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C-terminal motif within Sec7 domain regulates guanine nucleotide exchange activity via tuning protein conformation

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ABSTRACT

ADP-ribosylation factors (Arfs) play key roles in controlling membrane traffic and organelle structures. The activation of Arfs from GDP to GTP binding form is triggered by the guanine exchange factors (GEFs). There are six families of Arf-GEFs with a common guanine exchange catalytic domain (Sec7 domain) and various mechanisms of guanine exchange activity regulation. A loop region (loop>J motif) just following the helix J of Sec7 domain was found conserved and important for the catalytic activity regulation of Arf-GEFs. However, the molecular detail of the role the loop>J motif plays has been yet unclear. Here, we studied the catalytic domain of Sec7p, a yeast trans-Golgi network membrane localized Arf-GEFs, and found that the loop>J motif is indispensible for its GEF catalytic activity. Crystallographic, NMR spectrum and mutagenesis studies suggested that the loop>J motif with a key conserved residue Ile1010 modulates the fine conformation of Sec7 domain and thereby regulates its guanine exchange activity.

1. Introduction

ADP-ribosylation factors (Arfs), with a small guanine-nucleotide-binding (G) domain, play key roles in controlling membrane traffic and organelle structures via a cycle of GTP binding and GTP hydrolysis [1]. The GTP binding of Arf triggers its N-terminal myristoylated amphipathic helix exposing to bind membrane and thereby recruiting effectors onto membrane. The GTP hydrolysis, which is activated by GTPase activating proteins (GAPs), converts Arf into an inactive GDP binding form. The transition from GDP to GTP binding form of Arf is intrinsically slow, which needs to be accelerated by the guanine exchange factors (GEFs). There are six mammalian Arf proteins (Arf1–6) and at least 15 GEF proteins in human [2]. The specific interactions between Arfs and GEFs are fine controlled both temporally and spatially, which are important for vesicular transportation.

Although highly divergent in their overall sequences, all the Arf-GEFs share a common catalytic domain with ~200 residues, which is referred to as the Sec7 domain based on its homology to the yeast Arf-GEF, Sec7p [3]. Based on the specificities on Arfs, domain organizations and sensitivities to the fungal secretion inhibitor brefeldin A (BFA), the Arf-GEFs can be classified into seven families including GBF1 (Golgi-specific brefeldin A-resistance factor 1), BIG (brefeldin A-inhibited GEF), PSD (Pleckstrin homology (PH) and Sec7 domain), IQSEC (IQ motif and PH domain in addition to Sec7 domain, also called as BRAGs, brefeldin A-resistant Arf-GEFs), Cytohesin (consisting of a short N-terminal amphipathic helix followed by a Sec7 domain and a C-terminal PH domain), FBXO8 (an F-box motif in addition to Sec7 domain) and Sec12 (the specific GEF for Sar1 and not containing a Sec7 domain) [4].

The guanine nucleotide exchange activities of GEFs are highly regulated via various mechanisms. For the GEFs of Cytohesin family, ARNO (Arf nucleotide-binding site opener) and Grp1, they are auto-inhibited in cytosol by which the linker region between Sec7 and PH domains and a C-terminal amphipathic helix physically block the docking sites of Arfs and can be recruited to membrane by Arl4 (Arf like 4) and active Arf6 (GTP form) [5–7]. The interactions between Arf6 and PH domain as well as the C-terminal helix and thereby membrane binding by PH domain activate the guanine nucleotide exchange activity [5,8]. For the GEF of IQSEC/BRAG family, BRAG2 is constitutively active in cytosol but strongly potentiated ~2000-fold when its C-terminal tandem PH domain binds to negatively charged membrane [8]. Recently, a bacterial Arf-GEF RalF was discovered and activated by ~1000-fold when its terminal tandem CAP domain binds to membrane [9]. For the GEF of BIG family, yeast Sec7p, a trans-Golgi network (TGN) membrane localized protein [10], is involved in the ER (endoplamic reticulum)-Golgi anterograde traffic and Golgi biogenesis [11,12]. Sec7p is auto inhibited in cytosol via intra molecular interaction

