Role of maternal mRNA degradation in whole-body cellular phenotypes caused by DNA polymerase α deficiency

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Introduction

The potential roles of essential genes in organismal biology and disease in multicellular organisms can be difficult to assess due to immediate embryonic lethality of null mutations. For example, mutations in subunits of DNA polymerase α (Pol α), one of the primary eukaryotic DNA polymerases, result in immediate cell cycle arrest in yeast and *Arabidopsis*. We have found that the presence of wild-type maternal mRNA can sustain the viability of a null mutation in the B subunit of Pol α, resulting in the ability to detect a series of pleiotropic cellular phenotypes (Fig. 1), including nuclear atypia in gastrointestinal cells, apoptotic nuclear fragmentation in the neurons of the brain and eyes, as well as DNA damage and cell death were found in neuronal cells of the brain, eyes, and spinal cord. The causative mutation of the phenotype was a frameshift resulting in a premature stop codon in *pola2*, which encodes the B subunit of Pol α. Loss of *pola2* caused the accumulation of cells in S-phase and reduced DNA synthesis in *hht* larvae. The extended 120-168 hpf survival of the *hht* fish stands in striking contrast with the lethality of the corresponding mutants in yeast and *Arabidopsis.* The gradual disappearance of wild-type maternal *pola2* in homozygous mutant embryos provided an opportunity to study the effects of diminishing DNA synthesis on DNA damage, cell death, and tissue-dependent cytological deformities.

Results

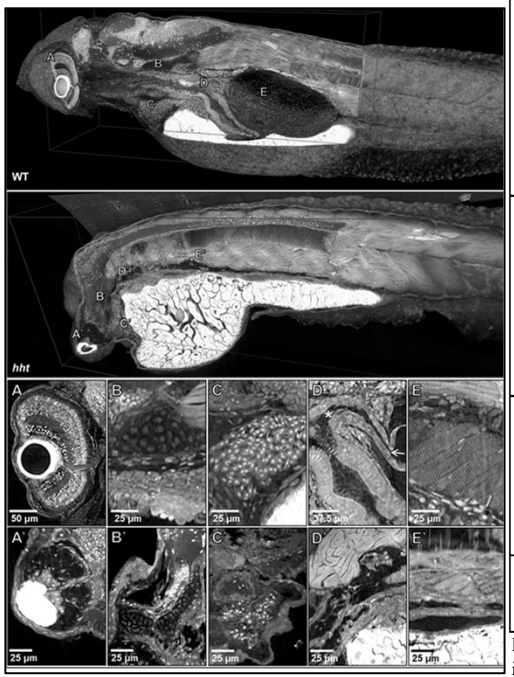

Figure 1. Characterization of 3D pathological features in wild-type larval and *huli hutu* mutant specimens.
Top: Cutout visualization of both wild-type and *huli hutu* larval (five dpf) zebrafish stained with PTA showing detail in many soft tissue structures. *Bottom*: Cell types and structures that can be visualized include neuronal cells in the eye (**A**), cartilaginous rudiments of the squamous patch on the dorsal (arrow) pharynx (**B**), nucleated red blood cells (**C**), intact pneumatic duct (* to arrow) and goblet cells in the gut (**D**), and cross-striations of bands of muscles encircling the swim bladder (**E**).

