How to Run a Journal Club With More Than 100 Students

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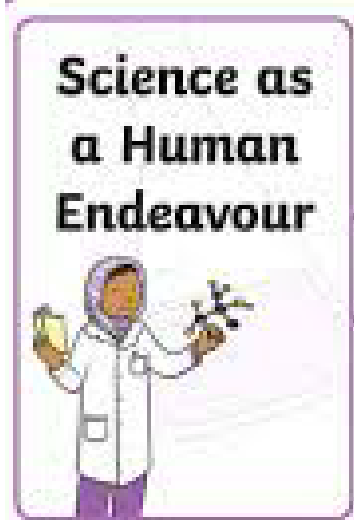
The Context of the Class:

- Upper-level Molecular Biology class
- 120+ students
- Juniors, seniors, and graduate students
- Wide variety of scientific backgrounds (biology, chemistry, engineering)
- Class meets twice weekly for 75 minutes each
- Optional recitations, 50 minutes each week

Our Goals:



- Give students experiences reading and analyzing papers from the primary science literature.

- Provide both asynchronous and synchronous opportunities for students to talk about the papers.

- Show students that scientific hypotheses, results, and models are created by a diverse group of scientists, working in teams.

Preparation:

- How do you choose ‘good’ papers?

  - Papers from the 1990’s/2000’s are often good
  - Avoid Cell/Science/Nature (they’re too long)
  - Material should synergize with course content
  - We choose 3 papers of increasing difficulty, spread throughout the semester

Preparation:

- What can we do for students with little experience reading the primary literature?

- Paper scavenger hunt with first paper
- Happens 3 weeks before the first journal club
- Requires students to examine *all* parts of the paper
- Students discuss strategies that they've used to read papers
- We also talk about how to interpret a figure

Student engagement before the journal club:

- We have students annotate the paper using Perusall (other platforms, like hypothes.is, also work)

  - Students are assigned into small groups (10-12 students)
  - They can annotate a pdf of the paper asynchronously
  - Visiting the paper multiple times allows them to make comments, ask questions, respond to their classmates' comments/questions, and upvote comments
  - This assignment can be graded by Perusall, the instructor, or not graded
  - Provides the instructor with a window into student thinking

An example of a Perusall annotation

3. Results

3.1. Spermatid's extracts promote TNR expansion in vitro

We developed an active nuclear extract from sonication resistant spermatids (steps 10–16, Fig. 1A) that supports a variety of DNA repair processes including NHEJ (Supplementary Fig. 1) or topoisomerase activity. These steps are associated with the chromatin-remodeling process and we have previously shown that they promote TNR expansion [22]. To detect TNR expansion, we used the pBL302 reporter plasmid harboring 22 CAG repeats which stabilizes a hairpin located upstream of the CAN1 gene, as described by Stevens et al. [27] When incubated with active nuclear extracts, CAG expansion above a 26 repeats threshold leads to the insertion of a new out-of-frame initiation site for CAN1 that generates an inactive protein (Fig. 1C). Therefore, once transformed into the BL1425 yeast strain and replicated onto canavanine plates, expansions confer resistance to this drug as shown (Fig. 1B). TNR expansion from canavanine plates selection are then validated by electrophoresis of PCR products using primers flanking CAG repeats (Fig. 1D) in order to determine the percentage of true positives in each reaction. TNR expansion frequency is then established relative to the control reporter plasmid not incubated with the extract.

