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Cell cycle independent role of Cyclin E during neural cell fate specification in *Drosophila* is mediated by its regulation of Prospero function

Christian Berger [a,1], Ramakrishnan Kannan [b,1], Sudharani Myneni [b], Simone Renner [a], L.S. Shashidhara [b,c,*], Gerhard M. Technau [a,*]

[a] Institute for Genetics, University of Mainz, D-55099 Mainz, Germany
[b] Center for Cellular and Molecular Biology, Uppal Road, 500 007 Hyderabad, India
[c] Indian Institute of Science Education and Research, 411008 Pune, India

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ABSTRACT

During development, neural progenitor cells or neuroblasts generate a great intra- and inter-segmental diversity of neuronal and glial cell types in the nervous system. In thoracic segments of the embryonic central nervous system of *Drosophila*, the neuroblast NB6-4t undergoes an asymmetric first division to generate a neuronal and a glial sublineage, while abdominal NB6-4a divides once symmetrically to generate only 2 glial cells. We had earlier reported a critical function for the G1 cyclin, CyclinE (CycE) in regulating asymmetric cell division in NB6-4t. Here we show that (i) this function of CycE is independent of its role in cell cycle regulation and (ii) the two functions are mediated by distinct domains at the protein level. Results presented here also suggest that CycE inhibits the function of Prospero and facilitates its cortical localization, which is critical for inducing stem cell behaviour, i.e. asymmetric cell division of NB6-4t. Furthermore our data imply that CycE is required for the maintenance of stem cell identity of most other neuroblasts.

Introduction

The diversity of cell types in the central nervous system (CNS) is generated by stem cells that are multipotent, exhibit a high mitotic index and have the capacity to self-renew by avoiding cell cycle exit and differentiation. This involves a tight control of proliferation of the precursor cells as deregulation leads to an early death (under-proliferation) or to the generation of tumors (overproliferation) (Chia et al., 2008; Doe, 2008; Egger et al., 2008; Knoblich, 2008). In the mammalian telencephalon neuroepithelial cells and radial glia self-renew, while producing basal neuronal and/or glial progeny cells (Götz and Huttner, 2005). Whereas the self-renewal capacity in the developing mammalian brain is restricted to these two populations of precursor cells, nearly all stem cells of the *Drosophila* CNS (called neuroblasts, NBs) exhibit self-renewal capacities during embryonic and postembryonic stages. *Drosophila* NBs divide in an asymmetric manner to self-renew and to produce a chain of smaller ganglion-mother cells (GMC), which typically divide once to generate neurons and/or glial cells.

The NB6-4 gives rise to one of the best-studied neuroblast lineages and has provided several clues on the mechanism by which the mode of cell division is regulated during neurogenesis. In thoracic segments of the embryonic CNS of *Drosophila*, NB6-4 (NB6-4t) undergoes an asymmetric first division to generate a neuroblast and a glial precursor cell (Figs. 1A, B). In NB6-4t, Prospero (Pros) is cortically localized and inherited only by the glial-producing daughter cell (Freeman and Doe, 2001). In the daughter cell, Pros is nuclear (Fig. 1D) and is required for the transcriptional maintenance of *gcm* expression, which in turn confers glial identity (Akiyama-Oda et al., 1999; Bernardoni et al., 1999; Freeman and Doe, 2001; Ragone et al., 2001). The glial precursor cell divides two more times to give rise to three glia, while the neuroblast divides several times in a stem cell mode to generate 5–6 neurons (Fig. 1A and Berger et al., 2005; Ragone et al., 2001; Schmidt et al., 1997). In abdominal segments, Pros is nuclear and cortical in NB6-4 (NB6-4a) prior to the first division (Fig. 1E). NB6-4a undergoes one symmetric division to give rise to only 2 glial cells (Figs. 1A, C) both expressing Pros (Fig. 1F). We had earlier reported that CyclinE (CycE) is expressed in NB6-4t (Fig. 1G), but not in NB6-4a. This difference is due to repression of CycE in NB6-4a by the Hox proteins AbdA and AbdB (Berger et al., 2005; Kannan et al., in press). In *CycE* loss of function situations, NB6-4t resembles the abdominal variant and generates only glial cells. Conversely, ectopic expression of CycE confers the stem-cell mode of division to NB6-4a, leading to the

* Corresponding authors. L.S. Shashidhara is to be contacted at fax: +91 20 25899790. G.M. Technau, fax: +49 6131 3924584.
E-mail addresses: ls.shashidhara@iiserpune.ac.in (L.S. Shashidhara), technau@uni-mainz.de (G.M. Technau).
[1] Authors contributed equally.

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generation of a neuronal sublineage in addition to the glial cells (Berger et al., 2005).

Our earlier result further suggested that the role of CycE in specifying the NB6-4t stem cell fate (leading to the production of neurons in addition to glia) might be independent of its role in cell cycle regulation. Here we provide genetic proof for this hypothesis and we show that distinct domains of the protein mediate these two functions of CycE. Our results also provide preliminary insights