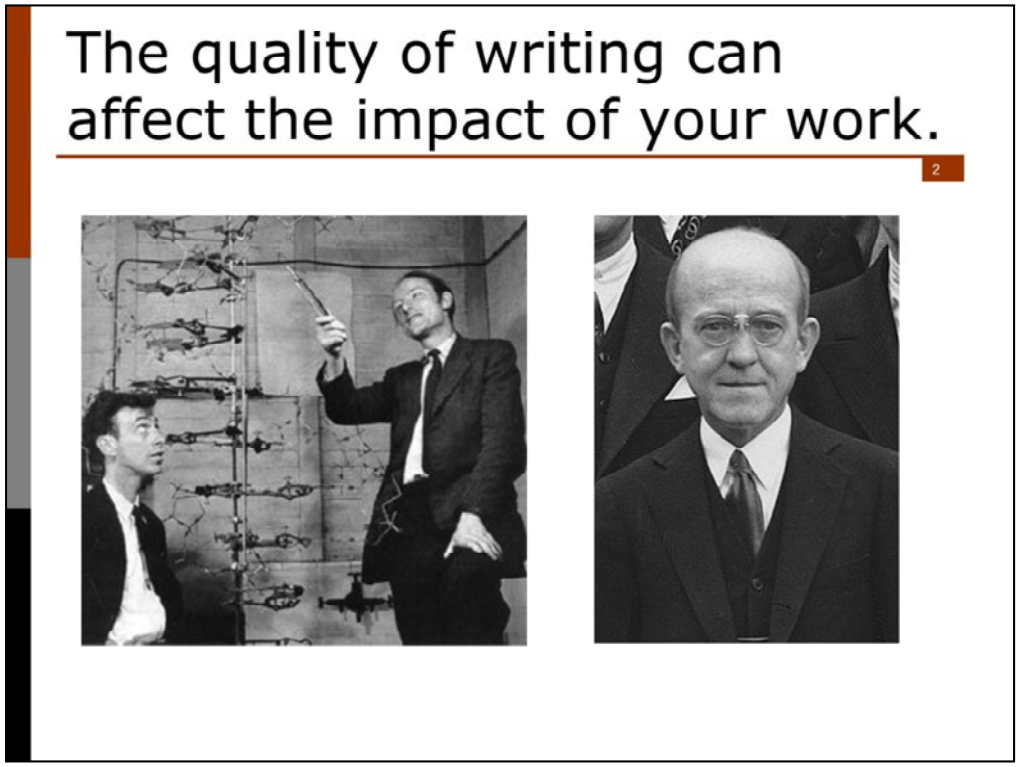
Scientific Writing

20.109
Fall 2014

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).



Watson & Crick, 1953: discovered the structure of DNA

Oswald Avery, Colin MacLeod, Maclyn McCarty, 1944: discovered that DNA is responsible for passing on heritable traits

-Long

-Difficult to read

-No claims of importance

-No confidence in work

The goal of scientific writing is to communicate ideas.

"The purpose of a scientific paper is to communicate results and analysis to the wider scientific community. The better a paper is written, the more readers it will attract and the more citations it is likely to receive."

Bredan & van Roy (2006) EMBO 7:846-9.

The IMRD structure helps you communicate effectively.

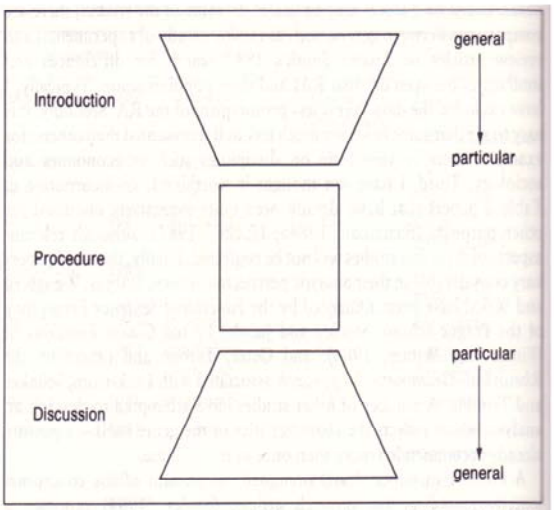


Figure 7 Overall organization of the research paper (Hill et al., 1982).

