Chapter 8
Translational Control in the *Caenorhabditis elegans* Germ Line

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Abstract Translational control is a prevalent form of gene expression regulation in the *Caenorhabditis elegans* germ line. Linking the amount of protein synthesis to mRNA quantity and translational accessibility in the cell cytoplasm provides unique advantages over DNA-based controls for developing germ cells. This mode of gene expression is especially exploited in germ cell fate decisions and during oogenesis, when the developing oocytes stockpile hundreds of different mRNAs required for early embryogenesis. Consequently, a dense web of RNA regulators, consisting of diverse RNA-binding proteins and RNA-modifying enzymes, control the translatability of entire mRNA expression programs. These RNA regulatory networks are tightly coupled to germ cell developmental progression and are themselves under translational control. The underlying molecular mechanisms and RNA codes embedded in the mRNA molecules are beginning to be understood. Hence, the *C. elegans* germ line offers fertile grounds for discovering post-transcriptional mRNA regulatory mechanisms and emerges as great model for a systems level understanding of translational control during development.

Keywords RNA regulatory network • Post-transcriptional RNA regulation • Translational control • RNA-binding protein • Polyadenylation • Deadenylation • RNA decay • miRNA

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8.1 Introduction

Development depends on the coordinated execution of gene expression programs in a spatial and temporal manner. These programs are regulated at the transcriptional and post-transcriptional level. The *Caenorhabditis elegans* germ line has emerged as a model for both the requirement and complexity of post-transcriptional gene expression control. The importance of post-transcriptional control is reflected at the simplest level by the sheer number of RNA regulatory proteins identified that play important roles in the development and function of the germ line. By contrast, transcription seems to be the primary mechanism for regulating gene expression in most somatic tissues.

Possible explanations for the dominance of translational control are the germ line's special organization, its unique functionality and the short time frame available for gametogenesis and early embryogenesis in *C. elegans*. The adult germ line is a syncytial tube-like organ, which harbors germ cells of two different cell cycles and many stages of differentiation. A pool of mitotically dividing cells is located at its distal most end. Germ cells undergoing the meiotic program are spatially arranged in the remaining tissue in a distal to proximal fashion (see Lui and Colaiácovo 2012). In adult hermaphrodites diakinesis-stage oocytes remain associated with the germline syncytium until they fully cellularize. Differentiated oocytes at the very proximal end complete meiotic progression with oocyte maturation are ovulated, fertilized and undergo the meiotic divisions (see Kim et al. 2012). While sperm production happens in the hermaphrodite prior to oogenesis, spermatogenesis is a continuous process in the male with spermatocytes cellularizing before diakinesis (see Chu and Shakes 2012). Consequently, germ cells have to undergo two different types of cell divisions, mitosis of undifferentiated cells and meiosis of two sex-specific differentiation programs, which occur in a spatially and temporally distinct manner while sharing a common cytoplasm. These constraints demand a system that supports cytoplasmic gene regulation, such as translational control.

In addition to the germ line's spatial character, a number of functional criteria argue for the importance of post-transcriptional regulation. From diakinesis until after the completion of the first mitotic division, all chromosomes are highly condensed. Consequently, the transcriptional machinery has restricted access to the genome, therefore limiting the reach of transcriptional control in these stages (see Robertson and Lin 2012). Also, female meiosis is completed after oocyte–sperm fusion and maternally donated mRNA and protein factors continue to direct early embryogenesis, as zygotic gene transcription does not begin in somatic blastomeres until the four-cell stage (see Robertson and Lin 2012). Furthermore, a fundamental characteristic of germ cells is their totipotency, allowing the zygote to differentiate into all somatic tissues after fertilization. Ectopic germline expression of master transcription factors necessary for specific somatic differentiation programs, or the elimination of certain post-transcriptional regulators leads to a loss of germ cell fate identity and the formation of neuronal, muscle, or gut cells in the germ line (Ciosk et al. 2006; Tursun et al. 2011). It is conceivable that general transcriptional

activity is tightly controlled to avoid unwanted cell fate commitment. Lastly, post-transcriptional control may offer a speed advantage. In transcriptional control an input signal has to be transmitted into the nucleus, the appropriate mRNA has to be at first synthesized, matured, quality controlled, and exported to the cytoplasm. In contrast, an input signal received by the cytoplasmic post-transcriptional control machinery accesses directly a pool of pre-made cytoplasmically localized mRNAs, awaiting translation.

8.2 Fundamentals of Translational Control

8.2.1 Life of an mRNA and the Concept of mRNPs

From its birth until its death, an mRNA passes through many different activity states and subcellular territories. One can globally divide the life of an mRNA into nuclear and cytoplasmic phases. In the nucleus the pre-mRNA is transcribed, co-transcriptionally modified at each end and spliced, before the mature mRNA is exported through the nuclear pores. In the cytoplasm, the mRNA's lifetime is marked by its translation and degradation. Over the past two decades a picture has emerged in which cytoplasmic RNA regulatory events are important gatekeepers that control the amount of protein synthesis in nearly all stages of germ cells development.

In the different subcellular territories, diverse proteins associate with any given mRNA, forming biochemically definable entities termed mRNA-protein particles (mRNPs). In general, activity state-specific mRNPs are considered as functional units and accompany the mRNA throughout its life. In these units mRNAs are structurally modified or transported within the cell or a syncitial tissue. More importantly, mRNPs represent integration platforms for developmental control. The nature of an mRNP is defined by its protein components and its subcellular location (discussed in Sect. 8.4.3). Proteins of an mRNP contact the RNA either indirectly or directly as designated RNA-binding proteins. Different families of RNA-binding proteins are encoded in the worm genome, of which many show either germline-specific expression or germline-enriched expression. Some of the best-studied examples are discussed in Sect. 8.4.1.

The mRNA itself provides the *cis*-regulatory information that is decoded by RNA-binding proteins recognizing structural and/or sequence-specific elements. A typical mature mRNA consists of unique parts encoded in the DNA sequence and non-encoded generic parts. DNA-encoded parts are the *O*pen *R*eading *F*rame (ORF), which serves as the protein synthesis template, and the 5′ and 3′ *un*translated *r*egions (UTRs), which flank the ORF (Wilkie et al. 2003). Both UTRs can possess valuable information that influences the mRNA's capacity to serve as a protein synthesis template. Regulatory features of the 5′UTR that have negative effects on protein production are short upstream open reading frames (uORFs) and stem-loop structures or binding sites that are recognized by RNA-binding proteins. 5′UTR structures that can have positive effects on protein production are internal

ribosome entry sites (IRESes) (Jackson 2005). Although a number of cases underlining the importance of these features have been described in yeast, *Drosophila* and mammals, examples in the worm remain to be identified. Features of the 3′UTR include interaction sites for sequence-specific RNA-binding proteins and microRNA-containing RNA silencing complexes. Numerous examples of 3′UTR-mediated controls exist in the *C. elegans* germ line and are discussed in Sect. 8.5. Most importantly, two structures that are not encoded in the DNA but are present on every mRNA are the 5′cap structure and the 3′ poly(A) tail. Both structures are added to the mRNA in the nucleus as part of the mRNA maturation process and are essential for stability and translatability (discussed in Sects. 8.3.1 and 8.3.2). It is the combination of all these mRNA-intrinsic features that influence the capability and strength of post-transcriptional regulation. The synergy or antagonism of multiple features and the availability of the *trans*-acting RNA regulators form the basis for mRNA-specific translational effector networks, organized in larger mRNP units, which is the topic of Sects. 8.4.2 and 8.4.3.

8.2.2 Events in the Cytoplasm: mRNA Quantity Versus Translatability

In the cytoplasm, an mRNA encounters three different fate choices. (1) It may enter directly the translating pool of mRNAs, giving rise to a protein. (2) It may enter the mRNA decay pathway, being removed permanently from the translating pool. (3) It may be subject to translational repression, withholding the mRNA from the translational pool without degradation. Although mechanistically different, mRNA degradation and stable repression may lead to similar amounts of protein produced from a single mRNA. While the balance of general RNA decay and active translation establishes a steady-state concentration of bulk protein produced, post-transcriptional regulation offsets this balance, allowing for larger differences between mRNA and protein amounts. For example, regulated mRNA degradation can lead to a local change in mRNA abundance and consequently establishes a subcellular gradient of mRNA available for translation. The effect of this quantitative difference can be magnified by additionally regulating the translational accessibility of an mRNA via qualitative features, such as the length of the 3′ poly(A) tail. Stable repression of an mRNA without degradation is a delicate task and is employed for protein production in a temporal and/or spatial manner. In a syncytial tissue mRNA repression is often a prerequisite for intracellular mRNA localization or transport. Once the mRNA reaches its destination the repression has to be reversed and the mRNA is activated. In complex tissues, such as the germ line, it is the interplay of mRNA translation, storage/transport and degradation that is the basis for the cytoplasmic phase of post-transcriptional gene regulation; this interplay dictates when, where, how, and to which extent synthesis of a particular protein is carried out.

8.2.3 Concept of Translational Control: Basic Properties and Control Possibilities

Strictly speaking, translational control is the regulation of ribosomal activity and, therefore, is not identical to post-transcriptional mRNA regulation. However, the operational definition of translational control includes any activity that affects the amount of protein output for an mRNA by either stimulating or reducing ribosome association. The majority of known regulatory mechanisms are repressive ones, arguing that the default state of most mRNAs is likely geared towards optimal translation. The process of translation is divided into three different phases: initiation, elongation, and termination. The initiation phase comprises all steps required for the assembly of a translationally competent 80S (S, Svedberg) ribosome on the start codon, i.e., 40S ribosome subunit recruitment, scanning and 60S subunit joining. During the elongation phase the 80S ribosome moves along the mRNA and synthesizes the polypeptide chain. When the ribosome encounters the stop codon, the termination phase is initiated, leading to a disassembly of the 80S ribosome and liberation of the newly synthesized protein (for more detail, see Mathews et al. 2007).

Although translation can be regulated at any of the three phases, the majority of regulatory events described so far target the initiation phase (Fig. 8.1). Translation initiation is a multistep act, reflected in the large number of factors taking part in this process. Across species, the six translation initiation factors are formed from many more individual components than the elongation or release factors. In *C. elegans*, more than 40 putative translation initiation factor components are encoded in the genome, in contrast to only six elongation factor components and two release factors. The individual initiation factors form large protein complexes, each fulfilling a distinct function during the initiation phase. Hence, the full complement of eIFs and their availability provides the basis for a high protein synthesis capacity. Two structural components of every mRNA contribute to efficient translation, the cap-structure and the poly(A) tail. Most importantly, their synergy for translational initiation is much more than the sum of each individual feature (Tarun and Sachs 1995; Wells et al. 1998). The basis for this observation is the proposed formation of the closed loop connection between cap- and tail-structures (Fig. 8.1a). Consequently, controlling the accessibility to, and the quality of the 5′cap and the 3′tail represent key mechanistic entry points in translational control. Each entry point is utilized in the *C. elegans* germ line and specific examples are discussed in Sects. 8.3.1 and 8.3.2.

