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Original research

# Regulators of alternative polyadenylation operate at the transition from mitosis to meiosis

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ABSTRACT

In the sexually reproductive organisms, gametes are produced by meiosis following a limited mitotic amplification. However, the intrinsic program switching cells from mitotic to meiotic cycle is unclear. Alternative polyadenylation (APA) is a highly conserved means of gene regulation and is achieved by the RNA 3′-processing machinery to generate diverse 3′UTR profiles. In *Drosophila* spermatogenesis, we observed distinct profiles of transcriptome-wide 3′UTR between mitotic and meiotic cells. In mutant germ cells stuck in mitosis, 3′UTRs of hundreds of genes were consistently shifted. Remarkably, altering the levels of multiple 3′-processing factors disrupted germline's progression to meiosis, indicative of APA's active role in this transition. An RNA-binding protein (RBP) Tut could directly bind 3′UTRs of 3′-processing factors whose expressions were repressed in the presence of Tut-containing complex. Further, we demonstrated that this RBP complex could execute the repression post-transcriptionally by recruiting CCR4/Twin of deadenylation complex. Thus, we propose that an RBP complex regulates the dynamic APA profile to promote the mitosis-to-meiosis transition.



## 1. Introduction

Meiosis, which reduces a diploid germ cell to a haploid gamete, is the fundamental event for sexual reproduction. Germ cells normally go through a limited mitotic amplification before entering meiosis. Although the external cues, such as nutrient conditions for yeast or retinoids for mammal, that trigger meiosis have been identified (Honigberg and Purnapatre, 2003; Bowles et al., 2006; Koubova et al., 2006), the internal program that switches germ cells from mitotic to meiotic track is largely unknown. Meiosis initiation remains the major obstacle for *in vitro* reconstitution of gametogenesis (Sun et al., 2014).

Alternative polyadenylation (APA) of gene transcripts represents an important layer of regulation during normal and pathological development. APA events generate different 3′UTRs (untranslated regions) of mRNA which may change the localization, stability, translation efficiency of the transcripts (An et al., 2008; Sandberg et al., 2008; Andreassi and Riccio, 2009; Mayr and Bartel, 2009; Pinto et al., 2011), or even mediate protein-protein interactions without changing the protein coding regions (Berkovits and Mayr, 2015; Mayr, 2016). From yeast to human, 50%–70% of the genome exhibit the usage of APA (Mangone et al., 2010; Ozsolak et al., 2010; Wu et al., 2011; Derti et al., 2012; Smibert et al., 2012; Ulitsky et al., 2012; Hoque et al., 2013). Characteristic 3′UTR profiles are

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associated with specific tissues or developmental stages. For example, testis-specific mRNAs tend to have much shorter 3′UTR than the brain-specific ones in general (Smibert et al., 2012; Ulitsky et al., 2012); genome-wide progressive 3′UTR lengthening was observed during cell differentiation or embryo development (Ji et al., 2009), whereas 3′UTR shortening in somatic reprograming (Sandberg et al., 2008; Ji and Tian, 2009; Shepard et al., 2011). APA has also been shown in association with diseases (Mayr and Bartel, 2009; de Klerk et al., 2012; Jenal et al., 2012; Batra et al., 2014; Masamha et al., 2014). Nevertheless, the causal relationship and mechanistic connection between APA and developmental regulation remain to be clarified.

3′UTR of different length is generated by around 20 factors of RNA 3′-processing machinery coordinating in the sequential steps from recognizing the polyadenylation sites, executing 3′-end cleavage after recognition, to adding polyA tails at the cleaved end (Di Giammartino et al., 2011; Zheng and Tian, 2014). Manipulating the protein levels of the core factors in 3′-processing machinery led to alternative polyA site usage (Takagaki et al., 1996; Takagaki and Manley, 1998; Kubo et al., 2006; Kim et al., 2010; Jenal et al., 2012; Martin et al., 2012; Lackford et al., 2014; Masamha et al., 2014; Zheng and Tian, 2014). Conceivably, the 3′-processing factors are the major targets to accomplish APA regulation under various conditions. If so, how are they regulated?

RNA-binding proteins (RBPs) can interact with both RNAs and various other proteins, which can connect the upstream signals and their target RNAs. In a human cell line, protein occupancy profiling showed that 3′UTRs contained widespread contacts with RBPs (Baltz et al., 2012), which regulate APA by competing with 3′-processing factors for binding sites on RNA (Shi, 2012; Zheng and Tian, 2014). Further, it is also possible that RBPs post-transcriptionally modulate the expression of 3′-processing factors to influence APA globally.

