Construction of expression plasmids for the fusion protein of Sendai virus, and their expression in *E. coli* cells and eucaryotic cells

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To examine the properties and the role of the fusion protein (F) of Sendai virus at the molecular level, a plasmid, pUC-F, was constructed by inserting cDNA for the F protein into a pUC vector. Upon induction of *E. coli* cells transformed with pUC-F, a new protein was obtained, which was identified as Fo on Western blot analysis. The cDNA fragment for the F gene was excised from pUC-F and inserted into an eucaryotic expression vector, pSVL, to yield pSVL-F. COS-1 cells transfected with pSVL-F gave a band on SDS-gel electrophoresis which corresponded to the size of the Fo protein.

Recombinant DNA; Sendai virus; Fusion protein; Eucaryotic expression; Procaryotic expression

1. INTRODUCTION

In the envelope of Sendai virus, a prototype of the paramyxoviruses, two glycoproteins, i.e., F protein and HN, are present as integral proteins. The F protein is known to participate in virus penetration, hemolysis and cell fusion, while HN exhibits both hemagglutinating and neuraminidase activities [1,2]. In infected cells, the F protein is synthesized as an inactive precursor protein (Fo), which is cleaved at a specific site by a protease present in host cells to form an active form consisting of two fragments, namely F1 and F2, linked through a disulfide bond [1,3–5]. The N-terminal region of F1 is very hydrophobic and is suggested to mediate fusion by interacting with adjacent membranes, while its C-terminal region, which is also especially hydrophobic, is thought to be a membrane anchorage [6].

To answer questions concerning the properties of the F protein such as its role in the process of membrane fusion and the function of the signal sequence in its transport during its integration into the membrane, it is important to have a system in which only the F protein is expressed. In this paper, we report the construction of expression plasmids for the F gene in *E. coli* as well as in eucaryotic cells, and their expression in these cells.

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Abbreviations: SV5, simian virus 5; F, fusion protein; HN, hemagglutinin/neuraminidase protein; P, phosphoprotein; NP, nucleocapsid protein; M, matrix protein; L, large protein; IPTG, isopropyl-β-D-thiogalactoside

2. MATERIALS AND METHODS

The portion of Sendai virus encoding the F gene was derived from pUC8-A35 and pUC8-A136 (Shioda and Shibuta, unpublished). Monkey COS-1 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum at 37°C, 5% CO_2. For transient expression of the F protein in eucaryotic cells, COS-1 cells were grown to 80% confluency in a 12-well tissue culture dish and then transfected with 5 μg of a plasmid by means of calcium phosphate precipitation methods [7]. After incubation at 37°C for 40 h, the cells were labeled for 4 h with [^{35}S]methionine (10 μCi/600 μl). The labeled lysates were immunoprecipitated with antiserum from Sendai virus-infected guinea pig as described previously [8], and the precipitate formed was analyzed by 12.5% SDS-PAGE [9]. Sendai virus-infected COS-1 cells were also labeled for 4 h with [^{35}S]methionine from 20 h postinfection.

3. RESULTS AND DISCUSSION

3.1. *Construction of an expression plasmid, pUC-F*

To construct an expression plasmid for the F protein in *E. coli*, pUC8-A35 and pUC8-A136 were used, of which the former carries the cDNA portion corresponding to the N-terminal half of the F gene (F_N), while the latter carries that corresponding to the C-terminal half (F_C). As schematically shown in fig.1, the F_N portion present in pUC8-A35 was cloned into pUC19 (pUC19-F_N) through 4 steps of manipulation including the isolation of a subclone, pUC19-BH, while the F_C portion present in pUC8-A136 was cloned into pUC18

