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Elevated temperature triggers human respiratory syncytial virus F protein six-helix bundle formation

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ABSTRACT

Human respiratory syncytial virus (RSV) is a major cause of severe lower respiratory tract infection in infants, immunocompromised patients, and the elderly. The RSV fusion (F) protein mediates fusion of the viral envelope with the target cell membrane during virus entry and is a primary target for antiviral drug and vaccine development. The F protein contains two heptad repeat regions, HR1 and HR2. Peptides corresponding to these regions form a six-helix bundle structure that is thought to play a critical role in membrane fusion. However, characterization of six-helix bundle formation in native RSV F protein has been hindered by the fact that a trigger for F protein conformational change has yet to be identified. Here we demonstrate that RSV F protein on the surface of infected cells undergoes a conformational change following exposure to elevated temperature, resulting in the formation of the six-helix bundle structure. We first generated and characterized six-helix bundle-specific antibodies raised against recombinant peptides modeling the RSV F protein six-helix bundle structure. We then used these antibodies as probes to monitor RSV F protein six-helix bundle formation in response to a diverse array of potential triggers of conformational changes. We found that exposure of 'membrane-anchored' RSV F protein to elevated temperature (45–55 °C) was sufficient to trigger six-helix bundle formation. Antibody binding to the six-helix bundle conformation was detected by both flow cytometry and cell-surface immunoprecipitation of the RSV F protein. None of the other treatments, including interaction with a number of potential receptors, resulted in significant binding by six-helix bundle-specific antibodies. We conclude that native, untriggered RSV F protein exists in a metastable state that can be converted in vitro to the more stable, fusogenic six-helix bundle conformation by an increase in thermal energy. These findings help to better define the mechanism of RSV F-mediated membrane fusion and have important implications for the identification of therapeutic strategies and vaccines targeting RSV F protein conformational changes.

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Introduction

Human respiratory syncytial virus (RSV) is an enveloped negative-sense ssRNA virus of the pneumovirus group in the paramyxoviridae family (Collins et al., 2001). RSV is a major cause of pediatric respiratory disease, resulting in severe lower respiratory tract infection in infants, immunocompromised patients, and the elderly (Collins et al., 2001). There are no effective vaccines available for RSV. Currently approved antiviral therapy for RSV infection is limited to ribavirin, a broad-spectrum antiviral drug with limited efficacy. Prophylactic treatment with the anti-RSV monoclonal antibody, palivizumab, is effective in reducing the severity of disease but is only recommended for high-risk patients (Johnson et al., 1997; Groothuis et al., 1993; Pollack and Groothius, 2002). Therefore, development of new therapies for the treatment of RSV infection is a high priority.

RSV enters cells by binding to the target cell surface and fusing its envelope with the plasma membrane of the target cell. There are three viral glycoproteins on the envelope surface: the attachment glycoprotein (G), the fusion (F) protein, and the small hydrophobic (SH) protein (Heminway et al., 1994). However, several lines of evidence indicate that only F, the fusion protein, is both necessary and sufficient for virus entry and cell fusion (Karron et al., 1997; Kahn et al., 1999; Teecharpornkul et al., 2001). Thus, the critical role of the F protein in virus entry, along with the fact that F is highly conserved and is a major antigen for neutralizing antibodies, makes it an attractive therapeutic target (Johnson et al., 1987; Eckert et al., 1999; Collins et al., 2001; Zhao et al., 2000).