3.2. In vitro TNR expansion frequency is driven by negative supercoiling

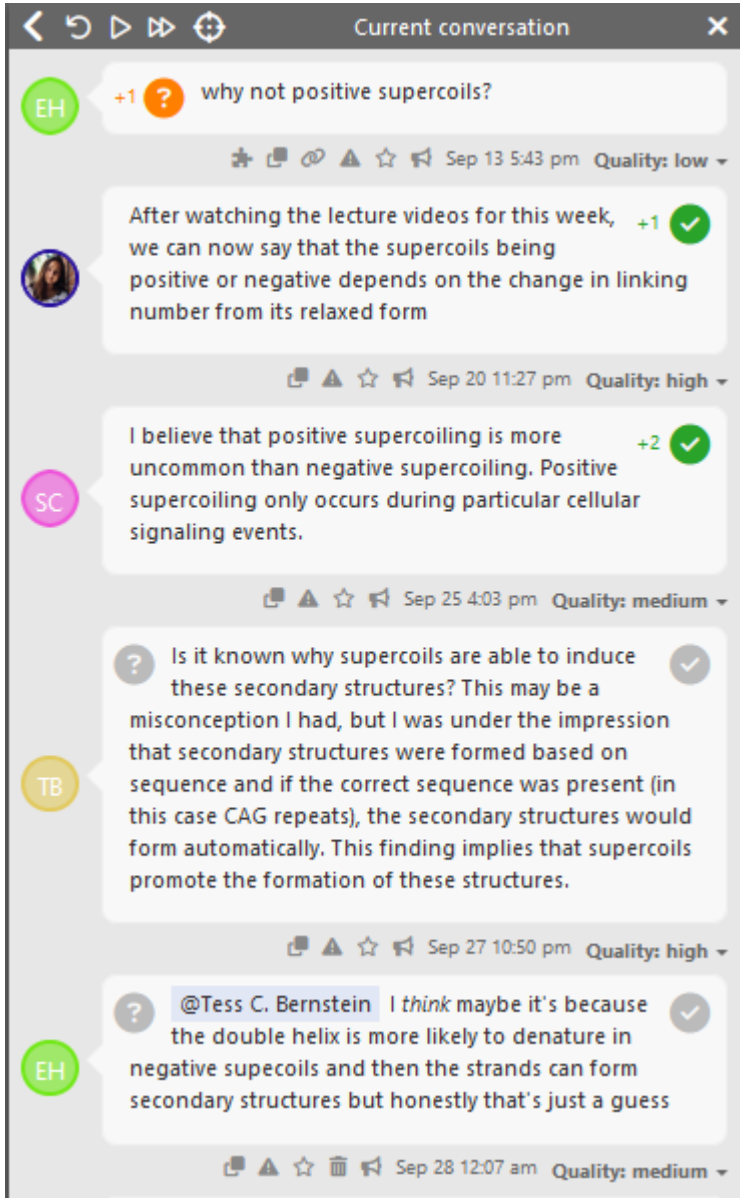
TNR expansion is driven by the capacity of trinucleotidic repeats to form secondary structures, such as hairpin [9,10,16]. Chromatin reduction of PCR products whereas no such decrease was observed at CAG repeats when the relaxed pBL302 was used as a template. It is worth noting that an incomplete elimination of PCR product was expected from S1 nuclease digestion since this enzyme may create nicks at other sites resulting in elimination of supercoils and extrusion of the hairpin.

Having confirmed stabilization of a hairpin loop at CAG repeats in the supercoiled pBL302, we sought to establish whether this topological form could promote greater TNR expansion than the relaxed version of pBL302 using the TNR *in vitro* assay described above. As shown in Fig. 2D, use of the supercoiled pBL302 resulted in a 1.5-fold increase in the CAG TNR expansion over the relaxed form. Hence, free negative supercoils alone may increase TNR expansions by promoting the formation of secondary structures such as hairpins at CAG repeats hat is not present in the relaxed form.