Introduction gives the context, justification, and focus.

5

- Start broadly
  - Living organisms sense and respond to their environments using an array of signal transduction systems. Better understanding of natural signaling, as well as "rewiring" systems to produce new biological functions and potential biotechnological applications, are goals of synthetic biology.
- Identify what is (un)known
  - However, the added complexity significantly complicates rational design of synthetic signal transduction pathways.
- Explain how you will address the unknown at the end
  - [These data suggest] that these bacterial components might be able to interact with plant HK components. We tested this hypothesis by heterologous expression of PhoB and OmpR in Arabidopsis.

The M&M allows replication or interpretation of your work.

6

Fluorometric GUS assays
Fourteen-day-old plants or plant tissue containing the T-DNAs …were incubated for 16 h in water (control), or water and t-zeatin. Total protein extraction and fluorometric measurements of GUS activity were performed on a DynaQuant 200 fluoro-meter (Hoefer Inc, San Francisco, CA), according to the methods described earlier (Gallagher, 1992). The 4-methylumbelliferone (4-MU) was used as a standard.

- Provide the right level of detail
- List the methods in logical order
- Use proper grammar

The Results tells a story about your data.

7

To confirm that the observed induction correlates with the cytokinin signal, we statistically analyzed our data with linear regression. A highly significant relationship was observed between cytokinin dose and GUS activity ($n=119$, $F=37.99$, $P=1.02 \times 10^{-8}$, $R^2=0.24$) (Figure 6C).

- Select data carefully
- Provide context
- Describe illustrations

Legends allow illustrations to stand on their own.

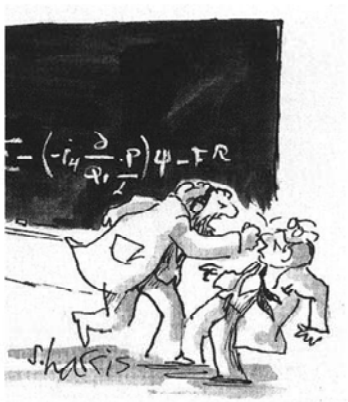
8

Figure 6. Design and function of the synthetic eukaryotic signal transduction system. (C) Linear increase in GUS activity (nmoles 4-MU mg^{-1} protein h^{-1}) with t-zeatin concentration. 4-MU, 4-methylumbelliferone.

- Describe experiment
- Explain abbrev, symbols
- Do not interpret or describe data

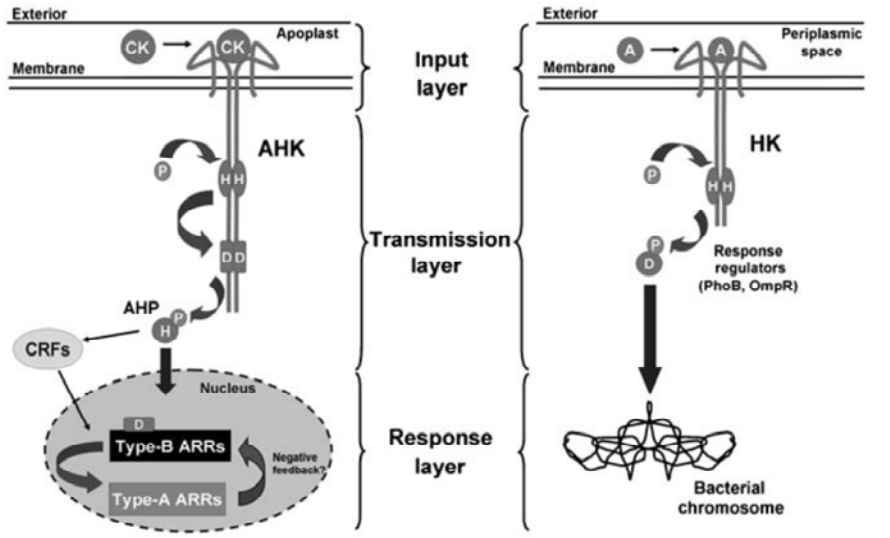
The Discussion is an argument about your data.

