Keeping development on time: insights into post-transcriptional mechanisms driving oscillatory gene expression during vertebrate segmentation

Monica C. Blatnik,
The Ohio State University, Department of Molecular Genetics, Columbus, Ohio, 43210-1132, United States

Thomas L. Gallagher,
The Ohio State University, Department of Molecular Genetics, Columbus, Ohio, 43210-1132, United States

Sharon L. Amacher
The Ohio State University, Department of Molecular Genetics, Columbus, Ohio, 43210-1132, United States

Abstract

Biological time keeping, or the duration and tempo at which biological processes occur, is a phenomenon that drives dynamic molecular and morphological changes that manifest throughout many facets of life(Kageyama et al., 2012; Shimojo et al., 2008; Van Norman et al., 2013). In some cases, the molecular mechanisms regulating the timing of biological transitions are driven by genetic oscillations, or periodic increases and decreases in expression of genes described collectively as a "molecular clock". In vertebrate animals, molecular clocks play a crucial role in fundamental patterning and cell differentiation processes throughout development. For example, during early vertebrate embryogenesis, the segmentation clock regulates patterning of the embryonic mesoderm into segmented blocks of tissue called somites, which later give rise to axial skeletal muscle and vertebrae. Segmentation clock oscillations are characterized by rapid cycles of mRNA and protein expression. For segmentation clock oscillations to persist, the transcript and protein molecules of clock genes must be short-lived. Faithful, rhythmic, genetic oscillations are sustained by precise regulation at many levels, including post-transcriptional regulation, and such mechanisms are essential for proper vertebrate development.

Graphical Abstract
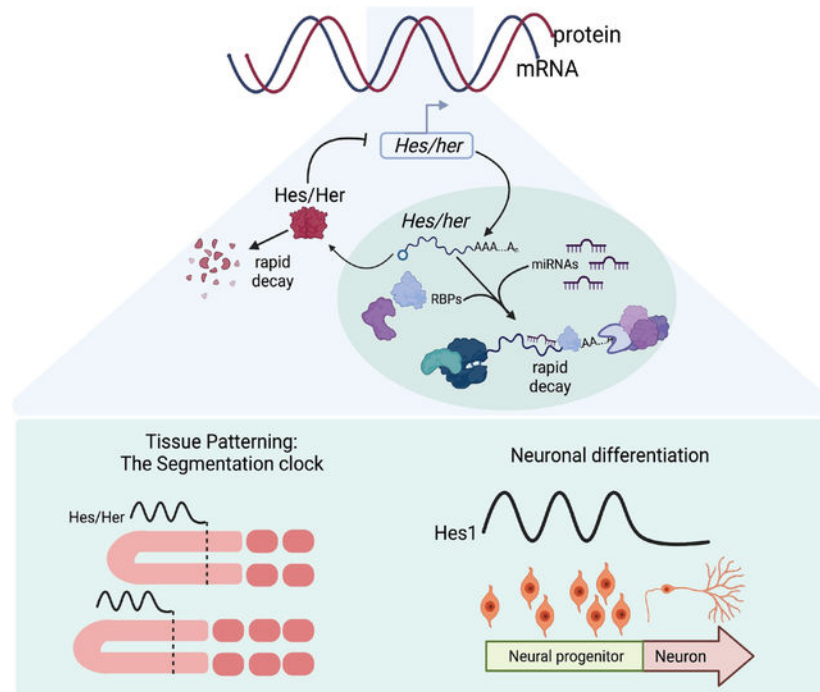
1. Introduction

Many rhythmic biological processes are controlled by a molecular "clock," in which one or more genes are expressed in an oscillatory manner with a predictable period(Oates et al., 2012; Zhang et al., 2014). Such biological clocks have been implicated in periodic root branching in *Arabidopsis*(Moreno-Risueno et al., 2010), the molting cycle in *C. elegans*(Kim et al., 2013), circadian rhythms in all diurnal and nocturnal species(Patke et al., 2020), the timing of mitosis to tightly regulate cell proliferation and tissue morphogenesis(Evans et al., 1983; Murray, 2004), and tissue patterning during somitogenesis, a fundamental vertebrate developmental process(Kageyama et al., 2012). While some oscillatory networks, like in the Kai cyanobacterial circadian clock and circadian redox clock, are driven solely by dynamic post-translational modifications(Milev et al., 2018; Snijder & Axmann, 2019), other genetic oscillators are often regulated by a negative feedback loop, in which a core oscillator gene encodes a transcriptional repressor that inhibits expression of downstream oscillators, including its own gene; this ultimately confers cell-autonomous control of genetic oscillations(Bessho, Miyoshi, et al., 2001; Brend & Holley., 2009; Patke et al., 2020; Shimojo et al., 2008; Webb et al., 2016). At the beginning of each gene expression cycle, the core oscillating gene promoter is activated and produces oscillatory gene mRNA and protein. As oscillatory protein levels rise and reach a critical threshold, oscillatory gene transcription is inhibited, preventing further production of mRNA and protein. Over time as oscillatory mRNA and protein are degraded, transcriptional repression is released, allowing for another cycle of expression to begin. Collectively, this form of autoregulation can generate a self-sustained negative feedback loop(Bessho, Miyoshi, et al., 2001; Brend & Holley., 2009; Patke et al., 2020; Shimojo et al., 2008) (Figure 1a). Whereas circadian clocks oscillate with a period of 24 hours to

regulate the daily rhythms of many organisms and their organ systems(Patke et al., 2020; Reinke H & G., 2019), ultradian clocks with periods of less than 24 hours, like the vertebrate segmentation clock, can cycle on the order of minutes and require rapid and robust gene regulation.

Vertebrate segmentation, or somitogenesis, is a critical developmental process in which the embryonic mesoderm is sequentially divided into segments, or somites, along the head-to-tail axis of all developing vertebrate embryos. Somitic cells organize to form the dermomyotome and sclerotome, which collectively form the mature trunk dermis, axial skeletal muscle, and vertebral column(Christ et al., 1978; Christ et al., 1986; Keynes & Stern, 1988).The sequential formation of somites is governed by a genetic oscillator called the segmentation clock, which is a network of genes that iteratively undergo waves of expression in the presomitic mesoderm (PSM) to establish boundaries between adjacent somites. Segmentation clock period is species-specific and varies across vertebrates (zebrafish, ~30 minutes; chick, ~90 minutes; mice, ~2 hours; human, ~4–5 hours)(Matsuda, Hayashi, et al., 2020; Matsuda, Yamanaka, et al., 2020; Palmeirim et al., 1997), and at the core of the segmentation clock are transcriptional repressors encoded by the *Hes/her* gene family whose expression is oscillatory among all vertebrates examined to date(Bessho, Sakata, et al., 2001; Oates & Ho, 2002; Palmeirim et al., 1997) (Figure 1b). *Hes/her* genes sustain cell autonomous clock oscillations through an autoregulatory negative feedback loop, which is widely regarded as the evolutionarily conserved pace-making unit of the segmentation clock(Bessho, Sakata, et al., 2001; Oates & Ho, 2002; Palmeirim et al., 1997). Additionally, other canonical Notch, Wnt, and Fgf pathway genes also oscillate in the PSM and have been implicated in initiating and synchronizing segmentation clock oscillations to propagate waves of clock gene expression across the PSM(Ay et al., 2014; Krol et al., 2011; Webb et al., 2016). An analogy to waves of clock gene expression is the "stadium wave", where spectators, representing individual cells, generate coordinated rhythmic waves by briefly standing up (transcriptional activation), raising their arms (transcript/protein production), sitting down (transcriptional repression), and lowering their arms (transcript/ protein decay) with a defined periodicity. In zebrafish embryos, Notch signaling and oscillatory Notch ligand-encoding genes like *deltaC*, help coordinate oscillation phase between neighboring cells (Delaune et al., 2012; Mara et al., 2007; Soza-Ried et al., 2014). Similarly, the chick and mouse Notch pathway gene *Lunatic Fringe (LFNG/Lfng)* oscillates in the PSM and is important for synchronizing segmentation clock dynamics and patterning the anterior skeleton(Okubo et al., 2012; Shifley et al., 2008). Like Notch pathway genes, some Fgf and Wnt pathway genes are periodically expressed, though the specific genes that oscillate in each pathway vary between species(Hubaud & Pourquie, 2014; Krol et al., 2011; Mara & Holley, 2007). In a segmenting embryo, cells proliferate in the tailbud and eventually become anteriorly displaced toward the posterior PSM(Kanki & Ho, 1997; Mara & Holley, 2007; Mara et al., 2007). Once cells are in the posterior PSM, robust, cell-autonomous oscillatory expression of segmentation clock genes initiates. As the tailbud extends and somites form, PSM cells interpret their shifting axial position relative to these anterior and posterior landmarks using opposing signaling gradients or "wavefronts": Fgf/Wnt signaling, which originates posteriorly from the tailbud, and retinoic acid signaling, which originates anteriorly from formed somites. Together, these gradients provide dynamic

positional information to PSM cells(Aulehla & Pourquie, 2010; Hubaud & Pourquie, 2014). Although not well understood, the period of molecular oscillation increases in the anterior PSM (Shih et al., 2015; Soroldoni et al., 2014). In a region of the anterior PSM termed the "determination front", where levels of both Fgf/Wnt and retinoic acid signaling are low, PSM cells transition from a presomitic to somitic state, molecular oscillations cease, and a mature somite is segmented from the PSM by the formation of a somite boundary(Aulehla & Pourquie, 2010; Hubaud & Pourquie, 2014) (Figure 1c).

In order for molecular oscillations to regulate the timing of somite formation, rates of each step in the oscillation, from intitial transcription and translation to decay, must be precisely regulated to ensure the correct size and number of somites are produced for the respective organism(Gomez et al., 2008; Holley et al., 2000; Keynes & Stern, 1988; Lewis, 2003; Matsuda, Hayashi, et al., 2020; Palmeirim et al., 1997; Schroter & Oates, 2010). The pace of genetic oscillations required to sustain clock periodicity has been investigated computationally and experimentally, whereby the rates of different steps in the gene expression pathway, from transcription, to splicing, to translation and decay, have been mathematically modeled and/or experimentally perturbed to determine the relative contribution of each step on clock periodicity(Ay et al., 2014; Giudicelli et al., 2007; Lewis, 2003). Computational and modeling studies predict that transcriptional and translational time delays (the amount of time from transcription or translation initiation to the emergence of a mature mRNA or protein, respectively) and degradation rates of both transcript and protein are the parameters that have the largest influence on clock period(Ay et al., 2014; Giudicelli et al., 2007). Experimental evidence from in vivo studies assessing the impact of timing of mRNA production, splicing, protein synthesis, and protein degradation closely mirrors in silico predictions(Hirata et al., 2004; Hoyle & Ish-Horowicz, 2013; Palmeirim et al., 1997; Takashima et al., 2011). Importantly, it is widely appreciated that transcriptional regulation alone is not sufficient to produce genetic oscillations. Real-time in vivo segmentation clock reporters designed to recapitulate clock dynamics must not only contain critical transcriptional regulatory regions that drive oscillatory expression, but must also contain features, typically 3'UTR sequences and protein motifs, that destabilize reporter mRNAs and proteins, respectively(Aulehla et al., 2008; Delaune et al., 2012; Masamizu et al., 2006; Yoshioka-Kobayashi et al., 2020) These observations underscore that multiple post-transcriptional regulatory mechanisms promote proper oscillatory expression.

Defects in segmentation clock gene expression that are sufficient to disrupt tissue-level clock periodicity can result in defects in somite patterning, which is a phenotypic readout commonly employed in genetic and reporter-based studies that have characterized the molecular mechanisms regulating clock period. Loss of segmentation clock gene function across multiple vertebrate species results in severe segmentation defects, characterized by irregular somite boundaries and fused vertebrae and ribs in juvenile and adult animals(Bessho, Sakata, et al., 2001; Choorapoikayil et al., 2012; Henry et al., 2002). Human congenital skeletal defects, like spondylocostal dysostosis, have been attributed to mutations in the human segmentation clock genes *HES7*, *DLL3*, and *LFNG*; therefore, segmentation clock oscillations are critical for muscle and axial skeletal organization(Bulman et al., 2000; Sparrow et al., 2006; Sparrow et al., 2008). Previously published reviews have provided comprehensive examinations of cellular

signaling pathways and transcriptional regulation involved in coordinating segmentation clock oscillations(Hubaud & Pourquie, 2014; Kageyama et al., 2012; Oates, 2020; Oates et al., 2012). For this review, we provide perspective on the current understanding of post-transcriptional mechanisms governing the segmentation clock and highlight those that may be shared with other developmental timing and oscillatory gene expression processes.

2.   3'UTR-mediated regulation of segmentation clock gene transcripts

mRNA stability, localization, and translation are commonly modulated by cis-regulatory elements or structural motifs present within the untranslated regions (UTRs) of mRNAs(Jambor et al., 2015; Lau et al., 2010; Lecuyer et al., 2007; Sandberg et al., 2008; Vejnar et al., 2019; Wu & Bartel, 2017). Importantly, trans-acting factors that bind 3'UTR elements can influence mRNA fate depending on the affinity of the trans factor to the mRNA and the availability of other interacting factors belonging to large, multivalent complexes that work in concert to degrade, stabilize, translocate, and/or regulate translation of an mRNA(Arvola et al., 2020; Atasoy et al., 1998; Azuma-Mukai A, 2008; Bulbrook et al., 2018; Chou et al., 2006; Enwerem et al., 2021; Landthaler et al., 2008; Moraes et al., 2006; Park-Lee et al., 2003; Pullmann et al., 2007). Multiple studies investigating post-transcriptional control of oscillating genes have demonstrated the importance of 3'UTR-mediated regulation, uncovering specific cis-elements and potential trans factors that modulate oscillatory gene transcript stability. Rapid mRNA clearance is crucial for the persistence of genetic oscillations, and studying the role of specific regulatory motifs within oscillatory gene mRNA 3'UTRs helps define post-transcriptional mechanisms that promote molecular oscillations.

