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Real-time flagellar gene expression

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Abstract

Time traces of GFP fluorescence expressed under the control of flagellar promoters have been used to order flagellar gene expression events in real time.

Significance and context

The study of flagellar assembly in *Escherichia coli* began as a classical genetics question, but remains of biochemical interest today. One modern twist has been to study the order of expression of flagellar genes using DNA microarrays or *lacZ* reporter assays. Kalir *et al*. revisit the ordering problem with an *in vivo* assay for the expression of genes under the control of flagellar promoters using green fluorescent protein (GFP). Their results are more precise than those from previous studies and agree remarkably well with the known structure of the flagellum.

Key results

The authors transfected *E. coli* with plasmids encoding the amino-acid sequence of GFP, under the control of the promoter from a given flagellar operon. They diluted cultures out of stationary phase (in which bacteria do not manufacture new flagella) and measured GFP fluorescence over time from this zero point. After subjecting the time traces to a clustering algorithm, the authors ordered the clusters according to time of activation of the fluorescence curves. The cluster containing *fliL* and *fliE* - operons coding for the basal, intracellular components of the flagellum - reached high fluorescence levels first. Intermediate flagellar components were next; extracellular components were last, along with the 'navigation' elements *meche* and *mocha*, which operate in chemotaxis.

Links

The algorithm for ordering fluorescence traces is available at the home page of Uri Alon.

Conclusions

Kalir *et al*. conclude that the expression sequence of flagellar operons follows the flagellar structure itself. But this may not be the whole story. Previous work has shown that a flagellum can assemble even when expression order is scrambled. Kalir *et al*. suspect that the synchrony seen here may hinge on the dilution step of their experiment, in which the population of cells goes through a concerted change in medium conditions which may activate wholesale flagellar production.

Reporter's comments

Kalir *et al*. appear to have nailed down the expression order of flagellar operons. There are caveats, however. If the authors used a different clustering or ordering scheme, for example, or retooled the experiment to eliminate the dilution step, would they get the same results?

Table of links

Science

Uri Alon

References

1. Kalir S, McClure J, Pabbaraju K, Southward C, Ronen M, Leibler S, Surette MG, Alon U: Ordering genes in a flagella pathway by analysis of expression kinetics from living bacteria. Science. 2001, 292: 2080-2083. 0036-8075