AKAP SIGNALLING COMPLEXES: FOCAL POINTS IN SPACE AND TIME

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Abstract | Multiprotein signalling networks create focal points of enzyme activity that disseminate the intracellular action of many hormones and neurotransmitters. Accordingly, the spatio-temporal activation of protein kinases and phosphatases is an important factor in controlling where and when phosphorylation events occur. Anchoring proteins provide a molecular framework that orients these enzymes towards selected substrates. A-kinase anchoring proteins (AKAPs) are signal-organizing molecules that compartmentalize various enzymes that are regulated by second messengers.

SCAFFOLD PROTEINS
Proteins that augment cellular responses by recruiting other proteins to a complex/scaffold. They usually contain several protein–protein-interaction domains.

G-PROTEIN-COUPLED RECEPTOR
(GPCR). A seven-helix transmembrane-spanning cell-surface receptor that signals through heterotrimeric GTP-binding and -hydrolysing G-proteins to stimulate or inhibit the activity of a downstream enzyme.

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Where and when enzymes become active have profound implications on the cellular processes that they control. Consequently, sophisticated molecular mechanisms have evolved to regulate the spatial and temporal organization of signal-transduction pathways. SCAFFOLD and anchoring proteins target protein kinases and phosphatases to distinct subcellular environments where these enzymes control the phosphorylation state of neighbouring substrates. The movement of enzymes into and out of these complexes contributes to the temporal regulation of signalling events. Prototypical examples of these signal-organizing molecules are the A-kinase anchoring proteins (AKAPs), which compartmentalize the cyclic-AMP-dependent protein kinase (protein kinase A (PKA)) and other enzymes. So, although AKAPs coordinate cAMP-responsive events, they also integrate and disseminate other intracellular signals. Here, we discuss two emerging principles in AKAP signalling: the combinatorial assembly of different enzymes on the same AKAP backbone; and the dynamic reorganization of AKAP complexes.

cAMP signalling pathways

cAMP was identified over 50 years ago as a diffusible intracellular second messenger that is produced in response to hormone action[1]. We now know that the release of cAMP into the cytoplasm is initiated by the occupancy of G-PROTEIN-COUPLED RECEPTORS (GPCRs) at the plasma membrane by several different ligands, including adrenocorticotropin, glucagon and adrenaline[2]. The ligand-bound GPCR catalyses the exchange of GDP for GTP on the α-subunit of the associated HETEROTRIMERIC G PROTEIN, which results in the activation of the α-subunit and its dissociation from the βγ-dimer[3] (FIG. 1). Both the α- and βγ-subunits can then initiate or inhibit distinct intracellular signalling cascades. The α-subunit of the G_s subtype activates adenylyl cyclase, which converts ATP to cAMP[4] (FIG. 1). GPCR-mediated downstream signalling is terminated by the intrinsic GTPase activity of the α-subunit, which hydrolyses GTP to GDP[3]. The net effect is the intracellular generation of cAMP at points that emanate from the plasma membrane.

cAMP was initially considered to be a second messenger that diffused freely throughout the cell with a theoretical range-of-action of 220 μm (REF. 5). However, advances in live-cell imaging have visualized gradients, rather than a uniform intracellular distribution, of cAMP, which indicates that this second messenger accumulates at specific sites within cells[6–8]. Several proteins, such as CYCLIC-NUCLEOTIDE-GATED CHANNELS[9], PHOSPHODIESTERASES[10] and guanine nucleotide-exchange proteins activated by cAMP (EPACs)[11], bind to and are activated by cAMP. The localized activation of such cAMP-binding proteins would therefore enable this ubiquitous second messenger to be used to propagate diverse responses.

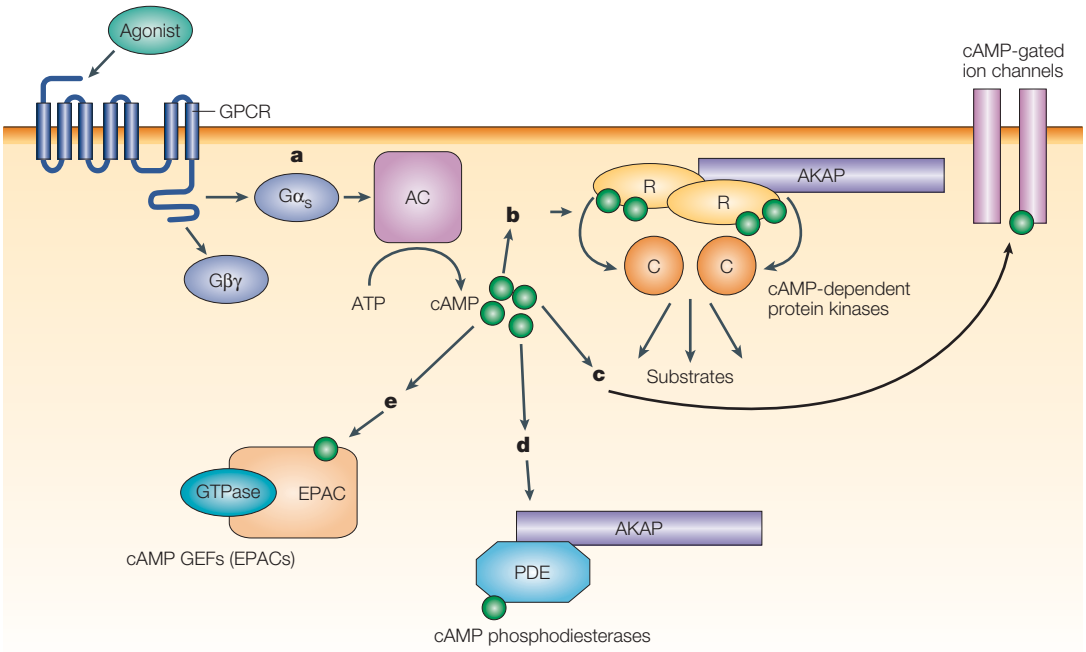

Figure 1 | **Cyclic AMP signalling pathways.** Agonist stimulation of (**a**) $G\alpha_s$-containing G-protein-coupled receptors (GPCRs) activates adenylyl cyclase (AC), which catalyses the synthesis of cyclic AMP from ATP[2,3]. cAMP binds to the regulatory (R) subunits of protein kinase A (PKA; **b**), which causes a conformational change that releases the active catalytic (C) subunit. The catalytic subunit of PKA can then phosphorylate nearby substrates[13–15]. PKA represents the main intracellular effector of cAMP signalling. However, cAMP can also activate nucleotide-gated ion channels[9] (**c**), phosphodiesterases (PDEs) such as PDE4 (REF. 10) (**d**), and guanine nucleotide-exchange factors (GEFs) that are known as exchange proteins activated by cAMP (EPACs; **e**)[11]. AKAP, A-kinase anchoring protein.

HETEROTRIMERIC G PROTEIN
A protein complex of three proteins (Gα, Gβ and Gγ) Whereas Gβ and Gγ form a tight complex, Gα is part of the complex in its inactive, GDP-bound, form but dissociates in its active, GTP-bound, form. Both Gα and Gβγ can transmit downstream signals after activation.

CYCLIC-NUCLEOTIDE-GATED CHANNEL
(CNGC). Conserved protein family with six predicted transmembrane helices that can form cation-conducting channels and is activated by the binding of cyclic nucleotides such as cAMP and cGMP.

PHOSPHODIESTERASES
Enzymes that can hydrolyse cAMP to 5′-AMP.

HOLOENZYME
An enzyme that consists of more than one subunit, each of which usually carries out a different function. Holoenzymes often exist as more than one isoform.