Reporter-based studies of segmentation clock transcript 3'UTRs have been conducted to analyze the expression dynamics of segmentation clock mRNAs in several vertebrate genetic model systems(Davis et al., 2001; Gallagher et al., 2017; Hilgers et al., 2005; Nitanda et al., 2013; Riley et al., 2013; Tietz et al., 2020; Wahi et al., 2017). This was first demonstrated in *Xenopus laevis* embryos for the segmentation clock gene *hairy2a*, whereby the expression patterns produced from reporter constructs containing different regions of the *hairy2a* gene were analyzed to identify the minimal regions required to recapitulate the endogenous striped *hairy2* expression pattern(Davis et al., 2001). Results from these experiments revealed that the *hairy2a* 3'UTR was necessary to reconstitute the endogenous expression pattern and drive rapid decay of reporter transcripts. Similar findings have been reported in zebrafish, mouse, and chick embryos(Kawamura et al., 2016; Riley et al., 2013; Tietz et al., 2020; Wahi et al., 2017), and more recent studies have identified specific cis-regulatory elements within segmentation clock gene mRNA 3'UTRs that influence transcript stability(Riley et al., 2013; Tietz et al., 2020; Wahi et al., 2017). These studies have motivated future experiments aimed at assessing the role of specific mRNA regulatory factors on segmentation clock mRNA decay and translation, and thus, the tempo of genetic oscillations.

2.1 miRNA-mediated regulation of oscillatory expression

miRNAs are well-described small non-coding RNA molecules that negatively regulate gene expression by promoting mRNA decay and/or repressing translation of their target transcript(Behm-Ansmant et al., 2006; Naeli et al., 2022; Pillai et al., 2004; Pillai et al., 2005). miRNA-mediated regulation has been implicated among a broad range of developmental processes, including the maternal to zygotic transition in zebrafish and *Xenopus* embryos(Giraldez et al., 2006; Lund et al., 2009), muscle differentiation(Goljanek-Whysall et al., 2011), and development of multiple organ systems(Ason et al., 2006; Bhaskaran et al., 2009; Zhao et al., 2007). Previous studies have investigated the role of miRNA-mediated segmentation clock regulation by identifying miRNAs expressed in the PSM of developing embryos and analyzing expression of reporter genes containing segmentation clock gene 3'UTRs in the presence and absence of specific miRNAs(Riley et al., 2013; Wahi et al., 2017). *miR-125a-5p* is expressed in the PSM of chick and mouse embryos, and binding sites for the *miR-125a-5p* seed sequence are present in the 3'UTR of the oscillating gene *Lunatic fringe* (*Lfng*)(Riley et al., 2013; Wahi et al., 2017). Expression of a reporter gene containing either the chick or mouse *Lfng* 3'UTR was strongly downregulated following exogenous overexpression of *miR-125a-5p*, and this effect was abrogated upon mutation of the putative *miR-125* binding sites in both 3'UTRs(Riley et al., 2013). In chick embryos, morpholino-mediated interference of the *miR-125a-5p:LFNG* 3'UTR interaction(Choi et al., 2007) resulted in defects in somite patterning and oscillatory expression of endogenous segmentation clock genes(Riley et al., 2013). Surprisingly, CRISPR/Cas9 mutagenesis of *miR-125a-5p* in mouse embryos had no observed effect on endogenous segmentation clock gene oscillatory expression, and homozygous mutant embryos were morphologically wild type(Wahi et al., 2017). Collectively, these data suggest that, while *miR-125a-5p* is able to regulate *Lfng* mRNA stability, closely related miRNAs or other trans-acting factors may compensate for loss of function of *miR-125a-5p* and bind the *Lfng* 3'UTR to regulate transcript decay. While the exact role of miR-mediated regulation is not fully understood, computational models suggest that *miR-125a-5p*-dependent decay is important for minimizing fluctuations and fine-tuning *Lfng* oscillatory expression(Jing et al., 2015). Future experimental work assessing the impact of mutating the miRNA seed sequences in the endogenous *Lfng* 3'UTR will address the impact of miRNA-mediated regulation on *Lfng* expression and vertebrate segmentation.

Hes/her oscillatory expression is also involved in regulating neurogenesis, whereby oscillatory expression of the mammalian *Hes1* gene maintains a multipotent neural progenitor fate and facilitates proliferation of neural stem cells(Shimojo et al., 2008). During neuronal differentiation, *Hes1* oscillations are terminated through downregulation of *Hes1* expression, promoting expression of proneural genes(Hatakeyama et al., 2004; Ohtsuka et al., 1999), and 3'UTR analyses have revealed that miRNA-mediated mRNA decay is important for regulating *Hes1* expression in multiple animal models. Specifically, deletion of the seed-complementary sequence for *miR-9* in a mouse *Hes1* 3'UTR-containing luciferase reporter gene, which matches endogenous *Hes1* oscillatory expression, significantly increased Luciferase protein expression and reduced the number of reporter gene oscillations, compared to reporters carrying the wild-type *Hes1* 3'UTR sequence(Bonev et al., 2012). In addition, direct interference between *miR-9* and the endogenous *Hes1*

3'UTR miR-9 seed sequence using oligonucleotides that bind the 3'UTR increased endogenous *Hes1* mRNA levels, while *miR-9* expression was unaffected(Bonev et al., 2012). Futhermore, overexpression of *miR-9* showed a dampening effect on endogenous *Hes1* oscillatory expression in murine neural progenitor cells. Damped *Hes1* oscillatory expression ultimately leads to increased expression of proneural genes, causing premature neuronal differentiation(Ishibashi et al., 1995; Tan et al., 2012). *miR-9* overexpression and downregulation has been shown to disrupt oscillatory expression of zebrafish *her6* and *Xenopus hairy1* (*hairy-related genes*) in the neural progenitor cells of each species(Bonev et al., 2011; Soto et al., 2020), indicating that *miR-9*-mediated regulation is highly conserved and important for sustained oscillatory expression of neural stem cell maintenance genes.

To ensure cell-autonomous oscillatory expression, HES1 protein, in addition to repressing its own expression, also represses transcription of *pri-miR-9*, forming a double negative feedback loop(Bonev et al., 2012). As a result, peak levels of HES1 protein correspond to low transcription of *pri-miR-9*, and conversely, *pri-miR-9* expression is high when HES1 protein levels are lowest, resulting in anti-phase oscillatory expression between HES1 protein and *pri-miR-9* transcripts.Thus, *Hes1* and *pri-miR-9* are observed to oscillate out of phase, which in turn sustains neural progenitor fate. In mouse and chick PSMs, mature *miR-125a-5p* is expressed uniformly throughout the PSM, suggesting this miR is not expressed in an oscillatory manner(Riley et al., 2013). However, many mature miRNAs are known to have relatively high stability, whereas primary miRNAs are considered short-lived intermediates(Gantier et al., 2011). This is observed for mature *miR-9*, which progressively accumulates over multiple cycles of *pri-mir-9* transcriptional activation and is speculated to reach a critical threshold to help ensure precise inhibition of *Hes1* expression during neuronal differentiation(Bonev et al., 2012). Thus, it would be interesting to determine whether *pri-miR-125a* is in fact transcribed in an oscillatory manner, further elucidating the mechanism of *miR-125a-5p*-mediated oscillatory *Lfng* expression.

Despite the impact of miRNA overexpression and depletion on *Lfng* and other oscillating segmentation clock gene transcripts, miRNA-mediated regulation is not universal for all segmentation clock mRNAs nor all vertebrate species. In cells expressing a luciferase reporter gene containing the mouse *Hes7* 3'UTR, overexpression of *mir-125a-5p* does not affect *Luciferase* mRNA or protein levels, compared to expression changes observed using the *Lfng* 3'UTR(Riley et al., 2013). This indicates that different segmentation clock gene mRNAs are subject to distinct mechanisms of post-transcriptional regulation. Furthermore, despite the presence of predicted miRNA target sites in the zebrafish *her1* 3'UTR, embryos that are deficient in Dicer-dependent miRNA processing have a normal *her1* expression pattern(Gallagher et al., 2017). A broader investigation into additional post-transcriptional regulatory factors, such as the RNA binding proteins discussed below, will further elucidate the molecular mechanisms involved in maintaining genetic oscillations during development.

2.2 Analyzing 3'UTR-dependent segmentation clock gene mRNA decay dynamics using inducible reporter assays

Oscillating gene transcript expression is dynamic. Because transcripts are repeatedly and rapidly transcribed and degraded, discerning newly-transcribed mRNAs from mRNAs that

are actively being translated or have been marked for decay becomes a major challenge, particularly in whole embryo lysates. Thus, analysis of steady-state segmentation clock mRNA levels can confound interpretations of oscillating gene transcript dynamics. The advent of vertebrate PSM cell culturing methods, in which stem cells are differentiated into PSM cells or PSM explants are cultured in vitro, have allowed techniques such as nuclear or transcriptional run-on assays in combination with transcriptional inhibitors to be used to measure segementation clock mRNA decay rates (Diaz-Cuadros et al., 2020; Hubaud et al., 2017; Matsuda, Yamanaka, et al., 2020; Webb et al., 2014). However, analysis of segmentation clock gene transcript dynamics in whole embryos using chemical inhibitors can be complicated by: 1) poor penetration or difficult delivery of transcriptional inhibitors to segmenting embryos, and 2) the rapid nature of oscillatory expression, particularly in zebrafish, that is on a time scale that is not amenable to inhibitor treatments, which can require 24 hours of treatments for effective transcriptional inhibition. Using inducible reporter assays has thus been instrumental in investigating mechanisms regulating segmentation clock transcript decay, as rapid and specific induction and inhibition of reporter mRNA in whole embryos can overcome potential secondary effects introduced by global transcription inhibition (Figure 2a).

Inducible segmentation clock reporters were first introduced into chick embryos using the Tet-Off system(Gossen et al., 1995; Hilgers et al., 2005). Using this technique, it was observed that the chick *LFNG* 3'UTR confers rapid transcript degradation, a feature observed for mouse *Lfng* as well(Nitanda et al., 2013). Early in vivo studies of segmentation clock reporter dynamics relied on electroporation of Tet-Off inducible reporter constructs into segmenting embryos, followed by reporter quantification post-induction. Transient introduction of reporter constructs is subject to variability due to mosaicism; therefore, transgenic lines carrying stably-integrated reporter constructs have recently been developed in zebrafish to better quantify mRNA half-lives conferred by the zebrafish *her1* and *dlc* 3'UTRs in segmenting embryos(Tietz et al., 2020). These experiments revealed that both transcript 3'UTRs impose rapid degradation of reporter transcripts, consistent with 3'UTR analyses conducted in other vertebrates(Davis et al., 2001; Nitanda et al., 2013; Riley et al., 2013), and suggest that strong destabilizing cis-regulatory elements reside in segmentation clock transcript 3'UTRs.

2.3 RNA binding protein motifs and segmentation clock mRNA stability

RNA binding proteins (RBPs) that bind to their cognate binding elements in 3'UTRs can recruit protein complexes that modulate mRNA stability and translation(Casolaro et al., 2008; Chou et al., 2006; Pullmann et al., 2007; Shyu et al., 1991; Vasudevan & Steitz, 2007). RNA-binding protein motifs are prevalent in 3'UTR sequences; therefore, partitioning a full-length 3'UTR into smaller regions for functional reporter analyses identifies critical elements that are necessary and sufficient to promote mRNA decay (Figure 2b).

Using this deletion strategy for the *her1* 3'UTR to generate lines carrying stably-integrated inducible reporter constructs, we discovered that the last 179 nts of the full-length 725 nt *her1* 3'UTR is both necessary and sufficient to rapidly destabilize reporter transcripts(Tietz et al., 2020). Comparison of the last 179 nts of the *her1* 3'UTR and the full-length *dlc*

and *her7* 3'UTRs for candidate RNA-binding protein motifs, revealed that AU-rich elements (AREs) and Pumilio Response Elements (PREs) were shared among all three transcript 3'UTRs. Importantly, these elements are absent in regions of the *her1* 3'UTR that lack decay-promoting activity in reporter assays. Both motifs are associated with well-described negative regulators of mRNA expression, often promoting decay and repressing translation of their target transcripts(Arvola et al., 2020; Bulbrook et al., 2018; Enwerem et al., 2021). AU-rich binding proteins (ARE-BPs) are a large family of RBPs that can both stabilize and promote decay of mRNA targets(Chou et al., 2006; Vasudevan & Steitz, 2007), and in the case of decay, ARE-BPs have been shown to recruit specific members of the CCR4-Not (CNOT) complex to initiate deadenylation of their target transcripts(Lai et al., 2003). Well-characterized destabilizing ARE-BPs include the ARE/poly(U)-binding/ degradation factors 1 (AUF1), tristetrapolin (TTP), and KH-type splicing regulatory protein (KSRP)(Briata et al., 2005; Gratacos & Brewer, 2010; Lykke-Andersen & Wagner, 2005; Sanduja et al., 2011). Similar to ARE-binding proteins, Pumilio proteins are also known to regulate mRNA stability through recruitment of the CNOT complex to target transcript 3'UTRs(Arvola et al., 2020; Enwerem et al., 2021; Goldstrohm et al., 2006; Joly et al., 2013; Van Etten et al., 2012; Weidmann et al., 2014). Single mutation of the ARE or PRE in the *her1* 3'UTR reporter moderately stabilized reporter transcripts, whereas mutation of both elements dramatically stabilized reporter transcripts and led to >8-fold increased half-life relative to the unmodified full-length *her1* 3'UTR reporter(Tietz et al., 2020). Results from reporter assays suggest that the ARE and PRE cooperatively promote decay, and that both ARE and PRE-mediated decay is crucial for normal *her1* post-transcriptional regulation.

