New kids on the block: The Popeye domain containing (POPDC) protein family acting as a novel class of cAMP effector proteins in striated muscle

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Abstract

The cyclic 3',5'-adenosine monophosphate (cAMP) signalling pathway constitutes an ancient signal transduction pathway present in prokaryotes and eukaryotes. Previously, it was thought that in eukaryotes three effector proteins mediate cAMP signalling, namely protein kinase A (PKA), exchange factor directly activated by cAMP (EPAC) and the cyclic-nucleotide gated channels. However, recently a novel family of cAMP effector proteins emerged and was termed the Popeye domain containing (POPDC) family, which consists of three members POPDC1, POPDC2 and POPDC3. POPDC proteins are transmembrane proteins, which are abundantly present in striated and smooth muscle cells. POPDC proteins bind cAMP with high affinity comparable to PKA. Presently, their biochemical activity is poorly understood. However, mutational analysis in animal models as well as the disease phenotype observed in patients carrying missense mutations suggests that POPDC proteins are acting by modulating membrane trafficking of interacting proteins. In this review, we will describe the current knowledge about this gene family and also outline the apparent gaps in our understanding of their role in cAMP signalling and beyond.

Keywords:
1. Popeye domain containing
2. cAMP signalling
3. heart disease
4. stress-induced bradycardia
5. atrioventricular block
6. limb-girdle muscular dystrophy
7. protein interaction
1. The cAMP signalling pathway

The second messenger cyclic adenosine 3'-5' monophosphate (cAMP) activates one of the most important signalling pathways in the eukaryotic cell, which is involved in a large variety of cellular responses. cAMP was first discovered by Earl W. Sutherland through his work on the glycogenolytic activity of epinephrine in liver homogenates [1].

cAMP signalling involves a complex protein network, which controls second messenger production and limits diffusion resulting in compartmentalized activation of effector proteins and thereby achieving specificity.

1.1 β-adrenergic receptors

Activation of β-adrenergic receptors (βARs) in the heart results in a rapid rise in cAMP levels and modulate cardiac contractility (inotropy), relaxation (lusitropy), heart rate (chronotropy), conductivity (dromotropy) and cohesion (adhesiotropy) [2].
The two cardiac βAR subtypes, β1 (β1AR) and β2 (β2AR), induce a rapid rise in cellular cAMP levels, however, different cellular responses are the result of their activation. For example, sustained activation of β1AR is causing myocyte apoptosis, while β2AR activation is considered to be cardioprotective. Consistent with the differing physiological responses induced by these two receptor subtypes, both display a differential subcellular distribution in ventricular myocytes (Fig. 1). Visualizing cAMP production with the help of transgenic mice, which express a fluorescent cAMP sensor in cardiac myocytes revealed that β1AR-stimulation induces cAMP, which propagates throughout large parts of the cell. In contrast, β2AR-induced cAMP synthesis is confined to transverse tubules (t-tubules), which are extensions of the sarcolemma that penetrate into the interior of striated muscle cells allowing efficient excitation-contraction coupling.

1.2 Adenylate cyclases
A family of enzymes, which are termed the adenylyl cyclases (ACs), catalyze cAMP synthesis in response to activation of βARs and other hormone receptors. There are nine different membrane-bound and one soluble AC isoform.
In the heart, the major isoforms found in cardiac myocytes are AC5 and AC6, while AC2 and AC9 being expressed at very low levels {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. The different AC isoforms are probably not interchangeable and the two most abundant isoforms, AC5 and AC6, are acting from opposite ends to adapt to cardiac stress. The loss of AC5 by gene targeting increases longevity and protects against cardiac stress {ADDIN EN.CITE {ADDIN EN.CITE.DATA}} and a similar result is obtained after overexpression of AC6 {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. Interestingly, in cardiac pacemaker cells, which are located in the sinoatrial node (SAN), the Ca\textsuperscript{2+}-activated isoforms AC1 and AC8 isoforms are specifically expressed along with those isoforms, which are also present in working cardiac myocytes {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. Consistent with the preferential expression of AC8 in the SAN, forced expression causes an increase in heart rate (HR), a reduction in heart rate variability (HRV) and leads to an uncoupling of HR and HRV from autonomic control {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}.

1.3 Phosphodiesterases
The only enzymes that degrade cyclic nucleotides are the cyclic nucleotide phosphodiesterases (PDEs), which are probably the most important determinants of cAMP compartmentalization. There are 21 genes forming the PDE superfamily, which are grouped into 11 distinct PDE families, PDE1–11 {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. All PDEs harbour a C-terminal catalytic domain of about 270 AA, which is 20–25% identical in isozymes belonging to different subfamilies. It is estimated that more than 50-100 different PDE isoforms exists, which are generated by differential splicing, or through the use of alternative transcription start sites {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. Splicing generates PDE isoforms, which differ from each other in their protein interaction partners, affinity for cyclic nucleotides, reaction kinetics, regulatory mechanisms, and subcellular localization {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. 

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PDEs also differ in their nucleotide specificity: PDE1-3, 10, and 11 hydrolyse both cAMP and cGMP, PDE4, 7, and 8 degrade only cAMP, and PDE5, 6, and 9 are cGMP-specific.
In the heart, four PDE families are responsible for cAMP degradation: PDE1, PDE2, PDE3, and PDE4. The expression level of these isoforms in the heart is species-specific. In rodents, PDE4 is the major isoform, contributing up to 60% of the total cAMP hydrolytic activity, whereas PDE3 accounts for only 20 to 30% of PDE activity. Basal pacemaking in the SAN depends on PDE3 and PDE4. However, the catecholamine-induced increase in pacemaking frequency is not affected by
blocking either enzyme, which suggests that the catecholamine-induced increase in pacemaking is independent of these isoforms \{ADDIN EN.CITE \{ ADDIN EN.CITEDATA \}\}. PDE1A is expressed at high levels in the SAN \{ADDIN EN.CITE \{ ADDIN EN.CITEDATA \}\}. PDE1A and AC1/8 are both Ca\textsuperscript{2+}-activated, linking cAMP production and degradation with the Ca\textsuperscript{2+}-clock mechanism, which together with the membrane clock is responsible for triggering cardiac pacemaking in the SAN \{ADDIN EN.CITE \{ ADDIN EN.CITEDATA \}\}. PDE1A is expressed at high levels in the SAN. PDE1A and AC1/8 are both Ca\textsuperscript{2+}-activated, linking cAMP production and degradation with the Ca\textsuperscript{2+}-clock mechanism, which together with the membrane clock is responsible for triggering cardiac pacemaking in the SAN.

