HP1 Gene Family Proteins and Their Impact on Meiotic Recombination

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Abstract

Genome architecture, governed by a highly diverse yet exquisitely arranged set of proteins, dictates a wide variety of cellular function. Among the starkest modifications of the genome architecture is the tightly-wound heterochromatin (HC) regions, which are maintained by a distinct and well-researched collection of proteins that dictate the region's physical structure. Of these, the HP1a gene has been widely researched and is known for its profound impact on preserving regions of HC (i.e. centromeres) that forego meiotic recombination. Nevertheless, the HP1 gene family consists of at least 3 highly conserved genes – HP1a, HP1b, and HP1c – that have varying degrees of functional understanding in the cell. Noting their common lineage, we are looking to further clarify what role, if any, each of the primary members of the HP1 gene family have on meiotic recombination. Through recessive marker scoring for each of the three HP1 paralogs, we measured the rate of crossing over (CO). Analysis of CO density in HP1a homologs shows stark discrepancies compared to the wildtype recombination rate, albeit no distinct pattern seems apparent. In the HP1b mutants, in spite of low sample count, meiotic crossover rates are the same as wildtype across the length of the chromosome. This corroborates existing evidence for localization to euchromatic regions in early development. Analysis of COs in HP1c mutants indicates a drastic shift in recombination rate, noting a sharp, decreased incidence of CO to distal of *black* and a sharp increase proximally. In summary, mutations in individual HP1 paralogs lead to distinctive CO rates and maintain a lack of CO around the centromere, though the mechanistic background is undefined. Further replication of these trials as well as double and triple mutant crosses would be valuable in to clarifying the role of HP1 homologs in meiotic recombination.

There are multiple HP1 paralogs, but each interacts distinctly with chromatin

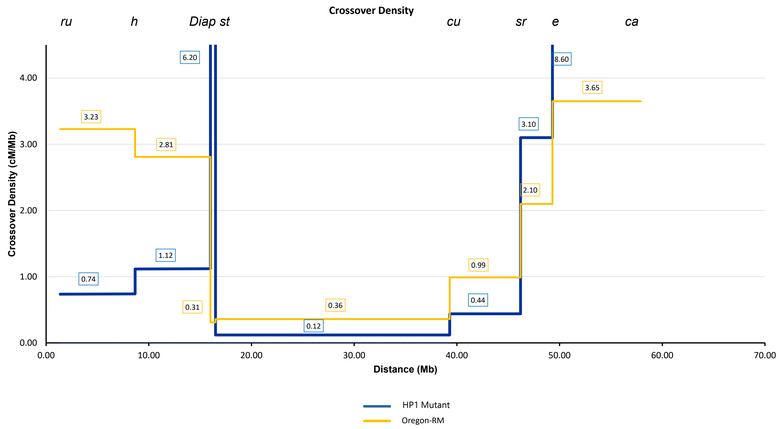
- HP1 homologs are found across all eukaryotes, with 21 novel variants within the *Drosophila* genus, yet HP1a, HP1b, and HP1c are found across all cell types and developmental stages.
- Despite their consistent presence and similar structure, HP1a, HP1b and HP1c maintain different locations and functions in terms of the nucleus.
    - HP1a is highly concentrated with heterochromatin (HC) and canonically associated with *su(var)3-9* at H3K9me2/3.
    - HP1b maintains affinity, albeit decreased relative to HP1a, for HC as well as euchromatin.
    - HP1c associates selectively with euchromatin, particularly regions of high transcriptional activity.
- HC formation is broadly dependent on HP1a, utilizing both the hinge and chromoshadow regions of the protein to form critical histone PTMs important to genome architecture.
- Expansion of HP1a can variably produce transient regions of HC in the pericentromere, which is known to reduce the incidence of CO.
- Noting the similarity in structure and common origin, modifications, namely, reductions in HP1 isoforms could have the potential to modify the structure of the heterochromatin and thus change CO rates.
- This relationship between HP1a,b,c and CO events has yet to be analyzed.

