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Functional Genomic Analysis of the Periodic Transcriptome in the Developing *Drosophila* Wing

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SUMMARY

The eukaryotic cell cycle, driven by both transcriptional and posttranslational mechanisms, is the central molecular oscillator underlying tissue growth throughout animals. Although genome-wide studies have investigated cell-cycle-associated transcription in unicellular systems, global patterns of periodic transcription in multicellular tissues remain largely unexplored. Here we define the cell-cycle-associated transcriptome of the developing *Drosophila* wing epithelium and compare it with that of cultured *Drosophila* S2 cells, revealing a core set of periodic genes and a surprising degree of context specificity in periodic transcription. We further employ RNAi-mediated phenotypic profiling to define functional requirements for more than 300 periodic genes, with a focus on those required for cell proliferation in vivo. Finally, we investigate uncharacterized genes required for interkinetic nuclear migration. Combined, these findings provide a global perspective on cell-cycle control in vivo, and they highlight a critical need to understand the context-specific regulation of cell proliferation.

INTRODUCTION

Cell-cycle progression is controlled by a combination of transcriptional and posttranslational regulatory events (Morgan, 2006). Transcriptionally, the retinoblastoma protein/E2F pathway directly regulates expression of *cyclin E* and other target genes to drive the G1/S transition (Duronio and O'Farrell, 1995; Duronio and Xiong, 2013; Dyson, 1998; Geng et al., 1996; Ohtani et al., 1995). In quiescent mammalian cells, overexpression of E2F can induce S phase entry (Johnson et al., 1993), and in *Drosophila*, ectopic E2F can accelerate cell-cycle progression (Neufeld et al., 1998). Similarly, overexpression of G1 cyclins results in truncated G1 phases (Johnson et al., 1993; Ohtsubo and Roberts, 1993; Resnitzky et al., 1994) and is reported to induce mammary gland tumors in mice (Smith et al., 2006; Wang et al., 1994). These studies collectively demonstrate the importance of proper cell-cycle-associated transcription and thus raise a critical question: How much of the genome is periodically transcribed in a cell-cycle-associated manner?

To address this issue, a paradigm for understanding global cell-cycle-associated transcription has emerged from studies of synchronized cells in humans (Cho et al., 2001; Whitfield et al., 2002), budding yeast (Cho et al., 1998; Spellman et al., 1998), fission yeast (Oliva et al., 2005; Rustici et al., 2004), bacteria (Laub et al., 2000), and plant cell culture (Menges et al., 2003). Together these studies have identified hundreds of periodic genes, a large number of which are involved in cell-cycle-specific processes and expressed at peak levels when their functions are required. However, to date, global analyses of periodic transcription have focused on single-cell systems, and the potential intricacies of the periodic transcriptome in complex multicellular tissues remain poorly understood.

Unlike single-cell cultures, cell division in a developing tissue has to be coordinated with the developmental control of growth, patterning, and morphogenesis. In the vertebrate neural tube, for example, nuclei migrate during cell-cycle progression such that mitotic events are confined to the apical epithelial surface (Sauer, 1935). This process, interkinetic nuclear migration (IKNM), is proposed to be essential for the maintenance of tissue architecture in complex epithelia (Nakajima et al., 2013), and it is also important for determining the cell fate of neural progenitors in vertebrates (Cappello et al., 2006; Del Bene et al., 2008; Murciano et al., 2002; Xie et al., 2007). Nevertheless, despite the ubiquity of this conserved mitotic cell behavior (Meyer et al., 2011), the mechanisms linking nuclear cell-cycle progression to IKNM remain unclear, and the potential contributions of periodically expressed genes to the regulation of IKNM and other tissue-specific processes have received little direct attention.

In *Drosophila*, the adult wings and other appendage structures are derived from imaginal discs, monolayer epithelial sacs that undergo rapid and continuous proliferation during larval development (Cohen, 1993). Although wing growth is directed by patterning signals, cell division occurs ubiquitously without an obvious spatial pattern until late in development (Garcia-Bellido and Merriam, 1971; Johnston and Edgar, 1998). In the present study, in order to gain insight into global aspects of cell-cycle-associated transcription and the role of periodic genes in wing development, we profiled gene expression in G1 and G2/M phase wing disc cells isolated using a dissociation-fluorescence-activated cell sorting (FACS) protocol. By directly comparing the cell-cycle-associated transcriptome of wing disc cells with that of cultured S2 cells, we identified both common and context-dependent periodic genes. These genes were further tested for their function in tissue development, cell proliferation, cell-cycle phasing, and mitosis in the developing wing. The vast majority of genes identified using this approach were not