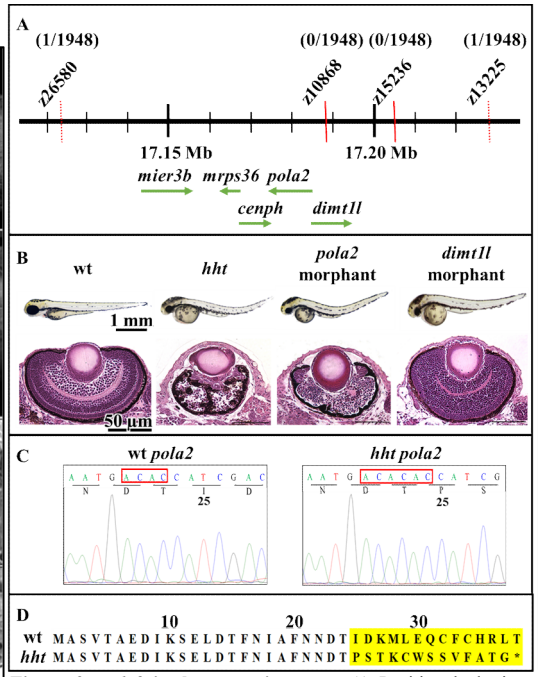

Figure 2. *pola2* is the causative gene. A) Positional cloning identified five candidate genes, *mier3b*, *mrps36*, *cenph*, *pola2*, and *dimtl1* (GRCz11). **B)** Gross and histological examination revealed striking similarity between *hht* and *pola2* morphant, **C)** 2-nucleotitde AC insertion detected in *hht* caused **D)** a frameshift mutation resulting in a premature stop codon

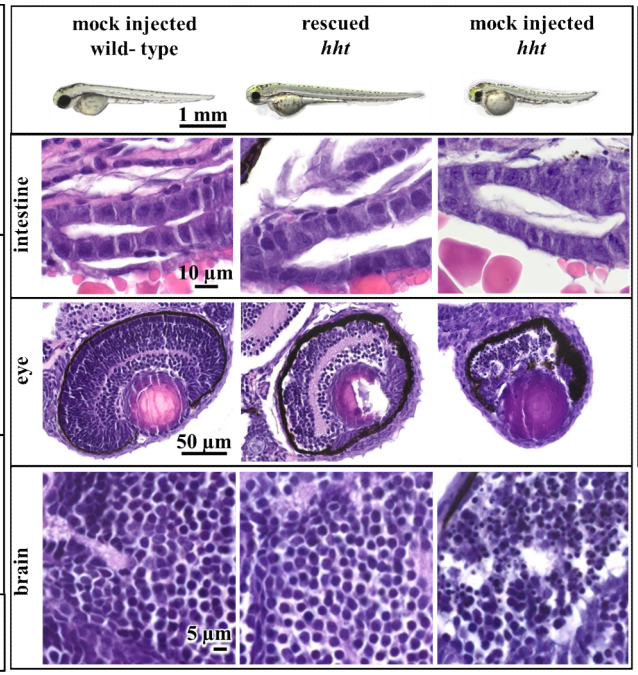

Figure 3. Injection of wild-type mRNA partially rescues *hht*. Rescued *hht* mutants exhibit a straight body and a normal yolk. Sizes of the eyes and head are intermediate between wild-type and *hht* mutants. Detailed examination by histology showed a normal appearance of cells in the intestine of rescued fish. The eyes are organized into layers, much like that of wild-type eyes. Both the eyes and brain show no evidence of nuclear fragments.

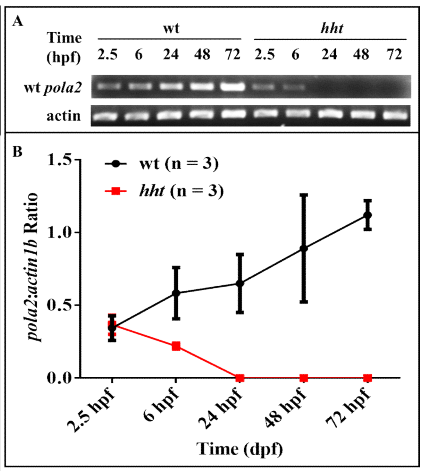

Figure 4. Wild-type *pola2* mRNA is detected in *hht* embryos. A) Wild-type *pola2* transcripts were detected in *hht* by allele-specific primers at 2.5 and 6 hpf. Wild-type transcripts were not detectable in *hht* after 24 hpf. **B)** Wild-type *pola2* transcripts were normalized to *actin1b*. Wild-type *pola2* transcripts in *hht* embryos were present at comparable levels to wild-type embryos at 2.5 hpf but were significantly reduced compared to wild-type embryos at 6 hpf ($p < 0.05$)

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Conclusion & Discussion

- The cell-type specific phenotype of nuclear fragmentation and atypia is caused by a null mutation in *pola2.*
- Presence of wild-type maternal *pola2* mRNA supports the survival of *hht* larvae up to 7 dpf.
- The tissue-dependent cellular phenotypes caused by *pola2* deficiency in *hht* mutants may be attributed to differences in cell proliferation or DNA repair in various cell types