Review

Ulrich Kück*, Daria Radchenko and Ines Teichert

STRIPAK, a highly conserved signaling complex, controls multiple eukaryotic cellular and developmental processes and is linked with human diseases

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Abstract: The striatin-interacting phosphatases and kinases (STRIPAK) complex is evolutionary highly conserved and has been structurally and functionally described in diverse lower and higher eukaryotes. In recent years, this complex has been biochemically characterized better and further analyses in different model systems have shown that it is also involved in numerous cellular and developmental processes in eukaryotic organisms. Further recent results have shown that the STRIPAK complex functions as a macromolecular assembly communicating through physical interaction with other conserved signaling protein complexes to constitute larger dynamic protein networks. Here, we will provide a comprehensive and up-to-date overview of the architecture, function and regulation of the STRIPAK complex and discuss key issues and future perspectives, linked with human diseases, which may form the basis of further research endeavors in this area. In particular, the investigation of bi-directional interactions between STRIPAK and other signaling pathways should elucidate upstream regulators and downstream targets as fundamental parts of a complex cellular network.

Keywords: Hippo; kinases; striatin; striatin-interacting phosphatases and kinases complex.

Introduction

In a changing environment, organisms adapt to a multitude of extracellular stimuli and cues. Intracellular signal transduction pathways forming interconnected regulatory

networks are now known to underlie the transmission and modulation of such signals. These networks, controlled by diverse regulators at different cellular levels, are highly conserved across eukaryotic organisms.

Among the signaling components that have received increased attention in the last decade is the STRIPAK complex, which was initially identified in mammals by mass spectrometry (MS) analysis (Goudreault et al., 2009). Here we will provide a summary of the key studies leading to the discovery of striatin, the major regulatory phosphatase subunit of the STRIPAK complex. Further, the components and architecture as well as the assembly and structural insights of the STRIPAK complex will be comprehensively reviewed. Another focus will be the control of developmental processes as a result of bi-directional interaction of STRIPAK with other components involved in conserved signaling pathways. Although we address all aspects of the current STRIPAK research, we mainly focus on results obtained in insect, fungal and mammalian systems. For further reading, other review articles on the STRIPAK complex should provide any remaining information (Hwang and Pallas, 2014, Kück et al., 2016, Shi et al., 2016).

Discovery of striatin

Striatin is the eponymous component of the STRIPAK complex, and was first detected as an abundant rat brain synaptosomal protein of 110 000 M_r . In particular, striatin is visible in the dorsal part of the striatum as well as in motor neurons, although it is absent in axons, but is highly abundant in dendritic spines (Castets et al., 1996; Goudreault et al., 2009). Dendritic spines are tiny, actin-rich protrusions that extend from dendrites and are the sites of most of the excitatory synapses in the mammalian CNS. Further analysis using peptide sequencing identified a cDNA coding for a 780-amino acids protein, containing a caveolin-binding motif, a coiled-coil structure, a Ca^{2+} -calmodulin-binding domain, and multiple WD40 repeats. Successive analyses in mammals found paralogs

*Corresponding author: Ulrich Kück, Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Universitätsstr. 150, D-44780 Bochum, Germany, e-mail: ulrich.kueck@rub.de

Daria Radchenko and Ines Teichert: Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Universitätsstr. 150, D-44780 Bochum, Germany

of striatin, which were termed STRN3 (SG2NA) and STRN4 (Zinedin), while the originally described striatin is referred to as STRN1. Of note is that striatin homologs have not only been found in brain, but also in other tissues such as liver and cardiac muscle. As outlined later, striatins are regulatory subunits of protein phosphatase PP2A (Moreno et al., 2000). These subunits are classified into four protein families, B, B', B'', and B''', where striatins represent the B''' family (Sents et al., 2012).

In *Drosophila melanogaster* a striatin homolog was identified in a genetic screen for mutations that affect the dorsal closure in the fly embryo. There, the striatin homolog was named *connector of kinase to AP-1 (cka)* and is known to play a role in the JUN N-terminal kinase (DJNK) signal transduction pathway. Interestingly, CKA homologs from *Caenorhabditis elegans* and mammals can activate the DJNK signal transduction pathway in *D. melanogaster*, indicating that they are structural and functional homologs (Chen et al., 2002).

In non-animal systems, the striatin homolog was first identified in a screen for developmental proteins from the sexually propagating fungus *Sordaria macrospora*. This ascomycetous fungus was used to generate a genetic library of about 100 sterile mutants, showing a defect in sexual fruiting body formation. Complementation analysis of mutant *pro11* revealed a mutated gene, later named *pro11*, which has a truncated open reading frame. The PRO11 protein shows a striatin-typical domain structure with a conserved coiled-coil region close to its N-terminus, a calmodulin binding site, and a C-terminal domain with seven WD40 repeats (Pöggeler and Kück, 2004). Most remarkably, the sterile *pro11* mutant of *S. macrospora* regained fertility after transformation with a mouse striatin cDNA. After this early discovery of a functional striatin homolog in *S. macrospora*, many other homologs were identified in a huge variety of fungi (see for a review Kück et al., 2016), and a recent molecular evolutionary comparison showed that homologs of striatin or other STRIPAK subunits are encoded in genomes of a broad variety of microbial eukaryotes. This includes slime molds, which are believed to belong to the early-emerging eukaryotes during evolution (Nguyen et al., 2018).

Components and architecture of STRIPAK

The first evidence for a striatin-containing multiprotein complex came from affinity purification and MS (AP-MS) studies focused on the human PP2A complex.

This biochemical characterization defined the major subunits of the human STRIPAK complex, which are the structural (PP2AA) and catalytic (PP2Ac) subunits of PP2A, the B''' regulatory subunits of PP2A (striatins), striatin-interacting proteins STRIP1/2, Mob3/phocein, cerebral cavernous malformation 3 (CCM3), the sarcolemmal membrane-associated protein (SLMAP), and the coiled-coil protein suppressor of I κ B kinase- ϵ (IKK ϵ), designated SIKE (Goudreault et al., 2009). A comprehensive overview of STRIPAK subunits is provided in Table 1, which further lists the synonymous designations of homologous subunits. In animals in general and in mammals in particular, there are several variants of the STRIPAK complex due to the existence of isoforms and paralogs of many STRIPAK subunits. For example, STRN1, -3, and -4 are human striatin paralogs, and their diversity may reflect the many cellular functions of STRIPAK in diseases of higher eukaryotes (see below). Homologs of all these subunits were found in invertebrates, while diverse eukaryotic microbes possess only a single striatin protein.

A recently discovered core component of STRIPAK is SIKE, a suppressor protein of IKK ϵ , which is not conserved by sequence similarity between closely related species (Reschka et al., 2018). First discovered in mammalian systems (Goudreault et al., 2009), this protein was identified later in the yeast *Schizosaccharomyces pombe* (Singh et al., 2011), and more recently, in filamentous fungi (Reschka et al., 2018, Elramli et al., 2019). To date, all STRIPAK-associated small coiled-coil proteins identified contain one to four predicted coiled-coil regions.

