of *nanos* and *pumilio*, it would not be surprising to find that *nanos* is also involved in these processes.

In the nematode *Caenorhabditis elegans*, homologs of *nanos* and *pumilio* are also involved in multiple steps of germline development. Homologs of *pumilio* have been identified in

Figure 1



Models of Nanos and Pumilio interaction in *Drosophila* and *C. elegans*. **(a)** Formation of the ternary complex of Nanos, Pumilio and *hunchback* mRNA that represses translation of the mRNA. Pumilio protein binds specifically to the *hunchback* mRNA, allowing it to recruit Nanos by specific protein–protein interaction. This interaction may be assisted by the non-specific interaction of the Nanos zinc-finger domain (CCHC) with the *hunchback* mRNA, which may also contribute to the stability of the resulting ternary complex. **(b)** The putative Nos-3–FBF–mRNA complex in *C. elegans*. Nos-3 and FBF may interact directly, as shown on the left, or indirectly via an intermediate component (X), as shown on the right. FBF binds specifically to the 3′ untranslated region of *fem-3* mRNA via its RNA-binding domain; this protein–RNA interaction is facilitated by non-specific binding of Nos-3 to the RNA via its zinc-finger domain. The requirement for an additional component in the indirect model may explain why FBF was not found to interact with Nanos-1 or Nanos-2. ORF, open reading frame; NRE, Nanos response element. See text for details.

organisms ranging from yeast to vertebrates [7–10]. In *C. elegans*, as many as eight *pumilio*-like genes and three *nanos*-like genes — *nos-1*, *nos-2* and *nos-3* — have been identified, all of which appear to be required for regulating different aspects of germline development [8,10,11]. Subramaniam and Seydoux [10] have recently shown that *nos-1* and *nos-2* are required redundantly for mitotic quiescence of primordial germ cells in starved animals, for the incorporation of primordial germ cells into the embryonic gonad, and for maintenance of the germline during larval development. These functions are very similar to those of *Drosophila nanos* in the mitotic quiescence of embryonic germ cells and their migration into the somatic gonad, and in the maintenance of germline stem cells during oogenesis.

Subramaniam and Seydoux [10] have further shown that a subset of five *pumilio*-like genes in *C. elegans* are required redundantly for the same processes of germline development as *nos-1* and *nos-2*, suggesting that the Nanos-like and Pumilio-like proteins in *C. elegans* also work together to control the same processes of germline development. Similar results on *nos-1* and *nos-2* have recently been reported by Kraemer *et al.* [11], whose phenotypic analysis has revealed an additional role of *nanos* genes in the spermatogenesis-oogenesis switch that occurs during the lifetime of hermaphrodite nematodes. The redundant or overlapping function of the Nanos-like and Pumilio-like proteins in *C. elegans* is not unique, as it has recently been found that the *Drosophila pumilio* gene encodes two functionally interchangeable protein isoforms that are redundantly required for embryonic patterning, but both are required to provide a sufficient dose of Pumilio for germline development [6].

The *Drosophila* and *C. elegans* results show that evolutionarily distinct organisms share the *nanos*/*pumilio*-mediated mechanism for germline development, but not for embryonic patterning or spermatogenesis–oogenesis switching. Thus, the role of these two gene families in germline development most likely reflects their ancestral function, while their patterning role in *Drosophila* and the switching role in *C. elegans* are likely to be more recently acquired and specific to only subgroups of organisms [2]. But Nanos and Pumilio in *Drosophila* — or their corresponding homologs in other organisms — nevertheless have parallel functions in both ancestral or more recently evolved developmental processes, indicating that two proteins may directly interact in a common translational repression mechanism.

The close functional interaction between Nanos and Pumilio is supported by the results of molecular analyses. In the case of *Drosophila*, two 32 base-pair sequences — the Nanos response elements — were identified in the 3′-untranslated region of *hunchback* mRNA that are necessary and sufficient for Nanos-dependent translational repression [12]. Despite the genetic evidence that *nanos* is a regulator of Hunchback translation, a direct physical relationship between *hunchback* mRNA and Nanos protein had not been demonstrated. Instead, biochemical experiments showed sequence-specific binding of Pumilio to the Nanos response element sequences in *hunchback* mRNA [13]. This binding represses *hunchback* translation by promoting the deadenylation of *hunchback* mRNA, a modification that apparently makes the mRNA less stable [14]. It therefore remained unclear precisely how Nanos contributes to *hunchback* repression.

