# Writing exercise 10: Experimental Narratives

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### You will want to describe your experiments in a logical way:

* Aim (what you intended to do/show with the experiment)
* Method (what the experiment was, how you performed it)
* Results (what you showed/discovered)
* Significance (what the results mean, a logical transition to the next experiment)

### Examples from a published paper1: (aim, method, results, significance highlighted)

To gain insight into the cellular processes controlled by c-di-GMP in streptomycetes, we overexpressed either the active DGC CdgB from *S. coelicolor* (Tran et al., 2011) or the active PDE YhjH from *E. coli* (Pesavento et al., 2008). Strikingly, overexpression of both CdgB and YhjH blocked the generation of aerial mycelium by *S. venezuelae* (Figure 1B). However, scanning electron micrographs (SEMs) revealed that, whereas overexpression of CdgB blocked development, resulting in a classical bald phenotype, overexpression of the PDE YhjH in fact promoted sporulation, but the colonies appeared bald to the naked eye because aerial mycelium formation had been bypassed (Figure 1C). As judged by heat resistance, the spores made by the YhjH overexpression strain were as robust as those of the wildtype (WT) (Figure S1A available online). Moreover, overexpression of catalytically inactive versions of YhjH or CdgB had no effect on *S. venezuelae* development (Figure S1B). These data suggest that intracellular levels of c-di-GMP influence the timing of development. In particular, they suggest that increased c-di-GMP levels delay differentiation, arresting the colonies in the vegetative growth stage, whereas decreased levels of the second messenger accelerate development, favoring sporulation.

### Exercise 10A.

Read each example and identify the aim, method, result, and significance parts of the author’s description. Can you improve the logical flow of the narrative?

**Sample 10.1** 1

As first reported by Li[8](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9529316/#B8) and Fix et al,[9](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9529316/#B9) we found that concurrent expression of RexA and RexB in *E. coli* sensitizes the cells to UV irradiation ([Fig. 2A](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9529316/figure/f2/)). In strain LT732, the RexA, RexB, and the CI857 repressor proteins are expressed from the bacterial chromosome in single copy from the phage *P*RM and *P*LIT promoters ([Fig. 1B](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9529316/figure/f1/)), in the absence of any other phage functions. Strain LT1964 is identical to strain LT732 except for a mutation in the −10 region of the *P*LIT promoter, thereby reducing the level of RexB relative to RexA in LT1964. This change in the ratio of the RexB and RexA proteins increases LT1964 sensitivity to UV-mediated DNA damage ([Fig. 2A](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9529316/figure/f2/)). Note that strains with null mutations in either or both *rex* genes have UV survival curves such as those of wild type *E. coli* ([Fig. 2B](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9529316/figure/f2/)). Thus, it is the relative change in RexA and RexB expression levels that causes increased UV sensitivity.

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| Suggested improvements? |

**Sample 9.2** 2

In the genome of *E. coli*, the *hiuH* gene is located 109 bp upstream of *msrP* and 1,114 bp upstream of *msrQ* ([Fig. 1A](https://journals.asm.org/doi/10.1128/jb.00449-21?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed#F1)). The expression of *hiuH*, *msrP*, and *msrQ* has been reported to be under the control of the HprSR two-component system ([13](https://journals.asm.org/doi/10.1128/jb.00449-21?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed#core-B13), [16](https://journals.asm.org/doi/10.1128/jb.00449-21?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed#core-B16)), with an HprR box located upstream of *hiuH* (87 to 70 bp before the *hiuH* start codon). These observations suggest that *hiuH*, *msrP*, and *msrQ* might belong to the same operon. This hypothesis was explored further by reverse transcription-PCR (RT-PCR) using converging pairs of primers located within each of the three genes: after RNA reverse transcription, PCR amplifications were observed between *hiuH* and *msrP* and between *msrP* and *msrQ*, showing a tricistronic organization ([Fig. 1B](https://journals.asm.org/doi/10.1128/jb.00449-21?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed#F1)). As positive and negative controls, we used chromosomal DNA and total RNA, respectively, as templates for PCR amplifications with the same pairs of primers. Together, these experiments strongly suggest that *hiuH*, *msrP*, and *msrQ* are part of the same operon.

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| Suggested improvements? |

**Sample 9.3** 2

A previous study characterized the critical role of DnaA boxes 6 and 7 on the *oriC* of *B. subtilis* in orchestrating DNA unwinding and replication initiation ([16](https://journals.asm.org/doi/full/10.1128/msystems.00221-24?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org#core-B16)). We therefore hypothesized that replication initiation could be blocked by inducing dCas9 expression in the presence of single guide RNAs (sgRNAs) specifically designed to target the inactivated nuclease to these boxes and thereby hinder DnaA binding ([23](https://journals.asm.org/doi/full/10.1128/msystems.00221-24?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org#core-B23)) ([Fig. 1A](https://journals.asm.org/doi/full/10.1128/msystems.00221-24?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org#F1)). To this end, we constructed *B. subtilis* strains carrying a xylose-inducible dCas9 as well as constitutively expressed sgRNAbox6–7 designed to target DnaA boxes 6 and 7. In addition, strains encoding sgRNAs designed to target DnaA boxes 1 and 2 or 3 and 4 (sgRNAbox1–2 and sgRNAbox3–4, respectively) were constructed as controls ([Fig. 1B](https://journals.asm.org/doi/full/10.1128/msystems.00221-24?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org#F1); Table S1) ([16](https://journals.asm.org/doi/full/10.1128/msystems.00221-24?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org#core-B16)). This system allows conditional expression of dCas9 in the presence of xylose and tight repression when glucose is available.

To assess the impact of this blockade on replication initiation, we examined cell growth during the early logarithmic phase under dCas9 induction or repression conditions. Cells with dCas9 in the absence of sgRNA [wild-type (WT)-dCas9] exhibited normal growth behavior, indicating that dCas9 expression alone does not affect bacterial proliferation ([Fig. 1C](https://journals.asm.org/doi/full/10.1128/msystems.00221-24?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org#F1)). Furthermore, cultures repressed by glucose, containing either sgRNAbox1–2, sgRNAbox3–4, or sgRNAbox6–7 showed subtle growth variations compared with the control, while inducing dCas9 with sgRNAbox6-7 led to a significant decrease in colony forming units (CFU) per milliliter after 3 h compared to the WT ([Fig. 1C](https://journals.asm.org/doi/full/10.1128/msystems.00221-24?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org#F1)). Notably, targeting adjacent boxes by inducing the CRISPRi system did not inhibit growth ([Fig. 1C](https://journals.asm.org/doi/full/10.1128/msystems.00221-24?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org#F1)). This indicates specific competition between DnaA and dCas9 for binding to DnaA boxes 6 and 7.

The formation of the dCas9-sgRNAbox6–7 complex could specifically block the formation of DnaA filaments, preventing the opening of the replication bubble associated with initiation. These results confirm the high specificity of DnaA binding to boxes 6 and 7 and demonstrate the potential of the CRISPRi system to modulate critical steps in replication initiation, as previously reported ([16](https://journals.asm.org/doi/full/10.1128/msystems.00221-24?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org#core-B16)).

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| Suggested improvements? |

### Exercise 10B.

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Look at your thesis thus far (or any other piece of writing). Go through it, and examine your narrative/whether you have described experiments in a logical order. Consider how your writing can be improved and make the appropriate edits.

### References

1. Tschowri N, Schumacher MA, Schlimpert S, et al. Tetrameric c-di-GMP mediates effective transcription factor dimerization to control *Streptomyces* development. *Cell.* 2014;158(5):1136-1147.
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