RBP-mediated post-transcriptional regulation is implicated in several developmental processes, and misregulation of RBP function can lead to drastic developmental defects(Brinegar & Cooper, 2016; Colegrove-Otero et al., 2005; Dash et al., 2016; Giudice & Cooper, 2014; Prashad & Gopal, 2021). Pumilio function is critical during mouse embryonic development, and loss of function of either *Pum1* or *Pum2* results in defects in neurogenesis(Siemen et al., 2011; Zhang et al., 2017), and *Pum1/Pum2* double knockout leads to embryonic lethality during gastrulation(Lin et al., 2018), precluding an analysis of PUM requirement during segmentation. Because the RNA binding domain, or Pumilio Homology Domain is highly conserved among many species analyzed to date, from *Drosophila*, to fish, to mammals, and binds to a specific, well-defined PRE sequence, candidate PUM-regulated mRNAs can be bioinformatically predicted through the presence of PRE motifs(Goldstrohm et al., 2018). In contrast, ARE-BPs can recognize a variety of AU-rich sequences distinct from the defined canonical ARE and thus, are harder to predict bioinformatically. Many genes in the ARE-BP superfamily are expressed during embryogenesis across vertebrates(Briggs et al., 2018; Collins et al., 2019; White et al., 2017), and loss of function studies in mouse embryos have demonstrated the requirement of multiple ARE-BPs for proper development of several tissues and organ systems(Beck et al., 1998; Bell et al., 2006; Katsanou et al., 2009; Stumpo et al., 2009). Furthermore, orthologs of many ARE-BP-encoding genes are expressed in the PSM across multiple vertebrate species, suggesting that ARE-BPs may play a conserved regulatory role in segmentation clock gene expression (Table 1). It is interesting to note that for the chick, mouse and human *LFNG* 3'UTRs, there is at least one canonical ARE (UAUUUAU) present, with the

chick *LFNG* 3'UTR containing two AREs(Hilgers et al., 2005). While these similarities may allude to potential shared mechanisms of segmentation clock mRNA decay, the difference in the strength and number of motifs among different species' clock transcript 3'UTRs may contribute to observed differences in mRNA decay rates. Consistent with this idea, recent massively parallel reporter assays show that ARE and PRE presence and number are frequently associated with rapid transcript decay in other contexts(Rabani et al., 2017; Siegel et al., 2022). It is interesting to consider whether the presence and strength of destabilizing 3'UTR elements in segmentation clock gene transcripts contributes to species-specific oscillation periods.

2.4 Functional role of segmentation clock transcript 3'UTR-mediated post-transcriptional regulation on somitogenesis

Reporter-based studies have advanced our understanding of molecular mechanisms that are important for 3'UTR-dependent mRNA regulation. However, whether disruption of 3'UTR-mediated post-transcriptional regulation of segmentation clock transcripts affects clock period and somitogenesis is not fully understood. This was serendipitously addressed in one study using mouse embryos, initially conducted to observe the effect of increasing transcriptional delay of *Hes7* by lengthening the gene(Fujimuro et al., 2014). Knock-in of an exogenous 10 kb DNA fragment from a human intron into a locus just downstream of the endogenous *Hes7* stop codon and directly upstream of the *Hes7* 3'UTR disrupted oscillatory *Hes7* mRNA expression and severely reduced Hes7 protein levels in mouse embryos homozygous for the insertion. Homozygous mutant neonates also exhibited segmental defects in their vertebrae and ribs, similar to phenotypes observed in *Hes7* null mice(Bessho, Sakata, et al., 2001). However, upon further examination of the knock-in allele, it was discovered that the inserted human intron was retained in mature *Hes7* transcripts and led to premature poly-adenylation within the retained human intron, ultimately producing a *Hes7* transcript that lacked the endogenous 3'UTR. The replacement of the endogenous *Hes7* 3'UTR with an exogenous human sequence resulted in a 30% decrease in *Hes7* mRNA and near undetectable levels of HES7 protein(Fujimuro et al., 2014), suggesting that loss of critical regulatory elements in the *Hes7* 3'UTR interfered with proper *Hes7* oscillatory expression and somite patterning. More specifically, the miRNAs and/or RBPs needed to refine mRNA oscillatory expression would be unable to promote decay or regulate translation, leading to disruption of the negative feedback loop. Introducing motif-specific mutations in endogenous 3'UTR sequences and analyzing segmentation clock mRNA and protein expression will more directly address the role of cis-regulatory element-dependent mRNA regulation on the tempo of clock oscillations.

3. Stimulators of mRNA decapping and deadenylation are required for sustained segmentation clock gene mRNA oscillations

In the final stages of an mRNA lifetime in eukaryotic cells, translation is terminated and transcript degradation occurs by either endonucleolytic cleavage (directed by small RNA species, like miRNAs and siRNAs)(Gu et al., 2018) or 3' and 5' end processing, followed by exonucleolytic degradation. Deadenylation is widely regarded as the rate-limiting and first step to occur in deadenylation-dependent mRNA decay, followed shortly by either 3'

to 5' exonucleolytic decay by the exosome or, more commonly, removal of the 5'm^{7}G cap and 5' to 3' Xrn1-mediated exonucleolytic decay(Muhlrad et al., 1994; Yamashita et al., 2005; Zheng et al., 2008). Both of these processes, if left unhindered and unaided, would occur at a rate determined only by the length of the poly(A) tail and relative strength of the decapping and deadenylation complex protein interactions with the mRNA(Steiger et al., 2003). However, for transcripts targeted for rapid decay, specific activators and RNA binding proteins facilitate rapid transcript turnover by recruiting or increasing activity of decapping and deadenylation complexes(Fenger-Gron et al., 2005; Muhlrad et al., 1994; Nissan et al., 2010; Shyu et al., 1991). In fact, overexpression of a dominant negative form of Cnot7, a member of the CCR4-NOT deadenylase complex, in zebrafish embryos disrupts segmentation clock transcript oscillatory expression and somite patterning(Fujino et al., 2018). Due to the dynamic expression of segmentation clock transcripts, it is reasonable to predict that activators of mRNA decay or factors that sequester mRNAs from the translation machinery are likely important for ensuring normal clock periodicity is maintained. A few such factors, described below, have been identified and characterized with respect to their role in segmentation clock post-transcriptional regulation.

3.1 Highly conserved deadenylation activators promote decay of segmentation clock-associated transcripts

The RNA binding protein Celf1 (CUGBP (CUG binding protein) Elav-like Family Member 1), also known as Embryo Deadenylation ElemeNt Binding Protein (EDEN-BP), is an activator of deadenylation and is known to promote rapid mRNA decay of its target transcripts(Cibois et al., 2013; Cibois et al., 2010; Gautier-Courteille et al., 2004; Moraes et al., 2006; Rattenbacher, 2010). It preferentially binds to GU-rich elements but has also been shown to bind AU-rich elements(Moraes et al., 2006; Paillard et al., 2002; Vlasova et al., 2008). Loss of Celf1 activity drastically increases the abundance of polyadenylated ARE-containing Celf1-target mRNAs in vitro, likely due to direct interaction between Celf1 and the deadenylase Poly(A)-specific ribonuclease (PARN)(Moraes et al., 2006). In *Xenopus* embryos, Celf1-dependent deadenylation is active during early embryogenesis and *celf1* expression is enriched in the paraxial mesoderm and PSM during somitogenesis(Gautier-Courteille et al., 2004). Both in vitro and in vivo UV cross-linking experiments demonstrated that Celf1 protein directly binds the 3'UTR of *rbpj* [*recombination signal binding protein for immunoglobulin Kappa J region*, also known as *suppressor of hairless*, *su(H)*](Gautier-Courteille et al., 2004). *rbpj*, which does not oscillate, is an important modulator of segmentation clock gene expression in *Xenopus*, and segmentation is impaired upon direct interference between the Celf1:*rbpj* mRNA interaction(Cibois et al., 2010). The *rbpj* 3'UTR confers rapid Celf1-dependent deadenylation of reporter transcripts, and morpholino-mediated knockdown of *celf1* in *Xenopus* embryos increases stability of endogenous *rbpj* mRNA(Gautier-Courteille et al., 2004). In contrast, *Xenopus* oscillating genes *hairy2a* and *esr9* are not direct targets of Celf1, suggesting that other mechanisms of segmentation clock transcript decay exist to collectively promote segmentation clock mRNA oscillations.

One class of ARE-binding proteins, encoded by the *zfp36* gene family (factors also known as TTP or Tis-11), was found to negatively impact expression of oscillatory genes *esr5* and

hairy2a and disrupted somite patterning when overexpressed in *Xenopus* embryos(Treguer et al., 2013). Human ZFP36 and its related proteins, ZFP36L1 and ZFP36L2, are known to directly bind mRNA and repress translation by promoting deadenylation-dependent mRNA decay(Carballo et al., 2000; Moore et al., 2018; Mukherjee et al., 2014). ZFP36 can promote deadenylation through recruitment of the CNOT deadenylase complex, via direct interaction with CNOT9, or recruitment and activation of PARN(Bulbrook et al., 2018; Lai et al., 2003). Direct interactions have also been observed between human ZFP36 and the decapping factors, DCP1A and DCP2, and this interaction enhances decapping of ARE-containing mRNAs in vitro(Fenger-Gron et al., 2005). It is important to note that in contrast to segmentation defects that arise from *zfp36* overexpression, morpholino-based knockdown of *zfp36* expression in *Xenopus* embryos does not cause overt segmentation defects(Treguer et al., 2013), and this may be due to redundant functions of other RBPs and/or incomplete *zfp36* knockdown. Nevertheless, it is intriguing to consider the impact of Zpf36-mediated turnover of segmentation clock transcripts, and whether other RNA binding proteins may act in parallel to enhance deadenylation and promote mRNA decay.

3.2 Pnrc2, an enhancer of decapping, is required for segmentation clock transcript turnover

In wild-type embryos, a striped pattern of segmentation clock mRNA expression is observed in the PSM at a fixed point in time, which arises due to coordinated oscillations of adjacent cells along the anterior-posterior axis. In a forward genetic screen carried out in zebrafish to identify regulators of segmentation clock gene expression, the *tortuga* deficiency allele was recovered, which when homozygous, displays defects in expression of segmentation clock transcripts *her1*, *her7*, *dlc*, and other segmentation clock-related transcripts(Dill & Amacher, 2005). Rather than typical, striped expression, *tortuga* mutant embryos exhibit uniform *her1* and *dlc* mRNA expression throughout the PSM. This misexpression phenotype arises due to a defect in clearance of segmentation clock transcripts that occurs when function of *proline-rich nuclear receptor coactivator 2* (*pnrc2*), a gene deleted in the *tortuga* deficiency, is lost(Gallagher et al., 2017). In human cultured cells, PNRC2 has been described as a mediator between the nonsense-mediated mRNA decay (NMD) and mRNA decapping complexes, specifically via its interactions with the NMD factor UPF1 and decapping complex protein DCP1A(Cho et al., 2013; Cho et al., 2009; Cho et al., 2015; Lai et al., 2012; Mugridge et al., 2016). Regions of the human PNRC2 protein important for PNRC2:DCP1A and PNRC2:UPF1 interactions show high sequence conservation with zebrafish Pnrc2(Gallagher et al., 2017). In human cell culture, specific mutations within regions encoding the conserved SRC-homology (SH3) domain and NR-box of human PNRC2 disrupt binding to DCP1A and UPF1, respectively, which in turn leads to stabilization of reporter mRNA(Lai et al., 2012). Similarly, rescue experiments demonstrate that expression of zebrafish Pnrc2 containing the orthologous mutations within the SH3 domain and NR-box does not rescue *her1* expression defects when introduced into *pnrc2* mutant embryos, in contrast to wild-type Pnrc2 which fully restores wild-type *her1* expression in *pnrc2* mutant embryos(Tietz et al., 2020). Morpholino-mediated depletion of *upf1* enhances the effects of *pnrc2* depletion on *her1* expression, suggesting that Upf1 facilitates Pnrc2-mediated decay(Gallagher et al., 2017). However, additional biochemical

evidence is needed to confirm whether direct interactions between Pnrc2 and other mRNA proessing and decay factors are required for segmentation clock transcript decay.

Although loss of Pnrc2-dependent segmentation clock mRNA decay in zebrafish embryos increases transcript stability and abundance, corresponding protein levels do not appear to be increased, compared to wild type embryos(Gallagher et al., 2017; Tietz et al., 2020). Consistent with normal segmentation clock protein expression, *pnrc2* mutant embryos form normal segments despite excess *her1* mRNA, in contrast to earlier *her1* overexpression studies that resulted in somite patterning defects(Giudicelli et al., 2007; Takke & Campos-Ortega, 1999). Exogenous overexpression of *her1* mRNA through microinjection or heat-shock induction(Giudicelli et al., 2007; Takke & Campos-Ortega, 1999) may overwhelm the mRNA decay and/or translational repression machinery, resulting in increased translation of segmentation clock protein and disruptions in clock periodicity. Additionally, it is unclear whether exogenously expressed *her1* transcripts contain the complete suite of cis-elements that are required to fully recapitulate endogenous *her1* post-transcriptional regulation. By contrast, accumulation of endogenous *her1* transcripts as a result of loss of Pnrc2-mediated mRNA decay has alluded to the importance of translational regulation of segmentation clock transcripts. Elucidating the translation status and poly-adenylation state of accumulated transcripts in *pnrc2* mutant embryos will determine whether stabilized transcripts exist as decay intermediates or are actively translationally repressed by yet unindentified translational regulatory factors.

4. Translational regulation of oscillatory gene expression

In order for segmentation clock oscillations to be sustained, the proteins encoded by core segmentation clock genes need to be degraded rapidly so that negative feedback loop-mediated oscillatory expression is maintained(Hirata et al., 2004). The importance of HES7 protein instability in the mouse segmentation clock was demonstrated in mouse embryos that express a mutant HES7 protein that has an increased half-life, but otherwise functions like wild-type HES7 protein(Hirata et al., 2004). Mouse embryos expressing mutant *Hes7* exhibited normal oscillations during early somitogenesis; however, after forming 3–4 normal somites, mutant embryos had fused somites, coinciding with segmentation clock gene mRNA and protein expression defects. On a molecular level, one would predict that stabilizing segmentation clock protein would prolong transcriptional repression of segmentation clock gene transcription. This would effectively dampen oscillations with each successive period and eventually perturb segmentation clock periodicity, resulting in somite patterning defects. The emergence of morphological phenotypes observed in the *Hes7* mutant mouse embryos demonstrates a direct relationship between protein stability and segmentation clock periodicity. In contrast, increases in endogenous segmentation clock mRNA stability, as observed upon loss of Pnrc2-mediated decay in zebrafish embryos, do not lead to overt segmentation defects. These observations indicate a robust post-transcriptional mechanism of mRNA regulation exists to fine-tune expression of oscillatory gene transcripts.

