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A functional Bucky ball-GFP transgene visualizes germ plasm in living zebrafish

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ABSTRACT

In many animals, the germline is specified by maternal RNA-granules termed germ plasm. The correct localization of germ plasm during embryogenesis is therefore crucial for the specification of germ cells. In zebrafish, we previously identified Bucky ball (Buc) as a key regulator of germ plasm formation. Here, we used a Buc antibody to describe its continuous germ plasm localization. Moreover, we generated a transgenic Buc-GFP line for live imaging, which visualizes germ plasm from its assembly during oogenesis up to the larval stages. Live imaging of Buc-GFP generated stunning movies, as they highlighted the dynamic details of germ plasm movements. Moreover, we discovered that Buc was still detected in primordial germ cells 2 days after fertilization. Interestingly, the transgene rescued *buc* mutants demonstrating genetically that the Buc-GFP fusion protein is functional. These results show that Buc-GFP exerts all biochemical interactions essential for germline development and highlight the potential of this line to analyze the molecular regulation of germ plasm formation.

1. Introduction

Multicellular organisms separate their soma from reproductive germ cells, which are specified in many species by the inheritance of cell fate determinants called germ plasm (reviewed in Ewen-Campen et al., 2010; Houston and King, 2000; Juliano et al., 2010; Schisa, 2012; Strome and Lehmann, 2007; Voronina et al., 2011). Germ plasm assembles during oogenesis forming a membrane-free compartment called the Balbiani body, which is observed in the oocytes of many species including humans and zebrafish (Heasman et al., 1984; reviewed in Dosch, 2015; Kloc et al., 2004; Lindeman and Pelegri, 2010; Raz, 2003). Although germ plasm has a critical role for germ line development, the molecular mechanisms by which it induces germ cell formation are still largely unknown.

Bucky ball (Buc) is a crucial regulator of germline development in zebrafish, since *buc*–/– oocytes fail to form a Balbiani body (Bontems et al., 2009; Marlow and Mullins, 2008). Fascinatingly, overexpression of Buc reprograms somatic cells *in vivo* into primordial germ cells (PGCs), which so far has not been reported for other vertebrate genes (Bontems et al., 2009). In addition, injecting Buc-GFP mRNA labels germ plasm during oogenesis and embryogenesis identifying Buc as the first germ plasm organizer in vertebrates. However, injection of mRNA encoding a Buc-GFP-fusion is (I) technically challenging in early oocytes, (II) creates unspecific background, (III) requires at least an hour to develop a fluorescent signal, and (IV) influences germline development limiting its application as a germline reporter. Existing germline reporters in zebrafish have the advantage of being biologically inactive *i.e.* they do not interfere with germline development (Blaser et al., 2005; Krøvel and Olsen, 2002; Leu and Draper, 2010; Onichtchouk et al., 2003). Unfortunately, they do not label germ plasm as a subcellular compartment and thus, a different approach is required to monitor germ plasm in living zebrafish.

Here, we used the Buc protein to follow germ plasm dynamics in zebrafish. We first characterized the endogenous localization of Buc with a novel antibody validating its suitability as a germ plasm marker. Then we generated a transgenic line expressing Buc-GFP under control of its own promotor (*tg*(*buc:buc-egfp*), hereafter called Buc-GFP). The Buc-GFP signal perfectly recapitulated germ plasm localization at high resolution in living oocytes and embryos. In addition, we detected fluorescence in germ cells 2 days post fertilization in the Buc-GFP line showing that Buc continuously highlights germ plasm from the oocyte up to larval stages. Interestingly, the transgene rescued the mutant phenotype demonstrating genetically that Buc-GFP is also functional and performs all biochemical interactions essential for germline development like the endogenous protein.

Abbreviations: Buc, Bucky ball; GFP, green fluorescent protein; hpf, hours post fertilization; PGC, primordial germ cell.

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Thus, the Buc-GFP line provides an excellent entry point for further studies dissecting the molecular regulation of germ plasm in the zebrafish. As Buc homologs are present in many vertebrate genomes, the protein might also be a versatile germline marker for these species.

2. Results

2.1. A novel Bucky ball antibody

Using a novel, polyclonal anti-Buc antibody we analyzed the localization of endogenous protein to validate its applicability as a germ plasm marker. We focused on the Balbiani body, the germ plasm aggregate in early oocytes, where we previously discovered Buc-GFP upon overexpression (Bontems et al., 2009). Immunostainings of early stage IB oocytes confirmed Buc in the Balbiani body (Fig. 1A). We then analyzed *buc* mutant oocytes to determine antibody specificity. *buc*^{p106} generates a nonsense mutation after 601 amino acids, and *buc*^{p43} after position 361. In both alleles Buc showed no signal, whereas Vasa was still detected around the nucleus suggesting that the novel antibody monitors Buc protein localization (Fig. 1B, C) (Braat et al., 1999a; Knaut et al., 2000).

