Functional Roles of the E3 Ubiquitin Ligase HYD in *Drosophila* Tissues

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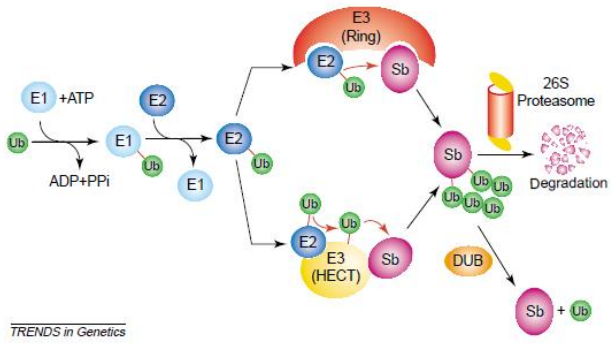
ABSTRACT

Drosophila tumor suppressor HYD is required for the regulation of cell proliferation, growth and differentiation. HYD is involved in cell processes at different levels: it can regulate gene expression by binding to its promoters, it binds to some RNA for gene silencing and it participates in protein degradation due to its ubiquitin-ligase activity. Analysis of *Drosophila hyd* mutants revealed that HYD functions depend on tissue and stage of development: misexpression in larval somatic tissues causes over-proliferation, defects of meiosis and spermatid differentiation in spermatogenesis, and massive cell death with occasional germline overproliferation during oogenesis.

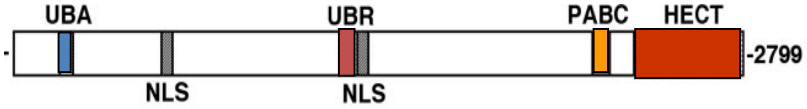
UBIQUITIN-PROTEASOME SYSTEM



The Ubiquitin-Proteasome System (UPS) is an important regulator of cell signaling and proteostasis, which are essential to a variety of cellular processes. E3 ubiquitin ligases play key role in UPS functioning. Ubiquitin ligases (E3 enzymes) transfer ubiquitin from ubiquitin-conjugating (E2) enzymes to target proteins. By determing the selection of target proteins, modification sites on those target proteins, and the types of ubiquitin modifications that are formed, E3 enzymes are key specificity factors in ubiquitin signaling.

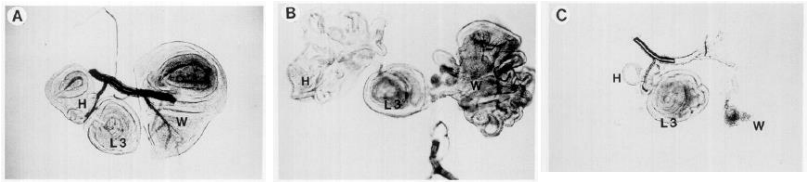
One of the most interesting E3 ligases is drosophila tumor suppressor HYD (hyperplastic disc).

HYD STRUCTURE



- **UBA** - Ubiquitin-associated domain
- **NLS** - Nuclear localization signal
- **UBR -** Ubiquitin recognition **-** zinc finger-like domain
- **PABC** - Poly(A)-binding protein C-terminal domain)
- **HECT -** Homologous to the E6-AP carboxyl terminus – E3 ligase catalytic domain

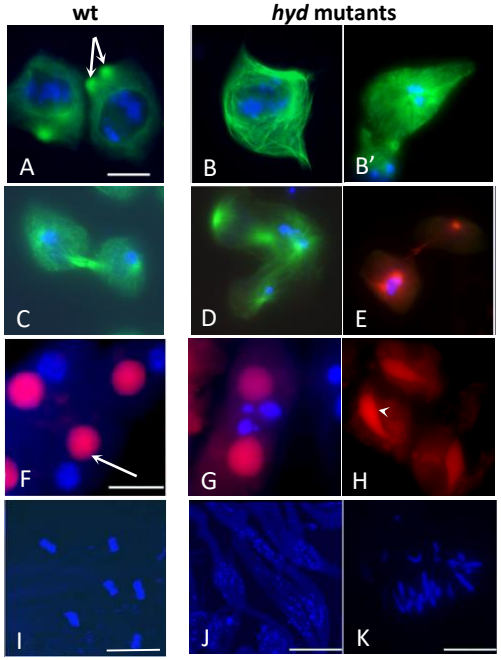
ROLE OF HYD IN SOMATIC TISSUES



It was originally identified by a temperature-sensitive mutation that causes imaginal disc overgrowth in mutant larvae raised at a restrictive temperature. Within a single larvae discs may be overgrown or small/absent [1].
(A) Wild –type wing (W), haltere (H), leg discs (L3).
(B) Overgrown wing and haltere discs from a *hyd* mutant.
(C) Small/absent wing disc. Very small haltere disc.
Photos taken at the same scale.

In eye imaginal disc HYD function differs from what is expected for conventional tumor suppressor behaviour [2]. In eye disc homozygous *hyd* mutant clones induce non-autonomous overproliferation of nearby tissue [2].