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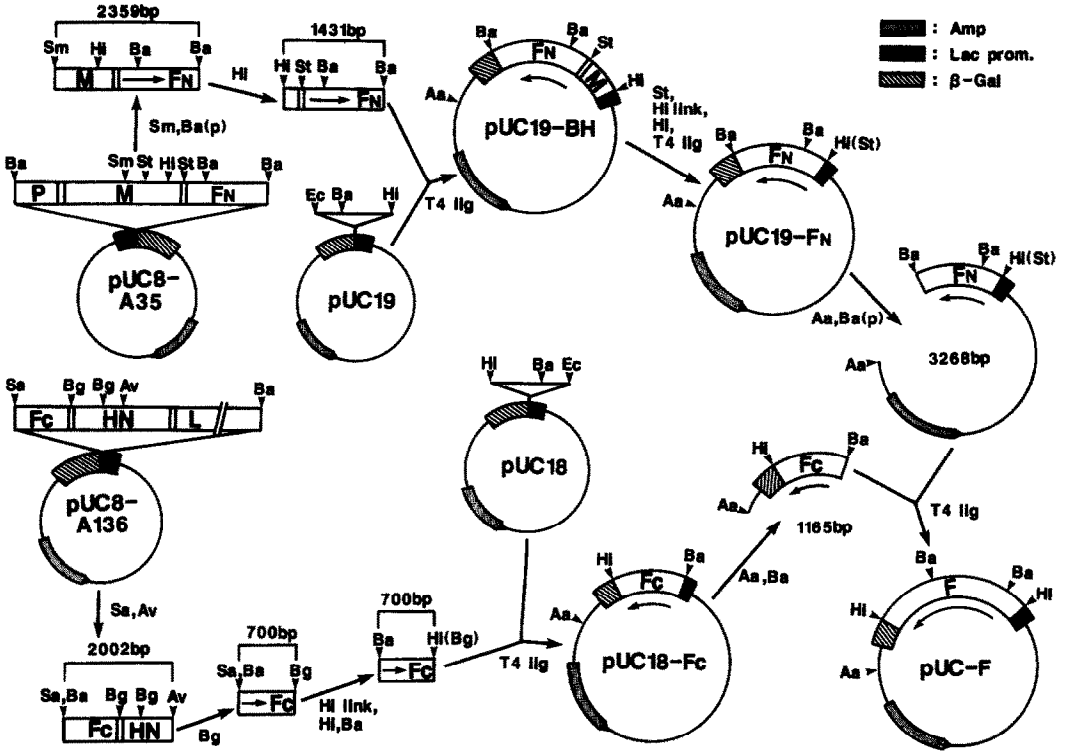

Fig.1. Schematic representation of the procedure for constructing the expression plasmid, pUC-F. Symbols: Aa, *Aat*II; Av, *Ava*I; Ba, *Bam*HI; Bg, *Bgl*I; Ec, *Eco*RI; Hi, *Hin*dIII; Sa, *Sal*I; Sm, *Sma*I; St, *Sty*I; Ba(p), partial *Bam*HI digestion; Hi link, *Hin*dIII linker; T4 lig, T4 DNA ligase.

($pUC18\text{-}F_C$) through 4 steps of manipulation. Both $pUC\text{-}F_N$ and $pUC\text{-}F_C$ were then cleaved with *Aat*II and *Bam*HI, and the large fragment (3268 bp) originating from the former and the small fragment (1165 bp) from the latter were religated to obtain pUC-F.

3.2. *Expression of the F protein in* E. coli *cells*

E. coli JM 109 was transformed with pUC-F and an ampicillin-resistant transformant was selected, which was named *E. coli* F-TR. After induction of this transformant with 1 mM IPTG, a crude cell lysate was prepared, and proteins in the lysate were then analyzed by SDS-PAGE. As shown in fig.2A, a new protein band having a molecular mass of 60 kDa was observed for induced *E. coli* F-TR cells (lane 1), but not for uninduced cells (lane 2). To identify this new band as the F protein, Western blot analysis was carried out using antiserum from Sendai virus-infected guinea pig [9,10]. As shown in fig.2B, a strong band was detected at a position corresponding to 60 kDa (lane 1) with a lysate prepared from induced *E. coli* F-TR cells. In contrast, a lysate prepared from uninduced *E. coli* cells did not give any bands (lane 2). This result indicated that the new protein was closely related to the Sendai virus F protein. It is noteworthy that the molecular mass of the new protein (60 kDa) was slightly smaller than that of Fo (66 kDa) detected in Sendai virus-infected cells, but was very close to the predicted molecular mass of the

