Role of the carboxyl-terminal Fib2 domain in fibronectin matrix assembly

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SUMMARY

A truncated form of fibronectin consisting of the N-terminal 70 kDa and C-terminal 37 kDa regions, designated r70F2, retained the ability to assemble into the extracellular matrix when expressed in cultured fibroblasts (Ichihara-Tanaka et al. (1992) FEBS Lett. 299, 155-158). To elucidate the role of the C-terminal 37 kDa region in fibronectin matrix assembly, we expressed a panel of mutant forms of r70F2 with various deletions and amino acid substitutions in mouse L cells. Although substitution of Ser for two Cys residues in the C-terminal dimer-forming segment led to a marked reduction in the matrix assembly activity of r70F2, the resulting monomeric r70F2 still retained a low, but significant activity to assemble into the matrix. Neither the N-terminal 70 kDa nor the C-terminal 37 kDa regions, when expressed as monomeric forms, exhibited any residual activity, suggesting that the core domain of the 37 kDa region consisting of I10 through I12 modules, termed Fib2 domain, is actively involved in the matrix assembly of r70F2. In support of the role of Fib2 domain, the proteolytic fragment derived from the 37 kDa region inhibited the assembly of r70F2. Furthermore, en bloc deletion of the Fib2 domain or deletion of the I10 through I12 modules from r70F2 resulted in a marked decrease in the matrix assembly activity. Since deletion of any one of the three type I modules led to a much lesser decrease in activity, it seems likely that a cluster of the three type I modules in the Fib2 domain, but not any one in particular, serves as a functional unit for the matrix assembly of r70F2. Further supporting the active role of the Fib2 domain, a recombinant homodimer of the 37 kDa region was found to be incorporated into the deoxycholate-insoluble matrix. These results, taken together, indicate that the Fib2 domain per se has an intrinsic ability to assemble into the matrix and is actively involved in the matrix assembly of fibronectin.

Key words: fibronectin, extracellular matrix, type I module, fibrillogenesis

INTRODUCTION

Fibronectin (FN) is a major component of connective tissues and various body fluids. It is a multifunctional, adhesive protein and has been implicated in a wide variety of cellular events including cell adhesion, migration, differentiation and growth (Hyne, 1990). FN is synthesized and secreted by many cell types as a disulfide-bonded dimer of almost identical subunits comprised of three types of internally homologous modules, termed types I, II, and III (Petersen et al., 1983). A portion of the secreted FN is captured at the cell surface and assembled into an elaborate three-dimensional network of the extracellular matrix, although the detailed molecular mechanisms of this process are poorly understood.

Most studies concerning FN matrix assembly reported to date have focused on the identification of FN domains involved in this process (McKeown-Longo and Mosher, 1985; McDonald et al., 1987; Quade and McDonald, 1988; Nagai, 1991; Schwarzbauer, 1991; Sottile et al., 1991; Ichihara-Tanaka et al., 1992; Morla and Ruoslahti, 1992). Through extensive screening of a panel of FN-derived proteolytic fragments as well as monoclonal/polyclonal anti-FN antibodies for their inhibitory effects on FN matrix assembly, at least three distinct regions of FN have been shown to be involved in this process. The first domain thus identified was the N-terminal 70 kDa region, of which the first five type I modules (I1 through I5) play a critical role (McKeown-Longo and Mosher, 1985; Quade and McDonald, 1988; Sottile et al., 1991; Schwarzbauer, 1991). Novel non-integrin receptor(s) have been reported to bind to this region (Limmer et al., 1991; Blystone and Kaplan, 1992; Moon et al., 1994), but their detailed molecular as well as functional properties have not yet been fully characterized. The cell-binding domain consisting of the three type III modules, III8, III9, and III10, is also considered to play a role in FN matrix assembly, possibly through binding of soluble FN molecules at the cell surface (McDonald et al., 1987; Fogerty et al., 1990; Nagai et al., 1991; Darribere et al., 1992). Recently, the first type III module (III1) was also implicated in this process (Chernousov et al., 1991; Darribere et al., 1992; Morla and Ruoslahti, 1992), possibly by promoting FN multimer formation (Morla et al., 1994). Besides these three regions, the extreme C-terminal short segment containing two Cys residues responsible for interchain disulfide linkages was shown to be crucial for efficient FN matrix deposition (Schwarzbauer, 1991; Sottile and Mosher, 1993).
In an attempt to elucidate the molecular mechanisms of FN matrix assembly, we previously expressed a panel of recombinant proteins modeled after the N-terminal 70 kDa or C-terminal 37 kDa region of FN. An oligonucleotide-directed mutagenesis (Kunkel et al., 1989, 1992) of the 37 kDa region, when expressed alone, was barely incorporated into the matrix, although the 70 kDa region directly linked to the C-terminal 37 kDa region, hereafter referred to as r70F2, was highly active in assembling into the matrix (Ichihara-Tanaka et al., 1992). The 37 kDa region, when expressed alone, was incorporated into the matrix to a much lesser extent than r70F2 (Ichihara-Tanaka et al., 1990, 1992), indicating that both N-terminal 70 kDa and C-terminal 37 kDa regions are required for efficient incorporation of FN into the extracellular matrix.

