Week 2 – Problem Set

Name: Kayla Carmichael

You sequence DNA from several Meier-Gorlin syndrome patients and discover a recurrent mutation in an uncharacterized gene. The mutation is not detected in control samples and introduction of this mutation into Zebrafish recapitulates the dwarfism phenotype of Meier-Gorlin patients. You name the new gene *MGS24* and set out to investigate its function.

(1) Based on your understanding of Meier-Gorlin syndrome (MGS), in which stage(s) of DNA replication could the MGS24 protein likely function? (Select ALL correct answers.) (3 pts)

- [x] Replication initiation
- [x] Replication elongation
- [x] Replication termination

(2) Explain your answer to (1) in no more than 75 words. (1 pt)
Be sure to address the function of MGS24 and connect what you propose back to what is known about MGS.

Other commonly mutated genes which result in Meier-Gorlin syndrome is ORC1, CDC6, MCM7, PSF2, etc. These genes all encode proteins which are either part of loading the CMG helicase onto DNA or are part of the helices itself. If MGS24 is found to be apart of the helicase, it could impact any aspect of replication (ie. initiation, elongation, termination) because there is no indication of its possible interactions with other replisome proteins.

You perform a CRISPR knockout of *MGS24* in U2OS cells and confirm the MGS24 protein is no longer expressed. The cells are viable, but there is an increased population of S phase cells, suggesting DNA replication is *slowed but not blocked*. Note that this is new data and could contradict previous models, including your answer to question (1).

(3) Which of the following answers and justifications could explain these effects? (Select ALL correct answers.) Note that the strength of the conclusion (must/cannot vs could) is important here. (6 pts)

- [ ] *MGS24* cannot be required for any part of DNA replication because DNA replication is essential for cell viability
- [ ] *MGS24* must be involved in initiation because all previously identified MGS patient mutations affect initiation
- [x] *MGS24* cannot be involved in initiation because no DNA synthesis takes place during initiation

- [x] *MGS24* could be involved in elongation because this is when most DNA synthesis takes place
- [ ] *MGS24* could be involved in termination because failure to replicate even a short stretch of DNA prevents cells from exiting S phase
- [x] *MGS24* could be involved in termination because failure to recycle replisomes slows elongation

You decide to perform LC-MS/MS to identify proteins that interact with MGS24.

(4) Which of the following statements are true about the experimental strategy/approach to isolate MGS24 and associated proteins? (Select ALL correct answers.) (4 pts)

- [x] This could be achieved by isolating MGS24 from yeast because DNA replication is highly conserved in eukaryotes
- [ ] Immunoprecipitation (IP) of MGS24 from human cells would also identify binding partners of MGS24
- [x] It would be essential to introduce a tag (FLAG, HIS, etc.) into the endogenous MGS24 protein in order to isolate the protein complex
- [ ] If SDS-PAGE was performed using human cell lysates then the band corresponding to MGS24 could be cut out and analyzed by mass spec in order to detect its binding partners

The proteomic analysis from (4) identifies CDC45 and the single-stranded DNA binding protein RPA in the experimental sample but not in the appropriate control. You therefore decide to test whether MGS24 is part of a complex containing both CDC45 and RPA.

(5) Could a co-immunoprecipitation experiment tell you whether MGS24, CDC45, and RPA, all form a complex together? (pick one; 1 pt)

- [ ] Yes
- [x] No

(6) Explain your answer to (5) in no more than 100 words. (1 pt)

Co-immunoprecipitation works by flagging an isolating a protein from a mixture then using mass spectrometry to further identify proteins that are interacting with the target protein. Co-IP is a good starting place to determine possible protein-protein interactions; however, it cannot determine whether those interactions are direct nor simultaneous. This means that CDC45 and RPA do interact with MGS24 in some manner; however, it may be through a protein intermediary or in a sequential manner rather than in a complex.

(7) Could a gel filtration experiment tell you whether MGS24, CDC45, and RPA, all form a complex together? (pick one; 1 pt)

- [x] Yes
- [ ] No

(8) Explain your answer to (7) in no more than 100 words. (1 pt)

Gel filtration works by passing a protein mixture over a resin containing small beads with pores of a particular size. Smaller proteins will elute slower due to them being trapped within the beads; whereas, larger proteins are capable of passing through the space between beads. If MGS24, CDC45, and RPA elute at the same rate (thus behaving as one, larger protein) one can assume that they are in complex with one another. If they are not in complex with one another, they would elute a different rates due to their varying sizes.

(9) Additional experiments reveal that MGS24 forms a complex with either CDC45 or RPA but never both. This suggests that MGS24 can associate with CMG but be displaced by RPA when CMG is actively unwinding. To test this hypothesis, you perform optical trapping and confocal microscopy using the same setup as in Fig 5E-F of Wasserman et al 2019 Cell except with human proteins and MGS24 also fluorescently labeled. In no more than 100 words, explain which result you would expect to see if the hypothesis is correct. (2 pts)

If this hypothesis is correct, that implies that MGS24 does not associate with the CMG helicase while it is actively "unwinding" the DNA (ie. elongation). Figure 5E and 5F demonstrates CMG helicase's diffusion capabilities when in the presence of double-stranded DNA. If MGS24 was fluorescently tagged, one would expect to see an additional oscillating fluorescence when CMG helices is in its diffusive state. The additional fluorescence pattern would match that of CMG helicase because MGS24 and CMG helicase are in complex. When CMG helicase encounters single-stranded DNA, the additional fluorescence should disappear because RPA has displaced the MGS24 protein.