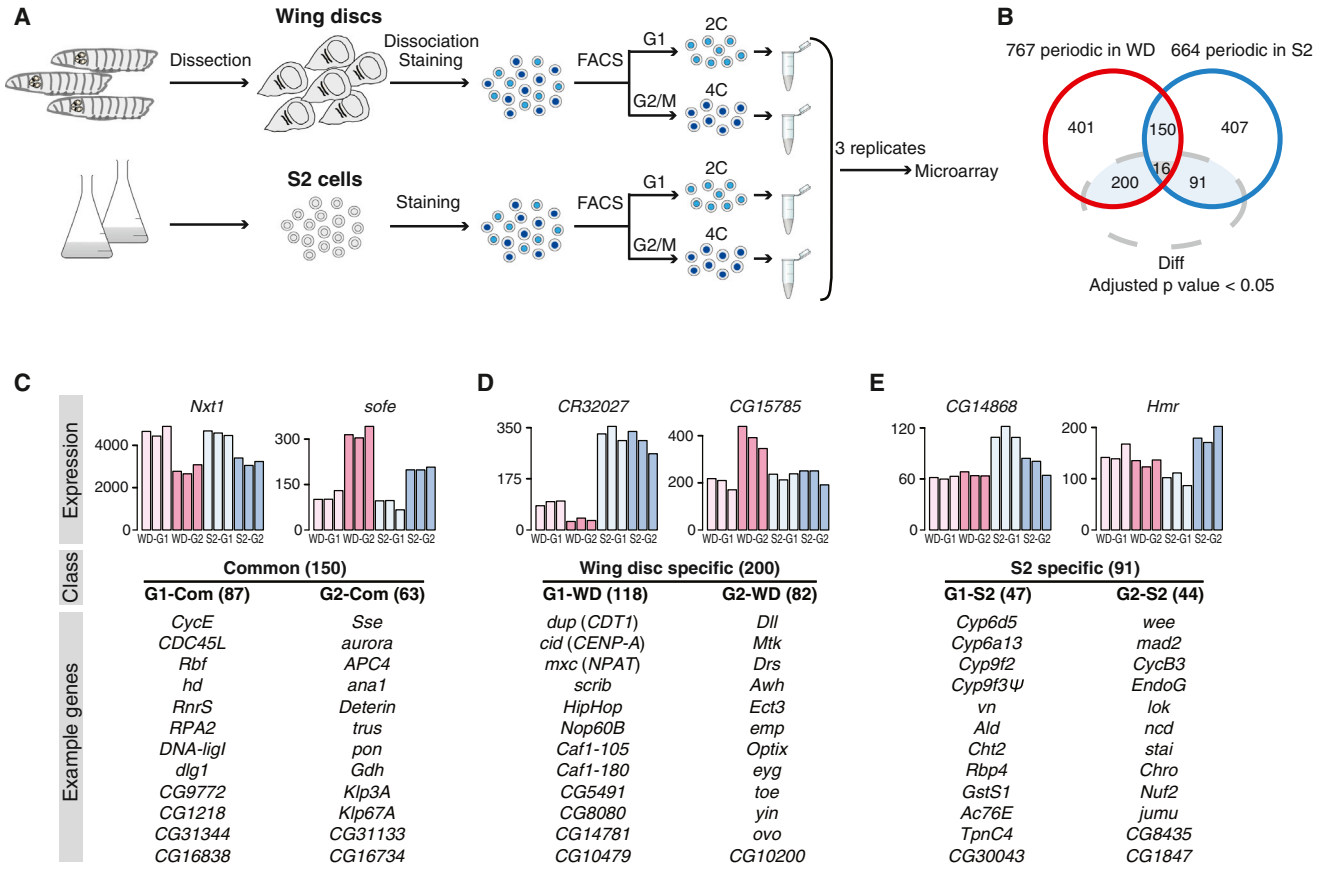

Figure 1. Cell-Cycle-Associated Transcription Is Robust and Context Specific

(A) Schematic representation of the integrative FACS-microarray analysis for identifying cell-cycle phase-dependent transcription in wing disc epithelial cells and S2 cells.

(B) Venn diagram illustrating sets of periodic transcripts in wing discs (WD) and S2 cells (S2), as well as the most differentially expressed genes (Diff) based on t statistics with a Benjamini-Hochberg adjusted p value of 0.05. For simplicity, the opposite gene group was manually segregated from the common category.

(C–E) Categorization of periodic transcripts into six main classes. Representative expression data and example genes are shown for the G1 and G2 common (C), wing disc specific (D), and S2 specific (E) classes. The numbers of transcripts in each class are listed in parentheses.

See also Figures S1 and S2 and Table S1.

revealed in a previous S2 RNAi screen in vitro (Björklund et al., 2006). Notably, we also implicate two periodic genes in the control of mitotic nuclear position during IKNM, highlighting the importance of understanding the regulation of cell-cycle progression in a context-dependent manner.

RESULTS

Global Analysis of Cell-Cycle-Associated Transcription

To define the global cell-cycle-associated transcriptional profile in the developing wing, we first developed a physical and enzymatic disruption protocol to rapidly dissociate whole discs into a suspension of single cells. Compared with the conventional 2–4-hr-long enzymatic protocol for imaginal disc dissociation, our method recovered approximately three times more live cells (~11,000 isolated live cells per wing disc) within a shorter time (20 min compared with 120 min). Using this approach, dissociated wing disc cells were stained live for DNA content and then sorted into G1 and G2/M populations by FACS (Figures S1A–S1C available online). To compare cell-cycle-associated transcription in the wing epithelium with that observed in cell culture, we performed a parallel series of FACS experiments in cultured *Drosophila* S2 cells (Figure 1A; Figures S1D–S1F). From the sorted G1 and G2/M populations, RNA samples were subjected to microarray analysis. Three biological replicates were examined for each condition and evaluated using a moderated t statistic (Smyth, 2004) to define the most significantly periodic genes in both wing discs and S2 cells (Figure 1B; adjusted p value < 0.05).

Based on the statistical analysis described above, we identified more than 700 cell-cycle-associated genes in wing discs and more than 600 in S2 cells (Figure 1B). The intersection of these sets included 150 genes with similar patterns of periodic expression in both cell types (defined as common genes in Figure 1C and Table S1A). Intriguingly, 200 genes were periodic exclusively in wing disc cells, and 91 were periodic only in S2 cells (defined as wing disc specific and S2 specific in Figures 1D and 1E; Table S1A). Furthermore, 16 genes displayed inverse periodic behavior; these genes were periodic in both cell types but peaked in different phases (and were thus defined as