However, the principal intracellular target for cAMP is PKA[12]. The PKA HOLOENZYME is a tetramer that consists of two catalytic (C) subunits that are held in an inactive conformation by a regulatory (R) subunit dimer[13–15]. There are two PKA subtypes: the type-I holoenzyme contains RI subunits, whereas the type-II holoenzyme consists of RII subunits[16,17]. For both PKA subtypes, the holoenzyme dissociates when cAMP binds to the R subunits, thereby releasing the active C subunits (FIG. 1). The C subunits are broad-spectrum serine/threonine kinases that could potentially target numerous proteins, so signalling specificity is achieved through compartmentalization of the PKA holoenzyme[18]. Accordingly, AKAPs target PKA to distinct subcellular locations to position the enzyme at regions of cAMP production and to confine phosphorylation to only a subset of potential substrates[19].

Properties of AKAPs

The first AKAP to be characterized was microtubule-associated protein-2 (MAP2; FIG. 2), which was initially identified because it co-purified with RII from brain extract[20]. The AKAP family has subsequently grown to include more than 50 structurally diverse, but functionally similar, members that are classified by their ability to co-purify with PKA catalytic activity from tissues. Despite their diversity, AKAP orthologues have been identified in a range of species, including fission yeast, nematode worms, fruitflies, mice and humans. All AKAPs share certain common properties: first, they contain a PKA-anchoring domain; second, they have unique localization signals; and third, they have the ability to form complexes with other signalling molecules. Each of these functions will be discussed below. At present, the AKAP nomenclature generally uses molecular weight to denote each anchoring protein (as outlined in TABLE 1). However, this method of classification is cumbersome as there are several splice variants of the same AKAP that have different molecular weights. Furthermore, other anchoring proteins — such as gravin, pericentrin and Rab32 — were named before it was realized that they bind PKA. We have attempted to present this information logically in TABLE 1.

PKA-anchoring domain. AKAPs contain an amphipathic helix of 14–18 residues, which binds to the N-terminal dimerization domain of the R subunit of PKA[21–23]. The NMR solution structure of such a complex has revealed that the hydrophobic face of the AKAP amphipathic helix lies across the four-helix bundle that is formed by the R-subunit dimer, thereby allowing extensive hydrophobic interactions between both proteins[24]. Although most of the AKAPs that have so far been identified bind to RII (REF. 19), several RI-specific AKAPs have been characterized[25]. There are also dual-affinity AKAPs such as D-AKAP1 (REF. 26) and D-AKAP2 (REF. 27), which can anchor both types of R subunit.

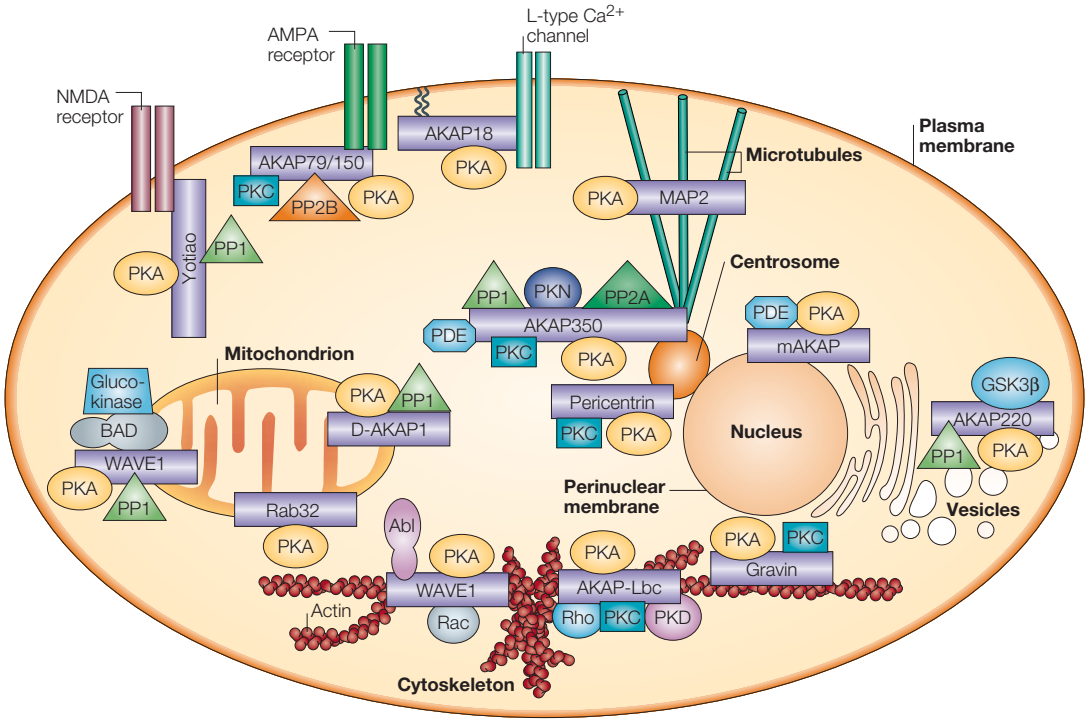

Figure 2 | **AKAP signalling complexes create focal points for signal transduction.** A-kinase anchoring proteins (AKAPs) target protein kinase A (PKA) to specific compartments, including the plasma membrane, mitochondria, cytoskeleton and centrosome[19]. Within a compartment, the same AKAP can associate with different substrates, as shown by the interaction of AKAP79/150 with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptors[77] and the KCNQ2 (K^+ voltage-gated channel, subfamily Q, member-2) channels[94] at the plasma membrane. Alternatively, different AKAPs within the same compartment can assemble distinct signalling complexes, as illustrated by the AKAP18 and AKAP79 complexes. In addition to binding to PKA, AKAPs can interact with other signalling molecules. One category includes signal-termination enzymes such as phosphatases that counterbalance kinase activity, or phosphodiesterases (PDEs) that degrade cyclic AMP and limit PKA activation. Protein phosphatase-1 (PP1) is found in complexes that are organized by yotiao[39], Wiskott–Aldrich verprolin-homology protein-1 (WAVE1)[36], AKAP220 (REF. 57) and D-AKAP1 (REF. 125); PP2A is contained within the AKAP350 complex[54]; and PP2B binds to AKAP79 (REF. 46). Muscle-specific AKAP (mAKAP)[97] and AKAP350 (REF. 107) have been shown to associate with a cAMP-specific phosphodiesterase, PDE4D3. AKAPs also interact with kinases and enzymes from other signalling pathways. For example, protein kinase C (PKC) interacts with AKAP79 (REF. 45), AKAP350 (REF. 55), AKAP-Lbc[108], gravin[53] and pericentrin[126]; protein kinase N (PKN) interacts with AKAP79 (REF. 45); protein kinase D (PKD) interacts with AKAP-Lbc[108]; Rho interacts with AKAP-Lbc[63]; and glycogen synthase kinase-3β (GSK3β) interacts with AKAP220 (REF. 58). WAVE1 organizes two compartment-specific signalling complexes. At the cytoskeleton, the binding partners of WAVE1 include Rac (REF. 61), WAVE-associated Rac-GTPase activating protein (WRP; REF. 61) and Abelson kinase (Abl; REF. 60). By contrast, the binding partners of WAVE1 at the mitochondrion are BCL2-antagonist of cell death (BAD) and glucokinase[36]. MAP2, microtubule-associated protein-2; NMDA, *N*-methyl D-aspartate.

