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Review

The sorting of a small potassium channel in mammalian cells can be shifted between mitochondria and plasma membrane

Charlotte von Charpuis [a], Tobias Meckel [a], Anna Moroni [b], Gerhard Thiel [a,*]

[a] Plant Membrane Biophysics, TU Darmstadt, Schnittspahnstrasse 3, 64287 Darmstadt, Germany
[b] Department of Biology and CNR IBF-Mi, and Istituto Nazionale di Fisica della Materia, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy

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ABSTRACT

The two small and similar viral K^+ channels Kcv and Kesv are sorted in mammalian cells and yeast to different destinations. Analysis of the sorting pathways shows that Kcv is trafficking via the secretory pathway to the plasma membrane, while Kesv is inserted via the TIM/TOM complex to the inner membrane of mitochondria. Studies with Kesv mutants show that an N-terminal mitochondrial targeting sequence in this channel is neither necessary nor sufficient for sorting of Kesv the mitochondria. Instead the sorting of Kesv can be redirected from the mitochondria to the plasma membrane by an insertion of ≥2 amino acids in a position sensitive manner into the C-terminal transmembrane domain (TMD2) of this channel. The available data advocate the presence of a C-terminal sorting signal in TMD2 of Kesv channel, which is presumably not determined by the length of this domain.

1. Introduction

Viruses have frequently served as tools in molecular and cellular biology to discover unknown cellular processes. For their replication viruses must first efficiently transport their genome together with auxiliary proteins into the host cells. Once inside they need to highjack the biosynthetic machinery of the host for their own replication and protein synthesis. Since viruses are not autonomous, they depend in virtually every step of their infection and replication cycle on the assistance from the host. The circumstance that viruses efficiently use or reprogram in this context cellular functions for their own benefit has frequently led to the discovery of cellular pathways and mechanisms, which were not known before. For example only by studying entry of the Simian virus 40 (SV4) into their host it was discovered that mammalian cells use an endocytotic pathway, which involves caveolae [1]. The fact that plants can transport proteins between neighboring cells via plasmodesmata with the help of movement proteins, was only discovered by following the systemic spread of the tobacco mosaic virus (TMV) [2]. Also the discovery for a direct transfer of proteins between the ER and mitochondria comes from studies with viruses, which show that Vpr from HIV1 and UL37 from human cytomegalovirus can be transported between the two organelles in the region of the so-called mitochondria associated membranes (MAMs) [3].

In order to exploit transport and biosynthetic pathways in the host, viruses use the "molecular language" of their host in that they effectively mimic cellular targeting and recognition signals. Again the analysis of these signals in viral proteins has provided not only information on the viruses but also about the equivalent mechanisms in cells. A prominent example is a di-acidic signal in the cytoplasmic tail of the vesicular stomatis virus glycoprotein (VSV-G), which stimulates export of the protein from the endoplasmic reticulum into the secretory pathway [4]. Only after this principle was understood for the viral protein it was found that the same di-acidic signals are common also in eukaryotic membrane proteins where they regulate in the same way an export from the ER [5,6].

In the spirit of uncovering cellular processes by investigating viral mechanisms, we use here the information on the sorting of two small viral K^+ channels to better understand the process of dual sorting [7]. It is well established that the membranes of mitochondria contain several proteins, which resemble in structure and function those in the plasma membrane. Among these are several different K^+ channels. In recent years Kv1.3, BK, IKCa and TASK-3 type channels were reported to function not only in the plasma membrane but also in the inner membrane of mitochondria [8–11].

The genome of the mitochondria lacks any obvious genes for K^+ channels. Hence it must be assumed that mitochondrial K^+ channels proteins are like all other K^+ channels nuclear coded and that the proteins are imported into the mitochondria. As yet the mechanism, which is responsible for the sorting and trafficking of similar

* Corresponding author at: Department of Biology, TU Darmstadt, Schnittspahnstrasse 3, 64287 Darmstadt, Germany. Tel.: +49 6151 166050.
E-mail address: Thiel@bio.tu-darmstadt.de (G. Thiel).

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membrane proteins and in particular of the aforementioned K^+ channels to these different cellular destinations is not fully understood. It is commonly believed that mitochondrial proteins, which are not encoded in the organelles, are translated on free ribosomes in the cytosol. From there they travel with the help of chaperons to specific translocases, the TIM/TOM complex, at the mitochondria. The latter catalyzes the transfer of the polypeptide into the target membranes of the mitochondria [12]. The majority of proteins, which are resident in the plasma membrane, are in contrast translated in a co-translational manner. This means they are synthesized at the endoplasmic reticulum (ER) and targeted via the signal recognition particle (SRP) as nascent polypeptide attached to the ribosome into the ER. The latter step employs the Sec61 translocon complex, which is supporting the co-translational insertion of the nascent protein into the ER membrane [13–15].

