Discussion

20.109
Spring 2015

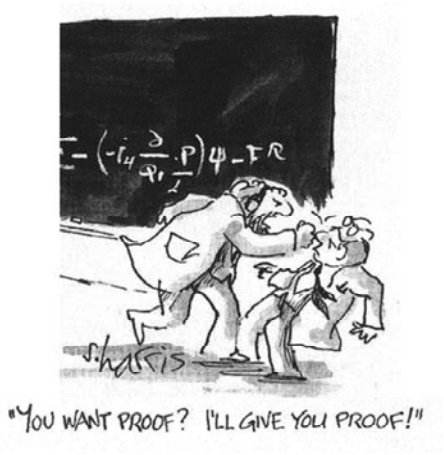
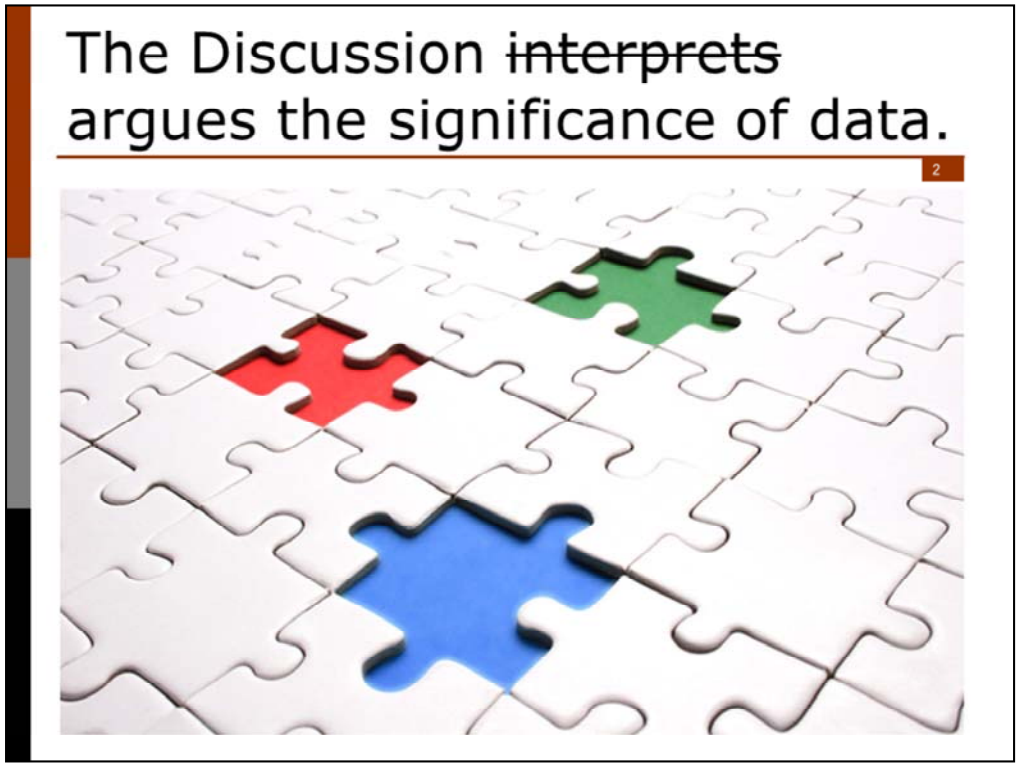