8.2.4 Two Aspects of Translational Control: Global Versus Gene-Specific

Translational control can be classified into two different modes, global and gene-specific. (1) Global regulation affects all cap-carrying mRNAs in a cell or tissue by targeting the functionality of eIFs or ribosomal subunits, mostly via post-translational

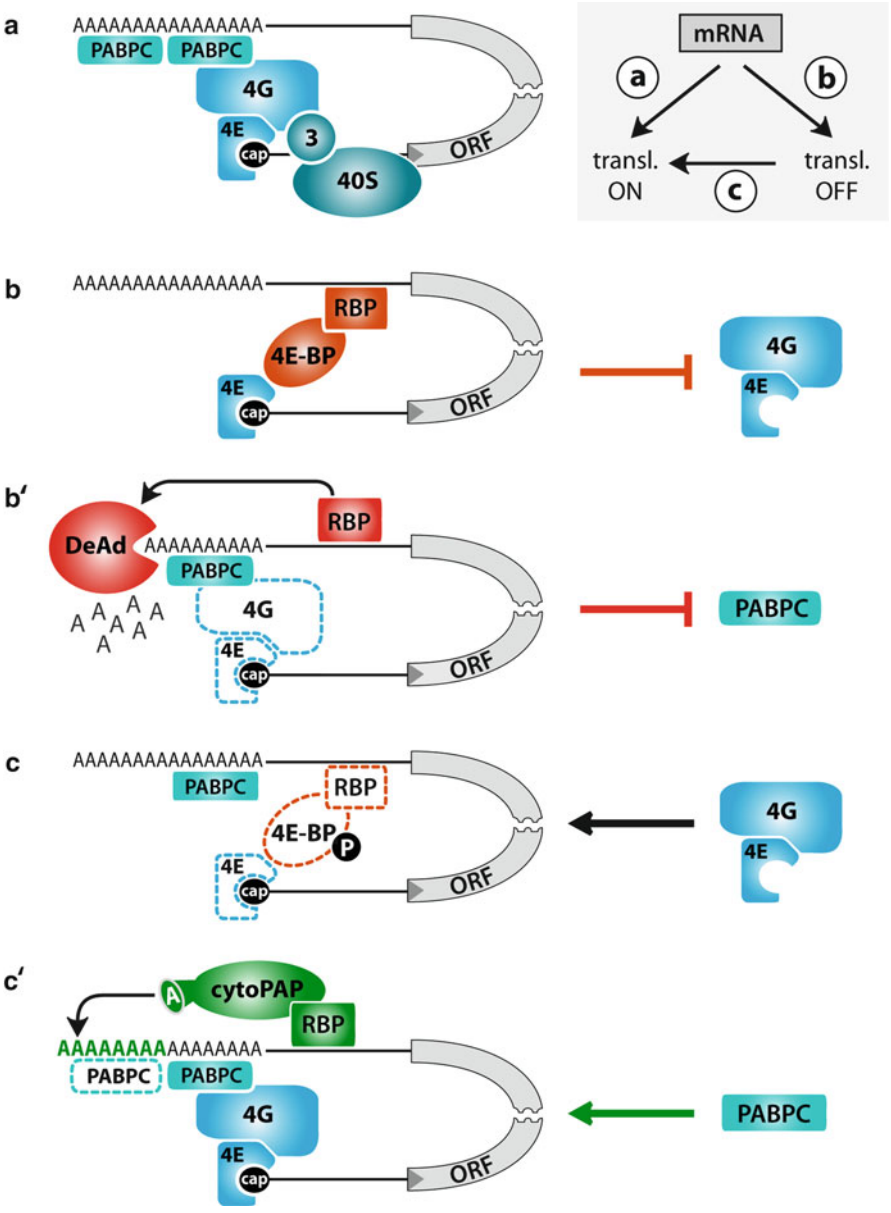

Fig. 8.1 Schematic representation of translation initiation, repression, and re-activation. (**a**) Cap-mediated translation initiation. Translation initiation factor eIF4G (4G) binds to eIF4E (4E) and the cytoplasmic poly(A)-binding protein (PABPC), facilitating circularization of the mRNA's tails. The central position of eIF4G in the closed loop aids translational initiation by indirectly recruiting the small ribosomal subunit (40S) to the mRNA via eIF3 (3). ORF, open reading frame; arrowhead in ORF indicates start codon. (**b**) Interfering with the placement of functional eIF4G results in translational repression. Specifically recruited RNA-bound proteins

modifications (Gebauer and Hentze 2004). This profound type of regulation is used in stress situations, such as toxic exposure or starvation, to quickly shut down new protein synthesis in order to dictate an appropriate stress response. Detailed mechanistic studies regarding this type of regulation have not been conducted in *C. elegans*, but indications for its existence can be found. For example, in response to heat stress, key eIFs are phosphorylated, which correlates with a general reduction in the abundance of translating ribosomes (Nousch and Eckmann, unpublished data). A different and more indirect indication is the presence of large cytoplasmic RNP structures in response to environmental stresses (Jud et al. 2008). The protein composition of these structures resemble in their protein components and dynamics stress granules of yeast or mammalian cells, which are thought to contain repressed translation initiation complexes (Buchan et al. 2011; Kedersha et al. 2005). (2) Gene-specific regulation affects only individual or a defined group of mRNAs. Here, not a canonical translation factor, but rather the mRNA itself is the direct target of regulation, utilizing sequence-specific RNA-binding proteins to recruit activating or repressive protein complexes.

8.3 Mechanistic Aspects of Translational Control in the Germ Line

8.3.1 Cap-Mediated Regulation

The 5′cap structure is a modified guanine nucleotide that protects the mRNA from 5′ to 3′ exonucleolytic decay (Furuichi et al. 1977; Shimotohno et al. 1977). In the majority of eukaryotes, mRNAs carry a guanosine that is mono-methylated at position seven (m7G), whereas a class of small nuclear RNAs carries a tri-methylated guanosine (TMG) with two methyl-groups at position two and one at position seven (Reddy et al. 1992). Either 5′cap is present on mRNAs in *C. elegans*, which depends on the nuclear history of RNA synthesis. mRNA produced by the canonical pre-mRNA maturation processes carries an m7G cap, while *trans*-spliced mRNAs to splice-leader sequences carries a TMG cap (Lasda and Blumenthal 2011). Importantly, *trans*-splicing is prevalent in worms and generates stereotyped 5′end sequences that replace, in the most extreme cases such as the *gld-3* mRNA (Eckmann et al. 2002),

Fig. 8.1 (continued) (RBP) guide eIF4E-binding proteins (4E-BP) to exclude eIF4G, forming alternative eIF4E/4E-BP complexes. (**b′**) Alternatively, RBPs may enhance the activity of deadenylases (DeAd), which indirectly limit the number of PABPCs associated with the poly(A) tail, therefore lowering the probability of eIF4G binding. (**c**) Translational re-activation of repressed mRNA. 4E-BP repressive complexes are destabilized and displaced from the mRNA by phosphorylation. (**c′**) Shortened poly(A) tails are re-elongated by cytoplasmic poly(A) polymerases (cytoPAP), which are stimulated upon mRNP remodeling. The translational initiation machinery efficiently recognizes a translationally re-activated mRNA, which is able to attract more PABPC

the entire gene-encoded 5′UTR. This leaves limited or no room for gene-specific 5′UTR translational control sequences, and perhaps explains why the 3′UTR-mediated translational control mechanisms are more prevalent (Merritt et al. 2008).

The cap structure is the docking point for a unique group of proteins, the cap-binding family of proteins (Rhoads 2009). In the cytoplasm, eIF4E recognizes specifically the 5′cap and assists translation initiation as part of eIF4F, a larger protein complex recruiting the small 40S ribosomal subunit to the mRNA (Mathews et al. 2007) (Fig. 8.1a). Besides eIF4E, two additional proteins build the core of eIF4F: the scaffolding protein eIF4G, which provides the structural basis for the complex and is the essential bridging factor to form a closed loop structure; and eIF4A, an RNA helicase that unwinds the 5′UTR, aiding the 40S ribosomal scanning process to locate the initial start codon (Fig. 8.1a). Homologs for all three factors are encoded in the *C. elegans* genome, with five paralogs of eIF4E, two of eIF4A and one for eIF4G (Table 8.1). The latter exists as several isoforms presumably due to alternative splicing events, adding to further modes of regulation (Contreras et al. 2008). Although any eIF4 complex member represents in principle a target for regulation, it is the availability or functionality of eIF4E that is modulated most commonly across organisms. Thus, it is not surprising that the best-studied translation factors in *C. elegans* are the five eIF4E paralogs, *ife-1* to *ife-5*, possessing diverse cap selectivity. IFE-3 and IFE-4 bind preferentially to the m7G cap, whereas IFE-2, IFE-3, and IFE-5 bind to both, m7G and TMG caps (Jankowska-Anyszka et al. 1998; Keiper et al. 2000; Miyoshi et al. 2002). All paralogs are expressed in the germ line, with the exception of IFE-4, which is produced in muscles and neurons (Dinkova et al. 2005). Only one of the IFEs is absolutely essential; loss of IFE-3 activity results in embryonic lethality (Keiper et al. 2000). This suggests that IFE-1, IFE-2, and IFE-5 may compensate for each other and that IFE-3 is either used for bulk or essential embryonic mRNAs. Consistent with this notion, more subtle and specific defects have been reported for the other paralogs. IFE-1 and IFE-2 have roles limited to germline development. Loss of *ife-1* activity leads to defective gametogenesis in males and hermaphrodites (Henderson et al. 2009), suggesting that IFE-1 functions to guide general differentiation programs. IFE-2 has an even more defined role. It regulates meiosis in hermaphrodites, because *ife-2* mutants display severe chromosome segregation defects at elevated temperature (Song et al. 2010). Both factors have in common that they are important for efficient translation of specific mRNA subsets. For example, in the adult hermaphrodite, *oma-1* and *mex-1* mRNAs are not efficiently translated in the *ife-1* mutant and *msh-5* mRNA in the *ife-2* mutant. This suggests that *C. elegans* utilizes different cap-binding isoforms to positively regulate small groups of mRNAs, which appear functionally connected. It remains to be determined how the different IFEs define their target mRNAs and if additional RNA-binding factors help to select the mRNA.

Translational repression via the 5′cap prevents the assembly of a functional eIF4F complex (Fig. 8.1). This is achieved by controlling the availability of a single eIF4F component or by blocking essential interactions among eIF4F complex members. For example, the same peptide motif in eIF4E that physically contacts eIF4G is also recognized by a group of regulatory 4E-binding proteins

Table 8.1 Components of the eIF4F complex and a selection of general translation factors implicated in germline development

Translation factors and regulators	Protein	*C. elegans* name	WB gene ID	Remarks
eIF4F complex	eIF4E	IFE-1	F53A2.6	Affinity to m7G, TMG (Jankowska-Anyszka et al. 1998; Keiper et al. 2000)
		IFE-2	R04A9.4	Affinity to m7G, TMG (Jankowska-Anyszka et al. 1998; Keiper et al. 2000)
		IFE-3	B0348.6	Affinity to m7G (Jankowska-Anyszka et al. 1998; Keiper et al. 2000)
		IFE-4	C05D9.5	Affinity to m7G, expressed in muscle and neurons (Keiper et al. 2000; Dinkova et al. 2005)
		IFE-5	Y57A10A.30	Affinity to m7G, TMG (Keiper et al. 2000)
	eIF4A	INF-1	F57B9.6	Embryonic lethal by RNAi (Simmer et al. 2003)
			F57B9.3	Larval arrest by RNAi (Simmer et al. 2003)
	eIF4G	IFG-1	M110.4	Prevents increase of GL apoptosis (Contreras et al. 2008)
General translation factors	eIF5A	IFF-1	T05G5.10	Promotes GL proliferation (Hanazawa et al. 2004)
	eIF5B	IFFB-1	Y54F10BM.2	Promotes GL proliferation and differentiation (Yu et al. 2006)
	eEF1A	EEF-1A.1 (EFT-3, GLP-3)	F31E3.5	Required for all germ cell mitosis (Kadyk et al. 1997)
	PABPC	PAB-1	Y106G6H.2	Promotes GL proliferation (Ciosk et al. 2004; Maciejowski et al. 2005; Ko et al. 2010)
		PAB-2	F18H3.3	Promotes fertility (Ceron et al. 2007)
Translational control regulator	4E-BP	SPN-2 (PQN-45)	F56F3.1	Promotes embryogenesis (Li et al. 2009), oogenesis Guven-Ozkan et al. 2010
		PGL-1	ZK381.4	Promotes GL proliferation and gametogenesis (Amiri et al. 2001
	PAIP	ATX-2	D2045.1	Required for sp–oo switch (Ciosk et al. 2004)

For the translation initiation factor eIF4F all *C. elegans* factors are shown. General translation factors are included, provided a specific germline function is known
GL germ line, *WB* Wormbase