Sonoda and Wharton [15] have recently shown that Nanos suppresses *hunchback* translation by directly binding to Pumilio and the *hunchback* mRNA to form a ternary complex, as demonstrated by a modified yeast three-hybrid assay and by *in vitro* reconstitution. Interestingly, only RNA-bound Pumilio is capable of binding to Nanos. Mutational mapping showed that the bases in the center of the Nanos response element are critical for Pumilio binding.

The eighth — and last — repeat of the Pumilio RNA-binding domain is important for recruitment of Nanos, and a zinc-finger domain near the carboxyl terminus of Nanos is crucial for its binding to Pumilio. The non-specific RNA binding activity of the Nanos zinc-finger domain may help to stabilize the resulting ternary complex (Figure 1a). Arrizabalaga and Lehmann [16] have recently shown that Nanos's zinc-finger domain is essential for all its known functions in embryonic patterning, embryonic germline development and germline stem-cell maintenance during oogenesis. As the primary function of the Nanos zinc-finger domain during *Drosophila* embryogenesis is to interact with Pumilio, the observations of Arrizabalaga and Lehmann [16] suggest that Nanos is likely to function in all the other processes by forming a ternary complex involving Pumilio and an mRNA to be repressed.

The direct interaction between Nanos and Pumilio appears to be conserved during evolution. Previous studies showed that the spermatogenesis–oogenesis switch in the *C. elegans* hermaphrodite involves the translational repression of *fem-3*, a key regulator of the switch, by a Pumilio-like protein known as *fem-3*-binding factor 1 (FBF-1) [8]. FBF-1 represses the translation of *fem-3* mRNA by binding to a regulatory element in its 3′ untranslated region, just as Pumilio binds to the Nanos response elements in the 3′ untranslated region of *hunchback* mRNA [8]. In their recent study, Kraemer *et al.* [11] have reinforced this mechanistic parallel by showing that Nos-3 interacts with FBF in yeast two-hybrid and *in vitro* assays (Figure 1b). Interestingly, unlike the *Drosophila* case, the Nos-3–FBF interaction does not require *fem-3* mRNA; this may reflect a true mechanistic divergence between the two organisms. Somewhat surprisingly, neither Nos-1 or Nos-2 bound to FBF in either the yeast-two hybrid or *in vitro* protein binding assays [11]. This could be because FBF needs *fem-3* mRNA — or a different mRNA — to interact with Nos-1 and Nos-2. Alternatively, Nos-1 and Nos-2 may partner a different Pumilio-like protein, in an interaction that may or may not require a substrate mRNA. Despite these possibilities, the functional conservation of the Nanos/Pumilio-mediated mechanism between *Drosophila* and *C. elegans* is clearly evident.

To understand fully the developmental function of this evolutionarily conserved translational repression mechanism, it will be important to learn more about its target mRNAs. For the species-specific functions that have been characterized, posterior patterning in *Drosophila* and spermatogenesis–oogenesis switching in *C. elegans*, *hunchback* and *fem-3* mRNA, respectively, are known to be the main, if not the only, targets. Previous studies of *hunchback* and *fem-3* functions have already provided a good understanding of the developmental processes in which they are involved. But for the ancestral function of Nanos and Pumilio homologs in early germline development, the target mRNAs remain to be identified.

The results of Deshpande *et al.* [4] indicate that, in *Drosophila* germline development, *Sxl* is a main target of Nanos/Pumilio regulation, albeit not a direct one. As mentioned above, *nanos*⁻ embryonic germ cells transcribe *Sxl* mRNA prematurely. Somewhat surprisingly, removal of *Sxl* activity from the *nanos*⁻ mutant germ cells can alleviate the germ-cell defects in migration, mitotic arrest and transcriptional quiescence, while premature expression of *Sxl* can phenocopy the *nanos*⁻ defects, both in a non-sex-specific manner. These findings suggest that Sxl has a novel role in early germline development. As the early germ-cell function of Nanos and Pumilio is likely to be ancestral and well-conserved, the identification of the direct targets of their regulation in these cells, as well indirect targets such as *Sxl*, should significantly advance our understanding of germline development.

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