1.4 Protein kinase A and AKAP proteins
The downstream targets remained unknown for a long time after cAMP has been discovered until protein kinase A (PKA) was discovered \{ADDIN EN.CITE \{ ADDIN EN.CITEDATA \}\}. The new kids on the block
PKA is a tetramer consisting of two regulatory and two catalytic subunits. There are three isoforms of the catalytic subunit called Ca, Cb and Cγ and a total of four regulatory subunits, R1α, R1β, R1Iα and R1Iβ.

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The PKA holoenzyme associates with A-kinase anchoring proteins (AKAPs), which is a large and diverse group of proteins. It is estimated that there are >30 different AKAPs, which are structurally diverse. However, a unifying principle is the presence of a 14-18 amino acids long protein kinase A binding domain, which forms an amphipathic helix mediating binding to the regulatory subunits of PKA.
Approximately 15 AKAPs have been identified in the heart, which play an important role in calcium-induced calcium release (AKAP18α, AKAP18γ, AKAP79), repolarization (Yotatio, D-AKAP2) and stress-response (AKAP-Lbc, mAKAPβ, D-AKAP1).
1.5 Exchange protein directly activated by cAMP

It was long thought that PKA and cyclic nucleotide-gated ion channels such as the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels would be the only effector proteins involved in cAMP signalling. However, 1998 EPAC1 and EPAC2 were identified as novel mediators of cAMP signalling \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. Apart from having one (EPAC1) or two (EPAC2) cAMP-binding domains, EPAC proteins also have a GEF domain via which the exchange of GDP/GTP of RAP proteins is induced \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. Both, EPAC1 and -2 are expressed in cardiac myocytes, however they are localized to different compartments. EPAC1 is mostly localized to the nuclear envelope, while EPAC2 is found along t-tubules \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. These isoform-specific subcellular localizations agree with the proposed regulatory role of EPAC2 for ryanodine receptor (RyR) function \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \} and of EPAC1 in modulating gene transcription, hypertrophy signalling and being part of a mAKAP/PKA/PDE4/ERK5 complex at the nuclear envelope \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. EPAC1 was also described to be present at the plasma membrane and in mitochondria \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. Recent data suggest that one of the mitochondrial functions of Epac1 is to mediate apoptotic signalling \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. The role of EPAC1 at the plasma membrane may also include the control of membrane trafficking of the gap junction protein connexin 43 (Cx43) \{ADDIN EN.CITE \}. 

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2. Discovery of the Popeye domain containing (POPDC) genes

With the goal to clone novel genes involved in heart development, two groups independently identified the first member of the Popeye domain containing (POPDC) gene family \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. Based on sequence homology, two additional members of the gene family were subsequently identified. In vertebrates, the POPDC family consists of \textit{Popdc1} (also known as \textit{Bves}), \textit{Popdc2} and \textit{Popdc3} \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. POPDC genes are abundantly expressed in the heart and skeletal muscle \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. Apart from striated muscle tissue, POPDC family members are also expressed in smooth muscle tissue in different organs (lung, gastrointestinal tract, bladder, uterus), epithelial cells (skin, cornea, esophagus, pyloric epithelium), and neurons of the autonomous and central nervous system \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. In the adult heart, expression of \textit{Popdc1} and \textit{Popdc2} is confined to cardiac myocytes and is absent from non-muscle cell type \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. \textit{Popdc1} and \textit{Popdc2} are differentially expressed in different parts of the heart. In the adult heart, \textit{Popdc1} is expressed at higher levels in the atria than the ventricles and expression is also elevated in the cardiac conduction system (CCS) which includes the SAN, the atrioventricular node (AVN), the His bundle, the bundle branches and the Purkinje fibres. \textit{Popdc2} on the other hand is expressed at equal levels in atria and ventricles but also displays a higher expression level in the CCS \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \} \}. A detailed expression pattern of \textit{Popdc3} is currently not available.

2.1 POPDC proteins

2.1.1 Protein structure
POPDC genes encode medium-sized transmembrane proteins (human POPDC1: 360 amino acids, predicted MW: 41.5 kDal; human POPDC2: 364 amino acids, predicted MW: 40.4 kDal; human POPDC3: 291 amino acids, predicted MW: 33.8 kDal) (Fig. 2). The amino terminus, of POPDC proteins is short and consists of only 27-39 residues. One or two N-glycosylation sites have been mapped and appear to be functional { ADDIN EN.CITE { ADDIN EN.CITE.DATA } }. Glycosylation of POPDC proteins is extensive and the apparent molecular weight of the major form of POPDC1 in the heart is approximately 55 – 58 kDal { ADDIN EN.CITE { ADDIN EN.CITE.DATA } }. Interestingly, the apparent molecular weight of POPDC1 is organ-specific and is approximately 70 kDal in skeletal muscle { ADDIN EN.CITE { ADDIN EN.CITE.DATA } }. POPDC proteins have three transmembrane domains. In the cytoplasmic part of the protein is the Popeye domain (Pfam domain: PF04831) localized (Fig. 2). The Popeye domain is a 150 amino acid long, evolutionarily conserved protein domain, which displays a strong structural similarity to the cAMP binding domains of the regulatory subunit of PKA { ADDIN EN.CITE { ADDIN EN.CITE.DATA } }. However, sequence similarity to the cAMP binding domains of PKA or EPAC is very low. While the Popeye domain is unique and only found in POPDC proteins, non-POPDC proteins with the highest sequence similarity are the catabolite activator proteins (CAPs), also
known as cAMP receptor proteins (CRPs). This family of transcription factors, which are exclusively found in bacteria, are involved in metabolic control and cyclic AMP functions as an allosteric effector by increasing the affinity of CRP or CAP for DNA {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. In this regard, it is noteworthy that for example in satellite cells, POPDC proteins are present in the nucleoplasm. {ADDIN EN.CITE} 

