Exploring Role of GleIF4A in Novel Translation-Initiation Mechanism in *Giardia lamblia* Through CRISPRi and Morpholino Mediated Gene Knockdown

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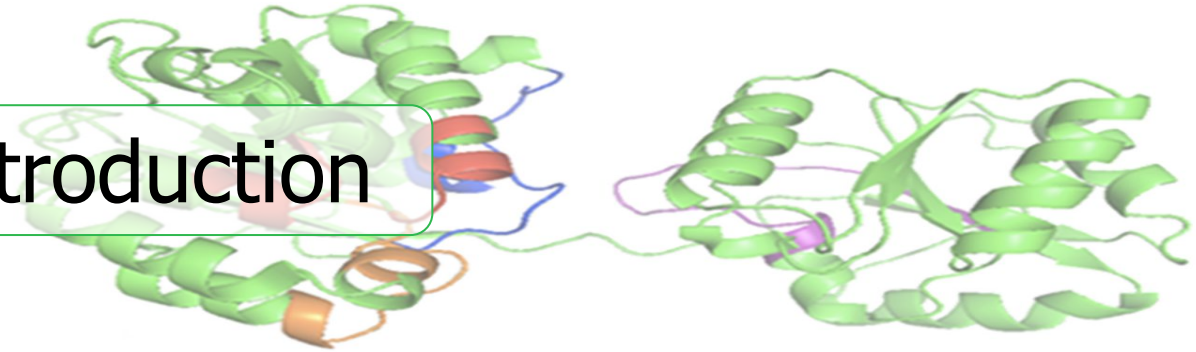
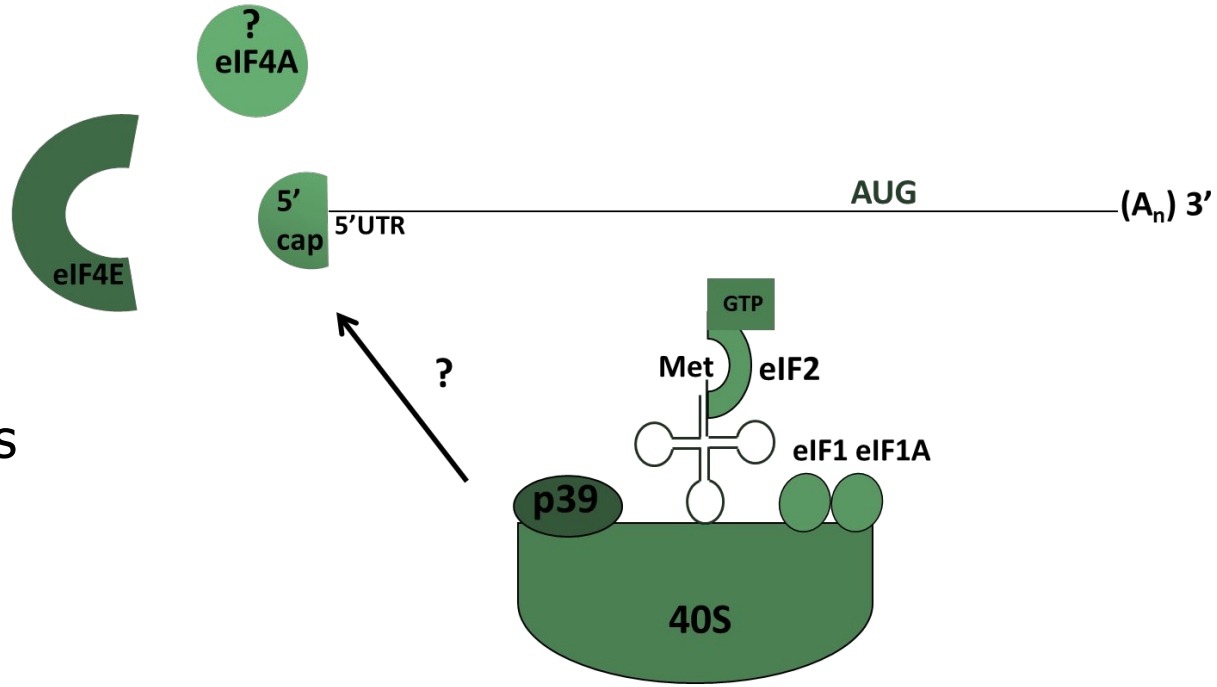
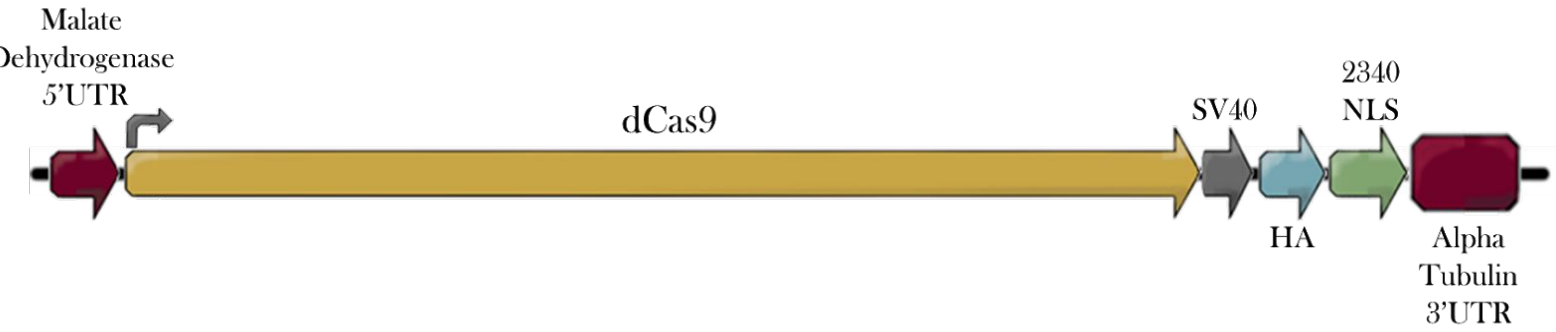
Introduction