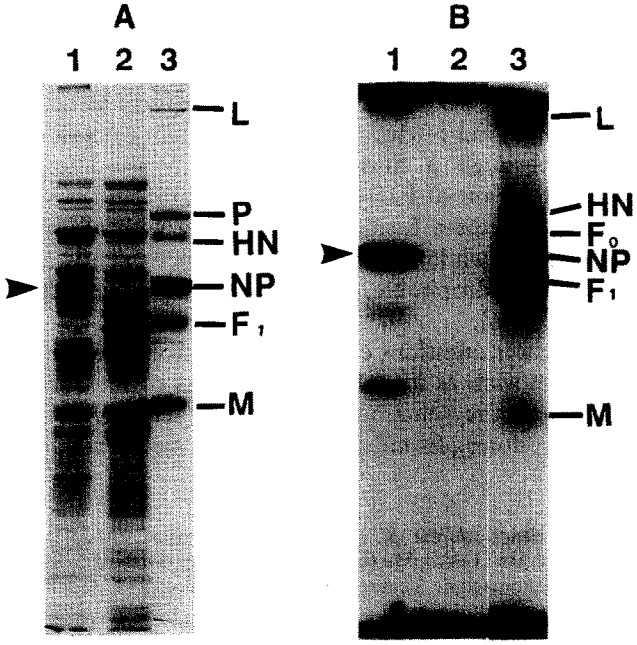

Fig.2. Analysis of the Sendai virus F protein produced in *E. coli* F-TR. (A) Coomassie brilliant blue staining of an *E. coli* cell lysate and Sendai virus particles prepared from 10-day-old embryonated chicken eggs. (B) Western blot analysis of an *E. coli* cell lysate and Sendai virus particles. 1, induced *E. coli* cells; 2, uninduced *E. coli* cells; 3, Sendai virus particles.

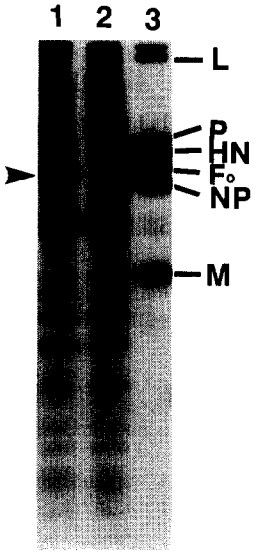

Fig.3. Immunoprecipitation with antiserum from Sendai virus-infected guinea pig of the F protein synthesized in pSVL-F transfected- or Sendai virus-infected COS-1 cells. 1, pSVL-F-transfected cells; 2, pSVL-transfected cells; 3, Sendai virus-infected cells.

F protein (61.5 kDa) deduced from the nucleotide sequence [6]. This indicates that the new protein synthesized in *E. coli* cells was unglycosylated Fo, which is reasonable since *E. coli* cells lack a system that glycosylates the Fo protein at its 4 N-linked glycosylation sites. It will be worthwhile obtaining a large amount of the purified Fo protein from *E. coli* F-TR to examine its properties as well as to prepare a specific antiserum against the Fo protein.

3.3. *Transient expression of the F gene in COS-1 cells*

For the expression of the F protein in eucaryotic cells, an expression vector pSVL (Pharmacia, LKB Biotechnology) was employed [11]. The F gene cDNA (1.7 kbp) was excised from pUC-F by *Hin*dIII digestion, filled in with DNA polymerase I to create a blunt end and then inserted into the *Sma*I site of pSVL to yield pSVL-F. The pSVL-F as well as pSVL were introduced into monkey COS-1 cells by the calcium phosphate method, and [^{35}S]methionine-labeled proteins immunoprecipitated with antiserum were analyzed as described in section 2. As shown in fig.3, a 66 kDa protein band was detected for COS-1 cells transfected with pSVL-F (lane 1). As this band was specifically precipitated with anti-Sendai virus antiserum and it was not detected in cells transfected with pSVL (lane 2), we concluded that this protein is the F protein. From its molecular mass, it is conceivable that the F protein expressed in COS-1 cells is a glycosylated and uncleaved form, which is similar to that synthesized in virus-infected cells. This indicates that the cleavage of the Sendai virus F protein is not inhibited by either the presence of other Sendai virus proteins or the cellular factor(s) induced on virus infection. In contrast to this, Paterson et al. [12] reported that in CV-1 cells infected with SV5 viruses or recombinant SV-F viruses, the F protein was present as a cleaved form. These results suggest the importance of having a proper combination as to the specificity of the host protease and the amino acid sequence of the cleavage site of the Fo protein. Thus, for examination of the function of the biologically active form of the F protein, namely the F1 fragment, in COS-1 cells, it is necessary to construct an expression plasmid containing a cDNA fragment exclusively corresponding to the F1 portion, e.g. pSVL-F1.

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