**2.1.2 Cyclic nucleotide binding properties**

Two invariant sequence motifs (FL/IDSPEW/F and FQVT/SL/I) in the putative binding pocket of the Popeye domain are linked by a non-conserved sequence, which is variable in length in different Popeye domains. Both, the DSPE and FQVT motifs are crucial for cAMP binding and are strongly conserved in evolution (Fig. 2). Charge-to-alanine mutations of D200 of POPDC1 and the homologous residue D184 of POPDC2 strongly impair cAMP binding {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. Mutation of the ultra-conserved serine 201 to phenylalanine also led to an impairment of cAMP binding of POPDC1 as did the Glu203 to Ala and Val217 to Phe mutations {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. Measurement of the cyclic nucleotide affinity with the help of recombinant POPDC protein in a competitive radioligand binding assay, lead to an estimate of the cyclic nucleotide binding affinity, which is approximately 118 nM for cAMP and approximately 40-fold lower (5 μM) for cGMP. Similar affinity data were independently obtained with the help of a bimolecular Förster-resonance energy transfer (FRET) assay {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. The carboxy-terminal domain (CTD) of POPDC proteins is variable in sequence and length and subject to alternative splicing. While being divergent between POPDC family members, the CTD displays isoform-specific conservation. Proteomic analysis suggests, that the CTD of each POPDC isoform is subject to β1AR-dependent phosphorylation, which may be important in modulating the biological activity of POPDC proteins {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}.
Interestingly, POPDC isoforms differ in the number of residues, which are getting phosphorylated in response to β1AR activation. POPDC1 appears to be the most targeted isoform with a total of 9 phosphorylation sites, POPDC2 has four sites and POPDC3 contains a single residue, which is phosphorylated in response to adrenergic agonists.
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2.2 Defining POPDC function in animal models

2.2.1 Drosophila
Loss-of-function experiments have been performed in a number of organisms. In Drosophila, a single POPDC gene (bves) is present in the genome. Due to the complexity of the genomic region, P-element mobilization did not result in the identification of a null mutant for bves. As an alternative approach, heat shock-induced expression of an antisense transcript caused early lethality with embryos displaying abnormal gastrulation movements and aberrant gut closure. No further analysis of organ formation and in particular no analysis of heart and skeletal muscle structure and function has been performed in the transgenic flies.

2.2.2 Zebrafish
Morpholino-mediated knockdown of popdc2 in zebrafish caused a severe muscular dystrophy phenotype, which is characterized by the disruption of the myotendinous junction. A similar phenotype was observed in case of popdc1 morphants and in the popdc1S201F knock-in (KI) mutant. In the heart, many popdc1 morphants and a sizable fraction of the popdc1S201F KI mutant displayed a pericardial effusion and a similar phenotype was also observed in case of popdc2. While a pericardial effusion is often interpreted as a sign of myocardial pumping deficiency, an alternative interpretation was recently provided.
POPDC proteins have an important function in organizing tight junction formation and the lack of popdc1 in zebrafish by morpholino-mediated knockdown causes a defective barrier formation of the skin leading to a fragility of the embryo when challenged by hyperosmotic conditions. The inability to maintain osmotic homeostasis is probably the reason for the pericardial effusion phenotype, as it was rescued by raising the osmolality of the culture medium {\ADDIN EN.CITE <EndNote><RecNum>9392</RecNum><DisplayText>[57]</DisplayText><record><rec-number>9392</rec-number><foreign-keys><key app="EN" db-id="rt59wpfsdrwves59gpr5fwaafzr2rraxa0ft" timestamp="0">9392</key><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wu, Y. C.</author><author>Liu, C. Y.</author><author>Chen, Y. H.</author><author>Chen, R. F.</author><author>Huang, C. J.</author></authors></contributors><auth-address>From the Institute of Zoology, College of Life Science, National Taiwan University, Taipei 10673, Taiwan.</auth-address><titles><title>Blood Vessel Epicardial Substance (Bves) Regulates Epidermal Tight Junction Integrity through Atypical Protein Kinase C</title><alt-title>The Journal of biological chemistry</alt-title><perdidcal><full-title>J Biol Chem</full-title></perdidcal></titles><periodical><full-title>J Biol Chem</full-title><abbr-1>The Journal of biological chemistry</abbr-1></periodical><pages>39887-97</pages><volume>287</volume><number>47</number><dates><year>2012</year><pub-dates><date>Nov 16</date></pub-dates></dates><isbn>1083-351X (Electronic); 0021-9258 (Linking)</isbn><accession-num>23019331</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/23019331</url></related-urls></urls><custom2>10.1074/jbc.M112.372078</custom2>
The molecular basis for the osmotic homeostasis phenotype in popdc1 morphants probably relates to an impaired accumulation of tight junction proteins (claudin) in the epithelium, which is caused by an aberrant localization of atypical protein kinase C (aPKC).

Support for the role of POPDC1 in cell-cell contact formation stems from the observation that another important protein, which directly or indirectly interacts with POPDC1 is zonula occludens 1 (ZO1) and also a cardiac arrhythmia phenotype was present in each of these zebrafish models. Loss-of-function experiments in an epithelial cell line caused a loss of ZO1 from the cell surface and reduced trans-epithelial resistance, suggesting an important function of POPDC1 in tight junction formation in epithelial cells.

Apart from the osmotic instability leading to a pericardial effusion phenotype in popdc1 and popdc2 morphants and popdc1S201F mutants, also a cardiac arrhythmia phenotype was present in each of these zebrafish models. The first arrhythmia phenotype seen is an AV-block, which is already present at 2-3 days post-fertilization (dpf) in popdc1 mutants and develops slightly later in popdc2 morphants. At 5 dpf close to 40%
of the popdc2 morphants displayed a cardiac arrhythmia phenotype \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \}\}. The popdc1S201F mutant displayed a similar phenotype as the popdc1 morphant, however in this case the frequency of animals displaying an arrhythmia phenotype was much lower and amounted to only 5%. However, the number of animals displaying a phenotype could be tripled when the animals were challenged by isoproterenol treatment \{ADDIN EN.CITE \}

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2.2.3 Xenopus

Loss-of-function experiments of POPDC1 using morpholinos were also performed in Xenopus and in contrast to what has been observed in zebrafish and in the mouse (see below) an early gastrulation phenotype was observed \{ADDIN EN.CITE \}.

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a regulator of epithelial movement during early Xenopus laevis development</title><secondary-title>Proc Natl Acad Sci U S A</secondary-title></title><periodical><full-title>Proc Natl Acad Sci U S A</full-title><abbr-title>Proceedings of the National Academy of Sciences of the United States of America</abbr-title></periodical><pages>614-9</pages><volume>103</volume><number>3</number><dates><year>2006</year></dates><accession-num>16407138</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&amp;db=PubMed&amp;dopt=Citation&amp;list_uids=16407138</url></related-urls></urls></record></Cite></EndNote>

It is possible that differences in the expression pattern of POPDC family members between model systems may be responsible for the differences in timing of phenotype development. Interestingly, the early gastrulation phenotype in *Xenopus* is similar to what has been described in *Drosophila*.

