The Structure of the Mammalian Signal Recognition Particle (SRP) Receptor as Prototype for the Interaction of Small GTPases with Longin Domains*

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The eukaryotic signal recognition particle (SRP) and its receptor (SR) play a central role in co-translational targeting of secretory and membrane proteins to the endoplasmic reticulum. The SR is a heterodimeric complex assembled by the two GTPases SRα and SRβ, which is membrane-anchored. Here we present the 2.45-A structure of mammalian SRβ in its Mg2+/GTP-bound state in complex with the minimal binding domain of SRα termed SRX. SRβ is a member of the Ras-GTPase superfamily closely related to Arf and Sar1, while SRX belongs to the SNARE-like superfamily with a fold also known as longin domain. SRX binds to the P loop and the switch regions of SRβ-GTP. The binding mode and structural similarity with other GTPase-effector complexes suggests a co-GAP (GTPase-activating protein) function for SRX. Comparison with the homologous yeast structure and other longin domains reveals a conserved adjustable hydrophobic surface within SRX which is of central importance for the SRβ-GTP:SRX interface. A helix swap in SRX results in the formation of a dimer in the crystal structure. Based on structural conservation we present the SRβ-GTP:SRX structure as a prototype for conserved interactions in a variety of GTPase regulated targeting events occurring at endomembranes.

Nascent chains of secretory and membrane proteins are targeted via the ribonucleoprotein particle SRP and its receptor (SR) to the translocation machinery within the endoplasmic reticulum (ER) membrane (1, 2). The function of the SRP system is described by the SRP cycle (3, 4). SRP recognizes N-terminal hydrophobic signal sequences as soon as they emerge from the ribosomal polypeptide exit tunnel. The complex is targeted to the SR at the membrane, and the ribosome-nascent chain (RNC) complex is transferred to the translocon. Upon GTP hydrolysis in SRP and SR the complex dissociates.

The eukaryotic SR consists of the two GTPases SRα and SRβ (1, 5). SRα is a multidomain SRP GTPase with a characteristic low affinity for nucleotide (~10 μM) and is stable in the nucleotide-free form (6–8). The C-terminal part of SRα (FtsY in archaea and eubacteria) contains the stable NG domain, which binds to the respective NG domain of SRP (8). Several structures of the isolated NG domains revealed the basis for the SRP GTPase cycle (for reviews, see Refs. 2–4 and 9), and the complex of the two NG domains shows a remarkably symmetric heterodimer with the nucleotides in direct contact at the center of the interface (10, 11). The N-terminal part of SRα is responsible for tethering SRα to the ER membrane bound SRβ (12). The interaction localizes to the globular SRX domain of SRα comprising the first 130 residues (13). SRX has been described as effector for SRβ and only binds to the GDP-bound form of the GTPase (13, 14). The SRX domain belongs to the SNARE-like superfamily including the N-terminal domains of non-syntaxin SNAREs, also known as longin domains (13, 15). Longin domains have been proposed to regulate a variety of membrane trafficking processes (16). Members of this superfamily with known three-dimensional structures include the SNAREs Sec22b (17) and Ykt6 (18), the component SEDL of the transport protein particle (TRAPP) (19), and the clathrin adaptor proteins AP-α and AP-μ (20, 21).

SRβ is a classical small Ras-GTPase most similar to Arf (ADP-ribosylation factor) and Sar1 (secretion-associated and Ras-related 1) proteins with an accordingly low Kd of ~30 nm for GTP (6, 22). Phylogenetically, SRβ together with Arf and Sar1 separated from other small Ras-GTPases already in the earliest branching event indicating the functional importance of an ancestral SRβ in eukaryotic evolution (23). Typical for small GTPases, SRβ is characterized by five conserved sequence elements (G1–G5), which form the nucleotide-binding site (24–26). The so-called switch I (residues 85B to 96B in SRβ) to distinguish between SRβ and SRX we use the subscript letters B and X, respectively, interswitch (97B to 115B) and switch II (116B to 130B) regions are known to change most in conformation during a GTPase cycle (26). In the switch II region of SRβ, the position of a critical catalytic residue (Gln-61 in Ras, Gln-71 in Arf) is occupied by a histidine (His-119B), which is conserved among the SRβ and in the Sar1 family (His-79). A special feature of SRβ is its predicted membrane spanning helix, which is unique but dispensable for SR function (27). In comparison, proteins of the Arf and Sar1 family have an extra N-terminal helix that becomes either myristoylated in Arf (28) or is preceded by an N-terminal hydrophobic patch in Sar1 (29). The GTPases are anchored in the GTP-bound state to their target membrane.

