I extremely enjoyed learning about the preparatory methods i.e origin activations, that our DNA carries out to enable successful DNA replication.

When contemplating the consequences of under-replication as stated in the lecture, I also thought about the presence of Okazaki fragments that are produced in lagging strands. What could be the potential consequences of unbound Okazaki fragments for cell division?

I am so glad to hear that you enjoyed learning about DNA replication, and thank you for sending in your question. This is a really good question!

Let me talk a little about Okazaki fragments first, for context for other readers.

Okazaki fragments, as you say, are the DNA fragments created on the lagging strand. DNA has two strands, one known as leading and the other known as lagging. The difference is to do with the direction in which they are replicated - the leading strand 'leads' and is easy to copy, whereas the lagging strand 'lags' in small fragments - this makes it a bit trickier to replicate.

Why is it trickier? The replication machinery travels in the opposite direction to the lagging strand replication mechanism, so the DNA cannot all be replicated in one go. You can think of this like trying to copy an alphabet from A to Z, but starting at Z. The replication machinery (the letters) move backwards from Z. At select points, the replication mechanism (the pen) is able to start writing, but it can only write A to Z. Each time it starts, the pen ends up back at Z and can't do any more, so you need to get a new pen to start from further up the alphabet. It stops when it hits a part that has already been copied. You would end up with some disjointed parts of alphabet like this:

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Original alphabet: ABCDEFGHIJKLMNOPQRSTUVWXYZ

Pen 1 writes:                          STUVWXYZ

Pen 2 writes:               IJKLMNOPQR

Pen 3 writes:      ABCDEFGH
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To begin with, these fragments are stuck to the original alphabet, and in order. In order to make a complete alphabet, something needs to seal the three fragments together. This brings me back to your original question.

Sticking Okazaki fragments together is where DNA ligase comes in. DNA ligase is a protein which sticks (ligates) bits of DNA together. It has some help from other proteins, but let's just focus on the ligase for now. Once the lagging strand is copied, DNA ligase seals all the Okazaki fragments, so that the lagging strand is one long DNA strand. This means when the two DNA strands split apart to replicate again before the next cell division, both are one long DNA molecule and stay intact.

However, if the lagging strand Okazaki fragments are not sealed by DNA ligase, this would cause a problem before the next cell division. When the unsealed lagging strand separates from the other DNA strand to allow replication to occur, the replication will only be able to continue for the length of the Okazaki fragment. Once it reaches the end, the replication machinery will not know where to find the next end, and will stop replicating. This effectively looks like a double strand DNA break (a very severe kind of DNA damage). If replication were to continue somehow, you would end up with a series of double stranded Okazaki fragments, all disconnected from one another. Short bits of double stranded DNA floating around are not recognised as part of our genome. Our cells have a

system to remove this DNA and degrade it. The end result would be huge genome loss or rearrangement, which cannot be survived by the cell.

Actually, this consequence is so severe that our cells have processes in place to stop things getting this bad. At the point at which the small gaps were noticed from DNA ligase not working, a damage report system would stop the cell dividing, to allow the damage to be fixed.

In budding yeast (which have a very similar DNA replication system to humans), DNA ligase is encoded by the *CDC9* gene. This gene is essential in yeast, which means if you delete it the yeast cannot live. This highlights how important the ligase is to allow DNA to replicate! In yeast, we also have something called temperature-sensitive mutants of genes. The *cdc9-1* version (allele) of the ligase gene makes a ligase protein which changes shape at high temperatures and stops working. By putting the yeast at a medium temperature, we can see how what happens when they have some functional ligase (but far less than they'd normally have). Using the *cdc9-1* mutant in the Next-generational sequencing studies I talked about in my session would be one way to see the effect of having some unbound Okazaki fragments on DNA replication - we don't know the full answer yet! (Like I said, this was a great question, right at the cutting edge of what we are doing now!).

For more information about ongoing exciting research on DNA replication, please have a look at the website of the Replication Lab, where I did my DPhil research: https://www.path.ox.ac.uk/content/replication-lab.

Best wishes,

Ana