2.2.4 Mouse

*Popdc1* and *Popdc2* null mutants in mice were engineered by homologous recombination. While both null mutants were viable and survived into adulthood, specific pathologies were observed, when the animals were challenged. *Popdc1* null mutants displayed a regeneration phenotype in skeletal muscle after cardiotoxin injection { ADDIN EN.CITE <EndNote><Cite><Author>Andrée</Author><Year>2002</Year><RecNum>2686</RecNum><DisplayText>[60]</DisplayText><record><rec-number>2686</rec-number><foreign-keys><key app="EN" db-id="rt59wpfssdprwves59gpr5fwafzf2rxaa0ft" timestamp="0">2686</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Andrée, B.</author><author>Fleige, A.</author><author>Arnold, H. H.</author><author>Brand, T.</author></authors></contributors><titles><title>Mouse Pop1 is required for muscle regeneration in adult skeletal muscle</title><secondary-title>Mol Cell Biol</secondary-title></titles><periodical><full-title>Mol Cell Biol</full-title><abbr-title>Molecular and cellular biology</abbr-title></periodical><pages>1504-12</pages><volume>22</volume><number>5</number><dates><year>2002</year></dates><url>1504-12</url></record></EndNote>}. The regeneration was retarded relative to wildtype skeletal muscle, however the underlying molecular mechanisms are currently unknown. Induction of ischemia/reperfusion injury to Langendorff-perfused hearts caused a significantly lower functional recovery in the *Popdc1* null mutant compared with wild type, while infarct size was larger { ADDIN EN.CITE <EndNote><Cite><Author>Alcalay</Author><Year>2013</Year><RecNum>9820</RecNum><DisplayText>[61]</DisplayText><record><rec-number>9820</rec-number><foreign-keys><key app="EN" db-id="rt59wpfssdprwves59gpr5fwafzf2rxaa0ft" timestamp="1400602215">9820</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Alcalay, Y.</author><author>Hochhauser, E.</author><author>Kliminski,
Isolated cardiac myocytes from *Popdc1* null mutants displayed impaired Ca\(^{2+}\)-transients, increased vulnerability to oxidative stress and absence of pharmacologic preconditioning. Further studies using myocytes treated with *Popdc1* siRNA revealed that *Popdc1* is required for cardiac myocyte survival.
Loss of *Popdc1* causes an increase in the expression of the proapoptotic BCL2 interacting protein 3 (*Bnip3*), which may explain the increased vulnerability when null mutant hearts are subjected to ischemia/reperfusion injury.

As mentioned before, *Popdc1* and *Popdc2* display high-level expression in the CCS. Implantation of telemetric ECG devices revealed normal heart rate and a normal electrocardiogram at baseline. However, when mouse mutants were subjected to physical exercise, mental stress or isoproterenol injection, the heart rate became highly variable and the SAN pacemaker was pausing for different lengths of time. Interestingly, the pathological phenotype, which is present in both *Popdc1* and *Popdc2* null mutants was not present in young mice, however, at 5–8 months of age, mutants displayed a severe stress-induced bradycardia with episodes of sinus node dysfunction. The age-dependency of phenotype development in the mutants is reminiscent of the sick sinus syndrome (SSS), which is the most frequent reason for pacemaker implantation and most prevalent in the elderly in the absence of any other heart disease.
2.3 Association of POPDC1 mutations with heart disease

While the association of heart and muscle phenotypes in animal models carrying loss-of-function mutations was demonstrated in several species, the question, whether POPDC mutations are also disease-causing in patients remained unanswered until recently.

2.3.1 Mutation in POPDC1 causes muscular dystrophy and cardiac arrhythmia

A family of Albanian origin living in a remote location in the southern part of Italy was found to carry a recessive missense mutation in POPDC1 (S201F) and to suffer from limb-girdle muscular dystrophy (LGMD) and type II atrioventricular block in individuals, which carried the mutation in homozygosis.

LGMD is a heterogeneous group of muscle diseases, which is caused by mutations in a large number of different genes. However, many LGMD-causing mutations affect proteins localized to the plasma membrane such as dystroglycan, sarcoglycans, caveolin-3 (CAV3), and dysferlin (DYSF) or the nuclear envelope (lamin A/C (LMNA)).
While several LGMDs only manifest in skeletal muscle, others also involve the heart and cause arrhythmias or DCM phenotypes. The missense mutation in POPDC1 discovered in the Italian family substitutes serine 201 by phenylalanine. Serine 201 is invariant and is part of the DSPE motif, which is thought to be directly involved in cyclic nucleotide binding.
hydrophobic amino acid phenylalanine is likely to interfere with cAMP gaining access to its binding pocket. Measurements of cAMP affinity revealed a drop in cAMP affinity by approximately 50% \cite{BrandTetal2017}. Significantly, zebrafish mutants carrying the homologous mutation \textit{popdc1S191F} displayed both, heart and skeletal muscle pathologies, which were reminiscent of the patient's phenotypes \cite{BrandTetal2017}. Most importantly, the impaired nucleotide binding affected the membrane localization of the mutant POPDC1 protein. In the patient’s skeletal muscle biopsies as well as skeletal muscle tissue of zebrafish mutants carrying the homologous mutation, the majority of the mutant POPDC1 protein was mislocalized and no longer present at the plasma membrane but in a peri-nuclear domain. Surprisingly, also the subcellular localization of POPDC2 was abnormal. The POPDC1\textit{S201F} mutation is rare and sequencing of an additional 104 patients with similar clinical phenotype did not reveal any additional carrier \cite{BrandTetal2017}.