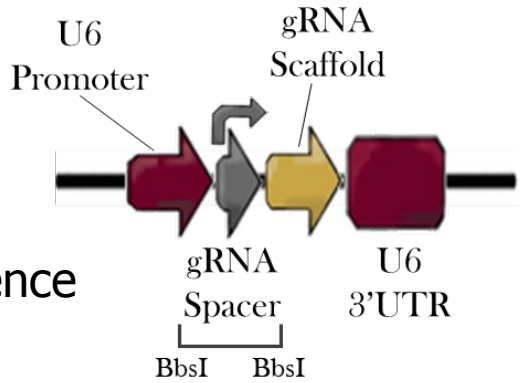
- eIF4A in most eukaryotes functions as helicase to unwind long 5’ UTR
- *Giardia* possesses short, unstructured 5’ UTR that do not appear to impede the PIC
- GleIF4A role is somewhat unknown due to an apparent lack in ribosome scanning in Giardia
- *Giardia* also lack eIF4G which plays the role of a scaffolding protein to recruit eIF4A in eukaryotes
- Previous research with 4A-3i (initiation factors) has shown novel protein-protein interaction
- Through CRISPRi and Morpholino we begin to explore the role of GleIF4A



Methods: CRISPRi



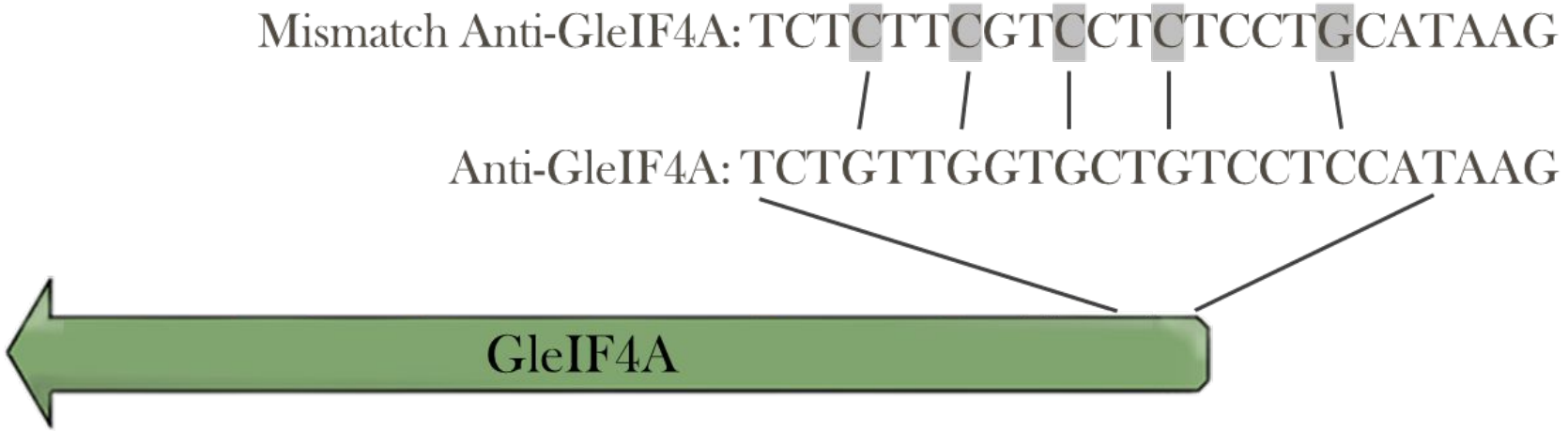
- Nuclear localization signals SV40 and 2340 are used to tag amino acid sequences for importation into the nucleus of Giardia
- dCas9 or deactivatedCas9 will sterically interfere with the translation initiation complex and block translation



- gRNA acts as a guide to bring dCas9 to GleIF4A +270 or +484 nucleotide sequence which blocks translation
- Puromycin selection allows the transfected CRISPR plasmid to permanently remain in the cell

Target	Sequence	On-Target	Off-Target
Non-specific	CAAAGGGTCTTCGAGAAGACCT		
GleIF4A (+270)	CAAAGGGGAGAGAATAATGGCCTG	72.5	99.9
GleIF4A (+484)	CAAAAAGGACCACCATCTTCACAT	71.9	100.0