The importance of translational regulation of gene expression, particularly at the nexus between translational repression and mRNA decay, is well appreciated(Decker & Parker,

2012). A well-described model of translational regulation posits that RBPs and/or miRNAs that suppress expression of target mRNAs can inhibit their translation and shuttle transcripts to cytoplasmic loci, such as processing bodies (P-bodies), which consist of several ribonucleoprotein components, including mRNA processing and decay complex proteins(Decker & Parker, 2012; Hubstenberger et al., 2017). Transcriptomic analysis of purified P-bodies showed that mRNAs enriched in P-bodies collectively encode proteins that act as regulatory switches among different biological processes, including RNA processing, cell division, differentiation, and development(Hubstenberger et al., 2017). P-body enriched mRNAs are also correlated with poor translation efficiency, compared to mRNAs that are not enriched in P-bodies(Hubstenberger et al., 2017). Intuitively, these findings are not surprising, since the dynamics of cell division and fate specification processes, like somitogenesis and neurogenesis, are characterized by rapid and highly regulated gene expression transitions. Translational repression has been described as a method of regulation of the plant circadian clock(Juntawong & Bailey-Serres, 2012; Missra et al., 2015), and may also be an efficient method of facilitating rapid downregulation of segmentation clock gene expression. Computational studies aimed at understanding the critical parameters needed to sustain autoinhibitory transcriptional feedback loops, such as the Hes/Her network, have showed that a translational time delay is particularly important for modulating oscillation period(Ay et al., 2014; Murray et al., 2021). Specifically, a mathematical model that includes translational time delays to accurately model oscillation dynamics is consistent with the idea that there are translational repressive factors that help to refine the negative feedback loop so that oscillations are maintained properly(Murray et al., 2021). Importantly, experimental evidence derived from studies investigating cis-regulatory elements that promote decay of oscillatory gene transcripts have alluded to putative translational regulatory factors(Bonev et al., 2012; Riley et al., 2013; Tietz et al., 2020).

In addition to their association with mRNA decay, RBPs, like Pumilio and ARE-BPs, and miRNAs also have well-described roles in translational regulation. miRNAs have been shown to regulate translation at multiple steps, including translation initiation and elongation(Fabian et al., 2010). Pumilio proteins are known to regulate translation by directly inhibiting binding of PABP (poly(A)-binding protein) to a target mRNA(Chritton & Wickens, 2011; Van Etten et al., 2012; Weidmann et al., 2014). Additionally, ARE-BPs including TIA1 (*Tia1 Cytotoxic Granule-associated RNA Binding Protein*) and TIAR [encoded by *TIAL1* (*Tia1 Cytotoxic Granule-associated RNA Binding Protein-like 1*)], have been found to inhibit translation initiation of immune response and cancer associated mRNAs(Dixon et al., 2003; Gueydan et al., 1999; Piecyk et al., 2000). Expression analysis of segmentation clock gene mRNA and protein levels upon miRNA mis-regulation in mouse embryos(Riley et al., 2013; Wahi et al., 2017), and loss of Pnrc2-mediated decay in zebrafish embryos(Gallagher et al., 2017; Tietz et al., 2020) suggest translation of segmentation clock mRNAs is tightly regulated. Further exploration into translational regulatory control mechanisms of segmentation clock transcripts will fill the current knowledge gap that exists in our understanding of post-transcriptional regulation of oscillatory gene expression.

Conclusion and Perspectives

Rapid changes in gene expression are hallmarks of many developmental processes, some of which are observed as early as a few hours post fertilization, such as the maternal to zygotic transition (MZT)(Vastenhouw et al., 2019). Prior to the onset of zygotic transcription, the development of metazoan embryos, particularly for animals that develop externally, relies on maternally provided gene products. As development proceeds, zygotic genome activation requires rapid clearance of maternal transcripts, marking the initiation of MZT(Vastenhouw et al., 2019). In *Xenopus* oocytes, Celf1/EDEN-BP suppresses expression of maternally deposited transcripts by promoting rapid deadenylation(Ezzeddine et al., 2002). Human CELF1/CUGBP1 protein sequence is 88% identical to *Xenopus* EDEN-BP, and recombinant human CELF1/CUGBP1 can directly bind and rapidly deadenylate *Xenopus* maternal transcripts in *Xenopus* egg extracts(Ezzeddine et al., 2002; Paillard et al., 2003). Post-transcriptional mechanisms of maternal mRNA decay have also been explored on a global scale in zebrafish embryos, in which a massively parallel reporter-based study investigating 3'UTR elements that drive rapid degradation of maternally provided transcripts identified three predominant motifs driving decay of a subclass of transcripts: *miR-430* seed sequences, AREs, and PREs(Rabani et al., 2017). It is interesting to posit that these large-scale mRNA decay programs have been co-opted for use in other developmental processes which require robust and rapid modulation of mRNA expression, like in the case of the segmentation clock, and that the functions of key mRNA regulatory proteins are highly conserved. Post-transcriptional regulation is a key mechanism to ensure proper developmental transitions, and critical regulatory factors may be re-utilized throughout embryogenesis to quickly clear progenitor-associated gene products and facilitate progression into more differentiated states. Future studies will further define the post-transcriptional regulatory program that ensures proper control of developmental timing and to what extent these mechanisms are conserved.

Genetic oscillations are utilized throughout development to ensure that timing of tissue growth and patterning is properly coordinated. In this review, we have summarized evidence of post-transcriptional control of segmentation clock gene expression from studies conducted across vertebrates, revealing robust regulation of mRNA expression. The combination of 3'UTR-interacting factors, deadenylation activators, and decapping enhancers facilitates precise regulation of mRNA oscillations, which in turn promotes oscillatory expression, a critical feature for the maintenance of stem cell fate. In segmenting embryos, *Hes/her* oscillations initiate in the posterior PSM and continue as cells are displaced anteriorly. Once cells are positioned at the determination front in the anterior PSM, oscillatory expression ceases, coinciding with pre-somitic to somitic cell differentiation and the formation of a somite boundary(Gomez et al., 2008; Shih et al., 2015). Dynamic expression of *Hes1* is also associated with progenitor fate in neural stem cells, and the termination of *Hes1* oscillatory expression promotes neuronal differentiation(Shimojo et al., 2008). The question of whether the cessation of genetic oscillations is a consequence or cause of cell differentiation, and how post-transcriptional regulators play a role in this transition, is a topic of interest among researchers studying stem cell determination processes(Hatakeyama et al., 2004; Momiji & Monk, 2009; Ohtsuka

et al., 1999; Shimojo et al., 2016; Shimojo et al., 2008; Van Norman et al., 2013). Future studies aimed at uncovering the post-transcriptional mechanisms involved in regulating genetic oscillations will provide further insight into the regulation of cell fate specification across development.

Acknowledgments

References

Arvola RM, Chang CT, Buytendorp JP, Levdansky Y, Valkov E, Freddolino PL, & Goldstrohm AC (2020). Unique repression domains of Pumilio utilize deadenylation and decapping factors to accelerate destruction of target mRNAs. Nucleic Acids Res, 48(4), 1843–1871. 10.1093/nar/gkz1187 [PubMed: 31863588]

Ason B, Darnell DK, Wittbrodt B, Berezikov E, Kloosterman WP, Wittbrodt J, Antin PB, & Plasterk RH (2006). Differences in vertebrate microRNA expression. Proc Natl Acad Sci U S A, 103(39), 14385–14389. 10.1073/pnas.0603529103 [PubMed: 16983084]

Atasoy U, Watson J, Patel D, & Keene J (1998). ELAV protein HuA (HuR) can redistribute between nucleus and cytoplasm and is upregulated during serum stimulation and T cell activation. J Cell Sci, 3145–3156. 10.1242/jcs.111.21.3145 [PubMed: 9763509]

Aulehla A, & Pourquie O (2010). Signaling gradients during paraxial mesoderm development. Cold Spring Harb Perspect Biol, 2(2), a000869. 10.1101/cshperspect.a000869 [PubMed: 20182616]

Aulehla A, Wiegraebe W, Baubet V, Wahl MB, Deng C, Taketo M, Lewandoski M, & Pourquie O (2008). A beta-catenin gradient links the clock and wavefront systems in mouse embryo segmentation. Nat Cell Biol, 10(2), 186–193. 10.1038/ncb1679 [PubMed: 18157121]

Ay A, Holland J, Sperlea A, Devakanmalai GS, Knierer S, Sangervasi S, Stevenson A, & Ozbudak EM (2014). Spatial gradients of protein-level time delays set the pace of the traveling segmentation clock waves. Development, 141(21), 4158–4167. 10.1242/dev.111930 [PubMed: 25336742]

Azuma-Mukai A,OH, Mituyama T, Qian ZR, Asai K et al. (2008). Characterization of endogenous human Argonautes and their miRNA partners in RNA silencing. Proc. Natl. Acad. Sci. USA, 105, 7964–7969. [PubMed: 18524951]

Beck AR, Miller IJ, Anderson P, & Streuli M (1998). RNA-binding protein TIAR is essential for primordial germ cell development. Proceedings of the National Academy of Sciences, 95(5), 2331–2336. 10.1073/pnas.95.5.2331

Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P, & Izaurralde E (2006). mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. Genes & Development, 20, 1885–1898. 10.1101/gad.1424106 [PubMed: 16815998]

Bell SE, Sanchez MJ, Spasic-Boskovic O, Santalucia T, Gambardella L, Burton GJ, & Turner M (2006). The RNA binding protein Zfp36l1 is required for normal vascularisation and post-transcriptionally regulates VEGF expression. Developmental Dynamics, 235(11), 3144–3155. 10.1002/dvdy.20949 [PubMed: 17013884]

Bessho Y, Miyoshi G, Sakata R, & Kageyama R (2001). Hes7: a bHLH-type repressor gene regulated by Notch and expressed in the presomitic mesoderm. Genes Cells, 6(2), 175–185. 10.1046/j.1365-2443.2001.00409.x [PubMed: 11260262]

Bessho Y, Sakata R, Komatsu S, Shiota K, Yamada S, & Kageyama R (2001). Dynamic expression and essential functions of Hes7 in somite segmentation. Genes Dev, 15(20), 2642–2647. 10.1101/gad.930601 [PubMed: 11641270]

Bhaskaran M, Wang Y, Zhang H, Weng T, Baviskar P, Guo Y, Gou D, & Liu L (2009). MicroRNA-127 modulates fetal lung development. Physiol Genomics, 37(3), 268–278. 10.1152/physiolgenomics.90268.2008 [PubMed: 19439715]

Bonev B, Pisco A, & Papalopulu N (2011). MicroRNA-9 reveals regional diversity of neural progenitors along the anterior-posterior axis. Dev Cell, 20(1), 19–32. 10.1016/j.devcel.2010.11.018 [PubMed: 21238922]

Bonev B, Stanley P, & Papalopulu N (2012). MicroRNA-9 Modulates Hes1 ultradian oscillations by forming a double-negative feedback loop. Cell Rep, 2(1), 10–18. 10.1016/j.celrep.2012.05.017 [PubMed: 22840391]

Brend T, & Holley. SA. (2009). Expression of the oscillating gene her1 is directly regulated by hairy/enhancer of split, T-box, and suppressor of hairless proteins in the zebrafish segmentation clock. Developmental dynamics: an official publication of the American Association of Anatomists 238(11), 2745–2759. 10.1002/dvdy.22100 [PubMed: 19795510]

Briata P, Forcales SV, Ponassi M, Corte G, Chen CY, Karin M, Puri PL, & Gherzi R (2005). p38-dependent phosphorylation of the mRNA decay-promoting factor KSRP controls the stability of select myogenic transcripts. Mol Cell, 20(6), 891–903. 10.1016/j.molcel.2005.10.021 [PubMed: 16364914]

Briggs JA, Weinreb C, Wagner DE, Megason S, Peshkin L, Kirschner MW, & Klein AM (2018). The dynamics of gene expression in vertebrate embryogenesis at single-cell resolution. Science, 360(6392). 10.1126/science.aar5780

Brinegar AE, & Cooper TA (2016). Roles for RNA-binding proteins in development and disease. Brain Res, 1647, 1–8. 10.1016/j.brainres.2016.02.050 [PubMed: 26972534]

Bulbrook D, Brazier H, Mahajan P, Kliszczak M, Fedorov O, Marchese FP, & Dean JLE (2018). Tryptophan-Mediated Interactions between Tristetraprolin and the CNOT9 Subunit Are Required for CCR4-NOT Deadenylase Complex Recruitment. Journal of molecular biology, 430(5), 722–736. 10.1016/j.jmb.2017.12.018 [PubMed: 29291391]

Bulman MP, Kusumi K, Frayling TM, McKeown C, Garrett C, Lander ES, Krumlauf R, Hattersley AT, Ellard S, & Turnpenny PD (2000). Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. Nat Genet, 24(4), 438–441. 10.1038/74307 [PubMed: 10742114]

Carballo E, Lai WS, & Blackshear PJ (2000). Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood, The Journal of the American Society of Hematology, 95(6), 1891–1899. 10.1182/blood.V95.6.1891

Casolaro V, Fang X, Tancowny B, Fan J, Wu F, Srikantan S, Asaki SY, De Fanis U, Huang SK, Gorospe M, Atasoy UX, & Stellato C (2008). Posttranscriptional regulation of IL-13 in T cells: role of the RNA-binding protein HuR. J Allergy Clin Immunol, 121(4), 853–859 e854. 10.1016/j.jaci.2007.12.1166 [PubMed: 18279945]

Cho H, Han S, Choe J, Park S, Choi S, & Kim Y (2013). SMG5-PNRC2 is functionally dominant compared with SMG5-SMG7 in mammalian nonsense-mediated mRNA decay. Nucleic Acids Research, 41(2), 1319–1328. 10.1093/nar/gks1222 [PubMed: 23234702]

Cho H, Kim K, & Kim Y (2009). Human proline-rich nuclear receptor coregulatory protein 2 mediates an interaction between mRNA surveillance machinery and decapping complex. Molecular Cell, 33(1), 75–86. 10.1016/j.molcel.2008.11.022 [PubMed: 19150429]

Cho H, Park OH, Park J, Ryu I, Kim J, Ko J, & Kim YK (2015). Glucocorticoid receptor interacts with PNRC2 in a ligand-dependent manner to recruit UPF1 for rapid mRNA degradation. Proc Natl Acad Sci U S A, 112(13), E1540–1549. 10.1073/pnas.1409612112 [PubMed: 25775514]

Choi WY, Giraldez AJ, & Schier AF (2007). Target protectors reveal dampening and balancing of Nodal agonist and antagonist by miR-430. Science, 318(5848), 271–274. 10.1126/science.1147535 [PubMed: 17761850]

Choorapoikayil S, Willems B, Strohle P, & Gajewski M (2012). Analysis of her1 and her7 mutants reveals a spatio temporal separation of the somite clock module. PLoS One, 7(6), e39073. 10.1371/journal.pone.0039073 [PubMed: 22723933]

Chou CF, Mulky A, Maitra S, Lin WJ, Gherzi R, Kappes J, & Chen CY (2006). Tethering KSRP, a decay-promoting AU-rich element-binding protein, to mRNAs elicits mRNA decay. Mol Cell Biol, 26(10), 3695–3706. 10.1128/MCB.26.10.3695-3706.2006 [PubMed: 16648466]

Christ B, Jacob HJ, & Jacob M (1978). On the formation of the myotomes in avian embryos. An experimental and scanning electron microscope study. Experientia, 34(4), 514–516.

