Dualistic Nature of Adhesive Protein Function: 
Fibronectin and Its Biologically Active Peptide Fragments 
Can Autoinhibit Fibronectin Function

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ABSTRACT 
Fibronectin and certain polypeptide regions of this adhesive glycoprotein mediate 
cell attachment and spreading on various substrates. We explored the theoretical prediction 
that this adhesive protein could become a competitive inhibitor of fibronectin-mediated 
processes if present in solution at appropriately high concentrations. Fibronectin function was 
inhibited by purified plasma fibronectin at 5-10 mg/ml, by a 75,000-dalton cell-interaction 
fragment of the protein at 0.5-1 mg/ml, and even by two synthetic peptides containing a 
conserved, hydrophilic amino acid sequence at 0.1-0.5 mg/ml. Inhibition of fibronectin-
dependent cell spreading was dose dependent, noncytotoxic, and reversible. It was competitive in nature, since increased quantities of substrate-adsorbed fibronectin or longer incubation periods decreased the inhibition. A peptide inhibitory for fibronectin-mediated cell 
spreading also inhibited fibronectin-mediated attachment of cells to type I collagen, but it did 
not affect concanavalin A-mediated spreading.

These results demonstrate the potential of a cell adhesion molecule and its biologically 
active peptide fragments to act as competitive inhibitors, and they suggest that fibronectin 
may act by binding to a saturable cell surface receptor.

Cell adhesion to other cells or to substrates is a complex 
process which is still poorly understood at the molecular level (e.g., references 1 and 2). Adhesive events mediated by fibro-
nectin and other extracellular attachment proteins provide 
experimental systems for analyzing polypeptide domains that 
mediate binding and adhesive functions (3-9). For example, 
a region of fibronectin termed the “cell-binding” region, which 
interacts with the cell surface to mediate cell attachment and 
spreading on substrates, has been identified and localized to 
polypeptide fragments (10, 11). Even a synthetic peptide from 
this region of fibronectin can mediate cell attachment to a 
plastic substrate (12).

Theoretically, a cell adhesion protein might become a spe-
cific inhibitor of its own function if it were bound in excess 
to a cellular receptor. Saturation of receptors by soluble 
adhesive molecules might prevent receptor interactions with 
substrate- or cell-attached adhesive molecules. A rough anal-
ogy might be the prezone effect in immunoprecipitation, in 
which an excess of antibody can saturate sites on antigens and 
prevent the formation of aggregates.

We tested this theoretical model with soluble plasma fibro-
nectin and certain peptide fragments of this glycoprotein. 
These molecules were found to be competitive, reversible 
inhibitors of fibronectin function. Our results suggest that 
adhesive proteins can have a dualistic nature, mediating pos-
tive or negative effects depending on their concentrations 
and whether they are in solution or attached to a substrate.

MATERIALS AND METHODS

Fibronectin and Fibronectin Fragments: 
Plasma fibronectin was isolated from human plasma (National Institutes of Health Blood Bank), either 
by sequential gelatin-Sepharose and heparin-Sepharose affinity chromatography 
with elution and washing by 4 M urea as described (13, 14) or by elution under 
nondenaturing conditions at pH 5.5 from a gelatin-Sepharose column (15), and 
centrifuged at 25,000 g for 15 min; results with either type of preparation were 
similar. As shown previously (11), such fibronectin preparations migrated as a 
homogeneous doublet band in SDS polyacrylamide gels.

The 75,000-dalton cell-binding fragment was generated by tryptic digestion 
of human plasma fibronectin and purified exactly as described previously (11). 
A 60,000-dalton collagen-binding fragment was generated by digestion of 
human plasma fibronectin by 5 μg/ml α-chymotrypsin (Worthington Biochem-
In a cell-spreading assay used routinely to quantitate fibronectin biological activity (11, 16, 17), BHK cells spread nearly completely on tissue culture substrates precoated with 3 μg/ml human plasma fibronectin. As shown in Fig. 1 and quantitated in Fig. 2, this fibronectin-mediated spreading was progressively inhibited by increased concentrations of plasma fibronectin added to the adhesion medium. Inhibition appeared maximal at ~10 mg/ml; various preparations displayed maximal inhibition at 6–12 mg/ml of soluble fibronectin. Although spreading was severely inhibited, some cells appeared to display abortive spreading, with increased phase...
density of cytoplasm according to phase-contrast microscopy, but with poor elaboration of peripheral lamellae (Fig. 1).

