Note

Direct Evidence for Postmeiotic Transcription During *Drosophila melanogaster* Spermatogenesis

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ABSTRACT

Extensive gene expression during meiosis is a hallmark of spermatogenesis. Although it was generally accepted that RNA transcription ceases during meiosis, recent observations suggest that some transcription occurs in postmeiosis. To further resolve this issue, we provide direct evidence for the *de novo* transcription of RNA during the postmeiotic phases. These results strengthen the newly emerging notion that postmeiotic transcription is dynamic and integral to the overall process of spermatogenesis.

SPERMATOGENESIS is a fundamental developmental process producing male sex cells, the spermatozoa. In *Drosophila melanogaster*, this process occurs in three phases: a diploid mitotic phase, which increases cell numbers and size; a second, diploid meiotic phase characterized by intense transcriptional activity and important structural changes; and a final postmeiotic haploid phase of sperm morphogenesis and maturation. Early experiments using autoradiography demonstrated RNA transcription during mitosis and early meiosis but no transcriptional activity during postmeiosis (OLIVIERI and OLIVIERI 1965). On the basis of this and other similar studies, it has generally been accepted that the postmeiotic phase is devoid of transcriptional activity and led to the prevailing notion that proteins required later during the postmeiotic stages were translated from mRNAs produced during meiosis and stored in the cytoplasm (SCHÄFER *et al.* 1995). Two contemporary studies have challenged this view, one using a targeted gene expression approach demonstrating mRNA accumulation in postmeiotic spermatids (BARREAU *et al.* 2008) and our recent microarray analysis estimating substantial genome-wide expression during postmeiosis (VIBRANOVSKI *et al.* 2009). This latter study demonstrated that 20–30% of testis genes transcribed are over expressed during postmeiosis in comparison to meiosis. However, both studies failed to provide clear-cut direct evidence for postmeiotic transcription: both studies measured, either qualitatively or quantitatively, mRNA in postmeiotic cells but did not provide direct evidence for the production of nascent RNA. In addition, in the first study, only a small subset of genes was analyzed and the second study measured bulk mRNA levels from dissected tissues and therefore expression at the cellular level remains unclear. Here, we directly visualized RNA transcripts in intact testes using 5-bromouridine (BrU) and we describe their cellular and subcellular distributions during spermatogenesis (Figure 1).

As expected, in intact testis, strong BrU signals were observed in somatic cells of the outer sheath, in presumptive nucleoli of primary spermatocytes (Figure 1A). We also observed a surprisingly strong BrU signal in developing spermatid bundles during postmeiosis (Figure 1B). Additionally, BrU incorporation in isolated spermatocytes (Figure 1C) and in isolated postmeiotic spermatid bundles (Figure 1D) routinely displayed strong BrU signal near spermatid nuclei. The latter result provides direct evidence for *de novo* RNA synthesis in postmeiosis. Those cells were isolated from an intact testis prior to addition of BrU and thus eliminating the possibility that intercellular transport of RNA molecules from surrounding somatic testis sheath cells in intact testes was responsible for the observed BrU signals in

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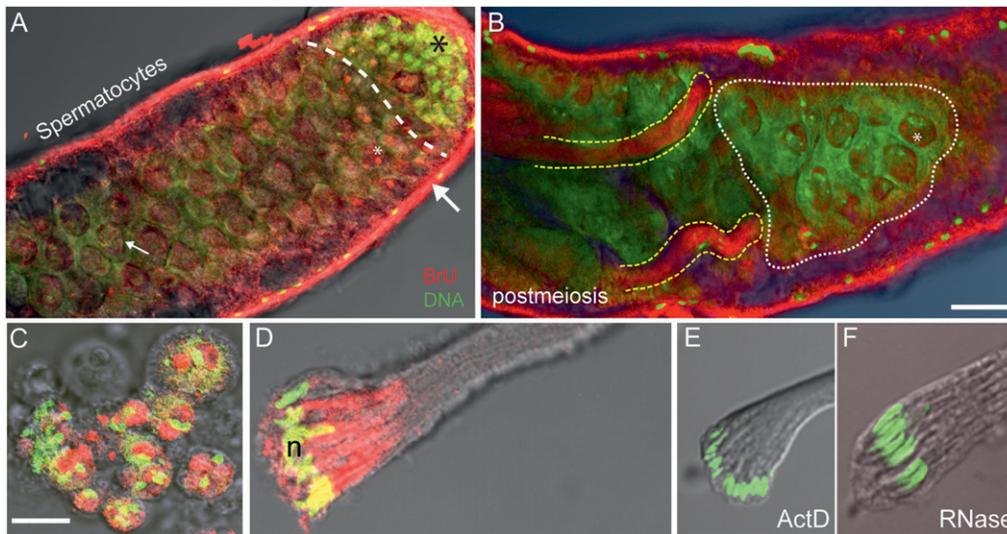postmeiotic cells. The BrU signal was reduced in the presence of actinomycin D, a general inhibitor of RNA synthesis (Figure 1E), confirming that the BrU signal in these cells was dependent on RNA synthesis. In addition, a virtual complete loss of BrU signal in Rnase-treated postmeiotic spermatid bundles provided further confirmation that the BrU signal was a consequence of RNA presence (Figure 1F). Statistical analysis confirmed BrU signal inhibition by actinomycin D and Rnase compared to control signal (Fisher exact test, $P \leq 0.0005$). Taken together, these results strongly support the conclusion that robust RNA transcription is prevalent in the postmeiotic phases.