Photo credit: Theresa Walunas, http://www.keyboardbiologist.net/knitblog/

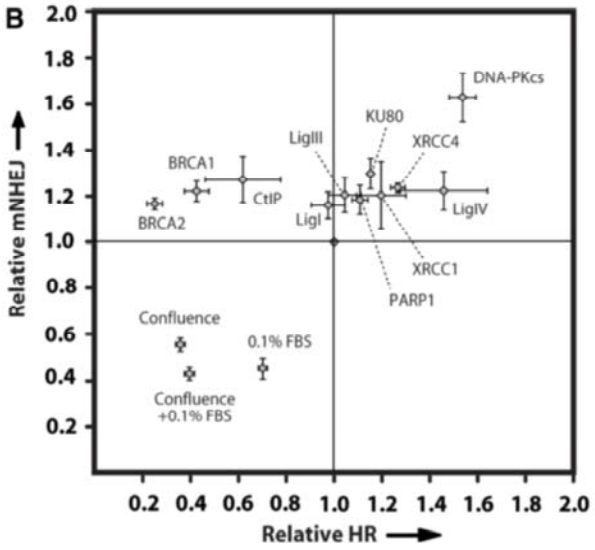
Unless otherwise stated, all samples are from Antunes et al. Mol Sys Biol 5:270 (2009).



The Discussion makes an argument about your contributions (blue) to the field, while still acknowledging your caveats (red, green).

Source: http://www.pdac.co.uk/wp-content/uploads/2012/03/how-puzzle.jpg

Analyze the Bindra et al. (2013) Discussion.



The Discussion resolves issues of the Introduction.

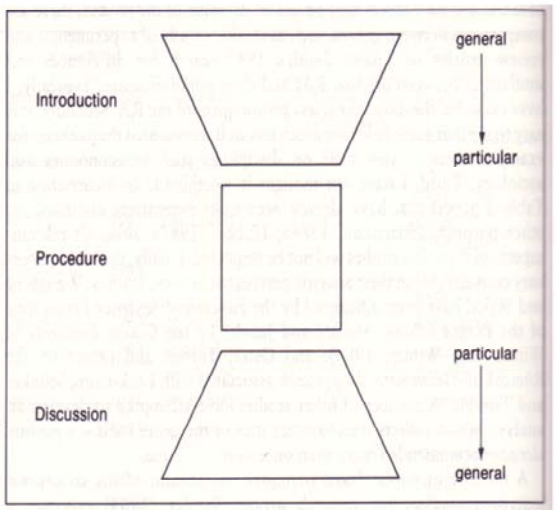


Figure 7 Overall organization of the research paper (Hill et al., 1982).

Begin by reminding reader key findings.