SRβ does not hydrolyze GTP (30, 31) and also not in complex with SRα or SRX alone (Refs. 13 and 22 and this study). Like for other small Ras-GTPases, a GTPase-activating protein (GAP) and a guanine nucleotide exchange factor (GEF), which stimulate the low intrinsic GTPase activity and the release of GDP, respectively, are necessary to drive the GTPase cycle (26, 32). RNCs interact with SRβ in its GTP-bound state (22). GTP binding to SRβ is stimulated by the translocon and is required to induce signal sequence release from SRP (33). The GAP function for trypsin-digested SR heterodimers that retain SRβ and the N-terminal

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The atomic coordinates and structure factors (code ZHS2) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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2 The abbreviations used are: SRP, signal recognition particle; SR, SRP receptor; GAP, GTPase-activating protein; ER, endoplasmic reticulum; RNC, ribosome-nascent chain; TRAPP, transport protein particle; Arf, ADP-ribosylation factor; Sar1, secretion-associated and Ras-related 1; GEF, guanine nucleotide exchange factor; r.m.s.d., root mean square deviation; SNARE, soluble NSF attachment protein receptor(s) (where NSF indicates N-ethylmaleimide-sensitive factor).
fragment of SRα including SRX has been attributed to the RNC complex (22). Interestingly, a GAP function of the RNC for the isolated SRβ could not be found (30, 31). In the yeast system, the GEF activity for SRβ has been assigned to the two orthologues (Sbh1p, Sbh2p) of the Sec61β subunit of the translocon (34) and point mutations in the cytoplasmic loops of the yeast translocon severely affect the co-translational translocation pathway (35). However, the molecular details for the initiation of GTP hydrolysis and the release of GDP in SRβ remain so far unclear.

Here we describe the crystal structure of the mammalian SRβ-GTP: SRX receptor complex. The comparison of the structure with the yeast homolog provides detailed insights into the family of the eukaryotic SR. The homology to other small GTPases and the underlying principles of regulation together with previous biochemical data allows attributing a co-GAP function to SRX for SRβ-GTP. We analyze the fundamentals of the longin domain family and suggest the interaction of small GTPases and longin domains to be important for targeting of large complexes or vesicles to the endomembrane system.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The His6-tagged N-terminal 176 amino acids from human SRα (including SRX, residues 1–130) together with mouse SRβ lacking the N-terminal transmembrane region (here referred to as SRβ, residues 58–269) were expressed as a bi-cistronic construct from vector pET16b (Stratagene) in BL21(DE3) Escherichia coli cells (Stratagene). The protein was purified via affinity tag purification using Ni2+-loaded chelating Sepharose Fast Flow beads (Amersham Biosciences). The protein was further purified via ion exchange chromatography (Q- and SP-Sepharose) and finally via size exclusion chromatography (Superdex 200, Amersham Biosciences) using a low salt buffer (10 mM Tris/HCl pH 8.0, 150 mM NaCl, 5 mM MgCl2, a n d 1 mM dithiothreitol). The same buffer was also used for crystallization trials.

