Information processing in recurrent gene regulatory networks

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Introduction

In order for a cell to survive, it must adapt accordingly to the environment it is in. The cellular environment is inherently dynamic due to constant changes in the surrounding conditions. Therefore, cells require continuous processing of information to adapt to these changes. Adaptation can be achieved by regulating expression of certain genes. Studies in *E. coli* suggested that genes can be classified as either "reservoir" or "readout" genes. The reservoir contains cyclical pathways, while the readout, which is found downstream of the reservoir genes, acts in a linear fashion: information kept in the reservoir is transmitted into the readout in an orderly fashion, which avoids fatal interference (Figure 1). Because of this genetic organization, it is postulated that the cell is able to exhibit "memory" i.e. the cell is capable of recognizing an input from the past. The goal of this project is to try and prove this idea of genetic organization and to validate the role of recurrence in the processing of information by gene regulatory networks

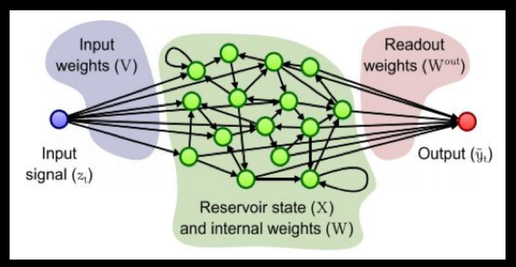

Figure 1[1] – depiction of the cyclical pathways

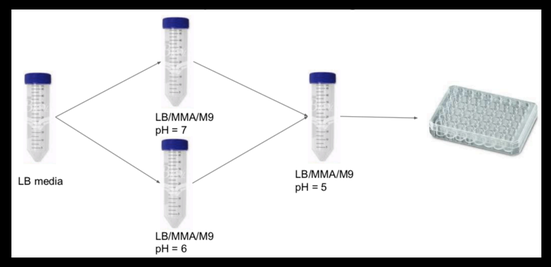

Figure 2- general outline of the experiment

Methods

1) First, we had to built a library of regulatory networks of synthetic recurrent genes in E. coli. We concentrated on six genes of interest: gadX, gadE, asnB, flhD, ompR and omrA. These genes were chosen as some are found in the reservoir state (gadX, gadE, flhD and ompR) while others are in the readout state (asnB and omrA). Furthermore, these genes respond to environmental changes which are easy to manipulate in the lab (eg. pH and osmolarity), thus making the experimental process easier to conduct.

2) Four plasmids were constructed and were used to transfect bacterial strains: PgadX-PgadE, PgadX-PasnB, PompR-PflhD and PompR-PomrA. To obtain these products, we had to insert the promoters (P) of the genes of interest into the pMS201 plasmid. Measurement of gene activity required adding a fluorescent marker (GFP/RFP) to the promoters of the genes of interest in order to obtain a signal reporter of the promoter activity.

- PgadX-PgadE: transcriptional activators. Control the expression of pH-inducible genes[2]
- PgadX-PasnB: asnB catalyses ATP-dependant conversion of aspartate into asparagine[3]
- PompR-PflhD: ompR plays a role in osmoregulation by altering the concentration of porines (ompC and ompF)[4]. flhD is a regulatory protein[5].
- PompR-PomrA: ompR controls the transcription of omrA, a small piece of RNA which is important for controlling the protein composition on the membrane of *E. coli* cells[6]

3) To see how gene activity changes, we incubated the *E. coli* within different medias (LB, MMA and MM9) which had different pH/osmolarity. Once incubated with these medias, they were placed in a 96-well microfluidics plate and were analysed by a spectrofluorometer (outline shown in Figure 1)