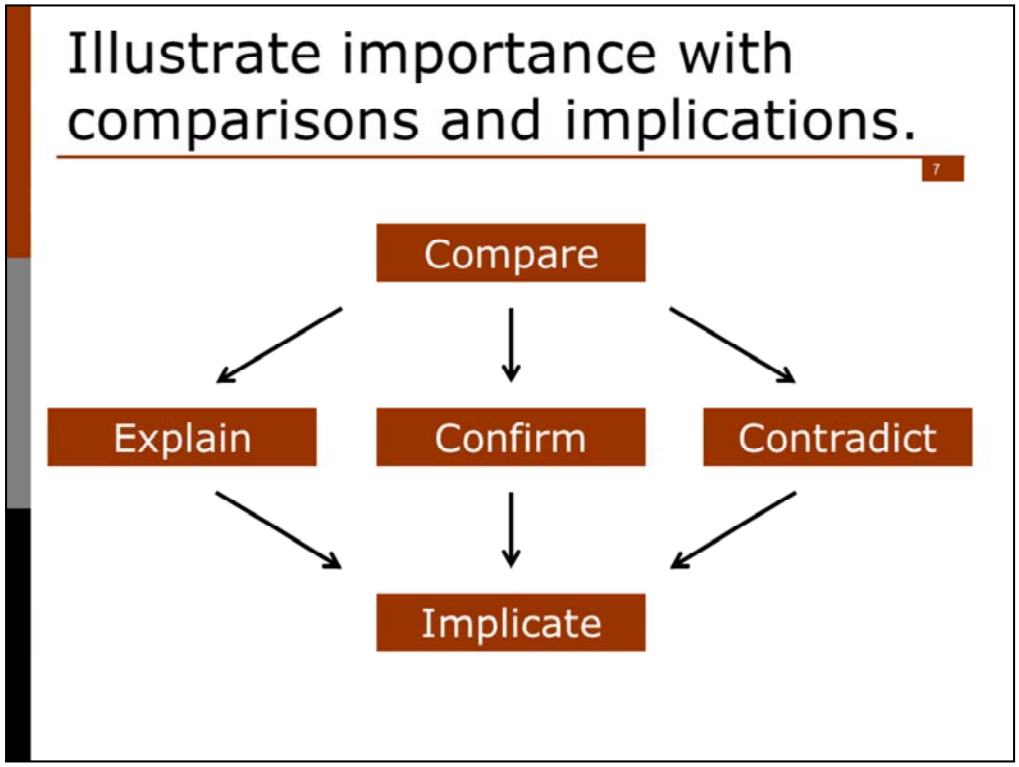
5

Here, we present the development of a novel NHEJ repair assay and a ligand-inducible system to control site-specific DNA cleavage in mammalian cells. Our NHEJ assay provides a robust and reproducible measure of mutagenic NHEJ repair, and it can be rapidly integrated into cell lines using a unique FACS-based enrichment strategy. Furthermore, we demonstrate that our NHEJ assay can be combined with an established HR assay to measure both pathways simultaneously in living cells. Our inducible DSB system is unique because it can be integrated into cell lines, with extremely high levels of cleavage-induced DSB repair, and also tightly controlled DSB induction rates. We have validated our combined DSB repair assay and inducible DSB system in a focused siRNA study of key DNA repair genes, which has yielded important insights into the dynamic balance between NHEJ and HR repair in cells. In addition, we have applied our system to the study of DSB repair in growth-arrested and serum-deprived cells. These experiments have revealed that mutagenic NHEJ repair is repressed under these conditions, whereas cNHEJ repair seems to be intact.

More effective is a reminder of the focus and justification, too.

6

Despite numerous NHEJ assays, we still lack understanding of the mechanism of the sub-pathways and the roles of individual DSB repair proteins. Here, we present the development of a novel NHEJ repair assay and a ligand-inducible system to control site-specific DNA cleavage in mammalian cells. Our NHEJ assay provides a robust and reproducible measure of mutagenic NHEJ repair, and it can be rapidly integrated into cell lines using a unique FACS-based enrichment strategy. Furthermore, we demonstrate that our NHEJ assay can be combined with an established HR assay to measure both pathways simultaneously in living cells. Our inducible DSB system is unique because it can be integrated into cell lines, with extremely high levels of cleavage-induced DSB repair, and also tightly controlled DSB induction rates. We have validated our combined DSB repair assay and inducible DSB system in a focused siRNA study of key DNA repair genes, which has yielded important insights into the dynamic balance between NHEJ and HR repair in cells. In addition, we have applied our system to the study of DSB repair in growth-arrested and serum-deprived cells. These experiments have revealed that mutagenic NHEJ repair is repressed under these conditions, whereas cNHEJ repair seems to be intact.



The significance of your data rests upon the integration of your data with the network of already accepted biological facts. To demonstrate this integration, you must compare your data with those of other (published or unpublished – cite accordingly). There are three outcomes of the comparison:

- Your data explains previous data.
- Your data confirms previous data.
- Your data contradicts previous data. What could account for the difference, and how would you resolve it?

Regardless of the outcome, state the implication of the comparison, e.g. a deeper understanding of a biological phenomenon.

A stronger paragraph is one that starts with a claim.

8

Our novel assay contributes to the ongoing debate about the roles of DSB repair proteins in NHEJ repair. Interestingly, although both the Mre11 and PARP-1 proteins also seem to play a role in non-canonical NHEJ (25,26), we did not detect any reductions in mutagenic NHEJ in our assay (data not shown and Figure 4B, respectively). We also did not detect any effects on mutagenic NHEJ repair after siRNA knockdown of several key genes implicated in non-canonical NHEJ, including LigIII and XRCC1. Although these findings are surprising at prima facie, they are nonetheless consistent with recent reports that these proteins may not be absolutely required for non-canonical NHEJ repair (12–15). Iliakis and colleagues (56) have reported that DNA ligases may have redundant functions, which could explain why knockdown of LigIII alone did not produce a phenotype…Further studies are necessary to determine the molecular mechanisms by which mutagenic repair is repressed under these conditions.

