Formation of the embryonic gonad.

PGC migration

In *Drosophila melanogaster*, the embryonic gonad is made up of two cell types, the primordial germ cells (PGCs) and the somatic gonadal precursor cells (SGPs). As the PGCs and SGPs are specified at distant sites, gonad formation requires directed migration, recognition and sustained association between these two cell types. Specification of the SGPs, which are mesodermal in origin occurs during mid-embryogenesis, and depends on the action of zygotic patterning determinants. In contrast, PGCs contrast, are specified earlier at the blastoderm stage, on the external surface of the embryo, and are formed under the control of maternal determinants located at the posterior pole of the egg during oogenesis and the BMP signaling pathway in the zygote. The PGCs must find their way from their site of formation on the outside surface of the embryo at the posterior pole to the mesodermally derived somatic gonadal precursor cells (SGPs) to form the primitive embryonic gonad. The migratory journey is initiated during gastrulation when the PGCs are brought into the interior of the embryo by the midgut invagination. They must then pass through the midgut and make their way through the mesoderm to reach the SGPs, which are located in parasegments (PS) 10-13. After the PGCs and SGP come into contact, they first align with each other across several parasegments and them coalesce into the embryonic gonad so that the PGCs are on the inside and the SGPs are on the outside. they line up with each other along the mesoderm

This migratory journey is orchestrated by a novel 'non-canonical' *hedgehog* (*hh*) signaling pathway which inveigles the PGCs to migrate towards the SGPs (Fig.1:ectopic *hh* induces mismigration). The attractive cues provided by *hh* are complemented by a repulsive "signal" that helps guide the migrating PGCs at critical junctures in the appropriate directions. The repulsive signal is generated by two lipid phosphate phosphatases, *wunen* and *wunen-2*.

The deployment of a classical morphogen as a guidance molecule poses many interesting questions. One of these is the mechanism of signal transduction. Like other signaling pathways, *hh* is known to signal over long distances. Moreover, at this stage of development there are many sources of Hh not only in the ectoderm, but also in the mesoderm parasegments anterior to PS10. What distinguishes the Hh ligand directing PGC migration from Hh ligands that are responsible for non-autonomous fate specification? One mechanism is the potentiation of *hh* signals from the SGPs mediated by *hmgcr*. *hmgcr* is required for the transmission of the Hh lingand (Fig. 2). In the classical view the SGPs are thought to secrete the guidance molecules into the extracellular space generating a diffusion gradient which inveigles the PGCs to migrate towards the source. However, long distance signaling by the Hh pathway is known to be mediated by special cytoplasmic extensions called cytonemes. Cytonemes containing the Hh ligand extend from *hh* expressing cells and are met by cytonemes containing the Patched receptor that extend from *hh* receiving cells. Is this the mechanism that is used to guide the migrating PGCs? In the canonical *hh* signaling pathway, reception of the Hh signal by the receiving cell induces a signaling cascade that results in transcriptional response. However, directed cell migration requires modulating the function of the cytoskeleton, not transcriptional activation. What the nature of the non- canonical pathway that remodels the cytoskeleton so that PGCs can move towards the SGPs (Fig. 3)?

<u>Selected Publications:</u>

1) hedgehog signaling in germ cell migration. Deshpande G, Swanhart L, Chiang P, Schedl P.Cell. 2001 Sep 21;106(6):759-69. doi: 10.1016/s0092-8674(01)00488-3.PMID: 11572781

2) HMGCoA reductase potentiates hedgehog signaling in Drosophila melanogaster. Deshpande G, Schedl P.Dev Cell. 2005 Nov;9(5):629-38. doi: 10.1016/j.devcel.2005.09.014. PMID: 16256738

3) The hedgehog pathway gene shifted functions together with the hmgcr-dependent isoprenoid biosynthetic pathway to orchestrate germ cell migration. Deshpande G, Zhou K, Wan JY, Friedrich J, Jourjine N, Smith D, Schedl P.PLoS Genet. 2013;9(9):e1003720. doi: 10.1371/journal.pgen.1003720. Epub 2013 Sep 12.PMID: 24068944

