**GROUP 1 - INTRODUCTION**

1. Start your presentation by reminding the class what biofilms are and why they may be important to study (brainstom within your group for this last question). Tell us the names of the well-studied bacterial pathogens that make biofilms.
2. Make the transition to TB and remind us all why studying M.smegmatis may be important.
3. For slide 3, discuss the last sentence of the first paragraph (“Understanding the molecular events involved….”) in your small group and paraphrase in your own words. This sentence summarizes why the author’s felt this study was important – convince the class why this study is important!
4. Talk about the two different GroEL forms in mycobacteria – GroEL1 and GroEL2 (ignore mention of the third form). (Remind the group about Hsp60 proteins.) You do not need to present the information regarding the E.coli GroE gene, but you should use this info in your small group discussion to assist in remembering the role of the Hsp60 proteins!
5. Discuss within your small group the rationale behind including the data in paragraph 3 (“For those bacteria…”), but you do not need to discuss in the group presentation.
6. Lastly, provide a final slide with bullet points outlining what the paper intends to prove (this is all in the last paragraph of the introduction). Start with a bullet point that reminds the class how we got from our last paper to this paper – what did the Kim et al folks discover that made this paper possible?

**RESULTS**

**GROUP 2 - Bxb1 Lysogens are Defective in BioFilm Formation through Inactivation of GroEL1**

1. Slide 1 should briefly outline the background of the integration of Bxb1 into the M.smeg genome as was discussed in the Kim et al. paper (First few sentence of the first paragraph of this section and Fig. 1)
2. Describe what the researchers observed in Fig. 2A. What are the controls? Which panel contains the Bxb1 lysogens? What are the smaller boxes?
3. Describe what they observed in the different panels of 2B. Discuss in your small group the experiment that was performed to generate each panel. Ignore any of the sentences that talk about the Figure S1 or S2. This is supplemental data and I figured we already had enough data to look at! Provide a one to two sentence summary of what was learned from the set of experiments in Figure 2.

**GROUP 2 - GroEL1 is Required for Biofilm Maturation Not for Attachment**

1. Read through the first paragraph of this section up until the sentence “To determine if GroEL1 is required for surface attachment”. Compare what they are telling you in the first section of this paragraph to the results shown in Fig3A and Fig 3D. You may want to put Fig 3D on the same slide to make your point more clearly. When describing Fig. 3A first tell us what the experiment was. Then tell us what the control is and compare the experimental data (deltagroEL1) to the control for each panel (Day 3 – Day 7). Then tell us how they set up the experiment for Fig. 3D, describe the x and y axis, and then discuss the data – again comparing the control to the experimental data. You will need to read through the Materials and Methods to understand how Fig. 3D was generated.
2. Next describe what the researchers were trying to determine (in your own words) for Fig. 3B. Compare what they report in the text of the paper with the image in Fig. 3B. Were you convinced of their argument after looking at Fig. 3B?
3. Lastly, talk about figure 3C. How did they generate this figure? What does the arrow on the left side indicate? What two things are being compared? Does this figure make their argument more or less convincing?

**GROUP 3 – GroEL1 is Required for Regulation of Mycolate Biosynthesis in Biofilm Maturation**

This is a tough set of figures! Work through the questions below and try to dissect out the most relevant data. Try to break down the scientific jargon into a more easy to understand set of statements.