Ectopic expression of the amphipathic helix can inhibit interactions between AKAPs and R subunits, as shown by the Ht31 peptide, which is a fragment of AKAP-Lbc that encompasses its RII-binding domain[21,28]. Bioinformatic design of an optimal RII-anchoring antagonist has generated a 17-residue peptide that is known as AKAP-*IS*, which binds to RII with greater affinity than Ht31 and can disrupt PKA anchoring inside cells[29]. An RI-selective anchoring antagonist has also been prepared that shows a 100-fold preference for RI over RII (REF. 30). These isoform-specific peptide inhibitors of AKAP–PKA interactions will allow discrimination between cellular events that are mediated by PKA types I and II.

Localization signals. Compartmentalization of individual AKAP–PKA units occurs through specialized targeting domains that are present on each anchoring protein (FIG. 2). As data have accumulated on a plethora of AKAP-targeting mechanisms, two general trends have emerged: first, several AKAPs can be targeted to the same subcellular compartment; and second, splice variants that arise from the same *AKAP* gene can be differentially targeted. For example, AKAP79/150 is anchored to the plasma membrane through sequences that bind phospholipids[31]. Gravin (which is also known as AKAP250; see TABLE 1) is also targeted to the plasma membrane, but it is thought to require an N-terminal myristoyl group as well as phospholipid-binding sequences[32]. The α- and β-isoforms of AKAP18, however, are recruited to membranes through myristoyl and dual palmitoyl groups[33] (FIG. 2). These subtle differences in membrane-targeting mechanisms could therefore direct each AKAP to distinct compartments

Table 1 | **Summary of AKAP nomenclature and potential binding partners***

Common name	Alternate names or splice variants	Proteins in complex	References
D-AKAP1	sAKAP84 AKAP121 AKAP149 AKAP1‡	Protein kinase A (PKA) Protein phosphatase-1 (PP1)	26 125
AKAP150 (rat/mouse)	AKAP75 (bovine) AKAP79 (human) AKAP5‡	PKA Protein kinase C (PKC) PP2B *N*-methyl D-aspartate (NMDA) receptor α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor Postsynaptic protein of 95 kDa (PSD-95) Synapse-associated protein-97 (SAP97) KCNQ2 channel L-type voltage-gated Ca^{2+} channels Aquaporin water channels	43,44,129 45 46 77 77 77 77 94 50 51
mAKAP	AKAP6‡	PKA Phosphodiesterase 4D3 (PDE4D3)	128 97
AKAP18	AKAP15 AKAP7‡	PKA Voltage-gated Ca^{2+} (Ca_V) channels Voltage-gated Na^+ (Na_V) channels	42 42,95 96
AKAP350	Yotiao AKAP450 CG-NAP AKAP9‡	*Yotiao:* PKA PP1 NMDA receptor KCNQ1 channel Inositol-1,4,5-trisphosphate receptor *AKAP350:* PKA Protein kinase Cε (PKCε) Protein kinase N (PKN) Casein kinase-1 (CK1) PP2A Intracellular Cl^- channels (CLIC) γ-tubulin-complex protein-2 and -3 (GCP2, GCP3) *AKAP450:* PDE4D3	 39 39 39 70 71 40 55 54 56 54 73 74 107
D-AKAP2	AKAP10‡	PKA	27
AKAP220	AKAP11‡	PKA PP1 Glycogen synthase-3β (GSK3β)	57 57 58
Gravin	AKAP250 SSeCKS (mouse) AKAP12‡	PKA PKC β_2-adrenergic receptor (β_2-AR)	53 53 64
AKAP-Lbc	AKAP13‡	PKA Rho 14-3-3 PKC Protein kinase D (PKD)	63 63 101,102 108 108
Microtubule-associated protein-2 (MAP2)		PKA Tubulin	20 130
Pericentrin		PKA PKC	37 126
WAVE1	SCAR (suppressor of cAMP receptor)	*Cytoskeleton:* PKA, Abl Rac WAVE-associated Rac-GAP protein (WRP) Actin-related protein-2/3 (Arp2/3) Abl interactor-1 (Abi1) p53-inducible messenger RNA-121 (PIR121) NCK-associated protein-125 (NAP125) *Mitochondria:* PKA, PP1, Glucokinase and BAD	 60 59 61 62 66 66 66 36

*This table lists alternative names for the AKAPs that are shown in FIG. 2 and described in the text. ‡Highlights the Gene Nomenclature Committee name. Potential binding partners, which are sorted according to splice variants or subcellular localization where applicable, are also given in this table. Owing to space constraints, only the binding partners that appear in FIG. 2 and/or the text are listed here. AKAP, A-kinase anchoring protein; BAD, BCL2-antagonist of cell death; BCL2, B-cell lymphoma-2; GAP, GTPase-activating protein; KCNQ1/2, K^+ voltage-gated channel, subfamily Q, member-1/2; WAVE, Wiskott–Aldrich verprolin-homology protein-1.

of the plasma membrane. A similar situation might arise on the surface of mitochondria. D-AKAP1 is targeted here through a conventional mitochondrial-targeting sequence[34]. By contrast, Rab32 and Wiskott–Aldrich verprolin-homology protein-1 (WAVE1) are attached by the prenylation of cysteine residues at the C terminus[35] and interaction with BCL2-antagonist of cell death (BAD)[36], respectively (FIG. 2). Interestingly, pericentrin and AKAP350/450/CG-NAP (hereafter collectively referred to as AKAP350) achieve targeting to the CENTROSOME through a conserved protein-interaction module that, appropriately, is known as the pericentrin-AKAP350 centrosomal targeting (PACT) domain (FIG. 2)[37,38]. Although pericentrin and AKAP350 both contain the same targeting domain, they share little homology otherwise, and are therefore likely to drive PKA-mediated phosphorylation of different substrates at the centrosome.

In other instances, the gene for a single anchoring protein might encode a number of targeting sequences that can be used to differentially target AKAP variants. For example, protein–protein interactions direct the so-called yotiao splice variant of AKAP350 to synaptic membranes, where it binds to the cytoplasmic tail of the NR1 subunit of the *N*-methyl D-aspartate (NMDA) receptor[39], whereas longer isoforms (AKAP350, AKAP450 and CG-NAP) are tethered to the centrosome through the C-terminal PACT domain[38,40,41] (FIG. 2). Similarly, the *AKAP18* mRNA undergoes alternative splicing to yield at least three isoforms (α, β and γ) that have distinct subcellular localizations: the α-variant accumulates at the lateral membrane of polarized epithelial cells; the β-variant is trafficked to the apical membrane of polarized epithelial cells; and the γ-variant shows a largely cytoplasmic distribution[33,42]. In contrast to the α- and β-variants, the N terminus of the γ-isoform lacks the lipid modification sequence, which therefore accounts for its cytoplasmic distribution[33]. So, differential targeting of PKA–AKAP complexes provides a molecular framework to sustain tightly localized pools of the kinase.

Other signalling molecules in AKAP complexes. Perhaps the most biologically significant property of AKAPs is their ability to colocalize PKA with other signalling enzymes. These multivalent anchoring proteins often form complexes that include enzymes for both signal transduction and signal termination, which therefore generates a locus to regulate the forward and backward steps of a given signalling process. The idea of multivalent anchoring proteins was first proposed for the AKAP79 family, which comprises a group of three structurally similar orthologues: human AKAP79, murine AKAP150 and bovine AKAP75 (henceforth referred to as AKAP79/150)[43,44]. AKAP79/150 is enriched in the POSTSYNAPTIC-DENSITY fractions of neuronal lysates and is present in the DENDRITIC SPINES of neurons[44]. In 1995, AKAP79 was shown by yeast two-hybrid assay to interact with the A subunit of the Ca^{2+}/calmodulin-dependent phosphatase PP2B[45]. One year later, protein kinase C (PKC) was also found to be a component of the AKAP79/150 signalling complex[46]. At that time, the simultaneous anchoring of these three signalling enzymes — PKA, PP2B and PKC — was thought to generate a locus for the integration of distinct second-messenger signals at postsynaptic membranes[47,48]. Functional studies have largely confirmed this idea by showing that the AKAP79/150 signalling complex controls the phosphorylation status and facilitates the regulation of various ion channels, including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA)- and NMDA-type glutamate receptors, L-type Ca^{2+} channels, M-type K^+ channels and aquaporin water channels[49–52].