4) Role of the ABC transporter Mdr49 in Hedgehog signaling and germ cell migration. Deshpande G, Manry D, Jourjine N, Mogila V, Mozes H, Bialistoky T, Gerlitz O, Schedl P.Development. 2016 Jun 15;143(12):2111-20. doi: 10.1242/dev.133587. Epub 2016 Apr 27.PMID: 27122170

Documents from 2001/2 "Controversy"

1 "Commentary" Manuscript
https://scholar.princeton.edu/sites/default/files/schedllab/files/c_commentary_manuscript_l_et_al.pdf

2 Report to *Cell* by Dr. Dinardo
https://scholar.princeton.edu/sites/default/files/schedllab/files/a_dinardo_report_to_cell_red.pdf

3 Report to *Cell* by Deshpande et al.
https://scholar.princeton.edu/sites/default/files/schedllab/files/b_deshpande_et_al_report_to_cell_red.pdf

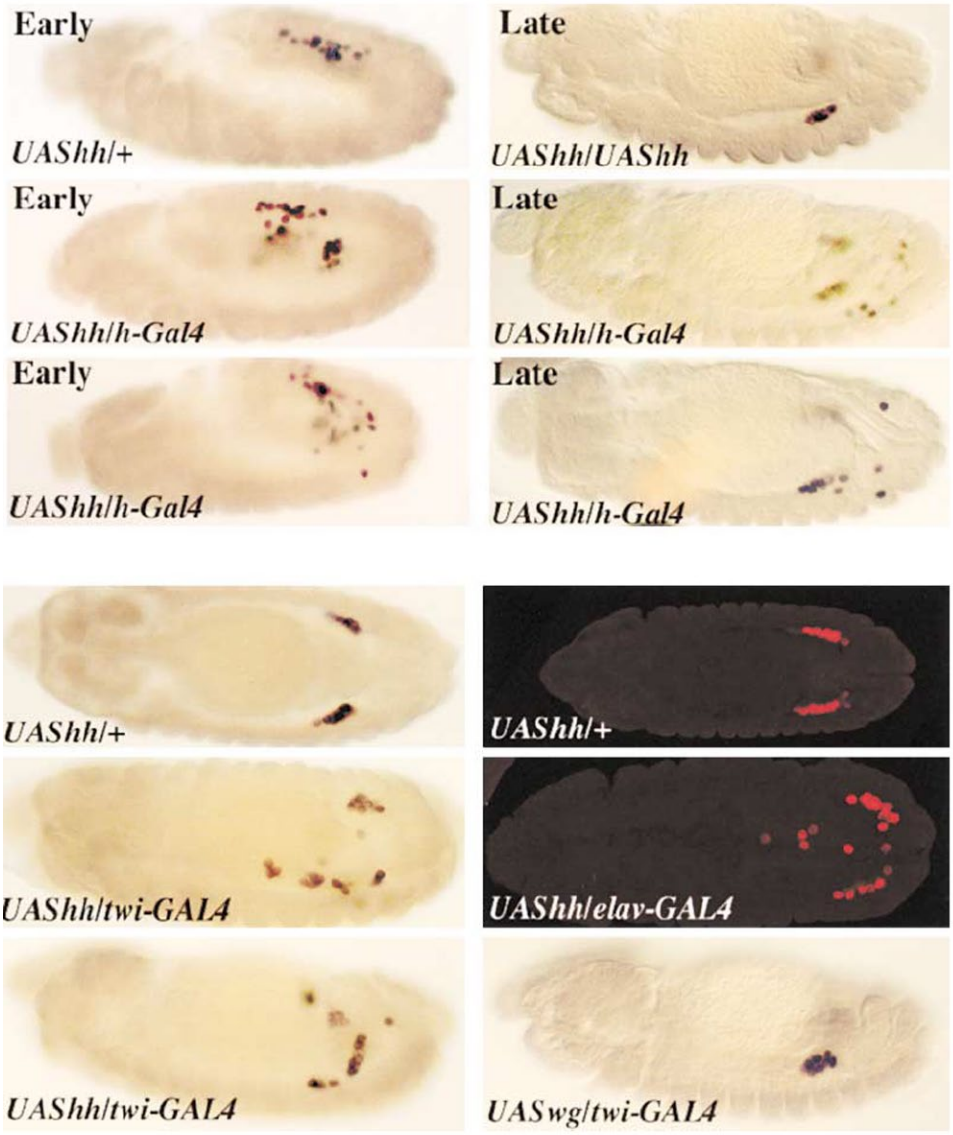

Fig. 1 Ectopic expression of Hh disrupts PGC migration

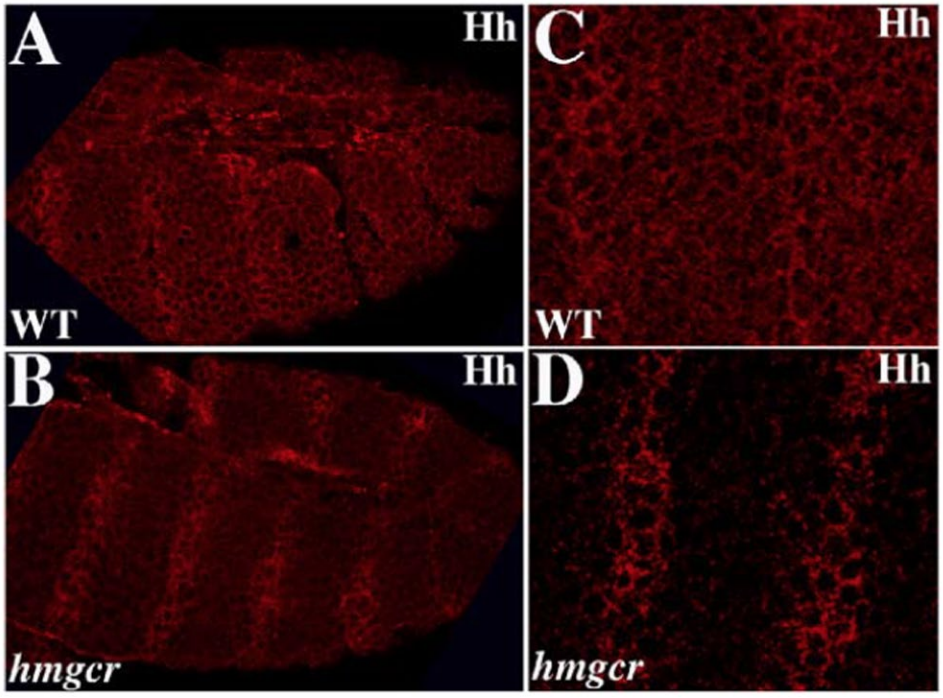

Fig. 2 *hmgcr* is required for releasing from *hh* expressing cells.

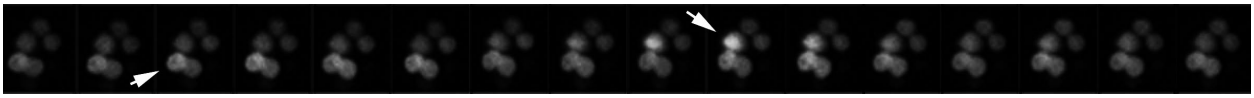

Fig. 3 Ca^{2+} influxes in migrating PGCs. Frame every 6 sec.

PGC fate specification

In flies, PGCs are formed after the onset of the minor wave of ZGA, which begins at nuclear cycle (NC) 8. Like many other organisms, transcription is downregulated when the PGCs are specified. Ongoing transcription is turned off by *germ-cell less* (*gcl*) while genes activated during the major wave of ZGA (NC14) are kept off by combined action of *polar granule component* (*pgc*) and *nanos* (*nos*) (Fig. 4). Broad downregulation of transcription is reflected in the phosphorylation status of the RNA polymerase II CTD domain and the absence of H3meK4, a modification associated with active transcription. Another feature common to worms, flies and mammals, PGCs arrest cell cycle in G2. Moreover, genes implicated in PGC specification in model invertebrates (flies or worms), like *nos*, *vasa,* and *piwi,* are also conserved in higher animals and appear to have a similar germline function. While many of the characteristics of PGCs that distinguish them from the soma are widely shared amongst different animal species, there is one striking dichotomy, namely whether the mechanism driving specification is “epigenesis” or “preformation.” In epigenesis, specification is non-autonomous and depends upon cell-cell signaling. In preformation, specification is autonomous and is driven by determinants that are localized in the presumptive PGCs. Mammals utilize epigenesis. In pre-implantation embryos, a combination of inductive Wingless (Wg) and Bone morphogenetic protein (BMP) signals from the extra embryonic ectoderm and visceral endoderm acts to induce

