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The mammalian and yeast translocon complexes comprise a characteristic Sec61 channel

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ABSTRACT

In eukaryotes, protein translocation across and insertion into the membrane of the endoplasmic reticulum (ER) is facilitated by a protein-conducting channel, the Sec61 complex or translocon. In our previous electrophysiological studies, we characterized the mammalian Sec61 channel from *Canis familiaris*. Here we extended these initial results to the Sec61 channel from the yeast *Saccharomyces cerevisiae* and compared the basic electrophysiological properties of both channel preparations with respect to the gating behaviour, distribution of channel open states, ionic conductance, approximated pore dimensions, reversal potential and selectivity as well as voltage-dependent open probability. We found that the Sec61 complexes from both species displayed conformable characteristics of the highly dynamic channel in an intrinsically open state. In contrast, the bacterial Sec61-homologue, the SecYEG complex from *Escherichia coli*, displayed under the same experimental conditions significantly different properties residing in an intrinsically closed state. We therefore propose that considerable differences between the respective eukaryote and prokaryote protein-conducting channel units and their regulation exist.

1. Introduction

The initial step in the biogenesis of approximately 30% of eukaryotic proteins is their integration into the membrane or their transport into the lumen of the endoplasmic reticulum (ER) [3]. Protein integration or transport into the ER can occur co- or post-translationally and typically requires signal peptides at the amino terminus of the precursor proteins and a protein translocase in the ER membrane. This protein translocase includes Sec61α (Sec61p in yeast), Sec61β (Sbh1p), and Sec61γ (Sss1p) as core components [8,10,20].

In our previous electrophysiological analysis of the Sec61 complex in canine pancreatic microsomes or reconstituted into proteoliposomes, we characterized mammalian Sec61 complexes containing either a mixture of undefined nascent precursor polypeptides in transit (cotranslational transport), or a defined presecretory polypeptide (co- and posttranslational transport). The observed conductance properties were almost identical under these various conditions and support the concept of an oligomeric Sec61 complex with a channel opening at the subcomplex interface [28].

Here we extended this initial study to the Sec61 complexes from yeast microsomes, demonstrating that these are also highly dynamic channels with a multitude of conductance states. Furthermore, upon refined analysis of channel properties, we observed that the channels from *Canis familiaris* and *Saccharomyces cerevisiae* displayed analogous, if not identical, characteristics with respect to ionic conductance, pore size, ionic selectivity, gating characteristics and voltage-dependent open probability. Moreover, the channel of the mammalian Sec61 complex resides in an intrinsically open state. In contrast to this, the channel of the purified bacterial Sec61-homologue, the SecYEG complex from *Escherichia coli*, resides in an intrinsically closed state.

2. Materials and methods

2.1. Materials

CHAPS, deoxy Big CHAP, and digitonin were purchased from Calbiochem, Darmstadt, Germany. Purified phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine) were from Sigma, egg yolk phospholipids were from Avanti Polar lipids, and L-α phosphatidylcholine (egg) came from Larodan Fine Chemicals.

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2.2. Preparation of microsomes and proteoliposomes

Rough microsomes were prepared from dog pancreas as previously described [28]. Yeast microsomes were prepared according to established procedures [21] from the protease-deficient strain MC2 (MATa, ura3–52, leu2–3112, trp1, prc1–407, prb1–112, pep4–3) kindly provided by C. Stirling (University of Manchester).

2.3. Nycodenz gradient flotation

One hundred microliters of proteoliposomes was covered with a discontinuous Nycodenz (Axis Shield, Oslo) gradient (0.5 ml 20%, 1 ml 10%, 1 ml 5%, 0.5 ml buffer) in 50 mM K-Hepes, 200 mM K-Acetat, 10 mM DTT, 12.5% glycerol, pH 7.5. The gradients were centrifuged for 1 h at 200,000*g* in a MLS50 rotor and separated into 10 fractions as indicated. Subsequently the protein content of the fractions was precipitated with TCA. The pellet was washed with ice-cold acetone and dried at 45 °C. Samples were analyzed by SDS–PAGE.

2.4. In vitro translocation assay

SecYEG was reconstituted into proteoliposomes (lipid composition: PE/PG/PC/CL = 12/3/3/2; mimicking the composition of the *E. coli* total Lipid Extract from Avanti) essentially as described previously using either 6% BigCHAP deoxy (BC) or 80 mM Mega-9 (M9) to dissolve the liposomes and Calbiosorb Adsorbent (Calbiochem) to remove the detergent [5]. In order to monitor the functionality of the SecYEG complex translocation of proOmpA into SecYEG proteoliposomes was assayed as described earlier [27], except that protease protected proOmpA was detected by Western blot using an antibody raised against proOmpA [7].