Crystallization and Data Collection—Initial crystallization conditions were obtained from hanging drop vapor diffusion at 20 °C using the WIZARD sparse matrix screen (Emerald BioStructures) and a protein concentration of 12 mg/ml by mixing the protein in a 1:1 ratio with reservoir. Crystals grew within 4–6 weeks over a reservoir containing 100 mM sodium citrate, pH 5.5, 2.0 M (NH4)2SO4, and in the presence of 100 mM guanidinium chloride. The leaf-shaped crystal plates belong to space group I222 and diffract to 2.45 Å. The asymmetric unit contains one SRβ-GTP:SRX heterodimer corresponding to a solvent content of 55% and a Matthews coefficient of 2.7 Å3/Da. Crystals were flash-frozen in liquid nitrogen using 20% (v/v) glycerol as cryo-protectant. Diffraction data were measured at the European Synchrotron Radiation Facility in Grenoble, France at beamline ID14–4. Data were collected at 100 K

**TABLE 1**

| Data collection |  |
|-----------------|--|
| Wavelength (Å)  | 0.979  |
| Resolution range (Å) | 50–2.45 |
| Completeness (%) | 99.4 (100.0) |
| Rmerge (%) | 5.7 (42.4) |
| m | 25.0 (3.3) |
| Space group | I222 |
| Unit cell dimensions (Å) | a = 68.1 |
| | b = 118.3 |
| | c = 120.4 |

**Refinement statistics**

| Resolution range (Å) | 20–2.45 |
| Total reflections | 18,124 |
| Working set | 17,202 |
| Test set | 922 |
| Number of refined atoms | Protein 2500 |
| | Mg2+ GTP 35 |
| | Water molecules 104 |
| B-factor (Å2) | 56.5 |
| R-factor (%)* | 19.3 |
| Rfree (%)* | 23.2 |
| r.m.s.d. bond length (Å) | 0.01 |
| r.m.s.d. bond angle (°) | 1.55 |
| Ramachandran plot (%) | |
| Most favored region | 90.9 |
| Additionally allowed region | 8.8 |
| Generously allowed region | 0.4 |
| Disallowed region | 0.0 |

* Values in parentheses are for the highest resolution shell: 2.49–2.45 Å.

**FIGURE 1. Overall structure and SRX topology.** A, the structure of the mammalian SRβ-GTP-SRX monomer is shown as a ribbon diagram with SRβ in cyan, SRX in green, and Mg2+-GTP as a ball-and-stick model. The termini and secondary structure elements are labeled. Disordered parts of the complex are indicated by dashed lines. B, SRX topology. Secondary structure elements are shown in green (β-strands) and magenta (α-helices). The termini and secondary structures are labeled.
and a wavelength of 0.979 Å and processed using the HKL program package (36). Data statistics are listed in Table 1.

Structure Determination and Analysis—The structure was determined by molecular replacement using the β-subunit of the yeast SRβ-GTP:SRX complex (Protein Data Bank code: 1NRJ) as a search model in AMoRe (37). Model building was done with program O (38). The model including 104 water molecules was refined at 2.45 Å resolution using the CNS package (39) to an R-factor of 19.3% and an R-free factor of 23.2% (Table 1). Residues 41X-47X, 131X-176X, 208B-219B, and 248B-254B are not ordered and therefore missing in the model. All structural figures were created with program PyMOL. Program DALI (40) was used to search the Protein Data Bank for proteins similar to SRβ and the angle between the two α1X helices within the SRβ-GTP:SRX "dimer" was determined with program DYNDOM (41). Fig. 5 was done with the program ALSCRIPT (42).

RESULTS AND DISCUSSION

Overall Structure—The overall structure of the refined SRβ-GTP:SRX model is depicted in Fig. 1A. SRβ is a typical small GTPase and features a classical Rossmann fold with a central six-stranded (β1n–β6n) mixed β-sheet packed in between five helices. SRβ reveals highest similarity to the GTP-bound structures of Sar1 in complex with Sec23/Sec24 (43) (r.m.s.d.: 1.30 Å over 143 Cα-positions) and Arf1 (44) (r.m.s.d. of 1.50 Å over 150 Cα-positions) reflecting their evolutionary neighborhood (23). Besides the N-terminal membrane anchoring regions, the most striking structural difference between SRβ and Arf or Sar1 is an insertion between helix α4B and strand β6n (37 residues compared with Sar1). Helix α4 is extended by two turns and protrudes from the protein core as described earlier (13). The insertion is partially disordered and no particular function has been attributed to it so far. In the SRβ-GTP:SRX complex SRβ is in a state not competent for GTP hydrolysis as the catalytic histidine residue (His-119B) is pointing away from the active site (see below).