(4E-BPs) (Gebauer and Hentze 2004). Hence, 4E-BPs compete with eIF4G for eIF4E and act as global translational repressors, as they do not assist 40S ribosome recruitment. Stable and mRNA-specific repression is further assisted by 3′UTR-associated RNA-binding proteins. Thereby the 5′cap is stably bound by an eIF4E/4E-BP complex, which forms an alternative closed loop structure with the 3′UTR-anchored RNA-binding protein, leading to a decrease in protein production and the formation of a 4E-BP-poisoned mRNP (Rhoads 2009) (Fig. 8.1b). Although examples exist from other organisms, no general 4E-BP translation regulator has yet been described in *C. elegans*. However, SPN-2, a 4E-BP regulator involved in gene-specific regulation, is active in the germ line as a maternally expressed factor (Li et al. 2009). SPN-2, also known as PQN-52, interacts with all IFEs, except IFE-4, and with the RNA-binding proteins OMA-1/2, which are highly abundant in oocytes. Two mRNA targets of an IFE/SPN-2/OMA complex have been identified (Li et al. 2009; Guven-Ozkan et al. 2010). *zif-1* mRNA, encoding a substrate-binding subunit of an E3 ubiquitin ligase, and *mei-1* mRNA, encoding a subunit of the microtubule-severing enzyme katanin. Oocyte repression of *zif-1* mRNA ensures high protein levels of PIE-1, a P lineage-specific transcriptional repressor donated maternally to the early embryo and a protein target of ubiquitin-mediated decay in somatic blastomeres (Strome 2005) (also see Wang and Seydoux 2012). Robust and fast elimination of the katanin subunit MEI-1 is important for the fertilized embryo to switch from an oogenic meiotic spindle to a first mitotic spindle, which is accomplished in two parallel steps in the early embryo (Clark-Maguire and Mains 1994a, b; Srayko et al. 2000) (also see Kim et al. 2012, Chap. 10). While MEI-1 is degraded via ubiquitin-mediated proteolysis (Bowerman and Kurz 2006), *mei-1* mRNA is translationally repressed to prevent new protein synthesis (Li et al. 2009). The effectiveness of this mechanism is underscored by numerous 4E-BPs in other organisms, such as Maskin or Cup. Maskin is important for the maturation of *Xenopus* oocytes (Stebbins-Boaz et al. 1999), whereas Cup has a role in axis formation in *Drosophila* embryos (Nelson et al. 2004). A reversal of 4E-BP-mediated repression is envisioned to depend on its phosphorylation status (Barnard et al. 2005) (Fig. 8.1c). In conclusion, 5′cap-mediated translational repression is a frequent mechanism during oocyte maturation and early embryogenesis, however, less often observed during post-embryonic *C. elegans* germline development.

mRNA decapping leads to immediate 5′–3′-directed RNA degradation. *C. elegans* homologs of the yeast enzymes and associated factors involved in decapping and 5′–3′ degradation are summarized in Table 8.2. Although the major decapping enzyme DCAP-2 is present in germ cells, no specific role in germline development has been identified (Lall et al. 2005). Interestingly, the important decapping enhancer PATR-1 is only weakly expressed during *C. elegans* germline development (Boag et al. 2008). In the absence of an additional decapping enhancer, it appears that specific mRNA-degradation has a minor role in post-transcriptional mRNA regulation in *C. elegans*.

Table 8.2 Factors of the mRNA deadenylation and decay machinery

RNA processing	Factor or complex	*C. elegans* name	WB gene ID
Deadenylation	CCR4-NOT	CCR-4	ZC518.3
		CCF-1	Y56A3A.20
		LET-711 (NTL-1)	F57B9.2
		NTL-2	B0286.4
		NTL-3	Y56A3A.1
		NTL-4	C49H3.5
		NTL-9	C26E6.3
	PAN2/PAN3	PANL-2	F31E3.4
		PANL-3	ZK632.7
	PARN	K10C8.1	K10C8.1
5′–3′ decay	Decapping enzyme	DCAP-2	F52G2.1
	Dhh1p	CGH-1	C07H6.5
	Pat1p	PATR-1	F43G6.9
	Lsm complex	LSM-1	F40F8.9
		GUT-2 (LSM-2)	T10G3.6
		LSM-3	Y62E10A.12
		LSM-4	F32A5.7
		LSM-5	F28F8.3
		LSM-6	Y71G12B.14
		LSM-7	ZK593.7
	Xrn1p	XRN-1	Y39G8C.1
		XRN-2	Y48B6A.3

Deadenylation and 5′–3′ decay proteins with sequence similarity to biochemically defined yeast or human factors
WB Wormbase

8.3.2 Poly(A)-Tail Length Control

The most dynamic structure of an mRNA is its poly(A) tail. Nuclear polyadenylation is a co-transcriptional default process that liberates the RNA from its site of transcription and assists mRNA export (Sachs and Wahle 1993). In the cytoplasm, the homopolymeric A-tail is removed as part of the natural mRNA decay pathway (Decker and Parker 1993). However, the poly(A) tail is also a platform for regulatory translational control mechanisms that exploit its two cytoplasmic functions, enhancing mRNA stability and translatability (Mathews et al. 2007). In particular, the length of the poly(A) tail is an indicator of the mRNA's fate. A short tail makes an mRNA less attractive for translation initiation and renders it rather unstable, whereas a long tail stimulates translation initiation and stabilizes an mRNA (Munroe and Jacobson 1990; Decker and Parker 1993). The molecular basis for this phenomena is a sequence-specific RNA-binding protein that decorates the mRNA's tail, cytoplasmic poly(A)-binding protein (PABPC) (Otero et al. 1999). A single PABPC molecule binds approximately 20 adenosines and interacts directly with the eIF4F

complex, via eIF4G, assisting closed-loop formation (Wells et al. 1998; Baer and Kornberg 1980, 1983) (Fig. 8.1a). Hence, a long poly(A) tail is able to recruit more PABP molecules than a short poly(A) tail, enhancing the probability of frequent translational initiation. Two PABPC proteins are present in *C. elegans*, PAB-1 and PAB-2 (Table 8.1). Only *pab-1,* not *pab-2,* seems essential for germline development. Loss of *pab-1* results in sterile animals with a strong germline proliferation defect, indicating that PAB-1-related poly(A)-tail metabolism is critical for germ cell divisions (Ciosk et al. 2004; Maciejowski et al. 2005; Ko et al. 2010).

The process of poly(A)-tail shortening is termed deadenylation and is carried out by deadenylases (Garneau et al. 2007) (Fig. 8.1b′). Three major deadenylation complexes have been described so far: the CCR4-NOT complex, the PAN2/PAN3 complex and PARN. All complexes show conservation from yeast to humans and, with the exception of the PAN2/3 complex, have been linked to mRNA-specific translational control (Garneau et al. 2007). Homologs of all three complex components can be found in the *C. elegans* genome, of which the majority remains uncharacterized (Table 8.2). Although the CCR4-NOT complex is the major cytoplasmic deadenylase in *Drosophila* and yeast (Garneau et al. 2007), it is also involved in mRNA-specific translational regulation, consistent with reported functions of its individual components during *C. elegans* germline development. The absence of CCF-1, the *C. elegans* homolog of POP2, which is one of the two catalytic subunits from the presumed worm CCR4-NOT complex, results in pachytene-arrested germ cells without differentiation into sperm or oocytes (Molin and Puisieux 2005). Upon RNAi knockdown of CCR-4, the second deadenylase of the presumed complex, more subtle defects are observed that are limited to the stability of specific mRNAs (Schmid et al. 2009). While a partial reduction of function of the *C. elegans* NOT1 homolog, LET-711, affects the first mitotic division of the one-cell embryo, a strong loss of function results in sterile germ lines (DeBella et al. 2006). Together these suggest that CCF-1/CCR-4/LET-711-mediated deadenylation is important for meiotic progression, germ cell differentiation and early embryogenesis. Given the unique phenotypic defects of each complex member, it is also likely that some mRNAs that are keys for a specific process in germ cell development are differentially affected by the activity of individual deadenylase complex components. Yet, a formal biochemical demonstration of the existence of a CCR4-NOT complex is still missing in *C. elegans*.

While shortening of the poly(A) tail is the initial step of canonical mRNA degradation, the removal of an mRNA is not an obligatory fate. mRNAs with a shortened tail can escape degradation and persist as stable yet translationally silenced molecules. The underlying molecular mechanisms are less clear; nevertheless, it is most likely assisted by specific mRNA-associated proteins that package the mRNA into translationally dormant mRNPs. mRNA-associated factors that recruit the presumed CCF-1/CCR-4/LET-711 deadenylase to specific mRNAs and enhancing poly(A)-tail shortening are slowly emerging and discussed in Sect. 8.5.

Translational activation of deadenylated mRNAs requires remodeling of the repressed mRNP and the resynthesis of a longer poly(A) tail. Cytoplasmic poly(A) polymerases (cytoPAPs) elongate the homopolymeric A-tail and reintroduce the mRNA into the translating pool (Fig. 8.1c′). A crucial aspect of cytoPAPs is that,

contrary to nuclear PAP, the enzyme lacks a predictable RNA-binding domain (Eckmann et al. 2011). They associate with mRNA targets via other mRNP components and, therefore, are also referred to as noncanonical poly(A) polymerases (Wang et al. 2002). cytoPAPs are conserved across species with two representatives described in *C. elegans*, GLD-2 and GLD-4 (Wang et al. 2002; Schmid et al. 2009). As their gene name indicates—*gld* stands for *g*erm*l*ine *d*evelopment defective—both proteins are implicated in many germline functions, ranging from germ cell fate decisions to meiotic progression, gametogenesis and early embryogenesis. The molecular composition of both cytoPAP complexes is discussed in detail in Sect. 8.4.2.

Interestingly, developmentally regulated mRNAs show complex poly(A) tail dynamics in the germ line, which are further exploited in the early embryo, illustrating a tight connection between cytoplasmic deadenylation and polyadenylation (see Sect. 8.5). The effectiveness, versatility, and the ability to fine-tune protein synthesis, rather than just establishing an all-or-non situation, make poly(A)-tail length control probably the most widely used mechanism in germline development to control the exact amount of protein synthesis at each stage.

8.3.3 miRNA-Mediated Regulation

Gene expression regulation by microRNAs (miRNA) has emerged as a widespread mechanism. miRNAs belong to the group of noncoding RNAs, are small in size (20–25 nt), and are generated from local hairpin structures by the action of two RNA endonucleases, Drosha and Dicer (Kim et al. 2009b). A mature miRNA is ultimately loaded into the RNA-induced silencing complex (RISC), which contains Argonaute-family proteins. As part of the RISC complex, miRNAs serve as RNA-recognition devices. They base-pair with complementary mRNA sequences preferentially located in the 3′UTR, leading to the subsequent translational repression or degradation of the mRNA (Fabian et al. 2010). Although miRNAs were first identified in *C. elegans* as regulators of developmental timing (Lee et al. 1993; Wightman et al. 1993), a direct involvement in germline development is still lacking. However, some indications exist: The absence of either miRNA processing factors, *drsh-1* (Drosha) or *drc-1* (Dicer), leads to sterile animals (Denli et al. 2004; Knight and Bass 2001) (Table 8.3). RNAi-mediated down-regulation of ALG-1 and ALG-2, two of 24 worm Argonaute (Ago) proteins, results in weak germ cell differentiation defects (Grishok et al. 2001), and a specific micro-RNA family is required for DNA damage response in germ cells (Kato et al. 2009).