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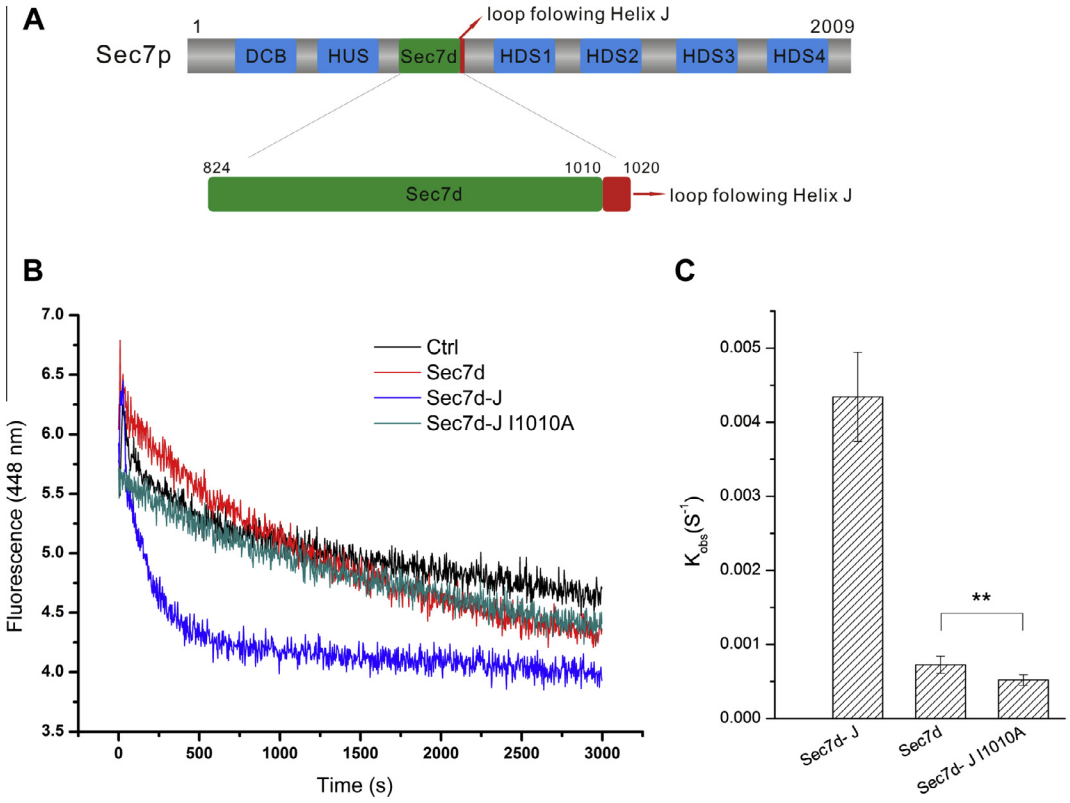

Fig. 1. Loop>J motif is indispensible for the GEF activity of Sec7d. (A) Domain arrangement of Sec7p. DCB, dimerization cyclophilin-binding domain; HUS, homology upstream of Sec7d; HDS1–4, homology downstream of Sec7d. (B) Representative time courses for Arf1-ΔN17 nucleotide exchange catalyzed by Sec7d (red), Sec7d-J (blue) and Sec7d-J (dark cyan). The measurement without GEF proteins is shown as a control (black). (C) Observed rate constants for Arf1-ΔN17 nucleotide exchange catalyzed by GEF proteins. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

between the Sec7 domain and its C-terminal tandem HDS1 domain and is activated when recruiting onto TGN membrane via the interaction between HDS1 domain and the active Arf1 (GTP form) [13,14].

The current knowledge of GEFs activity regulation mechanisms suggests important roles of tandem elements in addition to the C-terminus of Sec7 domain. Recently, a novel C-terminal loop (called as loop>J) immediately following helix J of BIG2 Sec7 domain was found regulating the binding of Arf and guanine nucleotide exchange catalysis activity [15]. Considering the high conservation of loop>J, the regulation of guanine nucleotide exchange activity by loop>J would be ubiquitous within Arf-GEF families. However, the molecular details of how loop>J controls the guanine nucleotide exchange activity of Arf-GEFs are poorly understood yet. Here, we selected another member of BIG family, yeast Sec7p, to study whether the loop>J motif (1008–1020) of its Sec7 domain (Sec7d, see Fig. 1A) affects its guanine nucleotide exchange activity. Our present studies showed that the loop>J motif could modulate the conformation and stability of Sec7d and thereby regulate its catalytic activity.