The SRX domain (Fig. 1, A and B) belongs to the mixed α/β class proteins sharing topology (βαβαββαα) and fold of the SNARE-like protein superfamily including the N-terminal domains of non-syntaxin SNAREs (longin domains). The fold is defined by a three-layer architecture with a central five-stranded antiparallel β-sheet packed against helix α1X on the concave side of the β-sheet and two C-terminal anti-
parallel helices α2X and α3X on the other side (secondary structure numbering is according to the Structural Classification of Proteins (SCOP) nomenclature, which is different to the nomenclature used for the yeast structure). At the N terminus the two anti-parallel β-strands β1X-β2X are connected by a conserved β-hairpin. Helix α1X locates almost perpendicular to the β-strands on the concave side and connects the peripheral β-strands of the β-sheet (β2X and β3X). The helix flanking loop regions are not conserved and only partially visible in the structure. Strands β3X, β4X, and β5X are connected by short β-hairpin structures. The central strand β5X is followed by the long helix α2X, the α2X-α3X loop in the plane of the β-sheet, and the C-terminal helix α3X running anti-parallel to helix α2X. Helix α2X is kinked and wraps around the convex side of the β-sheet like a clamp and helix αLX is inserted in the α2X-α3X loop.

The mammalian SRβ-GTP:SRX complex forms a crystallographic dimer due to an interaction of the SRX domains involving a domain swap of helix α1X (~50° rotation around helical N terminus) and the formation of a continuous trans β-sheet (Fig. 2). Here, strands β3X-β4X of one “monomer” merge and align anti-parallel across the dimer interface. Dimerization leads to an additional buried interface of ~1000 Å² between the two SRβ-GTP:SRX monomers. To analyze the oligomerization state of mammalian SRβ-GTP:SRX in solution, we performed a sedimentation equilibrium experiment by analytical ultracentrifugation and determined a KS of 270 μs for the dimer (data not shown). We cannot directly conclude from this result in solution to the state of the complete SR complex at the membrane, since full-length SRαβ is anchored to the membrane in vivo. The SRβ-GTP:SRα complexes (without the transmembrane region) showed a tendency for aggregation and the KS could not be determined. Therefore, the physiological relevance for the dimerization of SRβ-GTP:SRX is not clear. The dimer might be as well enforced by crystal packing. The crystal symmetry favors the domain swap of the flexibly linked helix α1 (see below) due to steric hindrance. The simultaneous formation of the trans β-sheet stabilizes oligomerization by main chain hydrogen bonding. Interestingly, the comparison with the yeast structure (monomer, see below) showed that the domain-swapped helix α1X of the second SRX molecule of the mammalian receptor superimposes with its corresponding position in the yeast monomer. Therefore, a “monomeric” mammalian receptor complex is used for further analysis.

The SRβ-GTP:SRX Interface—The SRβ-GTP:SRX interface involves the predominant effector-binding region of Ras-like GTPases (45) (Figs. 1 and 3). The buried surface between SRβ-GTP and SRX is 1850 Å², which is similar to the yeast structure and other GTPase-effector complexes (13). SRβ contributes to the interface with its G1 element (P loop, GLCDSGKT), switch I, interswitch, and switch II regions. The complete switch I region snugly binds into a hydrophobic groove of SRX and spans the whole interface. This groove is situated between the amphipathic helix α1X and the hydrophobic concave surface of the SRX β-sheet. Although the protein interface forms a continuous surface, three regions of SRX organized in three layers contribute to the interface (Fig. 3): (i) helix α1X, (ii) the β-hairpin between strands β1X and β2X, and (iii) the α2X-α3X loop including the short helix αLX.

In the top layer, the amphipathic helix α1X binds the switch I and II regions and the P loop of SRβ. The side chain of the conserved Asn-30X forms hydrogen bonds to the side chain Gln-91B (not conserved) and the main chain of Thr-92B in switch I. One helical turn further, Arg-34X forms a salt bridge to Asp-72B in the P loop bridging the active site and forming a hydrogen bond to the side chain of Thr-92B. Three residues of helix α1X (Ile-33X, Leu-37X, and Leu-38X) are part of a hydrophobic pocket, which accommodates Ile-94B and the aliphatic part of Gin-91B.

in the center of the interface. Leu-38X forms an additional hydrophobic interaction with Leu-122B of switch II.