The lack of signal could suggest that both mutations might cause protein null-alleles. Although overexpressed Buc^{p43} fused to GFP was recognized (Fig. S1), the antibody did not detect endogenous Buc in Western blots (data not shown) as described for Velo1, the *Xenopus* Buc homolog (Claussen and Pieler, 2004; Nijjar and Woodland, 2013). Thus, it remains unknown whether the *buc* mutations represent protein null-alleles or whether mislocalization dilutes Buc to background levels.

2.2. Endogenous Buc localizes to the germ plasm during oogenesis

We then examined protein localization during zebrafish oogenesis to corroborate Buc as a suitable germ plasm marker. Indeed, Buc localized in stage IA oocytes to the Balbiani body (Fig. 2A) as shown in zebrafish and *Xenopus* (Heim et al., 2014; Nijjar and Woodland, 2013). During stage IB, we discovered that it migrated to the vegetal pole as previously described for other germ plasm components (Fig. 2B–D) (Kosaka et al., 2007). At stage II, Buc spread along the vegetal cortex and was undetectable at stage III (Fig. 2E–G), thus identifying Buc as a germ plasm component in the oocyte.

2.3. A transgenic Buc-GFP line labels germ plasm in living oocytes

To follow the dynamics of germ plasm in living zebrafish, we created a transgenic line expressing a Buc-GFP fusion. The exon–intron structure of Buc-transgenes is important for correct expression (Heim et al., 2014). We therefore isolated its genomic locus and created a C-terminal fusion with eGFP in a Tol2-transposon vector for efficient transgenesis (Fig. 3A) (reviewed in Kawakami, 2007).

We raised fluorescent F1-embryos, which inherited maternal Buc-GFP from injected G0-adults. We dissected ovaries of transgenic

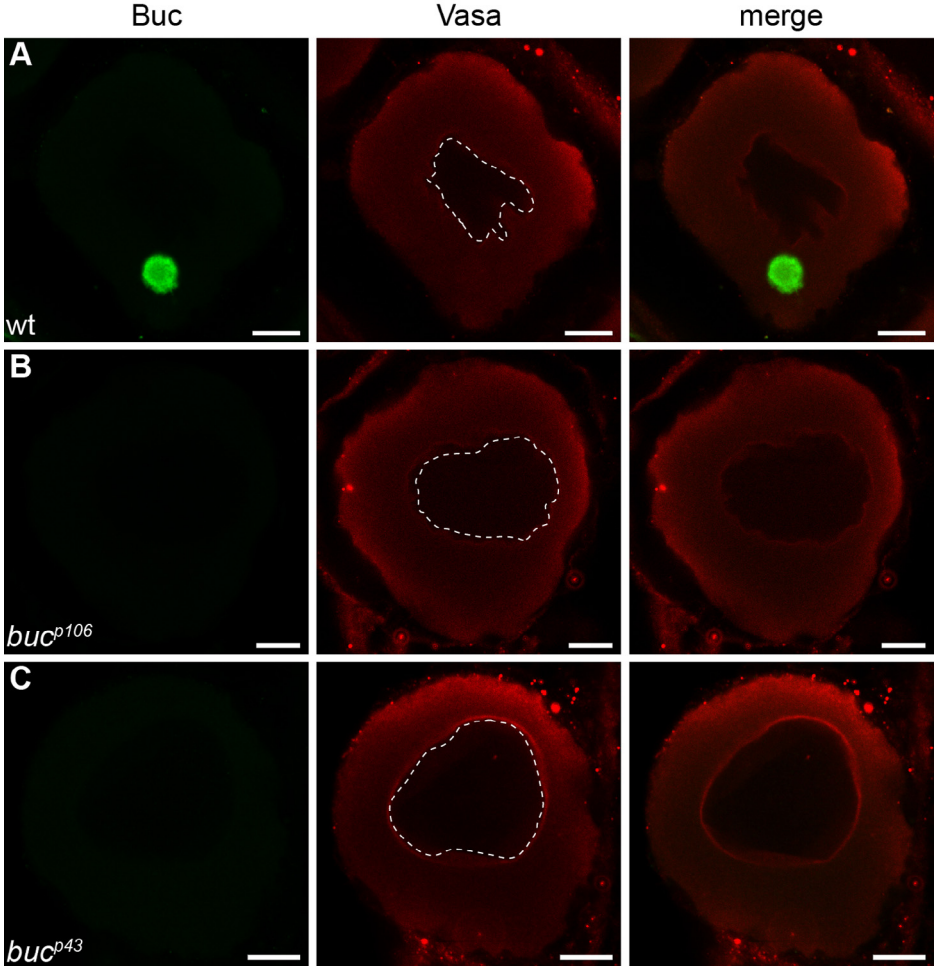

Fig. 1. Buc antibody specificity. Confocal images of stage IB oocytes. Buc (green) labels the Balbiani body in wild type oocytes (A) but not in mutants *buc*^{p106} (B) and *buc*^{p43} (C). By contrast, perinuclear Vasa (red) is not changed. Dashed line outlines the nucleus. Lateral views, animal to the top. Scale bar: 10 μm.