2. Materials and methods

2.1. Protein expression and purification

The cDNA of Sec7p and Arf1 were amplified from *Saccharomyces cerevisiae* genome. Sec7d (a.a. 824–1010, containing additional three residues of loop>J motif) and Sec7d with the C-terminal fusion of the loop>J motif (a.a. 824–1020, referred hereon as Sec7d-J) were sub-cloned into a pET32a vector (Novagen) with a modification of adding a GB1 (IgG domain B1 of streptococcus Protein G)-6xHis tag encoding sequence at the 5′ end of the inserted gene. Arf1 with N-terminal 17 residues deleted (referred hereon as Arf1-ΔN17) was cloned into a pGEX-6P-1 vector (GE Healthcare). The plasmids were transformed into *Escherichia coli* BL21 (DE3) strain (Novagen). Transformed cells were cultured at 37 °C in LB medium containing 0.1 mg/ml ampicillin or M9 medium containing 1 g/L $^{15}NH_4Cl$ and 0.1 mg/ml ampicillin for expressing ^{15}N labeled proteins. When the OD_{600} of cultured medium reached ~0.8, protein expression was induced by adding IPTG to a final concentration of 0.3 mM. After 20 h inducation at 16 °C, cells expressing Sec7d or Sec7d-J were harvested by centrifugation (6500×*g*) and disrupted by sonication in the lysis buffer, 50 mM Tris, pH 8.0, 300 mM NaCl, and 20 mM imidazole. Fusion proteins with the N-terminal GB1-6xHis tag were purified by affinity chromatography using Ni-NTA column (GE Healthcare) and the GB1-6xHis tag was cleaved by PreScission protease (GE Healthcare) at 4 °C for 16 h. Cells expressing Arf1-ΔN17 were harvested and disrupted in PBS buffer containing 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM KCl and 137 mM NaCl. The GST fused Arf1-ΔN17 proteins were purified by affinity chromatography using Glutathione Sepharose 4B column (GE Healthcare) and the GST tag was cleaved by PreScission protease (GE Healthcare) at 4 °C for 16 h. Further purifications of Sec7d, Sec7d-J and Arf1-ΔN17 were performed via gel-filtration chromatography using Superdex 75 column (GE Healthcare) with the buffer containing 20 mM Tris pH 8.0, 150 mM NaCl. For the purification of ^{15}N labeled proteins, the buffer for gel-filtration was changed to 10 mM $Na_2HPO_4{\cdot}NaH_2PO_4$, pH 6.6 and 100 mM NaCl.

2.2. Crystallization and data collection

Purified Sec7d and Sec7d-J were concentrated to 10 mg/ml for crystallization by hanging drop vapor diffusion method. Single

crystals appeared within 3 days at the condition of 20% PEG MME550, 50 mM $MgCl_2$, 100 mM Tris–Cl pH 8.5. The crystals were flash frozen into liquid nitrogen with the cryo-protectant that contains 20% glycerol in addition to the reservoir solution. Single wavelength (0.97913 Å) diffraction data set was collected to 1.5 Å at the beam-line BL17U, Shanghai Synchrotron Radiation Facility (SSRF). The diffraction data was processed using HKL2000 [16].

2.3. Structure determination and refinement

The crystal structure of Sec7d was solved by molecular replacement using MOLREP [17] from CCP4 package [18]. The starting model is the structure of the Sec7 domain of unbound ARNO (PDB code, 1R8M) [19]. There are two Sec7d molecules in one asymmetric unit. The initial phase was further improved by density modification and applying non-crystallographic symmetry using DM package [20]. The model was automatically built by using ARP/wARP [21], manually modified using Coot [22] and refined using REFMAC5 [23]. The final model was refined to 1.5 Å with R_{work} of 21.1% and R_{free} of 23.7%. The statistics of data collection and structure refinement were summarized in Table 1. All the structure figures were illustrated by using PyMOL (www.pymol.org).