In the central layer, SRX exclusively interacts with the switch I region of SRβ. The central hydrophobic pocket is completed by Val-14X and the aliphatic part of Lys-10X. The conserved β-hairpin between β1X and β2X contributes a number of hydrophobic interactions, which are surrounded by a hydrophobic rim. All hydrophobic interactions are established by main chain atoms of the β-hairpin, which contains a conserved glycine (Gly-12X) at the tip. The carbonyl oxygen of Lys-10X forms a hydrogen bond to the amide nitrogen of Ile-94B. The carbonyl oxygen of Gly-11X approaches the Mg²⁺ binding site in SRβ and forms a hydrogen bond with the side chain of Ser-93B, which is essential for Mg²⁺ coordination. Residues Gly-12X to Val-14X form a short stretch of an anti-parallel trans β-sheet with residues Gin-91B to Asp-89B of switch I.

In the third layer, SRX binds to the switch I and interswitch regions of SRβ. Interactions are formed by the α2X-α3X loop including the short helix αLX. Three hydrophobic side chains (Ala-103X, Leu-104X, and Leu-107X) from helix αLX interact with residues Phe-79B, Val-80B, Leu-83B, and the hydrophobic methyl group of Thr-84B from switch I as well as with Ala-99B and Ile-100B from the interswitch region. Hydrophilic interactions are established by main chain atoms of Ala-103X and Leu-
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107\textsubscript{X}, which hydrogen bond to the main chain of Ser-98\textsubscript{X} and the guanidinium group of Arg-88\textsubscript{X}, respectively. The layer is completed by the conserved glycine in strand 4\textsubscript{X}d on the convex side of the \( \beta \)-sheet. A 15-residue insertion changes the conformation of the loop between helices 3\textsubscript{Xy} and strand 3\textsubscript{Xy} (Fig. 4A). Yeast SRX shows two major insertions (Fig. 4A). A 20-residue insertion elongates the central \( \beta \)-sheet by introducing the sixth \( \beta \)-strand (\( \beta \)-strands \( \beta \textsubscript{Xy} \)) between helix \( \alpha \textsubscript{Xy} \) and strand \( \beta \textsubscript{4Xy} \) (\( \beta \)4\textsubscript{Xy} corresponds to strand \( \beta \textsubscript{3X} \) in our structure), which apparently stabilizes the position of helix \( \alpha \textsubscript{Xy} \) and thereby prevents dimer formation. While helix \( \alpha \textsubscript{X} \) and the \( \beta \textsubscript{1X} \)-\( \beta \textsubscript{2X} \) hairpin in the interface superimpose very well, the central \( \beta \)-sheet and the connected helices \( \alpha \textsubscript{2X} \) and \( \alpha \textsubscript{3X} \) do not. Differences increase with distance from the SR\( \beta \)-GTP:SRX interface.

The observed structural differences between mammalian and yeast SRX are reflected by the low degree of conservation on the sequence level (14.2% identity, Fig. 5). Low sequence conservation is a general feature of the SRX family (13). One functionally important exception is the conserved Gly-12\textsubscript{X} in the \( \beta \textsubscript{1X} \)-\( \beta \textsubscript{2X} \) hairpin (Figs. 3 and 5). It facilitates the \( \beta \)-hairpin turn and a bulky side chain would sterically interfere with binding of SR\( \beta \). Position and amphipathic character of the important helix \( \alpha \textsubscript{X} \) are conserved. Asn-30\textsubscript{X} is conserved between human and yeast and interacts with SR\( \beta \) by hydrogen bonding to the switch I region. A polar residue one turn further appears to occupy a crucial position within helix \( \alpha \textsubscript{X} \). Arg-34\textsubscript{X} forms a salt bridge with Asp-72\textsubscript{B} in the P loop and thereby influences the position of the catalytic histidine (His-119\textsubscript{B}) with respect to the active site of SR\( \beta \) (Fig. 4b). Although this salt bridge is not conserved, a polar interaction is observed in the yeast structure between Ser-35\textsubscript{X} and Gln-47\textsubscript{By} within the P loop, suggesting a similar role.

