Final report

Development and function of the ovarian stem cell niche in Drosophila K 117010

The main aim of our studies was to uncover fundamental phenomena regulating of niche development and function. Therefore, we applied the ovarian germ line stem cell niche of the fruit fly (*Drosophila melanogaster*) to model the general regulatory mechanisms acting in the niches. To investigate these mechanisms, we analysed the origin and the differentiation of the different cell types of the adult germ line stem cell niche and performed a detailed cell biological analysis of small ovaries (sov) gene function in stem cell niche formation, stem cell maintenance, and stem cell differentiation.

The adult niche cell types develop on an inductive manner from non-committed, uniform intermingled cells originating from three different embryonic abdominal segments

The adult germ-line stem cell niche is composed by three somatic cell types. Terminal filament, cap, and escort cells all are descendants of the so called intermingled (IC) embryonic cells. ICs differentiate from three different embryonic abdominal segments (Ps10-, Ps11, Ps12), thus it was conceivable that ICs represent a mixed population of already committed cell types. The different subsets of ICs may serve as ancestors of different cell lineages of the GSC niche cell types. Alternatively, ICs are uniform and naïve cells and their later commitment and differentiation are regulated by yet unknown induction effects that occur during the larval stages. To show which from the two developmental strategies determines the development of the terminal filament, cap, and escort cells, we performed a series of lineage tracing and clonal analysis experiments.

We have investigated the expression pattern of a large number of Gal4 sources and selected three specific Gal4 activator transgenes by which the embryonic gonads, the Ps10-12 segments, and a subset of the intermingled cells can be marked and the cell lineage tracing experiments could be performed. We have tested several lineage tracing methods and selected found Ubi-FRT-stop-FRT-GFPnls to be the most effective one and we used it in the later experiments. We extended the conventional lineage tracing method with the Gal4/Gal80^{ts} system, which enabled a precise temporal control of the clone induction and a quantitative analysis of the lineage tracing experiment. By this method, fluorescently marked embryonic gonadal cells were generated in a wery short period during embryonic development and the fate of their progenies was examined.

Tracing of the marked embryonic lineages revealed that the segmental origin of the cells has no effect on the development of the ovarian somatic niche cells. This result indicates that even though the intermingled cells (ICs) originated form three different abdominal segments they are non-committed,

uniform embryonic cells and the ovarian niche cell types develop under the control of inductive developmental strategy.

Sov is required for GSC maintenance and differentiation

Sov was previously identified in our laboratory in a large-scale RNAi-based reverse genetic screen for genes involved in germ cell development. Sov encodes for a C2H2 zinc finger protein with multiple nuclear localization signals indicating that sov is a putative transcription factor. We demonstrated that the differentiation of the germ line stem cell's progeny is not initiated in sov mutants, but a permanent proliferation of the stem cells generates small-sized stem cell-like tumours. We showed that the undifferentiated germ cells fail to express the differentiation factor bag of marbles (bam). Forced expression of bam in sov mutant tumorous niches was sufficient to induce germ cell differentiation indicating that sov acts upstream of bam. In the sov mutants, in addition to the tumorous niches, we detected niches which have lost germ line stem cells indicating that sov is required for stem cell maintenance. Direct visualisation of niche cell markers and subsequent quantitative analysis demonstrated that somatic cells of the germ line stem cell niche were also lost in the sov mutants indicating that sov function is required for niche cell survival.

sov is required cell-autonomously for GSC maintenance

To narrow the temporal and spatial requirement of sov in germ cell development, tissue-specific RNAi and clonal analyses were performed. Depletion of sov in the germ line resulted in a progressive loss of GSCs indicating that sov is required cell-autonomously in the germ line for GSC maintenance. The cell-autonomous requirement for sov in germ cell maintenance was confirmed by analysis of sov mutant germline clones. Taken together, our data show that sov is required for GSC maintenance intrinsically in the germline. Remarkably, loss of sov in the GSCs did not induce tumour formation, indicating that the differentiation defect observed in sov mutants is not germ-cell-autonomous.

Sov is required in escort cells for GSC maintenance, germ cell differentiation and EC survival

To silence sov in a cell-type-specific manner, transgenic fly lines were used to drive the expression of sov-shRNAs in various subsets of the somatic niche cells. Silencing of sov in the terminal filament and cap cells did not result in niche defects whereas specific silencing of sov in the escort cells phenocopied the hypomorphic sov mutant phenotypes. We observed a reduction in escort cell numbers when sov was silenced specifically in the escort cells of the niche, indicating that sov is required for escort cell survival in a cell-autonomous manner. We investigated how germline behaviour is affected sov function in the escort cells. We found that sov is required in the escort cells in a non-cell-autonomous manner for GSC maintenance and differentiation.

