Ca^{2+} signalling checkpoints in cancer: remodelling Ca^{2+} for cancer cell proliferation and survival

H. Llewelyn Roderick‡ and Simon J. Cook‡*

Abstract | Increases in cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) represent a ubiquitous signalling mechanism that controls a variety of cellular processes, including proliferation, metabolism and gene transcription, yet under certain conditions increases in intracellular Ca^{2+} are cytotoxic. Thus, in using Ca^{2+} as a messenger, cells walk a tightrope in which $[Ca^{2+}]_i$ is strictly maintained within defined boundaries. To adhere to these boundaries and to sustain their modified phenotype, many cancer cells remodel the expression or activity of their Ca^{2+} signalling apparatus. Here, we review the role of Ca^{2+} in promoting cell proliferation and cell death, how these processes are remodelled in cancer and the opportunities this might provide for therapeutic intervention.

Driver mutation
A mutation that contributes intimately to tumorigenesis and is selected for during tumour evolution, as opposed to a passenger mutation, which confers no selective advantage and is 'along for the ride'.

**Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1PD, UK.*
‡Laboratory of Molecular Signalling, Babraham Institute, Babraham Research Campus, Babraham, Cambridge CB22 3AT, UK.
Correspondence to H.L.R.
e-mail: llewelyn.roderick@bbsrc.ac.uk
doi:10.1038/nrc2374

Changes in the levels of intracellular calcium (Ca^{2+}) provide dynamic and highly versatile signals that control a plethora of cellular processes[1], although their importance is perhaps most strikingly exemplified by their role in life-and-death decisions. Consequently, Ca^{2+} needs to be used in an appropriate manner to determine cell fate; if this balancing act is compromised, pathology may ensue.

Tumorigenesis occurs as a result of mutations that confer a set of cancer-specific hallmarks, including self-sufficiency in growth signals and evasion of apoptosis[2]. Many cancer-causing genes encode protein kinases; indeed, the protein kinase domain is the most commonly found functional domain in known cancer genes[3]. As protein kinases occupy apical positions in signal-transduction cascades, integrate with many other signalling pathways and regulate the activity or abundance of transcription factors, the cellular effects of aberrant protein kinase activity are wide-ranging.

The same is true of Ca^{2+} signalling, which integrates with other signal-transduction cascades to control a variety of processes including gene expression[4–6]. Ca^{2+} signalling is required for cell proliferation in all eukaryote cells, but some transformed cells and tumour cell lines exhibit a reduced dependency on Ca^{2+} to maintain proliferation[7,8]. Recent years have seen a growing appreciation of the extent to which components of Ca^{2+} signalling pathways are remodelled or deregulated in cancer (BOX 1). Whether these changes are drivers[9,10] that are required to sustain the transformed phenotype remains to be established. Here, we review the core components of the Ca^{2+} signalling system (the Ca^{2+} toolkit), focus on the role of Ca^{2+} in two crucial aspects of the cancer phenotype — control of cell proliferation and cell death — and consider examples of how the Ca^{2+} toolkit is remodelled in tumour cells and the significance this has for the maintenance of the cancer phenotype. Finally, we consider whether any therapeutic opportunities are afforded by the remodelling of Ca^{2+} signalling in cancer.

The Ca^{2+} toolkit

Every cell expresses a unique complement of components from a Ca^{2+} signalling toolkit that enables it to generate intracellular Ca^{2+} signals of a particular amplitude, time course and intracellular location[11,12] (FIG. 1). This Ca^{2+} signalling fingerprint encodes information that allows Ca^{2+} to control diverse cellular processes in a specific manner. In resting cells, the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) is maintained at approximately 100 nM, but through mobilization from intracellular stores (such as endoplasmic reticulum (ER), Golgi or lysosomes[13–15]) or entry across the plasma membrane, $[Ca^{2+}]_i$ can increase to >1 μM[1,11,13,16]. The Ca^{2+} toolkit is extensive and includes environmental sensors (for example, plasma membrane receptors that detect changes in the level of circulating hormones); signal transducers (such as G proteins and phospholipase C isoforms (PLCs)[17]); signal-generating channels such as inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) on intracellular stores[18] and store-operated

At a glance

- Changes in Ca^{2+} levels are versatile and dynamic signalling events that control diverse cellular events over a wide range of timescales.
- Tumour cells are characterized by their acquisition of different physiological traits that allow them to proliferate independently of growth signals and avoid appropriate cell death.
- The Ca^{2+} signalling 'toolkit' — that is, the proteins involved in regulating Ca^{2+} signalling — is often remodelled in tumour cells to sustain proliferation and avoid cell death.
- Ca^{2+} signalling proteins and organelles are emerging as additional cellular targets of oncogenes and tumour suppressors.
- Ca^{2+} signalling pathways remodelled in cancer provide novel opportunities for therapeutic intervention.

or second messenger-operated channels on the plasma membrane (for example, ORAI1 and transient receptor potential (TRP) channels respectively)[1,16,19]; ER-localized Ca^{2+} storage proteins (such as calreticulin, GRP78 (also known as heat-shock protein 5 (HSPA5)) and calsequestrin); signal terminators that serve to return intracellular Ca^{2+} levels to pre-stimulation levels, such as the ER- and plasma membrane-localized Ca^{2+} pumps (SERCA and PMCA, respectively), plasma membrane exchangers (Na^+–Ca^{2+} exchanger (also known as SLC8A1)), mitochondria and cytosolic buffer proteins; and Ca^{2+} sensors and effectors such as calmodulin (CaM) and its downstream targets, including CaM kinase (CaMK)[20] and calcineurin (otherwise known as protein phosphatase 2B)[21] and protein kinase C (PKC). Specificity in decoding Ca^{2+} signals can be provided by the affinity of the Ca^{2+} sensor as well as its intracellular location[22]. In this way, the duration, amplitude and intracellular location of a particular Ca^{2+} signal can specifically regulate cell function[11]. A cell's complement of these proteins will reflect its unique physiological requirements and role, but this may change as cells undergo phenotypic changes, such as those experienced during growth and proliferation[11].