SRX as Effector for SR\( \beta \)−SRX occupies large parts of a typical GAP binding site (45) as it interacts with the P loop and the switch regions of SR\( \beta \)-GTP resulting in the stabilization of switch II. However, in the SR\( \beta \)-GTP:SRX complex the catalytic histidine (His-119\textsubscript{B}) in switch II of SR\( \beta \) (Gln-61 in Ras, Gln-71 in Arf) is in a "resting" position pointing away from the active site (Fig. 4b), the characteristic arginine finger of a GAP (46) is not present, and the complex is stable when bound to GTP. Therefore, the SR\( \beta \)-GTP:SRX complex is not a GTPase:GAP complex, and for the stimulation of GTP hydrolysis an additional binding partner is needed. The RNC has been shown to stimulate GTP hydrolysis of SR\( \beta \)-GTP:SRX (22). However, the RNC does not act as a GAP for SR\( \beta \)-GTP alone (30). Therefore, the SRX domain can be assigned as co-GAP

**FIGURE 5. Alignment of longin domain sequences.** The alignment is based on structures (bold sequences) or secondary structure predictions (nonbold type). Known structures include human and yeast SRX, mouse Sec22b (Protein Data Bank code: 1FIO, chain A), yeast Ykt6 (1H8M, mouse SEDL (1H8Q), human AP2-\( \omega \)2 (1GWS, chain A), and AP2-\( \omega \)2 (1GWS, chain M). The structures of c-COP (hCOP2), h-COP (hCOPD), and the SNARE hVamp7 are not known. Sequence numbering and secondary structure assignment are shown for human SRX above the aligned sequences (\( \beta \)-strands in green, \( \alpha \)-helices in orange). The secondary structure is indicated in all sequences. The conserved glycine in the \( \beta \)1X-\( \beta \)2X loop is marked in red. The residue causing a conserved anomaly in strand 3\textsubscript{Xy} is indicated in green, and the critical polar position in 3\textsuperscript{X} hydrogen bonding to the P loop in SR\( \beta \) is highlighted in blue.
for SRβ which fulfills one part of the GAP function by stabilizing switch II. Examples for a split GAP function have been reported before. The GAP for the α-subunit of a heterotrimeric G protein (G_{αq}) also stabilizes the switch regions, but the arginine finger is supplied in cis by an additional domain of the GTPase (47). A unique feature of the Arf1:ArfGAP1 structure is the exclusive stabilization of the switch II region (48). The switch I region is recognized by the heptameric coat protein complex (COPI) (49), which is found to stimulate GTP hydrolysis (48). Most likely an arginine finger is needed to trigger GTP hydrolysis in Arf1 (48), which might be the case as well in SRβ.

The co-GAP function can be envisaged by a comparison of SRβ-GTP:SRX with the structure of the Ras-GDP-AIF₃:RasGAP transition-state complex (50). When SRβ is superimposed with Ras, the loop of RasGAP containing the arginine finger (Arg-789_RasGAP) fits between SRβ and SRX (Fig. 6A). The only sterical clash concerns the arginine finger itself, which would interfere with the salt bridge between Arg-34X and Asp-72B (Fig. 6, A and B). In addition, the Ras-GDP-AIF₃:RasGAP complex contains a second arginine (Arg-903_RasGAP) in close proximity to Arg-34X (Fig. 6B). Arg-903_RasGAP forms a salt bridge to Glu-63Ras in the switch II region of Ras thereby stabilizing the switch II region. In SRβ-