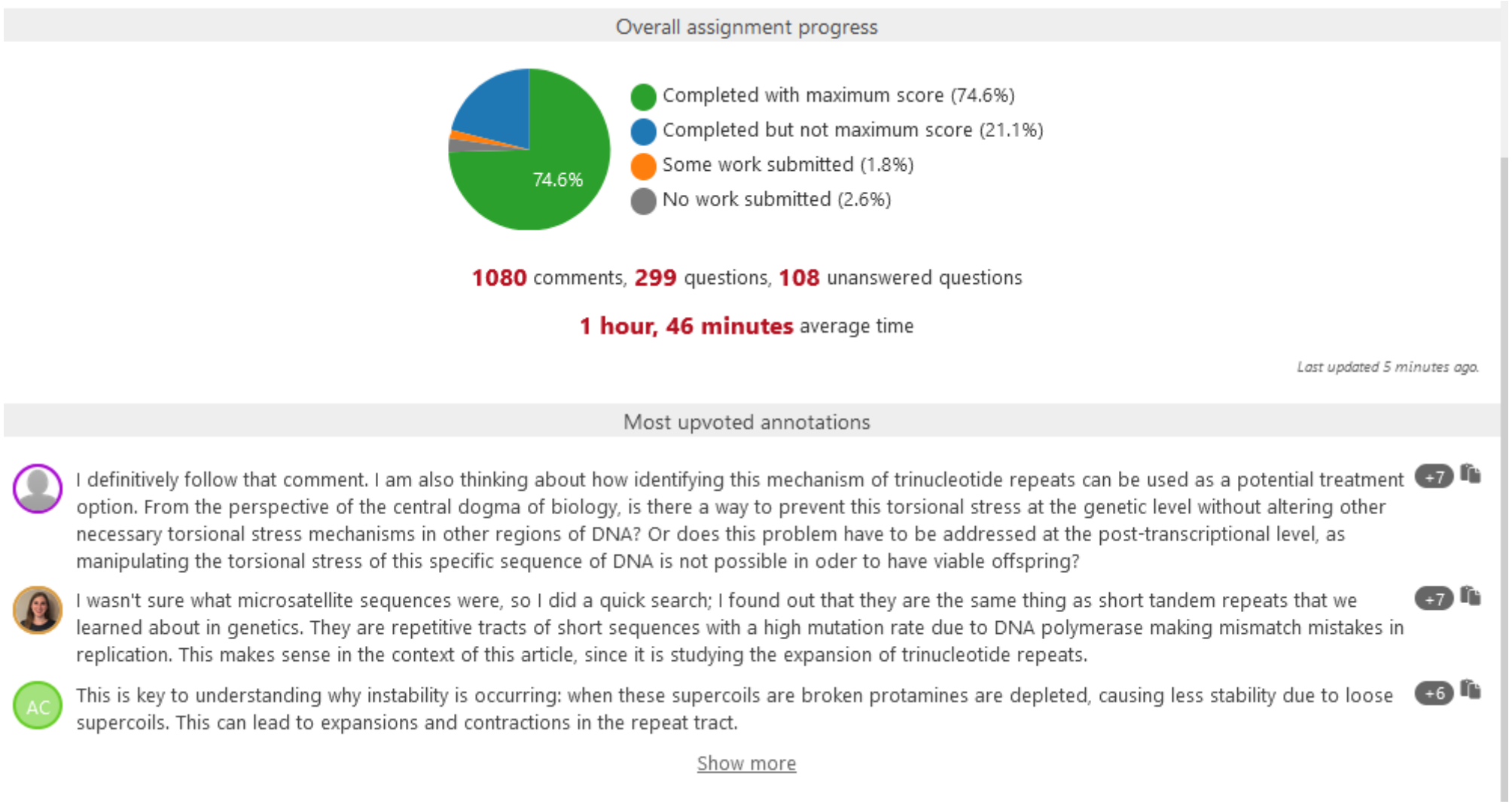
3.3. Topoisomerase II inhibition increases TNR expansion frequency

The results from the experiments describe above show that TNR expansions are enhanced by free negative supercoils. We observed that topoisomerase II activity from spermatids nuclear extracts progressively removes supercoils during incubation (not shown). We reasoned that inhibition of topoisomerase II activity in spermatids nuclear extract should stabilize the supercoiled form of the plasmid during incubation leading to increased TNR expansion.