Claim

Evidence

Analysis

Cite the literature to support your argument.

9

Our novel assay contributes to the ongoing debate about the roles of DSB repair proteins in NHEJ repair. Interestingly, although both the Mre11 and PARP-1 proteins also seem to play a role in non-canonical NHEJ (25,26), we did not detect any reductions in mutagenic NHEJ in our assay (data not shown and Figure 4B, respectively). We also did not detect any effects on mutagenic NHEJ repair after siRNA knockdown of several key genes implicated in non-canonical NHEJ, including LigIII and XRCC1. Although these findings are surprising at prima facie, they are nonetheless consistent with recent reports that these proteins may not be absolutely required for non-canonical NHEJ repair (12–15). Iliakis and colleagues (56) have reported that DNA ligases may have redundant functions, which could explain why knockdown of LigIII alone did not produce a phenotype…Further studies are necessary to determine the molecular mechanisms by which mutagenic repair is repressed under these conditions.

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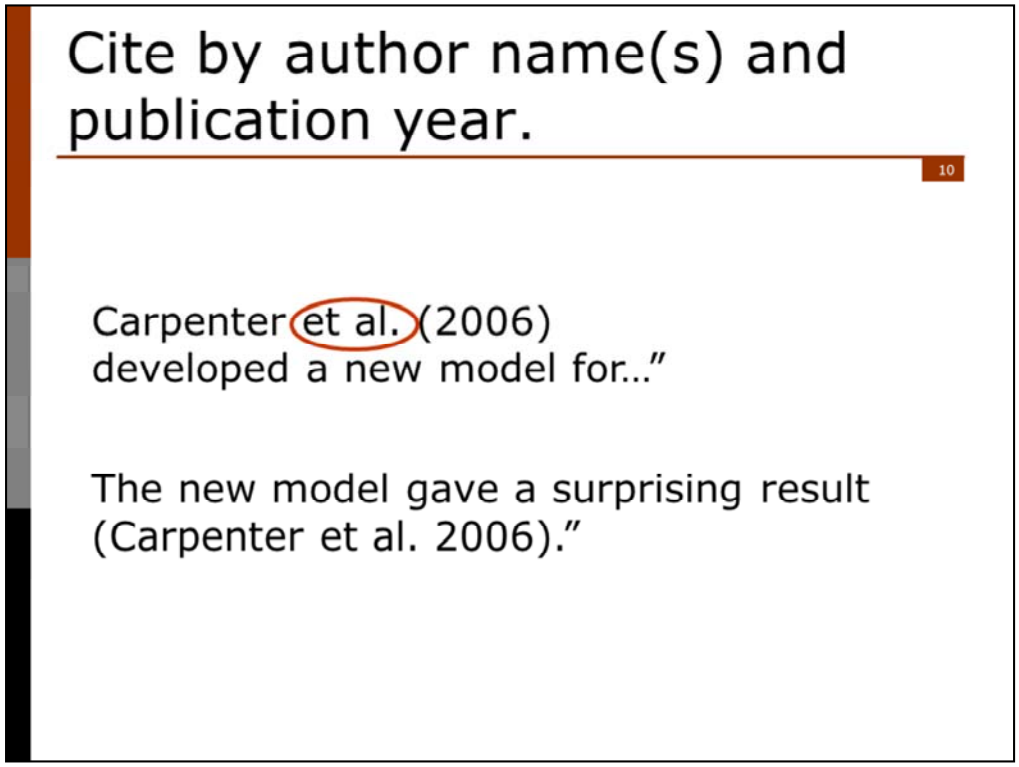
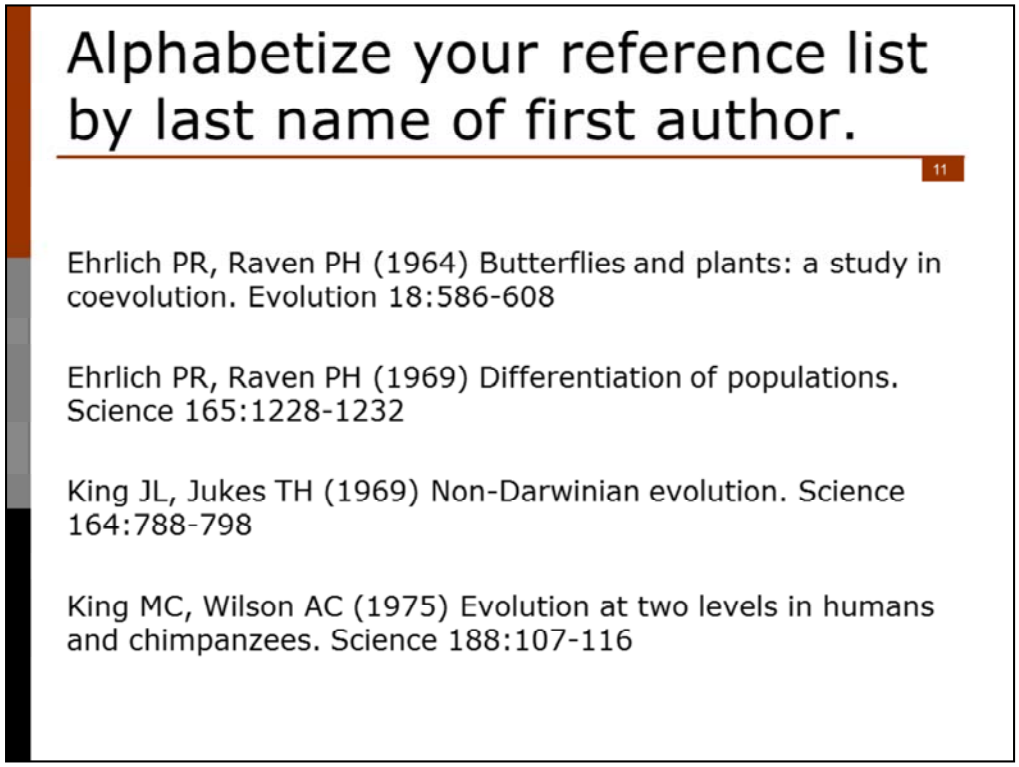