Significant components of the STRIPAK complex are germinal center kinases (GCK), which in mammalian systems can substantially vary, reflecting the different cellular functions of STRIPAK during eukaryotic development. STRIPAK kinases are members of the GCK family (Kyriakis, 1999). These are a subdivision of the Ste20-like kinases, which were named after the Ste20p kinase from *Saccharomyces cerevisiae*. GCKs are much more numerous in metazoan kinomes than in yeast and are involved in a multitude of essential processes. Being evolutionarily conserved, GCKs are present in both higher and lower eukaryotes. In mammals, eight (I-VIII) GCK subfamilies are known, each containing two to four members (Table 2). Some members of these subfamilies transmit extracellular signals to mitogen-activated protein kinase (MAPK) cascades, others act as signaling hubs within conserved eukaryotic complexes (Delpire, 2009). Although human GCKs and their counterparts from *D. melanogaster* have been extensively characterized and new GCK targets, regulators, and interaction partners are being identified, there are still some gaps in our current understanding of

Table 1: STRIPAK subunits in selected eukaryotes.

Subunit	Function	<i>Hs</i>	<i>Dm</i>	<i>Nc</i>	<i>Sm</i>	<i>An</i>	<i>Sc</i>	<i>Sp</i>
Striatin	PP2A regulatory subunit	STRN1/3/4 ^a	Cka	HAM-3	PRO11	StrA	Far8 ^b	Csc3
PP2AA	PP2A scaffolding subunit	PP2AA α / β	Pp2A-29B	PP2A-A ^c	PP2AA	SipF	Tpd3	Paa1
PP2Ac	PP2A catalytic subunit	PP2Ac α / β	Mts	PP2Ac	PP2Ac1	SipE	Pph21/22, Ppg1	Ppa3
STRIP	Cell size control	STRIP1/2 ^d	CG11526	HAM-2	PRO22	SipC	Far11	Csc2
Mob	Vesicular trafficking	Mob3 ^e	Mob4	MOB-3	SmMOB3	SipA	–	–
SLMAP	Membrane anchoring	SLMAP	CG17494	HAM-4	PRO45	SipD	Far9/10	Csc1
GCKIII	Germinal center kinase (GCKIII)	Stk24/25, Mst4	Hpo, Msn, GckIII	? ^f	SmKIN3, SmKIN24	? ^f	? ^f	–
Small coiled-coil protein	?	SIKE/FGFR1OP2	FGOP2	NCU04324	SCI1	SipA	Far3/7	Csc4p
CCM3	Tethering of GCKIIIs	CCM3	CG5073	–	–	–	–	–

In the yeasts *S. cerevisiae* and *S. pombe*, STRIPAK homologues are named FAR and SIP complexes, respectively. Table modified from diverse references (Goudreault et al., 2009), (Pracheil et al., 2012), (Frost et al., 2012), (Bloemendal et al., 2012), (Ribeiro et al., 2010) and (Hwang and Pallas, 2014). *An*, *A. nidulans*; *Dm*, *D. melanogaster*; *Hs*, *H. sapiens*; *Nc*, *N. crassa*; *Sc*, *S. cerevisiae*; *Sm*, *S. macrospora*; *Sp*, *S. pombe*.

^aMembers of the striatin family are termed STRN1 (striatin), STRN3 (SG2NA), and STRN4 (Zinedin).

^bFar8 lacks the C-terminal WD propeller domain of other homologues of striatin.

^cPP2A-A has also been termed PPG-1 (Dettmann et al., 2013; Fu et al., 2011).

^dSTRIP1/2 have also been known as FAM40A and FAM40B, respectively (e.g. Bai et al., 2011).

^eAlso named phocein.

^fThe homologous of STRIPAK kinases in *N. crassa* and *S. cerevisiae* are SID-1, MST-1, and Sps1, respectively. However, evidence is still lacking that they are subunits of STRIPAK.

Table 2: Ste20-like kinases in mammals and selected ascomycetes.

Family	Subfamily	Mammals	Ascomycetes			
		<i>Hs</i>	<i>Sp</i>	<i>Sc</i>	<i>An</i>	<i>Nc</i>
Germinal center kinases (GCK)	GCK-I	MAP4K1/2/3/5	Cdc7p	Cdc15p	SepH	CDC-7
	GCK-II	MST1/2				
	GCK-III	MST3/4, YSK1	Nak1p	Kic1p	AnPod6	POD-6
	GCK-IV	MAP4K4/6, NRK, TNIK				
	GCK-V	LOK, SLK	Ppk11p	Sps1p	AnMst1	MST-1
	GCK-VI	SPAK, OSR1				
	GCK-VII	MYO3A/B	Sid1p		SepL	SID-1
	GCK-VIII	TAO1/2/3				
p21 activated kinases (PAK)	PAK-I	PAK1/2/3	Shk1p	Ste20p	AnSte20	STE-20
	PAK-II	PAK4/5/6	Shk2p	Cla4p	AnCla4	CLA-4

Hs, *H. sapiens*; *Nc*, *N. crassa*; *Sc*, *S. cerevisiae*; *Sp*, *S. pombe*. Note, there is no subdivision in subfamilies in fungal Ste20-like kinases.

Modified after Delpire, 2009, Boyce and Andrianopoulos, 2011.

their mechanism and function. GCKs are known to regulate key developmental processes such as cytoskeleton organization, cell cycle and apoptosis. These kinases were intensively investigated in some unicellular and filamentous ascomycetes (Table 2) and, for example, a total of four conserved GCKs were described for *S. pombe*, which are not divided in further subfamilies.

Of special interest is that the key exception of the overall conservation of STRIPAK subunits seems to be CCM3, which was found only in animal systems. CCM3 has no known functional domain, but yet appears to have the function of binding GCKs to STRIPAK. Although CCM3 is

evolutionary conserved within animal systems, it has not yet been detected in microbial eukaryotes.

In all eukaryotes, core subunits of STRIPAK are structurally conserved, having characteristic functional domains as depicted in Figure 1. For example, striatins share four conserved domains, a caveolin-binding domain, a coiled-coil domain, a calmodulin-binding domain, and WD40 repeat domains. Overall, all STRIPAK subunits are characterized by several kinds of domains, some of which mediate their interaction with each other. Thus, the composition of STRIPAK complexes seems to be very similar in diverse eukaryotes. However, insights into

Figure 1: Primary structure of subunits of the STRIPAK complex. The general structure of all subunits is similar in all eukaryotes. Modified in part from Frost et al. (2012). Abbreviations: CC, coiled-coil; CM, calmodulin binding domain; CV, caveolin binding domain; FHA, forkhead associated domain; MOB1, monopolar spindle-one-binder (phocein); STKc, catalytic domain of serine threonine kinase; TM, trans-membrane domain; HEAT, Huntingtin, elongation factor 3 (EF3), protein phosphatase 2A (PP2A), and the yeast kinase TOR1; PP2Ac, catalytic domain of protein phosphatase 2A; WD40, WD or beta-transducin repeat sequence. For references see text.

the assembly and structure of the complex itself are just emerging.