Nine other proteins and glycoproteins that were tested at 10 mg/ml as controls for nonspecific effects of protein added to the assay system did not affect fibronectin-mediated spreading of these cells (Table I). Proteins without inhibitory effects in this assay included fetuin and fibrinogen.

**Inhibition of Fibronectin-mediated Cell Spreading by a Cell-binding Fragment**

A purified 75,000-dalton fragment of fibronectin which displays nearly intact molar activity compared with native fibronectin for mediating cell spreading (11) became inhibitory when incubated in increased concentrations with BHK cells (Figs. 1 and 3). Inhibition by this polypeptide fragment was maximal at 0.5–1.0 mg/ml, which is equivalent to a concentration of \( \sim 10^{-3} \) M peptide. Half-maximal inhibition occurred at \( 2.4 \times 10^{-6} \) M 75,000-dalton fragment. Fig. 3 also indicates that another fragment of fibronectin of 60,000 daltons containing the collagen-binding domain had no effect

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**Inhibition of Fibronectin-mediated Cell Spreading by Increased Concentration of Added Plasma Fibronectin**

Cells were placed into dishes precoated with 3 μg/ml plasma fibronectin and albumin and incubated in the presence of the indicated concentrations of added plasma fibronectin. A total of 600 cells was scored for spreading for each point; bars indicate standard deviation (n = six fields).

**TABLE I**

| Peptide                          | Amino acid sequence                                          | Concentration for half-maximal inhibition |
|----------------------------------|--------------------------------------------------------------|------------------------------------------|
| Synthetic peptide I              | Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro                     | 120                                      |
| Synthetic peptide II             | Gly-Arg-Gly-Asp-Ser-Pro-Cys                                  | 32                                       |
| Synthetic peptide III            | Cys-Gln-Asp-Ser-Glu-Thr-Arg-Thr-Phe-Tyr                     | >500                                     |
| Adrenocorticotropicin            |                                                               |                                          |
| Aprotinin                        |                                                               | >500                                     |
| Insulin chain A                  |                                                               | >500                                     |
| Luteinizing hormone              |                                                               | >500                                     |
| Fibrinopeptide A                 | Ala-Asp-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Val-Arg     | >500                                     |
| Fibrinopeptide B                 | pGlu-Gly-Val-Asn-Asp-Glu-Val-Glu-Val-Glu-Val                 | >500                                     |
| Angiotensin I                    | Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu                      | >500                                     |
| Bradykinin                       | Arg-Pro-Pro-Glu-Phe-Ser-Pro-Phe-Arg                         | >500                                     |
| Melanocyte-stimulating hormone   | Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro           | >500                                     |
| Neurotensin                      | pGlu-Leu-Val-Glu-Asn-Pro-Arg-Val-Pro-Leu-Leu                 | >500                                     |
| Oxytocin                         | Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly                          | >500                                     |
| Tuftsin                          | Thr-Lys-Pro-Arg                                             | >500                                     |
| --                               | Arg-Gly-Pro-Phe-Pro-Ile                                      | >500                                     |
| --                               | Gly-Pro-Arg-Pro                                             | >500                                     |
| --                               | Arg-Asp                                                     | >500                                     |
| --                               | Gly-Asp                                                     | >500                                     |

*Activities of peptides were determined for BHK cells on a substrate coated with 3 μg/ml plasma fibronectin in a 45-min cell-spreading assay performed as described in Materials and Methods. Also tested were mixtures of free amino acids at the same molar concentrations as the amino acids present in 500 μg/ml of either synthetic peptide I or synthetic peptide II; both mixtures were devoid of inhibitory activity.

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**Inhibitors of Cell Adhesion**

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on fibronectin-mediated cell spreading. This fragment alone had no intrinsic activity in mediating cell spreading (data not shown).