Christ B, Jacob M, Jürgen H, Beate J, & Wachtler B (1986). Myogenesis: A Problem of Cell Distribution and Cell Interactions. Somites in Developing Embryos, 118, 261–275.

Chritton JJ, & Wickens M (2011). A role for the poly(A)-binding protein Pab1p in PUF protein-mediated repression. J Biol Chem, 286(38), 33268–33278. 10.1074/jbc.M111.264572 [PubMed: 21768112]

Cibois M, Gautier-Courteille C, Kodjabachian L, & Paillard L (2013). A gene regulation network controlled by Celf1 protein-rbpj mRNA interaction in Xenopus somite segmentation. Biol Open, 2(10), 1078–1083. 10.1242/bio.20135629 [PubMed: 24167718]

Cibois M, Gautier-Courteille C, Vallee A, & Paillard L (2010). A strategy to analyze the phenotypic consequences of inhibiting the association of an RNA-binding protein with a specific RNA. RNA, 16(1), 10–15. 10.1261/rna.1742610 [PubMed: 19933768]

Colegrove-Otero LJ, Minshall N, & Standart N (2005). RNA-binding proteins in early development. Crit Rev Biochem Mol Biol, 40(1), 21–73. 10.1080/10409230590918612 [PubMed: 15804624]

Collins JE, White RJ, Staudt N, Sealy IM, Packham I, Wali N, Tudor C, Mazzeo C, Green A, Siragher E, Ryder E, White JK, Papatheodoru I, Tang A, Fullgrabe A, Billis K, Geyer SH, Weninger WJ, Galli A, . . . Busch-Nentwich EM. (2019). Common and distinct transcriptional signatures of mammalian embryonic lethality. Nat Commun, 10(1), 2792. 10.1038/s41467-019-10642-x [PubMed: 31243271]

Dash S, Siddam AD, Barnum CE, Janga SC, & Lachke SA (2016). RNA-binding proteins in eye development and disease: implication of conserved RNA granule components. Wiley Interdiscip Rev RNA, 7(4), 527–557. 10.1002/wrna.1355 [PubMed: 27133484]

Davis RL, Turner DL, Evans LM, & Kirschner MW (2001). Molecular targets of vertebrate segmentation: two mechanisms control segmental expression of Xenopus hairy2 during somite formation. Dev Cell, 1(4), 553–565. 10.1016/s1534-5807(01)00054-5 [PubMed: 11703945]

Decker CJ, & Parker R (2012). P-bodies and stress granules: possible roles in the control of translation and mRNA degradation. Cold Spring Harb Perspect Biol, 4(9), a012286. 10.1101/cshperspect.a012286 [PubMed: 22763747]

Delaune EA, Francois P, Shih NP, & Amacher SL (2012). Single-cell-resolution imaging of the impact of Notch signaling and mitosis on segmentation clock dynamics. Dev Cell, 23(5), 995–1005. 10.1016/j.devcel.2012.09.009 [PubMed: 23153496]

Diaz-Cuadros M, Wagner DE, Budjan C, Hubaud A, Tarazona OA, Donelly S, Michaut A, Al Tanoury Z, Yoshioka-Kobayashi K, Niino Y, Kageyama R, Miyawaki A, Touboul J, & Pourquie O (2020). In vitro characterization of the human segmentation clock. Nature, 580(7801), 113–118. 10.1038/s41586-019-1885-9 [PubMed: 31915384]

Dill KK, & Amacher SL (2005). tortuga refines Notch pathway gene expression in the zebrafish presomitic mesoderm at the post-transcriptional level. Developmental biology, 287(2), 225–236. 10.1016/j.ydbio.2005.07.032 [PubMed: 16236276]

Dixon DA, Balch GC, Kedersha N, Anderson P, Zimmerman GA, Beauchamp RD, & Prescott SM (2003). Regulation of cyclooxygenase-2 expression by the translational silencer TIA-1. J Exp Med, 198(3), 475–481. 10.1084/jem.20030616 [PubMed: 12885872]

Enwerem III, Elrod ND, Chang CT, Lin A, Ji P, Bohn JA, Levdansky Y, Wagner EJ, Valkov E, & Goldstrohm AC (2021). Human Pumilio proteins directly bind the CCR4-NOT deadenylase complex to regulate the transcriptome. RNA, 27(4), 445–464. 10.1261/rna.078436.120 [PubMed: 33397688]

Evans T, Rosenthal ET, Youngblom J, Distel D, & Hunt T (1983). Cyclin: A protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell, 33(2), 389–396. 10.1016/0092-8674(83)90420-8 [PubMed: 6134587]

Ezzeddine N, Paillard L, Capri M, Maniey D, Bassez T, Aït-Ahmed O, & Osborne HB (2002). EDEN dependent translational repression of maternal mRNAs is conserved between Xenopus

and Drosophila. Proceedings of the National Academy of Sciences, 99(1), 257–262. 10.1073/pnas.012555499

Fabian MR, Sonenberg N, & Filipowicz W (2010). Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem, 79, 351–379. 10.1146/annurev-biochem-060308-103103 [PubMed: 20533884]

Fenger-Gron M, Fillman C, Norrild B, & Lykke-Andersen J (2005). Multiple processing body factors and the ARE binding protein TTP activate mRNA decapping. Mol Cell, 20(6), 905–915. 10.1016/j.molcel.2005.10.031 [PubMed: 16364915]

Fujimuro T, Matsui T, Nitanda Y, Matta T, Sakumura Y, Saito M, Kohno K, Nakahata Y, & Bessho Y (2014). Hes7 3′ UTR is required for somite segmentation function. Scientific Reports, 4(1), 1–9. 10.1038/srep06462

Fujino Y, Yamada K, Sugaya C, Ooka Y, Ovara H, Ban H, Akama K, Otosaka S, Kinoshita H, Yamasu K, Mishima Y, & Kawamura A (2018). Deadenylation by the CCR4-NOT complex contributes to the turnover of hairy-related mRNAs in the zebrafish segmentation clock. FEBS Lett, 592(20), 3388–3398. 10.1002/1873-3468.13261 [PubMed: 30281784]

Gallagher TL, Tietz KT, Morrow ZT, McCammon JM, Goldrich ML, Derr NL, & Amacher SL (2017). Pnrc2 regulates 3'UTR-mediated decay of segmentation clock-associated transcripts during zebrafish segmentation. Dev Biol, 429(1), 225–239. 10.1016/j.ydbio.2017.06.024 [PubMed: 28648842]

Gantier MP, McCoy CE, Rusinova I, Saulep D, Wang D, Xu D, Irving AT, Behlke MA, Hertzog PJ, Mackay F, & Williams BR (2011). Analysis of microRNA turnover in mammalian cells following Dicer1 ablation. Nucleic Acids Res, 39(13), 5692–5703. 10.1093/nar/gkr148 [PubMed: 21447562]

Gautier-Courteille C, Le Clainche C, Barreau C, Audic Y, Graindorge A, Maniey D, Osborne HB, & Paillard L (2004). EDEN-BP-dependent post-transcriptional regulation of gene expression in Xenopus somitic segmentation. Development, 131(24), 6107–6117. 10.1242/dev.01528 [PubMed: 15548579]

Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, & Schier AF (2006). Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science, 312(5770), 75–79. 10.1126/science.1122689 [PubMed: 16484454]

Giudice J, & Cooper TA (2014). RNA-binding proteins in heart development. Adv Exp Med Biol, 825, 389–429. 10.1007/978-1-4939-1221-6_11 [PubMed: 25201112]

Giudicelli F, Ozbudak EM, Wright GJ, & Lewis J (2007). Setting the tempo in development: an investigation of the zebrafish somite clock mechanism. PLoS Biol, 5(6), e150. 10.1371/journal.pbio.0050150 [PubMed: 17535112]

Goldstrohm AC, Hall TMT, & McKenney KM (2018). Post-transcriptional Regulatory Functions of Mammalian Pumilio Proteins. Trends Genet, 34(12), 972–990. 10.1016/j.tig.2018.09.006 [PubMed: 30316580]

Goldstrohm AC, Hook BA, Seay DJ, & Wickens M (2006). PUF proteins bind Pop2p to regulate messenger RNAs. Nat Struct Mol Biol, 13(6), 533–539. 10.1038/nsmb1100 [PubMed: 16715093]

Goljanek-Whysall K, Sweetman D, Abu-Elmagd M, Chapnik E, Dalmay T, Hornstein E, & Munsterberg A (2011). MicroRNA regulation of the paired-box transcription factor Pax3 confers robustness to developmental timing of myogenesis. Proc Natl Acad Sci U S A, 108(29), 11936–11941. 10.1073/pnas.1105362108 [PubMed: 21730146]

Gomez C, Ozbudak EM, Wunderlich J, Baumann D, Lewis J, & Pourquie O (2008). Control of segment number in vertebrate embryos. Nature, 454(7202), 335–339. 10.1038/nature07020 [PubMed: 18563087]

Gossen M, Freundlieb S, Bender G, Muller G, Hillen W, & Bujard H (1995). Transcriptional activation by tetracyclines in mammalian cells. Science, 268(5218), 1766–1769. 10.1126/science.7792603 [PubMed: 7792603]

Gratacos FM, & Brewer G (2010). The role of AUF1 in regulated mRNA decay. Wiley Interdiscip Rev RNA, 1(3), 457–473. 10.1002/wrna.26 [PubMed: 21956942]

Gu K, Mok L, & Chong MM (2018). Regulating gene expression in animals through RNA endonucleolytic cleavage. Heliyon, 4(11). 10.1016/j.heliyon.2018.e00908

Gueydan C, Droogmans L, Chalon P, Huez G, Caput D, & Kruys V (1999). Identification of TIAR as a protein binding to the translational regulatory AU-rich element of tumor necrosis factor α mRNA. Journal of Biological Chemistry, 274(4), 2322–2326. 10.1074/jbc.274.4.2322 [PubMed: 9890998]

Hatakeyama J, Bessho Y, Katoh K, Ookawara S, Fujioka M, Guillemot F, & Kageyama R (2004). Hes genes regulate size, shape and histogenesis of the nervous system by control of the timing of neural stem cell differentiation. Development, 131(22), 5539–5550. 10.1242/dev.01436 [PubMed: 15496443]

Henry CA, Urban MK, Dill KK, Merlie JP, Page MF, Kimmel CB, & Amacher SL (2002). Two linked hairy/Enhancer of split-related zebrafish genes, her1 and her7, function together to refine alternating somite boundaries. Development, 129(15), 3693–3704. https://www.ncbi.nlm.nih.gov/pubmed/12117818 [PubMed: 12117818]

Hilgers V, Pourquié O, & Dubrulle J (2005). In vivo analysis of mRNA stability using the Tet-Off system in the chicken embryo. Developmental biology, 284(2), 292–300. 10.1016/j.ydbio.2005.05.021 [PubMed: 15993405]

Hirata H, Bessho Y, Kokubu H, Masamizu Y, Yamada S, Lewis J, & Kageyama R (2004). Instability of Hes7 protein is crucial for the somite segmentation clock. Nature Genetics, 36(7), 750–754. 10.1038/ng1372 [PubMed: 15170214]

Holley SA, Geisler R, & Nusslein-Volhard C (2000). Control of her1 expression during zebrafish somitogenesis by a delta-dependent oscillator and an independent wave-front activity. Genes Dev, 14(13), 1678–1690. https://www.ncbi.nlm.nih.gov/pubmed/10887161 [PubMed: 10887161]

Hoyle NP, & Ish-Horowicz D (2013). Transcript processing and export kinetics are rate-limiting steps in expressing vertebrate segmentation clock genes. Proc Natl Acad Sci U S A, 110(46), E4316–4324. 10.1073/pnas.1308811110 [PubMed: 24151332]

Hubaud A, & Pourquie O (2014). Signalling dynamics in vertebrate segmentation. Nat Rev Mol Cell Biol, 15(11), 709–721. 10.1038/nrm3891 [PubMed: 25335437]

Hubaud A, Regev I, Mahadevan L, & Pourquie O (2017). Excitable Dynamics and Yap-Dependent Mechanical Cues Drive the Segmentation Clock. Cell, 171(3), 668–682 e611. 10.1016/j.cell.2017.08.043 [PubMed: 28942924]

Hubstenberger A, Courel M, Benard M, Souquere S, Ernoult-Lange M, Chouaib R, Yi Z, Morlot JB, Munier A, Fradet M, Daunesse M, Bertrand E, Pierron G, Mozziconacci J, Kress M, & Weil D (2017). P-Body Purification Reveals the Condensation of Repressed mRNA Regulons. Mol Cell, 68(1), 144–157 e145. 10.1016/j.molcel.2017.09.003 [PubMed: 28965817]