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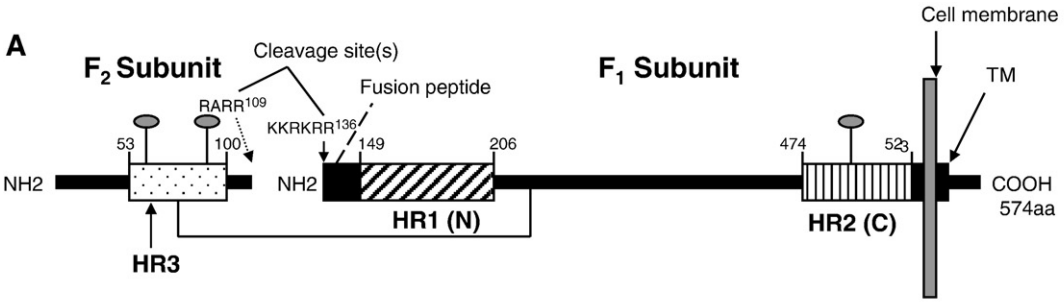
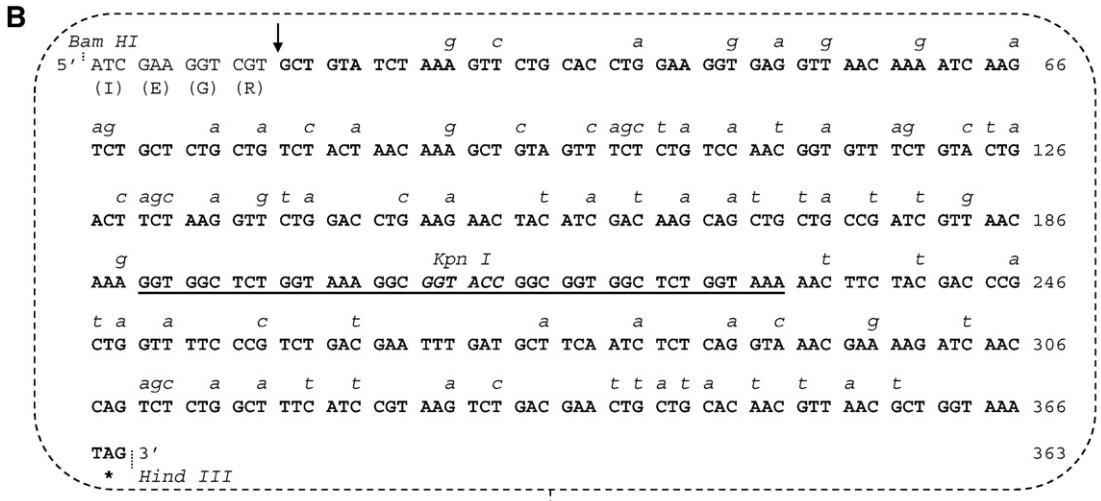
The RSV F protein is synthesized as a 70-kDa precursor (F0) that is processed by proteolytic cleavage in the trans-Golgi complex to yield two disulphide-linked subunits: F1 and F2 (Fig. 1A; Anderson et al., 1992; Collins and Mottet, 1991; Collins et al., 1984). The mature F protein is expressed as a homotrimer on the cell surface (Calder et al., 2000). The ectodomain of the F1 transmembrane subunit contains two heptad repeat regions, HR1 and HR2, and an N-terminal fusion peptide (Fig. 1A) (Collins et al., 2001). Processing of the F0 precursor has been proposed to generate a "metastable" F1/F2 complex (Zhao et al., 2000). In one proposed model, receptor binding triggers conformational changes in the metastable F protein, resulting in insertion of the N-terminal fusion peptide into the target cell membrane. The HR1 and HR2 heptad repeat regions then interact to form a six-helix bundle structure (Carr et al., 1997; Eckert and Kim, 2001). According to this model, formation of the six-helix bundle structure facilitates membrane fusion by bringing the viral and cellular membranes into close apposition (Chan and Kim, 1998; Eckert and Kim, 2001; Russell et al., 2001). The presence of heptad repeat regions that are capable of associating to form a six-helix bundle structure is a feature of several enveloped virus fusion proteins, suggesting a common membrane fusion mechanism (Lamb, 1993; Hernandez et al., 1996; Melikyan et al., 2000; Skehel and Wiley, 2000;





C

PEPTIDE	SEQUENCE	LENGTH	MOL. MASS
N	^{153}AVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK^{209}	57 aa	6.13 kDa
C	^{476}NFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGK^{520}	45 aa	5.11 kDa
NC	AVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKG GSGKGGTGGGSGKNFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGK	116 aa	12. 21 kDa

Fig. 1. Construction of RSV fusion (F) protein heptad repeat peptide immunogens. (A) Heptad repeat (HR) domains and other structural features in the RSV fusion protein. Key domains of the F1 subunit are shown, including the heptad repeats, HR1 (or N region) and HR2 (or C region), the fusion peptide (FP), and the transmembrane domain (TM). In the F2 subunit, the location of an additional predicted heptad repeat, HR3, is shown. Cleavage sites designate the location of furin cleavage sites in F2 and the F2–F1 junction, respectively. The location of conserved glycosylation sites are denoted by lollipop-shaped structures in F2 and F1. (B) Codon optimization of HR1/HR2 cDNA in an RSV peptide expression construct. The nucleotide sequence of the codon-optimized HR1/HR2 cDNA is shown. The wild-type RSV A2 strain F gene nucleotides that were replaced to permit optimal expression in *E. coli* are italicized. The HR1 region (nt 13–187, encoding F residues 153–209) and HR2 region (nt 232–366, encoding F residues 476–520) are joined by a linker region (underlined sequence). Also shown are the locations of restriction enzyme cloning sites and the IEGR- factor-Xa cleavage sequence. The individual HR1 and HR2 cDNA clones have sequences identical to the corresponding regions shown here. The cDNAs expressing RSV peptides NC, N, or C were cloned into the *Bam*HI-*Hin*dIII window of the pET-32a protein expression plasmid. (C) Sequence of recombinant RSV peptides (N, C, or single chain NC peptide) generated in the study. The lengths and predicted molecular weights of the peptides are shown.