The carboxy-terminal 37 kDa region is comprised of the fifteenth type III module (I₁₁), tenth through twelfth type I modules (I₀-I₁₂), and the extreme C-terminal dimer-forming segment with two Cys residues (see Fig. 1). Despite the well-defined role of the carboxy-terminal dimer-forming segment, it is not clear whether the core domain of this region, i.e. the Fib2 domain consisting of the I₁₁ and I₀-I₁₂ modules, is also involved in FN matrix assembly. In the present study, we examined the C-terminal 37 kDa region in FN matrix assembly by expressing a series of mutant forms of r70F2 with various deletions and amino acid substitutions in both the Fib2 domain and the dimer-forming segment (Fig. 1). Our results showed that the Fib2 domain per se has an intrinsic ability to assemble into the matrix as its own dimer and accelerates the matrix assembly of FN in collaboration with the N-terminal 70 kDa region.

### MATERIALS AND METHODS

#### Materials

DNA modifying and restriction enzymes were obtained from Takara Shuzo (Kyoto, Japan) and Nippon Gene (Toyama, Japan). Oligonucleotide primers were synthesized on an Applied Biosystems model 391 DNA synthesizer (Foster City, CA). ECL Western blotting analysis reagents were from Amersham (Buckinghamshire, England). Gelatin-Celulofine was from Seikagaku Kogyo (Tokyo, Japan). Heparin-Sepharose was from Pharmacia LKB Biotechnology (Uppsala, Sweden).

#### cDNA construction and site-specific mutagenesis

Expression vectors for mutant forms of r70F2 with amino acid substitutions and deletions of type I modules within the Fib2 domain were constructed by oligonucleotide-directed mutagenesis (Kunkel et al., 1987) using a cDNA clone, pSKF2, encoding the C-terminal 37 kDa region as a template. pSKF2 was constructed by inserting a 1907 nucleotide Sal/HindIII fragment excised from pHCF2D (Ichihara-Tanaka et al., 1990) to ClaI/HindIII-cleaved pBluescript SK(−), whose AccI site had been cleaved and filled-in to generate an NruI site. An oligonucleotide (5′TAAAGGCGATGAAAGCTCTCAATTGG

GCTATAACATTAG) was used as a primer to introduce two T to A mutations for substitution of Ser for both Cys²⁴²⁷ and Cys²⁴³¹ within the C-terminal dimer-forming segment. The amino acids are numbered from the N-terminal pyroglutamate residue in the mature protein (Petersen et al., 1989). The resulting plasmid, pSKF2mono, was digested with NruI and NspV, and a 1502 nucleotide fragment was ligated to HindIII/NspV-cleaved pAI70F2, the expression vector for r70F2 (Ichihara-Tanaka et al., 1992), along with a 1743 nucleotide HindIII/NruI fragment prepared from pAI70F2, yielding the expression vector for r70F2monod. Similarly, the 1503 nucleotide NruI/NspV fragment from pSKF2mono was inserted into the HindIII/NspV-cleaved pAI70F2 along with a 1451 nucleotide HindIII-Ncol fragment from pAI70F2 and a 287 nucleotide Ncol-Sal fragment from pCF26 (Ichihara-Tanaka et al., 1992). For the expression of deletion mutants of r70F2 lacking each one or all of the type I modules within the Fib2 domain, pSKF2 was mutagenized with the following primers: 5′AAATCTTGTAGTCCACATCTGGCAGCTTGTACCTCGGTAGTTGGG3′ for deletion of I₁₀ module (Cyš²²⁶⁶, Tp³¹¹); 5′CTTCCCATCTGAAACACACTCAGTAGAATCATC−

