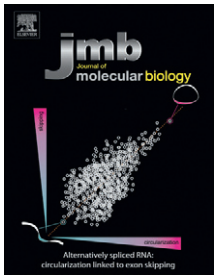

Structure and Mechanism of Dimer–Monomer Transition of a Plant Poly(A)-Binding Protein upon RNA Interaction: Insights into Its Poly(A) Tail Assembly

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Abstract

Poly(A)-binding proteins (PABPs) play crucial roles in mRNA biogenesis, stability, transport and translational control in most eukaryotic cells. Although animal PABPs are well-studied proteins, the biological role, three-dimensional structure and RNA-binding mode of plant PABPs remain largely uncharacterized. Here, we report the structural features and RNA-binding mode of a *Citrus sinensis* PABP (CsPABPN1). CsPABPN1 has a domain architecture of nuclear PABPs (PABPNs) with a single RNA recognition motif (RRM) flanked by an acidic N-terminus and a GRPF-rich C-terminus. The RRM domain of CsPABPN1 displays virtually the same three-dimensional structure and poly(A)-binding mode of animal PABPNs. However, while the CsPABPN1 RRM domain specifically binds poly(A), the full-length protein also binds poly(U). CsPABPN1 localizes to the nucleus of plant cells and undergoes a dimer–monomer transition upon poly(A) interaction. We show that poly(A) binding by CsPABPN1 begins with the recognition of the RNA-binding sites RNP1 and RNP2, followed by interactions with residues of the β2 strands, which stabilize the dimer, thus leading to dimer dissociation. Like human PABPN1, CsPABPN1 also seems to form filaments in the presence of poly(A). Based on these data, we propose a structural model in which contiguous CsPABPN1 RRM monomers wrap around the RNA molecule creating a superhelical structure that could not only shield the poly(A) tail but also serve as a scaffold for the assembly of additional mRNA processing factors.

Introduction

Most eukaryotic mRNAs are posttranscriptionally modified by processes such as capping, splicing, 3′-end cleavage, polyadenylation and deadenylation [1,2]. The process of polyadenylation involves the addition of a poly(A) tail to the 3′-end of mRNAs generating a scaffold for the binding of poly(A)-binding proteins (PABPs), the main regulatory proteins that interact with the poly(A) tail [1,2].

PABPs play multiple roles in posttranscriptional gene regulation, controlling not only the 3′-end processing but also the stability, nuclear export, translational activity and decay of mRNAs [1–4]. Two evolutionary conserved PABPs exist in most eukaryotic cells: the cytoplasmic PABPCs and the nuclear PABPNs [1,2].

PABPC1 is the most studied variant among the PABPC family members; it contains four copies of nonidentical RNA recognition motifs (RRMs) in the N-terminus and a Pro-rich domain required for oligomerization and interaction with other proteins in the C-terminus [5–7]. In contrast, eukaryotic cells seem to have only one nuclear PABP (PABPN1), characterized by a coiled-coil N-terminus, a single internal RRM domain and a C-terminal region with a nuclear localization signal (NLS). The PABPN1 NLS