Crystal structure and biophysical analyses of striatin showed that the protein has a parallel dimeric conformation with a large bend (Chen et al., 2014). The coiled-coil domain from striatin forms a stable core complex with PP2AA in a 2:2 stoichiometry. Essential for this interaction and thus for the assembly of STRIPAK is the homodimerization of striatin. In particular, the coiled-coil domain seems to be necessary for the assembly of STRIPAK as it mediates the oligomerization of striatins. As shown in Figure 2, this minimal core complex is able to associate with GCKs when assisted by adaptor proteins. More recently, this architecture and substructure

Figure 2: Physical interactions of subunits of the animal STRIPAK complex.

Adaptor, cerebral cavernous malformation 3 (CCM3), adaptor connecting striatin with GCKs; GCK, germinal center kinase; all other abbreviations are given in the legend of Figure 1. Modified from Chen et al. (2014).

of STRIPAK was refined in a study that investigated the assembly of Hippo kinase MST2-containing STRIPAK complex (Tang et al., 2019). Using human cell cultures, the authors propose that a PP2AA/PP2Ac-bound striatin contacts MST2, which then is loaded in a phosphorylation-dependent manner to STRIP1 and to a SIKE-SLMAP heterodimer. Interestingly, a very similar assembly process has recently been proposed for a fungal STRIPAK complex (Elramli et al., 2019). Using a diverse set of STRIPAK mutants from the ascomycete *Aspergillus nidulans*, these investigators predict three heteromeric sub-complexes, which are (i) MOB3 and striatin, (ii) SIKE and SLMAP, and (iii) PP2Ac, PP2AA and STRIP1/2. As shown in Figure 3, these sub-complexes finally form the functional STRIPAK complex. Common to all the structural models is the prediction that striatins act as a central scaffold in the STRIPAK complex, directly binding all other subunits. So far however, the direct or indirect interaction of kinases with striatin has not yet been ultimately solved. In mammalian systems, adaptor proteins promote the interaction of MST3/4 with striatin (Chen et al., 2014). However, MST1/2 pull-down experiments indicate a direct interaction (Tang et al., 2019). Similarly, pull-down as well as yeast-two-hybrid experiments provided evidence for a direct interaction of fungal MST1/2-like kinase SmKIN3 and MST3/4-like kinase SmKIN24 with striatin (Frey et al., 2015).

Figure 3: Model of the assembly of the STRIPAK complex and its role in fungal development (adopted from Elramli et al., 2019).

The STRIPAK complex is assembled from three sub-complexes, where striatin acts as a scaffold at the nuclear envelope. Striatin prevents the shuttling of the JNK homologous MAPK under non-stress conditions and further promotes the phosphorylation of the ERK homologous MAPK, which in fungi is part of the pheromone response pathway (PR). For clarity reasons, only the designations from the human STRIPAK complex were used.

Cellular localization

To elucidate the function of STRIPAK components, cellular localization studies were performed in diverse organisms. A comprehensive overview is provided in Table 3, which summarizes most of these studies reporting distinct subunits of STRIPAK. For example, localization of STRIPAK subunits at the nuclear envelope has been described in mammalian cells, insects, worms, and unicellular as well as multicellular fungi (reviewed by Hwang and Pallas, 2014). Moreover, studies in fungi revealed that localization of STRIPAK to the nuclear envelope depends on striatin and STRIP subunits (Dettmann et al., 2013; Nordzieke et al., 2015; Elramli et al., 2019). An exception is the *S. cerevisiae* Far complex, where the SLMAP paralogs Far9p and Far10p are required for assembly of the complex (Pracheil and Liu, 2013). These results indicate that the yeast Far complex has adapted a different function from other STRIPAK complexes (Frost et al., 2012). Finally, STRIPAK at the nuclear envelope has also been shown to control MAPK trafficking, as will be described later.

Single subunits of STRIPAK have also been found at other cellular locations. For example, striatin and STRIP have been detected at the nuclear envelope and the Golgi, while SLMAP homologs reside at mitochondria and the endoplasmic reticulum (Byers et al., 2009; Frost et al., 2012; Nordzieke et al., 2015). Switching of SLMAP between nuclear envelope and mitochondria is likely conveyed by different tail anchor domains. These C-terminal transmembrane domains direct co-translational integration into target membranes (Byers et al., 2009). These different localizations of STRIPAK subunits led to the hypothesis that STRIPAK bridges different organelles, i.e. the nuclear envelope, the Golgi, and the spindle pole body (SPB) or centrosome (the SPB is the fungal equivalent to the centrosome). This bridging function may facilitate communication between organelles during cell division (Frost et al., 2012). Recently, *D. melanogaster* striatin has been located at autophagosomes, and a function of STRIPAK in autophagosome trafficking has been recently proposed (Neisch et al., 2017).

In conclusion, a conserved localization of the STRIPAK complex was observed at the nuclear envelope, where it likely coordinates conserved cellular and developmental processes. However, further investigations are required to address the question of how different localizations of single subunits may relate to different functions of STRIPAK sub-complexes.

STRIPAK connects several cellular signaling complexes

Eukaryotic developmental processes, such as growth, movement, cell division and differentiation, are governed by intertwined signaling networks. These networks are important for maintaining homeostasis as they allow a suitable response to intra- and extracellular stimuli, including nutrients, stressors and pheromones. Some modules of these networks evolved as paralogs, which can partially adopt the function of each other in different pathways. Other modules have developed independently but show functional interaction during biological processes.

Although considerable efforts have been made to identify upstream regulators and downstream targets of the STRIPAK complex, our understanding of the mechanistic function of the STRIPAK complex is still fragmentary. STRIPAK complexes have the capacity to regulate phosphorylation of diverse proteins, some of which are members of associated signaling complexes. As described below, STRIPAK interacts with conserved signaling pathways and mediates remodeling of the cytoskeleton.

The yeast STRIPAK homolog counteracts the TORC2 complex

In budding yeast *S. cerevisiae*, the STRIPAK homolog is the Far complex, which is involved in pheromone-induced cell cycle arrest, vacuolar protein sorting, and cell fitness. Far8p is thought to be the striatin homolog, although it lacks a large part of the C-terminal WD 40 repeat domains of striatins, which are important for the function of striatin in eukaryotic multicellular development (Kemp and Sprague, 2003; Frost et al., 2012; Pracheil et al., 2012). The Far complex directly affects signaling by the TORC2 complex. TOR (target of rapamycin) is a highly conserved serine-threonine protein kinase. In yeast, it exists in two structurally and functionally distinct complexes, namely TORC1 and TORC2. Yeast Tor2 kinase, the main organizer of TORC2, is an important regulator of multiple cellular functions, including cell growth or actin polymerization (Bartlett and Kim, 2014). A genetic screen with yeast showed that a mutation of FAR11, the homolog of STRIP1/2, suppresses lethality of TORC2-deficient mutants. Further, a *far11* deletion mutant restores phosphorylation of two substrates of the TORC2 complex. Thus, Far antagonizes TORC2 signaling by promoting dephosphorylation of TORC2 substrates (Pracheil and Liu, 2013).