Confocal imaging and 3D reconstruction of testes demonstrated the subcellular localization of BrU signal within developing spermatocytes (supporting information, Figure S1, A and B). Additional 3D reconstructions of isolated elongating spermatid bundles suggested periodic phases ("waves") of postmeiotic transcription where blocks of BrU signal were observed between intervening regions devoid of signal (Figure 2). We observed this general phenomenon in repeated in-

dependent experiments and, although our data do not fully explain the origin or the temporal dynamics of this process, they do raise the intriguing possibility that RNA is produced by active transcription in spermatid nuclei and then actively transported down the bundle.

In summary, our work extends previous studies because the use of BrU incorporation in isolated spermatids provides direct evidence for the production of nascent RNA in postmeiotic cells. Therefore, it further strengthens the newly emerging notion that postmeiotic transcription is dynamic and integral to the overall process of spermatogenesis in *D. melanogaster.*

In addition to obvious potential functions during the latter stages of sperm maturation, are there other consequences for RNA products of postmeiotic transcription? One intriguing possibility is that a subset of these RNAs is packaged into mature sperm and delivered to the egg at fertilization. Indeed, microarray studies indicate robust mRNA signals in purified Drosophila (personal communication, S. RUSSELL, S. DORUS and T. L. KARR) and mammalian sperm (KRAWETZ 2005).
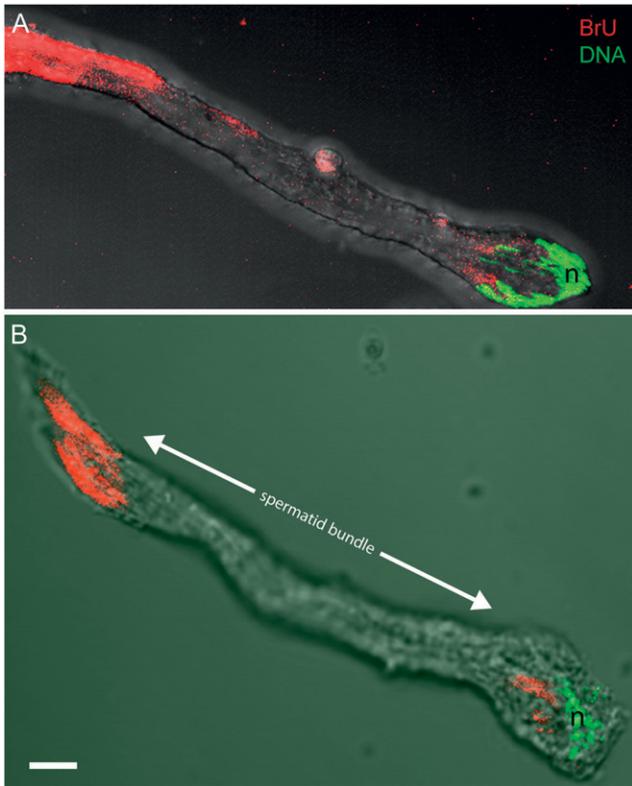
FIGURE 2.—Merged confocal and transmitted light images of isolated spermatid bundles labeled with BrU. (A) 3D reconstruction of the apical end of a single postmeiotic spermatid bundle (note, ~10% of the entire bundle is shown). The characteristic grouping of nuclei (n) denotes the apical end of the bundle. BrU staining is apparent both in the apical region near nuclei and along the growing spermatid axonemes. Note the absence of BrU signal in the middle region (between arrowheads) of the bundle. (B) Full view early stage of an elongating spermatid showing BrU signal at both apical and distal ends. Bar 10 μm.

Our results suggest that transcripts observed in mature sperm might arise from all three major phases of spermatogenesis including the postmeiotic phase. Finally, the postmeiotic transcriptional dynamics suggested by our results further strengthen the notion that haploid gamete gene expression may have impor-

tant consequences for the evolution of sperm competition and sperm cooperation by generating variation in and among sibling spermatozoa (PARKER and BEGON 1993; IMMLER 2008).

