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Short Communication

The Arabinose C Gene Product of *Escherichia coli* B/r is Hyperlabile in a Cell Free Protein Synthesis System

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Summary. Extreme instability of the *ara* activator (*ara*C gene product) of *Escherichia coli* in a cell free protein synthesis system explains why a mutant activator that produces constitutivity of the arabinose operon *in vivo* does not do so *in vitro*.

The arabinose operon of *Escherichia coli* is positively regulated *in vivo* and *in vtiro* by the product of the *ara*C gene, *ara* activator (Sheppard and Englesberg, 1967; Greenblatt and Schleif, 1971). Mutations in the C gene producing resistance to the induction inhibitor D-fucose are designated C^c and frequently lead to constitutive synthesis of the enzymes of the operon. Greenblatt and Schleif (1971) found the one C^c activator they tested was fucose resistant and constitutive *in vivo* but *in vitro* was fucose resistant and *not* constitutive.

We find *ara* activator is unstable when isolated from cells (half life about 4 to 20 hrs.) but is partially stabilized by arabinose (half life about 10 to 40 hrs.). Other workers have also seen stabilization of *ara* activator by arabinose (Jack Greenblatt, unpublished experiments; Yang and Zubay, 1973). This observation suggests C^c activator could be constitutive in a cell free protein synthesis system, but rapidly becomes inactive in the absence of stabilizing arabinose. Our data presented below shows this to be the case.

When added to the cell free protein synthesis system *ara* activator is very unstable both at 0° C and at 37° C unless arabinose is present. Fig. 1 shows the loss of stimulating activity of C^c activator protein in the cell free protein synthesis in the presence and absence of arabinose. The protein synthesizing system itself is very stable at 37° C as shown by its undiminished activity whenever activator is added. The system containing C^c activator and arabinose loses its ability to synthesize ribulokinase with a half-life of about 15 min measured either by restoration of missing 3′, 5′ cyclic AMP or addition of chloramphenicol to a complete system. However if arabinose is omitted from the system and restored at various times, ribulokinase synthetic activity is lost with a half-life of about 1.2 min. Wild type activator is similarly unstable.

Without arabinose or fucose this C^c protein in a very active cell free system does lead to L-ribulokinase synthesis at 10–15% of levels found in the presence of arabinose, presumably synthesis initiated before the activator's untimely death. No activity is detected with wild type activator in the absence of arabinose. Thus

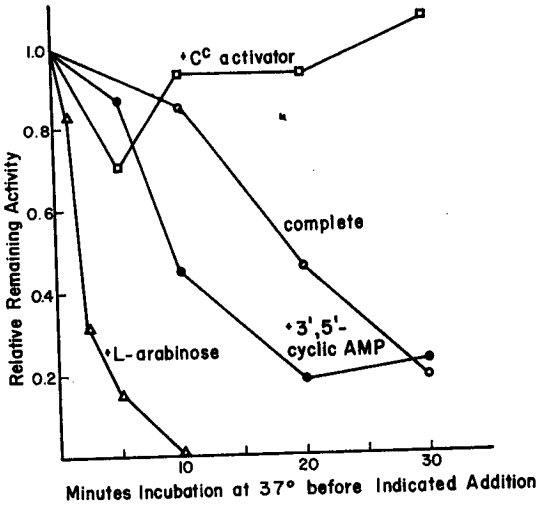

Fig. 1. Loss of L-ribulokinase synthesizing activity of the cell free protein synthesis system as a function of time at 37° C. The cell free protein synthesis system and L-ribulokinase assay are as described by Lis and Schleif (1973). Complete mixtures, or samples lacking L-arabinose Δ, 3′, 5′-cyclic AMP ●, or C^c protein □, were incubated at 37 °C, and at the indicated times the missing component added to complete the systems. Samples were incubated for 30 min after completion. Data is plotted to show the activity of samples completed at various times, relative to the activity of samples complete from zero time. The instability of C protein in the complete system (○) is plotted as the relative residual capacity for synthesis, measured as the difference between synthesis achieved at various times (stopped by 100 μg/ml chloramphenicol) and that at 70 min incubation

the arabinose requirement for full induction *in vitro* of this C^c activator protein is due to its instability in the absence of arabinose.

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