Table 3: Localization of STRIPAK subunits.

Organism	Component	Localization	Specificity	Reference
<i>A. nidulans</i>	STRIPAK	Nuclear envelope		Elramli et al., 2019
<i>A. nidulans</i>	Striatin	ER, nuclear envelope		Wang et al., 2010
<i>C. elegans</i>	Far111 (striatin)	ER, centrosomes (during nuclear division)		Maheshwari et al., 2016
CGR8 embryonic stem cells (mouse)	STRIP2	Nucleus/perinuclear		Sabour et al., 2017
CO57 (African green monkey kidney),	SLMAP	Nuclear envelope/ER and mitochondria	Dependent on tail anchor domain (2 isoforms)	Byers et al., 2009
C2C12 cells (mouse)				
<i>D. melanogaster</i>	Cka (striatin)	Autophagosomes		Neisch et al., 2017
<i>F. verticillioideus</i>	Fsr1 (striatin)	Endomembrane system, probably ER		Zhang et al., 2018
HeLa cells (human)	SLMAP	Nuclear envelope (interphase), centrosomes (mitosis)		Frost et al., 2012
HeLa cells (human)	STRN3 (striatin), STRP1 (STRIP)	Golgi		Frost et al., 2012
Neuro2A cells (mouse)	SG2NA (striatin)	ER, mitochondria, PM	Several localizations, dependent on interactions with Akt (protein kinase B) and DJ-1 (antioxidant protein) and on cellular stress	Tanti and Goswami, 2014
<i>N. crassa</i>	STRIPAK	Nuclear envelope	Localization of all other subunits dependent on HAM3 (striatin) and HAM2 (STRIP)	Dettmann et al., 2013
NIH3T3 cells (mouse)	SG2NA (striatin)	ER		Jain et al., 2017
<i>S. cerevisiae</i>	Far complex (STRIPAK homolog)	Nuclear envelope	Localization of all other subunits dependent on Far9 and Far10	Pracheil and Liu, 2013
<i>S. pombe</i>	PP2A (Paa1)	Actomyosin ring		Singh et al., 2011
<i>S. pombe</i>	SIP complex (STRIPAK homolog)	Spindle pole body (analogous to mammalian centrosome)	Asymmetric localization of some components; no interdependence	Singh et al., 2011
<i>S. macrospora</i>	GPI1	Mitochondria	MOB3 interaction partner	Frey et al., 2015
<i>S. macrospora</i>	PRO11 (striatin), SCI1 (SIKE)	Nuclear envelope		Reschka et al., 2018
<i>S. macrospora</i>	PRO22 (STRIP)	Intermembrane system, tubular vacuoles		Bloemendal et al., 2010
<i>S. macrospora</i>	PRO45 (SLMAP)	Nuclear envelope and mitochondria	Dependent on PRO11 (striatin) and PRO22 (STRIP)	Nordzieke et al., 2015
<i>S. macrospora</i>	PRO45 (SLMAP)	Spindle pole bodies	in $\Delta pro11$	Nordzieke, 2014

STRIPAK controls MAPK localization in filamentous fungi

MAPK pathways transduce input signals to factors in the nucleus for altering gene transcription. For the filamentous fungus *Neurospora crassa*, the STRIPAK complex is known to localize to the nuclear envelope and regulate the nuclear import of MAPK MAK-1 in a MAK-2-dependent manner (Dettmann et al., 2013). While MAK-2 (ERK homologous MAPK) is the homolog of the yeast pheromone response (PR) pathway MAPK, MAK-1 (JNK/p38 homologous MAPK) is a homolog of the yeast cell wall integrity (CWI) pathway MAPK. In *N. crassa*, both kinases are essential for cell-cell communication and fusion (Fischer et al., 2019).

Similar results were recently obtained for *A. nidulans*. Homologs of MAK-1 and MAK-2 were investigated in mutants lacking distinct subunits of the STRIPAK complex (Elramli et al., 2019). From this set of data, an interesting mechanistic model was postulated that predicts that STRIPAK promotes phosphorylation of MAK-2/ERK, a kinase of the PR pathway, and modulates its shuttling into the nucleus (Figure 3). There, the kinase phosphorylates transcription factors controlling processes such as sexual and asexual development and the biosynthesis of secondary metabolites. At the same time, nuclear transport of the CWI pathway MAPK MAK-1/JNK/p38 is constrained in the absence of stress conditions, thereby maintaining proper stress signals in the cytoplasm.

Crosstalk between STRIPAK and Hippo pathway

The Hippo pathway was first described in *D. melanogaster*. Mutant screening in *D. melanogaster* identified a GCK that

acts as a negative regulator of tissue growth. Loss of function in this kinase resulted in increased tissue growth and decreased apoptosis, and the corresponding mutants were named Hippopotamus because of their bulky build (Harvey et al., 2003; Wu et al., 2003). Later, the Hippo kinase was identified as a part of a large signaling network, which is conserved from humans to eukaryotic microbes (Pan, 2007, 2010). A fungal homolog of HIPPO is the septation initiation network (SIN), first discovered in fission yeast *S. pombe* (Guertin et al., 2000; Simanis, 2015). Meanwhile, SIN homologs were described in various unicellular and filamentous fungi. To compare designations of SIN subunits with those from fly and humans, Table 4 provides a comprehensive overview of the literature and designates synonymous subunits.

In humans, the Hippo pathway core consists of GCK-II kinase MST1/2 (also known as STK4/3) associated with the WW-domain-containing scaffold protein Salvador SAV1 and the nuclear dbf2-related (NDR) kinase large tumor suppressor (LATS) 1/2, which is associated with the kinase activator MOB1 (Pan, 2010) (Table 4). Recent data provide compelling evidence of GCKs being one of the major links between Hippo and STRIPAK. Five of eight animal GCK subfamilies (GCK-I, II, III, IV and VIII) listed in Table 2 and graphically presented in Figure 4 were shown to functionally interact with either the Hippo pathway, the STRIPAK complex, or with both (Couzens et al., 2013; Madsen et al., 2015; Meng et al., 2015; Thompson and Sahai, 2015; Meng et al., 2016). Hippo subunit MOB1 was described as an interacting partner and a substrate of PP2A (Moreno et al., 2001) (Figure 4, pathway b). Structural and biochemical studies went on to discover a key mechanism of MST1/2 activation in which GCK-II kinases bind via their C-terminal linker to a forkhead-associated (FHA) domain of the STRIPAK subunit SLMAP, thereby enabling their dephosphorylation by PP2Ac. SAV1 inhibits phosphatase activity of PP2A via binding to the STRIPAK core, thereby counteracting dephosphorylation

Table 4: Components of the septation initiation network (SIN) complex, the homologue of the Hippo pathway in fly and humans.