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GENETICS

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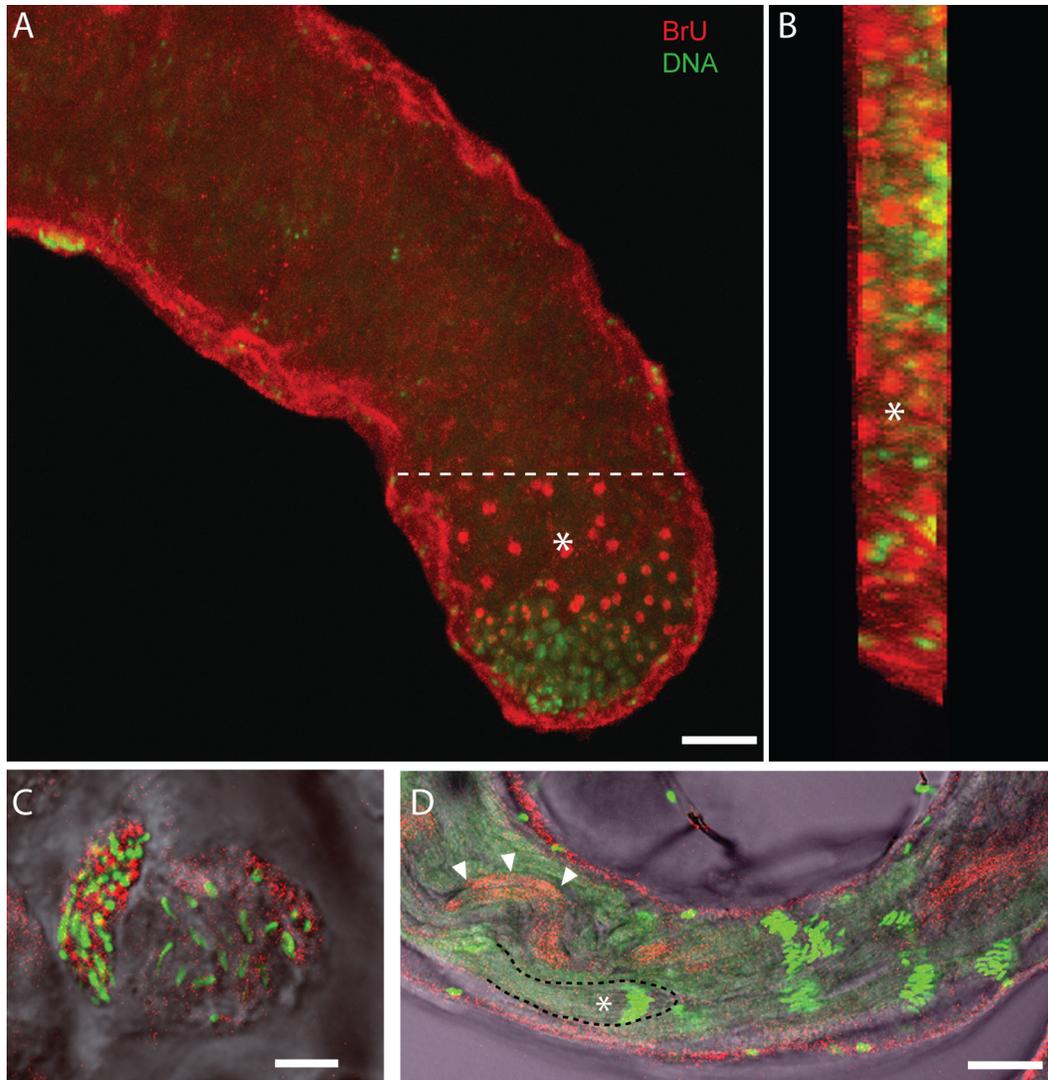and Timothy L. Karr**

FIGURE S1.—BRU incorporation in the Drosophila testis. (A) 3D reconstruction of intact testis showing apical end of testis with characteristic meiotic cell transcription (asterisk). (B) View of the apical-most region rotated 90 degrees edge on below the dashed line in panel A. Spherical BrU objects (asterisk) located interiorly are clearly within developing germ cells. (C) End view of two early spermatid bundles showing strong BrU signal in close proximity to the elongating spermatid nuclei. (D) Elongating spermatid bundles in an intact testis. Note differences in BrU signal in bundles from undetectable (asterisk in bundle outlined by black stippling) immediately adjacent to bundle with clearly observable BrU signal (arrowheads). Scale bars indicated: 50, 5 and 100 in panels A, C and D, respectively.