Subsequently, other AKAP signalling complexes have been defined. These include gravin, which targets PKA and PKC to the NEUROMUSCULAR JUNCTION[53]; AKAP350, which organizes PKA, PKCε, the Rho-activated protein kinase N (PKN), casein kinase-1 and the protein phosphatases PP1 and PP2A[54–56]; and AKAP220, which assembles a complex containing PKA, PP1 and glycogen synthase kinase-3β (GSK3β), a serine/threonine kinase from the Wnt pathway[57,58] (FIG. 2). Other AKAP complexes mediate various cytoskeletal events. WAVE1 binds several enzymes in addition to PKA, including the Abelson (Abl) tyrosine kinase, the GTPase Rac, the GTPase-ACTIVATING PROTEIN (GAP) WAVE-associated Rac-GAP protein (WRP), and the Arp2/3 COMPLEX[59–61] (FIG. 2). The dynamic assembly of this complex at sites from which LAMELLIPODIA extend occurs in response to growth-factor signals that activate Rac[62]. Analogous signalling networks participate in the formation of actin STRESS FIBRES, as AKAP-Lbc, which is a longer transcript of the *Lbc* oncogene, anchors PKA and functions as a Rho-selective GUANINE NUCLEOTIDE-EXCHANGE FACTOR (GEF)[63]. Activation of AKAP-Lbc and subsequent activation of Rho leads to the formation of stress fibres in fibroblasts[63] (FIG. 2).

Each of the examples that have been cited above indicates that certain AKAPs can provide a molecular platform to allow the convergence of signalling pathways. There can be crosstalk between these pathways — for instance, PKA and PKC can phosphorylate the same substrates within the complex that is formed between gravin and the β_2-adrenergic receptor (β_2-AR) complex (a GPCR that is activated by the hormone adrenaline)[64,65]. By contrast, enzymes in the WAVE1 complex can process several signals in parallel, as PKA seems to operate independently of the other enzymes contained in this complex. This latter model indicates that the intracellular terrain might be composed of discrete pools of anchored kinases and phosphatases that are surrounded by select substrates. If this is the case, each constellation of enzymes must be positioned to efficiently receive activation signals from other parts of the cell.

Combinatorial assembly of AKAP complexes

As the list of binding partners for AKAPs increases (see TABLE 1 for some potential binding partners), it is apparent that a single anchoring protein can interact with only a subset of its possible interacting proteins

CG-NAP
Centrosome-and-Golgi-localized protein-kinase-N-associated protein.

CENTROSOME
The main microtubule-organizing centre of animal cells.

POSTSYNAPTIC DENSITY (PSD). A multiprotein complex that contains the membrane, regulatory and scaffolding proteins that are required for efficient synaptic signalling in the postsynaptic neuron. It is particularly enriched in cytoskeletal proteins, which renders this complex resistant to solubilization by non-ionic detergents. In electron-microscopy preparations, this structure appears as an electron-dense region on the cytoplasmic face of the postsynaptic membrane.

DENDRITIC SPINES
Knob-like extensions of the dendritic surface which can receive synaptic input. The actin cytoskeleton within these structures undergoes constant remodelling, thereby giving rise to dynamic changes in the shape of dendritic spines.

NEUROMUSCULAR JUNCTION (NMJ). The place of contact between the terminal of a motor neuron and the membrane of a muscle fibre. Nerve impulses are transmitted across the gap by diffusion of a transmitter.

GTPase-ACTIVATING PROTEIN (GAP). Proteins that inactivate small GTP-binding proteins, such as Ras-family members, by increasing their rate of GTP hydrolysis.

Arp2/3 COMPLEX
A complex that consists of two actin-related proteins, Arp2 and Arp3, along with five smaller proteins. When activated, the Arp2/3 complex binds to the side of an existing actin filament and nucleates the assembly of a new actin filament. The resulting branch structure is Y-shaped.

LAMELLIPODIA
Thin, flat extensions at the cell periphery that are filled with a branching meshwork of actin filaments.

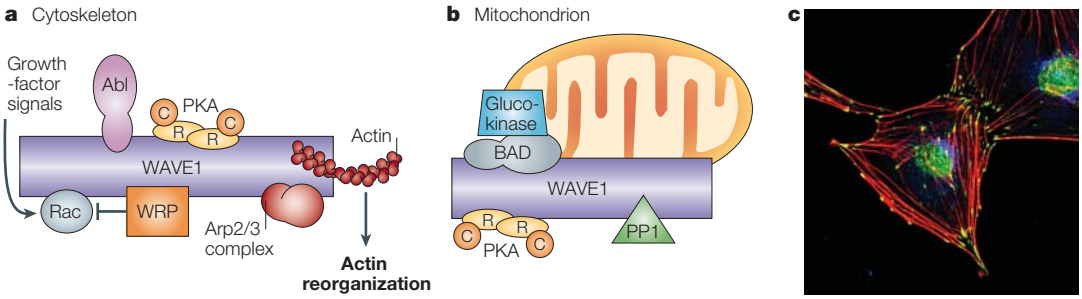

Figure 3 | **Tissue-specific AKAP complexes.** Wiskott–Aldrich verprolin-homology protein-1 (WAVE1) assembles signalling complexes in a tissue-specific manner. **a** | At neuronal growth cones, growth-factor signals stimulate WAVE1-bound Rac[59] and cause actin reorganization through activation of the actin-related protein-2/3 (Arp2/3) complex[62]. The WAVE-associated Rac-GTPase activating protein (WRP) opposes the activity of Rac[61]. The Abelson tyrosine kinase (Abl) is a known regulator of cytoskeletal dynamics. **b** | At mitochondria in hepatocytes, WAVE1-anchored protein kinase A (PKA; R and C denote the regulatory and catalytic subunits of PKA, respectively) phosphorylates BCL2-antagonist of cell death (BAD) to downregulate its apoptotic activity[36]. Dephosphorylation by the type-1 protein phosphatase (PP1) can reset this signalling pathway to basal conditions. In turn, the phosphorylation status of BAD affects glucokinase activity. **c** | Immunostaining of a Swiss 3T3 fibroblast shows WAVE (green) and the regulatory subunit (RII) of PKA (blue). The actin cytoskeleton has been labelled using Texas-Red-conjugated phalloidin. Areas of colocalization between WAVE and actin are indicated (yellow).

at any one time. Consequently, each anchoring protein has the potential to organize different enzyme combinations in a context-specific manner. This expands the repertoire of signals that can be processed through an AKAP complex.