ACATT3′ for deletion of I₁₁ module (Cyš³¹¹-Th³³³); 5′CCAGTATGCGTCTTCGGGAGCTGGTTCCACCCGGTTCACCTAC−

CTGAC3′ for deletion of I₁₂ module (Cyš³²⁵-Gly³⁷⁷). The nucleotide sequence of the mutagenized plasmids was verified by the dye-exoxy-mediated chain-termination method (Sambrook et al., 1989). Mutagenized cDNAs were excised by digestion with NruI and NspV, and inserted into the NruI/NspV-cleaved pAI70F2 along with the 1743 base HindIII/NruI fragment of pAI70F2.

#### Cell culture, DNA transfection and selection of stably transformed cells

Mouse L cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum. Expression vectors for r70F2 and its mutant forms described above were cotransfected into L cells with pKONeo by the calcium-phosphate precipitation method (Chen and Okayama, 1987). G-418-resistant colonies were randomly picked and expanded in 24-well culture dishes, and assayed for the expression of recombinant proteins by dot blot analysis as follows: transfected cells were grown to confluence in DMEM containing 10% FBS, and the conditioned medium was harvested 48 hours after the cells reached confluence. The medium was centrifuged at 15,000 rpm for 5 minutes, and 100 µl aliquots of the supernatant were spotted onto a nitrocellulose membrane. After being air-dried, the membrane was blocked with 5% non-fat milk for 1 hour, and then incubated with monoclonal anti-human FN antibody FN9-1 or FN8-12 for 1 hour. The bound antibody was detected with a peroxidase-conjugated antibody and peroxidase substrate (TMB). The bands were analyzed by SDS-PAGE followed by fluorography.

#### Metabolic labeling and affinity precipitation

Metabolic labeling with [³⁵S]methionine and affinity precipitation with either monoclonal anti-human FN antibody immobilized on Sepharose CL4B or gelatin-Celulofine were performed as described previously (Ichihara-Tanaka et al., 1990). Precipitated materials were analyzed by SDS-PAGE followed by fluorography.
Indirect immunofluorescence staining

Cells were grown to confluence in Lab-Tek chamber slides in DMEM containing 10% FN-depleted fetal bovine serum. The cells were labeled with monoclonal anti-human FN antibody FN9-1 or FN8-12 at room temperature for 30 minutes without fixation, washed three times with PBS containing 5% FN-depleted fetal bovine serum, and then incubated with rhodamine-conjugated goat anti-mouse IgG for 30 minutes. The labeled cells were washed three times with PBS containing 5% FN-depleted fetal bovine serum, fixed with 3% paraformaldehyde, and mounted in PBS containing 50% glycerol and 2.6% 1,4-diazabicyclo[2.2.2]octane. The labeled cells were examined with a Zeiss Axiophot epifluorescence microscope and photographed on Kodak-Tri-X films.

Isolation of deoxycholate-insoluble matrix

The deoxycholate-insoluble matrix was prepared as described by Sekiguchi et al. (1986). Briefly, confluent cells were washed three times with PBS and extracted twice with 50 mM Tris-Cl, pH 8.6, containing 145 mM NaCl, 0.5 mM EDTA, 0.5% sodium deoxycholate, and 1 mM PMSF at 4 °C for 10 minutes under slow gyratory agitation. The detergent-insoluble materials left on the culture dish were washed twice with a low salt buffer (2 mM Tris-Cl, pH 8.0, containing 0.5 mM EDTA and 1 mM PMSF) at 4 °C for 5 minutes, twice with a high salt buffer (20 mM Tris-Cl, pH 8.0, containing 1 M NaCl, 0.5 mM EDTA, and 1 mM PMSF) at 4 °C for 10 minutes, and once with the low salt buffer at 4 °C for 5 minutes. The insoluble materials remaining attached to the substratum were scraped off in 10 mM Tris-Cl, pH 7.2, containing 50 mM NaCl, 0.5 mM EDTA, 1 mM PMSF, and 0.05% SDS, and then lyophilized.

