Regulation of the *Escherichia coli* hfq Gene Encoding the Host Factor for Phage $Q_\beta$

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The host factor (HF-I) for phage $Q_\beta$ RNA replication is a small protein of 102 amino acid residues encoded by the *hfq* gene at 94.8 min on the *Escherichia coli* chromosome. The synthesis rate of HF-I at the exponential-growth phase is higher than at the stationary phase, and it increases concomitantly with the increase in cell growth rate. The intracellular level of HF-I is about 30,000 to 60,000 molecules per cell, the majority being associated with ribosomes as one of the salt wash proteins. Taken together, we suggest that HF-I is one of the growth-related proteins.

Since the establishment of an in vitro replication system of $Q_\beta$ phage RNA (8), detailed analysis of the molecular mechanism of RNA replication and the replication machinery involved in this process has been done. RNA replication involves a two-step reaction; i.e., plus-strand phage RNA (vRNA)-directed synthesis of cRNA of the minus-strand and cRNA-directed synthesis of vRNA. The basic replication machinery is composed of one phage-encoded protein (subunit $\beta$) and three host-encoded proteins, i.e., 30S ribosome protein S1 (subunit $\alpha$) and protein synthesis elongation factors EF-Tu (subunit $\gamma$) and EF-Ts (subunit $\delta$) (1). In addition, the synthesis of cRNA (the first-step reaction of RNA replication) requires an *Escherichia coli* protein which has hitherto been referred to as host factor I (HF-I) (4, 6, 19). In the absence of this factor, cRNA synthesis does not take place at all (7, 14), while the cRNA-directed vRNA synthesis proceeds in its absence. The molecular mechanism of HF-I action is, however, not clear yet except that purified HF-I alone binds specific regions of $Q_\beta$ phage RNA (2, 18).

Recently, we succeeded in cloning the HF-I gene (*hfq*) from the 94.8-min region of the *E. coli* chromosome (13). As an initial attempt to understand the function of HF-I, we analyzed in this study the synthesis rate, intracellular level, and distribution of HF-I in uninfected *E. coli* cells.

**Construction of expression plasmids.** A 1.5-kbp *BamHI*-PvuII DNA fragment carrying the intact *hfq* gene was isolated from plasmid pHFQ101, which carries the HF-I gene (13), and subcloned into pSPT18, a T7 promoter-containing expression vector, between *BamHI*-HindIII, to yield pHFQ343. A 0.76-kbp *RsaI*-HindIII fragment was subcloned into pSPT18 between HindII and HindIII to make an HF-I expression plasmid, pHFQ607. Methods for DNA cloning were as described by Sambrook et al. (17).

**Purification of HF-I and anti-HF-I.** Plasmid pHFQ607 was introduced into strain BL21(DE3), which carries the isopropyl-$\beta$-d-thiogalactopyranoside (IPTG)-inducible T7 RNA polymerase gene on its chromosome. HF-I was overproduced by IPTG induction of the transformant culture under the same conditions employed for induction of RNA polymerase subunits (9). Overexpressed HF-I was purified essentially as described by Kajitani and Ishihama (13). To remove HF-I-associated nucleic acids, DEAE column chromatography was repeated but in the presence of 6 M urea. Polyclonal monospecific anti-HF-I was produced in rabbits by injecting the purified HF-I. Western blot (immunoblot) analysis against an *E. coli* whole-cell lysate separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gave a single band, as stained with a rabbit immunoglobulin G (IgG) detection kit (PromoBlot; Promega).

**Cell culture and radioactive labeling.** Each stationary-phase culture of *E. coli* W3350 was transferred into 20 volumes of M9 medium supplemented with an appropriate carbon source at a concentration of 0.4% and with other additions as indicated for each experiment. Cells were grown at 37°C with shaking, and growth was monitored by measuring the turbidity with a Klett-Summerson photometer. For determination of the rate of protein synthesis, cells were pulse-labeled at various growth phases with [3H]leucine (Amersham) for the time indicated for each experiment. For determination of the intracellular content of HF-I, radioactive leucine was added shortly after the inoculation of preculture into a fresh medium and the cells were continuously labeled for various periods.

**Estimation of synthesis rate and intracellular content of HF-I.** HF-I protein in whole cell lysates was immunoprecipitated with anti-HF-I antibodies. The antigen-antibody complexes were recovered by adding Protein A-agarose (ImmunoPure; Pierce) and subjected to SDS-PAGE. This three-step method for the determination of proteins in crude lysates was employed for the determination of RNA polymerase subunits (5). As references, RNA polymerase subunits were determined simultaneously by using anti-holoenzyme antisera. After electrophoresis, gels were stained with Coomassie brilliant blue R250. For the determination of radioactivity associated with HF-I and RNA polymerase subunits, gel bands were excised and treated with 0.5 ml of tissue solubilizer (Solvable; DuPont) for 3 h at 50°C and the radioactivity was counted in a scintillation cocktail (Atomlight; DuPont). The differential rate of protein synthesis (the rate of synthesis of each protein relative to the rate of synthesis of total protein) was determined as described previously after correction for the leucine content of each protein and of the bulk of *E. coli* proteins (12).