Ishibashi M, Ang SL, Shiota K, Nakanishi S, Kageyama R, & Guillemot F (1995). Targeted disruption of mammalian hairy and Enhancer of split homolog-1 (HES-1) leads to up-regulation of neural helix-loop-helix factors, premature neurogenesis, and severe neural tube defects. Genes Dev, 9(24), 3136–3148. 10.1101/gad.9.24.3136 [PubMed: 8543157]

Jambor H, Surendranath V, Kalinka AT, Mejstrik P, Saalfeld S, & Tomancak P (2015). Systematic imaging reveals features and changing localization of mRNAs in Drosophila development. Elife, 4. 10.7554/eLife.05003

Jing B, Yuan J, Yin Z, Lv C, Lu S, Xiong H, Tang H, Ye G, & Shi F (2015). Dynamic properties of the segmentation clock mediated by microRNA. Int J Clin Exp Pathol, 8(1), 196–206. https://www.ncbi.nlm.nih.gov/pubmed/25755706 [PubMed: 25755706]

Joly W, Chartier A, Rojas-Rios P, Busseau I, & Simonelig M (2013). The CCR4 deadenylase acts with Nanos and Pumilio in the fine-tuning of Mei-P26 expression to promote germline stem cell self-renewal. Stem cell reports, 1(5), 411–424. 10.1016/j.stemcr.2013.09.007 [PubMed: 24286029]

Juntawong P, & Bailey-Serres J (2012). Dynamic Light Regulation of Translation Status in Arabidopsis thaliana. Front Plant Sci, 3, 66. 10.3389/fpls.2012.00066 [PubMed: 22645595]

Kageyama R, Niwa Y, Isomura A, Gonzalez A, & Harima Y (2012). Oscillatory gene expression and somitogenesis. Wiley Interdiscip Rev Dev Biol, 1(5), 629–641. 10.1002/wdev.46 [PubMed: 23799565]

Kanki JP, & Ho RK (1997). The development of the posterior body in zebrafish. Development, 124(4), 881–893. 10.1242/dev.124.4.881 [PubMed: 9043069]

Katsanou V, Milatos S, Yiakouvaki A, Sgantzis N, Kotsoni A, Alexiou M, & Kontoyiannis DL (2009). The RNA-binding protein Elavl1/HuR is essential for placental branching morphogenesis

and embryonic development. Molecular and Cellular Biology, 29(10), 2762–2776. 10.1128/MCB.01393-08 [PubMed: 19307312]

Kawamura A, Ovara H, Ooka Y, Kinoshita H, Hoshikawa M, Nakajo K, Yokota D, Fujino Y, Higashijima S, Takada S, & Yamasu K (2016). Posterior-anterior gradient of zebrafish hes6 expression in the presomitic mesoderm is established by the combinatorial functions of the downstream enhancer and 3'UTR. Developmental biology, 409(2), 543–454. 10.1016/j.ydbio.2015.11.010 [PubMed: 26596999]

Keynes RJ, & Stern CD (1988). Mechanisms of vertebrate segmentation. Development, 103(3), 413–429. https://www.ncbi.nlm.nih.gov/pubmed/3073078 [PubMed: 3073078]

Kim D, Grun D, & van Oudenaarden A (2013). Dampening of expression oscillations by synchronous regulation of a microRNA and its target. Nat Genet, 45(11), 1337–1344. 10.1038/ng.2763 [PubMed: 24036951]

Krol AJ, Roellig D, Dequeant ML, Tassy O, Glynn E, Hattem G, Mushegian A, Oates AC, & Pourquie O (2011). Evolutionary plasticity of segmentation clock networks. Development, 138(13), 2783–2792. 10.1242/dev.063834 [PubMed: 21652651]

Lai T, Cho H, Liu Z, Bowler M, Piao S, Parker R, Kim Y, & Song H (2012). Structural basis of the PNRC2-mediated link between mrna surveillance and decapping. Structure, 20(12), 2025–2037. 10.1016/j.str.2012.09.009 [PubMed: 23085078]

Lai WS, Kennington EA, & Blackshear PJ (2003). Tristetraprolin and its family members can promote the cell-free deadenylation of AU-rich element-containing mRNAs by poly (A) ribonuclease. Molecular and Cellular Biology, 23(11), 3798–3812. 10.1128/MCB.23.11.3798-3812.2003 [PubMed: 12748283]

Landthaler M, Gaidatzis D, Rothballer A, Chen P, & Soll S (2008). Molecular characterization of human Argonaute-containing ribonucleoprotein complexes and their bound target mRNAs. RNA, 14, 2580–2596. 10.1261/rna.1351608 [PubMed: 18978028]

Lau AG, Irier HA, Gu J, Tian D, Ku L, Liu G, …, & Feng Y. (2010). Distinct 3′ UTRs differentially regulate activity-dependent translation of brain-derived neurotrophic factor (BDNF). Proceedings of the National Academy of Sciences, 107(36), 15945–15950. 10.1073/pnas.1002929107

Lecuyer E, Yoshida H, Parthasarathy N, Alm C, Babak T, Cerovina T, Hughes TR, Tomancak P, & Krause HM (2007). Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. Cell, 131(1), 174–187. 10.1016/j.cell.2007.08.003 [PubMed: 17923096]

Lewis J (2003). Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator. Curr Biol, 13(16), 1398–1408. 10.1016/s0960-9822(03)00534-7 [PubMed: 12932323]

Lin K, Zhang S, Shi Q, Zhu M, Gao L, Xia W, Geng B, Zheng Z, & Xu EY (2018). Essential requirement of mammalian Pumilio family in embryonic development. Mol Biol Cell, 29(24), 2922–2932. 10.1091/mbc.E18-06-0369 [PubMed: 30256721]

Lund E, Liu M, Hartley RS, Sheets MD, & Dahlberg JE (2009). Deadenylation of maternal mRNAs mediated by miR-427 in Xenopus laevis embryos. RNA, 15(12), 2351–2363. 10.1261/rna.1882009 [PubMed: 19854872]

Lykke-Andersen J, & Wagner E (2005). Recruitment and activation of mRNA decay enzymes by two ARE-mediated decay activation domains in the proteins TTP and BRF-1. Genes Dev, 19(3), 351–361. 10.1101/gad.1282305 [PubMed: 15687258]

Mara A, & Holley SA (2007). Oscillators and the emergence of tissue organization during zebrafish somitogenesis. Trends Cell Biol, 17(12), 593–599. 10.1016/j.tcb.2007.09.005 [PubMed: 17988868]

Mara A, Schroeder J, Chalouni C, & Holley SA (2007). Priming, initiation and synchronization of the segmentation clock by deltaD and deltaC. Nat Cell Biol, 9(5), 523–530. 10.1038/ncb1578 [PubMed: 17417625]

Masamizu Y, Ohtsuka T, Takashima Y, Nagahara H, Takenaka Y, Yoshikawa K, Okamura H, & Kageyama R (2006). Real-time imaging of the somite segmentation clock: revelation of unstable oscillators in the individual presomitic mesoderm cells. Proc Natl Acad Sci U S A, 103(5), 1313–1318. 10.1073/pnas.0508658103 [PubMed: 16432209]

Matsuda M, Hayashi H, Garcia-Ojalvo J, Yoshioka-Kobayashi K, Kageyama R, Yamanaka Y, Ikeya M, Toguchida J, Alev C, & Ebisuya M (2020). Species-specific segmentation clock periods are due to differential biochemical reaction speeds. Science, 369(6510), 1450–1455. 10.1126/science.aba7668 [PubMed: 32943519]

Matsuda M, Yamanaka Y, Uemura M, Osawa M, Saito MK, Nagahashi A, Nishio M, Guo L, Ikegawa S, Sakurai S, Kihara S, Maurissen TL, Nakamura M, Matsumoto T, Yoshitomi H, Ikeya M, Kawakami N, Yamamoto T, Woltjen K, . . . Alev C. (2020). Recapitulating the human segmentation clock with pluripotent stem cells. Nature, 580(7801), 124–129. 10.1038/s41586-020-2144-9 [PubMed: 32238941]

Milev NB, Rhee SG, & Reddy AB (2018). Cellular Timekeeping: It's Redox o'Clock. Cold Spring Harb Perspect Biol, 10(5). 10.1101/cshperspect.a027698

Missra A, Ernest B, Lohoff T, Jia Q, Satterlee J, Ke K, & von Arnim AG (2015). A precedent for translational control of mRNAs in a core oscillator is found in the Arabidopsis circadian clock. The Plant Cell, 27(9), 2582–2599. 10.1105/tpc.15.00546 [PubMed: 26392078]

Momiji H, & Monk NA (2009). Oscillatory Notch-pathway activity in a delay model of neuronal differentiation. Phys Rev E Stat Nonlin Soft Matter Phys, 80(2 Pt 1), 021930. 10.1103/PhysRevE.80.021930 [PubMed: 19792174]

Moore MJ, Blachere NE, Fak JJ, Park CY, Sawicka K, Parveen S, Zucker-Scharff I, Moltedo B, Rudensky AY, & Darnell RB (2018). ZFP36 RNA-binding proteins restrain T cell activation and anti-viral immunity. Elife, 7. 10.7554/eLife.33057

Moraes KC, Wilusz CJ, & Wilusz J (2006). CUG-BP binds to RNA substrates and recruits PARN deadenylase. RNA, 12(6), 1084–1091. 10.1261/rna.59606 [PubMed: 16601207]

Moreno-Risueno MA, Van Norman JM, Moreno A, Zhang J, Ahnert SE, & Benfey PN (2010). Oscillating gene expression determines competence for periodic Arabidopsis root branching. Science, 329(5997), 1306–1311. 10.1126/science.1191937 [PubMed: 20829477]

Mugridge JS, Ziemniak M, Jemielity J, & Gross JD (2016). Structural basis of mRNA-cap recognition by Dcp1-Dcp2. Nat Struct Mol Biol, 23(11), 987–994. 10.1038/nsmb.3301 [PubMed: 27694842]

Muhlrad D, Decker CJ, & Parker R (1994). Deadenylation of the unstable mRNA encoded by the yeast MFA2 gene leads to decapping followed by 5'-->3' digestion of the transcript. Genes Dev, 8(7), 855–866. 10.1101/gad.8.7.855 [PubMed: 7926773]

Mukherjee N, Jacobs NC, Hafner M, Kennington EA, Nusbaum JD, Tuschl T, Blackshear PJ, & Ohler U (2014). Global target mRNA specification and regulation by the RNA-binding protein ZFP36. Genome Biol, 15(1), R12. 10.1186/gb-2014-15-1-r12 [PubMed: 24401661]

Murray AW (2004). Recycling the cell cycle: cyclins revisited. Cell, 116(2), 221–234. 10.1016/s0092-8674(03)01080-8 [PubMed: 14744433]

Murray PJ, Ocana E, Meijer HA, & Dale JK (2021). Auto-Regulation of Transcription and Translation: Oscillations, Excitability and Intermittency. Biomolecules, 11(11). 10.3390/biom11111566

Naeli P, Winter T, Hackett AP, Alboushi L, & Jafarnejad SM (2022). The intricate balance between microRNA-induced mRNA decay and translational repression. The FEBS journal. 10.1111/febs.16422

Nissan T, Rajyaguru P, She M, Song H, & Parker R (2010). Decapping activators in Saccharomyces cerevisiae act by multiple mechanisms. Mol Cell, 39(5), 773–783. 10.1016/j.molcel.2010.08.025 [PubMed: 20832728]

Nitanda Y, Matsui T, Matta T, Higami A, Kohno K, Nakahata Y, & Bessho Y (2013). 3′–UTR-dependent regulation of mRNA turnover is critical for differential distribution patterns of cyclic gene mRNAs. The FEBS journal, 281(1), 146–156. 10.1111/febs.12582 [PubMed: 24165510]

Oates AC (2020). Waiting on the Fringe: cell autonomy and signaling delays in segmentation clocks. Curr Opin Genet Dev, 63, 61–70. 10.1016/j.gde.2020.04.008 [PubMed: 32505051]

Oates AC, & Ho RK (2002). Hairy/E(spl)-related (Her) genes are central components of the segmentation oscillator and display redundancy with the Delta/Notch signaling pathway in the formation of anterior segmental boundaries in the zebrafish. Development, 129(12), 2929–2946. https://www.ncbi.nlm.nih.gov/pubmed/12050140 [PubMed: 12050140]

Oates AC, Morelli LG, & Ares S (2012). Patterning embryos with oscillations: structure, function and dynamics of the vertebrate segmentation clock. Development, 139(4), 625–639. 10.1242/dev.063735 [PubMed: 22274695]

Ohtsuka T, Ishibashi M, Gradwohl G, Nakanishi S, Guillemot F, & Kageyama R (1999). Hes1 and Hes5 as Notch effectors in mammalian neuronal differentiation. EMBO J, 18(8), 2196–2207. 10.1093/emboj/18.8.2196 [PubMed: 10205173]

Okubo Y, Sugawara T, Abe-Koduka N, Kanno J, Kimura A, & Saga Y (2012). Lfng regulates the synchronized oscillation of the mouse segmentation clock via trans-repression of Notch signalling. Nat Commun, 3, 1141. 10.1038/ncomms2133 [PubMed: 23072809]

Paillard L, Legagneux V, Maniey D, & Osborne HB (2002). c-Jun ARE targets mRNA deadenylation by an EDEN-BP (embryo deadenylation element-binding protein)-dependent pathway. J Biol Chem, 277(5), 3232–3235. 10.1074/jbc.M109362200 [PubMed: 11707455]

Paillard L, Legagneux V, & Osborne HB (2003). A functional deadenylation assay identifies human CUG-BP as a deadenylation factor. Biology of the Cell, 95(2), 107–113. 10.1016/s0248-4900(03)00010-8 [PubMed: 12799066]

Palmeirim I, Henrique D, Ish-Horowicz D, & Pourquie O (1997). Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. Cell, 91(5), 639–648. 10.1016/s0092-8674(00)80451-1 [PubMed: 9393857]