2.4. Guanine nucleotide exchange activity assay

0.5 mg Purified Arf1-ΔN17 was incubated with 1 mM Mant-GDP (Invitrogen) in the buffer (500 μL) containing 20 mM Tris, pH 8.0, 150 mM NaCl, 5 mM EDTA and 1 mM DTT overnight. Then 10 mM $MgCl_2$ was added to stabilize the loading of Mant-GDP onto Arf1-ΔN17. Excess Mant-GDP was removed by desalting chromatography using the buffer 20 mM Tris, pH 8.0 150 mM NaCl, 5 mM $MgCl_2$. The guanine nucleotide exchange reaction was initiated by adding Mant-GDP loaded Arf1-ΔN17 with a final concentration of 1 μM into the buffer containing 0.4 μM Sec7d proteins and 200 μM GDP. The kinetics of guanine nucleotide exchange was measured by monitoring the decrease of fluorescence caused by Mant-GDP releasing [5,24]. The fluorescence was excited at 356 nm and monitored at 448 nm. Kinetics data was collected using Varioskan Flash multimode reader (Thermo Fisher Scientific). Observed pseudo first order rate constants (K_{obs}) can be computed by fitting data to an exponential model [5].

Table 1
Statistics of data collection and structure refinement.

Sec7d	
Data collection and processing[a]	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	48.6, 69.0, 59.6
α, β, γ (°)	90, 106.7, 90
Wavelength (Å)	0.97913
Resolution (Å)	50–1.50 (1.53–1.50)
Total reflections	224,370
Unique reflections	58,905 (2927)
Completeness (%)	97.4
Redundancy	3.8 (38)
R_{merge} (%)[b]	6.2 (54)
$\langle I/\sigma(I)\rangle$	18.8 (2.4)
Refinement	
Resolution (Å)	36.3–1.5
R_{work} (%)[c]	21.1
R_{free} (%)[c]	23.7
RMSD of bonds (Å)[d]	0.0051
RMSD of angle (°)[d]	1.02
Average *B*-factor (Å^2)[e]	17.8

[a] Corresponding parameters for the highest-resolution shell are shown in parentheses.
[b] $R_{merge} = \sum_h\sum_i|I_{ih} - \langle I_h\rangle|/\sum_h\sum_i\langle I_h\rangle$, where $\langle I_h\rangle$ is the mean intensity of the observation I_{ih} reflection *h*.
[c] $R_{work} = \sum(||\mathbf{F}_p(obs)| - |\mathbf{F}_p(calc)||)/\sum|\mathbf{F}_p(obs)|$; R_{free} = *R* factor for a selected subset (5%) of the reflections that was not included in prior refinement calculations.
[d] RMSD indicates the root mean of the standard deviation.
[e] Averaged *B*-factors of corresponding atoms in both chains from one asymmetric unit are shown separately.

2.5. Protein thermal stability by thermal shift assay

The proteins were diluted to 0.2 mg/ml in the buffer 50 mM Tris, pH 8.0 and 150 mM NaCl. The fluorescent dye SYPRO Orange (Invitrogen) was added to the protein solution by ~1000-fold of dilution. 20 μL of mixture in a PCR tube was heated up from 25 °C to 95 °C with the step of 1 °C/min according to the reported protocol [25,26]. The fluorescence of the mixture was measured by using a RT-PCR device (Corbett 6600). The melting temperature (Tm) was estimated as the temperature corresponding to the minimum of the first derivative of the protein denaturation curve.

2.6. $^1H^{15}N$ heteronuclear single quantum coherence (HSQC) spectroscopy

^{15}N labeled proteins, which were prepared in the buffer containing 10 mM $Na_2HPO_4{\cdot}NaH_2PO_4$, pH 6.6 and 100 mM NaCl, was concentrated to 0.3 mM. 450 μL of labeled protein solution was mixed with 50 μL of D_2O and then subjected to HSQC spectrum collection at 25 °C, which was performed with the nuclear magnetic resonance spectrometer (Agilent DD2, 600 MHz).

2.7. Circular dichroism (CD) spectroscopy

0.1 mg/ml Protein in the buffer containing 10 mM $Na_2HPO_4{\cdot}NaH_2PO_4$, pH 6.6 and 100 mM NaCl were used to measure its CD spectrum. The spectra were recorded over the wavelength from 195 nm to 260 nm with a bandwidth of 1 nm and 0.5 s per step by using CD spectrometer (Chirascan-plus, Applied photphysics). The measurements were repeated three times and the spectrum data were corrected by subtracting the buffer control.