In summary, sov is required for EC survival in the adult niches, which ensures GSC differentiation and maintenance in a non-cell-autonomous manner

Sov promotes GSC differentiation by restricting Dpp-signalling activity in the niche

We determined sov's role in the communication among the different cell types of the germline stem cell niche. By a series of epistasis experiments, we were able to place sov function into the complex regulatory network of signalling events of the niche. We showed that sov acts downstream of the JAK/STAT, EGF and the Notch pathways and upstream of the TGFβ-pathway. By a combination of cell type-specific silencing, microscopic examination of various mutant niches and qRT-PCR based gene expression analysis, we demonstrated that sov activity is required in the escort cells to suppresses the activity of decapentaplegic (dpp), the Drosophila TGFβ- homolog. We proved that the mis-regulation of the transcription factor *engrailed* in the sov mutant escort cells leads to increased dpp production. Excessive Dpp inhibits the expression of bam in the germ cells, preventing the initiation of their differentiation.

Sov is a heterochromatin component

To identify the mechanisms by which Sov regulates stem cell niche, we first determined the subcellular localization of the GFP-tagged Sov protein expressed from a genomic fosmid construct. We detected ubiquitous Sov expression in the somatic and germ cells of the ovary, confirming our previous conclusion about the requirement of sov in these cell types. Sov localized in the nuclei and accumulated at nuclear foci. At these foci, Sov co-localized with Heterochromatin Protein 1a (HP1a), a central component of the heterochromatin suggesting a direct role for Sov in chromatin regulation. To identify Sov interacting proteins, we affinity-purified protein complexes from the Sov:GFP ovaries and performed mass spectrometry analysis of the samples. This method revealed the complete protein interactome of Sov. Spectral counting was used to estimate the relative abundance of individual proteins in the Sov:GFP and control samples. We found that Sov strongly associates with HP1a, indicating that sov affects stem cell development as a heterochromatin regulator.

Sov promotes heterochromatin formation by stabilization of heterochromatic domains

We performed several functional assays to investigate the precise role of sov in chromatin regulation. First, in the pericentromeric position effect variegation (PEV)-assay, we detected dominant suppressor effect of sov mutations on PEV. This result suggested that Sov promotes heterochromatin formation at the pericentromeric regions of the genome. The positive effect of sov on heterochromatin formation is gene-dose-dependent, as demonstrated by the enhancement of PEV with increasing gene copies of sov. Furthermore, PEV analysis revealed a dominant genetic interaction between HP1a and sov. Haplo-

suppression and triplo-enhancement of PEV by sov and its genetic and physical interaction with HP1a indicates that Sov functions as a structural component of the heterochromatin. Second, we tested the involvement of Sov in HP1a mediated gene silencing by a LacI/LacO transcriptional reporter assay. We found that sov is not required for HP1a-mediated repression of the reporter locus when HP1a is artificially tethered to the DNA, but rather promotes the recruitment of HP1a to the chromatin. To study the function of sov in the regulation of the dynamic properties of heterochromatin, we measured the immobile fraction of HP1a at the heterochromatin foci by fluorescent recovery after photobleaching (FRAP). Silencing of sov resulted in an increase of the mobile fraction in sov silenced germ cell nuclei. Increase of HP1a mobility indicates that sov stabilizes the heterochromatin domain by the enhancement of HP1a association with the chromatin polymer.

Sov promotes transcription in the heterochromatic genome regions

To examine the global effect of sov transcription regulation, we performed transcriptome analysis of sov mutant ovaries. For this, we compared mRNA-seq data of nosGal4>sovRNAi and nosGal4>wRNAi control ovaries. Of the 6,811 euchromatic genes expressed in the ovary, transcription was activated by more than two-fold at 161 genes (2.4%), whereas 146 genes had a more than two-fold decrease in mRNA levels (2.1%). Of note is that of the 203 expressed genes mapping to the heterochromatic regions of the genome, 26 were downregulated more than two-fold (15.1%), whereas no heterochromatic gene was upregulated. Over-representation of the heterochromatic genes in the downregulated gene set indicates that Sov preferentially promotes transcription in the heterochromatic genome regions.

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