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Research Report

A 3D model of Reelin subrepeat regions predicts Reelin binding to carbohydrates

Roger Panteri[a,*,1], *Alessandro Paiardini*[b,1], *Flavio Keller*[a]

[a]*Laboratory of Developmental Neuroscience, Università Campus Bio-Medico Via Longoni, 83, 00155 Rome, Italy*
[b]*Dipartimento di Scienze Biochimiche A. Rossi Fanelli and Centro di Biologia Molecolare del Consiglio Nazionale delle Ricerche, Italy*

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ABSTRACT

Reelin is a large molecule of the extracellular matrix (ECM) which regulates neuronal positioning during the early stages of cortical development in vertebrate species. The Reelin molecule can be subdivided into a smaller N-terminal domain, showing homology with F-spondin, and a larger C-terminal region containing 8 EGF-like repeats. The localization of Reelin in the ECM, its large dimensions and the modular organization of its primary structure led us to suppose a structure of its modules similar to domains commonly found in ECM proteins such as Agrin, laminins and thrombospondins. We therefore performed a sequence alignment and molecular modeling analysis to study the three-dimensional fold of the Reelin subrepeat regions. Our analysis produces a tentative model of the core region of the Reelin subrepeat sequences and suggests the presence in this 3D model of structural features common to polysaccharide-binding modules which are often found on proteoglycans of the ECM. These findings provide a conceptual framework for further experiments aimed at testing the functions of the EGF-like repeat regions of Reelin.

1. Introduction

Reelin is a large extracellular matrix (ECM) protein that is critical for the appropriate positioning of migrating post-mitotic neurons and the laminar organization of the cerebral cortex during central nervous system development (D'Arcangelo et al., 1995; Rice and Curran, 2001; Tissir and Goffinet, 2003). There is increasing evidence that Reelin is implicated in human diseases of neurodevelopmental origin, such as autism and schizophrenia (Fatemi, 2005). A well-established signaling pathway of Reelin involves binding to the lipoprotein receptors, VLDLR and ApoER2, followed by tyrosine phosphorylation of the intracellular adaptor Dab1, leading to cytoskeletal rearrangements and gene expression changes in the target neurons (Hiesberger et al., 1999). Reelin has also been shown to associate with α3β1 integrins and to modulate neuron–glia interactions (Dulabon et al., 2000). The mouse Reelin gene encodes a protein of 3461 amino acids with several features of ECM molecules, including a cleavable signal peptide, several potential glycosylation sites, a series of EGF-like repeats and a carboxy terminus containing several positively charged amino acids that are required for secretion (D'Arcangelo et al., 1995). The N-terminal region, of about 200 amino acid residues shows ~25% identity with F-Spondin, a

* *Corresponding author*. Fax: +39 06 22 54 14 56.
E-mail address: R.Panteri@unicampus.it (R. Panteri).
[1] These authors contributed equally to this work.

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protein secreted from the floor plate of the neural tube and promoting cell adhesion and neurite growth (Klar et al., 1992). The first 500 amino acids are followed by eight consecutive modules of 350 to 390 amino acids, each containing two related subrepeats A and B, separated by an EGF-like motif of ~30 amino acids (Fig. 1). From previous sequence analyses it emerged that Reelin subrepeats are homologous (D'Arcangelo et al., 1995; Ichihara et al., 2001), raising the possibility of structurally similar modules. Other proteins of the ECM, like Agrin, Perlecan, thrombospondins and laminins, have a multidomain structure in which individual modules have specific functions in cell–matrix interactions, ion channel trafficking or supramolecular assembly (Adams and Tucker, 2000; Dityatev and Schachner, 2003). Some of these small repeated domains were shown in functional studies to bind heparin, sulfatides and α-dystroglycan, thus mediating a series of interactions that are crucial to extracellular matrix assembly and the formation of neuronal connections (Talts et al., 1999; Yoshida et al., 1999; Lawler, 2000).

