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2D gel analysis of *E.coli* global protein change during GroELS depletion.

Chaperonin proteins help other proteins to fold. Although GroE (the *Escherichia coli* chaperonin) is abundant, calculation¹ suggests that there is only enough present in a cell to interact with 10% of newly synthesised proteins.

By replacing the native *groE* chromosomal promoter region with the *araC* Gene and *araBAD* promoter, a strain was created which depended on arabinose for continual viability and GroE production². Following arabinose removal, GroE levels halve with each generation. Growth remains exponential for about 1.5 hours and then continues at a decreased rate for about another hour, after which the cells abruptly lyse. This was found to be caused by a decrease in the activity of the lysine biosynthetic enzyme DapA. A product of this pathway, DAP is necessary for peptidoglycan synthesis, and thus a decrease in DapA led to cell lysis.

While the addition of DAP to growth medium prevents lysis, its addition to solid medium does not support colony formation, suggesting that GroE is essential for processes other than cell wall synthesis.

GroE depleted, DAP compensated cells form filaments, and we wished to study changes in protein levels in these cells by two dimensional electrophoresis. Cells were pulse labelled with ³⁵S-Methionine (100mCi/ml) and then separated in the first dimension on an ImmobilineTM drystrip 3-10NL 13cm, then run in the second dimension on a 10% polyacrylamide gel (1mm thick).

Unfortunately the filament phenotype when the cells were grown in Methionine deficient media was different than when the cells were grown in Lauria Broth (LB), our media of choice. Yet it was not possible to ³⁵S-Met. label the cells in LB, as there was already methionine in the media. A typical LB sample labelled for 15 minutes with 100mCi/ml ³⁵S-Met. gave no result when visualised on a Molecular Dynamics storage phosphor screen. Yet the same sample, when used in conjunction with EA-Wax and put on X-ray film for three days, gave excellent results. Individual proteins, both of high and low abundance are easily discernible and most importantly the results are reproducible. By using EA-Wax we are now able to determine global protein changes in *E.coli* when GroE depleted.

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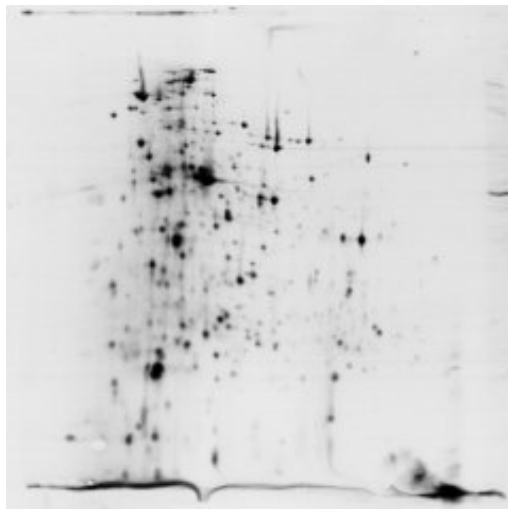
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Ref:

1 Lorimer, G.H. *FASEB J.* **10**, 5-9 (1996)

2 McLennan, N, and Masters, M. *Nature*, **392**, 139 (1998)



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