In *Drosophila* spermatogenesis, Bam (Bag of marbles) dosage determines how many mitotic cycles germ cells undergo before meiosis (Insco et al., 2009). However, the mechanism downstream of Bam that switches germ cells from mitosis to meiosis is unknown. We previously demonstrated that an RBP complex containing Tut (Tumorous testis), Bam, and Bgcn (Benign gonial cell neoplasm) is required for limiting the mitotic cycles (Chen et al., 2014). One of the molecular targets of this RBP complex is *mei-P26* whose expression is repressed in the presence of Tut-Bam-Bgcn. Interestingly, *mei-P26* 3′UTR varies in length in the testes between wild type and any of the mutants (*tut*, *bam*, or *bgcn*). However, restoring Mei-P26 expression is not sufficient to rescue the over-amplification defect of *tut*, *bam*, or *bgcn*, indicating the involvement of other genes and a more complicated scenario (Insco et al., 2012; Chen et al., 2014). This prompted us to examine the 3′UTR profile genome-wide and its relation to the germline development in wild type and in these RBP mutants.

In this study, we found by transcriptome RNA-seq that the 3′UTR patterns are distinct between mitotic and meiotic germ cells in *Drosophila* testis. Altering the levels of multiple 3′-processing factors disrupted the germ cell progression from mitosis to meiosis, implying APA's active role in this transition. We then obtained *in vitro* and *in vivo* evidence to show that Tut could directly bind the 3′UTRs of 3′-processing factors and suppress their expression. Further, we provided genetic and biochemical data to propose that the RBP complex of Tut-Bam-Bgcn regulates the components of 3′-processing machinery at the post-transcriptional level by recruiting CCR4/Twin of deadenylation complex. Thus, we discovered the upstream regulators of APA in *Drosophila* germline, and proposed the causal relationship between APA and meiosis entry.

## 2. Results

### 2.1. Distinct 3′UTR profiles of the germ cells from mitotic to meiotic stage

To explore the global 3′UTR pattern in relation to the transition from mitosis to meiosis, we performed transcriptome RNA-seq using the accurately staged germ cells, the mitotic spermatogonia (SG; small 2-to-8-cell cysts) and the meiotic spermatocytes (SC; big 16-cell cysts), by laser excision from wild-type *Drosophila* testes (Figs. S1A and B; Materials and methods). Additionally, we used *bam*, *bgcn*, and *tut* mutants to obtain germ cells retained at mitotic stage for comparison (Fig. S1C–E). The enrichment of the cell types in wild-type SG or SC samples was verified by the relative mRNA levels of a SG marker (*bam*) or two SC markers (*bol* and *sa*) obtained from the RNA-seq data (Figs. S1F and G).

Global 3′UTR analysis was based on RNA-seq data at over 100× coverage of the transcriptome. The 3′UTR profiles were compared between wild-type SG and SC, or between mutant SG (SG-*bam*, SG-*bgcn*, or SG-*tut*) and wild-type SC (Fig. 1A and B). In general, more than 600 genes showed 3′UTR shifts in their transcripts between SG (wild-type or mutant) and SC. Among these genes, the majority had more long 3′UTR forms in SG (wild-type or mutant) than in SC (Fig. 1A, blue dots). This polarization of 3′UTR profile was more prominent in the wild-type SG than in any of the mutant SG samples (Fig. 1A, compare the gene numbers on top of the blue and orange bars).

Since Tut, Bam, and Bgcn act in the same protein complex in SG differentiation (Insco et al., 2009; Chen et al., 2014) and if meanwhile they are involved in APA regulation, we expect that the 3′UTR patterns of these three mutant SG would consistently display the distinction from that of the meiotic cells (Fig. 1A). Indeed, among the genes showing distinct 3′UTR profiles between SG and SC, 274 genes were common in all three mutant SG samples (Fig. 1B). Pearson correlation analysis, which was based on the 3′UTR patterns of 1457 genes that exhibited the differences between wild-type SG, SG-*bam*, SG-*tut*, or SG-*bgcn* and wild-type SC, showed the close relationship of all mutant SG (Fig. 1C, clustering of SG-*bam*, SG-*tut*, and SG-*bgcn*). The distinction between mitotic SG and meiotic SC was also indicated by the relatively similar patterns of wild-type SG, SG-*bam*, SG-*tut*, and SG-*bgcn* in contrast to that of wild-type SC (Fig. 1C). Hence, the 3′UTR profiles are highly distinct between mitotic and meiotic germ cells.

### 2.2. Altering the levels of 3′-processing factors disrupts the mitosis-to-meiosis switch

Since we observed a global change of 3′UTR length in mitotic SG vs meiotic SC, we asked if RNA 3′-processing factors (components listed in Table S1), the major executors of APA, are involved in the transitional control from mitosis to meiosis. We tried to alter the levels of 3′-processing factors by RNAi or overexpression using SG-specific GAL4 driver (*bam*-GAL4), and scored the phenotype of SG overproliferation (Fig. 2, compare the accumulation of small germ cells brightly stained by DAPI in GFP control and others; only those exhibiting SG overgrowth are shown). Under normal developmental conditions in *Drosophila melanogaster*, SG mitotically amplify to 16-cell per cyst before entering meiosis, whereas many cysts containing much more than 16 cells were observed when we downregulated the levels of some 3′-processing factors (Fig. 2A, arrows). In the wild-type background, knockdown of Pabp2, Hrg, and Sym could induce significant SG overproliferation (Fig. 2A and C; see Table S1 for all 20 factors tested in the RNAi screen).

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