\subsection*{2.3.2 Putative association \textit{POPDC1} with other forms of cardiac disease}

An association of \textit{POPDC1} with other forms of cardiac arrhythmia was recently predicted by two studies using bioinformatics, which suggests that \textit{POPDC1} might act as a hub gene for atrial fibrillation (AF) \cite{BrandTetal2017} and as a novel determinant of the length of the QT interval, which is the time between depolarisation and repolarisation of the ventricular chambers \cite{BrandTetal2017}. An increase in the QT
time raises the risk of developing severe forms of ventricular arrhythmia. A down-regulation of POPDC1 and POPDC3, and to a lesser degree POPDC2 in heart failure has also been recently reported {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. POPDC1 variants were identified in patients with Tetralogy of Fallot (ToF) an important form of congenital heart disease (CHD) {ADDIN EN.CITE <EndNote><Cite><Author>Wu</Author><Year>2013</Year><RecNum>19299</RecNum><DisplayText>[69]</DisplayText><record><rec-number>19299</rec-number><foreign-keys><key app="EN" db-id="rt59fwpsdprwves59gpr5fwafzf2rrxao0ft" timestamp="1499941681" key="rt59fwpsdprwves59gpr5fwafzf2rrxao0ft" ref-type name="Journal Article">17</ref-type><contributors><author>Wu, M.</author><author>Li, Y.</author><author>He, X.</author><author>Shao, X.</author><author>Yang, F.</author><author>Zhao, M.</author><author>Wu, C.</author><author>Zhang, C.</author><author>Zhou, L.</author></contributors><address>Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, PR China.</address><titles><title>Mutational and functional analysis of the BVES gene coding region in Chinese patients with non-syndromic tetralogy of Fallot</title><secondary-title>Int J Mol Med</secondary-title><periodical><full-title>International journal of molecular medicine</full-title><abbr title="International journal of molecular medicine">IJMM</abbr><pages>899-903</pages><volume>31</volume><number>4</number><keywords><keyword>Base Sequence</keyword><keyword>Case-Control Studies</keyword><keyword>China</keyword><keyword>DNA Mutational Analysis</keyword><keyword>Humans</keyword><keyword>Membrane Proteins/*genetics/physiology</keyword><keyword>Molecular Sequence Data</keyword><keyword>Polymorphism, Single Nucleotide/*genetics/physiology</keyword><keyword>Sequence Alignment</keyword><keyword>Tetralogy of Fallot/*genetics</keyword><dates><year>2013</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>1791-244X (Electronic)&#xD;1107-3756 (Linking)</isbn><accession-num>23403794</accession-num><urls><related-url>https://www.ncbi.nlm.nih.gov/pubmed/23403794</related-url></urls><electronic-resource-num>10.3892/ijmm.2013.1275</electronic-resource-num></periodical></titles><dates><year>2013</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>1791-244X (Electronic)&#xD;1107-3756 (Linking)</isbn><accession-num>23403794</accession-num><urls><related-url>https://www.ncbi.nlm.nih.gov/pubmed/23403794</related-url></urls><electronic-resource-num>10.3892/ijmm.2013.1275</electronic-resource-num></record></Cite></EndNote> }. However, the discovered variants haven’t been introduced into animal models to confirm that they are disease-causing. The association of POPDC1 with ToF is therefore rather uncertain given the lack of CHD in the Popdc1 null mutant in mice.

2.4 Association of POPDC genes with cancer

Apart from their association with heart and skeletal muscle disease, a significant body of knowledge has accumulated in recent years, which indicates a strong association of a loss of POPDC gene function with various cancer types (reviewed in {ADDIN EN.CITE <EndNote><Cite><Author>Amunjela</Author><Year>2016</Year><RecNum>1

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Suppression of POPDC1 or POPDC3 expression correlates strongly with disease progression and poor clinical prognosis in different cancer forms including gastric cancer, lung cancer, hepatocellular carcinoma, breast cancer, etc.

Amunjela, J. N. Tucker, S. J. (2017). POPDC proteins as potential novel therapeutic targets in cancer. Drug Discov Today, 21(12), 1920-1927. doi:10.1016/j.drudis.2016.07.011.
The reduction in Popdc1 expression in the tumour is probably due to an increase in methylation. An investigation of pan-cancer hypermethylated CpG sites identified an enhancer, which is hypermethylated in at least 11 different cancer forms and is predicted to regulate POPDC1 expression. Recent work on a model of inflammation-induced colon carcinoma formation has lead to the discovery that POPDC1 controls c-Myc levels by forming a complex with the PR61α and c-Myc. PR61α is a regulatory subunit of protein phosphatase 2A (PP2A), which apart from other activities promotes c-Myc degradation by dephosphorylation of a critical residue. In agreement with this finding is the observation that a knockdown of Popdc1 causes increased c-Myc levels, whereas forced expression suppressed c-Myc.

3. POPDC interacting proteins in striated muscle

3.1. Popdc1 is a multi-compartment protein
POPDC proteins in striated muscle cells are localized to the plasma membrane and its derivatives in striated muscle including intercalated discs. The new kids on the block
In addition, POPDC1 and POPDC2 are also present at the nuclear envelope and nucleoplasm. Presently, it is unknown, whether the structure of any of these membrane compartments is affected in Popdc1 or Popdc2 null mutants with the exception of caveolae, which were described in the heart of Popdc1 null mutants to be reduced in number but larger in diameter. Interestingly, ultrastructural analysis of skeletal muscle biopsies of one of the patients carrying the POPDC1 S201F mutation revealed the presence of membrane discontinuities, which were similar to those observed in case of patients carrying anoctamine-5 (ANO5) mutations and possibly an indication for impaired repair of the muscle plasma membrane.
membrane. Consistent with these ultrastructural changes in patients and mouse mutants is the recent findings that POPDC proteins interact with Caveolin (CAV3) and Dysferlin (DYSF). Interestingly, POPDC1, ANO5, CAV3 and DYSF have all been genetically linked to LGMD suggesting that they possibly all active in the same disease pathway.

Membrane instability of the mutant muscle may also be related to Dystrophin, which is also an interaction partner of POPDC proteins. Membrane instability of the mutant muscle may also be related to Dystrophin, which is also an interaction partner of POPDC proteins.

Schindler, R.F. & Brand, T. (2016). The Popeye domain containing protein family - A novel class of cAMP effectors with important functions in multiple tissues. Prog Biophys Mol Biol. 2016 Jan 7.
Popdc1 interacts with the two-pore potassium channel TREK-1

The plasma membrane localization of POPDC proteins, the pacemaker phenotype in Popdc1 and Popdc2 null mutants, the AV-block in patients and in zebrafish mutants and the high expression levels of POPDC proteins in the cardiac conduction system all point to a role of POPDC proteins in cardiac action potential generation and/or cardiac conduction. The interaction of POPDC proteins with ion channels or other electrogenic proteins was recently tested, which resulted in the identification of the potassium channel TWIK-related K+ channel 1 (TREK-1) as a POPDC interacting protein \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \}\}. TREK-1 is a member of the two-pore domain potassium channel (K_2P) family, which is modulated by a large number of different stimuli including stretch, pH, temperature, phosphorylation, and interacting proteins \{ADDIN EN.CITE\}.

