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The Effect of *rpoH* for Heat Shock Gene Expression on Plasmid Transformation

Lloyd Yoo
Department of Microbiology & Immunology, UBC

The heat shock method of transformation is done by rapidly heating cold cells. The rpoH gene encodes an RNA polymerase that is highly expressed under heat shock and transcribes downstream heat shock proteins. Transformation efficiencies of two strains of bacteria were compared with the single difference between the two strains being a temperature sensitive mutation in the rpoH gene in order to assess whether any of the genes induced by the heat shock cascade were important for the transformation process. It was expected that there would be a much higher transformation efficiency in the wild type strain over the mutant strain if the heat shock proteins aided the transformation process. While the mutant strain was unable to grow in high temperatures that the wild type could survive in, the effect of the rpoH gene expression in aiding transformation efficiency was unclear. There was a slightly greater transformation efficiency of the wild type over the mutant strain, but it was not a dramatic difference. Therefore, either the rpoH gene was expressing well enough in the mutant strain or the downstream heat shock proteins do not have a strong effect in aiding transformation.

Bacterial plasmid transformation is a process by which genetic material, often a plasmid, is inserted into a bacterial cell. Though transformation does occur naturally, scientists have developed many techniques to cause DNA uptake by bacterial cells. In order for cells to take up genetic material, they must first be made competent so that they more readily take in the DNA through their membranes. Once cells are made competent they are transformed by keeping the cells and plasmids on ice for a period of time, followed by heat shock, then cold shock to induce plasmid uptake by the cells. The rapid change of temperatures is largely attributed for causing membrane fluidity changes allowing DNA molecules to enter the cell. However, the same temperature changes are also known to cause the expression of certain genes, some of which are transcription factors. The effect of these cascades on the transformation is largely unknown.

The rpoH gene encodes the RNA polymerase σ^{32}. This RNA polymerase is expressed at much higher levels upon heat shock and is thought to be responsible for the expression of heat shock proteins and the heat shock response (1,3,4,6). Therefore it was proposed that the effect of heat shock proteins and their cascades could be examined by transforming a wild type (WT) *Escherichia coli* and a mutant strain with a mutation in the rpoH gene. Two strains of bacteria were found with the only difference being a mutation in the rpoH gene. KY1445 strain was designated as the wild type, and KY1429 was the strain with a mutation in the rpoH gene, rendering it temperature sensitive (7). The expectation was that with a temperature sensitive rpoH gene, at high temperatures it would not express properly, and thus all the downstream heat shock proteins would not be expressed, or would be poorly expressed. The differences between the two strains, post transformation, would be compared by examining their respective transformation efficiencies. It was expected that the KY1429 strain would have a significantly lower transformation efficiency if the rpoH ws involved in the competence development.

MATERIALS AND METHODS

Bacteria and media. The isogenic bacterial strains used were *E. coli* KY1445 which has the genotype F-, *[araD139]$_{B/r}$, Δ(argF-lac)169, λ$^-$, flhD5301, Δ(fruK-yeiR)725(fruA25), relA1, rpsL150(strR), zhh-50::Tn10, rbsR22, Δ(fimB-fimE)632(::IS1), deoC1* and *E. coli* KY1429 which has the genotype F-, *[araD139]$_{B/r}$, Δ(argF-lac)169, λ$^-$, flhD5301, Δ(fruK-yeiR)725(fruA25), relA1, rpsL150(strR), zhh-50::Tn10, rpoH606(ts), rbsR22, Δ(fimB-fimE)632(::IS1), deoC1*. LuriaBertani (LB) Lenox media was used for all growth media in this experiment. 10.0 g/L tryptone, 5.0 g/L yeast extract and 5.0 g/L of NaCl in water for broth, and 15.0 g/L of agar for the LB plates. For plasmid uptake verification, Amp^R was used to select bacteria, and ampicillin was added to 0.1 mg/ml in the media.

Plasmids. The Amp^r containing plasmids pBR322 and pUC19 were grown up in *E. coli* and isolated by Invitrogen PureLink™ HiPure Maxiprep Kits and stored in water at 4°C. The plasmid DNA was supplied by MICB 447 student Nathan Plaa.

Viability test. Both strains were tested for viability following heat shocking for different time intervals. The cells were shocked at 42°C for 0 seconds, 45 seconds, 1 minute, 2 minutes and 4 minutes, then the heat shocked bacteria were spread plated on LB agar plates and grown up at 25°C over 48 hours. The number of resulting isolated colonies were counted.

