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## Transposon-mediated directed mutation in *E. coli*

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*Escherichia coli* cells deleted for the cyclic AMP (cAMP) receptor protein (Crp) gene (Δcrp) cannot utilize glycerol because cAMP-Crp is a required activator of the glycerol utilization operon, glpFK. We have previously shown that a transposon, Insertion Sequence 5 (IS5), can insert into the upstream regulatory region of the operon to activate the glpFK promoter and enable glycerol utilization. GlpR, which represses glpFK transcription, binds to the glpFK upstream region near the site of IS5 insertion and inhibits insertion. By adding cAMP to the culture medium in ΔcyaA cells, the cAMP-Crp complex, which also binds to the glpFK upstream regulatory region, inhibits IS5 hopping into the activating site. Control experiments show that the frequencies of mutations in response to cAMP were independent of parental cell growth rate and the selection procedure. These findings led to the prediction that glpFK-activating IS5 insertions can also occur in wild-type (Crp+) cells under conditions that limit cAMP production. Accordingly, IS5 insertion into the activating site in wild-type cells is elevated in the presence of glycerol and a non-metabolizable sugar analogue that lowers cytoplasmic cAMP concentrations. The resultant IS5 insertion mutants arising in this minimal medium become dominant constituents of the population after prolonged periods of growth. Thus, DNA binding transcription factors can reversibly mask a favored transposon target site, rendering a hot spot for insertion less favored. Such mechanisms could have evolved by natural selection to overcome environmental adversity. We have further shown that IS elements can insert upstream of the flagellar master regulator operon, flhDC, to activate transcription in a process that depends on viscosity (agar concentration). Documentation of these processes shows that IS elements can direct mutations (IS insertions) to specific sites in response to environmental stress.

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