Purification of proteolytic fragments

FN was purified from human plasma by gelatin affinity chromatography. The N-terminal 70 kDa fragment was released from human plasma FN by cathepsin D digestion and purified by gelatin affinity chromatography as described by Richter et al. (1981). The C-terminal 37 kDa fragment was released from human plasma FN by mild trypsin digestion as described previously (Sekiguchi and Hakomori, 1983). The trypsin digest was sequentially passed through columns of gelatin-Cellulofine and heparin-Sepharose, and the 37 kDa fragment in the flow-through fraction was purified on a Mono-Q column with a 0.1-0.5 M NaCl gradient in 20 mM Tris-Cl (pH 7.6) containing 0.5 mM EDTA, using a FPLC Chromatography System (Pharmacia, Uppsala, Sweden). Purified fragments were dialyzed against 10 mM ammonium acetate and then lyophilized.

SDS-PAGE and immunoblot analysis

SDS-PAGE was performed as described by Laemmli (1970). Recombinant proteins separated on a 6.5% polyacrylamide gel were transferred onto a nitrocellulose membrane and stained with monoclonal anti-human FN antibodies. The bound antibodies were visualized with either 125I-Protein A or ECL reagents as described previously (Ichihara-Tanaka et al., 1990).

RESULTS

Matrix assembly of monomeric recombinant FNs

Previously, we reported that r70F2, a truncated form of FN consisting of the N-terminal 70 kDa and the C-terminal 37 kDa regions, was highly capable of assembling into the extracellular matrix when expressed in mouse L cells, although the recombinant protein containing only the C-terminal 37 kDa region, designated rF2, was much less efficiently incorporated into the matrix, and that containing only the N-terminal 70 kDa region, designated r70, showed no matrix incorpora-
Fig. 2. Expression of monomeric forms of r70F2 and rF2 in L cells. Mouse L cells stably expressing r70F2, r70F2 mono, rF2, rF2 mono and r70 were labeled with [35S]methionine, and the recombinant proteins secreted into the culture medium were affinity-precipitated with gelatin-Cellulofine (for r70F2 and r70F2 mono) or with monoclonal anti-human FN antibody FN8-12 which recognizes the C-terminal Hep1/Fib1 domain (for r70) immobilized on Sepharose CL-4B. Precipitated proteins were solubilized with SDS-PAGE sample treatment buffer under nonreducing conditions, separated through a 6.5% acrylamide gel, and subjected to fluorography.

Positions of monomeric proteins are indicated by brackets. Arrows show the positions of heterodimers linked to mouse FN. Positions of r70F2 and rF2 homodimers are indicated by an open arrowhead and an asterisk, respectively. The positions of molecular mass markers are shown in the right margin.

Inhibition of the matrix assembly of r70F2 by proteolytic fragments

To further explore the roles of the N-terminal 70 kDa region and the core domain of the C-terminal 37 kDa region in the matrix assembly of r70F2, L cell transfectants stably expressing r70F2 were cultured for four days in the presence of exogenous proteolytic fragments derived from either the 70 kDa or the 37 kDa region. Immunofluorescence staining with monoclonal antibodies specific to human FN showed that the catheptic 70 kDa fragment strongly inhibited matrix assembly of r70F2 at concentrations as low as 0.05 mg/ml, although the tryptic 37 kDa fragment was only weakly inhibitory at the same concentration (Fig. 4). At an elevated concentration (i.e. 1 mg/ml), however, the matrix assembly of r70F2 was significantly inhibited by the 37 kDa fragment. For quantification of the inhibitory effects of these proteolytic fragments, the amount of r70F2 deposited in the detergent-insoluble matrix was estimated by immunoblot analysis (Fig. 5). Denaturing immunoblot analysis showed that the catheptic 70 kDa fragment inhibited the incorporation of r70F2 into the detergent-insoluble matrix by more than 90% at 0.05 mg/ml, while the tryptic 37 kDa fragment showed 44% and 82% inhibition at 0.05 and 1.0 mg/ml, respectively. These results further provide evidence that not only the 70 kDa region but also the Fib2 domain is actively involved in the matrix assembly of r70F2, although the contribution of Fib2 in the assembly process appears to be much less than that of the 70 kDa region.