Inhibition of Cell Spreading by Synthetic Peptides

A comparison of published (19–21) amino acid sequences from a limited portion of the cell-binding region of fibronectin is shown in Fig. 4. The sequences reveal a complete identity of amino acids between the three species, except for a conservative substitution of isoleucine for valine. We also focused on this region because it contains the point of greatest local hydrophilicity in the cell-binding domain, as determined for averages of decapeptide units based on the values of Hopp and Woods (reference 22; data not shown). These properties suggest that this region is evolutionarily conserved and particularly well exposed on the outer surface of the protein molecule because of its paucity of hydrophobic residues. Two synthetic peptides from this region were synthesized, one of which contained an additional carboxyl-terminal cysteine residue to permit coupling of the peptide to other proteins and its labeling by alkylation.

As shown in Figs. 5 and 6, a synthetic decapeptide (peptide I) from this region inhibited cell spreading. Inhibition was dose dependent, with half-maximal inhibition at 120 µg/ml. A conserved decapeptide region from the collagen-binding domain (peptide III) containing an identical amino acid sequence in comparisons of bovine and chicken fibronectin (23, 24) was not inhibitory (Fig. 6). A series of 15 other nonrelated peptides shown in Table I also did not produce such inhibition.

Figs. 5 and 7 show that another synthetic peptide (peptide II) containing five amino acids in common with the first peptide was also an effective inhibitor of fibronectin-mediated cell spreading. Half-maximal inhibition occurred at 32 µg/ml. Alkylation of this peptide with iodoacetic acid to block the free -SH group did not alter its inhibitory activity (Fig. 7).

The inhibition appeared to be competitive, since the inhibitory effect of synthetic peptide I was diminished in a dose-dependent fashion as the amount of fibronectin preadsorbed onto the substrate was increased by preincubation of the dishes with increased amounts of fibronectin (Fig. 8). As shown in Fig. 8, there was a 95% inhibition of spreading by 0.5 mg/ml synthetic decapeptide I at a low concentration of fibronectin in the adsorption step, but <25% inhibition at the highest concentration.

Fibronectin biological activity can also be quantitated by...
attachment assays by simply measuring the number of CHO cells that attach or cannot attach to a type I collagen substrate. A similar dose-dependent inhibition of fibronectin-dependent adhesion was observed (Fig. 9), indicating that the inhibitory effect is not unique to an assay based on cell spreading.

**Controls for Cytotoxicity**

None of the autogenous or synthetic inhibitors appeared to be cytotoxic by phase-contrast microscopy (Fig. 5). Viabilities as determined by trypan blue exclusion remained unaltered (e.g., 98.4 ± 0.7% for control cells, 98.0 ± 1.4% for cells with rounded morphology in the presence of 6 μg/ml plasma fibronectin, and 99.2 ± 0.7% for rounded cells in the presence of 0.3 mg/ml synthetic decapetide I).

Protein synthesis was also not substantially inhibited: the incorporation of 14C-labeled amino acids into BHK cells increased 33% in cells that had spread on fibronectin compared with parallel cultures in dishes with no fibronectin coating (uncoated = 28.2 ± 1.8 x 10^4 cpm per 35-mm dish, compared with 37.4 ± 6.6 x 10^4 cpm per dish on fibronectin-coated dishes; values indicate mean ± SD, n = 3). Cells that remained rounded in the presence of the highly inhibitory synthetic peptide II at a concentration of 0.5 mg/ml incorporated amounts of radiolabeled amino acids similar to those of the rounded control cells (24.2 ± 7.5 x 10^4 cpm per dish compared with 28.2 ± 1.8 x 10^4 cpm per dish in controls). Even a drastic inhibition of protein synthesis by treatment with cyclohexamide does not inhibit fibronectin-mediated spreading of BHK cells (reference 25; and data not shown).