- Interpret data
- Explain contribution to field
- Admit limitations and flaws



"YOU WANT PROOF? I'LL GIVE YOU PROOF!"

Analyze the Discussion of Antunes et al. (2009).



Analyze the Discussion section of Antunes et al. Engineering key components in a synthetic eukaryotic signal transduction pathway. Mol Sys Biol 5:270 (2009):

1. *What is the function of the first two paragraphs?*
2. *Highlight sentences that conveys the contribution to the field*
3. *Highlight sentences that describe caveats or define unsettled points.*
4. *How does the Discussion end in the last paragraph?*

Synthetic signal transduction systems will allow us to better understand the behavior of endogenous systems and produce new types of biological sensing and responses. Earlier work toward this end used modular components from endogenous signal transduction systems to change the input–output connectivity in yeast cells (Zarrinpar *et al*, 2003; Dueber *et al*, 2004), and rational changes in protein specificity were used to rewire a bacterial two-component signal transduction system (Skerker *et al*, 2008). In higher organisms, the complexity of signal transduction processes presents a considerable challenge to design synthetic systems. The signal transduction process can be viewed as three connected functional layers: input → transmission → response (Figure 1). However, eukaryotic signal transduction systems are not linear; each layer has multiple proteins that are themselves often composed of multiple functional domains and typically encoded by multigene families.

As these complex signal transduction systems are thought to have arisen from new combinations of protein domains (Bhattacharyya *et al*, 2006), we tested whether conserved modular domains from highly evolved bacterial systems could retain functionality in a eukaryotic system. The requirement for nuclear translocation of a phosphorylated carrier protein is a key difference between bacteria and plant HK signal transduction systems. We discovered that PhoB-GFP and OmpR-GFP can translocate to the plant cell nucleus in response to a cytokinin-induced HK signal. We used this discovery, detailed knowledge about phospho-PhoB's affinity for DNA, and known DNA-binding sites to re-design the bacterial RR for eukaryotic function. A eukaryotic transcriptional activator was added to the C-terminal end of PhoB and a signal-receptive transcriptional promoter designed for plant function. The synthetic PhoB-VP64 → *PlantPho::GUS* system responded to cytokinin-mediated HK activation and expressed the GUS reporter.

The signal-dependent nuclear translocation of bacterial RR seems remarkable because bacteria do not have a nuclear compartment. To our knowledge, this is the first example in plants of proteins from non-pathogenic bacteria showing signal-dependent nuclear translocation. Although some Avr proteins from plant pathogenic bacteria localize to plant cell nuclei, these proteins have been shown to contain nuclear localization signal (NLS) sequences (Kjemtrup *et al*, 2000). The effector domain of PhoB contains an arginine–lysine-rich region that may act as a cryptic NLS with phosphorylation-dependent 'uncovering' of the DNA-binding domain. However, mutations in this region did not alter the cellular partition of PhoB-GFP in the presence or absence of cytokinin (data not shown). Therefore, PhoB does not appear to have a canonical NLS sequence. Although a complete mechanistic interpretation for this signal-dependent nuclear translocation phenomenon awaits further experimentation, our work reveals aspects about the process. PhoB-GFP and OmpR-GFP fusions accumulate in the nucleus in a signal-dependent manner not consistent with diffusion. Although it may not be possible to establish an absolute size limit, small proteins <20–40 kDa are capable of nuclear diffusion, whereas larger proteins require transport through selectivity filters provided by phenylalanine-glycine (FG) repeats in proteins of the nuclear pore complex (Sun *et al*, 2008). Our bacterial RR-GFP fusions are ~55 kDa, suggesting that they cannot diffuse into the nucleus. In addition, after cytokinin treatment, we observed nuclear accumulation. As the Arabidopsis genome has no homology to PhoB's DNA-binding sequence, the signal-dependent nuclear accumulation cannot be explained by diffusion combined with DNA affinity. Collectively, these data suggest that some type(s) of transport mechanism(s) is involved (Figure 4E–H; Supplementary Figure S2).