2.5. Vesicles prepared for bilayer fusion

Vesicles for planar bilayer experiments were prepared by mixing (3:2 v/v) of the *C. familiaris* and *S. cerevisiae* rough microsomes with preformed liposomes (egg L-α phosphatidylcholine, 20 mg/ml, Larodan Fine Chemicals) in 50 mM KCl, 10 mM MOPS/Tris, pH 7.0, and additional 80 mM Mega-9 (nonanoyl-*N*-methylglucamide). A Branson sonifier equipped with a microtip (output control 4, 20% duty cycle) was used. After mixing, the sample was dialysed for 4 h at room temperature and then overnight at 4 °C against a buffer (5 L) containing 50 mM KCl and 10 mM MOPS/Tris, pH 7.0. This protocol was also applied for SecYEG proteoliposomes in cases where no successful bilayer fusions could be obtained directly after reconstitution. Incubation of 10 μl aliquots (typically 10 mg/ml protein, lipid/protein 2:1 [w/w]) of the proteoliposomes derived from RM vesicles with 200 μM puromycin and 250–500 mM KCl was performed for 15–30 min on ice. After 15 min, 1 μl aliquots were applied for bilayer fusion.

2.6. Planar lipid bilayers

Planar lipid bilayers were produced by the painting technique [18]. The resulting bilayers had a typical capacitance of 0.5 μF/cm^2 and a resistance of >100 GΩ. The noise was 3 pA (rms) at a 2 kHz bandwidth. An osmotic gradient was used for vesicle fusion [12]. Membrane potentials refer to the *trans* compartment. Recording and analysis of the data were performed as described. Voltage ramps were conducted with a rate of 10 mV/s. Ramps started from −50 to 0 mV and from 0 to +50 mV.

2.7. Calculation of the pore diameter

The pore size was calculated according to [11] (p. 352) as described in [12]:

$$d = \frac{\rho G}{\pi}\left(\frac{\pi}{2} + \sqrt{\left(\frac{\pi}{2}\right)^2 + \frac{4\pi l}{\rho G}}\right)$$

where d is the diameter of the pore, G is the conductance, l is the length of the constriction zone, assumed to be 1 nm, and ρ is the resistivity of the solution, 31.7 Ω cm for a 250 mM KCl solution; taking into account the correction factor of 5 [23], the value is 158.5 Ω cm.

3. Results

3.1. The electrophysiologically analyzed canine and yeast Sec61 complexes were active and transport competent

In a first set of experiments we determined whether the canine and yeast microsomes contained native Sec61 complexes which were active in protein translocation. The controls for the canine microsomes and the purified Sec61 complex from the same source are described in details elsewhere [28]. For yeast microsomes, yeast prepro-alpha-factor that lacked glycosylation sites was used as a model precursor polypeptide. The precursor was synthesized in the presence of buffer or in the presence of two different concentrations of yeast microsomes as indicated. Subsequently, the samples were subjected to protease treatment, SDS–PAGE and phosphorimaging (Fig. 1A). As expected, in the absence of microsomal membranes (−RM) the precursor remained unprocessed and was protease-sensitive in the presence and absence of detergent. Furthermore, in the presence of microsomes (+RM) the precursor was processed to mature pro-alpha-factor and imported into microsomes (i.e. was protected against externally added protease in the absence of detergent but not in its presence). Processing and import efficiency correlated with the microsome concentration. Thus the yeast microsomes, too, were active in protein translocation, i.e. contained active Sec61 complexes.

3.2. The mammalian and yeast Sec61 channels display similar electrophysiological properties

To compare the electrophysiological properties of the mammalian and yeast Sec61 channels, we carried out single-channel bilayer measurements with rough microsomes with undefined precursor polypeptides in transit (cotranslational translocation) prepared from *C. familiaris* and *S. cerevisiae* ER (see Section 2). Sec61 channel activity in the respective microsomes was induced by termination of translocation and dissociation of ribosome nascent chain complexes initiated by treatment with puromycin and 250 mM KCl [28]. The observed ion channel activity was clearly attributable to the Sec61 complexes, for the following reasons. (i) No ion channel activity was observed for RM (rough microsomal membrane vesicles) preparations from *C. familiaris* or *S. cerevisiae* without pretreatment with puromycin, i.e. with attached ribosomes. (ii) Rough microsomes from *C. familiaris* displayed the same basic channel properties as the RM preparations from *S. cerevisiae* (see below); in addition, they displayed the same basic channel properties as proteoliposomes containing the purified Sec61 complex as the only proteinaceous component that we used in our previous electrophysiological studies [28]. (iii) There was no channel activity when we used protein-free liposomes instead of rough microsomes (data not shown).

Electrophysiological procedures started with the formation of a stable lipid bilayer followed by the osmotic fusion of the respective rough microsomes under asymmetrical salt conditions (Table 2) [12]. Single-channel current recordings of Sec61 complexes from *C. familiaris* and *S. cerevisiae* consistently displayed complex gating patterns with a variety of subconductance open states (Fig. 2A–C and E–G). Upon closer inspection of the current amplitudes