Removal of the inhibitors resulted in a rapid re-initiation of spreading to final percentages approaching control values, further indicating the absence of cytotoxicity (Fig. 10). In fact, controls in which inhibitors remained present for a second 45-min assay period showed a modest recovery of the capacity to spread even in the continued presence of synthetic decapetide (Fig. 10; showing 5.7 ± 0.9% spread cells at 45 min compared with 22.2 ± 5.8% after an additional 45 min). There is particularly striking recovery of spreading in the
Reversibility of the autoinhibition by fibronectin (I) or by a synthetic peptide (II). Percentage of BHK cells with a flattened, spread morphology on a control fibronectin substrate after precoating with 3 μg/ml plasma fibronectin (A) or the same substrate plus 6 mg/ml soluble plasma fibronectin in adhesion medium (B), or after the same treatment with soluble fibronectin for 45 min, followed by three washes and a further incubation for 45 min in the absence of soluble fibronectin (C). Spreading of control BHK cells with 3 μg/ml plasma fibronectin (D), fibronectin plus 0.5 mg/ml synthetic peptide I (E), or after washing and incubation for a second 45-min period in the absence of inhibitory peptide (F). Control BHK cells were also incubated on fibronectin for two consecutive 45-min periods (G), plus the continuous presence of 0.5 mg/ml synthetic peptide I (H), or after three washes of the control cultures (no peptide) after 45 min and a second incubation for 45 min (I). Error bars indicate standard error, n = 6; 600 cells for each point.

FIGURE 11 Inhibition by a synthetic peptide of cell spreading mediated by a purified cell-binding fragment of fibronectin. The percentage of BHK cells with a spread morphology is indicated for control cells with no additions (A), compared with cells spreading on dishes coated with 1 μg/ml 75,000-dalton cell-binding fragment (75K) (B–D) in the presence of no additional peptide (B), 90 μg/ml synthetic peptide I (C), or 270 μg/ml synthetic peptide I (D). Cells were also examined using dishes coated with 2 μg/ml 75,000-dalton cell-binding fragment (E and F) in the presence of no additional peptide (E) or 270 μg/ml synthetic peptide I (F). Note that the inhibition by synthetic peptide is less when the cell-binding peptide is present at twice the concentration.

Effect on Spreading Mediated by a Fibronectin Fragment

It was possible that the inhibition occurred by interference with a binding site on fibronectin unrelated to the "cell-binding" site, e.g., with a heparin- or collagen-binding site. However, Fig. 11 shows that synthetic peptide I caused a similar dose-dependent inhibition of cell spreading that was mediated by the 75,000-dalton cell-binding fragment; this fragment is known to lack heparin-, collagen-, and fibrin-binding activities (11).

Effect of Concanavalin A-mediated Spreading

The lectin concanavalin A binds to cell surface glycoconjugates and mediates cell spreading (26, 27; Fig. 12). Such lectin-mediated spreading was not inhibited by synthetic decapeptide I in an experiment in which fibronectin-mediated spreading was inhibited completely (Fig. 12). It is important to note that no inhibition could be demonstrated even at low concentrations of adsorbed concanavalin A, where inhibition might be expected to be detected most easily.

DISCUSSION

Our major conclusions are (a) fibronectin-mediated adhesion as measured by a standard cell-spread assay can be inhibited by fibronectin itself; (b) similar inhibition is produced by a purified cell-binding fragment of 75,000 daltons and two synthetic peptides from this region, but not by a series of other proteins and peptides; (c) the inhibition appears to be
The decrease in effectiveness of this autogenous inhibition when more fibronectin was adsorbed onto the substrate or when the incubation time was prolonged may be explained by the multivalency, and therefore higher expected effective affinity, of molecules adsorbed onto a substrate. In fact, it had been suggested previously that fibronectin requires aggregation or adsorption onto a surface such as a culture dish or a bead in sufficiently high concentrations in order to interact with a low-affinity receptor (2, 30). Substrate-attached, multivalent ligands would be expected to compete more effectively for cellular receptors than monovalent or divalent ligands such as intact fibronectin, its cell-binding fragment, and peptides. Increased quantities of substrate-adsorbed fibronectin or longer incubation times would be expected to favor the formation of adhesive bonds with fibronectin on the substrate, thus permitting cell spreading even in the presence of soluble autogenous inhibitors.