Tissue-specific AKAP complexes. WAVE proteins (WAVE1, WAVE2 and WAVE3) coordinate different signalling complexes in both a tissue-specific and compartment-specific manner. Targeting of PKA is achieved through interaction with the WAVE1 isoform[60]. Proteomic approaches have identified numerous binding partners that are positive and negative regulators of WAVE function. WAVE1 activation requires Rac, although the precise mechanism is controversial. In one model, Rac mediates the release of an inhibitory complex that contains p53-inducible mRNA-121 (PIR121), NCK-associated protein-125 (NAP125) and Abl interactor-1 (Abi1)[66]. An alternative model proposes that Rac binding to PIR121 is the trigger for WAVE1 activation[67] (FIG. 3a). By contrast, it is clear that Rac signalling is terminated by the GAP activity of WRP[61]. Interestingly, the phenotype of WAVE1-knockout mice suggests that a direct link between WAVE1 and WRP is functionally important for sensorimotor and cognate behaviours[68]. A different proteomic approach using liver tissue has isolated WAVE1 as part of a mitochondrial complex that contains PKA, the type-1 protein phosphatase PP1, the pro-apoptotic protein BAD and the metabolic enzyme glucokinase[36] (FIG. 3b). This is consistent with previous findings that BAD activity is inhibited by PKA-mediated phosphorylation in response to growth and survival factors[69]. Furthermore, *Bad*-null mice are deficient in glucose homeostasis, which is consistent with the presence of glucokinase in the complex[36]. So, in one cellular context, WAVE1 assembles a neuronal signalling complex to control actin remodelling and neurite outgrowth but, in another cellular context, it packages enzymes that integrate the glycolytic and apoptotic pathways (FIG. 3a,b).

Compartment-specific AKAP complexes. Differential targeting of AKAP isoforms within the same cell provides another means to distribute PKA to compartment-specific substrates. For example, yotiao is associated with the plasma membrane, where it anchors PKA to surface proteins such as the NMDA receptor[39], the KCNQ1 (K^+ voltage-gated channel, subfamily Q, member-1) K^+ channel[70] and the inositol-1,4,5-trisphosphate receptor[71]. By contrast, the longer splice variants — AKAP350, AKAP450 and CG-NAP — are targeted to the Golgi apparatus[72] and the centrosome[40,41,54], where their binding partners include proteins that are unique to these compartments, such as intracellular Cl^- channels[73] and γ-tubulin-complex protein-2 and -3 (REF. 74).

A more sophisticated example of the combinatorial assembly of enzymes on the same AKAP backbone occurs at the postsynaptic densities of neurons where various AKAP79/150 complexes contribute to the regulation of synaptic ion channels (FIG. 4a–d). In this case, adjacent AKAP79/150 complexes assemble different enzyme combinations and are themselves recruited into different ion-channel protein networks.

AKAP-mediated cAMP-dependent phosphorylation events are crucial in controlling the activity and surface expression of AMPA-type glutamate receptors[75]. These channels are present at the terminals of excitatory synapses and are gated by the release of glutamate across the synaptic cleft[76]. In this context, a postsynaptic AKAP79/150 signalling complex that consists of PKA and PP2B is attached indirectly to the GluR1 subunit of the AMPA receptor[77]. Functional studies indicate that anchored PKA promotes basal phosphorylation of GluR1 on Ser845, whereas anchored PP2B dephosphorylates GluR1 at this site and causes downregulation of the current[78]. This Ser residue is an important site for the regulation of AMPA-receptor function during the induction of long-term synaptic depression, which is a molecular model for hippocampal learning and

STRESS FIBRE
Also known as 'actin microfilament bundles'. These are bundles of parallel filaments that contain F-actin and other contractile molecules, which often stretch between cell attachments as if under stress.

GUANINE NUCLEOTIDE-EXCHANGE FACTOR (GEF). A protein that facilitates the exchange of GDP (guanine diphosphate) for GTP (guanine triphosphate) in the nucleotide-binding pocket of a GTP-binding protein.

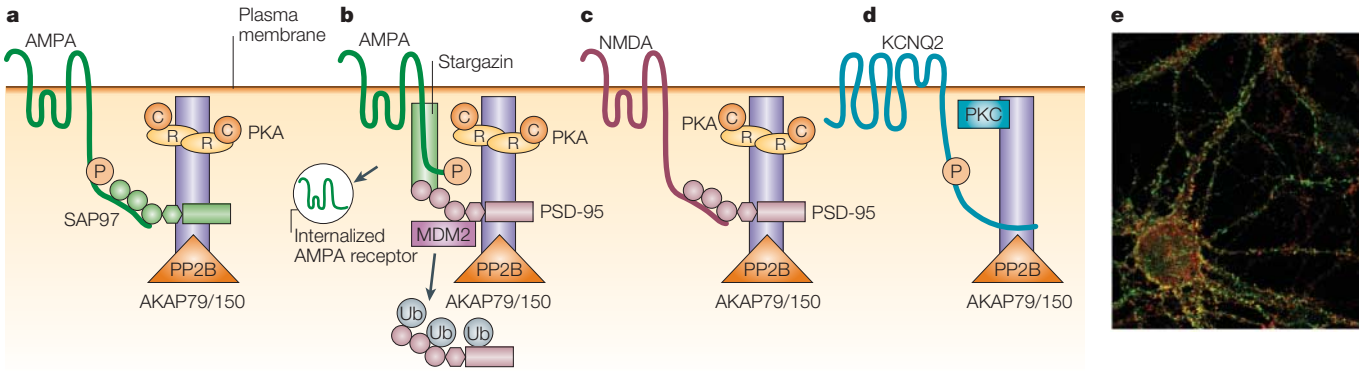

Figure 4 | **Substrate-specific AKAP complexes.** In neurons, AKAP79/150 assembles signalling complexes that have distinct complements of ion-channel substrates and kinases. **a** | Synapse-associated protein-97 (SAP97) can function as an adaptor between AKAP79/150 and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor[77]. This configuration brings AKAP79/150-anchored protein kinase A (PKA; R and C denote the regulatory and catalytic subunits of PKA, respectively) and protein phosphatase-2B (PP2B) into association with the substrate, the AMPA receptor. **b** | An alternative configuration that facilitates the PKA-mediated phosphorylation (P) of the AMPA receptor requires postsynaptic density protein of 95 kDa (PSD-95) and stargazin to function as a bridge between the AMPA receptor and the AKAP[127]. AKAP79/150-anchored PP2B activates the ubiquitin E3 ligase mouse double minute-2 (MDM2), which causes the ubiquitylation and degradation of PSD-95 (REF. 85). This ultimately results in internalization of the AMPA receptor. **c** | In addition, PSD-95 can link AKAP79/150 to *N*-methyl D-aspartate (NMDA) receptors[77]. **d** | AKAP79/150 interacts directly with the KCNQ2 channel and facilitates protein kinase C (PKC)-mediated phosphorylation of this channel[94]. **e** | A cultured neonatal rat hippocampal neuron that has been immunostained for AKAP150 (red) and PSD-95 (green). Areas of colocalization appear in yellow. KCNQ2, K^+ voltage-gated channel, subfamily Q, member-2.

memory acquisition[77–81]. The association is indirect, as postsynaptic density protein of 95 kDa (PSD-95), or synapse-associated protein-97 (SAP97), forms a protein bridge between AKAP79/150 and GluR1 (REF. 77) (FIGS 2b,4a). Not only do the two slightly different configurations (FIG 4a,b) contribute to the efficiency of PKA-phosphorylation events, but they also maintain the protein phosphatase PP2B in proximity to its substrates so that it can oppose the actions of PKA[82–84]. Recent studies indicate that AKAP-anchored PP2B might dephosphorylate and thereby activate a PSD-95-associated UBIQUITIN E3 LIGASE, mouse double minute-2 (MDM2)[85]. As a result, PSD-95 is ubiquitylated and removed by proteasomal degradation, the link between the channel and AKAP79/150 is broken, and the internalization of AMPA receptors is triggered[85] (FIG. 4b). Anchored PKA therefore sustains surface expression of AMPA receptors by phosphorylating them, whereas anchored PP2B signals internalization of the channel, and long-term synaptic depression is likely to ensue. However, there are probably further synaptic channel-anchoring protein networks, as PSD-95 connects AKAP79/150 to the NR2B subunit of the NMDA receptor (FIG. 2c). The plasticity of these protein-network configurations gives rise to distinct AKAP79/150 complexes that respond to glutamate release in different ways.