Preparation of competent cells by $CaCl_2$ method and transformation. Single colonies of KY1445 and KY1429 were each inoculated into 500ml of LB Lenox media and grown until a turbidity of just under 0.5 OD_{600} was reached. Culture broth centrifuged in pre-chilled Oakridge tubes at 2,700g for 10 minutes at 4°C. $CaCl_2 \bullet 2H_2O$ (0.1 M) and $MgCl_2$-$CaCl_2$ (80 mM $MgCl_2$, 20 mM $CaCl_2$) are both heat stable and thus autoclaved. DMSO was filter sterilized through a 0.45μ filter. Protocol 25 of Molecular Cloning was used for competent cell preparation (6). Transformation were done by cooling competent cells with plasmids on ice for 20 minutes followed by 42°C heat shock for 45 seconds then by cold shock on ice for 2 minutes.

RESULTS

The temperature sensitivity of the KY1429 mutant strain was inadvertently tested when single colonies of both the KY1445 and KY1429 strains were inoculated into LB Lenox broth that was not yet cooled to room temperature after being autoclaved. The temperature of the LB Lenox broth when incoulated was approximately 40-50°C and after being grown up at 25°C, 200 RPM, overnight, only the KY1445 strain showed turbidity as a result of growth. The broth containing the KY1429 strain was still clear. Ampicillin resistance for both strains was verified to ensure that only transformed cells would grow in the selective media. Both the KY1445 and KY1429 strains were streak plated onto LB+ampicillin plates and grown over 48 hours at 25°C. No colonies could be found on the LB+ampicillin plates after the incubation period.

A preliminary viability test was performed to determine if the heat shock would affect the viability of the temperature sensitive KY1429 mutant strain. Following the different heat shock treatments of 30 seconds, 1 minute, 2 minutes and 4 minutes at 42°C, the spread plated cells were grown over 48 hours at 25°C. The plates all exhibited too many colonies to count, and from a visual analysis, no distinguishable differences could be seen between the wild type and mutant strains.

The cells were then made competent by the $CaCl_2$ method to allow for plasmid transformation. Because the process of making competent cells results in some physiological differences, a viability test was repeated. First the competent cells were diluted 1:1x10^6 in LB media to reduce the colony count, which would allow a more comparable analysis.

The competent cells were then transformed with 0.16 μg/ml of pUC19 plasmid and 0.06 μg/ml of pBR322 plasmid, spread plated and grown over 48 hours at 25°C. The KY1429 had 25 and 7 colonies respectively for pBR322 and pUC19 transformations. The KY1445 had 62 and 123 colonies respectively for pBR322 and pUC19 transformations. There was some contamination detected in three of the four plates.

Table 1. The number of isolated colonies of competent cells on LB plates after varying time lengths of heat shocks at 42°C.

Duration of Heat Shock (seconds)	Observed colony count for KY1429 strain	Observed colony count for KY1445 strain
0	59	178
45	228	165
60	288	194
120	406	94
240	317	207

DISCUSSION

When examining the results of bacterial growth by colony count, there are some limitations. Because spread plating was employed, there is an estimated number of bacteria that are plated which can be calculated from the concentration of the stock of bacteria. However, although the stock was vortexed prior to taking an aliquot of bacteria to plate, there was a significant degree of difference from one aliquot to the next. This was apparent when the viability test was performed on the competent cells. The standard deviation of number of colonies for the KY1445 strain was about 44 and 129 for the KY1429 strain. It becomes difficult to determine differences between strains because the differences within the same strain are so large. This was likely not due to the heat shock process because the KY1429 temperature sensitive strain has the lowest colony count at 0 seconds of heat shock (negative control). There was no discernable correlation between viability and heat shock at 42°C for up to 4 minutes. However, the temperature sensitivity of the KY1429 strain was seen when both strains were inoculated in 40-50°C LB broth and only the KY1445 strain showed significant growth. From data, we can see that the KY1429 strain is temperature sensitive at temperatures higher than 42°C or longer than 4 minutes in 42°C. The lack of growth in the LB broth showed that many of the KY1429 cells were killed as a result of the initial high temperature, as there was little growth in the media after it had cooled down and was incubated at 25°C, 200 RPM. Therefore, the rpoH gene has some function regarding the viability of cells at high temperatures. Although rpoH is constitutively expressed in regular temperature conditions, of the three promoters for rpoH, only one is expressed in heat shock, which results in much higher levels of rpoH gene expression (2). Thus the viability is dependent on a high number of rpoH gene products.