A previous study from our laboratory reported the inhibition of cell attachment to collagen after preincubation of cells with a cell-binding fragment of cellular fibronectin; the final concentrations of fragment were quite low (<14 μg/ml). The inability of our and other laboratories to repeat this observation with equal concentrations of soluble plasma fibronectin or its cell-binding fragment supports the hypothesis that the cellular form of fibronectin can bind to cells with high apparent affinity because it is aggregated; a requirement for fibronectin to be aggregated or adsorbed to a substrate to bind effectively to its receptor has been suggested (2, 4, 28-30).

Our results, however, suggest the existence of direct interactions of soluble molecules with the putative fibronectin receptor, since soluble plasma fibronectin, its 75,000-dalton fragment, and highly soluble, hydrophilic synthetic peptides can all function effectively as inhibitors. The efficacy of synthetic peptides as inhibitors suggests that they may be useful probes of fibronectin-related adhesion in vivo. The synthetic decapeptide was specific in that it did not inhibit another cell-spreading event, i.e., lectin-mediated spreading, but it is important to note that the specificity of such peptides for fibronectin as compared with other attachment factors such as laminin remains to be determined.

Inhibition of normal rat kidney cell attachment by related synthetic peptides has been reported recently by Pierschbacher and Ruoslahti in work accepted for publication after the completion of our study (31). They discovered that the sequence Arg-Gly-Asp-Ser was the minimal sequence necessary to mediate cell attachment, and they were able to estimate the affinity constants of peptides containing this sequence for binding to the cell surface as 3-6 × 10-4 M. The inhibitory activities of synthetic peptides in their assay were less than for the synthetic peptides in our study, perhaps as a result of differences in assay conditions or cell types. Our experiments indicate that adhesion proteins can display either positive or negative activities depending on their concentrations and locations, i.e., in solution or bound to a substrate. The concentration of fibronectin in human plasma is 0.3 mg/ml; it is interesting that some inhibition was detected even at 1.5 mg/ml (Fig. 2). It is possible that similar antagonistic relationships might occur in vivo, and that weakening of adhesive interactions might result from events that increase the local concentrations of soluble adhesive proteins, e.g., in certain disease states or possibly even at localized regions on the cell surface near the sites of secretion of such molecules.

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Note Added in Proof: Plasma fibronectin and a mixture of its proteolytic fragments were recently found to produce a partial, tran-
sient inhibition of hepatocyte attachment to fibronectin (Johansson, S., and M. Höök, 1984, J. Cell Biol., 98:810–817).