3. Results

3.1. Loop>J motif is indispensible for the GEF activity of Sec7d

According the studies of C-terminal motifs of GEFs (GBF1, BIG2 and ARNO) [15], the loop>J motif was defined as the amino acids just following helix J and here refers to residues 1008–1020 in Sec7p. Both Sec7d (a.a. 824–1010) and Sec7d-J (a.a. 824–1020) were constructed and subjected into the guanine nucleotide exchange activity assay by monitoring the decrease of Mant-GDP fluorescence [5,24]. Without the existence of the last ten residues of the loop>J motif (a.a. 1011–1020), the guanine nucleotide exchange rate by Sec7d is only slightly higher than the background. Whereas, with the loop>J motif, the Sec7d-J exhibits significant guanine nucleotide exchange catalytic rate (Fig. 1B). The observed pseudo first order catalytic rate (K_{obs}) of Sec7d-J is over 5-folds of that of Sec7d (Fig. 1C). As a result, in consistency with GBF1, BIG2 and ARGO [15], the loop>J motif in Sec7p is also indispensible for its GEF activity.

3.2. Crystal structure of Sec7d

To understand the role of the loop>J motif in details, we tried to solve the crystal structures of both Sec7d and Sec7d-J and then compare their structural differences. However, Sec7d-J degraded to Sec7d during crystallization procedure and thus both constructs were crystallized in the same condition and yield the same

structure. The crystal structure of Sec7d was resolved at a resolution of 1.5 Å (Table 1).

Conserved in the family of Arf-GEFs [27–29], the overall structure of Sec7d comprises 10 α-helices that arrange in a super-helix fold with the N-portion (α-helix from A to G) and the C-portion (α-helix from H to J) (Fig. 2A). The hydrophobic groove formed by helices F–H for binding to Switch I of Arf, the glutamic finger Glu923 for GEF catalytic activity, and the conserved FG loop [10,19,30] are all registered in the structure of Sec7d as predicted.

Superposition of Sec7d by aligning secondary structures of N-portion (Fig. 2B), C-portion (Fig. 2C) or overall structure with respective parts of other Arf-GEFs (ARNO [27], Gea2 [29], BIG1 and BIG2 [15]) in the Arf-unbound state revealed that the root mean square deviations (RMSD) of N-portions or C-portions among Arf-GEFs are all much smaller than RMSDs of overall structures (Table 2), suggesting that both N-portion and C-portion of Arf-GEFs share a highly conserved conformation but exhibit the flexibility of their relative positions (Fig. 2B and C). The relative movement between N-portion and C-portion of Arf-GEFs had been disclosed as the domain closure that is involved in the guanine nucleotide exchange activity of Arf-GEFs [19,29]. Thus, the relative flexible position between Arf-GEF's N-portion and C-portion would be important for GEF activity.

3.3. Loop>J motif tunes the conformation of Sec7d

To gain further insights into how loop>J motif contributes to the GEF activity of Sec7d, we sought to use nuclear magnetic resonance (NMR) spectrometry to investigate whether the existence of loop>J motif affects the conformation of Sec7d. As we predicted, the $^{1}H^{15}N$ hetero-nuclear single quantum coherence ($^{1}H^{15}N$-HSQC) NMR spectrum of Sec7d-J showed obvious shifts of most peaks in comparison with the spectrum of Sec7d (Fig. 3A), therefore indicating a significant conformational changes occurred upon the interaction between the loop>J motif and the main body of Sec7d. As a result, the role of the loop>J in regulating the guanine nucleotide exchange activity of Arf-GEFs [15] (see also Fig. 1C) would be directly tuning the fine conformation of Sec7 domain.

3.4. The residue Ile1010 controls the conformational stability of Sec7d

In consistency with the study of BIG2/3A mutant by Lowery et al. [15], our Ala-scanning of loop>J motif mutagenesis studies revealed that the mutants I1010A, K1011A and L1012A of Sec7d-J exhibited obvious reduction of the guanine nucleotide exchange activity. Significantly, the mutation of Ile1010 to Ala eliminated the guanine nucleotide exchange activity of Sec7d-J almost completely (Fig. 1B and C). Sequence alignments of Arf-GEFs revealed that the residue Ile1010 in Sec7p is highly conserved across different families (Fig. 3B). Thus, Ile1010 would play a role like a hotspot in controlling the activity of Arf-GEFs.

Investigating the crystal structure of Sec7d, we found that the residue Ile1010 folds back into the hydrophobic cavity formed by helices F, H and J (Fig. 3C) and is involved in the hydrophobic interactions between helices J and F (Fig. 3D), which is conservative in other Arf GEFs, such as BIG2 [31] and ARNO [27]. Thus, the mutation of Ile1010 to Ala would break such interactions and thereby modulate the conformation of Sec7d-J, resulting a significantly reduced GEF activity.