cells within the posterior epiblast to become PGCs. By contrast, PGC specification in flies is the classic example of an exclusively preformation. Cell-autonomous factors localized to the posterior pole of the egg by an *oskar* dependent mechanism during the late stages of oogenesis are thought to be both necessary and sufficient for PGC specification in early embryos. During pole bud formation, the centrosomes/microtubule network associated with each incoming nucleus triggers the release of the localized PGC determinants from the posterior cortical cytoskeleton, and these factors are then incorporated into the newly formed PGCs during cellularization (Fig. 5). When these factors are not properly sequestered in the newly formed PGCs, PGC specification fails. While the importance of maternally localized determinants has known for decades, recent studies have shown that PGC specification in flies is not exclusively preformation as has long been thought. Instead, BMP signaling from the soma functions in conjunction with the *oskar* localized maternal determinants to specify PGC fate (Fig 6).

Selected Publications

1) Novel functions of nanos in downregulating mitosis and transcription during the development of the Drosophila germline. Deshpande G, Calhoun G, Yanowitz JL, Schedl PD.Cell. 1999 Oct 29;99(3):271-81. doi: 10.1016/s0092-8674(00)81658-x.PMID: 10555143

2) Overlapping mechanisms function to establish transcriptional quiescence in the embryonic Drosophila germline. Deshpande G, Calhoun G, Schedl P.Development. 2004 Mar;131(6):1247-57. doi: 10.1242/dev.01004. Epub 2004 Feb 11.PMID: 14960492

3) Germ Cell-less Promotes Centrosome Segregation to Induce Germ Cell Formation.

Lerit DA, Shebelut CW, Lawlor KJ, Rusan NM, Gavis ER, Schedl P, Deshpande G.Cell Rep. 2017 Jan 24;18(4):831-839. doi: 10.1016/j.celrep.2016.12.074.PMID: 28122234

4) Antagonism between *germ cell-less* and Torso receptor regulates transcriptional quiescence underlying germline/soma distinction. Colonnetta MM, Lym LR, Wilkins L, Kappes G, Castro EA, Ryder PV, Schedl P, Lerit DA, Deshpande G.Elife. 2021 Jan 18;10:e54346. doi: 10.7554/eLife.54346.PMID: 33459591

5) Preformation and epigenesis converge to specify primordial germ cell fate in the early Drosophila embryo. Colonnetta MM, Goyal Y, Johnson HE, Syal S, Schedl P, Deshpande G.PLoS Genet. 2022 Jan 5;18(1):e1010002. doi: 10.1371/journal.pgen.1010002. eCollection 2022 Jan.PMID: 34986144

Fig. 4 Induction of the female specific *Sex-lethal* gene in *nos* mutant male PGCs