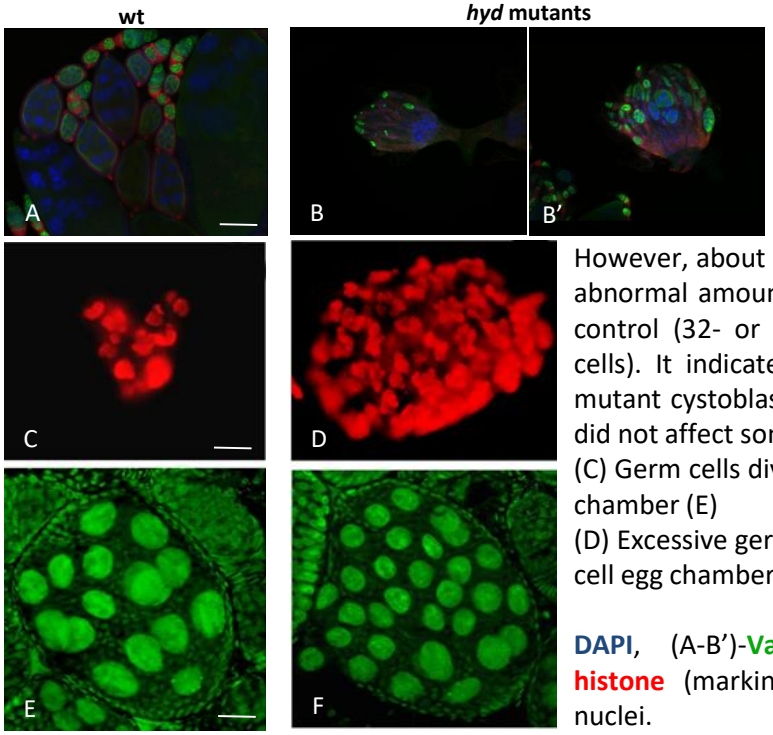
ROLE OF HYD IN STERMATOGENESIS



(A) In normal meiosis, a bipolar spindle structure with asters is formed, microtubules are anchored on spindle poles (arrows) and distribute around the nucleus.
(B-B') Mutant cells without poles and asters, microtubules are disintegrated and are misoriented in the cytoplasm.
(C) Normal cytokinesis
(D) Abnormal spindle structure in anaphase II. Two spindles in common cytoplasm and one of them lose chromosomes.
(E) Cytokinesis in *hyd* mutant cell with normal bipolar structure: chromosomes are contained in only one daughter cell, other daughter cells are separated with the centrosome.
(F) Normal onion stage. Nebenkern is marked by arrow.
(G) Abnormal chromosome condensation, two nebenkerns in one cell.
(H) Elongating nebenkern (arrowhead) in cells lacking chromosomes.
(I) Elongated cyst.
(J-K)Nuclei are scattered through the elongated cysts and have around instead of needle shape.

(A-K) –**DAPI,** (A-D) - **α-tubulin,** E - **α-tubulin,** (F-H) – **MitoTracker Red**
For (A-E, K) the scale bar represents 5 μm, for (F-H)-10 μm, for (I)-30 μm, for (J)- 20 μm.

ROLE OF HYD IN OOGENESIS



Main effect of *hyd* mutations in oogenesis was massive cell death:
(A) Normal ovary
(B) Mutant ovaries containing reduced number of egg chambers

However, about 5% of ovaries contained egg chambers with abnormal amount of germ cells – 2-4 times higher than in control (32- or 64-cell cysts in comparing to normal 16-cells). It indicates 1 or 2 extra rounds of cell division of mutant cystoblasts. It should be noted that *hyd* mutations did not affect somatic follicular cells:
(C) Germ cells divisions in wt germarium forming 16-cell egg chamber (E)
(D) Excessive germ cells divisions leading to formation of 32-cell egg chamber (F)

DAPI, (A-B')-**Vasa** (marking germline cells), **phospho-H3 histone** (marking dividing cells), (E,F)- **GFP** marker for nuclei.
(A-B')-Scale bar represents 40 μm, (C-D)- 20 μm.

CONCLUSION

Thus, in different tissues mutations of *hyd* lead to various consequences: overproliferation in somatic tissues, defects of meiosis and spermatid differentiation in drosophila spermatogenesis, and massive cell death with occasional overproliferation during oogenesis. So, HYD functions may differ depending on cell context. It was shown that HYD can regulate gene expression at transcriptional level [3] and translational level, owing to its unique feature – presence of PABC domain, commonly found in pABP proteins, whose function is related to mRNA translation. Also it is known that mammalian EDD plays a critical role in miRNA silencing [4] and participates in DNA damage response [5]. Interesting, that E3 ubiquitin ligase activity is dispensable for EDD function in miRNA silencing. The PABC domain of EDD is essential for its silencing function.
It is clear that HYD possess many apparently divergent roles in multiple pathways in different tissues.

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