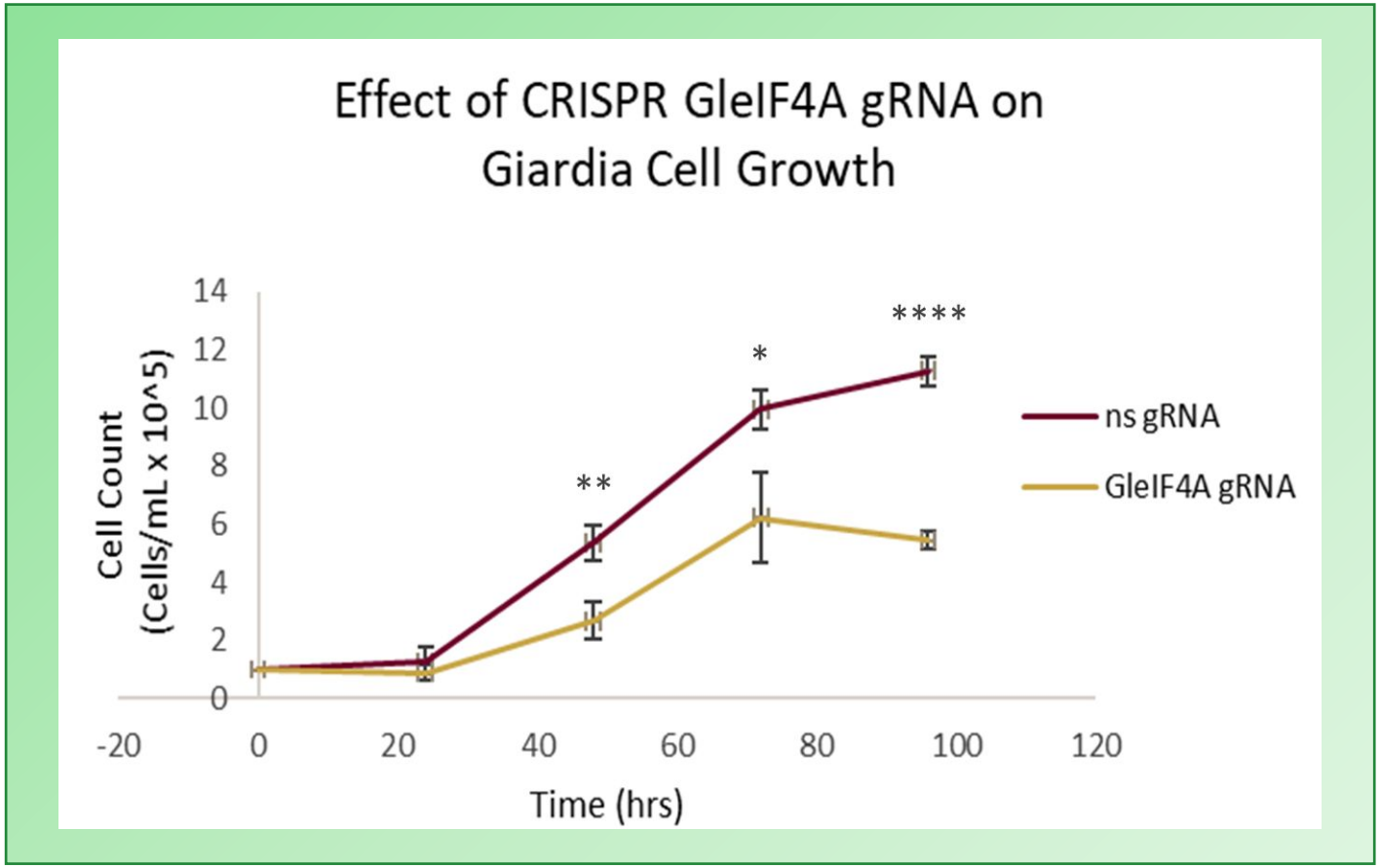
Methods: Morpholino



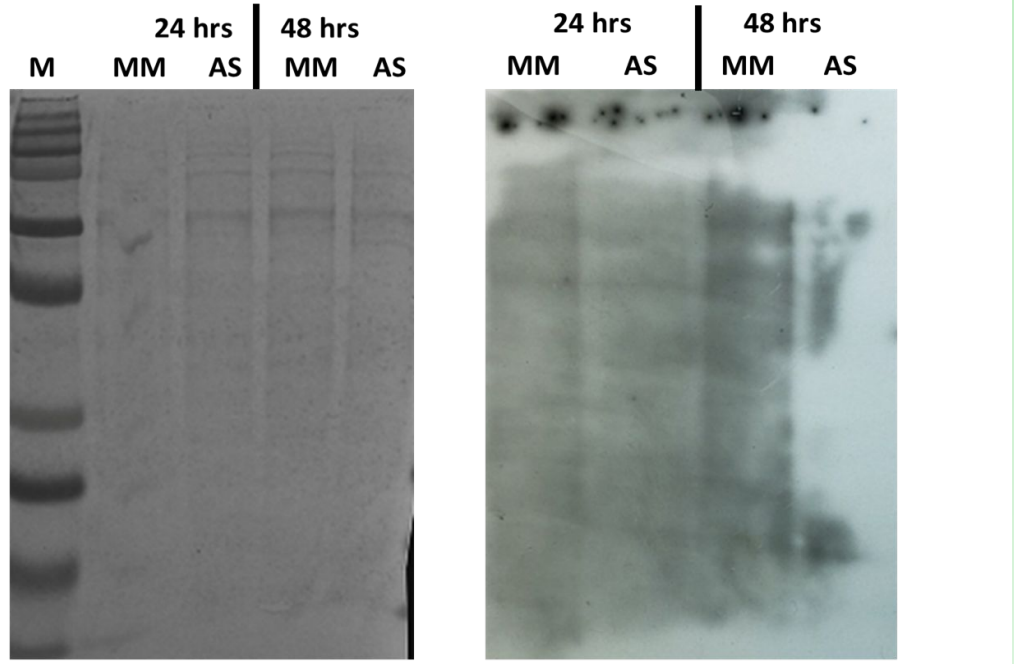
- Morpholinos are specific complementary oligonucleotide sequence to the mRNA of a gene that binds and prevents ribosomal scanning and translation of gene
- Mismatch anti-GleIF4A vs anti-GleIF4A shows the specificity needed to block translation of GleIF4A
- Morpholino transfection is transient and will only knockdown translation for 48 to 72 hours