FIGURE 6. Split GAP model for SRβ activation. The model is based on the superposition of the respective GTPases within SRβ-GTP:SRX and the Ras-GDP-AIF₃:RasGAP complex (gray, Protein Data Bank code: 1WQ1). A, SRβ-GTP:SRX is shown together with the finger loop of RasGAP containing the arginine finger Arg-789_RasGAP. The loop fits between SRβ and SRX, and the arginine finger interrupts the Arg-34X-Asp-72B salt bridge. B, superposition of the active sites. Arg-903_RasGAP occupies a similar position as Arg-34_X. In the activated Ras-RasGAP complex, the arginine binds to Glu-63Ras (position Ser-121X in SRX), and the catalytic Gln-61_Ras is bound to the nucleophilic water in the active site. C, model of GAP-activated SRβ-GTP:SRX. Arg-34_X could bind to Ser-121_X in a similar way as Arg-903_RasGAP to Glu-63Ras. His-119_X is rotated into the active site. The arginine finger could be provided by the RNC-SRP complex.
GTP:SRX the catalytic residue His-119B is hydrogen bonded to the corresponding residue of Glu-63Ras (Ser-121B) (Fig. 6B).

The comparison of SRβ-GTP:SRX with the Ras-GDP-ALF3-RasGAP complex suggests that upon the insertion of an arginine finger into the GTP binding pocket the salt bridge between Arg-34X and Asp-72B can be disrupted. The liberated Arg-34X may then swing from the P loop toward Ser-121B in switch II forming a hydrogen bond (Fig. 6C). His-119B would therefore be released, the catalytic water can be positioned, and hydrolysis occurs. Mutants in which the salt bridge is disrupted (Asp-72B → Gly and an Arg-34X → Ala) still form the SRβ-GTP:SRX complex (data not shown) indicating that the missing GAP is essential to stimulate GTP hydrolysis. The large conformational changes that are typically observed in the switch regions upon GTP hydrolysis are expected to disrupt the SRβ:SRX interface and lead to the dissociation of the SR complex (13). In the context of the SRP cycle this could happen either before or after signal peptide release from SRP.

Longin Domains Revisited—SRX belongs to the superfamily of SNARE-like proteins with the longin domain fold (15). Sequence homology within the superfamily is low (Fig. 5), but the structural homology is high (Fig. 7) as illustrated by the comparison of SRX with SEDL (19), with the SNAREs Sec22b (17) and Ykt6 (18), and the μ2 (N-terminal domain) and α2 adaptins (20). To determine conserved elements within the longin domain fold we prepared a structure based sequence alignment of structurally known longin domains and of important longin domain candidates (COPI, Nβ-COP, VAMP7; Fig. 5). Among longin domains with known structures, SRX reveals specific insertions like strand β3xy, whereas the mammalian structure is closer to other members of the superfamily.

Longin domains share the ββαββαα topology as described for SRX (Fig. 1B). The glycine residue (Gly-12x in SRX) in the β1-β2 hairpin is highly conserved (Fig. 5), and the hairpin adopts a similar conformation in all longin domain structures. Only in SEDL this glycine is exchanged.
for an aspartate, and the change is compensated by adjustments in the adjacent β-strands. Ykt6 comprises a unique insertion of three residues. Helix α1 is an essential component of the longin domains (see below). The amphipathicity of helix α1 is highly conserved, while there is no conservation on the sequence level and the length ranges from three (SRX) to six turns (Ykt6). The orientation of helix α1 with respect to the central β-sheet varies in the different longin domains (Fig. 7). Flexibility is reflected by elevated temperature factors in the loops connecting helix α1 to the β-sheet (not shown) and in the SRX structure the flexibility is responsible for dimer formation by the swap of helix α1X (Fig. 2).

A conserved β-sheet anomaly (down-up-down) is the insertion of a bulky hydrophobic residue (Leu-15X in SRX, Fig. 5) within strand β2. It seems to be important for stabilizing the protein core and indicates an evolutionary relationship between the longin domains. The C-terminal helix α3 differs in length and orientation between the individual structures and superimposes best for Sec22b, SEDL, and SRX. Helix α3 is truncated in the longin domains of the AP2 complex (Nβ2, α2), which according to secondary structure predictions is also the case in other AP complexes and the COPI complex (Nδ- and ε-COPI) (Fig. 5). Here, the longin domain fold is extended by a β-hairpin structure followed by another helix forming a fourth layer in the back of the longin domain fold (not shown). The length and the conformation of the loop regions vary significantly (Fig. 7).