The localization of Reelin in the ECM and its modular assembly prompted us to investigate, through sequence comparisons, secondary structure predictions and three-dimensional modeling, the structural organization of the subrepeat regions of the Reelin EGF-like repeats. The analysis was carried out on the human Reelin protein sequence and showed that Reelin subrepeats represent structurally similar modules with a high content of β-strands. Furthermore, in a surface cleft of the modeled 3D structure of a Reelin subrepeat region, we detected the presence of structural features common to polysaccharide-binding modules: the ASP-BNR box, a linear motif that frequently occurs in proteins that act on, or interact with, polysaccharides, and originally detected in the Reelin sequence by a combined sequence- and structure-based analysis (Copley et al., 2001) as well as several large aromatic residues. This 3D model, although being a model that approximates the true molecular structure, can be useful to identify conserved residues or structural motifs in Reelin subrepeat regions localized on exposed sites that may take part either in the oligomerization of Reelin molecules (Utsunomiya-Tate et al., 2000) or in its binding to surface receptors. These findings are thus of potential interest for understanding the structural organization of Reelin and for testing the function of the Reelin subrepeat modules.

2. Results

2.1. *Multiple alignment analysis and fold prediction*

To examine the conservation of residues and structural motifs within the Reelin subrepeat sequences we performed a multiple alignment analysis of the Reelin subrepeats using the ClustalW tool. The secondary structure predictions mapped onto the multiple alignment indicate a high content of β-strands which are spaced in a similar pattern within the Reelin subrepeats, suggesting that these Reelin modules are structurally similar units showing an all beta conformation (Fig. 2). The ASP-BNR box, previously identified in the Reelin sequence (Copley et al., 2001), shows a well-conserved consensus sequence with almost invariant residues (S/TXD-XGXXW, boxed sequence in Fig. 2). Since a database search performed with Psi-Blast, using each Reelin subrepeat as the query sequence, failed to find suitable structural templates to be employed in a homology modeling approach, extensive threading experiments were performed. Threading experiments are useful when simple sequence comparisons, even using sensitive profile based methods, fail to locate known structural homologues for a new sequence. This approach takes advantage of the use of additional information, such as incorporation of predicted secondary structure (Kelley et al., 1999) and energetic analysis of models derived from constraining the probe sequence in particular folds (Thiele et al., 1999). Since threading results can vary according to a number of factors, such as the algorithm used in the search and the length of the sequence, we submitted each Reelin subrepeat to two different servers, 3D-PSSM (Kelley et al., 2000) and Fugue (Shi and Blundell, 2001).

The threading results indicate for 8 of the 15 subrepeats analyzed a structure representative of a β-sandwich architecture as the top ranking hit in both of the servers used (Table 1); accordingly there is a good correspondence between the secondary structural elements typical of a β-sandwich architecture and those predicted for the Reelin subrepeats (Fig. 2).

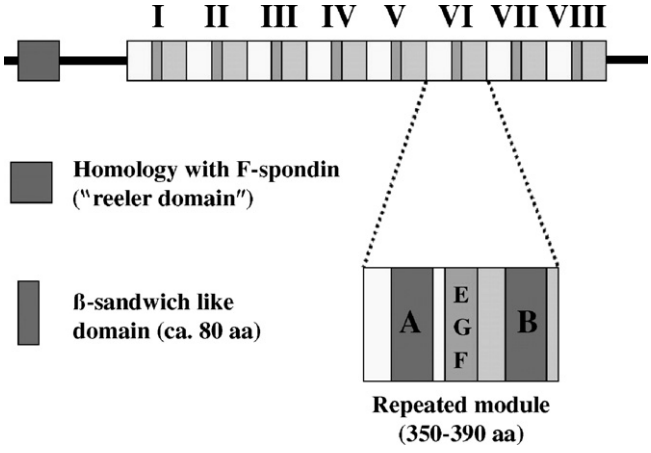

Fig. 1 – The structural organization of Reelin, showing the location of the predicted β-sandwich like domains.