Park-Lee S, Kim S, & Laird-Offringa I (2003). Characterization of the interaction between neuronal RNA-binding protein HuD and AU-rich RNA. J Biol Chem, 278(41), 39801–39808. 10.1074/jbc.M307105200 [PubMed: 12900401]

Patke A, Young MW, & Axelrod S (2020). Molecular mechanisms and physiological importance of circadian rhythms. Nat Rev Mol Cell Biol, 21(2), 67–84. 10.1038/s41580-019-0179-2 [PubMed: 31768006]

Piecyk M, Wax S, Beck A, Kedersha N, Gupta M, Maritim B, Chen S, Gueydan C, Kruys V, Streuli M, & Anderson P (2000). TIA-1 is a translational silencer that selectively regulates the expression of TNF-α. The EMBO journal, 19(15), 4154–4163. 10.1093/emboj/19.15.4154 [PubMed: 10921895]

Pillai RS, Artus CG, & Filipowicz W (2004). Tethering of human Ago proteins to mRNA mimics the miRNA-mediated repression of protein synthesis. RNA, 10(10), 1518–1525. 10.1261/rna.7131604 [PubMed: 15337849]

Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, Bertrand E, & Filipowicz W (2005). Inhibition of translational initiation by Let-7 MicroRNA in human cells. Science, 309(5740), 1573–1576. 10.1126/science.1115079 [PubMed: 16081698]

Prashad S, & Gopal PP (2021). RNA-binding proteins in neurological development and disease. RNA Biol, 18(7), 972–987. 10.1080/15476286.2020.1809186 [PubMed: 32865115]

Pullmann RJ, Kim H, Abdelmohsen K, Lal A, Martindale J, Yang X, & Gorospe M (2007). Analysis of Turnover and Translation Regulatory RNA-Binding Protein Expression through Binding to Cognate mRNAs. Mol Cell Biol, 27(18), 6265–6278. 10.1128/MCB.00500-07 [PubMed: 17620417]

Rabani M, Pieper L, Chew GL, & Schier AF (2017). A Massively Parallel Reporter Assay of 3' UTR Sequences Identifies In Vivo Rules for mRNA Degradation. Mol Cell, 68(6), 1083–1094 e1085. 10.1016/j.molcel.2017.11.014 [PubMed: 29225039]

Rattenbacher B, Beisang D, Wiesner DL, Jeschke JC, Von Hohenberg M, St. Louis-Vlasova IA, & Bohjanen PR. (2010). Analysis of CUGBP1 targets identifies GU-repeat sequences that mediate rapid mRNA decay. Molecular and Cellular Biology, 30(6), 3970–3980. 10.1128/MCB.00624-10 [PubMed: 20547756]

Reinke H, & G., A. (2019). Crosstalk between metabolism and circadian clocks. Nat Rev Mol Cell Biol, 20(4). 10.1038/s41580-018-0096-9

Riley MF, Bochter MS, Wahi K, Nuovo GJ, & Cole SE (2013). Mir-125a-5p-mediated regulation of Lfng is essential for the avian segmentation clock. Developmental cell, 24(5), 554–561. 10.1016/j.devcel.2013.01.024 [PubMed: 23484856]

Sandberg R, Neilson JR, Sarma A, Sharp PA, & Burge CB (2008). Proliferating cells express mRNAs with shortened 3' untranslated regions and fewer microRNA target sites. Science, 320(5883), 1643–1647. 10.1126/science.1155390 [PubMed: 18566288]

Sanduja S, Blanco FF, & Dixon DA (2011). The roles of TTP and BRF proteins in regulated mRNA decay. Wiley Interdiscip Rev RNA, 2(1), 42–57. 10.1002/wrna.28 [PubMed: 21278925]

Schroter C, & Oates AC (2010). Segment number and axial identity in a segmentation clock period mutant. Curr Biol, 20(14), 1254–1258. 10.1016/j.cub.2010.05.071 [PubMed: 20637625]

Shifley ET, VanHorn KM, Perez-Balaguer A, Franklin JD, Weinstein M, & Cole SE (2008). Oscillatory lunatic fringe activity is crucial for segmentation of the anterior but not posterior skeleton. Development, 135, 899–908. [PubMed: 18234727]

Shih NP, Francois P, Delaune EA, & Amacher SL (2015). Dynamics of the slowing segmentation clock reveal alternating two-segment periodicity. Development, 142(10), 1785–1793. 10.1242/dev.119057 [PubMed: 25968314]

Shimojo H, Isomura A, Ohtsuka T, Kori H, Miyachi H, & Kageyama R (2016). Oscillatory control of Delta-like1 in cell interactions regulates dynamic gene expression and tissue morphogenesis. Genes Dev, 30(1), 102–116. 10.1101/gad.270785.115 [PubMed: 26728556]

Shimojo H, Ohtsuka T, & Kageyama R (2008). Oscillations in notch signaling regulate maintenance of neural progenitors. Neuron, 58(1), 52–64. 10.1016/j.neuron.2008.02.014 [PubMed: 18400163]

Shyu A, Belasco J, & Greenberg M (1991). Two distinct destabilizing elements in the c-fos message trigger deadenylation as a first step in rapid mRNA decay. Genes & Development, 5(2), 221–331. 10.1101/gad.5.2.221 [PubMed: 1899842]

Siegel DA, Le Tonqueze O, Biton A, Zaitlen N, & Erle DJ (2022). Massively parallel analysis of human 3′ UTRs reveals that AU-rich element length and registration predict mRNA destabilization G3, 12(1). 10.1093/g3journal/jkab404

Siemen H, Colas D, Heller HC, Brustle O, & Pera RA (2011). Pumilio-2 function in the mouse nervous system. PLoS One, 6(10), e25932. 10.1371/journal.pone.0025932 [PubMed: 22016787]

Snijder J, & Axmann IM (2019). The Kai-Protein Clock-Keeping Track of Cyanobacteria's Daily Life. Subcell Biochem, 93, 359–391. 10.1007/978-3-030-28151-9_12 [PubMed: 31939158]

Soroldoni D, Jorg DJ, Morelli LG, Richmond DL, Schindelin J, Julicher F, & Oates AC (2014). Genetic oscillations. A Doppler effect in embryonic pattern formation. Science, 345(6193), 222–225. 10.1126/science.1253089 [PubMed: 25013078]

Soto X, Biga V, Kursawe J, Lea R, Doostdar P, Thomas R, & Papalopulu N (2020). Dynamic properties of noise and Her6 levels are optimized by miR-9, allowing the decoding of the Her6 oscillator. EMBO J, 39(12), e103558. 10.15252/embj.2019103558 [PubMed: 32395844]

Soza-Ried C, Ozturk E, Ish-Horowicz D, & Lewis J (2014). Pulses of Notch activation synchronise oscillating somite cells and entrain the zebrafish segmentation clock. Development, 141(8), 1780–1788. 10.1242/dev.102111 [PubMed: 24715465]

Sparrow DB, Chapman G, Wouters MA, Whittock NV, Ellard S, Fatkin D, Turnpenny PD, Kusumi K, Sillence D, & Dunwoodie SL (2006). Mutation of the LUNATIC FRINGE gene in humans causes spondylocostal dysostosis with a severe vertebral phenotype. Am J Hum Genet, 78(1), 28–37. 10.1086/498879 [PubMed: 16385447]

Sparrow DB, Guillen-Navarro E, Fatkin D, & Dunwoodie SL (2008). Mutation of Hairy-and-Enhancer-of-Split-7 in humans causes spondylocostal dysostosis. Hum Mol Genet, 17(23), 3761–3766. 10.1093/hmg/ddn272 [PubMed: 18775957]

Steiger M, Carr-Schmid A, Schwartz DC, Kiledjian M, & Parker R (2003). Analysis of recombinant yeast decapping enzyme. RNA, 9(2), 231–238. 10.1261/rna.2151403 [PubMed: 12554866]

Stumpo DJ, Broxmeyer HE, Ward T, Cooper S, Hangoc G, Chung YJ, Shelley WC, Richfield EK, Ray MK, Yoder MC, Aplan PD, & Blackshear PJ (2009). Targeted disruption of Zfp36l2, encoding a CCCH tandem zinc finger RNA-binding protein, results in defective hematopoiesis. Blood, 114(12), 2401–2410. 10.1182/blood-2009-04-214619 [PubMed: 19633199]

Takashima Y, Ohtsuka T, González A, Miyachi H, & Kageyama R (2011). Intronic delay is essential for oscillatory expression in the segmentation clock. Proc Natl Acad Sci U S A, 108(8).

Takke C, & Campos-Ortega JA (1999). her1, a zebrafish pair-rule like gene, acts downstream of notch signalling to control somite development. Development, 126(13), 3005–3014. https://www.ncbi.nlm.nih.gov/pubmed/10357943 [PubMed: 10357943]

Tan SL, Ohtsuka T, Gonzalez A, & Kageyama R (2012). MicroRNA9 regulates neural stem cell differentiation by controlling Hes1 expression dynamics in the developing brain. Genes Cells, 17(12), 952–961. 10.1111/gtc.12009 [PubMed: 23134481]

Tietz KT, Gallagher TL, Mannings MC, Morrow ZT, Derr NL, & Amacher SL (2020). Pumilio response and AU-rich elements drive rapid decay of Pnrc2-regulated cyclic gene transcripts. Dev Biol, 462(2), 129–140. 10.1016/j.ydbio.2020.03.017 [PubMed: 32246943]

Treguer K, Faucheux C, Veschambre P, Fedou S, Theze N, & Thiebaud P (2013). Comparative functional analysis of ZFP36 genes during Xenopus development. PLoS One, 8(1), e54550. 10.1371/journal.pone.0054550 [PubMed: 23342169]

Van Etten J, Schagat TL, Hrit J, Weidmann CA, Brumbaugh J, Coon JJ, & Goldstrohm AC (2012). Human Pumilio proteins recruit multiple deadenylases to efficiently repress messenger RNAs. J Biol Chem, 287(43), 36370–36383. 10.1074/jbc.M112.373522 [PubMed: 22955276]

Van Norman JM, Xuan W, Beeckman T, & Benfey PN (2013). To branch or not to branch: the role of pre-patterning in lateral root formation. Development, 140(21), 4301–4310. 10.1242/dev.090548 [PubMed: 24130327]

Vastenhouw NL, Cao WX, & Lipshitz HD (2019). The maternal-to-zygotic transition revisited. Development, 146(11). 10.1242/dev.161471

Vasudevan S, & Steitz JA (2007). AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. Cell, 128(6), 1105–1118. 10.1016/j.cell.2007.01.038 [PubMed: 17382880]

Vejnar CE, Abdel Messih M, Takacs CM, Yartseva V, Oikonomou P, Christiano R, Stoeckius M, Lau S, Lee MT, Beaudoin JD, Musaev D, Darwich-Codore H, Walther TC, Tavazoie S, Cifuentes D, & Giraldez AJ (2019). Genome wide analysis of 3' UTR sequence elements and proteins regulating mRNA stability during maternal-to-zygotic transition in zebrafish. Genome Res, 29(7), 1100–1114. 10.1101/gr.245159.118 [PubMed: 31227602]

Vlasova IA, Tahoe NM, Fan D, Larsson O, Rattenbacher B, Sternjohn JR, Vasdewani J, Karypis G, Reilly CS, Bitterman PB, & Bohjanen PR (2008). Conserved GU-rich elements mediate mRNA decay by binding to CUG-binding protein 1. Mol Cell, 29(2), 263–270. 10.1016/j.molcel.2007.11.024 [PubMed: 18243120]

Wahi K, Friesen S, Coppola V, & Cole SE (2017). Putative binding sites for mir-125 family miRNAs in the mouse Lfng 3′ UTR affect transcript expression in the segmentation clock, but mir-125a-5p is dispensable for normal somitogenesis. Developmental Dynamics, 246(10), 740–748. 10.1002/dvdy.24552 [PubMed: 28710810]

Webb AB, Lengyel IM, Jorg DJ, Valentin G, Julicher F, Morelli LG, & Oates AC (2016). Persistence, period and precision of autonomous cellular oscillators from the zebrafish segmentation clock. Elife, 5. 10.7554/eLife.08438

Webb AB, Soroldoni D, Oswald A, Schindelin J, & Oates AC (2014). Generation of dispersed presomitic mesoderm cell cultures for imaging of the zebrafish segmentation clock in single cells. J Vis Exp(89). 10.3791/50307

Weidmann CA, Raynard NA, Blewett NH, Van Etten J, & Goldstrohm AC (2014). The RNA binding domain of Pumilio antagonizes poly-adenosine binding protein and accelerates deadenylation. RNA, 20(8), 1298–1319. 10.1261/rna.046029.114 [PubMed: 24942623]

White RJ, Collins JE, Sealy IM, Wali N, Dooley CM, Digby Z, Stemple DL, Murphy DN, Billis K, Hourlier T, Fullgrabe A, Davis MP, Enright AJ, & Busch-Nentwich EM (2017). A high-resolution mRNA expression time course of embryonic development in zebrafish. Elife, 6. 10.7554/eLife.30860

Wu X, & Bartel DP (2017). Widespread Influence of 3'-End Structures on Mammalian mRNA Processing and Stability. Cell, 169(5), 905–917 e911. 10.1016/j.cell.2017.04.036 [PubMed: 28525757]

Yamashita A, Chang TC, Yamashita Y, Zhu W, Zhong Z, Chen CY, & Shyu AB (2005). Concerted action of poly(A) nucleases and decapping enzyme in mammalian mRNA turnover. Nat Struct Mol Biol, 12(12), 1054–1063. 10.1038/nsmb1016 [PubMed: 16284618]

Yoshioka-Kobayashi K, Matsumiya M, Niino Y, Isomura A, Kori H, Miyawaki A, & Kageyama R (2020). Coupling delay controls synchronized oscillation in the segmentation clock. Nature, 580(7801), 119–123. 10.1038/s41586-019-1882-z [PubMed: 31915376]