HU-331 is a potent inhibitor of topoisomerase II that act by specifically blocking he reaction from the binding step therefore



Perusall provides lots of useful metrics for the instructor



The In-Class Journal Club

- The goal is to keep students engaged, while providing them with multiple opportunities to engage with the paper

  - “Vent session” (5 minutes)
  - Setting the stage/background (5 minutes)
  - Figure assignment, work in small groups (15 minutes)
  - Presenter assignment, preparation (10 minutes)
  - Figure presentations (2-3 minutes per, total ~ 30 minutes)
  - Wrap-up and conclusions (5 minutes)

The Journal Club

- The “Vent Session” (5 minutes)

  - Students are divided into small groups (3-4 individuals)
  - Open discussion in their groups
  - This gives students a chance to discuss their experiences with the paper in a safe space
  - They often gripe for a few minutes and then start to talk about the most interesting/confusing parts of the paper

  - Instructor role: roam and listen

The Journal Club

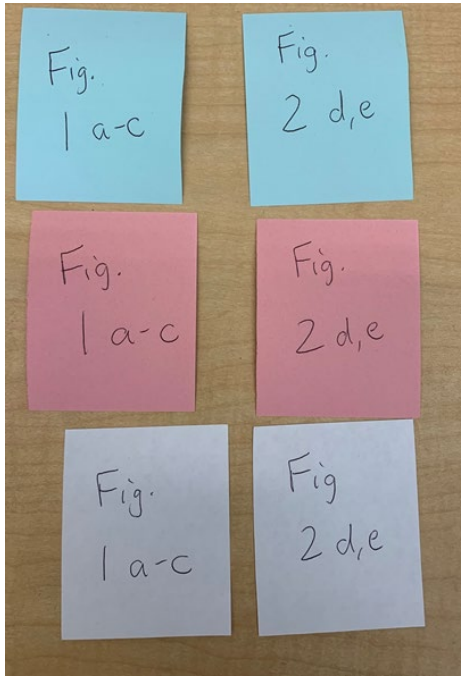
- Setting the stage/background (5 minutes)

  - Instructor establishes the context for the paper

    1. Why did we read this paper?
    2. Who are the authors and where are they from?
    3. Connections to class material

  - Instructor role: present

The Journal Club



- Figure assignment, work in small groups (15 minutes)

  - Groups are told that one person in the group will be presenting a figure to the class
  - They should answer 4 questions:
    1. What question were the authors trying to answer in this figure?
    2. What technique(s) did they use?
    3. Walk us through results of the experiment.
    4. What did the authors conclude from the data?

  - Instructor role: guide and support, encourage questions

The Journal Club

- Choose presenters, preparation (10 minutes)

  - Instructor assigns a person from each group to be the presenter

    - person whose birthday is next
    - person whose last name starts with letter closest to M
    - person who got up the latest today

    **should be something unrelated to academics and not single out individuals based on race, gender, or socioeconomic status

  - Instructor role: make sure all presenters are OK

The Journal Club

- Figure presentations (~ 30 minutes)

  - Assigned presenter walks the class through the figure

    - can do it from their seat or at the front of the room
    - helps to provide a microphone and laser pointer
    - can finish with questions that their group had

  - Instructor role: supplement information when necessary
    affirm and probe (when appropriate)

The Journal Club

- Wrap-up/conclusions (5 minutes)

  - Deal with final questions/points of confusion
  - Restate connection of the paper with the class material
  - Talk about what's happened since the paper was published

  - Instructor role: create closure and connect

Engaging with the authors

- For our final journal club, students work in groups to propose a ‘what’s next experiment’ (before class, submit in lieu of a final exam)
- We then meet with the authors (in person or on Zoom)
  - Contact the first and/or last author prior to the start of the semester
  - Ask if they would be willing to meet with the class and discuss the paper, their lab, and their career
  - Students submit questions that they want to ask the authors, instructor compiles these and sends them 2-3 days before the final meeting
  - Students drive the conversation during the meeting
  - Some students even reach out to the authors after the class!

