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The germline stem cells of *Drosophila melanogaster* partition DNA non-randomly

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Abstract

The Immortal Strand Hypothesis proposes that asymmetrically dividing stem cells cosegregate chromatids to retain ancestral DNA templates. Using both pulse-chase and label retention assays, we show that non-random partitioning of DNA occurs in germline stem cells (GSCs) in the *Drosophila* ovary as these divide asymmetrically to generate a new GSC and a differentiating cystoblast. This process is disrupted when GSCs are forced to differentiate through the overexpression of Bag of Marbles, a factor that impels the terminal differentiation of cystoblasts. When Decapentaplegic, a ligand which maintains the undifferentiated state of GSCs, is expressed ectopically the non-random partitioning of DNA is similarly disrupted. Our data suggest asymmetric chromatid segregation is coupled to mechanisms specifying cellular differentiation via asymmetric stem cell division.

Keywords: Stem cells; Chromosomes; Asymmetric division

Introduction

During mitosis, dividing cells segregate their replicated chromatids into each daughter to ensure the inheritance of the complete genome. This repeated replication of DNA presents a problem to a long-term dividing cell such as a stem cell (SC). If segregation is random, and DNA is copied from a previous copy, replication errors will accumulate in frequently dividing SCs and their progeny. The Immortal Strand Hypothesis (ISH) (Cairns, 1975) proposes that DNA is segregated non-randomly between recipient daughter cells, as a means through which SCs might lower their mutation load (Cairns, 2002). According to the ISH, asymmetrically dividing SCs cosegregate chromatids in order to retain ancestral DNA templates in the SC daughter (Fig. 1A). Given that DNA replication is semi-conservative, such chromosomes are distinguished because they contain one ancestral strand associated with a newer strand from the preceding round of DNA synthesis. This asymmetry in DNA molecule inheritance between daughter cells might also segregate differences in chromatin architecture to retain sequence fidelity and enzyme accessibility (Jablonka and Jablonka, 1982a, b)

Abbreviations: Bam, Bag of marbles; BrdU, 5-bromo-2-deoxyuridine; CldU, 5-chloro-2-deoxyuridine; Dpp, decapentaplegic; GSC, germline stem cell; HTS, Hu Li Tai Shao; IdU, 5-iodo-2-deoxyuridine; ISH, immortal strand hypothesis; PGC, primordial germ cell; SC, stem cell.

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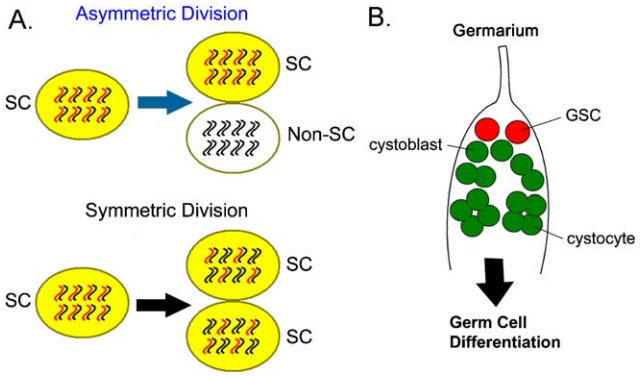

Fig. 1. The immortal strand hypothesis: (A) When SCs divide asymmetrically (blue), producing a daughter SC and a daughter non-SC, chromatids containing ancestral DNA templates (indicated in red) are segregated to SCs. DNA is replicated semi-conservatively, thus chromosomes contain newly synthesized strands (indicated in black) associated with ancestral template strands. When SCs divide symmetrically (black), chromosome segregation is random. (B) Schematic of the germarium, the region in which GSCs (red), cystoblasts (green single cells) and cystocytes (green clusters) reside. Note that the GSCs are the germ cells that occupy positions adjacent to the tip of the germarium. More differentiated cystocytes are further down from this area, forming cysts with 16 nuclei that mature into follicles.

for genes conferring pluripotency to SCs, and might allow non-SCs to adopt a novel chromatin architecture. Hence, the ISH also provides an attractive single-factor explanation for an epigenetic genome maintenance of the self-renewing SC and the concomitant differentiation of its non-SC offspring.