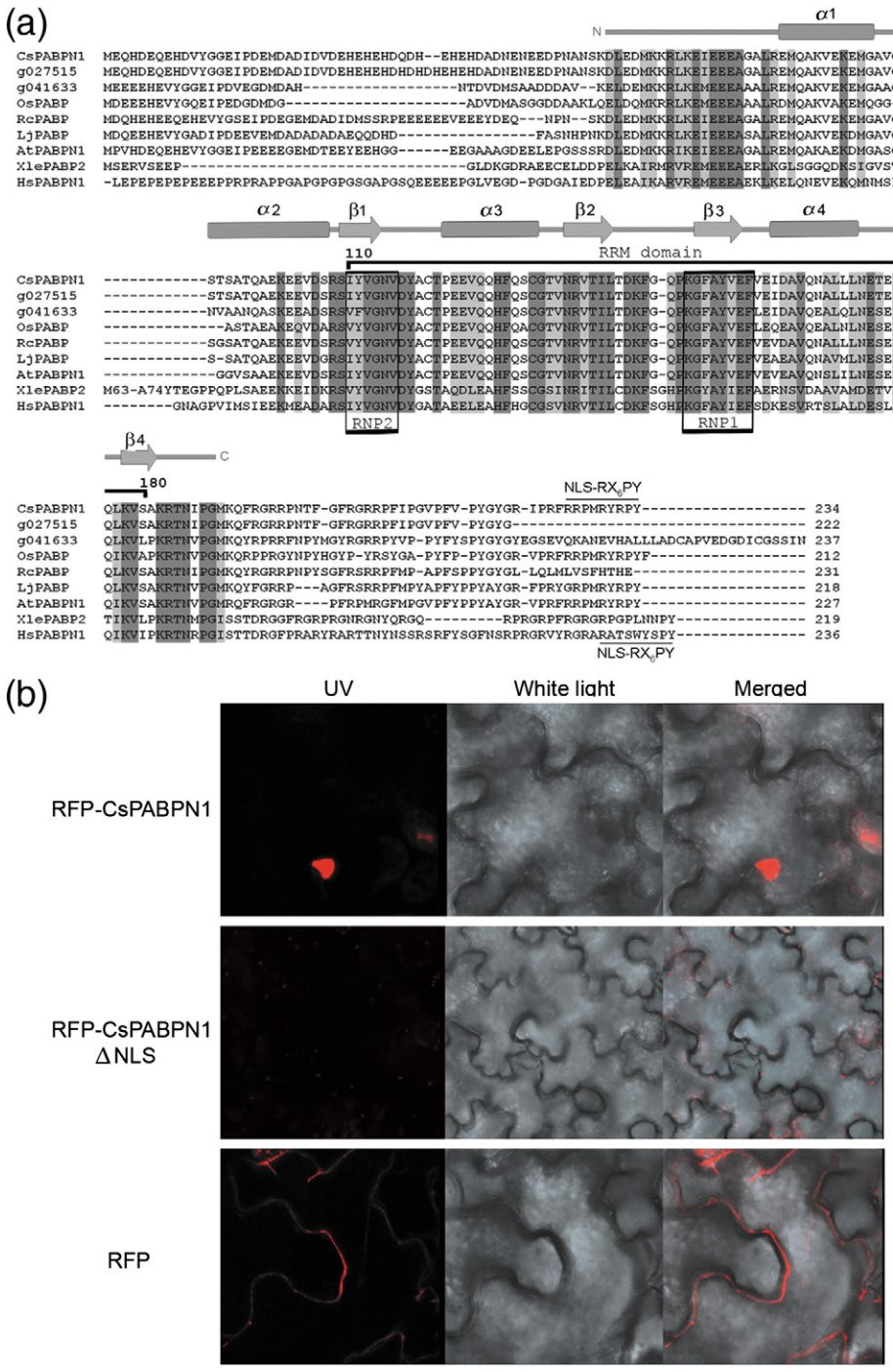

Fig. 1. CsPABPN1 is homologous to type II PABPs and localizes to the nucleus of citrus protoplasts. (a) Amino acid sequence alignment of CsPABPN1 from sweet orange "Pera" cultivar with type II PABPs from *C. sinensis* (orange1.1g027515m and orange1.1g041633m), *A. thaliana* (AtPABPN1), *R. communis* (RcPABP), *L. japonicus* (LjPABP), *Oryza sativa* (OsPABP), *X. laevis* embryonic (XlePABP2) and *Homo sapiens* (HsPABPN1). Identical and conserved residues are highlighted in dark and light gray, respectively. The RRM domain of CsPABPN1 comprising the residues I110 to V180 is indicated. The secondary structural elements determined by NMR are shown above the alignment. Cylinders represent α-helices and arrows represent β-strands. The two conserved RNA-binding sites, RNP2 and RNP1, are boxed. The putative NLS (NLS-RX_6PY) is highlighted in the C-terminal end of CsPABPN1 and HsPABPN1. (b) Subcellular localization of RFP-CsPABPN1 (upper panel) and RFP-CsPABPN1-ΔNLS (middle panel) fusion proteins in *N. benthamiana* cells showing that CsPABPN1 is nucleoplasm located and that the deletion of its putative PY-NLS motif affects the protein nuclear targeting. RFP only control is shown on the bottom panel.