**Analysis of HF-I localization.** *E. coli* W3350 cells were lysed...
under mild conditions and fractionated by glycerol density gradient centrifugation to the nucleoid and cytoplasm (15). Aliquots of each fraction were separated by SDS-PAGE, and the amounts of HF-I and RNA polymerase subunits were analyzed by Western blot analysis.

Synthesis rate of HF-I at various growth phases. As an initial attempt to understand the physiological role of HF-I in uninfected E. coli, we measured the rate of HF-I synthesis under various growth conditions by immunoprecipitation of pulse-labeled HF-I using the antibodies followed by gel electrophoresis of antigen-antibody complexes. For this purpose, HF-I was overexpressed under control of a phage T7 promoter, purified to homogeneity, and used to prepare antibodies in rabbits. In parallel with HF-I measurement, the synthesis rate of RNA polymerase subunits was measured as an internal reference.

An overnight culture of E. coli W3350 was diluted 20-fold with fresh medium, and the rates of radioactive leucine incorporation into HF-I and RNA polymerase subunits were determined at various phases of the cell growth. As shown in Fig. 1, the differential rate of synthesis of RNA polymerase core subunits was high at the exponential-growth phase and began to decrease concomitantly with the growth transition from log to stationary phase, confirming our previous determination (15). The synthesis rate of HF-I showed essentially the same pattern as that of RNA polymerase core enzyme (in particular of the α subunit): its rate was high at 0.04 to 0.06% of total proteins) at the exponential-growth phase but decreased to a level as low as 0.01 to 0.02%. The growth phase-coupled variation in synthesis rate was established for ribosomal proteins (16).

Synthesis rate of HF-I at various growth rates. The rates of synthesis of HF-I as well as of RNA polymerase subunits were also determined for cells growing at various rates. Cell growth rate was controlled by changing carbon sources. The rates of cell growth analyzed were (in doublings per hour) 0.22 on M9-0.4% acetate, 0.38 on M9-0.4% succinate, 0.70 on M9-0.4% glucose, and 1.46 on M9-0.4% glucose plus 50 μg of each of 20 amino acids per ml. The relative synthesis rates of both HF-I and RNA polymerase subunits increased at rates dependent on the cell growth rates, from 0.005 to 0.01% to 0.04 to 0.05% (Fig. 2).

These observations together suggest that the synthesis of HF-I is under the control of cell growth rate, as has been found for the synthesis of RNA polymerase and ribosomes (for reviews, see references 10, 11, and 16).

Intracellular content of HF-I. Structural components constituting the transcription and translation apparatus are metabolically stable under steady-growth conditions (12). As a result, the growth-dependent variations in the synthesis rates of RNA polymerase subunits and ribosomal proteins lead to concomitant variations in the intracellular content of these components (10, 11, 16). We then measured the intracellular level of HF-I in cells growing at various rates. For this purpose, cells were continuously labeled with radioactive leucine and the amounts of labeled HF-I and RNA polymerase were determined by using a combination of immunoprecipitation and gel electrophoresis. The variation pattern in the intracellular content of HF-I was essentially the same as that of RNA polymerase core subunits (data not shown). The HF-I level at the exponential-growth phase was about 0.05% of total proteins; during the transition into the stationary phase, it decreased and finally reached a level less than half of the maximum level. This result again confirms that HF-I is maintained at a level characteristic of the rate of cell growth.

The amount of RNA polymerase in the E. coli W3350 grown in M9-0.4% glucose is estimated to be about 3,000 molecules per cell (or 2,000 molecules per genome) (10, 11). From the relative levels of these two components, the number of HF-I
was analyzed by staining with this in inconsistent replication, indicating that HF-I is also involved in maintenance of the E. coli chromosome structure or control of DNA functions. However, basic proteins stick unspecifically to nucleic acids after cell lysis. Thus, the association of HF-I with ribosomes and the nucleoid is not a strong clue for the participation of HF-I in ribosome or nucleoid functions. In fact, overproduced HF-I was recovered as complexes with cellular DNA and RNA after DEAE-Toyopearl column chromatography.

From sedimentation analysis of purified HF-I, it was proposed that native HF-I exists in a form of hexamer (2, 6). Our analysis by the RFHR method of two-dimensional gel electrophoresis (20, 21) indicated that HF-I exists as a pentamer (data not shown). The net charge of the monomeric form of HF-I was estimated to be ~2.0, in good agreement with its amino acid sequence (13). On the other hand, the net charge of the oligomeric form of HF-I was calculated to be about ~5.6 (per monomer). This decrease in net charge of HF-I upon oligomerization may suggest that the oligomerization couples with structural modification. If this is the case in vivo, it is important to reveal which of the two alternative HF-I forms is involved in Qb RNA replication.

In concert with the indication that HF-I is one of the essential components for cell growth, HF-I homologs are present at least in all E. coli-related bacteria (3). This concept could be examined by gene disruption experiments.

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REFERENCES