In Xenopus oocytes, co-expression of any POPDC isoform and TREK-1 stimulates a twofold higher current \{ADDIN EN.CITE \{ADDIN EN.CITE.DATA \}\}, which is thought to be due to an enhanced membrane transport of the channel protein.
This effect was modulated by cAMP and was lost in the presence of the general PDE inhibitor theophylline. The interaction of POPDC proteins with TREK-1 is mediated by the cytoplasmic part of the protein. Based on the interaction of POPDC and TREK-1, a bi-molecular FRET sensor was constructed. The FRET ratio obtained at baseline decreased after the addition of isoproterenol or forskolin, suggesting that cyclic nucleotide-binding affects the interaction of POPDC1 with TREK-1. A cardiac specific knockout of Kcnk2, which encodes TREK-1, displays a stress-induced sinus bradycardia similar to the one observed in Popdc1 and Popdc2 null mutants, suggesting that the sinus bradycardia in POPDC mutants may in part be due to an impaired TREK-1 current.

3.3. POPDC1 might control membrane trafficking of interacting proteins
In support of a role of POPDC proteins in regulating membrane trafficking is the recent finding that the POPDC1S201F mutant protein displayed a reduction in membrane localization and at the same time an increased perinuclear expression of POPDC1.

Similar to the POPDC1S201F protein, POPDC2 was also mislocalized in the mutant muscle. Surprisingly, increase of cAMP levels caused a reduction in membrane localization of TREK-1 in the presence of POPDC1S201F, whereas a reduction in the cAMP-binding affinity of POPDC1S201F caused a reduction in membrane transport of itself and of POPDC2. These data suggest that the effect of cAMP on membrane trafficking of POPDC1
and associated proteins is complex. Therefore, more work is required to unravel how cAMP-binding of POPDC proteins affect protein-protein interaction and membrane trafficking.

3.3. POPDC1 interacting proteins in epithelial cells

A number of additional POPDC1 interacting proteins, which play in particular a role in epithelial cells, were recently described and include Zona occludens 1 (ZO1), guanine nucleotide exchange factor T (GEFT), vesicle-associated membrane protein 3 (VAMP3) and N-Myc downstream regulated gene 4 (NDRG4) \{ADDIN EN.CITE {ADDIN EN.CITE.DATA} \}. The PDZ protein Zonula occludens-1 (ZO1) \{ADDIN EN.CITE {ADDIN EN.CITE.DATA} \} functions as a scaffolding protein and interacts for example with tight junction proteins. Loss of POPDC1 in cultured epithelial cells reduces transepithelial resistance and junctional protein localization at the plasma membrane \{ADDIN EN.CITE {ADDIN EN.CITE.DATA} \}. In cardiac myocytes, ZO1 binds the carboxy terminus of Cx43 and thereby controls gap junction plaque size \{ADDIN EN.CITE {ADDIN EN.CITE.DATA} \}.
It is possible, that POPDC1 and ZO-1 are important for myocardial conduction. In this regard it is noteworthy that POPDC1 and POPDC2 are expressed in the intercalated disk. VAMP3 is a soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) protein and part of the vesicle docking and fusion complex.
Interestingly, VAMP proteins are involved in membrane trafficking of transmembrane proteins by modulating the recycling endosome compartment. Thus, the impaired membrane trafficking in POPDC mutants may involve SNARE protein-mediated membrane transport. POPDC1 interacts with NDRG4 and the knockdown of both genes affects membrane docking of VAMP3-labeled vesicles. GEFT, a guanine nucleotide exchange factor (GEF) docking for the Rho-family GTPases Rac1 and Cdc42 is another interaction partner of POPDC1. GEFT is expressed in cardiac and skeletal muscle, however, the interaction of POPDC1 and GEFT is largely unexplored.

4. Regulation of POPDC genes

4.1 Popdc1 expression is suppressed by EGF signalling

Little is known about the regulation of POPDC gene expression. In Drosophila, bves is suppressed in dorso-anterior follicle cells by the secretion of gurken, an epidermal growth factor (EGF)-like signalling molecule. Regulation of POPDC expression by EGF was also investigated in the gastric adenocarcinoma cell line SNU-216, which expresses all three POPDC genes.
promoter hypermethylation in gastric cancer</title><secondary-title>Carcinogenesis</secondary-title><title>Carcinogenesis</title><periodical><full-title>Carcinogenesis</full-title><abbr>Carcinogenesis</abbr><pages>1685-93</pages><volume>31</volume><number>9</number><edition>2010/07/16</edition><year>2010</year><pub-dates><date>Sep</date></pub-dates><isbn>1460-2180 (Electronic)&#xD;xD;0143-3334 (Linking)</isbn><accession-num>20627872</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&amp;db=PubMed&amp;dopt=Citation&amp;list_uids=20627872</url></related-urls></urls><electronic-resource-num>bgq144</electronic-resource-num><language>eng</language></record></Cite></EndNote> and in several breast cancer cell lines { ADDIN EN.CITE <EndNote>CiteAuthor>Amunjela</CiteAuthor><Year>2017</Year><RecNum>19359</RecNum><DisplayText>[75]</DisplayText><record><rec-number>19359</rec-number><foreign-keys><key app="EN" db-id="rt59fwpsdprwves59gr5fwafzf2rxaa0ft" timestamp="1505665888">19359</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Amunjela, J. N.</author><author>Tucker, S. J.</author></authors></contributors><auth-address>School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, United Kingdom. Electronic address: j.n.amunjela@abdn.ac.uk;&#xD;School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, United Kingdom. Electronic address: s.j.tucker@abdn.ac.uk.</auth-address><titles><title>POPDC1 is suppressed in human breast cancer tissues and is negatively regulated by EGFR in breast cancer cell lines</title><secondary-title>Cancer Lett</secondary-title></titles><periodical><full-title>Cancer Lett</full-title><pages>81-92</pages><volume>406</volume><keywords>Egfr</keywords><keywords>Her2</keywords><keywords>Popdc1</keywords><keywords>Popdc2</keywords><keywords>Popdc3</keywords><dates><year>2017</year><date>Oct 10</date></dates><isbn>1872-7980 (Electronic)&#xD;xD;0304-3835 (Linking)</isbn><accession-num>28807821</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/28807821</url></related-urls></urls><electronic-resource-num>10.1016/j.canlet.2017.08.002</electronic-resource-num></record></Cite></EndNote>}. Addition of EGF causes a repression of POPDC genes. However, POPDC2 differs from POPDC1 and -3 in kinetic and extent of suppression. Interestingly, also in cardiac myocytes, POPDC gene expression is regulated by EGF signalling { ADDIN EN.CITE { ADDIN EN.CITE.DATAD } }. Expression of all three vertebrate POPDC genes is lost, when cardiac myocytes were cultured in the presence of the EGF receptor antagonist tyrphostin. The EGF-like signalling molecule neuregulin is secreted by endocardial cells and responsible for the formation of the trabecular layer in the
developing ventricle {ADDIN \ EN.CITE \ {ADDIN \ EN.CITE.DATA }}. Neuregulin signalling suppresses apico-basal polarity and thereby myocytes destined to participate in trabecular layer formation acquire a mesenchymal-like state and leave the myocardial wall by a process reminiscent of EMT in epithelia. Possibly neuregulin signalling is responsible for the low level of Popdc1 expression in the trabecular layer in the midgestation mouse heart {ADDIN \ \ EN.CITE \ \ {ADDIN \ \ EN.CITE.DATA }}.