To verify this hypothesis, we performed thermal shift assays to investigate the thermal stabilities of Sec7d and its variants (Fig. 3E). The critical melting temperatures of Sec7d and Sec7d-J are similar as 45.5 °C while the mutant Sec7d-J I1010A exhibited a significant reduced value as 39.7 °C, revealing that the mutation of Ile1010 to Ala largely affects the structural stability of Sec7d-J. The higher initial fluorescence of Sec7d-J I1010A suggests that the mutation of Ile1010 to Ala induces the conformational changes of the domain, therefore yielding more hydrophobic regions exposed to the solvent.

The modulation of domain conformation by mutation I1010A was further investigated again by inspecting the $^{1}H^{15}N$-HSQC

Table 2
Structural RMSD (root mean square deviation) of Sec7 domains of different Arf-GEFs.

	ARNO	Gea2	BIG2	BIG1
$RMSD_{all}$/$RMSD_N$/$RMSD_C$ (Å)[a]				
Sec7p	1.341/1.210/1.008	1.388/1.167/1.114	1.273/1.067/1.197	1.690/1.018/1.365
ARNO		1.538/1.435/0.983	1.256/0.907/1.050	1.884/0.852/0.998
Gea2			1.346/1.167/1.060	1.919/1.276/1.494
BIG2				1.527/0.706/1.434

[a] $RMSD_{all}$/$RMSD_N$/$RMSD_C$ represent the root mean square deviations of overall, N-portion and C-portion structures of Arf-GEFs' Sec7 domains, respectively. The amino acid regions of N-portion and C-portion structures are 824–941 and 942–1010 for Sec7p (this study), 52–174 and 175–246 for ARNO (PDB code, 1PBV), 559–672 and 673–759 for Gea2 (PDB code, 1KU1), 640–756 and 762–828 for BIG2 (PDB code, 3L8N), and 695–811 and 812–885 for BIG1 (PDB code, 3LTL).

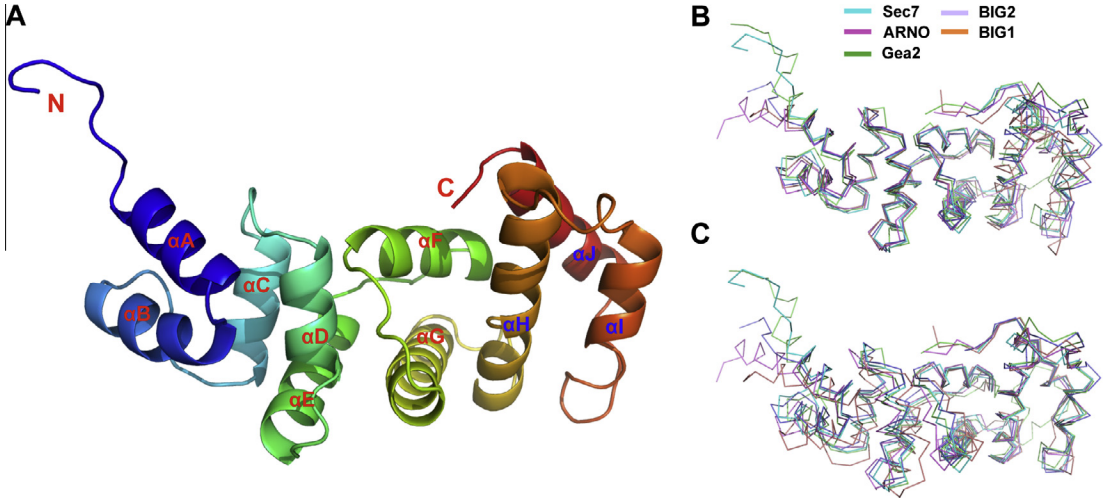

Fig. 2. Crystal structure of Sec7d. (A) Overall structure of Sec7d shown in cartoon with secondary structure labeled. (B and C) Structural superposition of Sec7 domains of Arf-GEF proteins by aligning their N-portion (B) and C-portion (C) structures, respectively. See also Table 2.