Experimental set-up for analyzing the impact on HP1a,b,c mutants on crossing over

- Viable stocks of *Drosophila melanogaster* with mutations for each of HP1a,b,c were acquired from the Bloomington Stock Center, IN, USA.
- In order to track meiotic recombination, crossover frequency was scored with the following recessive marker stocks:
    - *ru, h, Diap, st, cu, sr, e, ca* on *chr3*
    - *net, ho, dp, b, pr, cn* on *chr2*
- Individual flies were scored per the above phenotypes for each testcross, yielding:
    - HP1a: 491 flies with 189 COs
    - HP1b: 73 flies with 87 COs
    - HP1c: 707 flies with 308 COs

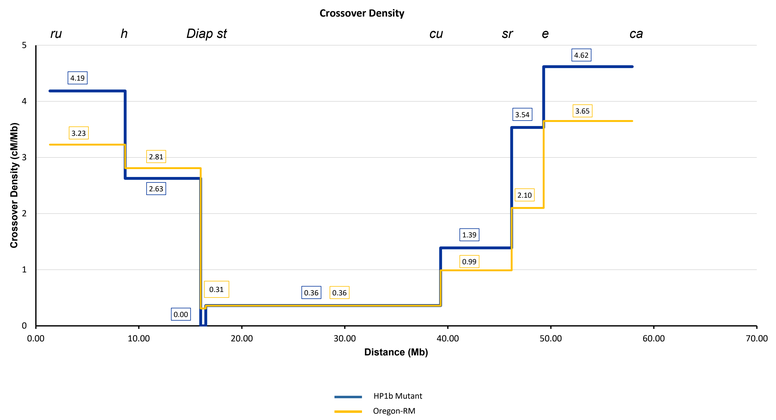
note: the arrival of COVID-19 hindered data collection levels for the HP1b testcross

CO density and distribution in HP1a mutants shows deviation from wildtype rate



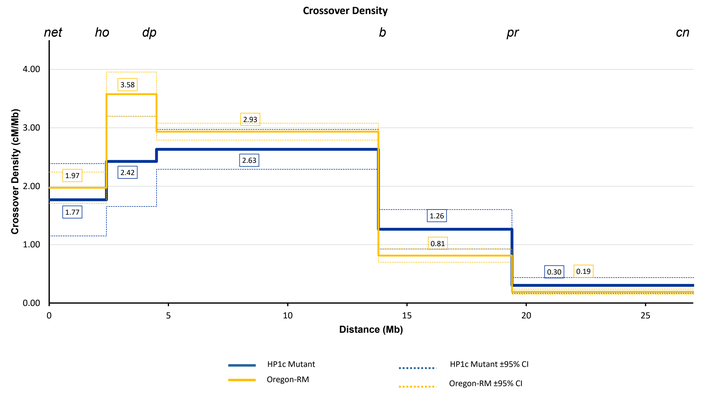
- CO rates at the distal end of *chr3L* are significantly lower than the wildtype, but significantly higher on the distal end of *chr3R.* This difference is difficult to explain, but it is possible that the requirement for *Hp1a* is different on different chromosome arms.
- CO rates on the *st-cu* interval between 16.5Mb and 39.9Mb indicate the persistence of reduced recombination rates in centromeric and pericentromeric regions.
- The sharp increase in recombination on the Diap-st interval between 16.0Mb and 16.5Mb contrasts decreased CO rates in surrounding intervals. We cannot rule out that errors were made while scoring the *Diap* or *st* markers.

Wildtype CO densities are conserved in HP1b mutants.



- CO rates are consistently within one SD across the length of chromosome 3.
- These results are preliminary due to the small sample size (n=73); scoring was interrupted by a lab shutdown due to COVID-19.
- Nearly identical CO rates in the centromeric and pericentromeric regions between 16.5Mb and 39.9Mb indicate fidelity of CO suppression in heterochromatic regions.

HP1c mutants show varying changes in CO densities



- Deviation in CO rates on *chr2L* are elevated when peripheral to the *black* marker at 13.8Mb yet decreased with proximal to the centromere from the same distance.
- Near-zero recombination rates on the *pr-cn* interval from 19.4Mb to 28.3Mb reaffirms CO suppression in HC-rich centromeric and pericentromeric regions.

Conclusions

- Distinct variation in CO rates in each of the HP1 homologs further differentiates their distinct roles in cellular function, despite their common origin.
- HP1a knockout trials seem to implicate a non-wildtype CO distribution while maintaining HC CO suppression. Further investigation and larger sample sizes are required to confirm or deny given trends and to parse out discrepancies.
- HP1b knockout trials do not show a demonstrable link to variation in meiotic recombination rates, which corroborates its mechanistic role as a transcriptional moderator.
- HP1c knockout trials have indicated a non-wildtype CO distribution, with a striking inflection point at 13.8Mb on *chr2L*. HP1c localization to selective euchromatin regions corroborates novel activity in the given region, but does not explain the specificity of inflection.

Future Directions

- Renewed trials of HP1a and HP1c with larger sample size would recapitulate current findings and diminish the incidence of manual error in phenotype collection.
- Double and triple mutant experiments, particularly HP1a + HP1b, may reveal redundant function in CO formation.
- Increased sample size, particularly for HP1b trials, would be valuable to confirm or deny existing trends.

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