1. BACKGROUND: To best understand this section, first read the last sentence of the first paragraph. This sentence summarizes the essence of what this section of the paper set to prove. Recall from the class discussion that the FAS-II complex is a group of proteins (a protein complex) that is involved in the biosynthesis of mycolic acids. KasA and KasB are two proteins that are part of the FAS-II complex. Therefore, by looking at KasA and KasB levels in the cell, we can perhaps get a look at whether formation of the FAS-II complex is somehow being affected by the mutant groEL1 gene. Figure 4A compared the protein profiles in two different cell lines – the wildtype M. smeg mc2155 and the Bxb1 lysogen cell line (ΔgroEL1). Essentially they extracted all of the proteins in the cell lines at a particular timepoint and run the gel in one direction under a defined set of conditions, then turned the gel and ran it again under a different set of conditions – thus separating all of the proteins that were expressed in the 2 cells at that particular moment. They then looked at the gels (using a computer!) to see if some spots were present in one gel and not the other, to determine if some spots were darker in one gel than the other, etc. The darker the band, the more proteins was present.
2. For your class presentation, briefly review the experiment for Fig. 4A (I outlined much of it for you above) and describe what they observed. Then describe what they are looking at in Figure 4B. Is this surprising given the results observed earlier in the paper in Figure 2?
3. In Figure 4C, the authors are now only looking at two proteins in this 2D-PAGE gel. They have isolated out KasA and KasB. KasB is the protein represented by the single dot on the far right of the blot. What they have noticed is that there are 2 different “versions” (isoforms) of KasA as indicated by the two dots sitting next to each other (the one with the arrow and the one immediately to the left of it). When deciphering this figure, ignore the sentence “The molecular basis for the difference….” The sentence “Interestingly, there is a marked…” will help you decipher the figure.
4. Use all of your newfound knowledge to discuss Figure 4D and ignore Fig. 4E.
5. Lastly, prepare a set of bullet points highlighting the major points of your section of the paper.

**GROUP 4 - GroEL1 Physically Associates with KasA during BioFilm Formation**

BACKGROUND: The authors were interested in learning more about the groEL1 gene and were especially interested in the fact that the entire gene function appears to knock out with the insertion of the Bxb1 phage that only interferes with the last (C-terminal) 18AA of the protein. Thus, is appears those 18AA may be critical! They also noticed that when they were trying to purify the GroEL1 protein they could get the protein to bind to the metal, nickel (Ni-affinity matrix). Furthermore, they could get the protein to detach from the nickel when they use the buffer imidazole (an organic compound commonly used to remove proteins from nickel columns – the imidazole can compete for the same binding sites the protein is using to attach to the nickel column).

1. For Fig. 5A, provide the class with some of the background I gave you above so they have context. Next discuss in your small group why you think they used two different concentrations of imidazole to try to elute the GroEL1 protein from the column. What may this tell them about the protein and its binding affinity (strength) for Nickel. Be sure you can explain the different constructs that were tested (and are listed at the top of the figure).
2. For Fig. 5B – the researchers started with three different protein extracts : Lane 1 is purified GroEL1 WT protein eluted from the Ni+ column (this is a pure protein preparation – note the single band in the Coomassie stained gel). Lane 2 is an extract of all proteins from wildtype M.smeg cells. Notice all of the black lines in the Coomassie-stained gel. Remember, each black line refers to a protein – the thicker the black band, the more protein. Yet, recall when you are running SO MANY proteins on a gel in one lane (as in this case) many proteins may be sitting on top of one another in the gel. Lane 3 is another extract of all proteins, but this time , they extracted all the proteins from the Bxb1 lysogens (ΔgroEL1). Again notice all of the bands in the Coomassie gel. Also recall that the Coomassie stain will stain all proteins . In the middle image, the gel is the same as the one above, but this time they probed the gel with a particular antibody that only recognized GroEL proteins (either GroEL1 or GroEL2). Would we expect this antibody to recognize proteins in all three lanes? The bottom image shows the same gel as the top again, but this time they probed the gel with antibody that is specific for GroEL2. What does it mean if they do not detect a band in lane 1? What does it mean when they do detect a band in Lanes 2 and 3? Lastly, put all of the data together and discuss the relationship of GroEL1 to GroEL2? Do they bind together? Are they separate proteins?
3. In a previous figure, the researchers show that one of the members of the FAS-II protein complex involved in mycolic acid biosynthesis (KasA) has an altered expression profile when the groEL1 gene is deleted. They then hypothesized that the mycolic acid profile in M.smeg may be very different in planktonic growth versus biofilm formation. They were wondering if there was some direct connection between the proteins, KasA and GroEL1. Given this background, see if you can decipher Fig. 5C. Ignore all of the Δ4308 mutants (last 2 lanes). You can also ignore Figure 5D.
4. Lastly, prepare a set of bullet points highlighting the major points of your section of the paper.

**GROUP 5 – Changes in the Mycolic Acid Profile during Biofilm Formation**

Background: For this figure, we are only going to focus on the main point and avoid some of the underlying information. Thus only focus on reading the first paragraph of this section until you hit the sentence “The individual species within this mass range…” Then skip the center part of the paragraph and start reading again at the sentence “Although the total mycolate content is greatly reduced…” and read until the end of the paragraph.