REFERENCES

1. Frazier, W. A., and L. Glaser. 1979. Surface components and cell recognition. Annu. Rev. Biochem. 48:491–523.
2. Grinnell, F. 1983. Cell attachment and spreading factors. In Growth and Maturation Factors. G. Guroff, editor. John Wiley and Sons, New York. 267–292.
3. Moneson, M. W., and D. L. Amrani. 1980. The structure and biologic activities of plasma fibronectin. Blood, 56:145–128.
4. Pearson, E., L. I. Gold, and A. Garcia-Pardo. 1980. Fibronectin: a review of its structure and biological activity. Mol. Cell. Biochem. 29:103–128.
5. Ruoslahti, E., E. Engvall, and E. G. Hayman. 1981. Fibronectin: current concepts of its structure and functions. Collagen Related Research. 1:95–112.
6. Hynes, R. O., and K. M. Yamada. 1982. Fibronectins: multifunctional modular glycoproteins. J. Cell Biol. 95:369–377.
7. Aplin, J. D., and R. C. Hughes. 1982. Complex carbohydrates of the extracellular matrix. Structure, interactions and biological roles. Biochim. Biophys. Acta. 694:375–418.
8. Yamada, K. M. 1983. Cell surface interactions with extracellular materials. Annu. Rev. Biochem. 52:761–799.
9. Mosher, D. F., editor. 1984. Fibronectin. Academic Press, Inc., New York. In press.
10. Pierschbacher, M. D., E. G., Hayman, and E. Ruoslahti. 1981. Location of the cell-attachment site in fibronectin with monoclonal antibodies and proteolytic fragments of the molecule. Cell. 26:259–267.
11. Hayashi, M., and K. M. Yamada. 1983. Domain structure of the carboxyl-terminal half of human plasma fibronectin. J. Biol. Chem. 258:3332–3340.
12. Pierschbacher, M. D., E. G., Hayman, and E. Ruoslahti. 1983. Synthetic peptide with cell attachment activity of fibronectin. Proc. Natl. Acad. Sci. USA. 80:1224–1227.
13. Engvall, E., and E. Ruoslahti. 1977. Binding of soluble form of fibroblasts surface protein, fibronectin, to collagen. Int. J. Cancer. 20:1–5.
14. Yamada, K. M. 1983. Isolation of fibronectin from plasma and cells. In Immunchemistry of the Extracellular Matrix. Vol. I. H. Furthmayr, editor. CRC Press, Boca Raton, Florida. 111–123.
15. Murka, S. L., R. C. Ingham, and D. Menache. 1982. Rapid methods for isolation of human plasma fibronectin. Thromb. Res. 27:1–14.
16. Hahn, L. H. E., and K. M. Yamada. 1979. Isolation and biological characterization of active fragments of the adhesive glycoprotein fibronectin. Cell. 18:1043–1051.
17. Grinnell, F., D. G. Hays, and D. Minter. 1977. Cell adhesion and spreading factor: partial purification and properties. Exp. Cell Res. 110:175–190.
18. Grinnell, F., and M. K. Feld. 1981. Adsorption characteristics of plasma fibronectin in relationship to biological activity. J. Biol. Chem. 257:9593–9597.
19. Pierschbacher, M. D., E. Ruoslahti, J. Sundelin, P. Lind, and P. A. Peterson. 1982. The cell attachment domain of fibronectin. Determination of the primary structure. J. Biol. Chem. 257:9593–9597.
20. Peterson, T. E., H. C. Thøgersen, K. Skorstengaard, K. Vibe-Pedersen, P. Sahl, L. Sottrup-Jensen, and S. Magnusson. 1983. Partial primary structure of bovine plasma fibronectin: three types of internal homology. Proc. Natl. Acad. Sci. USA. 80:137–141.
21. Schwartzbauer, J. E., J. W. Tamkun, I. R. Lemischka, and R. O. Hynes. 1983. Three different fibronectin mRNAs arise by alternative splicing within the coding region. Cell 35:421–431.
22. Hopp, T. P., and K. R. Woods. 1981. Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA. 78:3824–3828.
23. Hirano, H., Y. Yamada, M. Sullivan, B. de Crombrugghe, I. Pastan, and K. M. Yamada. 1983. Isolation of genomic DNA clones spanning the entire fibronectin gene. Proc. Natl. Acad. Sci. USA. 80:46–50.
24. Peterson, T. E., K. Skorstengaard, P. Sahl, K. Vibe-Pedersen, and H. C. Thøgersen. Primary structure of fibronectin. In Fibronectin. D. F. Mosher, editor. Academic Press, Inc., New York. In press.
25. Tarone, G., G. Galetto, M. Prat, and P. M. Comoglio. 1982. Cell surface molecules and fibronectin-mediated cell adhesion: effect of proteolytic digestion of membrane proteins. J. Cell Biol. 94:179–186.
26. Grinnell, F., and D. G. Hays. 1978. Induction of cell spreading by subtratum-adsorbed ligands directed against the cell surface. Exp. Cell Res. 110:275–284.
27. Aplin, J. D., and R. C. Hughes. 1981. Cell adhesion on model substrata: threshold effects and receptor modulation. J. Cell Sci. 50:89–103.
28. Klebe, R. J. 1974. Isolation of a collagen-dependent cell attachment factor. Nature (Lond.). 250:248–251.
29. Kleinman, H. K., R. J. Klebe, and G. R. Martin. 1981. Role of collagens in the adhesion of cells. J. Cell Biol. 88:473–485.
30. McAber, D. D., and F. Grinnell. 1983. Fibronectin-mediated binding and phagocytosis of polyionyrene latex beads by baby hamster kidney cells. J. Cell Biol. 97:1513–1523.
31. Pierschbacher, M. D., and E. Ruoslahti. 1984. The cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature (Lond.). In press.