By contrast, functional AKAP79/150 complexes that do not respond to cAMP can also be assembled. The M CURRENT is produced by a sub-threshold voltage-gated K^+ channel that stabilizes neuronal excitability[86,87]. MUSCARINIC suppression of M currents proceeds through a $G_{q/11}$-coupled signalling pathway that requires Ca^{2+}, phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$) and diacylglycerol (DAG), and involves phosphorylation processes[88,89]. Although Ca^{2+} and DAG are known to activate certain isoforms of PKC[90], obtaining evidence for a role for PKC in this process has proven difficult[91,92]. Recent electrophysiological studies show that a core component of the M current, the KCNQ2 channel, binds directly to AKAP79/150 (REF. 93), which, as discussed previously, can anchor PKC at the plasma membrane[46]. This provides a mechanism to orient PKC to sites at which it can efficiently phosphorylate Ser541 on the channel, which is the molecular basis for M-current inhibition by muscarinic agonists[94] (FIG. 4d). Furthermore, biochemical and electrophysiological experiments using mutant forms of the anchoring protein have indicated that PKA-anchoring sequences are not required for the modulation of KCNQ2 channels by AKAP79/150-bound PKC[94]. A range of synaptic AKAP79/150 signalling complexes can therefore be assembled on KCNQ2 channels, AMPA receptors or NMDA receptors — each of which uses a subset of the possible interacting proteins. A further implication of this arrangement is that these substrates might compete for AKAP79/150, and thereby modulate signal integration on the basis of cellular context and accessibility to the appropriate binding partners. Certainly, the unique topology of dendritic spines contributes to this plasticity by providing spatially segregated signalling compartments where a range of AKAP79/150-channel complexes can receive individual synaptic inputs.

UBIQUITIN PROTEIN LIGASE (E3)
An enzyme that functions together with a ubiquitin-conjugating enzyme (E2) to couple the small protein ubiquitin to Lys residues on a target protein, which marks that protein for destruction by the proteasome.

M CURRENT
A cationic current that is suppressed by the activation of muscarinic receptors and that participates in determining the sub-threshold excitability of neurons and their responsiveness to synaptic input.

MUSCARINIC RECEPTORS
Acetylcholine GPCRs that are activated by the prototypical agonist, muscarine, a compound isolated from the mushroom *Amanita muscaria*.

Dynamic reorganization of AKAP complexes

An emerging theme in AKAP regulation is the dynamic reorganization of the composition and function of AKAP signalling complexes. The recruitment or release

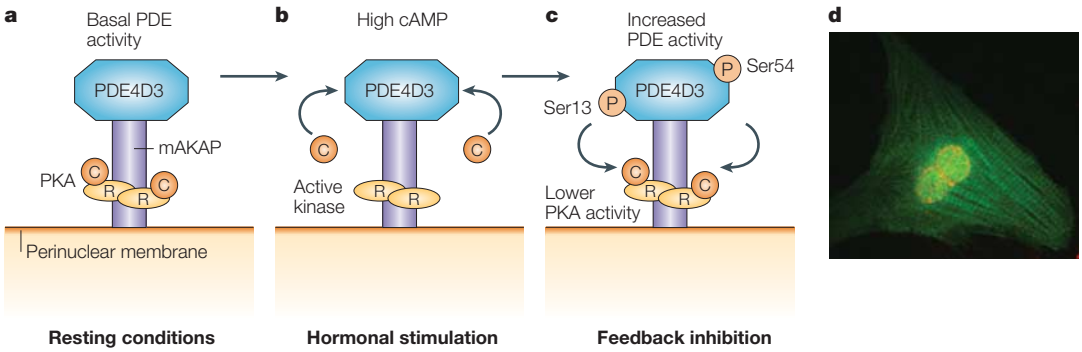

Figure 5 | **A cyclic AMP signalling module that is maintained by mAKAP.** Muscle-specific AKAP (mAKAP) anchors protein kinase A (PKA; R and C denote the regulatory and catalytic subunits of PKA, respectively)[128] and the cyclic AMP (cAMP)-specific phosphodiesterase 4D3 (PDE4D3)[97] at the perinuclear membrane of myocytes. **a** | Basal phosphodiesterase activity keeps cAMP levels in the vicinity of the anchoring protein low, which restricts the activity of anchored PKA[97]. **b** | PKA activity is triggered by a rise in local cAMP levels. **c** | PDE4D3 is a substrate for PKA-mediated phosphorylation on Ser13 and Ser54. Phosphorylation of Ser54 increases the activity of the phosphodiesterase twofold[10,98,99], which thereby enhances the degradation of cAMP that is required to sustain PKA activity and forms a negative-feedback loop between mAKAP-anchored PKA and PDE4D3 (REF. 97). Phosphorylation of Ser13 enhances the binding affinity of PDE4D3 for mAKAP[105], which thereby prevents the phosphodiesterase from diffusing away from the local cAMP gradient and so causes quicker termination of the cAMP signal. **d** | A cultured neonatal rat cardiomyocyte that has been immunostained for mAKAP (red) and actinin (green).

of AKAP-binding partners can alter the response to incoming signals or change the location of a signalling complex.

Dynamic localization of AKAP complexes. The location of an AKAP complex can be modulated by diverse mechanisms, one example of which is competition between binding partners in the WAVE1 complex. Actin and the RII subunit of PKA bind to overlapping regions on this anchoring protein in a mutually exclusive manner. This might give rise to situations in which PKA anchoring is favoured over WAVE1 tethering to the actin cytoskeleton or vice versa[60].

Modification of the targeting signal represents another method of altering the location of an AKAP complex. As mentioned above, the α- and β-isoforms of AKAP18 possess consensus sequences for MYRISTOYLATION and PALMITOYLATION at their N termini[33,42], which are required for membrane targeting. Protein palmitoylation is a dynamic and reversible process; depalmitoylation might therefore dissociate AKAP18α and AKAP18β from the plasma membrane and translocate PKA from this compartment. The functional consequence could be a decreased probability of phosphorylation of substrates such as the voltage-gated Ca^{2+} (Ca_V)[42,95] and Na^+ (Na_V)[96] channels. This could give rise to subpopulations of either channel that are no longer subject to regulation by PKA.

Finally, phosphorylation of the AKAP can modulate its subcellular localization. As discussed previously, the targeting of AKAP79/150 with the plasma membrane requires interaction of the AKAP with phospholipids — in particular, PtdIns(4,5)P_2 (REF. 31). This association can be abolished by the phosphorylation of AKAP79/150 by PKC. This releases the anchoring protein into the soluble fraction[31] and might cause the AKAP complex to move into microdomains where activating stimuli for PKA or PKC are present at sub-activation levels.