In non-vascular cells, the nuclear translocation largely required the signal-receptive Asp residue for both PhoB and OmpR (Figures 2 and 5; Supplementary Figure S2), implying that some aspect of the

phospho-protein is required for efficient nuclear transport. One possibility is suggested from the conformation change that PhoB undergoes with phosphorylation in bacteria (Ellison and McCleary, 2000; Bachhawat *et al*, 2005). If this or a similar conformation change takes place *in planta*, the receiver domain of PhoB becomes more exposed. As PhoB's receiver domain has homology to plant receiver domains, plant machinery could recognize and transport the phosphorylated PhoB to the nucleus. In response to exogenous cytokinins, cortical cells showed variable and sporadic nuclear localization of the mutant PhoB^{D53A}-GFP, and vascular cells accumulated PhoB^{D53A}-GFP to some extent (Figure 5C–F). These observations suggest that there could be various inefficient means by which PhoB is translocated to the nucleus, or that PhoB can be phosphorylated at other residues in plants.

In bacteria, PhoB is known to undergo a conformational change with phosphorylation that significantly increases affinity of this protein for its target DNA sequence, the *Pho* box (Blanco *et al*, 2002; Bachhawat *et al*, 2005). We engineered our eukaryotic PhoB-responsive promoter with four *Pho* boxes located upstream of a minimal transcriptional promoter (−46 *CaMV35S*) (Benfey *et al*, 1989). We chose four PhoB-binding sites based on other plant-inducible transcription systems that use prokaryotic DNA-binding proteins (Padidam, 2003; Moore *et al*, 2006). Experimentally determining the optimal number of Pho boxes in the *PlantPho* promoter may lead to an improved PlantPho system.

By combining PhoB-VP64 with the *PlantPho* promoter, we constructed a synthetic eukaryotic signal transduction system (PlantPho system). Activation of endogenous plant HKs with increasing concentrations of the cytokinin *t*-zeatin resulted in a near linear increase in GUS activity (Figure 6B and C). The PlantPho system showed high un-induced GUS levels with variability at each cytokinin level tested (Figure 6B and C). This may result from activation of the synthetic system by endogenous cytokinin along with accumulation of the highly stable GUS in the 2-week-old plants assayed. Also, because vascular tissues are highly sensitive to cytokinin (Moritz and Sundberg, 1996; Brugiere *et al*, 2003; Aloni *et al*, 2005; Hutchison *et al*, 2006; Kuroha *et al*, 2006; Mahonen *et al*, 2006), and entire plants were assayed, the vascular tissues could have high GUS levels even without induction. Consistent with this hypothesis, we observed that both PhoB-GFP and OmpR-GFP accumulated in the nucleus of vascular cells before exogenous cytokinin application (Figure 4; Supplementary Figure S2). As vascular cells already have some nuclear-localized PhoB before cytokinin application, a signal-dependent increase would be difficult to see in these cells. Our system depends on promiscuous cross talk (Supplementary Figure S7) and does not create a privileged signal transduction system, in which one input produces one specific response. As such, in addition to endogenous cytokinins, cross talk from other plant HK systems, such as ethylene (Grefen and Harter, 2004), could also contribute to the high background in GUS activity.