Another group of noncoding RNAs with germline functions are the endogenously produced small interfering RNAs, called 26G-RNAs (Han et al. 2009; Conine et al. 2010). This group of endo-siRNAs is 26 nt in length, preferentially starts with guanine, is exclusively present in the germ line, and shows perfect complementarity to their target transcripts. Two non-overlapping subsets of 26G RNAs are expressed during spermatogenesis (class I) and oogenesis (class II). Mapping of the 26G RNAs to the genome shows that they preferentially target protein-coding genes that are

Table 8.3 Factors involved in miRNA biogenesis and miRNA-mediated gene silencing

miRNA factor	Protein	WB gene ID	Germline phenotypes
Drosha	DRSH-1	F26E4.10	Reduced fertility (Denli et al. 2004)
Pasha	PASH-1	T22A3.5	Reduced brood size by RNAi
Dicer	DCR-1	K12H4.8	Abnormal oocytes (Knight and Bass 2001)
Dicer-related helicases	DRH-1	F15B10.2	Required redundantly with DRH-2 for GL and somatic RNAi (Tabara et al. 2002; Lu et al. 2009)
	DRH-2	C01B10.1	Required redundantly with DRH-1 for GL and somatic RNAi (Tabara et al. 2002; Lu et al. 2009)
	DRH-3	D2005.5	Abnormal chromosome arrangements in pachytene (Nakamura et al. 2007; She et al. 2009)
Argonaute	ALG-1	F48F7.1	Redundant with ALG-2 shows GC differentiation defects (Grishok et al. 2001)
	ALG-2	T07D3.7	Redundant with ALG-1 shows GC differentiation defects (Grishok et al. 2001)
GW182	AIN-1	C06G1.4	No apparent GL defect in mutants or by RNAi
	AIN-2	B0041.2	No apparent GL defect in mutants or by RNAi

Germline development-associated miRNA processing factors and miRNA-mediated translational silencing complexes are given. Factors of other non-coding RNAs, such as endo-siRNAs or piRNAs, are not included
GL germ line, *WB* Wormbase, *GC* germ cell

expressed during spermatogenesis or oogenesis/early embryogenesis, respectively. In their absence the abundance of putative target mRNAs increases, suggesting that 26G RNAs specifically repress mRNAs by enhancing their degradation. The two classes of 26G RNAs are suggested to be sorted into distinct RISC complexes, based on the observation that different Ago-proteins are essential for the presence of the different classes of 26G-RNAs in the worm. Whereas ERGO-1 is important for class II, the abundance of class I depends highly on the two paralogs ALG-3 and ALG-4 (Han et al. 2009; Conine et al. 2010). The functional relevance of class II 26G-RNA remains to be demonstrated, but class I 26G-RNAs are linked to gene regulation during spermatogenesis. This is based on the observation that the absence of *alg-3* and *alg-4* results in the production of defective sperm at elevated temperatures (Han et al. 2009; Conine et al. 2010).

8.4 Connections Between Global and Specific Regulators of Translational Control

8.4.1 RNA-Binding Protein Families in the Germ Line

Genome-wide gene expression analysis shows that in *C. elegans* RNA metabolism-associated proteins are significantly more abundant in the germ line than in the soma (Wang et al. 2009a). Not surprisingly, most RNA-associated protein families are involved in numerous aspects of germline development. This is further underscored

by evolutionary conservation of the relevant RNA-binding protein families and an expansion in number of individual protein family members in *C. elegans*, which is consistent with a further diversification of their biological roles. While some protein families are quite closely related in their overall protein architecture and function (e.g., PUF or STAR proteins), others show poor sequence similarities outside their family-defining structural domains and have diverse RNA regulatory functions (e.g., KH or Nanos-like proteins).

Although most RNA-binding proteins use a particular protein domain(s) to interact with an mRNA, they do so with varying degrees of specificity and affinity. One can roughly divide them into binders with high or low selectivity, which reflects on their molecular roles in mRNP complex formation. A high-selectivity binder has high RNA-affinity, close to the single-digit nanomolar range, and recognizes a well-defined target sequence motif (e.g., PUF proteins and the STAR protein GLD-1) (Bernstein et al. 2005; Stumpf et al. 2008; Ryder et al. 2004). The group of low-selectivity binders is quite broad. Proteins belonging to this group display RNA-affinities in an upper two-digit to a three-digit nanomolar range and can, but not always do, display some general interaction preferences to a loosely definable consensus sequence, such as AU-rich sequences bound by *Xenopus* CPEBs (Hake et al. 1998). Whereas high selectivity RNA-binding proteins, defined here as RNA selectors, are envisioned as the primary targeting unit in an mRNP complex, the low selectivity ones may help to narrow down the mRNA target pools further. Additionally, low affinity binder may stabilize the mRNP structure or provide further regulatory capacity to the mRNP (e.g., GLD-3).

In the following section some of the best-studied RNA-binding protein families are described in more detail. We have limited our discussion to four protein families. Other RNA-binding proteins and their characteristics are summarized in Table 8.4.

8.4.1.1 PUF Proteins

One of the most distinguished RNA selector family in the *C. elegans* germ line is composed of the PUF (*Pu*milio and *F*BF) proteins (Wickens et al. 2002), FBF-1 and FBF-2 (collectively referred to as FBF), and PUF-3 to PUF-12, of which PUF-9 is exclusively somatic (Nolde et al. 2007). All PUF proteins possess eight, ~40 aa long, consecutively arranged PUF-repeats, which form a single RNA-recognition platform. Extensive structural analysis revealed the global domain architecture of the staged PUF-repeats into an arched superhelix with an inner RNA-binding surface and an outer protein interaction surface (Edwards et al. 2001). Each individual PUF-repeat contacts a single RNA nucleotide, providing the molecular basis for its defined RNA target motif, the FBF-binding element (FBE) (Bernstein et al. 2005). Although the UGU core sequence of an FBE is essential for all tested PUF proteins to bind RNAs with high affinity, additional flanking nucleotides add to their selectivity and mode of recognition. For example, PUF-8 preferentially binds to an eight nucleotide long motif with the consensus sequence of 5′-**UGU**ANAUA-3′, whereas FBF proteins prefer a nine nucleotide long 5′-**UGU**RNNAUA-3′ (R, purine; N, any base)

Table 8.4 RNA-binding proteins involved in germ cell development

Protein family	Protein	WB gene ID	5′/3′ regulator[a]	GL protein expression[b]	GL function
PUF proteins	PUF-1 (FBF-1)	H12I13.4	3′	A	sp–oo; mit–mei
	PUF-2 (FBF-2)	F21H12.5	3′	A	sp–oo; mit–mei
	PUF-3	Y45F10A.2	3′		emb
	PUF-4	M4.2	3′		
	PUF-5	F54C9.8	3′	D	oo
	PUF-6	F18A11.1	3′		oo
	PUF-7	B0273.2	3′		oo
	PUF-8	C30G12.7	3′	A	sp–oo; sp mei
	PUF-9[c]	W06B11.2	3′		
	PUF-11	Y73B6BL.38	3′		
	PUF-12	ZK945.3	3′		
K homology (KH) proteins	GLD-1	T23G11.3	5′ and 3′	B	mit–mei; oo mei; GC identity
	GLD-3	T07F8.3	3′	C	sp–oo; mit–mei
	MEX-3	F53G12.5		C	mei; GC identity; prolif
CPEB proteins	CPB-1	C40H1.1	3′		sp mei
	CPB-2	C30B5.3	3′		
	CPB-3	B0414.5	3′	B	sp–oo; mit–mei; oo
	FOG-1	Y54E10A.4	3′	B	sp–oo; mit–mei
Nanos proteins	NOS-1	R03D7.7	3′		GC viability
	NOS-2	ZK1127.1	3′		GC viability
	NOS-3	Y53C12B.3	3′	U/D[d]	sp–oo; mit–mei
DEAD-box ATP-dependent RNA helicases	GLH-1	T21G5.3		U	prolif; gametogenesis
	GLH-2	C55B7.1		U	prolif; gametogenesis
	GLH-3	B0414.6		U	
	GLH-4	T12F5.3		U	prolif; gametogenesis
	CGH-1	C07H6.5		U	oo
	LAF-1	Y71H2AM.19			sp–oo
	VBH-1	Y54E10A.9		U	sp–oo; emb
Y-box proteins	CEY-1	F33A8.3			
	CEY-2	F46F11.2			
	CEY-3	M01E11.5			
	CEY-4	Y39A1C.3			
Other RNA-binding motif containing proteins	LARP-1	R144.7		U	sp–oo; oo
	OMA-1	C09G9.6	3′	D	oo
	OMA-2	ZC513.6	3′	D	oo
	RNP-8	R119.7	3′	D	sp–oo; oo
	CAR-1	Y18D10A.17		U	emb
	DAZ-1	F56D1.7		A	sp–oo; oo mei

RNA regulator protein families with evolutionary defined RNA-binding domains are listed. Most proteins are referred to in the main text. Y-box proteins (Boag et al. 2005) are also known as cold-shock domain proteins. The proteins LARP-1(Nykamp et al. 2008; Zanin et al. 2010) and CAR-1(Squirrell et al. 2006; Audhya et al. 2005; Boag et al. 2005) contain an La-type and LSM-like RNA-binding motif, respectively

GC germ cell. *WB* Wormbase. Reported germline(GL) functions: *sp–oo* sperm-to-oocyte switch, *mit–mei* mitosis-to-meiosis switch, *sp* spermatogenesis, *oo* oogenesis, *emb* early embryogenesis, *mei* meiotic progression, *prolif* proliferation

[a]5′ or 3′ end-mediated translational control regulation

[b]Protein distribution in the germ line. Type A to D and U are according to Fig. 8.2

[c]Expressed in the soma

[d]phosphorylated NOS-3

sequence (Wang et al. 2009b). To comply with the one nucleotide per PUF-repeat logic, the central nucleotide in the RNA-binding motif of FBF is flipped away from the protein and remains exposed, diversifying the binding repertoire of PUF proteins (Opperman et al. 2005). Functionally the PUF and FBF proteins are also quite diverse, which correlates with their distinct protein expression profile in the germ line (see Sect. 8.4.2). Both FBF proteins are essential for actively dividing germ stem cells (Crittenden et al. 2002), meiotic entry (Lamont et al. 2004), and the sperm-to-oocyte switch (Zhang et al. 1997). PUF-8 is important for germline proliferation (Ariz et al. 2009), the sperm-to-oocyte switch (Bachorik and Kimble 2005), and male meiotic progression (Subramaniam and Seydoux 2003). While PUF-5, -6, -7 assist oocyte differentiation and maturation (Lublin and Evans 2007), PUF-3 is essential for early embryogenesis (Sonnichsen et al. 2005). For the most part, PUF proteins are translational repressors (discussed in detail in Sect. 8.5.3); however, in certain circumstances they may also act as translational activators (further discussed in Sects. 8.4.2 and 8.4.3). We term such dual regulators here as translational effectors.

8.4.1.2 Nanos Proteins

A protein family closely connected to PUF proteins consists of three *Drosophila* Nanos-related proteins, NOS-1, NOS-2, and NOS-3. The defining criterion of these germ cell-enriched RNA-binding proteins is the presence of two consecutive C-terminal CCHC zinc fingers assumed to be important for RNA association. In analogy to *Drosophila* Nanos, they are considered as high affinity binders with little sequence specificity (Curtis et al. 1997). While together all three worm Nanos proteins assist postembryonic germ cell proliferation and germ cell survival in the later larval stages, individual family members have also additional functions. Maternal NOS-2 protein is important for efficient primordial germ cell incorporation into the somatic gonadal primordium (Jadhav et al. 2008). Furthermore, NOS-1 and NOS-2 are redundantly required to block primordial germ cell proliferation in the first larval stage, upon starvation (Jadhav et al. 2008). Essential roles of NOS-3 in germ cells adopting the meiotic or oogenic fate are revealed when other redundantly acting RNA regulators are eliminated (Hansen et al. 2004; Eckmann et al. 2004).

8.4.1.3 KH Proteins

The hnRNP K homology (KH) domain represents a versatile protein fold of ~70–100 aa in length. Depending on its detailed amino-acid composition, KH domains bind ssDNA, RNA and/or proteins with varying binding affinities. Structural analysis revealed that a single KH domain accommodates at a maximum four nucleotides (Valverde et al. 2008). Thus, additional flanking protein sequences are necessary to extend the nucleic acid-binding surface and to increase its RNA-binding affinity and selectivity. Alternatively, multiple KH domains are combined in one protein to expand its RNA-binding potential. Proteins that carry

either one or multiple KH domains are assigned to essentially all aspects of RNA metabolism. In *C. elegans*, three diverse KH domain proteins, GLD-1, GLD-3, and MEX-3, direct germline development.