There is some data to suggest the separation of older and newer chromosomes following DNA replication in vitro (Karpowicz et al., 2005; Lark et al., 1966; Merok et al., 2002) as well as in vivo (Potten et al., 1978, 2002; Smith, 2005; Shinin et al., 2006; Lark, 1967) and that it is coupled to the differentiation of the non-stem cell counterparts (Conboy et al., 2007). Though these studies are notable, cells which demonstrated asymmetric DNA segregation were not identified unequivocally as asymmetrically dividing SCs. As well, there are a significant number of studies that have failed to support the ISH both in SCs and other cell types as well (Neff and Burke, 1991; Kuroki and Murakami, 1989; Ito and McGhee, 1987; Kiel et al., 2007; Waghmare et al., 2008; Sotiropoulou et al., 2008). Thus, the ISH remains an active and controversial subject of research (Tajbakhsh, 2008).

The ovary of the fruit fly, *Drosophila melanogaster*, contains germaria with a germline stem cell (GSC) population that can be identified unambiguously (Ohlstein et al., 2004). Each germarium is known to possess either 2 or 3 SCs (Fig. 1B), that divide asymmetrically to give rise to daughter SCs and cystoblasts. The cystoblast progeny of GSCs undergo a further four divisions to produce a cyst containing 16 nuclei, which matures into a follicle, and which develops into a single egg (King, 1970). This asymmetric division of GSCs to produce a GSC and cystoblast daughter continues throughout the lifetime of the female fly. Here we demonstrate that asymmetric segregation of DNA occurs in the dividing GSCs. We show that this process ceases when differentiation is molecularly perturbed and that, unlike GSCs, the differentiated progeny of GSCs segregate DNA randomly.

Materials and methods

Fly stocks and dissection

Wild-type, w^{1118}, *c*587-*Gal*4; *UAS-Dpp*, *w*; *P*[*hsp*70-*bam*]11*d* and *P*[*hsp*70-*bam*]18*d* stocks were maintained at 25 °C. For retention experiments, BrdU stock (25 mg/ml in 40% EtOH) was applied to medium at a final concentration of 0.2 mg/ml. For pulse-chase experiments, female prepupae were selected and maintained at 25 °C on apple plates. Heat shock was performed one day prior to injections as described (Ohlstein and McKearin, 1997). Pupae were fixed to slides using double-sided scotch tape and injected at 3 days pupation with 1.0 mM BrdU (Sigma) dissolved in Ringer's buffer (pH 6.9), or with 1.0 mM BrdU (Sigma) together with 100 mM BrdU thymidine (Sigma) dissolved in Ringer's buffer (pH 6.9). Injections were done using 25° ground capillary needles directly into the abdomen of the pupa. Subsets of BrdU-injected pupae were injected 24 h following BrdU infusion, with 100 mM BrdU thymidine (Sigma) dissolved in Ringer's (pH 6.9). Pupae were maintained at 25 °C, ovaries were dissected in 10 mM phosphate-buffered saline (PBS) and fixed 12 min at room temperature with 5% formaldehyde diluted in PBS (Roche). Following fixation, ovaries were washed three times with PBS + 1.0% Triton X-100 (Sigma) and triturated using an 1c.c. syringe and 30G1/2 tip (Becton-Dickenson) to dissociate ovaries.

Immunostaining

The following antibodies were used: (1) rat monoclonal anti-BrdU Bu1/75 (Abcam, 1:500), (2) mouse monoclonal anti-pan-histone (Chemicon, 1:500), (3) rabbit polyclonal anti-VASA (courtesy of Paul Lasko, 1:2000), (4) mouse monoclonal anti-HTS 1B1 (courtesy of Howard Lipshitz, 1:1). Secondary 568 or 633 nm cross-adsorbed Alexa Fluor antibodies (Molecular Probes, 1:300) were used excepting BrdU secondary stain which was amplified using biotin-conjugated antibodies (Jackson, 1:250) followed by streptavidin-DTAF