Here, we show that synthetic eukaryotic systems can be produced by using conserved components from prokaryotic systems, taking advantage of the cross talk from conserved bacterial HK systems. Remarkably, this heterologous cross talk is so highly conserved that plant two-component signal transduction components can function in bacteria (Suzuki *et al*, 2001; Spichal *et al*, 2004; Romanov *et al*, 2005) and bacterial components in plants (this study). It is tempting to speculate that cross talk coupled with horizontal gene transfer is a conserved mechanism by which new signal transduction systems evolve. In this model, nascent systems are initially promiscuous and later become more specialized, not unlike the theory of new enzyme function (Kraut *et al*, 2003). On one hand, the ability to establish new connectivities from bacteria in a higher eukaryote is remarkable. It will be interesting to determine whether such adaptation of other conserved signal transduction components and/or components from other highly evolved systems can function in other eukaryotic systems. The Pho system itself would likely function in yeast, which has conserved HK components, whereas mammalian cells may require a better understanding of the nuclear translocation process. On the other hand, it is also equally clear that the system is far from optimal. The possibility of experimentally controlling signal transduction systems provides a useful tool for plant and other biological studies, as it provides a means to control input and response. This approach, along with a simple readout system (Antunes *et al*, 2006), may also allow us to develop plant sentinels that can detect chemical threats and pollutants (Looger *et al*, 2003).

The Discussion resolves issues of the Introduction.

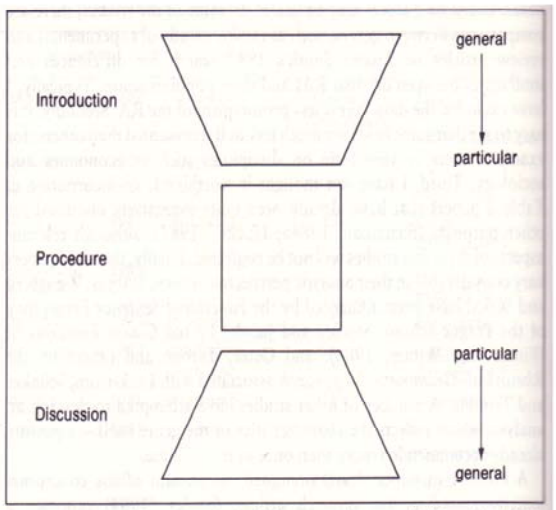


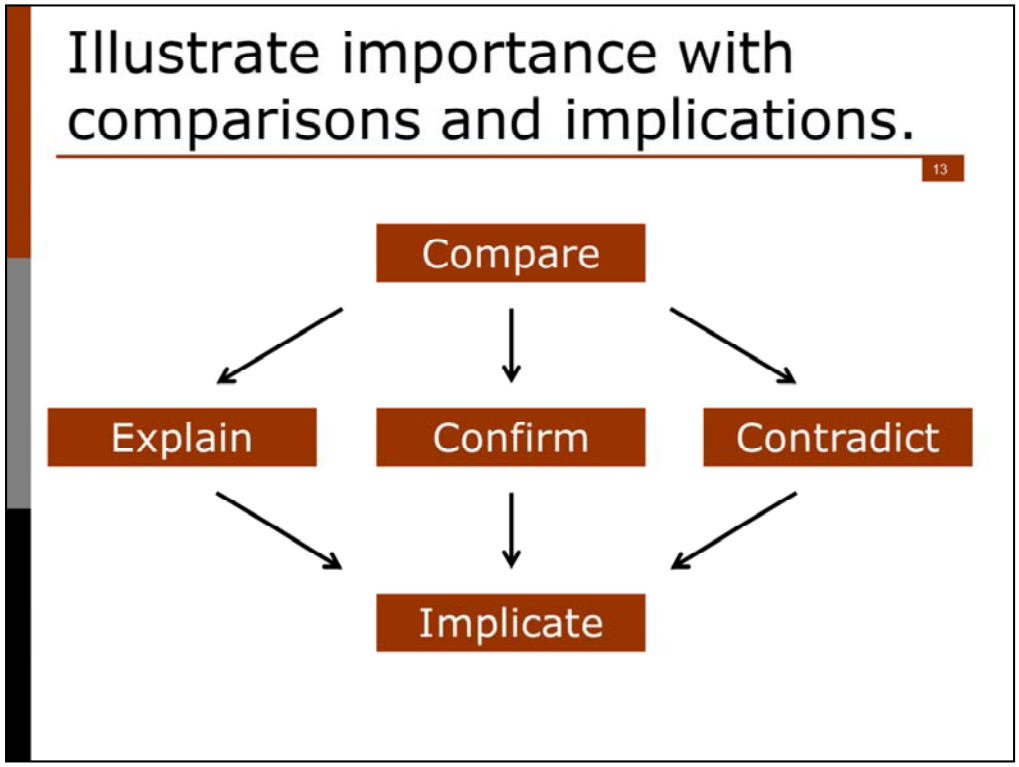
Figure 7 Overall organization of the research paper (Hill et al., 1982).