4.2 Popdc1 expression is suppressed by Netrin

Netrin-1, a laminin-related neuronal guidance molecule, which is also expressed in non-neural cell types, is a negative regulator of POPDC1 gene expression {ADDIN \ \ EN.CITE \ \ {ADDIN \ \ EN.CITE.DATA }}. Netrin-1 promotes cell migration and invasion by downregulation of BVES expression in human hepatocellular carcinoma {ADDIN \ \ EN.CITE \ \ {ADDIN \ \ EN.CITE.DATA }}. Netrin-1 modulates POPDC1 expression through PI3-kinase/AKT signalling and a specific antagonist of this signalling pathway is able to re-establish POPDC1 expression in the presence of Netrin-1. In several cancer types, gain of Netrin 1 expression has been observed, which may lead to tumour progression and possibly is associated with the suppression of POPDC1 expression.

4.3 Transcriptional regulation of POPDC genes
Apart from methylation little is known about transcriptional regulation of POPDC gene expression. No systematic study of the regulatory elements involved in the transcriptional regulation of Popdc1 and Popdc3 expression has been performed. However, it has been proposed that Popdc1 might be a target of Pax3 {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. Popdc2 is the isoform with highest level of expression in the heart {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. The promoter of the Popdc2 gene has recently been characterized and found to contain a binding site for the NK homeobox protein 2.5 (Nkx2.5), which overlaps with a binding site for Meis homeobox protein 1 (Meis1) {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. The Nkx2.5 binding site is part of a compact promoter, which is sufficient to drive cardiac expression in zebrafish and mouse embryos. Chromatin immunoprecipitation experiments using the mouse Popdc2 promoter revealed that Nkx2.5 occupied the binding site in differentiated cardiac myocytes, whereas Meis1 is present in anterior heart field mesoderm {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}.

5. Working Models of POPDC function

5.1 The switch model

Over the years several working models have been proposed to describe the functions of POPDC proteins. The first model that was proposed is the switch model (Fig. 3A) {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. This model proposes that interacting proteins are forming a complex with POPDC proteins. Such protein complexes could be demonstrated by a number of techniques such as pull-down, immunoprecipitation or FRET experiments and one such protein is TREK1 for which interaction with POPDC1 has been demonstrated by each of the aforementioned techniques {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. Upon binding of cAMP, it is likely that the Popeye domain alters its conformation as has been reported for all other cAMP binding proteins {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}.
Cyclic nucleotide binding may affect protein-protein interaction, which may also have an impact on the functionality of the interacting protein. Support for this model comes from FRET experiments using POPDC1 and TREK-1. The POPDC1/TREK-1 FRET signal is modulated upon raising cAMP levels. However, direct measurements of TREK-1 current in *Xenopus* oocytes, which were acutely stimulated with 8-Br-cAMP did not show any difference between agonist versus vehicle-treated oocytes. This negative outcome does not rule out the possibility that other proteins might be directly affected by the binding of cAMP to POPDC proteins.

5.2 The cargo model

The *cargo model* (Fig. 3B) describes a role of POPDC proteins in membrane trafficking of interacting proteins and this model is based on the finding that the membrane localization of TREK-1 in *Xenopus* oocytes is doubled if any of the POPDC proteins.
three POPDC isoforms are present. Moreover, in patients carrying the POPDC1<sup>S201F</sup> mutation and the corresponding zebrafish mutant display aberrant membrane localization of the mutant protein but also of POPDC2. POPDC proteins are not only found at the plasma membrane but are also present in vesicular structures in the sarcoplasm. While these vesicles haven't yet been further characterized, it can be envisioned that they are part of the recycling vesicle compartment. Possibly, cAMP alters the interaction of POPDC proteins with the transport machinery involved in vesicle transport. Moreover, as mentioned before, POPDC proteins interact with the SNARE protein VAMP3, which controls vesicle fusion with the plasma membrane. Thus, it is possible that in response to cAMP binding to POPDC proteins, vesicles are more likely to fuse with the plasma membrane resulting in an enhanced incorporation of the vesicle proteins into the plasma membrane.

5.3 The shielding model

The shielding model (Fig. 3C) is a variation of the switch model taking into account that POPDC proteins and also some of the interacting proteins are getting phosphorylated in response to βAR activation. Lundby et al. (2017) The new kids on the block
It can be envisioned that cAMP binding and phosphorylation of POPDC proteins may lead to conformational changes that affect access of PKA or other kinases to their substrates. Support for such a hypothetical mechanism stems from the electrophysiological measurement of TREK1 current in *Xenopus* oocytes, which is inhibited by cAMP and therefore blunting the effect of co-expression of POPDC proteins. However, TREK-1 current maintained an increased level when the same experiment was performed in the presence of a cAMP binding-deficient POPDC1 mutant. These results may indicate that POPDC proteins form a complex with TREK-1, which may prevent PKA from gaining access and being able to phosphorylate TREK-1.

