The role of histone crotonylation in neuronal function
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On a molecular level, histone lysine crotonylation is the addition of a crotonyl group, crotonyl-CoA, to an epsilon-amino group of a lysine residue on the histone tail. Crotonyl-CoA is commonly generated from conversion of the short-chain fatty acid crotonate via ACS52. The histone acetyltransferase will catalyze the addition of crotonyl from crotonyl-CoA to the histone. Adding crotonyl neutralizes the lysine’s positive charge, thus loosening the chromatin. Selective crotonylation readers will then bind to the crotonyl to influence the functional outcome. The histone deacetyltransferase will catalyze the removal of crotonyl from the histone. CDYL will hydrolyze crotonyl-CoA to beta-hydroxybutyryl-CoA.

Motivation for this Project
Fellows et al. published a Western blot indicating that in mice, histone crotonylation is most abundant in the brain and colon compared to other parts of the body. Since crotonyl is a metabolite of gut microbiota, this indicates that histone crotonylation in the brain may be due to the crotonyl travelling from the colon to the brain. Further research is needed to better understand the gut-brain axis and the function and regulation of histone crotonylation in the brain.

Guiding Questions
1. Can crotonate be used to stimulate histone crotonylation?
2. What is the role that histone crotonylation plays in neuronal function?
3. How does histone crotonylation affect the development of N2As?
4. How does manipulating the gut microbiome affect histone crotonylation in the brain?

Why It Matters
- Primary function: Positive regulation of transcription
- Unique structure: Rigid planar structure
- Greater reader specificity
- Distinct functions
- CDYL-mediated Kcr regulates stress-induced depression
- CDYL regulates neuroplasticity, seizure susceptibility, and neuronal migration
- Deficiency in ECH1 causes Leigh-like syndrome
- Kcr may be involved in BTBP-related abnormalities

References

In the original experiment, the mice were just given crotonate solutions that had an acidic pH around 3. In this optimization, we adjusted the pH of half of the crotonate solutions to 6.4, close to the normal pH of the drinking water, to see if acidity was discouraging the mice from drinking. Additionally, we used the mice’s regular drinking water to create solutions of low or high concentration, 1 g/L or 2 g/L respectively, and added Splenda to counteract any salty taste from the crotonate that may have caused the mice to drink less. The control solutions were the regular drinking water and a solution of just Splenda in the water.

We expected to see that the mice given water and Splenda would have less Kcr than the crotonate-treated mice and that the pH-adjusted crotonate-treated mice would have more Kcr than the untreated controls. The blots shows similar Kcr levels for the water and Splenda compared to the crotonate treatment, so the results were inconclusive for this optimization experiment.

We also conducted the crotonate experiment with an N2A culture. N2As were grown in a 12-well plate and treated with either PBS, crotonate solution with HEPES (a buffer to maintain the pH), or crotonate solution without HEPES, or were given no treatment. One plate received 5 mM crotonate and another plate received 10 mM crotonate. Then, after a time period of 1, 2, 4, or 6 hours, the N2As were lysed to extract their chromatin for a Western blot. This is the optimization of the original experiment, where the N2As in all wells were lysed after 3 hours; adding the extra time points allowed us to determine whether time changes the efficacy of the crotonate treatment.

The untreated N2As showed less Kcr than the treated N2As, whether given HEPES or not, which is what we expected to happen. This indicates that adding crotonate to N2As increases histone crotonylation. The 10 mM crotonate N2As generally had higher Kcr than the 5 mM crotonate N2As, indicating that increasing the crotonate treatment concentration also increases the amount of histone lysine crotonylation in the brain. Also, the 6 hr N2As have the same darkness as the 1, 2, and 4 hr N2As, so time doesn’t affect the efficacy of the crotonate treatment.

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