Protein feature	MEN	SIN					Hippo	
	<i>Sc</i>	<i>Sp</i>	<i>An</i>	<i>Nc</i>	<i>Sm</i>		<i>Dm</i>	<i>Hs</i>
STE kinase	Cdc15	Cdc7	SEPH	CDC-7	SMAC_04024		/	/
GCK	/	Sid1	SEPL	SID1	SmKIN3, SMAC_04490		Hpo	MST1 (STK4)/ MST2 (STK3)
GCK adaptor	/	Cdc14	SEPM	CDC-14	SMAC_06569		Sav?	SAV1?
NDR kinase	Dbf2p	Sid2	SIDB	DBF-2	SMAC_05230		Wts	Lats1/2
NDR kinase adaptor	Mob1p	Mob1	MOBA	MOB-1	SMAC_02346		Mats	MOB1

In baker's yeast, the SIN complex was named mitotic exit network (MEN) (Lee et al., 2001) (Modified from Radchenko, 2018). *An*, *A. nidulans*; *Dm*, *D. melanogaster*; *Hs*, *Homo sapiens*; *Nc*, *N. crassa*; *Sc*, *S. cerevisiae*; *Sm*, *S. macrospora*; *Sp*, *S. pombe*.

Figure 4: Signal modulation between STRIPAK complex and the GCK network.

Positive (solid arrows), and negative regulations (blunt-end dashed lines), physical translocations (dotted lines), as well as phosphorylation and dephosphorylation reactions (P+ and P-, respectively) are indicated. This model is based on *Homo sapiens* and *D. melanogaster* studies as referenced in this review. Different regulatory pathways are indicated by small letters ('a' to 'k'). The STRIPAK complex emerges as a negative regulator of the Hippo pathway by dephosphorylating the kinase activation-loop of MST1/2 (A) and kinase activator MOB1 (B) (Moreno et al., 2001; Couzens et al., 2013). MST1/2 can interact with the STRIPAK complex via binding to an FHA domain of SLMAP (C). This interaction recruits SAV1 to the STRIPAK core and enables reciprocal negative regulation of its phosphatase activity (D), by a yet unknown mechanism (Bae et al., 2017). The STRIPAK/Hippo crosstalk is extended with GCK-III and GCK-IV subfamily kinases, which are able to phosphorylate NDR kinases NDR1/2 (E) and LATS1/2 (F), the targets of MST1/2 (G, H) (Stegert et al., 2005; Meng et al., 2015; Tang et al., 2015; Zheng et al., 2017; Meng et al., 2016). Additionally, GCK-III kinase MST3 might regulate the Hippo pathway by phosphorylating GCK-VIII kinase TAO1 (I), which in turn acts as MST1/2 activator (J) (Boggiano et al., 2011; Ultanir et al., 2014). Members of GCK-I subfamily were not identified as direct STRIPAK or Hippo core interaction partners, nevertheless they have shown the activity in LATS1/2 phosphorylation (K) (Meng et al., 2015; Zheng et al., 2017). Since the catalytic subunit of PP2A acts as a negative regulator of GCK-II (A) and GCK-III (L) subfamily kinases (Moreno et al., 2001; Gordon et al., 2011), it may be hypothesized that the STRIPAK complex also regulates other GCK group members via dephosphorylation.

of the GCK-II activation loop. This mechanism is responsible for the reciprocal downregulation of STRIPAK and Hippo (Figure 4, pathways a, c, d) (Couzens et al., 2013; Bae et al., 2017). MST1/2 are capable of auto-activation by self-phosphorylation but can also be activated by upstream regulatory GCK-VIII kinases TAO1/2/3, whose phosphorylation state is dependent on the STRIPAK GCK-III kinases (Figure 4, pathways i, j) (Fallahi et al., 2016; Meng et al., 2016). Hippo pathway dysfunction was described in mammalian cancer cell proliferation, migration and metastasis (Harvey et al., 2013). In particular, loss of function of the Hippo kinase homolog in human cells is connected to carcinoma, adenoma and acute leukemia (Pan, 2010; Harvey et al., 2013; Richardson and Portela, 2017).

Crosstalk between STRIPAK and fungal homologs of the Hippo pathway

The core of SIN in fission yeast consists of the GCK Sid1p, associated with a GCK adaptor Cdc14p, and an NDR kinase Sid2p, associated with its activator Mob1p (Table 4). Deletion or loss-of-function mutations of SIN members in *S. pombe* result in a septation-deficient phenotype (Guertin et al., 2000). Moreover, overexpression of SIN subunits, as well as deletion or loss of function of SIN negative regulators leads to hyperseptation (Simanis, 2015). The STRIPAK homolog in *S. pombe* is the SIN-inhibitory phosphatase (SIP) complex, which acts as a negative regulator

of SIN. Among other subunits, SIP comprises the striatin homolog Csc3p, PP2A scaffolding, and catalytic subunits Paa1p and Ppa3p, as well as the SLMAP homolog Csc1p (Table 1). Like its metazoan counterpart SLMAP, Csc1p acts as a link between SIP and SIN. However, in *S. pombe*, Ppa3p does not directly dephosphorylate a GCK activation loop, but rather the SPB-targeted scaffold protein Cdc11p (Tomlin et al., 2002). Cdc11p is required for the localization of other SIN components to SPBs and its dephosphorylation by SIP negatively regulates the SIN pathway. The dephosphorylation of SIN results in its inactivation and dislocation from SPBs (Singh et al., 2011). In conclusion, in *S. pombe*, SIN is negatively regulated by the STRIPAK homolog SIP, which is comparable to downregulation of Hippo through human STRIPAK.

In filamentous fungi, all subunits of the SIN three-step kinase cascade were recently identified in *N. crassa* and their hierarchy was confirmed by *in vitro* phosphorylation assays (Table 4). GCK CDC-7, which is constitutively localized to SPBs, interacts with GCK SID-1 via the GCK adaptor CDC-14 and phosphorylates SID-1. Activated SID-1 then phosphorylates NDR kinase DBF-2, which is associated with the kinase adaptor MOB-1 (Heilig et al., 2013). Interestingly, CDC-7 can form a complex not only with SID-1, but also with GCK MST-1, and in a mutually exclusive manner. Subsequently, the CDC-7-bound MST-1 becomes enzymatically inactive. Active MST-1 is able to phosphorylate either of two NDR kinases, namely COT-1 or DBF-2. These features highlight GCK MST-1 as a link between two NDR pathways – a phenomenon that is similar to GCK-III kinases in metazoan (Figure 4E) (Heilig et al., 2014). In *N. crassa*, the main function of SIN in the promotion of septation and cytokinesis was confirmed by the phenotypes of SIN mutants. In detail, deletion of genes encoding CDC-7 and DBF-2 results in septation-deficient vegetative mycelium, while deletion mutants lacking SID-1 and its adaptor CDC-14 generate aseptate mycelium, which later reverts to a wildtype phenotype (Heilig et al., 2013). In *A. nidulans*, knowledge about SIN has been mainly derived from the screening of temperature-sensitive cytokinesis-deficient mutants (Harris et al., 1994). There, lack of the SIN NDR kinase SIDB and its activator MOBA causes complete loss of septation and conidiation (Kim et al., 2006). In *S. macrospora*, two STRIPAK-associated GCKs were described, namely SmKIN3 and SmKIN24 (Frey et al., 2015). Strains lacking the Hippo-related GCK SmKIN3 exhibit rare septation and early sexual development arrest. A more detailed analysis showed that strains carrying a point mutation in the ATP-binding domain of SmKIN3 exhibit severe developmental defects. More recently, SmKIN3 has been shown to