### 5.4 The sponge model

Finally, the sponge model (Fig. 3D) takes into account that POPDC proteins are abundantly present in the cardiac and skeletal muscle proteome. Assuming a high affinity for cAMP in the range of PKA, which however has thus far only been experimentally determined in case of POPDC1, suggests that a lot of the cellular cyclic nucleotide binding capacity is attributable to POPDC proteins. The sponge model also predicts that in case of a loss of function mutation such as POPDC1S201F, which reduces cAMP binding affinity by 50% and also alters the subcellular localization of the mutant protein, or a complete loss of protein expression as in case of the POPDC null mutants, should affect the diffusion rates and compartmentalization of cAMP. In this regard it is noteworthy that recent experimental and computational modelling approaches concluded that although the subcellular localization of adenylate cyclases and phosphodiesterases are important for the creation of cAMP nanodomains, they are by far not sufficient. Several studies have suggested that compartmentalised cAMP signalling requires a slower diffusion rate of cAMP.
6. Outlook

As in any other novel scientific field, many open questions remain to be addressed and it is the firm belief of the authors of this review that we currently are just at the beginning of our understanding of the role of POPDC proteins in cAMP signalling. An important question that needs to be answered is, whether POPDC proteins are directly interacting with other proteins of the cAMP signalling pathway and whether POPDC proteins modulate the enzymatic activity of these proteins. Thus, we need to address the question, whether POPDC proteins do physically interact with adenylate cyclases or phosphodiesterases. Equally important will be to answer the question, whether there are AKAP proteins, which bind to POPDC proteins. A number of AKAP proteins are localized to the same
compartments in cardiac myocytes as POPDC proteins and these candidates need to be tested. It remains to be determined whether PKA or EPAC isoforms show a physical interaction with POPDC proteins and whether interaction may affect their enzymatic activity. It has been shown that POPDC proteins are phosphorylated in response to βAR-dependent phosphorylation. However, whether POPDC proteins and PKA are part of the same signalosome is currently not known. POPDC proteins and HCN channels both bind cAMP and are involved in cardiac pacemaking. Nonetheless, no evidence has been obtained so far for a direct interaction of POPDC1 with HCN4 (the most abundant HCN isoform in the SAN). Patch clamp analysis of I_f in SAN myocytes of Popdc1 null mutants revealed that I_f was not affected at baseline or after βAR stimulation. Another important area to look further into is the recent finding of cell proliferation control in cancer cells. In skeletal muscle, the lack of Popdc1 affects

Brand T. et al. (2017) The new kids on the block
the ability of muscle regeneration {ADDIN EN.CITE
<EndNote><Cite><Author>Andrée</Author><Year>2002</Year><RecNum>2686</RecNum><DisplayText>[60]</DisplayText><record><rec-number>2686</rec-number><foreign-keys><key app="EN" db-id="rt59wpfsdprwves59gpr5fwafzf2rxaa0ft" timestamp="0">2686</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Andrée, B.</author><author>Fleige, A.</author><author>Arnold, H. H.</author><author>Brand, T.</author></authors></contributors><titles><title>Mouse Pop1 is required for muscle regeneration in adult skeletal muscle</title><secondary-title>Mol Cell Biol</secondary-title></titles><periodical><full-title>Mol Cell Biol</full-title><abbr-1>Molecular and cellular biology</abbr-1></periodical><pages>1504-12.</pages><volume>22</volume><number>5</number><dates><year>2002</year></dates><label>21829279</label><urls></urls></record></Cite></EndNote>}. However, currently nothing is known about the role of POPDC genes in cardiac regeneration.

Likewise, we do not understand how POPDC proteins mechanistically affect membrane trafficking of interacting proteins. Thus, the interaction of POPDC proteins with proteins involved in vesicle transport and membrane protein recycling will be an important avenue to be investigated. Electrophysiological defects have been observed in the heart of patients carrying POPDC1 mutations, in contrast structural defects were observed in case of skeletal muscle. It will be interesting to find out, whether mutations in POPDC genes are also able to cause structural defects in the heart such as dilated cardiomyopathy and likewise, whether skeletal muscle could also be functionally affected. We will also need to screen patient populations for the presence of mutations in any of the other POPDC isoforms, given their overlapping expression and function. It is likely that we might find mutations also in these family members.

Currently, no specific drugs are available to activate or inhibit POPDC protein function. Thus, functional analysis is solely dependent on the genetic approach of generating gain- or loss-of-function mutations. Over the years several specific agonists and antagonists have been characterized for PKA, EPAC and HCN channels {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}. None of these compounds have made it into the clinics, however, they are very useful as scientific tools allowing isoform-specific modulation of different effector proteins. At present, similar tools are not available in case of the POPDC protein family. Given the unique sequence of the Popeye domain and in particular of the cyclic nucleotide binding cassette {ADDIN EN.CITE {ADDIN EN.CITE.DATA}}, which does not resemble any other cAMP binding domains, it is very likely that specific small molecules could be defined, which will help to analyse the functional involvement of POPDC proteins in different aspects of cAMP signalling. Many more problems will need to be addressed to find out what the new kids on the block are up to.

Acknowledgements

{PAGE}
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References
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Figure 1. The cAMP signalling pathway in cardiac myocytes. In response to adrenergic stimulation via β1- or β2- adrenergic receptors (β1AR, β2AR), cAMP is synthesized by adenylyl cyclases (AC) and degraded by phosphodiesterases (PDEs). The effector proteins protein kinase A (PKA) and exchange protein activated by cAMP (EPAC) are part of signalosomes, which are organized by A protein kinase associated proteins (AKAPs). Target proteins, which are getting phosphorylated by PKA are often part of AKAP-dependent signalosomes. Popeye domain containing (POPDC) proteins are localized to many different subcellular compartments, some of which are also shared by other cAMP binding proteins. It is unknown whether POPDC proteins are part of the same signalosome complex as other cAMP effector proteins. Like PKA and EPAC proteins, POPDC proteins are also found in the nucleoplasm probably lacking the transmembrane domains (ΔTM-POPDC).
Figure 2. Structural modelling of the Popeye domain containing proteins. (A) Secondary structure prediction of POPDC proteins using Psipred [108]. In each isoform, a short (26-47 residues) extracellular domain (ECD) is present, which harbors one or two N-glycosylation motifs (asterisks) followed by three transmembrane domains (TM1-3). A large part of the cytoplasmic domain consists of the Popeye domain, which is close to 50% similar between family members. The carboxy-terminal domain (CTD) is most variable part of the POPDC proteins and distinct between isoforms. (B) 3-D models of POPDC1, POPDC2, and POPDC3. The models were produced with the help of the Phyre 2 web portal, which predicts 3-D protein structures on the basis of homology modelling
The structures were visualized using the CCP4 molecular graphics program. The new kids on the block
(C,D) Demonstration of evolutionary conservation of the Popeye domain with the help of the Consurf program. (C) Cartoon and (D) Spacefill model of the Popeye domain of human. Many invariant amino acids cluster around the PBC or are part of the lid, which is predicted to fold over the PBC when ligand is bound.
Figure 3. Different models of POPDC protein function. (A) Switch model. (B) Cargo model. (C) Shielding model. (D) Sponge model. See text for a further description and the experimental evidence supporting these different models of POPDC protein function.