Use "et al" for papers with more than 3 authors.
-"et" needs no period
-"al" needs a period

The citation style used here is Springer Basic.



First two references: Papers with the same first author are listed in chronological order.

Last two references: Articles whose first authors share the same last name are listed alphabetically by first initial.

The format used here is Springer Basic.

Briefly describe caveats and uncertain points.

12

Further studies are necessary to determine the molecular mechanisms by which mutagenic repair is repressed under these conditions. Furthermore, it will be interesting to determine whether specific non-canonical NHEJ repair sub-pathways (e.g. mutagenic NHEJ with or without microhomology usage) are regulated by serum stimulation and/or cell cycle phase.

End by reiterating overall conclusion and impact.

13

In conclusion, our novel DSB repair assay will be an important tool to help further elucidate NHEJ sub-pathways in the future. Furthermore, the combination of EJ-RFP with DR-GFP will allow investigators to study dynamic shifts between NHEJ and HR in a manner that was not possible previously. In addition, our novel inducible I-SceI assay has many potential applications, including the study of DSB repair patterns with larger siRNA libraries, analyses of DSB induction and resolution kinetics, and also the analysis of DSB repair in specific cell cycle phases. The robust signal associated with the EJ-DR assay, and the ease of ligand-inducible cleavage with ddSceGR, makes these systems amenable for high-throughput screening studies to identify novel DSB repair inhibitors. These studies are currently ongoing in our laboratory. Furthermore, this two-tiered approach to the control of I-SceI cleavage activity likely can be applied to other proteins, including other endonucleases and zinc-fingers, as a means to better control site-specific cleavage rates in mammalian cells.

In sum, the Discussion argues the significance of your data.

14