When comparing the number of colonies counted for the competent cell count and for the viability test, it can

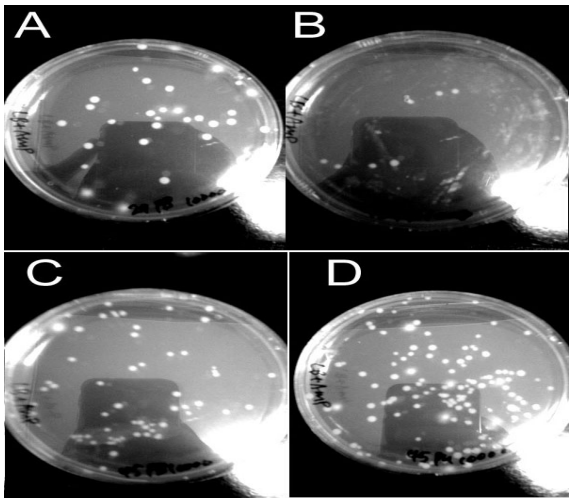

FIG 1. Transformed competent cells with different dilutions of plasmids. A. Strain KY1429 with 0.06 μg/ml of pBR322 plasmid. B. Strain KY1429 with 0.16 μg/ml of pUC19 plasmid. C. Strain KY1445 with 0.06 μg/ml of pBR322 plasmid. D. Strain KY1445 with 0.16 μg/ml of pUC19 plasmid.

be seen that the mutant KY1429 competent cells had a higher bacterial concentration. The $1{:}1x10^6$ dilutions of both strains of competent cells resulted in 184 colonies counted for the plates with KY1429 cells and 62 colonies for the KY1445 strain. Similarly, in the viability test of the competent cells, KY1429 cells had on average 260 colonies/plate against 170 colonies/plate for KY1445 cells. Due to the high degree of variation, it was difficult to determine the concentrations of the competent cells, but there seemed to be a higher concentration of cells in the KY1429 competent cells though both strains were made competent at an absorbance of 0.45 OD_{600} and 0.45 OD_{600} for KY1429 and KY1445 strains respectively. However, when observing the transformation results, there was a 2.5 fold higher number of colonies in the wild type strain for pBR322 transformations and a 18 fold higher number of colonies in the wild type strain for pUC19. This difference could be a direct result of the presence of the fully functional rpoH gene. The same diluted DNA was used for both strains of bacteria, thus it would be unlikely that such a large variety is due variations in DNA concentration. It should be noted however, that there was some fungal contamination on all the transformations except the plate with the KY1429 strain with pUC19. The contamination was likely from the sterilized water that was used to dilute the plasmid DNA. The contamination could have competed with the transformed bacteria for nutrients, however it should be noted that the plate with a KY1429 strain and pUC19 had no contamination, yet still had a significantly lower colony count compared to any of the other transformations. The difference that exists in colony number between the pBR322 and pUC19 transformations might be attributed in part to the concentrations of plasmid used, as the concentrations were determined by $OD_{260/280}$, the concentrations reflect the number of base pairs, but not necessarily moles of DNA. The differences in transformation levels may also be a direct correlation to the content or shape of the plasmid itself and the plasmid's ability to cause transformation.

In this experiment, the effect of the rpoH gene expression under heat shock was examined by looking at the resulting transformation efficiency of the cells. However, the results were very vague as a result of variability, which made it difficult to make a direct correlation between heat shock transformation and the rpoH gene expression. Despite the higher transformation efficiency, it does not seem to correspond directly with the dramatic increase of σ^{32} proteins that occurs as a result of heat shock. The σ^{32} factor is known to increase from 50 molecules per cell to 3000 molecules per cell after a heat shock from 30°C to 42°C[4]. Therefore, the effects of rpoH expression may be essential for cells surviving heat shock, but the proteins that results from heat shock cascades may not have as large of a role in the transformation process. The effects of proteins from heat shock cascades may have a role in increasing transformation efficiency of competent cells, but the results of this experiment are not conclusive enough to confirm this. It is unknown whether the rpoH was not inactivated enough in the mutant strain, or if the resulting downstream proteins did not have much of an effect on the transformation process.

FUTURE EXPERIMENTS

Due to the large level of variation found in this experiment, future experiments could include larger sample sets to confirm the results with higher confidence. Looking at gene expression downstream of the heat shock cascade, heat shock proteins such as hsp60 as well as SOS signal proteins such as recA, could also be a more accurate way of determining which proteins have functions that aid in transformation. Confirming the level of rpoH expression could be another consideration to make sure the downstream results correlate to an effect of rpoH expression.

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