The STAR protein GLD-1 is to date the most comprehensively characterized RNA selector of the *C. elegans* germ line. Its single KH domain is flanked by N-terminal and C-terminal sequences that form a unique structural arrangement, known as the *s*ignal *t*ransduction and *a*ctivation of *R*NA (STAR) domain (Vernet and Artzt 1997). This conserved maxi-KH domain can form functional homodimers (Chen et al. 1997) and contacts RNA in a sequence-specific manner with high affinity in the low nanomolar range (Ryder et al. 2004). Originally a six nucleotide-long recognition sequence was determined in vitro, which was subsequently extended to a 7-mer motif (UACUAAC) based on a much larger number of in vivo associated mRNA targets (Wright et al. 2011). GLD-1-binding motifs (GBMs) are present in both the 5′UTR or the 3′UTR of target mRNA (Lee and Schedl 2004). All verified mRNA targets are translationally repressed upon GLD-1 association, which in some cases also protects uORF-containing mRNAs from non-sense-mediated decay (Lee and Schedl 2004). Although detailed molecular mechanisms remain to be determined, the latter finding suggests that GLD-1 may inhibit translational initiation, 80S ribosome assembly or translation elongation (Mootz et al. 2004). It is also conceivable that the mechanism of GLD-1-mediated translational repression depends on the target mRNA and its associated factors. This is further underscored by mutations in GLD-1 that affect the regulation of a few mRNA targets but not others (Schumacher et al. 2005). Given that a large number of verified GLD-1 target mRNAs encode proteins involved in diverse biological roles (see Table 8.5), the importance of GLD-1 for germline development is easy to comprehend. It regulates the balance of proliferation vs. meiotic entry (Hansen et al. 2004), female meiotic progression (Francis et al. 1995a, b), physiological apoptosis (Schumacher et al. 2005), the sperm-to-oocyte switch (Jan et al. 1999), and maintenance of germ cell totipotency (Ciosk et al. 2006).

The multi-KH domain protein GLD-3, together with its paralog BCC-1, are the Bicaudal-C (BicC) protein family RNA regulators in *C. elegans* (Eckmann et al. 2002). They contain 5 KH domains arranged in tandem connected via very short amino acid linkers, whereby each individual domain adopts a classic KH fold (Eckmann et al. 2002; Nakel et al. 2010). Three-dimensional structural analysis has revealed that all five KH domains of GLD-3 have extensive contacts with each other, forming a tightly packaged protein core, which is in contrast to a previously assumed "beads on a string" organization. Consistent with GLD-3 having an extremely low affinity for homopolymeric guanidine RNA (Jedamzik and Eckmann, unpublished results), the typical GxxG RNA-contacting loops (G, glycine) of known RNA-binding KH domains are missing either one or both of the two glycine residues (Nakel et al. 2010). GLD-3 binds GLD-2, and GLS-1 (see below). The amino-terminal KH domain region of GLD-3 is the binding site for GLD-2 (Eckmann et al. 2004) and GLS-1 (Rybarska et al. 2009), thus the multi-KH domain arrangement in GLD-3 likely serves as a large protein interaction platform, rather than providing an RNA selector function. The inferred scaffolding and regulatory functions of GLD-3 in

Table 8.5 mRNAs targets of 3′ end-mediated translational control

mRNA	GL function	Repressor	Activator	*cis*-regulatory motif in 3′UTR
glp-1	GL proliferation	GLD-1	GLD-2[a]	TCCTAAC; ATCTCAC; GACTAAT (Wright et al. 2011)
fbf-1	Maintenance of GL stem cells, sp–oo switch	FBF-x		UGUAAUAUU; UGUGCCAUC (Lamont et al. 2004)
fbf-2	Maintenance of GL stem cells, sp–oo switch	FBF-x		UGUAAUAUU (Lamont et al. 2004)
cye-1	Promotion of mitosis	GLD-1		TACTTAC; AATTAAC; TACTCAT; ATCTCAC (Wright et al. 2011)
cki-2	Inhibition of mitosis	FBF-x	GLD-2[a]	UGUGAAUUU; UGUCCAUUU[b]; UGUGUUCUA[b]; TGTUUUUUU[a] (Kalchhauser et al. 2011)
gld-1	Entry into meiosis, mit–mei, meiotic progression, GL totipotency	FBF-x	GLD-2	UGUGCCAUA; UGUGCCAUA (Crittenden et al. 2002)
him-3	Synaptonemal complex component	FBF-x		UGUGCAAUG (Merritt and Seydoux 2010)
htp-1	Synaptonemal complex component	FBF-x		UGUAAAAUG (Merritt and Seydoux 2010)
htp-2	Synaptonemal complex component	FBF-x		UGUAACAUG; UGUACAAUG(Merritt and Seydoux 2010)
syp-2	Synaptonemal complex component	FBF-x		UGUAUCAUU (Merritt and Seydoux 2010)
syp-3	Synaptonemal complex component	FBF-x		UGUCGAAUG; UGUAAUAUU (Merritt and Seydoux 2010)
cep-1	GL apoptosis	GLD-1		n.d.
rme-2	Yolk receptor in oo	GLD-1		TACTAAA (Wright et al. 2011)
oma-1	oo maturation	GLD-1	GLD-2	TACTAAC (Wright et al. 2011)
oma-2	oo maturation	GLD-1	GLD-2	TACTAAC; CACTAAC (Wright et al. 2011)
lip-1	Germline proliferation	FBF-x		UGUAAAAUC; UGUGCCAUC (Lee et al. 2006)
mpk-1	oo maturation, GL apoptosis	FBF-x		UGUAUCAUA; UGUAAUAUA (Lee et al. 2007a)
fem-3	sp–oo switch	FBF-x	GLD-2[a]	UGUGUCAUU (Zhang et al. 1997)
fog-1	sp–oo switch	FBF-x	GLD-2[a]	UGUAAAAUC; UGUAUCAUG; UGUAUCAUU (Thompson et al. 2005)

(continued)

Table 8.5 (continued)

mRNA	GL function	Repressor	Activator	*cis*-regulatory motif in 3′UTR
tra-2	sp–oo switch	GLD-1	GLD-2[a]	4×UACUCA (Ryder et al. 2004)
mex-3	GL proliferation, GL totipotency, establishment of embryonic cell fates	GLD-1		n.d.
pal-1	Specification of cell fates in embryo (maternally provided)	GLD-1		n.d.
		MEX-3		AUAGAGCUUCUUUAUUUA; UUAGGAAAAAGUUUA (Pagano et al. 2009)
		PUF-8		UGUACAAAA (Mainpal et al. 2011)

Translationally controlled mRNAs that are confirmed mRNA targets of indicated translational effectors (indicated as Repressor or Activator) by either in vitro binding assays, gel shift assays, transgenic reporter construct expression studies, or by a combination of these methods. FBF-x experiments did not distinguish between FBF-1 and FBF-2. If no particular motif was described yet (n.d.), translational regulation of the mRNA was at least demonstrated to dependent on the entire 3′UTR

GL germ line, *sp–oo* sperm-to-oocyte, *mit–mei* mitosis-to-meiosis decision, *oo* oocyte

[a]Proposed target mRNAs based on their association with GLD-2 in genome-wide RNA target analysis via co-immunoprecipitation experiments

[b]Have only weak binding affinities for FBF-x

mRNP complexes are consistent with its elaborate expression pattern and its global involvement in germline development. Germ cells require GLD-3 function to initiate the meiotic fate, progress beyond meiotic pachytene, and to achieve proper meiotic chromosome segregation (Eckmann et al. 2002, 2004; Rybarska et al. 2009). Additionally, GLD-3 assists in specification of the male germline fate in hermaphrodites and males (Eckmann et al. 2002, 2004). Maternal GLD-3 regulates early embryogenesis and maintains germline survival in the post-embryonic germline (Rybarska et al. 2009; Eckmann et al. 2002). The functions of GLD-3 are carried out by two prevalent protein isoforms of GLD-3, GLD-3S, and GLD-3L, which are distinguished by unique C-termini. BCC-1 is most similar to GLD-3 in its amino-terminal KH domain arrangement but significantly differs in its C-terminus from GLD-3. Functional roles of BCC-1 remain unclear.

A different multi-KH domain protein is the evolutionarily conserved RNA-binding protein MEX-3 (Buchet-Poyau et al. 2007). Originally discovered as a maternal-effect lethal gene that regulates early embryonic cell fates, *mex-3* is also active in the postembryonic germ line (Draper et al. 1996; Mootz et al. 2004; Ciosk et al. 2004, 2006; Ariz et al. 2009). MEX-3 is a 3′UTR-associated translational repressor and prevents the premature accumulation of the maternally donated embryonic cell fate determinant PAL-1 in the growing oocytes (Draper et al. 1996; Mootz et al. 2004; Hunter and Kenyon 1996; Jadhav et al. 2008). MEX-3 contains two prototypical KH domains and a bipartite RNA sequence motif was defined as a consensus MEX-3 RNA-binding site, using an in vitro reiterative RNA selection approach. This MEX-3 recognition element (MRE) consists of two four-nucleotide-binding motifs, which are separated by 0–8 nucleotides: DKAGN$_{(0–8)}$UHUA (D, everything except a C; K, is a G or U; H, everything except G) (Pagano et al. 2009). Consistent with the notion that each individual KH domain of MEX-3 may contact one motif to achieve overall high RNA selectivity and affinity, the elimination of both motifs is more detrimental to MEX-3 RNA binding than compromising an individual motif of the MRE (Pagano et al. 2009). Although numerous candidate MEX-3 target mRNAs were suggested (Pagano et al. 2009), little is known about how MEX-3 functions with GLD-1 to maintain totipotency of germ cells (Ciosk et al. 2006) and functions with PUF-8 in germ cell proliferation (Ariz et al. 2009).

8.4.1.4 CPEB Proteins

*C*ytoplasmic *p*olyadenylation *e*lement-*b*inding (CPEB) proteins represent an evolutionarily conserved protein family with a stereotypic multi-domain organization (Mendez and Richter 2001). Two centrally placed RNA recognition motifs (RRMs) are followed by two consecutive zinc finger motifs of the CCCC- and CCHH-types, respectively. All four domains are required for RNA binding in *Xenopus* CPEB1, the best-characterized protein family member (Hake et al. 1998; Richter 2007). CPEB1 recognizes a 3′UTR located sequence motif, termed the cytoplasmic polyadenylation element (CPE), of the AU-rich consensus sequence U$_4$A$_{1–2}$U (Stebbins-Boaz et al. 1996; de Moor and Richter 1997). In *Xenopus* oocytes, CPE-mediated

translational control depends on the phosphorylation status of CPEB1: hypo-phosphorylated CPEB1 acts as a translational repressor, while hyper-phosphorylated CPEB1 acts as a translational activator, thereby CPEB1 represents a molecular switch of CPE-containing maternal mRNAs (Richter 2007). In *C. elegans*, the CPEB family is comprised of four proteins of yet undefined RNA-binding capacity, CPB-1 to CPB-4. As *C. elegans* 3′UTRs are in general quite AU-rich it is unclear how many mRNAs would serve as specific CPB targets, and if CPBs serve as a translational regulatory molecular switch. In contrast to the other RNA regulators discussed in this section, the mRNAs of most CPB proteins are either expressed abundantly or even exclusively in male germ cells (Luitjens et al. 2000). Consistent with this CPB-4/FOG-1 is essential for germ cells to adopt the male fate in hermaphrodites (Barton and Kimble 1990). Moreover, FOG-1 promotes germ cell proliferation in a dose-dependent manner (Thompson et al. 2005). CPB-1 aids meiotic progression of spermatogenic germ cells (Luitjens et al. 2000). Similar to its homologs in *Xenopus* and *Drosophila*, CPB-3 functions in early oogenesis, preventing excessive physiological germline apoptosis (Boag et al. 2005). Additional functions may include the regulation of the sperm-to-oocyte switch and the mitosis-to-meiosis decision (Hasegawa et al. 2006). No roles of CPB-2 have yet been reported.

8.4.2 Expression and Activity Domains of RNA Regulators

The overall organization of the adult hermaphrodite germ line is perfectly suited for correlating protein expression levels with germ cell fates. While mRNA and protein gradients can be analyzed in a spatially stretched out distal to proximal axis, this arrangement reflects in reality a gradient of high temporal resolution, with undifferentiated mitotic germ cells near the distal end and fully differentiated gametes at the proximal end. This cell biological advantage compensates for the downside of being unable to isolate developmentally staged germ cells for biochemical experiments. For ease of description we refer to the mitotic region and the early stages of meiotic prophase I (leptotene, zygotene, and pachytene) as the distal part of the adult germ line. Germ cells undergoing diplotene are confined to the loop region, a morphological hallmark of the germline tube. The proximal part of the germ line contains differentiated germ cells, which in the case of oocytes are in diakinesis or in the case of sperm have completed the meiotic divisions.