GTPase:Longin Domain Complexes at Endomembranes—The localization of longin domains at the endomembrane system correlates with the presence of small membrane-associated GTPases like the Arf and Sar1 proteins, which are the closest relatives of SRβ. The structural conservation and the co-localization strongly suggest that other GTPases:longin domain interactions may exist. Two hydrophobic patches flanking helix α1 were noticed previously in longin domain structures and were proposed as protein-protein interaction surfaces (17–19). Interestingly, these patches are conserved in structurally determined longin domains (Fig. 7). In the SRβ-GTP:SRX complex, SRβ intercalates its switch I region between helix α1X and the SRX β-sheet; one of the helix flanking hydrophobic patches is extended and forms a hydrophobic groove (Figs. 3 and 8). In free longin domain structures the hydrophobic groove is absent (Fig. 8). The opening of the groove can be envisaged by rolling the conserved amphipathic helix α1 onto the second hydrophobic surface patch on the other side of the helix. The flexibility of helix α1 is therefore a prerequisite for the interaction of longin domains with their respective GTPase.

While the conservation of the hydrophobic patches suggests a similar mode of GTPase:longin domain interaction, the low degree of conservation reflects the special adaptations of the individual systems. For example, in all known longin domains the equivalent position of Arg-34X within helix α1X (Figs. 3 and 4B) seems to be occupied by a charged or polar residue (Fig. 5). In the respective GTPases the same is true for the residue at the position equivalent or adjacent to Asp-72s in the P loop. Therefore, a polar contact between helix α1 and the P loop might be present in all GTPase:longin domain interactions. As discussed for the co-GAP function of SRX (see above), the residues corresponding to Arg-34X could also participate in the stabilization of the switch II regions of the respective GTPases. The mammalian SRβ-GTP:SRX complex can thus be regarded as a structural prototype for a GTPase:longin domain interaction. Although there is no direct experimental proof, to our knowledge this idea does not contradict any previous data.

Structures of longin domains other than SRX have been determined as monomers (Sec22b, Ykt6, SEDL) or in context of the AP adaptin “trunc” complex. All clathrin adaptor complexes (AP1, -2, -3, -4) and COPI share a tetrameric trunc organization that consists of two large, a medium, and a small subunit (51). COPI and AP complexes contain two copies of longin domains (Nδ- and ε-COPI, and AP-Nβ and -α, respectively). In the structure of the AP2 complex (20), the two longin domains form the core of the trunc with the respective α1 helices being in close proximity. Therefore, a tandem GTPase:longin domain interaction might be an important feature in all these complexes.

A Molecular Explanation for a Genetic Disease—The GTPase:longin domain concept offers a structural explanation for the occurrence of spondyloepiphyseal dysplasia tarda, an X-linked skeletal disorder characterized by a short trunk (52). Point mutations in the human SEDL protein seem to be involved in a defect in cartilage transport from the ER.
to the Golgi apparatus (53). The yeast homologue of SEDL (Tres20p) has been shown to be part of the highly conserved transport protein particle I (TRAPP I) that is required to tether ER-derived vesicles to the Golgi (54) and consists of ten subunits (55). When the structure of SEDL is superimposed with SRX in the SRβ-GTP:SRX complex (Fig. 9), the pathogenic Asp-47→Tyr mutation in human SEDL would be located on the protein surface within helix a1x in close proximity to the catalytic residue His-119x and the interacting Ser-121x of SRX (see Fig. 4f). There is no structure of the corresponding SEDL:GTPase complex; however, Ypt1p has been shown as the TRAPP interacting GTPase (55–57) and according to our model Gln-67 and Arg-69 in Ypt1p could form a favorable interaction with Asp-47$^{\text{SEDL}}$. Thus, the mutation most likely disturbs the GTPase regulation by interfering with the positioning of the catalytic residue.

**CONCLUSIONS**

The structure of the mammalian SRβ-GTP:SRX complex reveals the overall features of a GTPase-effector complex. Although the sequence identity is very low ([13] this study), the interaction is conserved between mammals and yeast. The mode of interaction, previous biochemical data (22, 30), and the structural comparison with the Ras:RasGAP complex (50) point to a co-GAP function of SRX for SR

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