

## Identification of stem cells during prepubertal spermatogenesis via monitoring of nucleostemin promoter activity.

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The nucleostemin (NS) gene encodes a nucleolar protein found at high levels in several types of stem cells and tumor cell lines. The function of NS is unclear but it may play a critical role in S-phase entry by stem/progenitor cells. Here we characterize NS expression in murine male germ cells. Although NS protein was highly expressed in the nucleoli of all primordial germ cells, only a limited number of gonocytes showed NS expression in neonatal testes. In adult testes, NS protein was expressed at high levels in the nucleoli of spermatogonia and primary spermatocytes but at only low levels in round spermatids. To evaluate the properties of cells expressing high levels of NS, we generated transgenic reporter mice expressing green fluorescent protein (GFP) under the control of the NS promoter (NS-GFP Tg mice). In adult NS-GFP Tg testes, GFP and endogenous NS protein expression were correlated in spermatogonia and spermatocytes but GFP was also ectopically expressed in elongated spermatids and sperm. In testes of NS-GFP Tg embryos, neonates, and 10-day-old pups, however, GFP expression closely coincided with endogenous NS expression in developing germ cells. Our results support the existence in neonatal testes of spermatogonial stem cells with long-term repopulating capacity. Furthermore, our data show that NS expression does not correlate with cell-cycle status during prepuberty, and that strong NS expression is essential for the maintenance of germline stem cell proliferation capacity. We conclude that NS is a marker of undifferentiated status in the germ cell lineage during prepubertal spermatogenesis.

### FACS analysis of testicular cells