Little data on de novo RNA synthesis activity of germ cells is available (Sheth et al. 2010; Schisa et al. 2001; Starck 1977; Starck et al. 1983). However, a comparison of the steady-state levels of mRNAs, deduced from the in situ hybridizations of many specific mRNAs generated by several laboratories, reveals the following general picture. Bulk transcriptional activity of the adult female germ line appears to be confined to the distal arm, especially to the more proximal mitotic region and early stages of prophase I (Schisa et al. 2001; Starck 1977), giving rise to essentially two prevalent mRNA expression patterns, a ubiquitous

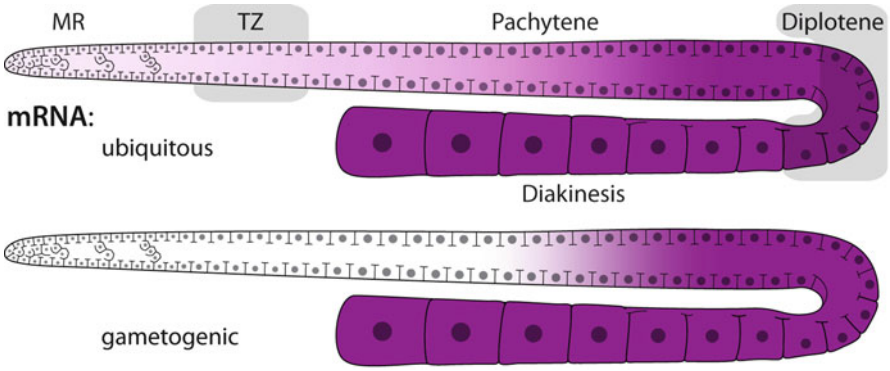

Fig. 8.2 Distribution of mRNAs in the adult germ line. The relative mRNA abundance in female germ cells is illustrated in magenta as observed by in situ hybridization experiments. A ubiquitous and a gametogenic expression pattern are depicted. Distal is top left and differentiated oocytes are most proximal. Previously made sperm is not shown. MR, mitotic region. For further details, see text, Sect. 8.4.2

and a gametogenic pattern, each sharing the common feature of abundant mRNA accumulation in oocytes (Fig. 8.2). Ubiquitously expressed mRNAs are present at lower levels in the more distal germ line when compared to their amounts in oocytes. Often the level increases before the loop region, which may correlate with an additional transcriptional burst in the late pachytene stage. By contrast, the gametogenic expression pattern is exclusively dominated by transcriptional activity in the pachytene region with no detectable mRNA in earlier developmental stages. In both cases, mRNAs are stockpiled in oocytes as maternal load for the early embryo, suggesting that such mRNAs are either important for early embryogenesis or they indirectly escape mRNA decay, as RNA degradation mechanisms may not be active in growing oocytes and their clearance is initiated upon fertilization (Seydoux and Fire 1994). A similar situation is present in the male germ line; mRNA is either produced in the mitotic region and/or in a second wave during late pachytene (Klass et al. 1982). However, little to no mRNA remains detectable in mature sperm, presumably due to exclusion of most cytoplasmic components in the last maturation steps of spermatogenesis or due to active mRNA degradation in the late stages of meiosis. Nevertheless, some RNAs may be in sperm and paternally donated to the zygote, like in other organisms (Bourc'his and Voinnet 2010).

In strong contrast to these simple and generic mRNA expression patterns, the derived protein expression patterns are far more diverse and complex, exemplifying the importance of translational control. A direct correlation between RNA and protein levels rarely exists. A better correlation is observed between protein amounts and their activities, although additional layers of regulation can occur. In addition, all protein patterns are presumably further shaped by the intrinsic stability of the encoded protein. However, little is known yet about regulated protein degradation during postembryonic germline development.

General and specific post-transcriptional RNA regulators have distinct protein expression patterns, often nicely paralleling their requirements for germline development (Table 8.4). While general RNA regulators are expressed in most germ cells, giving rise to a ubiquitous protein expression pattern across the germ line, specific RNA regulators can be selectively expressed, occupying distinct germline territories. This is especially true for RNA-binding proteins. In the case of translational repressors, five distinct protein expression patterns are prevalent in the adult hermaphrodite (Types A–D and U, Fig. 8.3): The PUF proteins FBF-1, FBF-2, and PUF-8 are predominantly expressed in the distal most part of the germ line, corresponding to the mitotic region and the initial stages of meiosis (Type A) (Crittenden et al. 2002; Lamont et al. 2004; Ariz et al. 2009). The expression pattern of the maxi-KH protein GLD-1 covers a larger region of the distal part extending to the germline loop, overlapping with the mitotic region and the early meiotic stages until diplotene (Type B) (Jones et al. 1996). MEX-3 expression is inverse to Type B, being abundant in the mitotic region, very low in early prophase, but abundant again in late prophase (Type C) (Mootz et al. 2004; Ciosk et al. 2006). PUF-5 and the zinc finger proteins OMA-1 and OMA-2 are restricted to meiotic stages beyond the germline loop, corresponding to the last two meiotic prophase stages, diplotene and diakinesis (Type D) (Detwiler et al. 2001; Lublin and Evans 2007).

NOS-3, a presumed translational co-repressor of FBF, is an example of a ubiquitously expressed germline protein (Type U) (Kraemer et al. 1999; Arur et al. 2011). Nevertheless, its temporal activity during female germline development is modulated at the post-translational level by MAP kinase (MPK-1) (Arur et al. 2011). Non-phosporylated NOS-3 is restricted to the distal part of the germ line, corresponding to a Type A pattern, whereas phosporylated NOS-3 (phospho-NOS-3) accumulates during pachytene and persists until diakinesis, similar to a Type D pattern (Fig. 8.3). The resulting overlap between non-phosporylated NOS-3 and FBF has functional consequences with respect to controlling the expression of the sex determining protein FEM-3 (Arur et al. 2011). At the molecular level, the interaction of FBF and NOS-3 is sensitive to the phosphorylation status of NOS-3, as FBF binds in vitro with higher affinity to non-phospho-NOS-3 than to phospho-NOS-3, suggesting that MPK-1-phosphorylated NOS-3 can no longer engage in the translational repression of *fem-3* (Arur et al. 2011). Although no other example of activity is currently available that demonstrates post-translational RNP-activity changes, it is conceivable that this type of regulation is prevalent. Especially, MPK-1 may appear as a master regulatory kinase for controlling the activity of RNA regulators as it is ubiquitously expressed and its activated form is abundant prior to the loop region and in the proximal part of the germ line, underscoring its many roles during germline development (Lee et al. 2007b; Arur et al. 2009, 2011; Lee et al. 2007a). Other kinases and different post-translational modifications likely also add to regulate mRNP activities.

Subtle deviations from the five dominant expression patterns exist and the relative amounts of a given RNA regulator may vary in detail among distinct germ cell stages. This may reflect dose-dependent requirements of germ cell fate regulation whereby protein amounts correlate directly with the activity of an RNA regulator.

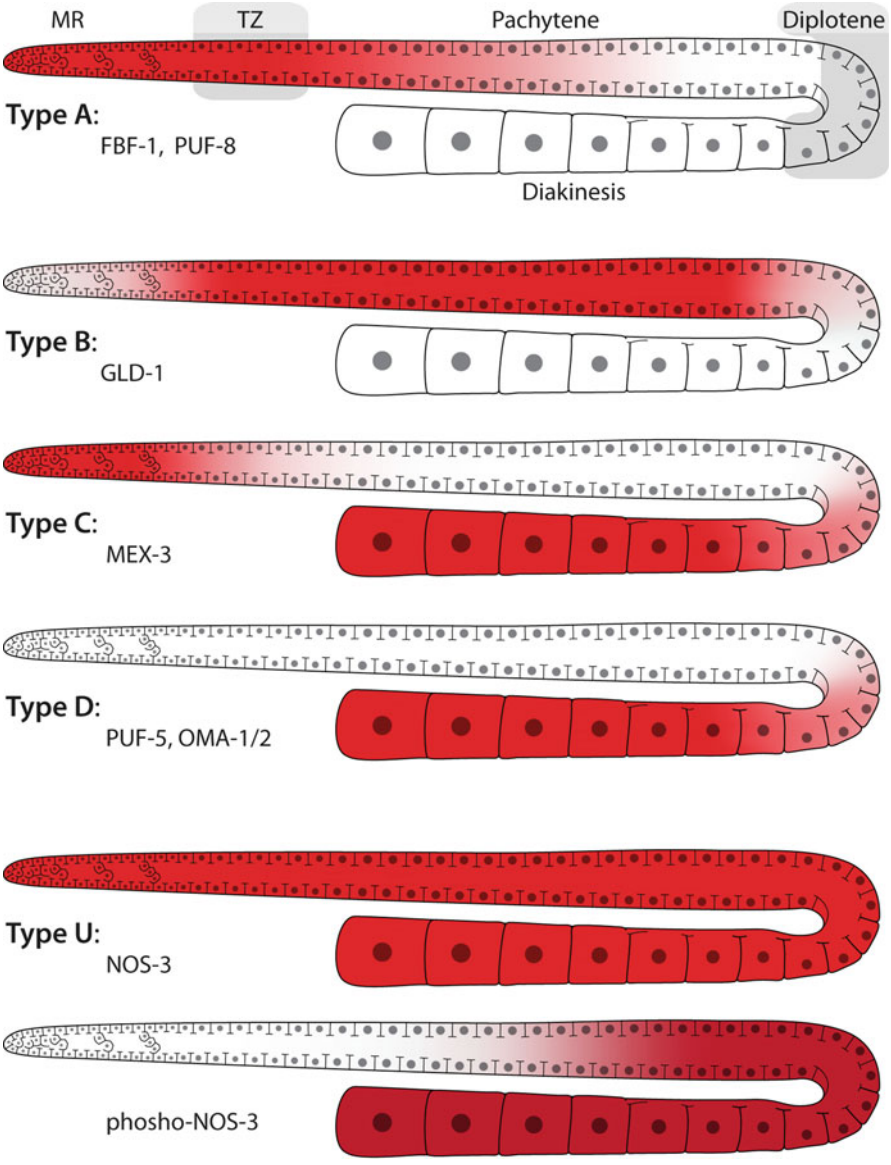

Fig. 8.3 Predominant distribution of translational repressors in the adult germ line. The relative protein abundance in female germ cells is illustrated in red. Orientation and labels as in Fig. 8.2. Five prevailing expression patterns can be distinguished: Type A—mainly restricted to the mitotic region, B—mainly restricted to very early prophase and the pachytene region, C—is an example of a complex expression pattern, which demonstrates that also combinations of Type A and Type D are possible, D—mainly limited to developing oocytes in diplotene and diakinesis, U—ubiquitous expression, which can be further limited by post-translational modifications to restrict protein activity such as phosphorylation (phospho). Deviations and other combination of these categories are possible. The sharpness of the boundaries needs to be adjusted for each RNA regulator in detail

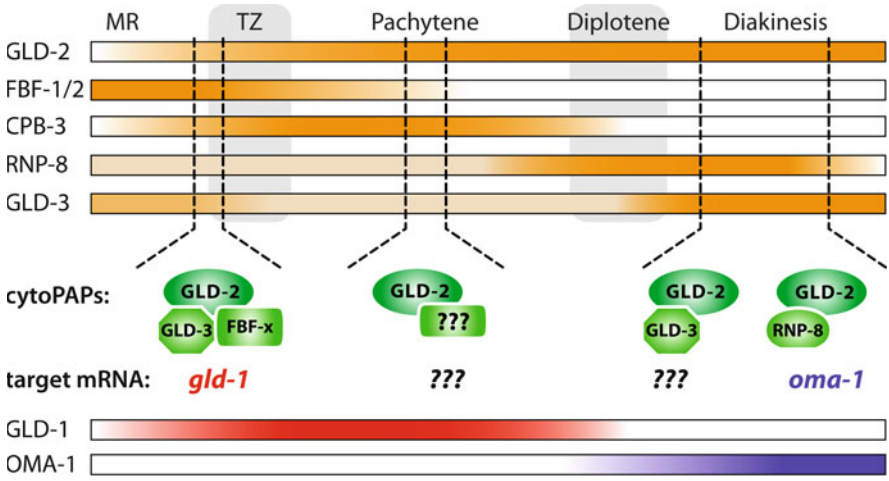

Fig. 8.4 Different translational activator complexes dominate specific regions in the adult germ line. The relative protein distribution across the female germ line for GLD-2 cytoPAP components, FBF-1, FBF-2, RNP-8, and GLD-3 is depicted in shades of orange. The corresponding developmental germ cell stage is indicated. Based on protein abundance an assembly of GLD-2/GLD-3/FBF-x is likely to be dominant in the mitotic region (MR) and very early meiosis (TZ, transition zone), and of GLD-2/GLD-3 and GLD-2/RNP-8 during diakinesis. Examples of established target mRNAs of the individual cytoPAPs are given. Translational activator complexes are contrasted with the expression domain of GLD-1 and OMA-1, two translational repressors. *gld-1* mRNA is translationally activated by GLD-2 cytoPAP complexes. *oma-1* mRNA is a target of GLD-1 repression and GLD-2 activation. For further details, see Sects. 8.4.3 and 8.5.3

Alternatively, the expression of the RNA regulator follows indirectly germ cell fates. A good example of the former type is the expression profile of GLD-1/STAR in the distal most part of the female germ line (Fig. 8.4), where a two-step increase of GLD-1 expression is observed: GLD-1 levels rise from virtually undetectable to low levels in mitotic germ cells closest to the transition zone. GLD-1 expression is further boosted to its highest expression level in the transition zone and plateaus in pachytene (Type B, Fig. 8.3). This level difference is critical: low levels of GLD-1 correlate with its known role in meiotic entry and high GLD-1 levels are consistent with its essential role in female meiotic progression. By contrast, raising the usually low amounts of GLD-1 during the development of the mitotic region, all distal germ cells enter meiosis at the expense of further mitotic activity (Crittenden et al. 2002; Hansen et al. 2004).