Effects of deletion of the Fib2 domain on the matrix assembly of r70F2

To narrow down the active site within the 37kDa region involved in FN matrix assembly, we expressed two types of deletion mutants of r70F2, i.e. r70F2(dI10-12) and r70DS, in L cells (see Fig. 1). r70F2(dI10-12) lacks I10-I12 modules in the Fib2 domain, while r70DS (DS stands for dimer-forming segment) lacks the entire 37 kDa region except for the N-terminal 70 kDa region and the C-terminal interchain disulfide bonds, the core domain of the 37 kDa region, i.e. Fib2, also contributed to the matrix assembly of r70F2.
Fig. 3. Incorporation of monomeric recombinant proteins into the extracellular matrix. L cells stably expressing r70, rF2, rF2mono, r70F2 and r70F2mono (denoted as L/70, L/F2, L/F2mono, L/70F2 and L/70F2mono) and control untransfected cells (denoted as L) were grown to confluence and labeled with monoclonal antibody FN9-1 (L/70) or FN8-12 (L, L/F2, L/F2mono, L/70F2 and L/70F2mono), followed by labeling with rhodamine-conjugated goat anti-mouse Ig antibody, as described in Materials and Methods. Bar, 10 μm.

Fig. 4. Inhibition of matrix assembly of r70F2 by proteolytic fragments. L cells stably expressing r70F2 were cultured for four days in the presence of the trypsin 37 kDa fragment (0.05 mg/ml or 1 mg/ml) or catheptic 70 kDa fragment (0.05 mg/ml) of FN. The cells were labeled with monoclonal antibody FN8-12 (for control untransfected L cells and those incubated with catheptic 70 kDa fragment) or FN9-1 (for the L cells incubated with trypsin 37 kDa fragment), then with rhodamine-conjugated goat anti-mouse Ig antibody. Bar, 10 μm.
To further dissect the active site within Fib2 domain, three deletion mutants of r70F2 lacking each of the three type I modules were expressed in L cells. Immunofluorescence staining of the stable transfectants with monoclonal antibody FN9-1 showed that all of the three mutant proteins were incorporated into the matrix to a lesser extent than control r70F2, although no significant differences were observed in the immunostaining patterns among these mutant proteins (Fig. 8). It is likely, therefore, that a cluster of the three type I modules in the Fib2 domain, but not any one in particular, serves as a functional unit for the matrix assembly of r70F2.

Incorporation of rF2 homodimer into the detergent-insoluble matrix

Since rF2 was secreted as both homo- and heterodimers, immunofluorescence staining of the transfected cells could not distinguish which type of dimer was incorporated into the matrix. To further confirm the involvement of the Fib2 domain in FN matrix assembly, we prepared the deoxycholate-insoluble matrix from L cells stably expressing rF2, and analyzed which type of dimer was deposited into the matrix. Immunoblot analysis with monoclonal antibody FN8-12 showed that the 70 kDa bands of homodimers were more prominent than those of heterodimers (Fig. 6). This result suggests that the Fib2 domain is crucial for the matrix assembly of rF2.

Incorporation of deletion mutants of r70F2 into the extracellular matrix

L cells stably expressing deletion mutants of r70F2 were grown to confluence and labeled with monoclonal antibody FN9-1, followed by labeling with rhodamine-conjugated goat anti-mouse Ig antibody as described in Materials and Methods. Bar, 10 \( \mu \)m.
showed that the homodimer of rF2, but not the heterodimer linked to mouse FN, was predominantly incorporated into the detergent-insoluble matrix (Fig. 9). Similarly, the detergent-insoluble matrix prepared from L cells stably expressing r70F2 contained more homodimer than heterodimer, although the r70F2 secreted to the medium contained both hetero- and homodimers in comparable quantities (see Fig. 2). These results provided additional evidence that the 37 kDa region per se possesses the ability to assemble into the matrix, and that homodimers rather than heterodimers of both rF2 and r70F2 were more favorably incorporated into the extracellular matrix.