The challenging question now is to understand how structurally similar membrane proteins can use one or the other sorting pathway for trafficking to their final destination. In the case of Kv1.3 it has been speculated that this channel could enter the mitochondria in an indirect manner via the plasma membrane. Such a mechanism may involve a retrograde transport of channel proteins via Golgi and ER from where the channel may then be transferred to the mitochondria at specific contact sites e.g. areas in which the ER and the mitochondria are in close proximity [9]. Recent data imply a different mechanism for dual sorting of channel proteins; they suggest that splicing variants of the same channel are targeted to either the plasma membrane or the mitochondria. In the case of the BK-channel it occurred that a 50 amino acid long splice insert in the C-terminus decides on the targeting of the channel between the plasma membrane and the mitochondria [11]. A distinct sorting signal in the C-terminus of the BK channel for mitochondrial targeting is fundamentally different from the canonical N-terminal signal, which is generally employed in targeting proteins to this organelle. Hence at this point a sorting signal has been identified in BK splicing isoforms but the mechanisms, which is underlying the dual sorting of the isoforms to their target membranes, remains unknown.

2. Materials and methods

2.1. Heterologous expression in HEK293

All localization studies were realized in human embryonic kidney (HEK) 293 cells as reported in Balss et al. [16].

2.2. Labeling of ER and mitochondria

After replacement of the cell culture medium with PBS the cell cultures were treated immediately before starting the CLSM session with MitoTracker® Red CMXRos or ER-Tracker™ Red (BODIPY® TR Glibenclamide) (Life Technologies GmbH, Frankfurt, Germany) to label the mitochondria or the ER respectively; MitoTracker® Red CMXRos was used at 25 nM and ER-Tracker™ Red at 1 μM. Mitochondria and ER were also labeled by transient expression of fluorescent proteins; the mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase fused to the N-terminus of the fluorescent protein mKate2 (COXVIII:mKate2) was used to label the mitochondria. For an ER labeling the retention sequence KDEL was fused to mKate2 (KDEL:mKate2). Both constructs were obtained from Evrogen (Moscow, Russia).

2.3. Isolation of mitochondria

Mitochondria were isolated from HEK293 cells expressing Kesv:GFP by incubating cells for 30 min at 4 °C in 0.3 M sucrose, 10 mM TES (pH 7.4) und 0.5 mM EGTA. After homogenization the suspension was centrifuged at $600 \times g$ for 5 min at 4 °C. The supernatant was again centrifuged ($6000 \times g$, 10 min, 4 °C) and mitochondria were isolated from the pallet with a percoll-gradient-centrifugation ($8500 \times g$, 10 min, 4 °C). Mitochondria were collected in the interphase between 30% and 60% percol and re-suspended in a buffer containing 50 mM PIPES–KOH (pH 7.4), 50 mM KCl, 2 mM $MgCl_2$, 2 mM EGTA, 2 mM ATP, 10 mM succinate, 50 μg/ml ceatinkinase and 10 μg/ml protease inhibitors.

2.4. Confocal laser scanning microscopy (CLSM)

Confocal microscopic analysis was performed using a Leica SP5II (Leica Microsystems GmbH, Mannheim, Germany) equipped with a HCX PL APO 63× NA 1.2 Water immersion objective. Fluorophore excitation and detection of emission was achieved using the following laser lines and emission bandpass settings, respectively: TagBFP (405 nm, 430–480 nm), EGFP (488 nm, 505–535 nm), mKate2 (561 nm, 600–700 nm), MitoTracker® Red CMXRos: (561 nm, 600–700 nm), ER-Tracker™ Red/BODIPY® TR Glibenclamide (561 nm, 600–700 nm).

2.5. Editing of primary data

For an improvement of the qualitative the primary microscopic data were processed with an algorithm combining the additive white Gaussian noise model with the Poisson distribution. In this manner the background noise could be reduced without producing artifacts or lost of information. The calculation was accomplished with the plugin "*Pure denoise*" (Poisson unbiased risk estimate) in the image-analysis program "ImageJ" [17]. To improve the denoise quality the cycle-spin setting was regulated up to 10 cycle spins. The noise estimation was performed automatically for the whole picture (global).

2.6. Yeast complementation

Experiments were performed as described in [16,18].

3. Results

3.1. Two similar viral K^+ channels are sorted to different intracellular targets

A key observation for the present paper was that large dsDNA viruses, which infect algae [19], have gene products with the structural and functional hallmarks of eukaryotic and prokaryotic K^+ channels [20]. The best-studied among the viral K^+ channel is the K^+ channel Kcv from *Pabramecium bursaria* Chlorella virus 1 (PBCV-1) [20,21]. Like eukaryotic K^+ channels this protein forms a functional tetramer [22,23]. Compared to other K^+ channel proteins, the monomer is small, consisting of only 94 amino acids [20,21]. Each monomer is made from two transmembrane domains (TMD), which are linked by a pore helix including a selectivity filter; the latter is present in all K^+ channels [20]. From a structural point of view Kcv represents essentially the pore module of all K^+ channels. In this context, it is not surprising that Kcv exhibits the basic properties of K^+ channels such as ion selectivity, gating and sensitivity to blockers [20].

After the initial discovery of Kcv in virus PBCV-1 many more K^+ channels have since been discovered in nearly 100 other members of the Phycodnaviridae that infect different hosts [24–28]. The most interesting protein with respect to protein sorting in this collection of viral channels is Kesv [28]. The latter protein is encoded by the *Ectocarpus siliculosus* virus 1 (EsV-1), another member of the Phycodnaviridae [28]. An alignment of the two channels in Fig. 1 shows