Zhang M, Chen D, Xia J, Han W, Cui X, Neuenkirchen N, Hermes G, Sestan N, & Lin H (2017). Post-transcriptional regulation of mouse neurogenesis by Pumilio proteins. Genes Dev, 31(13), 1354–1369. 10.1101/gad.298752.117 [PubMed: 28794184]

Zhang R, Lahens NF, Ballance HI, Hughes ME, & Hogenesch JB (2014). A circadian gene expression atlas in mammals: implications for biology and medicine. Proc Natl Acad Sci U S A, 111(45), 16219–16224. 10.1073/pnas.1408886111 [PubMed: 25349387]

Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, & Srivastava D (2007). Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1–2. Cell, 129(2), 303–317. 10.1016/j.cell.2007.03.030 [PubMed: 17397913]

Zheng D, Ezzeddine N, Chen CYA, Zhu W, He X, & Shyu AB (2008). Deadenylation is prerequisite for P-body formation and mRNA decay in mammalian cells. The Journal of cell biology, 182(1), 89–101. 10.1083/jcb.200801196 [PubMed: 18625844]
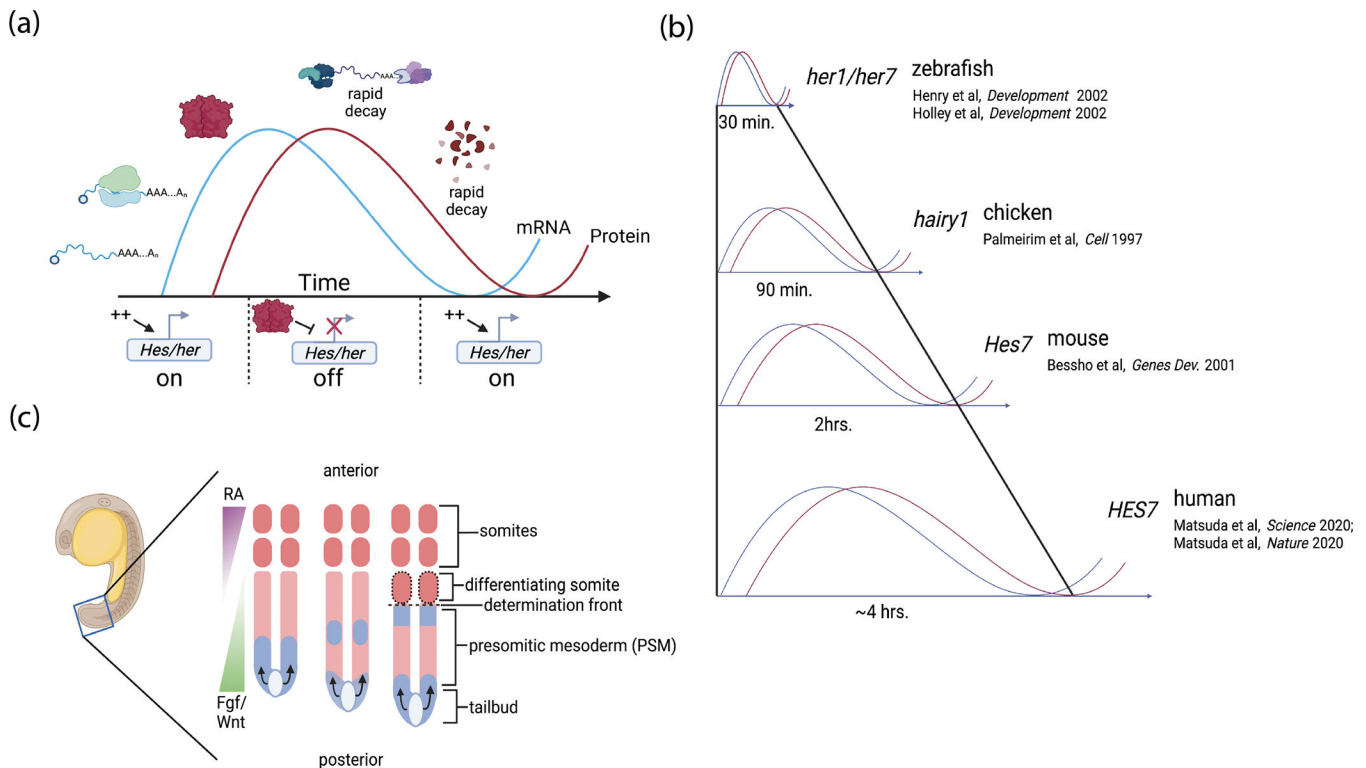
Figure 1.

Negative feedback loops and somitogenesis. (a) *Negative feedback loop autoregulation.* Core oscillators, often encoding transcriptional repressors, participate in negative feedback loops to sustain autoregulatory genetic oscillations. Within each gene expression cycle, segmentation clock mRNA and protein are produced in increasing amounts, and increased protein levels correspond to decreased transcriptional activation as segmentation clock protein inhibits its own expression. As segmentation clock mRNA and protein are both degraded, repression of segmentation clock gene expression is released, allowing for another cycle of expression to begin. Collectively, this forms a self-sustained negative feedback loop. (b) *Species-specific segmentation clock periods.* Clock periodicity varies widely across vertebrates. However, the tempo of oscillations corresponds to the timing of somite formation in all species. The examples shown are classic *hairy enhancer of split* orthologs, but other genes also oscillate. The tempo of oscillations is determined by species-specific biochemical rates of gene expression. (c) *Oscillating PSM cells receive positional information from anterior-posterior gradients.* Core segmentation clock oscillators, like zebrafish *her1*, are expressed in the PSM and tailbud. As cells in the tailbud proliferate, the tailbud extends and cells become displaced into the posterior PSM. Once in the posterior PSM, cells initiate robust oscillatory gene expression. These cell-autonomous oscillations appear as travelling waves across the PSM from posterior to anterior (shown in blue), and are coordinated by Notch-mediated cell-cell communication. At the determination front, which is established by opposing Fgf/Wnt and retinoic acid signaling gradients, cells transition from a presomitic to somitic cell state and a new somite boundary is formed. Although neglible over just one oscillation, PSM size changes over developmental time,

gradually shrinking as the tailbud ceases to proliferate and somite formation continues. Image created using Biorender.com.
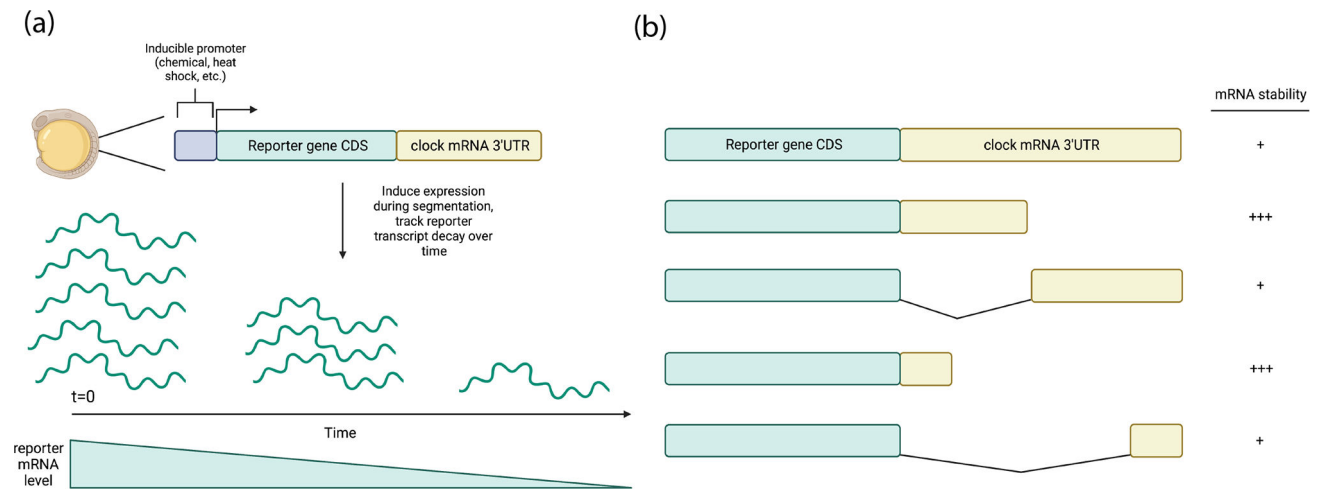
Figure 2.

In vivo reporter assays and 3'UTR deletion analysis. (a) *Inducible reporter systems and measuring mRNA decay rates.* Inducible reporter assays are utilized to measure and compare reporter transcript stability in the context of varied 3'UTR sequences. Depending on the promoter sequence used, these constructs can be chemically- or heat shock-induced in segmenting embryos, when the appropriate segmentation clock transcript regulatory factors are expressed. To calculate reporter transcript decay rates, RNA is extracted from embryos collected at regular intervals post induction and reporter mRNA is subsequently quantified across time points using real-time PCR. (b) *3'UTR fragmentation and reporter transcript decay analysis.* Because 3'UTR sequences are rich in motifs that may or may not influence stability, the generation of a set of reporters containing varying portions of a 3'UTR can help to identify smaller regions that influence reporter stability. Upon identification of minimal regions that influence reporter stability, motif analysis followed by mutagenesis of potential regulatory elements can uncover miRNA and/or RBP binding sites that are the primary regulators of mRNA stability. Image created using Biorender.com.
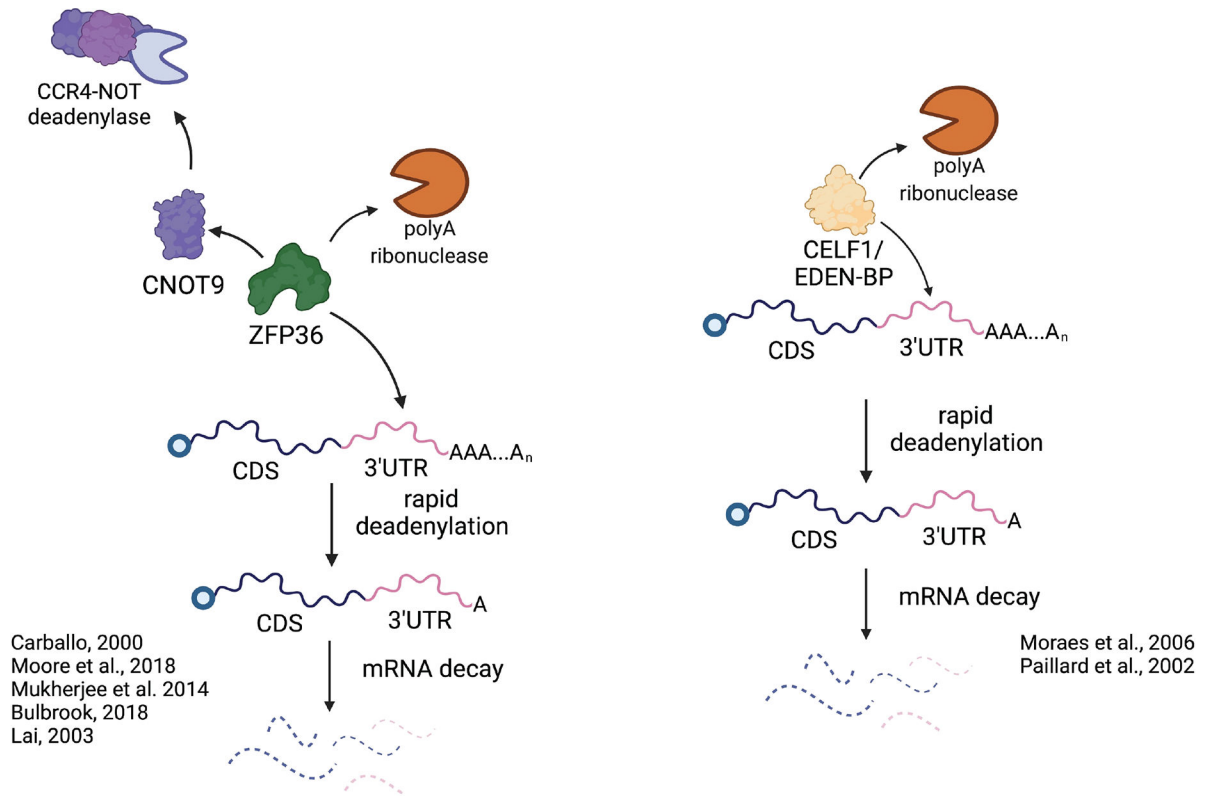
Figure 3.

Activators of deadenylation promote rapid mRNA decay. Both CELF1/EDEN-BP and ZFP36 proteins promote deadenylation-dependent decay by binding 3'UTRs of target transcripts and recruiting deadenylation factors. CELF1/EDEN-BP has been shown to directly bind transcript 3'UTRs and recruit the polyA ribonuclease (PARN) to promote rapid transcript deadenylation in *Xenopus* embryos. Additionally, ZFP36 proteins bind AU-rich elements within transcript 3'UTRs and promote deadenylation through recruitment of PARN or the CCR4-NOT complex via CNOT9. Both RNA binding proteins have been shown to regulate segmentation clock transcript stability, and further biochemical evidence will determine whether these precise interactions are also important in the context of segmentation clock transcript deadenylation and decay.

Table 1.

ARE-BP expression in vertebrate PSMs or cultured PSM cells

Species	ARE-BP encoding genes expressed in PSM (or cultured PSM cells)
Zebrafish (PSM)	*elavl1a, elavl1b, hnrnpd, hnrnpdl, khsrp, tia1, tia1l, tial1, zfp36l1a, zfp36l1b, zfp36l2* (Rauch et al, 2003; Thisse et al, 2004; Thisse et al, 2005; Wagner et al, 2018)
***Xenopus* (PSM)**	*elavl, hnrnpd, hnrnpdl, khsrp, tia1, zfp36l1, zfp36l2* (Gawantka et al Mech Dev 1998; Treguer et al, 2013; Briggs et al, 2018)
Mouse (PSM)	*Elavl1, Hnrnpd, Khsrp, Tia1, Tial1 Zfp36l1, Zfp36l2* (Diaz-Cuadros et al, 2020)
Human (Cultured PSM cells)	*ELAVL1, HNRNPD, KHSRP, TIA1, TIAL, ZFP36L1, ZFP36L2* (Diaz-Cuadros et al, 2020)