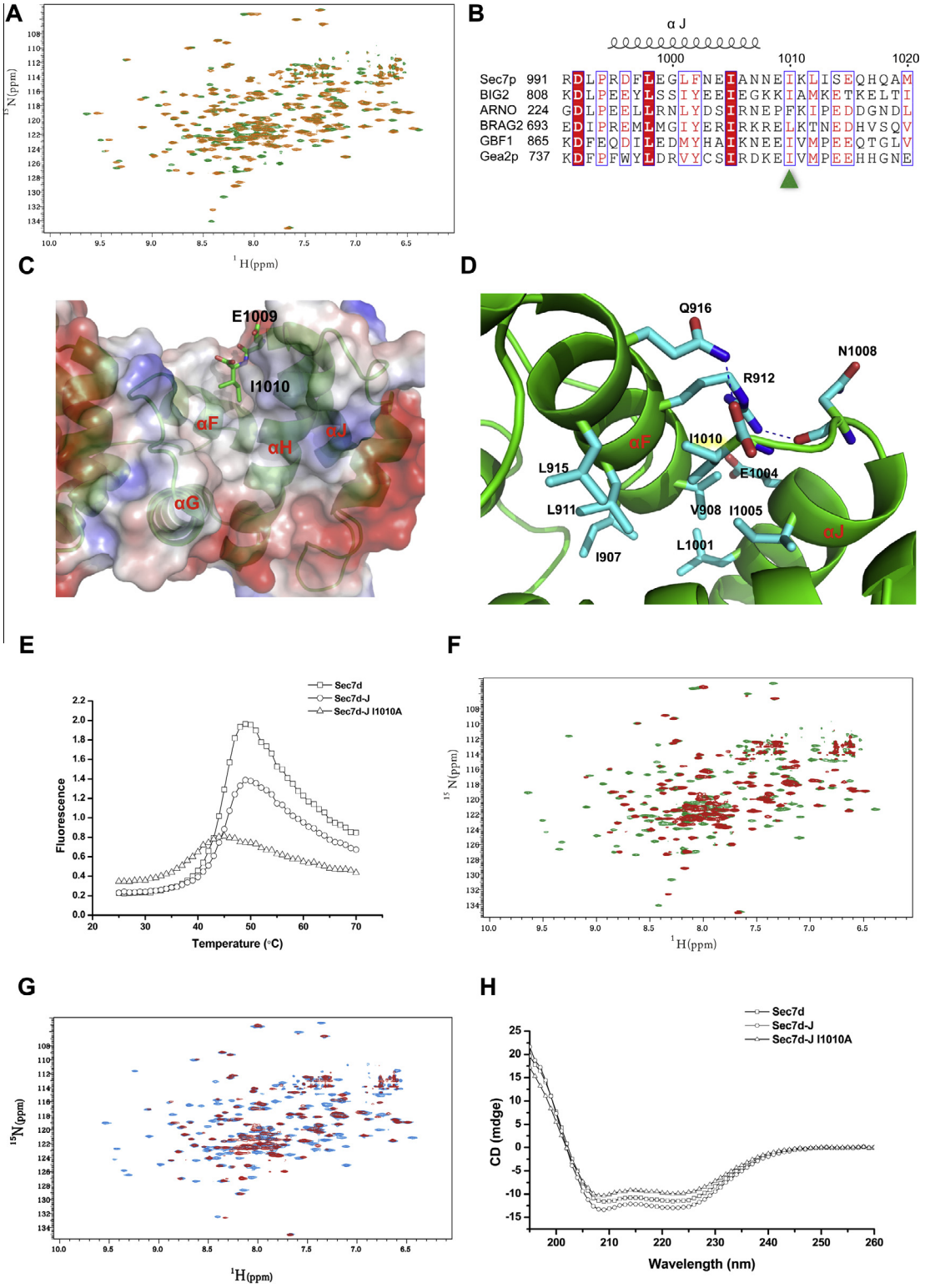

Fig. 3. Loop>J motif and Ile1010 control the conformation and stability of Sec7d. (A) $^{1}H^{15}N$-HSQC NMR spectrum overlay of Sec7d (orange) and Sec7d-J (green). (B) Sequence alignments of Arf-GEFs among different families around the helix J and loop>J motif region. The GenBank accession numbers for the amino acid sequences of these proteins are NP_010454.3 for Sec7p, NP_006411.2 for BIG2, NP_059431.1 for ARNO, NP_055684.3 for BRAG2, NP_001186308.1 for GBF1 and NP_010892.1 for Gea2p. The alignment was performed using ClustalW [34] and the figure was prepared using ESpript [35] (http://espript.ibcp.fr). (C) Surface representation of Sec7d structure around the hydrophobic groove region. The electrostatic potentials of Sec7d are mapped onto the surface with blue for positive and red for negative values. The ribbon of overall structure is overlapped into the surface representation. The loop>J motif with residues I1010 and E10009 are shown in sticks. (D) A zoom-in view showing the hydrophobic interactions between helices (F) and (J). (E) Thermo shift assay of Sec7d, Sec7d-J and Sec7d-J I1010A. (F) $^{1}H^{15}N$-HSQC NMR spectrum overlay of Sec7d-J (green) and Sec7d-J I1010A (red). (G) $^{1}H^{15}N$-HSQC NMR spectrum overlay of Sec7d (cyan) and Sec7d-J I1010A (red). (H) CD spectroscopy of Sec7d, Sec7d-J and Sec7d-J I1010A. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

NMR spectrum of Sec7d-J I1010A (Fig. 3F and G). In comparison with Sec7d and Sec7d-J, the mutant has its HSQC spectrum with reduced number of peaks and abundant of peaks overlapped together, suggesting the presence of significant unstructured elements in the mutant [32]. However, the circular dichroism (CD) spectrum showed that the I1010A mutant displayed α-helix dominated secondary structures that are exactly same as Sec7d and Sec7d-J (Fig. 3H). As a result, the mutation of Ile1010 to Ala does not alter the secondary structures of Sec7d but destabilize the tertiary conformation into a partially unfold state, which is called as molten globule state [33].

4. Discussions

In the present study, we further confirmed the indispensible role of the loop>J motif in the GEF activity of yeast Sec7p, which is consistent with that of GBF1, BIG2 and ARNO [15]. Superposition of all available structures of unbound Arf-GEFs' Sec7 domain revealed the flexibilities of the relative conformation between N-portion and C-portion regions of Sec7 domain and such conformational flexibility had been suggested functional important [19,29]. Although crystallographic data did not tell how the loop>J motif affects the conformation of Sec7 domain, the $^{1}H^{15}N$-HSQC NMR spectrum of Sec7d-J, in comparison to that of Sec7d, showed an obvious conformational change with the existence of loop>J motif. Thus, the mechanism of the loop>J motif regulating the GEF activity of Sec7 domain can be in relevant to conformation modulation, which was also commented by Lowery et al. [15].