An example of a Type A variation is FBF-1, as its protein expression in the mitotic region is not uniform. FBF-1 abundance is increased in the more centrally positioned mitotic cells compared to the distal and proximal flanking germ cells (Crittenden et al. 2002). Conversely, FBF-2 may be even more enriched in the distal-most germ cells of the mitotic region and less prevalent in the proximal part of the mitotic region (Lamont et al. 2004). The differences between both expression

patterns were attributed to a positive transcriptional response of *fbf-2* mRNA expression, stimulated by the *glp-1*/Notch signaling pathway (Lamont et al. 2004). Their different expression levels were also correlated with a distinct influence of each FBF protein on the size of the mitotic region (Lamont et al. 2004).

The protein expression patterns of many translational activators resemble those of the translational repressors (Table 8.4 and Fig. 8.4): DAZ-1, a conserved RRM-containing protein, follows a type A pattern, CPB-3 fits a type B pattern, and the RRM protein RNP-8 fits an extended modified type D pattern, as it is abundant in pachytene (Hasegawa et al. 2006; Maruyama et al. 2005; Kim et al. 2009a). The multi-KH domain protein GLD-3 expression is similar to a type C pattern (Eckmann et al. 2002). GLD-3, and RNP-8 are protein interactors of the ubiquitously expressed cytoPAP GLD-2 (Kim et al. 2009a; Wang et al. 2002) (Fig. 8.4). Further, GLD-3, RNP-8, and FBF stimulate GLD-2 poly(A) polymerase activity in vitro (Wang et al. 2002; Kim et al. 2009a). Thus, given their spatially distinct expression patterns it is likely that different cytoPAP complexes are formed, activating translation of specific mRNA targets at specific germline stages (Fig. 8.4). For example, it is proposed that distally expressed FBF and GLD-3 may control GLD-2 activity for promoting meiotic entry (Eckmann et al. 2004). A GLD-2/GLD-3 complex may be important for meiotic progression. GLD-3 and RNP-8 may control GLD-2 activity separately to promote the sperm or oocyte fate, respectively (Kim et al. 2009a), but together they may regulate maternal mRNA storage during oocyte maturation (Kim et al. 2010). A peculiarity of the GLD-2 expression pattern is its low abundance in the distal-most mitotic region, which may reflect a lower demand for GLD-2 in mitotic cells and/or that mitotic cells regulate GLD-2 in a specific manner. Certainly high levels of GLD-2 correlate with its essential roles in meiotic progression. GLD-4 and GLS-1, two components of the second poly(A) polymerase complex, are ubiquitously expressed (Schmid et al. 2009; Rybarska et al. 2009).

Interestingly, some RNA regulators also display sexually dimorphic expression patterns, suggesting fundamental differences in the requirement of the individual factors for male and female gametogenesis. An obvious example is the translational repressor GLD-1. Low levels of GLD-1 promote entry into meiosis and high levels promote female meiotic progression. Consistent with these functions, GLD-1 is weakly expressed in pre-meiotic male germ cells and remains low during very early stages of meiosis (Jones et al. 1996). Further, meiosis commitment defects are restricted to female *gld-1* mutant germ lines (Francis et al. 1995a). A second example is the protein expression profile of the RNA regulator GLD-3. Its Type C pattern appears inverse to the Type B pattern of GLD-1 in females (Eckmann et al. 2004) (Fig. 8.4). In the male germ line, GLD-3 accumulates steadily during all stages of male gametogenesis and maintains highest expression levels in metaphase of meiosis I and II (Eckmann et al. 2002). The lack of an apparent down-regulation of GLD-3 in male pachytene compared to female pachytene remains unclear but an artificial elevation of GLD-3 in female pachytene causes germ cells to arrest in meiosis (Jedamzik and Eckmann, unpublished results). Therefore, this sexual dimorphic expression pattern reflects presumably dose-dependent differences for RNA regulators controlling male and female meiotic progression.

8.4.3 Systems Biology of RNA Regulatory Networks

A remarkably and almost universal feature of germ cells is their overt reluctance to employ transcription factor networks to generate gene expression patterns, unlike somatic cells. Especially in *C. elegans* female germ cells, gene regulation appears rather permissively instructed at the DNA level. Transgenic reporter analysis of a dozen germline-enriched genes demonstrated that female protein expression patterns can be recapitulated to a large extent with the gene's cognate 3′UTR, while male germ cell differentiation genes rely largely on promoter-mediated gene regulation (Merritt et al. 2008). These experiments underscore the regulatory power of 3′UTRs and highlight the fact that mRNAs can encode all the information necessary to regulate their expression (Kuersten and Goodwin 2003). Within mRNAs, multiple protein-binding and regulatory sites (e.g., for miRNAs) together facilitate the formation of distinct mRNPs. Here, different RNP protein components may act on a single mRNA and single mRNP protein components may act on multiple mRNA species. The combinatorial coordination of *cis*-acting sites and *trans*-acting factors provide the structural framework of RNA regulatory networks that coordinate gene expression of germ cells. This is further facilitated by the extreme modularity of the protein–RNA interactions, which also provide the basis for evolutionary rewiring within the network to adapt cell fate decisions to developmental or environmental changes.

RNA regulatory networks are built of many different modules. Each module is composed of three integral components, which organize themselves into mRNP units. It is important to keep in mind that not all mRNAs of a given gene are regulated in the same way within the germ line. Rather, mRNPs are flexible operational entities that exchange their constituents, even within cellular territories. The organizational principle of an mRNPs may be viewed as three "layers" on top of the nucleating mRNA itself. At the heart of an mRNP unit is the RNA selector protein, which recognizes a *cis*-regulatory site and thereby flags the RNA target for regulation. The RNA selector represents the physical link between the mRNA and the mRNP, and may act in certain instances even as a seed for mRNP formation. The second principal mRNP components are mRNA-associated proteins that primarily assist the formation of larger and stable mRNP units, by having RNA affinity themselves and/or binding directly to the RNA selector. This second layer of proteins represents regulators of RNA selector capacity by further narrowing the RNA target group and provides the basis for the functional output of the mRNP (i.e., translational repression/activation or RNA stability). Further, they form an extended signaling input platform that integrates developmental controls into the formation, disassembly or remodeling of mRNPs. An example of this class includes RNA helicases. The third layer consists of mRNP-associated components that either enforce or change the activity of the mRNP. In this category belong RNA-modifying enzymes, such as deadenylases or poly(A) polymerases. Nevertheless, it is possible that the functions of two components are combined into a single molecule, like in the case of the RNA selector FBF. In addition to its established role as a translational repressor, FBF may

also have the capacity to act as a translational activator, based on the composition of the mRNP unit. Data on *gld-1* mRNA regulation suggest that it forms mRNPs that require FBF for their translational activation and repression (Suh et al. 2009). Hence, FBF-mediated regulatory functions appear to be dictated by the type of mRNP that is assembled on the mRNA target. This concept may even apply for many mRNPs that use RNA selectors for dual functions. Therefore, such RNA selectors are better designated as translational effectors.

The establishment of RNA regulatory networks in the germ line is strictly correlated with the germ cell fate and the developmental stage of the germ cells. Integral to the network is that mRNP protein components are themselves subject to translational control, forming a strongly connected web of regulators with sharp boundaries of expression territories throughout the germ line. Especially the central nodes of the network, the RNA selectors, are precisely controlled. As discussed in Sect. 8.4.2, the expression domains of FBF, GLD-1 and PUF-5 establish an interdependent regulatory system reaching from the distal to the proximal part of the germ line. FBF limits GLD-1 expression (Crittenden et al. 2002), whereas GLD-1 limits OMA-1/2 (Lee and Schedl 2004) and PUF-5 (Lee and Schedl 2001) expression (Fig. 8.4). The initiation of this sequential negative repression cascade is further enforced by negative auto-regulation and cross-regulation, as observed for FBF-1 and FBF-2 (Lamont et al. 2004). These small circuits may be in place to speed up the response time and reduce the cell-to-cell variation in protein levels that are due to fluctuations in their production rate.

A major aspect of RNA regulatory networks in the germ line is the high redundancy of its modules that govern germ cell fate decisions and the strong enforcement level once the decisions have been made. The two best examples are the sperm-to-oocyte decision and meiotic entry, which are both regulated by PUF proteins. Combinations of two out of three different PUF proteins, FBF-1, FBF-2, and PUF-8, translationally repress multiple players of the sex determination pathway, achieving a tight suppression of sperm promoting factors at several different levels of the gene hierarchy (see Zanetti and Puoti 2012). A minimum of two parallel-acting pathways ensures meiotic entry. Two genetically independently acting translational repressors (NOS-3 and GLD-1) form a negative feedback loop to repress mitotic genes, i.e., GLD-1 translationally represses Notch/*glp-1* mRNA (Marin and Evans 2003) and cyclin E/*cye-1* mRNA (Biedermann et al. 2009). Concomitantly, the two translational activators, GLD-2 and GLD-3, enforce spatially regulated meiotic entry by presumably activating yet unknown meiotic genes (Eckmann et al. 2004). The bimodality of this cell fate switch is further assisted by FBF, which translationally represses GLD-1 (Crittenden et al. 2002), cyclin-dependent kinase inhibitor (CKI-1) accumulation (Kalchhauser et al. 2011), and the ectopic expression of meiotic proteins, such as the synaptonemal complex components HIM-3, HTP-1, SYP-1, and SYP-2 (Merritt and Seydoux 2010) (Table 8.5). Once female germ cells have entered meiosis, abundant GLD-1 levels ensure meiotic progression. GLD-1 accumulation is promoted by the redundant action of the two distinct poly(A) polymerases GLD-2 and GLD-4/GLS-1 (Suh et al. 2006; Schmid et al. 2009). This tight connectivity of the RNA regulatory network nodes and the

enormous redundancy of the system allowed initially the identification of the key RNA selectors, as they are central to the network's gene expression capacity. Lastly, it becomes apparent that different mRNA species associated with defined RNA-binding protein components appear to encode functionally related proteins, leading to coordinated gene expression patterns.

An obstacle in elucidating the wiring of the RNA regulatory system is that simple forward genetic approaches have reached their limits in identifying mRNA targets of the network. Consequently, much more focused genetic screens are required (Schumacher et al. 2005) and biochemical approaches combined with modern molecular detection systems need to be pursued (Kim et al. 2010; Kershner and Kimble 2010; Wright et al. 2011). Although this is not limited to *C. elegans*, as most RNA-binding selector proteins in all species will have multiple targets, a few target mRNAs were identified in such screens by mutations that clustered in 3′UTRs. These gain-of-function mutations removed a key translational repressor site in *tra-2* (Goodwin et al. 1993) and *fem*-3 (Ahringer and Kimble 1991), which encode two essential sex-determination pathway components (see Zanetti and Puoti 2012). A fortuitous combination of the dose sensitive nature of the sex-determination pathway and an easy to score morphological phenotype enabled their discovery. Further, both genes appear to represent an Achilles' heels in a cell fate decision that presumably evolved recently and therefore is less complex. Together with the dose sensitivity of some RNA selectors and RNA-associated components, these examples also underscore that a quantitative assessment of the system is key in understanding the networks wiring. Although new technologies are available to tackle the RNA-target repertoire of RNA selectors, quantifications of the amounts necessary to build functional distinct mRNPs, its protein components and the number and strength of diverse *cis*-regulatory elements, remain a challenge for the future in understanding germline gene regulation.