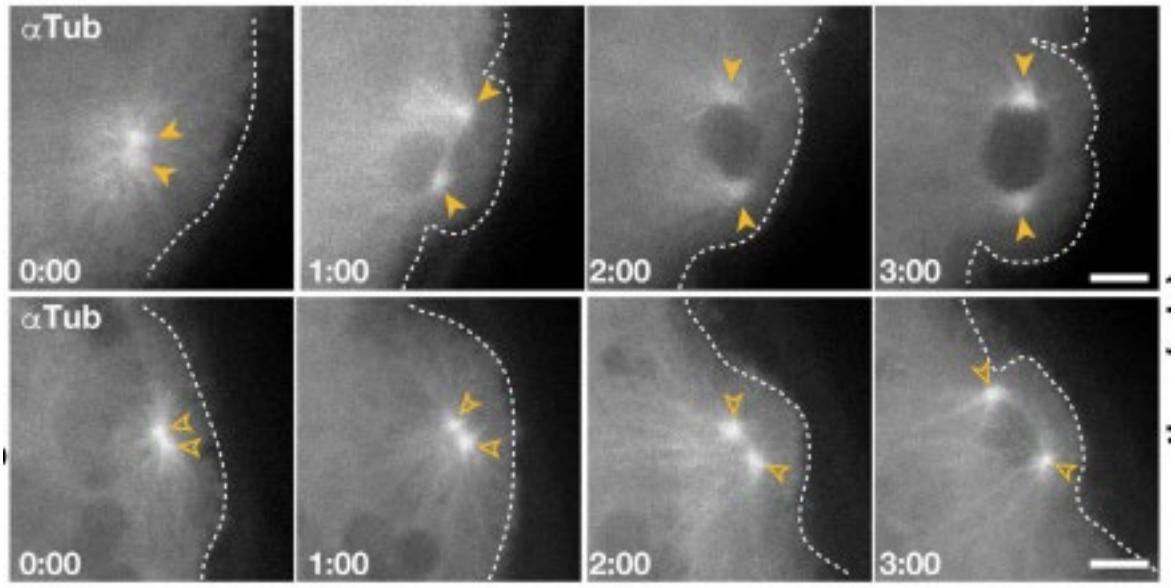

Fig. 5. Centrosome defects in *gcl* embryos during PGC cellularization (pole bud stage).

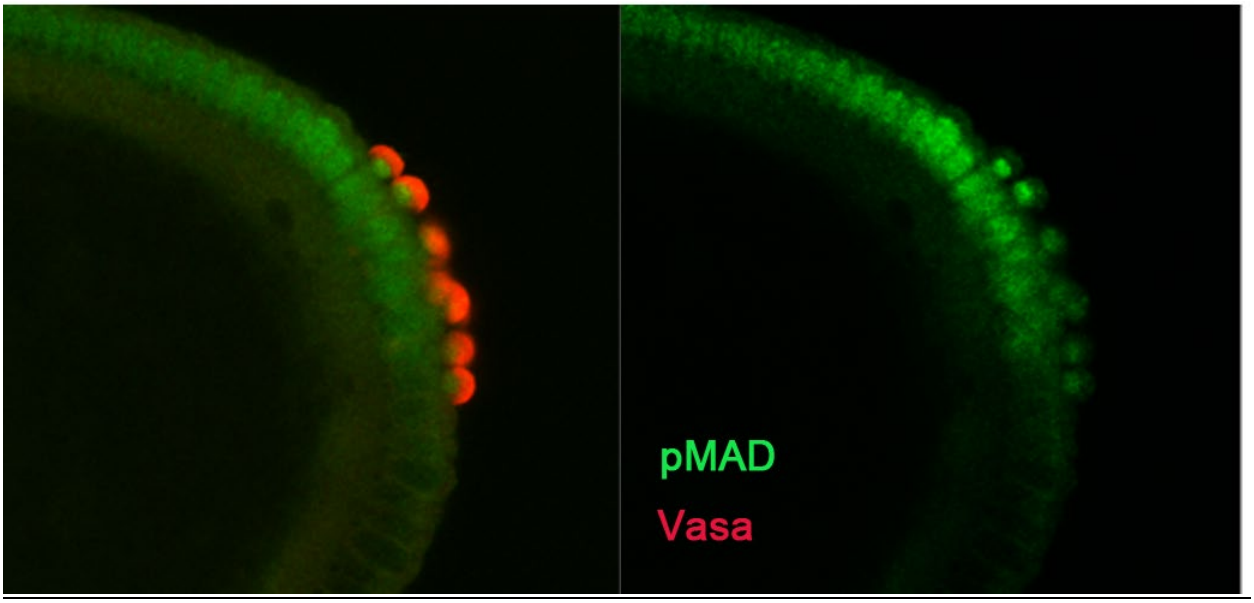

Fig 6 Nuclear localization of the BMP transcription factor pMAD in newly formed PGC nuclei induced by somatic BMP signals.