BipA is required for growth of *Escherichia coli* K12 at low temperature

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**Abstract** The *bipA* gene encodes a ribosome-associated GTPase postulated to be involved in regulatory functions in enteropathogenic *Escherichia coli*. Previous studies demonstrated that BipA is tyrosine phosphorylated in EPEC strains, but not in *E. coli* strain K12. Results presented here indicate that BipA function is required at low temperatures in *E. coli* K12, suggesting a regulatory role independent of phosphorylation and of pathogenicity.

**Keywords** *bipA* · *yihK* · Cold sensitivity · *Escherichia coli* K12

**Results and discussion**

Isolation of multicycop suppressors of the cold sensitivity of strain D10

To ascertain the cause of the observed cold sensitivity of strain D10, we isolated multicycop suppressors from a genomic library. DNA was prepared from strain MC4100 (Casadaban 1976), partially digested with *Sal*I, and size fractionated by agarose gel electrophoresis. DNA fragments between approximately 1 kb and 4 kb in size were ligated with pBR322 DNA that had been digested with *Bam*HI and dephosphorylated (Sambrook et al. 1989). The resultant library was transformed into D10 by electroporation, and transformants were selected on LB in the presence of ampicillin (125 mg/l) at 20°C. Although prolonged incubation led to high levels of background growth, cold-resistant colonies were easily distinguishable by their early appearance.

The cold-sensitive phenotype of strain D10 is suppressible by *bipA*

Approximately 12,000 transformants were analyzed for cold resistance and six such colonies were identified. These six colonies were purified and restreaked at 20°C to verify the cold-resistant phenotype. Plasmid DNA was purified from each isolate and retransformed into D10 to confirm that the cold resistance was plasmid encoded. All transformants displayed a cold-resistant phenotype, demonstrating that each contained a plasmid which suppressed the cold sensitivity of D10 (for example, pPPL7 in Fig. 2A).

The six plasmids were analyzed by restriction enzyme digestion; the size of the inserts ranged from approximately 2300 bp to 8200 bp and each exhibited a different restriction fragment pattern, demonstrating that each plasmid was an independent isolate. DNA sequence
Fig. 1A–C Strains D10 and AF600 (MG1655 Δ hip A::kan) are cold sensitive. A The indicated strains were streaked on LB agar plates and incubated at 20°C for 4 days. B Strains were grown overnight at 37°C in LB broth, then diluted to a starting A600 of approximately 0.02 (again in LB broth). Growth was allowed to proceed at 20°C in a BioScreen C Microbiology Reader from Labsystems as described previously (Flower 2001). Measurements were taken every 30 min, but for clarity only readings for each 5-h increment are shown. C Bacteria were grown overnight at 37°C, then subcultured to a starting A600 equal to approximately 0.02 in LB broth. Cultures were incubated in the BioScreen at 37°C and readings were taken every 30 min. Symbols: closed squares, MG1655; open squares, AF600; closed triangles, D10 (E. coli Genetic Stock Center); open triangles, D10 (Flower laboratory stock).

Fig. 2A–C Plasmid-borne hip A complements the cold-sensitive defect of D10 (Flower stock) and AF600. Plasmid pPPL7 was obtained in the screen for multicopy suppressors, and contains a 2300-bp fragment encoding hip A. A Strains were grown on LB agar with 125 mg/l ampicillin at 20°C for 4 days. B, C Strains were grown in LB broth with 100 mg/l ampicillin at 20°C in the BioScreen as described in Fig. 1. Again, measurements were taken every 30 min, but only every 5-h increment is shown. Symbols: closed squares (B), MG1655; open squares (B), AF600; closed triangles (C), D10 (E. coli Genetic Stock Center); open triangles (C); D10 (Flower stock). All points connected by solid lines indicate strains that carry control plasmid pBR322, data for strains with pPPL7 are shown with dashed connecting lines.