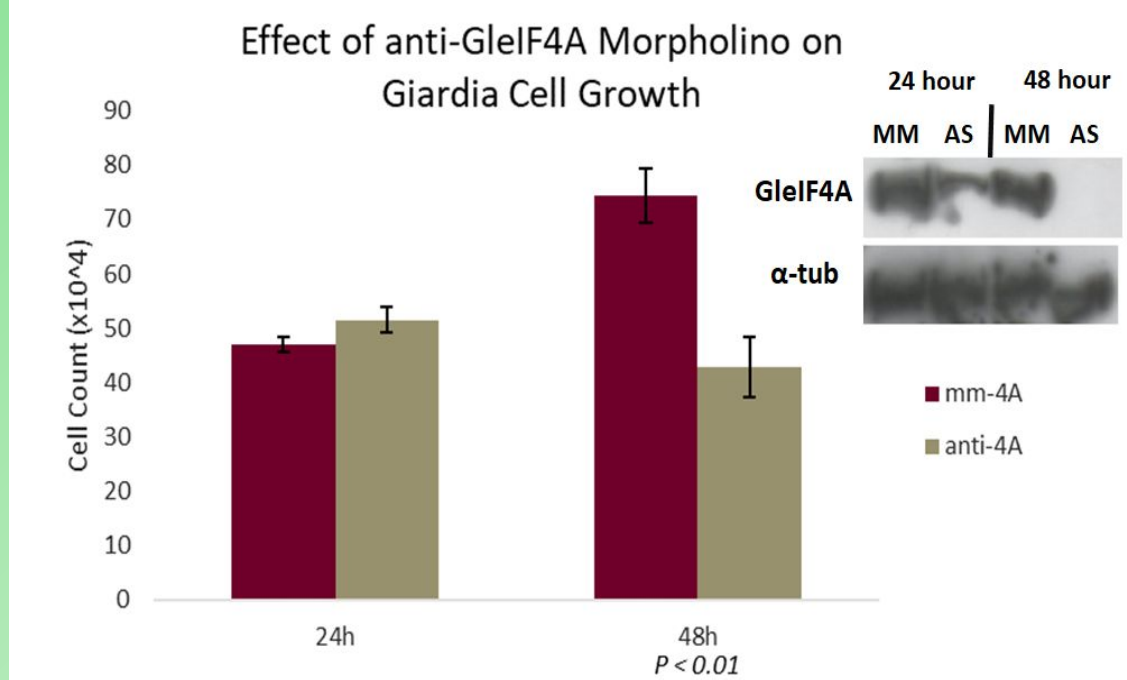
Results: CRISPRi

- Cell growth was monitored over 96 hours following subculture
- Experiment was conducted using biological triplicates
- CRISPRi with specific GleIF4A gRNA shows cell count reduction after 96 hours compared to non-specific gRNA



Morpholino (results)





- Giardia cell growth shows reduction in cell count intensified after 48 hours
- GleIF4A mismatch anti-strand and anti-strand results compared to alpha tubulin loading control Western Blot shows large reduction in protein after 48 hours
- Ponceau stain indicates that the same level of protein was loaded into each lane
- anti-puromycin antibody SUnSET assay shows overall reduction of protein synthesis after 48 hours

CONCLUSIONS

- Knockdown of GleIF4A has led to the reduction of cell growth and overall translation
- This could prove that GleIF4A plays a role in *Giardia lamblia* translation initiation despite the lack of ribosomal scanning
- Specific GleIF4A functional assays in the future could check for protein activity
- Helicase assay could monitor unwinding of 5' UTRs
- Future works could determine the mechanism GleIF3i plays with GleIF4A
  - **Colocalization** – to determine if 3i and 4A are localized within *Giardia*
  - **Mutant overexpression** - A mutant that antagonizes its coexpressed wild-type gene product, resulting in reduction of total activity
  - **Biotinylation** – addition of biotin to specific proteins that interact with 4A

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