**DISCUSSION**

The C-terminal 37 kDa region of FN is comprised of the Fib2 domain and the short dimer-forming segment containing two Cys residues. Although the importance of the Cys residues in FN matrix assembly has already been reported by Schwarzbauer (1991) and further confirmed in the present study, the role of the Fib2 domain has remained only poorly defined. Quade and McDonald (1988) showed that the N-terminally derived 70 kDa fragment inhibited FN matrix assembly on human fibroblasts, whereas the C-terminally derived 15 kDa fragment containing I10-I12 modules did not. Sottile and Mosher (1993) reported that recombinant full-length FN retained the ability to assemble into the matrix even after deletion of I10-I12 modules from the Fib2 domain. Despite these observations, our present findings strongly suggest that the Fib2 domain is actively involved in FN matrix assembly from the following reasons: (a) The mutation of two Cys residues to Ser within the dimer-forming segment significantly reduced the incorporation of r70F2 into the matrix, but the resulting monomeric protein still retained a weak, yet significant ability to assemble into the matrix. Neither the N-terminal 70 kDa region nor the 37 kDa region alone, however, was incorporated into the matrix when expressed as monomeric forms, indicating synergistic effects of the 70 kDa and 37 kDa regions on the matrix assembly of r70F2. (b) Incorporation of r70F2 into the matrix was inhibited by exogenously added 37...
kDa tryptic fragment as was the case with the N-terminally derived 70 kDa cathptic fragment, although the former fragment was less inhibitory than the latter. (c) Deletion of the whole Fib2 domain or of the three type I modules I10 through I12, from r70F2 resulted in a marked reduction of the matrix assembly activity of r70F2. (d) The 37 kDa region per se has intrinsic ability to assemble into the matrix, since the rF2 homodimer was incorporated into the detergent-insoluble matrix more readily than the rF2 heterodimer linked to mouse FN. These results, taken together, indicate that the C-terminal 37 kDa region synergistically potentiates the matrix assembly activity of r70F2. Moreover, it is noteworthy that the matrix assembly of exogenous FN added to cultured fibroblasts (Peters et al., 1990). Thus, plasma FN lacking both ED-A and ED-B modules coassembled into the same fibrils with endogenous cellular FN containing the extra modules, but each FN isoform segregated into discrete patches along the fibrils. Based on these observations, Peters et al. (1990) proposed that the orientation of FN in the fibrils imposes certain structural requirements so that like subunits associate with each other; i.e., plasma FN assembling preferentially with plasma FN and cellular FN with cellular FN.

Recently, the III1 module was reported to be involved in the matrix assembly of FN both in vitro (Chernousov et al., 1991; Morla and Ruoslahti, 1992) and in vivo (Darribere et al., 1992). A 75 amino acid recombinant fragment modeled after the III1 module was shown to induce spontaneous disulfide cross-linking of FN molecules into multimers (Morla et al., 1994). Although little is known about the role of the III1 module in FN matrix assembly, it may function as a binding site for self-association of FN or it may alter the conformation of FN so as to expose the binding site for self-association. It remains to be determined whether inclusion of the III1 module in r70F2 promotes matrix deposition and multimer formation of the recombinant protein.

The extracellular matrix is a supramolecular network consisting of various kinds of fibril-forming proteins such as collagens, laminin, and FN, as well as other components associating with these fibrils. Immunohistochemical studies have shown that most, if not all of these matrix constituents appear to colocalize on the same fibrillar meshwork at light microscopic resolution (Vaheri et al., 1978; Furcht et al., 1980; Little and Chen, 1982), suggesting that fibrils of individual components are further organized into thicker fibrils. It is possible, therefore, that the matrix assembly of FN may be influenced by fibrillogenesis of matrix components other than FN. Dzamba et al. (1993) reported that FN fibrillogenesis was significantly reduced in mouse fibroblasts with defective synthesis of type I collagen, although there have also been conflicting reports that FN matrix assembly is independent of collagen fibrillogenesis (Chen et al., 1978; Vaheri et al., 1978; Dzamba and Peters, 1991; Schwarzbauer, 1991). Since r70F2 contains the collagen-binding domain, incorporation of r70F2 into the matrix may well be influenced by the expression of endogenous type I collagen. Further studies of the interaction between FN fibrils and other components such as collagens are
necessary for better understanding of not only the molecular mechanism of FN matrix assembly but also the supramolecular organization of the extracellular matrix.

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REFERENCES


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