Dynamic regulation of enzyme activity. Protein phosphorylation can influence the activity of enzymes that are associated with AKAPs. A well-defined regulatory phosphorylation event occurs within the muscle-specific AKAP (mAKAP) signalling complex. This anchoring protein synchronizes the activities of PKA and a cAMP-specific phosphodiesterase, PDE4D3, in cardiomyocytes[97]. Under basal conditions, tonic phosphodiesterase activity helps to limit kinase activation by hydrolysing cAMP within the vicinity of PKA (FIG. 5a). After hormonal stimulation, however, the cAMP levels rise and overcome the local phosphodiesterase activity, which allows for activation of the kinase (FIG. 5b). The newly activated PKA phosphorylates PDE4D3 on Ser54 to increase the V_{MAX} of the enzyme 2–3 fold over basal conditions[10,98,99]. Consequently, cAMP metabolism is increased and this favours reformation of the PKA holoenzyme (FIG. 5c). The negative-feedback loop that is formed by these anchored enzymes emphasizes the importance of phosphodiesterase compartmentalization in the preservation of cAMP homeostasis and provides a means to propagate pulses of PKA activity in response to the ebb and flow of intracellular cAMP levels.

A recently discovered regulatory event involves the AKAP-Lbc signalling complex. As mentioned above, AKAP-Lbc has GEF activity and nucleates a $G\alpha_{12/13}$-mediated pathway that leads to the activation of Rho[63,100]. Genetic, proteomic and biochemical approaches have now identified 14-3-3 proteins as the link between these two pathways[101,102]. 14-3-3 proteins constitute a family of evolutionarily conserved dimerizing polypeptides that bind to specific motifs containing phosphorylated Ser or Thr residues. These proteins mediate diverse functional

MYRISTOYLATION
The covalent attachment of a hydrophobic myristoyl group to the N-terminal glycine residue of a nascent polypeptide.

PALMITOYLATION
The covalent attachment of a palmitate (16-carbon, saturated fatty acid) to a cysteine residue through a thioester bond.

V_{MAX}
The maximal rate of enzymatic activity.

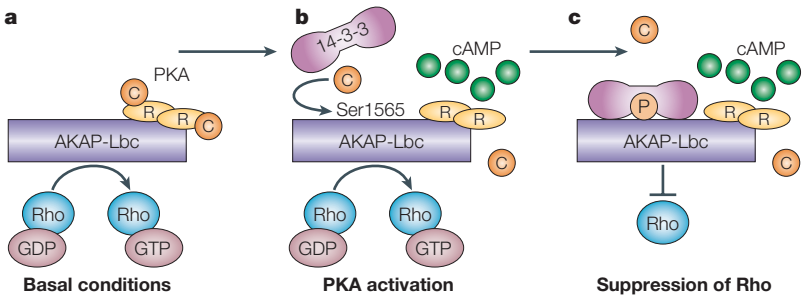

Figure 6 | **AKAP-Lbc and Rho signalling. a** | AKAP-Lbc can function as an exchange factor for the Rho family of small GTPases, and thereby stimulates the exchange of GDP for GTP[63,100]. **b** | The local production of cyclic AMP activates anchored protein kinase A (PKA; R and C denote the regulatory and catalytic subunits of PKA, respectively), which phosphorylates AKAP-Lbc on Ser1565. This phosphorylation event recruits 14-3-3 to AKAP-Lbc[101,102]. **c** | The binding of 14-3-3 to the anchoring protein inhibits its Rho-GEF activity[101,102], which thereby prevents the activation of Rho.

consequences, such as stabilization of an active/inactive conformation, interference with protein–protein interactions or restriction in subcellular localization[103]. Two groups have independently shown that PKA-mediated phosphorylation of Ser1565 of AKAP-Lbc is required to recruit 14-3-3 to the anchoring protein[101,102] (FIG. 6a,b). Cellular studies show that 14-3-3 binding inhibits the Rho-GEF activity of AKAP-Lbc (FIG. 6c), an effect that is accentuated by activation of PKA. The PKA-mediated recruitment of 14-3-3 therefore provides a molecular explanation for the evidence that cAMP functions to attenuate Rho action in various cell lines.

Dynamic recruitment and/or release of binding partners. Recent findings indicate that covalent modifications probably not only modulate the activity of anchored enzymes, but also alter the composition of the signalling complex. The recruitment of PDE4D3 to the mAKAP complex is regulated because the phosphodiesterase contains another PKA phosphorylation site at Ser13 (REF. 104). This residue lies within the mAKAP-binding domain[97] and *in vitro* binding studies show that phosphorylation of this residue increases the affinity of PDE4D3 for the anchoring protein[105]. Cellular experiments show that activation of PKA enhances the mAKAP–PDE4D3 interaction. This is further confirmed using phospho-mutants of PDE4D3 that mimic the unphosphorylated and phosphorylated Ser13, whereas disruption of the ability of mAKAP to anchor PKA diminishes the AKAP–PDE4D3 interaction[105]. The recent identification of PP1 within this complex gives rise to the possibility that binding of PDE4D3 to mAKAP is dynamically regulated by phosphorylation and dephosphorylation[106]. The same might be true for AKAP350, which also binds PKA, PP1 and PDE4D3 and maintains a multiprotein signalling complex at centrosomes[54,107]. So, cAMP-responsive events might also be dynamically regulated by anchored PKA and PDEs in other compartments and in other cell types.

In addition, the release of AKAP binding partners is a regulated process. Whereas phosphorylation-dependent recruitment of 14-3-3 suppresses the Rho-GEF activity of AKAP-Lbc[101,102], phosphorylation of this anchoring protein at a different site disrupts its association with another kinase, protein kinase D (PKD). This phosphorylation event is crucial to the role of AKAP-Lbc as a platform for the assembly of a PKD activation cascade[108] (FIG. 7a). AKAP-Lbc contributes to PKD stimulation in two ways. First, it recruits the upstream activating kinase PKCη[109] and orients it in close proximity to PKD. In addition, AKAP-Lbc might provide access to DAG, a crucial lipid activator of PKCη[90], through activation of anchored Rho. One of the downstream effects of Rho activation is stimulation of phospholipase C (PLC)[110], which generates DAG through hydrolysis of PtdIns(4,5)P_2 (REF. 111) (FIG. 7a). Second, AKAP-Lbc directs PKA-mediated phosphorylation of the anchoring protein itself at a Ser residue within the PKD binding domain (Ser2737), which releases the newly activated PKD enzyme into the cytoplasm (FIG. 7b).

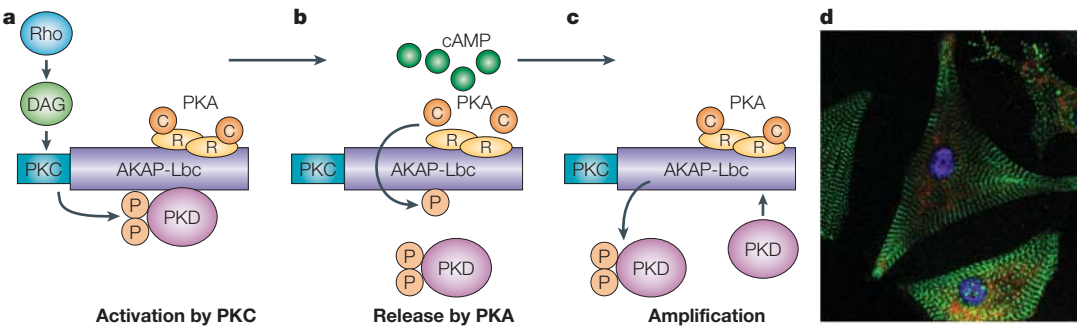

Figure 7 | **Activation and release of protein kinase D (PKD) from the AKAP-Lbc complex.** AKAP-Lbc organizes three enzymes — protein kinase A (PKA; R and C denote the regulatory and catalytic subunits of PKA, respectively), protein kinase C (PKC) and protein kinase D (PKD) — in a protein-kinase cascade. **a** | Rho activation leads to diacylglycerol (DAG) production[110], which activates the novel PKC isoform η (REF. 90). This kinase then phosphorylates two key Ser residues within the activation loop of the catalytic domain of PKD[109]. **b** | Local cyclic AMP production activates anchored PKA, which phosphorylates AKAP-Lbc at Ser2737. This consequently decreases the affinity of PKD for the anchoring protein and releases the newly activated enzyme into the cytoplasm[108]. **c** | The transient nature of the PKD interaction might allow the passage of several molecules through the activation complex, thereby resulting in amplification of PKD activity[108]. **d** | Cultured neonatal rat cardiomyocytes that have been immunostained for AKAP-Lbc (red) and actinin (green). Blue staining indicates the nuclei.