Begin by reminding reader of focus and key findings.

12

Synthetic signal transduction systems will allow us to better understand the behavior of endogenous systems and produce new types of biological sensing and responses...In higher organisms, the complexity of signal transduction processes presents a considerable challenge to design synthetic systems. The signal transduction process can be viewed as three connected functional layers: input → transmission → response (Figure 1). However, eukaryotic signal transduction systems are not linear; each layer has multiple proteins that are themselves often composed of multiple functional domains and typically encoded by multigene families.

As these complex signal transduction systems are thought to have arisen from new combinations of protein domains (Bhattacharyya *et al*, 2006), we tested whether conserved modular domains from highly evolved bacterial systems could retain functionality in a eukaryotic system. The requirement for nuclear translocation of a phosphorylated carrier protein is a key difference between bacteria and plant HK signal transduction systems. We discovered that PhoB-GFP and OmpR-GFP can translocate to the plant cell nucleus in response to a cytokinin-induced HK signal. We used this discovery, detailed knowledge about phospho-PhoB's affinity for DNA, and known DNA-binding sites to re-design the bacterial RR for eukaryotic function. A eukaryotic transcriptional activator was added to the C-terminal end of PhoB and a signal-receptive transcriptional promoter designed for plant function. The synthetic PhoB-VP64 → *PlantPho::GUS* system responded to cytokinin-mediated HK activation and expressed the GUS reporter.



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.

This paragraph structure helps highlight importance.

14

The signal-dependent nuclear translocation of bacterial RR seems remarkable because bacteria do not have a nuclear compartment. To our knowledge, this is the first example in plants of proteins from non-pathogenic bacteria showing signal-dependent nuclear translocation. Although some Avr proteins from plant pathogenic bacteria localize to plant cell nuclei, these proteins have been shown to contain nuclear localization signal (NLS) sequences (Kjemtrup *et al*, 2000). The effector domain of PhoB contains an arginine–lysine-rich region that may act as a cryptic NLS with phosphorylation-dependent 'uncovering' of the DNA-binding domain. However, mutations in this region did not alter the cellular partition of PhoB-GFP in the presence or absence of cytokinin (data not shown). Therefore, PhoB does not appear to have a canonical NLS sequence.

Claim

Evidence

Analysis

Caveats and uncertain points can be briefly described.

15

Although a complete mechanistic interpretation for this signal-dependent nuclear translocation phenomenon awaits further experimentation…

[It] is also equally clear that the system is far from optimal…

End by reiterating overall conclusion and impact.

16

Here, we show that synthetic eukaryotic systems can be produced by using conserved components from prokaryotic systems, taking advantage of the cross talk from conserved bacterial HK systems..[The] ability to establish new connectivities from bacteria in a higher eukaryote is remarkable. It will be interesting to determine whether such adaptation of other conserved signal transduction components and/or components from other highly evolved systems can function in other eukaryotic systems…The possibility of experimentally controlling signal transduction systems provides a useful tool for plant and other biological studies, as it provides a means to control input and response…

In sum, understand IMRD to improve scientific writing.

17

- Introduction: What did you know?
- M&M: What did you do?
- Results: What did you see?
- Discussion: What is the significance?

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