In vivo detection of lamellocytes in Drosophila melanogaster

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Sir,

In both vertebrates and invertebrates the blood cells undergo differentiation in spatially separated organs, in a characteristic time sequence [1-5]. Recently Drosophila became a powerful model organism for studying blood cell development and cellular events of innate immunity. The cellular elements of innate immunity, the blood cells or hemocytes in Drosophila, have been characterized by morphological criteria and molecular markers, including transcription factors [6,7] and cell-type specific immunological markers [8-10]. In Drosophila, at least three main classes of hemocytes can be discerned. The predominant class comprises small round cells with a phagocytic capacity, the plasmatocytes. A second class, the crystal cells, distinguished by pronounced crystal like inclusions in the cytoplasm, are involved in melanin deposition in wounds and around foreign objects. Finally, a class of large flat cells, the lamellocytes, appears when the larvae are infected by parasitoid wasps. Lamellocytes participate in the encapsulation of parasites and differentiate in response to mechanical assault and their development is finely regulated [11, 12, 5].

Hemocytes are distributed in three main hematopoietic compartments in the larva, the lymph gland, the sessile tissue and the circulation. The understanding of molecular events of hemocyte development in these compartments and the definition of signalling pathways that lead to differentiation and the immune response have been facilitated by genetic screens in
combination with functional tests and monitoring of hemocyte subsets. This analysis has been helped by the availability of reporter-gene constructs expressing marker molecules, including fluorescent proteins, \textit{in vivo}. The constructs make possible to analyze and follow the hemocyte development \textit{in vivo} by expressing fluorescent proteins in a cell-type specific manner. Plasmatocytes, lamellocytes and crystal cells can be visualized by appropriate constructs [11, 13-15]; however, lamellocyte specific marker for \textit{in vivo} use has not been described yet.

Recently we have defined a molecular marker for lamellocytes as Atilla-L1, and determined the localization of its gene in the genome [Laurinyecz et al., unpublished]. Database analysis revealed that an insertion of a MiET1 element [16] in this gene is available. The element contains a hsp70 promoter controlled \textit{GAL4} gene, and an \textit{EGFP} marker gene driven by the 3xP3 promoter. The \textit{Mi[ET1]CG6579[MB03539]} insertion is located in the \textit{atilla} gene (CG6579) [Laurinyecz et al., unpublished] at position 2L:12,177,639 in the ‘plus’ orientation in the 5’ untranslated region. Our analysis revealed that the stock carrying this insertion is suitable for \textit{in vivo} detection of lamellocytes in immune-challenged larvae (Fig. 1A, arrows) as well as in hemocyte overproducing mutants. The use of a panel of lamellocyte specific markers [10] showed that the expression of GFP is specific for/to lamellocytes. The 3xP3 promoter also shows an expression pattern similar to that of the \textit{eyeless} driver, which appears in the eye discus, brain, gut and anal plate (Fig. 1A, arrowheads) showing up as a background fluorescence appearing in all stocks carrying the MiET1 element [16]. The insertion blocks the expression of the Atilla protein encoded by the \textit{atilla} gene, but does not affect the expression of other, independent lamellocyte-specific markers [10], as L2 and L6 (Fig. 1B,C). These results confirm that the insertion allows \textit{in vivo} investigation of lamellocyte differentiation as well as helps genetic screens with the aim of dissecting signaling pathways.
of hemocyte development. We also found that the GAL4 gene in this insertion is inactive. This however allows the use of this insertion with independent GAL4 drivers in combination with UAS-RFP reporter construct thus making possible double-labelling of hemocyte populations in vivo with a clear distinction of lamellocytes.

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References


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Legend to Fig. 1

Lamellocytes in the $Mi\{ET1\}CG6579[MB03539]$ larva

(A) GFP expressing lamellocytes are visible through the cuticule of the wasp infested $Mi\{ET1\}CG6579[MB03539]$ larva. Arrows are pointing at lamellocytes, arrowheads point to organs showing background fluorescence characteristic for the MiET1 element [16] (scale bar: 100µm). (B,C) The GFP expression overlaps with the L2 (B) and L6 (C) antigen expression in the hemocytes of homozygous $Mi\{ET1\}CG6579[MB03539]; l(3)mbn^I$ larvae. Lamellocytes are visualized by indirect immunofluorescence. The GFP expression in lamellocytes is enhanced by rabbit anti-GFP staining and anti-rabbit Alexa-488 (green). The expression of lamellocyte specific antigens is detected with mouse anti-L2, anti-L6 and anti-mouse Alexa-568 (red). Arrows are pointing at/to double labelled lamellocytes (scale bars: 20µm).