8.5 Regulation of Specific Germ Cell Fate Decisions Via mRNP Activities

The emerging concepts of the detailed molecular mechanisms of translational control in the *C. elegans* germ line are derived from the studies on mRNAs that encode key germ cell fate determinants. The first mRNAs identified, *tra-2* and *fem-3* (Ahringer and Kimble 1991; Goodwin et al. 1993), turned out to be controlled by two distinct RNA regulatory machineries and became paradigms of GLD-1- and FBF-mediated translational repression (Zhang et al. 1997; Jan et al. 1999). Recently, a few additional targets were added to this list of well-studied mRNAs, which include *gld-1* and *glp-1* mRNA. Many more mRNA targets are currently being discovered in genome-wide studies using large-scale RNP immunopurifications, coupled to microarray discovery or next-generation RNA-sequencing techniques. Here, we will focus on a few select examples that will serve as paradigms of translational

control mechanisms. Other mRNAs subject to translational control with defined and validated *cis*-regulatory elements are listed in Table 8.5.

8.5.1 Multidimensional Translational Control of tra-2 mRNA

The sex determining gene *tra-2* is required for female cell fates (see Zanetti and Puoti 2012, Chap. 3). As *tra-2* activity must be lower than *fem-3* activity during spermatogenesis in hermaphrodites, *tra-2* mRNA is translationally repressed in a GLD-1-containing mRNP (Jan et al. 1999). Gain-of-function 3′UTR mutations in *tra-2* affect one or both of two direct repeat elements (DREs), which contain RNA-binding sites for the maxi-KH domain protein GLD-1 (Goodwin et al. 1993; Jan et al. 1999). At least two functional GLD-1 binding motifs (GBMs) are present in the *tra-2* 3′UTR, which are bound by GLD-1 from worm extracts (Clifford et al. 2000). A comparison of wild-type and GBM-lacking 3′UTR sequences shows that the poly(A) tail of wild-type *tra-2* mRNA is shorter than that of the non-repressed mRNA (Jan et al. 1997). Also, reporter RNA constructs that carry GBMs are rapidly deadenylated in *Xenopus* embryos (Thompson et al. 2000). Taken together, this suggests that GLD-1 may repress *tra-2* mRNAs via a poly(A) tail-mediated mechanism, e.g., by recruiting mRNA deadenylases. However, experimental tests of this mechanistic aspect of *tra-2* mRNA regulation in *C. elegans* germ cells have not yet been performed.

Certainly, additional factors are needed for *tra-2* translational regulation. Animals heterozygote for loss-of-function mutations in the RNA-dependent DEAD-box RNA helicase LAF-1 are sperm-to-oocyte switch defective and contain higher levels of TRA-2 protein (Goodwin et al. 1997). As the isolated mutations presumably affect LAF-1's ATPase activity or RNA-binding capacity, it has been suggested that LAF-1-mediated remodeling of GLD-1-containing mRNPs may be important for efficient *tra-2* mRNA translational repression. In addition, GLD-1/*tra-2* mRNPs contain FOG-2, a special type of F-box protein (Clifford et al. 2000). Although the *tra-2* 3′UTR can recruit FOG-2 and GLD-1 proteins from worm extracts, the binding of GLD-1 to *tra-2* mRNA is not dependent on FOG-2. Rather, FOG-2 may represent a unique co-factor that influences GLD-1-mediated translational repression, via defined protein interaction sites between FOG-2 and GLD-1. The true molecular function of both RNA-associated components remains unclear.

A second mechanism contributes to *tra-2* mRNA regulation, which illustrates the tight interplay of mRNP history with mRNA fates. This mechanism involves TRA-1, a conserved zinc finger transcription factor. TRA-1 binds to a sequence element in the *tra-2* 3′UTR adjacent to the GBMs and facilitates the nuclear export of *tra-2* mRNA (Graves et al. 1999). The export of TRA-1 protein and *tra-2* mRNA is interdependent and a deletion of the TRA-1-binding element in *tra-2* mRNA results in the nuclear accumulation of both TRA-1 protein and *tra-2* mRNA (Segal et al. 2001). Importantly, *tra-2* mRNA is not exported via the canonical RNA export pathway of polyadenylated mRNAs, but rather uses an alternative pathway

mediated by NXF-2, REF-1, and REF-2, which facilitates a more efficient translation regulation (Kuersten et al. 2004). Other conserved general RNA regulators, such as members of the exon junction complex affect the sperm-to-oocyte switch and likely regulate components of the sex determination pathway (Li et al. 2000). Together this suggests that the nuclear history of mRNA may directly influence its cytoplasmic fate.

8.5.2 *Reiterated Translational Control of glp-1 mRNA During Development*

glp-1 is important for maintaining proliferation in the mitotic region and its mRNA is of the ubiquitous type (Fig. 8.2, see Hansen and Schedl 2012). In addition to its post-embryonic germline function, *glp-1* is also essential for anterior cell fates in the early embryo (Austin and Kimble 1987; Priess et al. 1987). GLP-1 protein expression is therefore tightly controlled. *glp-1* mRNA is translationally repressed during multiple stages of germ cell development via different RNA selectors. In undifferentiated oocytes during early meiosis *glp-1* mRNA is responsive to GLD-1-mediated repression (Marin and Evans 2003), as it carries several GBMs in its 3′UTR (Wright et al. 2011). In oocytes, where GLD-1 is absent, members of the PUF-family, PUF-5 and PUF-6/7, suppress efficient GLP-1 protein synthesis (Lublin and Evans 2007). During early stages of embryogenesis, *glp-1* mRNA is subject to POS-1-mediated repression, a maternally provided zinc finger-containing protein (Ogura et al. 2003). This illustrates nicely that an mRNA can be repressed by different translational regulators at different points during germ cell development. However, detailed mechanistic insight into how *glp-1* mRNA is translationally repressed is still lacking.

8.5.3 *Distinct Poly(A)-Tail Length Control Mechanisms in PUF-Mediated mRNA Regulation*

fem-3 mRNA is a target of FBF-mediated translational repression (Zhang et al. 1997). FEM-3 protein expression must be lowered after initial spermatogenesis in hermaphrodites to facilitate oogenesis. The RNA selector FBF and the FBF-associated co-repressor NOS-3 translationally repress ubiquitously expressed *fem-3* mRNA, possibly in the pre-meiotic germ cells (Arur et al. 2011). One FBF-binding element (FBE) is present in the *fem-3* 3′UTR (Zhang et al. 1997), which was initially genetically defined as mutations that conferred a *fem-3* gain-of-function phenotype (Barton et al. 1987). Comparative Northern blot analysis of such *fem-3* mutations with wild-type clearly demonstrated that *fem-3(gf)* mRNAs possess a longer poly(A) tail (Ahringer and Kimble 1991). Together this suggests that FBF-mediated translational regulation involves poly(A)-tail shortening of *fem-3* mRNA

to inhibit FEM-3 accumulation in the mitotic region. As mentioned in Sect. 8.4.2, the co-repressor function of NOS-3 depends on its phosphorylation status.

A similar picture of poly(A)tail-mediated translational regulation emerged with *gld-1* mRNA, an FBF target mRNA that contains two FBEs in its 3′UTR (Crittenden et al. 2002). Intriguingly, the *gld-1(oz10)* allele is a deletion affecting the *gld-1* 3′UTR. It eliminates both FBEs and confers also a semi-dominant gain-of-function sperm-to-oocyte phenotype (Francis et al. 1995a; Jones and Schedl 1995). In agreement with this, more GLD-1 protein is expressed in the mitotic region, where FBF is active (Jones et al. 1996). Unfortunately, the interpretation of this result is hampered by a second-site mutation in the GLD-1 protein that causes an amino acid change of unknown consequence (Jones and Schedl 1995). Consistent with FBE-mediated translational repression, the elimination of FBF-1 causes precocious GLD-1 protein synthesis in mitotic germ cells (Crittenden et al. 2002). In vitro studies show that FBF-bound synthetic polyadenylated RNA is sensitive to a Pop2p-containing deadenylase complex purified from yeast (Suh et al. 2009). Compatible with a general ability of PUF proteins to physically associate with Pop2p-type deadenylases, FBF binds CCF-1 in vitro (Suh et al. 2009). Although a formal test of the CCF-1–FBF interaction in vivo is lacking, it is reasonable to envision that FBF-mediated translational repression causes a shortening of the poly(A) tail via the recruitment of a CCF-1/CCR-4/LET-711 complex.

Although evidence for a worm CCF-1/CCR-4/LET-711 deadenylase complex is currently lacking (see Sect. 8.3.2), it is interesting to note that *ccr-4* activity is linked to *gld-1* mRNA stability. This has been observed in the *gld-2 gld-4* double mutant, which lacks polyadenylation activity of GLD-2 and GLD-4 cytoPAPs (Schmid et al. 2009). Both GLD-type cytoPAPs are required for efficient GLD-1 protein synthesis after FBF-mediated translational repression. In the absence of both cytoPAPs, a destabilization of *gld-1* mRNA is observed, which can be prevented either by elimination of FBF or CCR-4; however, no protein accumulation of GLD-1 occurred (Schmid et al. 2009). Hence, translational activation is assumed to be a consequence of poly(A)-tail elongation of *gld-1* mRNA. Consistent with this view is that *gld-1* mRNA poly(A)-tail length is shortened in the absence of GLD-2 (Suh et al. 2006).

In summary, the emerging picture from these two examples is that a single FBE may require FBF and co-repressors for efficient translational repression. Multiple FBEs may recruit more efficiently FBF molecules to the mRNP and depending on the mRNP unit formed, FBF elicits deadenylation of the target mRNA or participates in its polyadenylation-mediated translational activation.

8.5.4 RNA Regulatory Networks in the Male Germ Line

The *C. elegans* XO male germ line produces sperm only. While most RNA regulators are shared between male and female germ cells, some are unique to either sex, largely correlating with the two different gametogenesis programs. Like their female

counterparts, spermatogenic cells utilize almost the exact same set of RNA regulatory factors to achieve the mitosis-to-meiosis decision and meiotic progression. However, the RNA regulatory network is not wired identically, for example, GLD-1 is not crucial for male meiotic progression (Francis et al. 1995a). Although the male germ line does not promote the switch to the oocyte fate, RNA regulators such as FOG-1 and GLD-3 are important for initiating and maintaining the sperm fate, respectively (Eckmann et al. 2002; Thompson et al. 2005). To maintain meiotic progression, CPB-1 (Luitjens et al. 2000), PUF-8 (Ariz et al. 2009) and the activity of both cytoPAPs, GLD-2 (Kadyk and Kimble 1998) and GLD-4 (Schmid et al. 2009), is essential, suggesting that poly(A)-tail metabolism plays also a central role in the male germ line. Furthermore, 5′cap-mediated regulation has been documented to assist male gametogenesis (Amiri et al. 2001). However, much less is known about the individual RNA regulatory components in the male, representing fertile ground for future research.

8.6 Concluding Remarks

The evolutionary conservation of the many germline RNA regulators across metazoans highlights the importance of post-transcriptional gene expression control. Research done on the *C. elegans* germ line allowed us to start grasping its magnitude and complexity in a developmental setting. However, our understanding of translational control at a systems level is far from complete and will require new tools. In vitro reconstitution assays need to be to setup to define the contribution of mRNP components in their respective environment. Also, in vitro translation assays to measure mRNP activities have to be developed. Our current knowledge of individual mRNPs, their composition and the interplay between different mRNPs has to be clearly expanded. Once individual mRNP units are defined and the *cis*-regulatory code of mRNAs is deciphered, a fully integrated view of all mRNP units into a developmentally changing RNA regulatory network will remain a last big challenge.

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