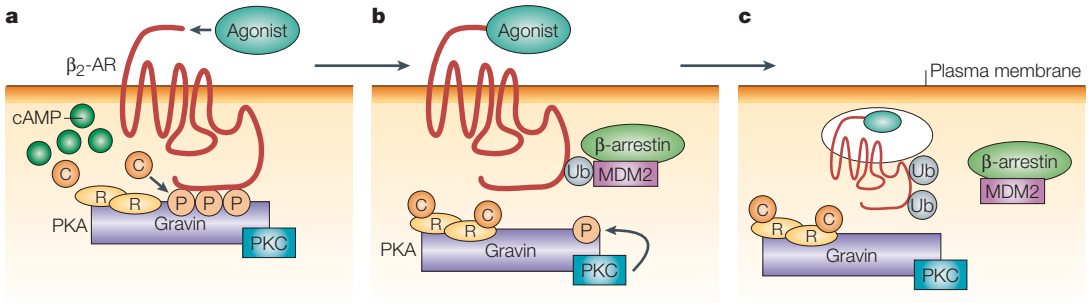

Figure 8 | **Downregulation of the β_2-adrenergic-receptor–gravin complex. a** | Agonist stimulation of the β_2-adrenergic receptor (β_2-AR) leads to activation of the kinases that are associated with gravin (protein kinase A (PKA; R and C denote the regulatory and catalytic subunits of PKA, respectively) and protein kinase C (PKC)). PKA-mediated phosphorylation of gravin initially strengthens the interaction between the anchoring protein and the receptor[65,117]. **b** | PKC-mediated phosphorylation of gravin causes it to dissociate from the receptor. Prolonged agonist stimulation leads to the recruitment of β-arrestin to the receptor, which functions as an adaptor protein for the E3 ubiquitin ligase mouse double minute-2 (MDM2). The recruitment of β-arrestin and MDM2 facilitates ubiquitylation of the receptor[117,119]. **c** | The ubiquitylated receptor is internalized and undergoes proteasomal degradation[119].

The transient nature of the PKD interaction allows the passage of many molecules through the activation complex (FIG. 7c). This is consistent with recent studies showing that activated PKD is recruited to membranes and can then translocate to other cellular sites of action[112–116].

Gravin is another example of an AKAP complex in which both recruitment and release of binding partners are controlled by phosphorylation events. Gravin targets PKA and PKC to the neuromuscular junction or the membrane cytoskeleton[53]. The gravin complex is dynamically recruited to the membrane through interactions with β_2-AR, as mentioned above[64,65,117,118]. Agonist stimulation initially strengthens the association of gravin with the β_2-AR[65,117] through PKA-mediated phosphorylation of Ser696, Ser698 and Ser772 on the anchoring protein (FIG. 8a). However, prolonged agonist stimulation also sets into motion a sequence of events that dismantles the signalling complex. This includes phosphorylation by PKC, ubiquitylation, proteasomal degradation and β_2-AR trafficking[117,119] (FIG. 8b). A key molecule in this downregulation process is the regulatory adaptor protein β-arrestin, which recruits MDM2 (REF. 119) (FIG. 8b,c). Dynamic regulation of β_2-AR surface expression might involve anchored kinases, the recruitment of MDM2 and proteasomal degradation. This sequence of events is strikingly similar to the agonist-dependent modulation of AMPA receptors by the AKAP79/150 signalling complex that was discussed earlier.

Conclusions and future perspectives

The original AKAP hypothesis postulated that spatial resolution of PKA-mediated phosphorylation events could provide an element of specificity to cAMP signalling. However, we now realize that AKAP complexes integrate and disseminate various intracellular signals. A further level of complexity arises from findings that individual AKAP complexes can assemble in a tissue-, compartment- or substrate-specific manner. Furthermore, we now appreciate that AKAP complexes are highly dynamic entities, as the location, enzyme activity or components of these units are continually adjusted in response to physiological stimuli. The challenge ahead is to discern the rules that control how a distinct complement of AKAP binding partners is assembled on a single anchoring protein to elicit a specific physiological response. Here, we list some of the emerging techniques that might enable us to achieve these goals.

Certainly, it will be necessary to refine protein-purification techniques and proteomic analyses such that they can routinely be used to dissect compartment- or tissue-specific AKAP complexes. These refined analytical techniques should allow the resolution of other complexes that are nucleated by WAVE1, gravin, AKAP350 and non-neuronal AKAP79/150, which has been identified in pancreatic β islets, epithelial cells, myocytes and T cells[50,78,120,121]. An extension of this theme would be to selectively displace individual AKAP binding partners and monitor signal flow through this modified complex. This approach requires the removal of an endogenous anchoring protein and rescue with a mutant form, which can be carried out by RNA INTERFERENCE or the generation of 'knock-in' animals. Such experiments examine an AKAP complex under a set of defined conditions and will provide a foundation for future studies that trace remodelling events dynamically.

Other lines of investigation will undoubtedly move toward real-time experiments that explore the impact of AKAP signalling networks in living cells. Promising experiments by Dell'Acqua and colleagues, using FLUORESCENCE RESONANCE ENERGY TRANSFER to examine PKA and PP2B recruitment to AKAP79, support the feasibility of this approach[122]. However, there is a need for additional fluorescent probes that allow the simultaneous detection of multiple binding partners. MULTIPLEX IMAGING WITH QUANTUM DOTS (luminescent nanoparticles that are characterized by narrow emission spectra, resistance to photobleaching and high quantum efficiency[123]) or the expression of Anthozoa green fluorescent protein (GFP)-like fluorescent proteins of different colours[124] are two ways to circumvent this problem, although, at present, there are limitations to both of these potentially useful approaches. We predict that the application of these new techniques will uncover further examples in which relocalization and turnover of AKAP complexes have an essential role in specifying the spatial and temporal organization of signalling events.

RNA INTERFERENCE
(RNAi). A form of post-transcriptional gene silencing in which expression or transfection of double-stranded RNA induces degradation, by nucleases, of the homologous endogenous transcripts, which mimics the effect of the reduction, or loss, of gene activity.

FLUORESCENCE RESONANCE ENERGY TRANSFER
(FRET). The non-radiative transfer of energy from a donor fluorophore to an acceptor fluorophore that is typically <80 Å away. FRET will only occur between fluorophores in which the emission spectrum of the donor has a significant overlap with the excitation of the acceptor.

MULTIPLEX IMAGING WITH QUANTUM DOTS
A method that allows the simultaneous imaging of multiple events in a single cell by attaching quantum dots of different sizes to different molecules (such as antibodies). Quantum dots are inorganic fluorescent nanocrystals with a broad excitation spectrum and a narrow emission spectrum. A mixture of quantum dots of different sizes that is excited using one wavelength will yield multiple fluorescent signals at discrete wavelengths.

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Competing interests statement

The authors declare no competing financial interests.

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