Fig. 6 – Focus on deciphering what the panels represent (A,B,C,D). I highly encourage you to write this on the ppt. slide so it is easy for the class to figure out. You do not have to cover the cover of mass spectrometry in detail, but you should remind us all that this was the experimental technique used for the figure and maybe give a one sentence, in your own words, description of what mass spectrometry is use for (i.e. “Mass spectrometry is an experimental tool used to analyze…….”). Then compare the panels and be sure to point out the controls. Focus on the major concepts – the comparison of wild-type M.smeg versus the lysogen, the comparison of planktonic growth versus log-phase, presence/absence of short chain mycolic acids.

**GROUP 6 – Mutants Defective in Mycolate Biosynthesis Are Also Defective in Biofilm Maturation**

Background: The researchers were convinced at this point in the paper that there was some connection between the production of short chain mycolic acids and biofilm formation. When short chain mycolic acids are not made (as is the case with the mutants) then a mature biofilm is not formed. As an extension, they were also convinced that mutation of groEL1 somehow affected the biosynthetic pathway of mycolic acid formation. They further hypothesized that other mutants defective in the mycolic acid biosynthetic pathway would also not form biofilms. They mutant that was available for them to test was one called InhAts. InhAts is an interesting mutant because it had been previously shown to have a different mycolic acid profile, therefore it may not make biofilms either.

Fig. 7A – Using the information above , compare and contrast the two panels. Which is the control? Compare the control to the experimental data. What did they find? Is the InhAts mutant incapable of producing biofilms?

I suspect the researchers were pleased with the results of 7A, but they still had a little problem. The InhAts mutant not only had an altered mycolate profile, but it was also previously observed to have an increase in KasA protein expression. So, there were really two questions here: 1) Was the defect in biofilm maturation a result of the InhA mutation?, OR 2) Was the biofilm defect related to the KasA overexpression. To answer this question, they cloned out the KasA gene, put it in a plasmid, put that recombinant plasmid into M.smeg (pMV261 is a bacterial strain containing a plasmid with no genes inserted – this is the control; pMV261:KasA is same bacterial cell line containing the plasmid pMV261 plasmid with the KasA gene inserted– this strain will have lots and lots of KasA protein expressed). They then compared the two in Figure 7B.

Fig. 7B – Compare/contrast the M.smeg with the overexpressed KasA to the control. What did the researchers observe? You can pull in the data from Fig. 7C to help explain the results.

Lastly, the researchers developed a model that goes something like this:

1. The transition from early biofilm formation to a mature biofilm requires the elevated synthesis of short chain fatty acids. GroEL1 is likely involved in this process (i.e. this is a GroEL1-dependent process).
2. GroEL1 binds with KasA and SMEG4308 and seems to be the critical component linking these things together – it is this complex that is also important for the synthesis of the short chain fatty acids. If you lose GroEL1, then the entire thing breaks down and you don’t get short chain fatty acids made.
3. The level of KasA in the cell seems to act like some sort of molecular clock such that the level of KasA is important in determining when the cell will switch over to making the short chain fatty-acids necessary for biofilm formation. When there is too much KasA, there is a significant delay in the transition to mature biofilm formation.
4. KasA is also interesting because it has been noted in a previous figure that there are 2 different forms of KasA in the cell.
5. Tying it all together, the researchers suggest that one form of the KasA is a precursor form and the other form is the active form necessary for production of the short chain fatty acids. When GroEL1 is present, it switches the KasA from the precursor form to the active form. When there is too much KasA, the GroEL1 protein cannot keep up and thus the biofilm maturation is severely delayed.
6. Lastly, they also notice that KasB inactivation also leads to a delay in biofilm maturation likely because KasA and KasB are strongly associated in the cell.

This model is described in the last paragraph of the Results section of the paper. Your final task is to take the model and diagram it out so we can visualize it better in class (think concept mapping). Put the main players in boxes and then connect the model with arrows and linking words. You may have to re-work this a few times to get it the way you like and something than can be easily discussed with the class. Feel free to use the post-it notes and the chalkboard when working to develop you pictorial model.