interact not only physically, but also genetically with the striatin homolog PRO11 (Radchenko et al., 2018).

Actin assembly is governed by the HIPPO/SIN and STRIPAK complexes

In eukaryotic cells, actin monomers exist as a free pool, each bound with one molecule of profilin and ATP. These monomers can be polymerized in two different ways: either linear or branched. Generation of branched actin filaments is governed by the actin-related protein (Arp) 2/3 complex, while linear polymerization is formin-dependent (Insall and Machesky, 2009). Actin assembly is a major factor providing cell polarization, mechanistic support, cytokinesis and cell movement.

In humans, misregulation of actin assembly at any time causes defects in cell polarity and migration, which in turn are associated with different cancer types and neurodegenerative diseases (Insall and Machesky, 2009). In *D. melanogaster*, different types of actin polymerization are required in different cell types for promoting a myriad of key cellular and developmental processes. Finally, during fungal development, it has been suggested that different functions correspond to differently polymerized actin filaments.

In vertebrates and invertebrates, formins and the Arp2/3 complex are filopodium formation regulators (Pellegrin and Mellor, 2005). Filopodia, also known as microspikes, are thin cytoplasmic projections of migrating cells, which appear when actively growing actin filaments push towards the plasma membrane. To date, two independent mechanisms for filopodia formation are known. The first mechanism involves formin Dia2, activated by the small GTPase Rho in filopodia (Rif), inducing the generation of thin elongated microspikes on the cell surface. The second mechanism is driven by the Arp2/3 complex and Rho GTPase Cdc42. The Arp2/3 complex binds slow-growing ends of daughter filaments and anchors them to the mother filament, thereby forming a highly branched structure that shapes the cell membrane into compact lamellipodia (Figure 5A). Overexpression of Rif in mammalian cell culture results in the formation of elongated cells with long and thin filopodia (Passey et al., 2004). In contrast, overexpression of Cdc42 results in rounded cells with short and thick filopodia. Remarkably, a similar effect was observed in fibroblasts with depletion of either Strip1 or Strip2. Of interest is that Strip1-deficient cells tend to flat spreading and the shape of Strip2-deficient cells is rather elongated, while both mutant cell lines

Figure 5: SIN/Hippo and STRIPAK regulate actin cytoskeleton organization in animals and filamentous fungi.

The model does not represent the localization of proteins/protein complexes, but only regulatory pathways. Arrows and blunt-end lines indicate activation and inhibition, respectively. Actin polymerization agents (Arp2/3 or Dia2/SepA) and associated GTPases (Cdc42 and Rif/RHO1) are indicated with blue and red font, respectively. Corresponding animal and fungal homologs with different nomenclature are enclosed in brackets. Actin structures, such as linear or branched filaments in animal cells, as well as actomyosin rings and actin cables in fungal cells are shown as dashed blue lines.

show a decrease in migration. These findings suggest that two STRIPAK sub-complexes, containing either Strip1 or Strip2, regulate actin cytoskeleton organization in an opposite manner, i.e. activating or inhibiting Rif-Dia2 and Cdc42-Arp2/3 activities, respectively (Figure 5A; Bai et al., 2011).

Another effect of Strip depletion is the malformation of the nervous system. In *D. melanogaster*, depletion of the single Strip homolog phenocopies overexpression of Arp2/3 during synapse development, which includes formation of presynaptic termini (boutons) between motor neuron and muscle cell. Besides primary boutons, which are always formed in wild-type neurons, generation of smaller satellite boutons is also possible. Satellite boutons emerge from the main nerve or bud from the primary bouton. During normal development, these satellites may

fulfil the function of fine synaptic signal modulation. Of note is that in mutants with increased satellite bouton formation, abnormally decreased signaling levels are observed. Overexpression of Arp2/3 or the Hippo kinase as well as depletion of Strip causes formation of multiple satellite boutons (Figure 5B). Moreover, knockdown of Hippo suppresses the Strip-deficient phenotype, thereby revealing the function of the STRIPAK complex as a negative regulator of the Hippo pathway. Finally, Hippo kinase may consecutively phosphorylate and inactivate the actin polymerase Enabled (Ena), which antagonizes Arp2/3 and Cdc42 activity (Figure 5B; Sakuma et al., 2016).

In fungi, proper actin assembly is essential for budding and cytokinesis in yeast and polarized growth, branching and septation in filamentous fungi. During the process of septation, hyphal filaments are divided into compartments

by cross-walls named septa. In most fungi, septa are perforated by a large central pore that allows the movement of cytoplasm and organelles between cells. Septation occurs in vegetative hyphae (trunk hyphae and lateral branches) as well as in sexual organs (ascogonia) (Figure 5C). Branching and septation in filamentous and dimorphic fungi are crucial for growth, nutrient acquisition, interaction with the environment, pathogenicity, as well as for asexual and sexual propagation (Harris, 2008; Mouriño-Pérez, 2013; Riquelme et al., 2018). Unlike metazoan, fungi possess a solid cell wall, which maintains a rigid cell shape and does not allow temporary protrusions or amoeboid motility. However, cell wall protrusions in fungi can be observed during budding or branching. The main branching regulator in *A. nidulans* and *N. crassa* is the conserved Rho GTPase Cdc42. In both ascomycetes, loss-of-function mutation of Cdc42 leads to compromised growth, aberrant branching and abnormal hyphal morphology (Virag et al., 2007; Araujo-Palomares et al., 2011). ARP-3 in *N. crassa*, the homolog of metazoan Arp3, colocalizes with actin patches on future branching sites and follows the contour of the emerging branch (Delgado-Alvarez et al., 2010). In this way, appearance of lateral branches resembles satellite bouton formation in neuronal cells from *D. melanogaster* (Figure 5B,C). Fungi possess fewer formin paralogs than animals. Consequently, depletion of fungal formins leads to more severe defects. In *S. pombe* and *S. cerevisiae*, the homologs of the actin polymerizing agent Dia2 are Cdc12p and Bni1p, respectively. These Dia2 homologs are required for the formation of the fungal-specific contractile actin ring as well as budding (Pollard, 2007). Filamentous ascomycetes, such as *A. nidulans*, *N. crassa*, and *S. macrospora*, only encode one formin, SepA. Deletion of *sepA* is lethal in *A. nidulans* (Sharpless and Harris, 2002). Heterokaryotic strains of *N. crassa* with only some nuclei lacking the SepA homolog BNI-1 show severe hyphal growth defects as well as loss of septation (Lichius et al., 2012). Moreover, SepA was reported as a SIN phosphorylation target in *A. nidulans* (Sharpless and Harris, 2002) and an interaction partner of the STRIPAK subunit PRO45 in *S. macrospora* (Nordzieke et al., 2015). Collectively, these reports indicate a SIN/STRIPAK-dependent regulation of septum formation in fungi (Figure 5C).