  - Instructor role: sit back and enjoy

Author perspective

- Discussion about science
- Development of creating paper
- Labs future research plans
- Career development

Details about the paper

1. What other promoter sequences/genes does CBX4 play a role in? While the researchers tested the RAMS11 binding to the CBX4 protein, we were wondering what other proteins that CBX4 interacted with. An analysis of other protein-protein interactions could display what functions CBX4 could have in repressive pathways. In addition, we would like to see other nucleic acid sequences which this CBX4 binds to. They say it has been observed in studies to bind to the TOP2a promoter, however with both activation and repressive activities we would hypothesize this protein is involved in the expression of many other genes.

2. How do you normalize where "metastatic" cells are being retrieved from patient to patient? Is it possible that the reason behind the different survival of the two patient cohorts (used to determine the RAMS11 as key lncRNA) is caused by differences in how this CRC metastasizes in different patients?

3. What are the differences between the CRISPR1 and CRISPR2 KO cell lines that could have caused in the difference in results for CRISPR1 KO cells and CRISPR2 KO cells in figures 2I/2J? One hypothesis we had was that during the process of RAMS11 KO with CRISPR, there could have been off-target effects that lead to a mutation in an additional gene in the CRISPR2 KO line. Also, are the different results between the two CRISPRs significant or noteworthy?

4. How does CBX4's binding affect topoisomerase inhibitors' binding to Top2alpha? Is it a competitive inhibitor or does it directly affect the inhibitor's function?

Questions about your lab's future research plans

1. Through this study, we've seen that lncRNA's ability to recruit chromatin modifiers to the promoter can alter expression of cancer-causing topoisomerases. In your lab, have you found that lncRNA's various functions, including acting as a microRNA sponge, can also serve as an effective therapeutic against these topoisomerases?

2. Were there other lncRNAs (RAMS) out of the 148 that you identified in the initial screens that you hope to (or are currently) looking more into? Could the fact that there were ones that seemed to be specific to mCRC be a path forward for treatment?

3. Have you observed any other lncRNAs participating in the development of other cancer types similar to how RAMS11 drives metastasis in CRC? If so, are the mechanisms similar or different?

4. In Figure 1B, RAMS11 went from underexpressed to overexpressed in metastatic tissues. At the bottom, it looks like there are some that do the opposite and go from overexpressed to underexpressed in metastatic tissues. Have you thought of investigating any of these lncRNAs further?

5. Are you looking at any new therapeutics? You mentioned locked nucleic acids - how promising do you think the application of LNAs as a cancer therapeutic is? Is your lab doing any additional work specifically with LNAs or other therapeutics for patients with CRC?

Questions about the scientific process in the paper

1. At which experiment did you have full confidence that you had proven RAMS11 to act in colorectal cancer? Were there specific small experiments that were not necessary looking back or were there some that proved to be extremely important?

2. Are there other characteristics beyond anchorage-independent growth, cellular proliferation, and invasiveness that are hallmarks of aggressive cancers?

3. How is the timeline of cancer determined in mice and how does this compare to humans?

4. How was the process in this paper? What did it look like "behind the scenes"- were there any obstacles encountered in any of the experiments, and were any of the results surprising?

5. There are lots of figures and data that were gathered during the process of this paper. How did the research team deal with the vast amount of data and organize it?

6. Did the project start off as something different than originally planned, and were results obtained that led to a shift in the experimental focus?

7. Why were the lung and spleen chosen to evaluate the amount of tumor growth (metastasis) rather than other organs such as the colon from which the cancer cells are harvested from?

Questions about your career and DEI efforts

1. What has your experience been like as a woman working in STEM? What obstacles/challenges have you encountered because of this? And what advice can you give to other women who want to pursue a career in STEM?

2. We wanted to know what it has been like for you as a woman of color in STEM. What are some challenges you faced?

3. Do you feel that the field of biological research has become more diverse and equitable over time? Have your efforts or those of your peers towards upholding those core values changed this year in light of the pandemic and movements for racial justice?

4. How have your experiences as a woman in STEM shaped the way in which you run your lab?

Feel free to contact us with questions and ideas!

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