

The expression of Pipe in *Blattella germanica* ovaries is essential for embryo development

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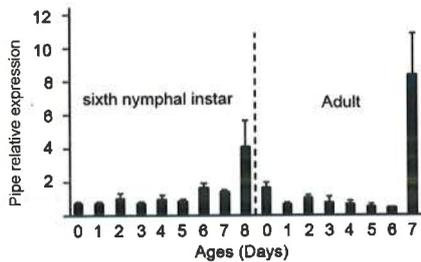
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In the holometabolous species *Drosophila melanogaster*, which has meristic ovaries, Pipe sulfotransferase is one of the last proteins involved in D/V polarity of the egg, which is expressed only in the ventral follicular cells thus activating a series of proteases acting in the perivitelline space between the embryonic membrane and the eggshell (Nilson and Schupbach, 1998; Sen et al. 1998; Cho et al., 2010). Pipe encodes ten different isoforms (Sen et al., 1998) and only one among them has been involved in embryonic DV axis polarity (Zhang et al., 2009). Therefore, Pipe provides a link between the ovarian and embryonic DV polarity, as Pipe mutant females produced dorsalized embryos (Sen et al., 1998).

Here, we report the function of Pipe in a hemimetabolous insect, the cockroach *Blattella germanica*, which has panoistic ovaries.

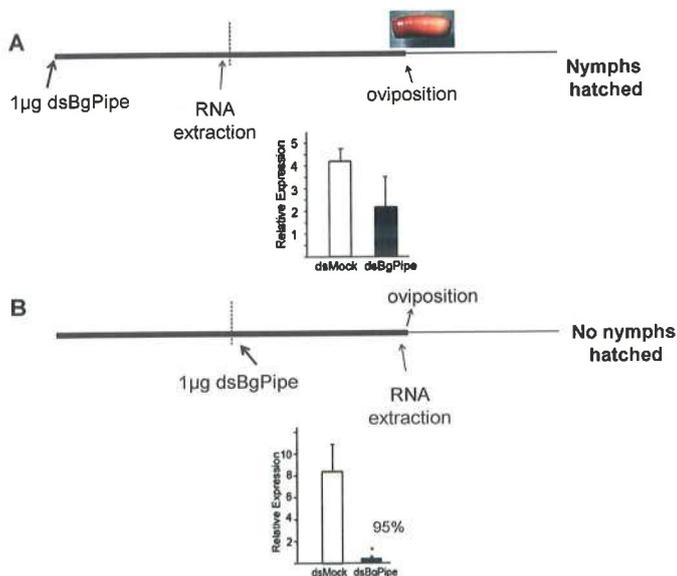
Pipe in *Blattella germanica* ovary

Expression of Pipe in panoistic ovaries of *B. germanica* shows a slight increase just before the moult to adult. A second, very prominent peak of expression, appears in ovaries from 7-day-old adults, just before chorion synthesis.



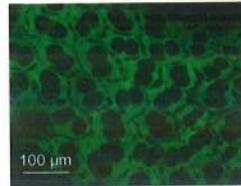
How important are these two peaks of expression?

(A) The strategy followed was injecting dsBgPipe in 0-day old sixth instar nymphs, and measuring the expression in ovaries from 8-day-old sixth instar nymphs. (B) The strategy followed was injecting dsBgPipe in 0-day old adult females, and measuring the expression in ovaries from 7-day-old adults.



Pipe is expressed in follicular cells

BgPipe mRNA appears localized in the cytoplasm of follicular cells in the basal ovarian follicles from 7-day-old females



■ DAPI ■ BgPipe mRNA

Depletion of BgPipe does not affect chorion formation



A: dsMock-treated females. B-C: dsBgPipe injected in 6-day old adult females. Scale bar: 5 µm. CL: columnar layer. ex: exochorion. FC: follicular cells. ie: inner-endo chorion. oe: outer-endo chorion. TP: tunica propria.

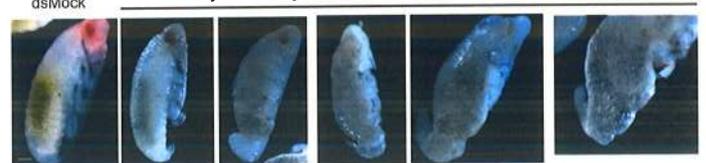
Effects of BgPipe depletion on embryo development

3-day-old embryos from dsBgPipe-treated females



3-day-old embryo. Depletion of BgPipe determines a deficient segmentation of the germ band, with the head and the abdomen not well differentiated or poorly developed. The embryo ventral region is in the upper part. Staining: DAPI.

14-day-old embryo from dsBgPipe-treated females



14-day-old embryo. In embryos from dsMock-treated females the tips of antennae and the hind legs almost reach the fifth abdominal segment. The eyes are well formed, increasing the pigmentation. Embryos from dsBgPipe-treated females that reached to develop (65%) show a great degree of affection. From individuals that look like controls, to those that have short antenna and legs, the eyes not well formed and/or poorly pigmented, an the abdomen incompletely segmented, with a most common that is the caudal region curved towards the dorsal part of the embryo. Scale bar: 200 µm.

The results obtained suggest that Pipe is a maternal gene, accumulated in the ovary in early adult stages and essential for the establishment of the axes in the embryo, and its depletion results lethal.

Acknowledgment

Support for this research was provided by the Spanish Ministry of Science and Innovation (Grant. BFU2011-22404 to MDP). NE received a pre-doctoral research grant (JAE-Pre) from the CSIC, and The European Social Fund (ESF).