STRIPAK directs development in eukaryotic microbes

In addition to the association of the STRIPAK complex with conserved signaling pathways, such as controlling yeast cellular processes and cell wall integrity or cell cycle

arrest, the yeast Far (STRIPAK) complex is also known to be involved in mitophagy. Mitophagy is a type of selective autophagy that is responsible for mitochondrial quality and quantity control. Mitophagy defects cause various diseases, such as different cancer types and neurodegenerative diseases. ATG32 is a mitophagy receptor in yeast that has to be phosphorylated in a distinct manner to induce mitophagy. More recent studies have shown that the Far complex is responsible for the regulated dephosphorylation of ATG32, thus counteracting the phosphorylation process and preventing extensive mitophagy (Furukawa et al., 2018).

In the two related filamentous ascomycetes *N. crassa* and *S. macrospora*, diverse (morphological) phenotypes were observed when mutants were investigated lacking distinct genes for STRIPAK subunits. The two most significant phenotypes observed were (1) lack of mature fruiting bodies, rendering these mutants sterile, as well as (2) their inability to generate hyphal (cellular) fusions (Xiang et al., 2002; Pöggeler and Kück, 2004; Bloemendal et al., 2010; Simonin et al., 2010; Bernhards and Pöggeler, 2011; Fu et al., 2011; Dettmann et al., 2013). Most significantly, all STRIPAK mutants display identical phenotypes, indicating that all STRIPAK subunits function in concert (Teichert et al., 2014; Gautier et al., 2018; Elramli et al., 2019). Remarkably, in some filamentous fungi, such as *Fusarium verticillioides* and *Epichloë festucae*, even pathogenicity or symbiotic interactions are controlled by STRIPAK (Green et al., 2016; Zhang et al., 2018).

STRIPAK directs neuronal and embryo development

As described, striatin is preferentially expressed in neurons in the striatum. However, little is as yet known about the precise function of striatin or the STRIPAK complex within the neurobiological context. A recent shRNA knockdown approach in primary striatal neuronal cultures showed the selective role for striatin in neuron maturation (Li et al., 2018). In detail, the knockdown approach resulted in reduced expression of striatin, followed by an increase in dendritic complexity as well as density of dendritic spines. Thus, this study highlights the regulatory role of striatin in neuronal development. Further investigation of the interaction of STRIPAK with cortactin-binding protein 2 (CBP2) has also demonstrated the role of STRIPAK subunits in neuronal development. CBP2 is a neuron-specific, F-actin-associated protein that regulates the formation and maintenance of dendritic spines. CBP2 has previously been associated with autistic spectrum disorder and was

suggested to be critical for neural development and function (Chen et al., 2012). Using imaging analysis of cultured hippocampal neurons, these authors also predicted that CBP2 targets STRIPAK to dendritic spines.

Another example of the developmental role of a STRIPAK subunit was recently demonstrated for Strip1. In mouse embryos, a Strip1 mutation disrupts migration of the mesoderm after the gastrulation epithelial-to-mesenchymal transition (EMT). Complete deletion of the gene for Strip1 resulted in profound disruptions in the organization of the mesoderm and its derivatives. Finally, these studies also revealed that the mesoderm migration defect is correlated with changes in actin cytoskeleton organization and decreased velocity of cell migration (Bazzi et al., 2017).

In *Drosophila*, the CLOCK/CYCLE transcription factors activate the transcription of period and timeless genes, which are essential components of the molecular clock controlling circadian rhythms. The CLOCK protein is highly phosphorylated and inactive in the morning, whereas hypophosphorylated active forms are present in the evening. The phosphorylation status is highly dependent on subunits of the *Drosophila* STRIPAK complex, namely the regulatory striatin subunit of PP2A and the interacting STRIP subunit (Andreazza et al., 2015).

Human and mammalian diseases

In recent years, the role of STRIPAK complexes in cellular and developmental processes of multiple model organisms has been extensively studied. However, little is currently

known about the role of STRIPAK complexes during mammalian development in general and pertaining underlying molecular mechanisms in particular. Notably, however, recent investigations have indicated that the dysregulation of subunits of the STRIPAK complexes correlates with numerous human diseases, including different cancer types and diabetes as well as cardiovascular and fatty liver diseases. A compilation is provided in Table 5.

In mammalian cells, the scaffold subunit of the STRIPAK protein phosphatase 2A (PP2AA) exists in two isoforms (α/β), for which mutations in the corresponding genes correlate with different forms of human carcinomas (Calin et al., 2000). These mutations include point mutations as well as exon deletions and occurred at low frequency. Mutations in other STRIPAK subunits also seem to correlate with distinct disease phenotypes. For example, in boxer dogs, a deletion in the striatin gene was associated with dilated cardiomyopathy (DCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC) (Meurs et al., 2013). Similarly, polymorphic variants of the human striatin (STRN1) gene have been associated with increased salt sensitivity of blood pressure. These results were further confirmed by knockdown experiments using the mouse striatin gene (Garza et al., 2015). Likewise, silencing of the striatin (STRN4) gene in mouse cell cultures suppresses proliferation, migration, and invasion of cancer cells (Wong et al., 2014). More recently, another transcriptionally controlled mechanism was predicted when RNA from patients showing intracranial aneurysm was used in association studies. Of note is that among downregulated genes in these samples was striatin (STRN). The authors therefore suggested that striatin may be associated with

Table 5: Mammalian diseases correlated with dysfunction of STRIPAK subunits.