Ala-scanning mutagenesis in Sec7d loop>J motif revealed a key residue Ile1010 that is highly conserved across Arf-GEFs families and mutation of Ile1010 to Ala abolishes the GEF activity of Sec7d almost completely. The contact between Ile827 in BIG2 (equivalent to Ile1010 in Sec7d) and switch I region of Arf1 from the modeled complex [15] should not fully explain such severe effect by mutation. Thus, besides affecting the Arf1 binding, other factors exist to contribute the important role of Ile1010. Structure analysis of Sec7d showed that the residue Ile1010 folds back into the hydrophobic cavity that is formed by helices F, H and J and Ile1010 contributes majorly to the hydrophobic interaction between helices F and J. As it had been observed that the 10 helices of Sec7 domain assemble into a super-helix fold mainly through hydrophobic and van der Walls interactions (less hydrogen bonds) among helices [27], the disturbance of helices interactions by mutation of Ile1010 would affect the overall conformation and stability of Sec7 domain. As was predicted, the thermal stability of Sec7-J I1010A decreased significantly in comparison to the wild type. Furthermore, the $^{1}H^{15}N$-HSQC NMR and CD spectrum of Sec7-J I1010A indicated that the mutation of Ile1010 to Ala converts the overall protein into a partial unfolded state with secondary structural elements kept but packing unstably. As a result, the residue Ile1010 at the C-terminal end of Sec7d plays a hotspot in controlling the conformational stability and thereby GEF activity of Sec7 domain. Disturbing the location (potentially by the conformational changes of loop>J motif) and amino acid type of this terminal residue would raise the overall effect on the conformation of Sec7 domain and regulate the GEF activity.

In summary, our experimental data suggested that, besides affecting Arf binding and GEF catalytic activity [15], the loop>J motif with a key conserved residue (Ile1010 in Sec7p) can tune the conformation and stability of Sec7 domain, which is another important factor for GEF activity regulation.

Accession codes

The coordinate of Sec7d crystal structure has been deposited in the Protein Data Bank (PDB) with the Accession code 4OIY.

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