References
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Results

- Subjecting gadX to changes in pH, we got the results shown in Figure 3

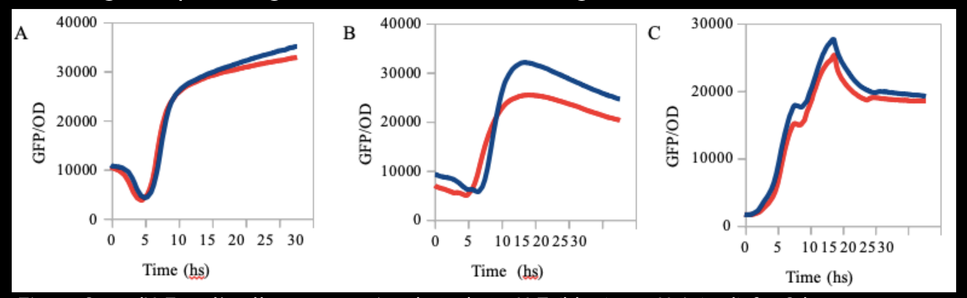

Figure 3- gadX *E. coli* cells were pre-incubated at pH 7 (blue) or pH 6 (red) for 3 hours and were subsequently placed in one of 3 kinds of pH 5 medium

- Subjecting ompR (D), gadE (E) and omrA (F) to environmental changes resulted in the results shown in Figure 4

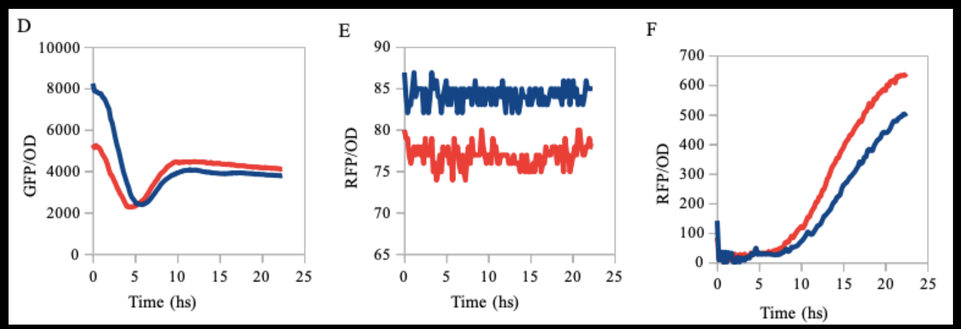

Figure 4- gadE was challenged with a change in pH. ompR and omrA were challenged with a change in osmolarity (adding 20% sucrose). All cells were grown in MMA

Discussion and Conclusion

i. By growing gadX *E. coli* in the different medias, surprisingly, there were different results. Specifically, the greatest difference was seen in MMA. In LB and MMA, expression of gadX falls abruptly. However, after incubation with pH5 mediums, activity increases dramatically until the bacteria reach a stationary phase. As for MM9 mediums, bacteria experience little lag phase. Following this, gadX expression is marked by 2 stages of sudden activation: one at 8h and the other several hours after. This may be due to a change in carbon sources used by the *E. coli* cells.
From these results, it can be seen that only a significant history-dependant behaviour was observed in the MMA medium. For the other two medias, the difference between pH7 and pH6 cells did not show enough of a difference

ii. On the other hand, gadE activity does not change when there is a challenge in pH. Activity remains constant throughout time. ompR expression is high at the start but then decreases until reaching its lowest at 5h. Activity then increases and remains constant at 10h. Finally, omrA activity increases, even when there is no change in osmolarity. However, it is expressed more when there is an increase in osmolyte concentration
From these results, there is little evidence of history dependant behaviour. ompR and omrA do not show enough of a difference and there is no difference between pH7 and pH6 cells for gadE. Perhaps the test should be conducted again, as research has shown that gadE activity depends on glutamate concentration[7]. Thus, addition of this amino acid could help with retrieving better results.

iii. In conclusion, we have demonstrated that history dependant behaviour is possible in the selected *E. coli* strains. gadX *E. coli* were the only ones that showed this, however by redefining and perfecting our methodology we could retrieve better results from gadE by using glutamate in the media. The application of this research can be used for better understanding neuronal networks in our brain as there is similarity between the feedback mechanisms in the two systems. By using genes, it is much easier to observe this recurrence as there are a few thousand genes compared to billions of neurons