STRIPAK subunit	Gene locus change	Disease/syndrome	Organism	Reference
PP2AA α/β	E64D	Lung carcinoma	Human	Calin et al., 2000
	E64G; frame shift	Breast carcinoma		
	R418W	Melanoma		
	Alternative splicing, exon 9 skipping	Breast carcinoma		
Striatin1	Downregulated	Intracranial aneurysm (IA)	Human	Wei et al., 2018
	8 bp deletion in the 3' UTR	Dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC)	Dog	Meurs et al., 2013
SG2NA/Striatin3	G/A point mutation 5' UTR	Salt-sensitive hypertension	Human	Garza et al., 2015
	Upregulated	Cancer and metastasis development	Mouse	Wong et al., 2014
YSK1	Upregulated	Type 2 diabetes, non-alcoholic fatty liver disease	Mouse	Amrutkar et al., 2016; Chursa et al., 2017
MST4	Upregulated	Prostate cancer and pituitary tumorigenesis	Human	Sung et al., 2003; Xiong et al., 2015
SLMAP	V269I, E710A and some other SNPs	Brugada syndrome/cardiac channelopathy	Human (Asians)	Ishikawa et al., 2012

the development of intracranial aneurysm via influencing the development of neuron projections of the corresponding cells (Wei et al., 2018).

RNA association studies were also conducted to study the function of STRIPAK-associated kinases. Overexpression and knockdown experiments with the *YSK1* (synonymous to *STK25*) gene highlighted its role in type 2 diabetes and non-alcoholic fatty liver diseases (Amrutkar et al., 2016; Chursa et al., 2017). In follow-up studies using *YSK1* transgenic and wild-type mice, a global quantitative phosphoproteomic analysis identified 21 proteins that were differentially phosphorylated, indicating these as potential downstream mediators of STRIPAK action (Chursa et al., 2017).

Similar association studies were also performed with the STRIPAK-related *MST4* (synonymous to *STK23*) kinase. Ectopic expression of the wild-type and kinase-inactive *MST4* gene in human prostate tumor cell lines showed that overexpression of *MST4* increases proliferation and tumorigenesis, while downregulation of this gene reverts this highly tumorigenic behavior. Taken together, these studies suggest a potential role of *MST4* in signaling pathways involved in prostate cancer progression. Moreover, microarray expression analysis with samples from human tumors revealed that the *MST4* gene is upregulated in all gonadotrope tumor samples investigated. Thus, *MST4* seems to be a novel candidate that is involved in human pituitary tumorigenesis (Xiong et al., 2015).

The STRIPAK subunit *SLMAP* is also known to be involved in pathophysiological processes. For example, association studies with samples from Asian patients showed that *SLMAP* mutations have an effect on cell surface expression of the pore-forming α -subunit of the cardiac sodium channel *hNav1.5* (Ishikawa et al., 2012). The corresponding gene is also known to underlie Brugada syndrome, a cardiac channelopathy, which is often accompanied by syncope and sudden cardiac death attributable to ventricular arrhythmias (Brugada and Brugada, 1992). The conclusion that *SLMAP* plays a pivotal role in Brugada syndrome was further substantiated by small interfering RNA experiments and whole-cell patch clamp recordings (Ishikawa et al., 2012).

Thus, despite the accumulating evidence that STRIPAK subunits are linked with mammalian diseases, we currently have very little mechanistic understanding of STRIPAK function in pathogenicity. New insights into mechanistic action may come from studies with cell cultures investigating different forms of PP2A phosphatase that regulates the NF- κ B pathway. This pathway consists of transcription factor nuclear factor kappa B (NF- κ B), together with at least three of its subunits (IKK β , IKK α , and RelA). NF- κ B

controls many cellular and organismal processes, including immune and inflammatory responses, cellular growth, and cell survival. Studies with protein phosphatase holoenzyme PP2A showed that only the regulatory subunit B''/striatin promotes the dephosphorylation of all three subunits of NF- κ B (Tsuchiya et al., 2017). In this context, it is interesting that transactivation of the glucocorticoid receptor (GR) transcription factor, which regulates genes controlling development, metabolism, and immune response, depends on striatin (STRN3). STRN3 itself is a prerequisite for the formation of a complex between the catalytic subunit of protein phosphatase 2A (PP2AC) with GR, which results in its downregulation. It was hypothesized that a functional trimeric protein phosphatase 2A complex in the nucleus dephosphorylates GR at serine 211, a known marker for GR transactivation (Petta et al., 2017).

Another upstream regulatory mechanism was recently described when 17 β -estradiol (E2) was investigated, which has a beneficial effect on the cardiovascular system. E2, which induces the phosphorylation of phosphoinositide 3-kinases (PI3Ks), increases striatin protein expression in a dose- and time-dependent manner in human umbilical vein endothelial cells (HUVECs). Interestingly, the treatment of HUVECs with the phosphatidylinositol-3 kinase inhibitor, wortmannin, abolished E2-mediated upregulation of striatin protein expression. Wortmannin, a fungal steroid metabolite, is a covalent inhibitor of PI3Ks, which are recruited to the membrane and that act upstream of the STRIPAK pathway. Thus, E2 seems to regulate striatin via the PI3K signal transduction pathway (Zheng et al., 2018). Collectively, the abovementioned examples provide the first mechanistic insights on how STRIPAK dysfunction might cause human diseases on the cellular level.

Future perspectives

Since the first description of striatin about 20 years ago, striatin and its associated proteins have been further investigated in a broad range of studies using diverse eukaryotic experimental and model systems. Subsequently, several structural and mechanistic models have been proposed to explain the role of the STRIPAK complex in diverse cellular and developmental processes occurring in both microbial as well as animal systems. Despite enormous progress in biochemically characterizing STRIPAK complexes, key questions still remain about the regulation of developmental processes as well as the cross-talk between STRIPAK and other conserved signaling pathways. For example, the mechanisms by which upstream

regulators and downstream targets affect signaling of the STRIPAK complex are not yet fully understood. In particular, it is unknown how the inactivation or inhibition of subunits of the STRIPAK complex influence the phosphorylation status of target proteins.

Phosphoproteomic studies may advance our current rudimentary understanding of how STRIPAK leads to the deregulation of cellular division processes in microbes or to the onset of diverse cancer types in mammals. Thus, gaining additional insights into its upstream regulators and downstream targets is crucial as a prelude to translating our basic knowledge of this complex and its signaling pathways into therapeutic interventions. Given the relevance of the STRIPAK complex for human diseases, such as cancer, this signaling pathway may thus provide promising drug targets for pharmaceutical development aimed at producing new therapies for distinct types of cancer.

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Bionotes

Ulrich Kück
Allgemeine und Molekulare Botanik,
Ruhr-Universität Bochum, Universitätsstr.
150, D-44780 Bochum, Germany
ulrich.kueck@rub.de

Ulrich Kück is a professor in the department of General and Molecular Botany of the Ruhr University Bochum. He has a long-standing interest in the genetics of fungi.

Daria Radchenko
Allgemeine und Molekulare Botanik,
Ruhr-Universität Bochum, Universitätsstr.
150, D-44780 Bochum, Germany

Daria Radchenko is a postdoctoral fellow and has studied fungal signal transduction pathways, related to the Hippo signaling complex.

Ines Teichert
Allgemeine und Molekulare Botanik,
Ruhr-Universität Bochum, Universitätsstr.
150, D-44780 Bochum, Germany

Ines Teichert leads an independent research group in the department of General and Molecular Botany and has a focus on development-dependent RNA editing.