Ca^{2+} and cell proliferation

Ca^{2+} has an important role throughout the mammalian cell cycle and is especially important early in G1, at the G1/S and G2/M transitions (BOX 2, FIG. 2). Indeed, changes in $[Ca^{2+}]_i$ have been detected as a cell passes through G1, G1/S and mitosis[23]. The requirement for Ca^{2+} signals is illustrated by the cessation of cell proliferation when extracellular Ca^{2+} is lowered from 1 mM to 0.1 mM[24]. Cells are most sensitive to depletion of extracellular Ca^{2+} in G1, in which Ca^{2+} is important for the expression of immediate-early genes, such as *FOS*, *JUN* and *MYC*, and later towards the G1/S boundary where Ca^{2+} is required for retinoblastoma (RB1) phosphorylation[25]. CaM is required for cell cycle progression through G1 and mitosis[26], and CaM antagonists or CaMK inhibitors block cell-cycle progression early or late in G1, whereas cells are much less sensitive after RB1 phosphorylation[24]. Inhibition of CaMK causes loss of cyclin D1 (CCND1) expression, increased expression of p27 (encoded by *CDKN1B*), inhibition of cyclin-dependent kinase 4 (CDK4) and CDK2, and G1 arrest[27–29] (FIG. 3).

Calcineurin also has a major role in the progression through G1 and S phases. Inhibition of calcineurin by cyclosporin A suppresses CDK2 activity by increasing expression of p21 (encoded by *CDKN1A*)[30], or reducing cyclin E (CCNE1) and cyclin A (CCNA2) levels[31]. In addition, calcineurin might be required for cyclin D1 expression during G1 (REF 32). Calcineurin also regulates the transcription factors that control the G1/S transition, including cAMP-responsive element binding protein 1 (CREB1), which binds to the cyclin D1 promoter[33] and the nuclear factor of activated T cells (NFAT). NFATs reside within the cytoplasm in an inactive, phosphorylated state but, following an increase in $[Ca^{2+}]_i$, activated calcineurin dephosphorylates NFAT proteins, allowing them to enter the nucleus and regulate expression of their target genes[34]. Although Ca^{2+} signals arising from either intracellular stores or the extracellular space can activate calcineurin, by supporting sustained signals, Ca^{2+} influx through plasma membrane channels such as the store-operated channel ORAI1 is principally responsible for engaging the NFAT pathway and inducing changes in gene expression[35]. TRPC6 and TRPV6 (TRP vanilloid family member 6) have both been shown to mediate NFAT-dependent gene transcription in primary and cultured prostate cancer cell lines[36,37]. Interestingly, TRPV6 is highly expressed in high-grade prostate cancer and is a marker of prognosis[38]. As TRPV6 is a constitutively active channel that is regulated by expression and cellular distribution, the increased expression observed in cancers will result in greater Ca^{2+} entry and therefore enhanced NFAT activation[39]. Links between NFAT and the cell-cycle machinery are starting to emerge. For example, overexpression of a constitutively active NFATC1 mutant in 3T3-L1 fibroblasts was sufficient to induce expression of MYC, cyclins D1 and D2 (CCND2) and a transformed phenotype[40], and NFATC1 has been shown to bind directly to an NFAT site in the *MYC* promoter[41]. As cyclin E and E2F are transcriptional targets of MYC, the NFAT–MYC connection provides a link between Ca^{2+} and calcineurin and the cell cycle.

Ca^{2+} and centrosome duplication. In addition to activation of CDKs, Ca^{2+} and CaMKII also control centrosome duplication and separation, allowing distribution of replicated chromosomes to daughter cells. Defects in this process can lead to aberrant mitotic spindles, genetic instability, aneuploidy and cancer. Centrosome duplication commences as cells exit G1 and enter S phase (FIG. 2), and cyclin E–CDK2 has a key role in the process, by activating ROCK2 (Rho-associated, coiled-coil containing protein kinase 2)[42] and monopolar spindle 1 (MPS1)[43], two protein kinases involved in centrosome duplication. The polo-like kinases and Aurora kinases are also involved in the centrosome cycle[44].

Ca^{2+} oscillations occur at the G1/S boundary (centrosome duplication) and the G2/M transition (centrosome separation), during which CaMKII localizes to centrosomes[45]. Indeed, chelation of intracellular

Box 1 | **Ca^{2+} and the hallmarks of cancer**

Tumour cells exhibit distinct hallmarks or acquired traits that lead to changes in their physiology and distinguish them from non-malignant cells[2]. These are the means by which tumour cells overcome inherent anticancer defence mechanisms and the genetic diversity found in human tumours represents different solutions to the selection pressure to acquire these traits. Changes in Ca^{2+} handling are relevant to or are involved in many of these cancer traits. In the text we consider the role of Ca^{2+} as a cell proliferation signal and the remodelling of survival pathways that this necessitates. Examples of other traits not covered in the main text are given below:

- Insensitivity to anti-growth signals. Ca^{2+} is crucial for controlling the balance of proliferation and differentiation of some cells. Normal keratinocytes differentiate as Ca^{2+} levels increase whereas transformed keratinocytes show little differentiation at any Ca^{2+} concentration[185]. Even transforming growth factor β, a growth inhibitor for many epithelial cells, requires the Ca^{2+}-binding protein S100A11 for inhibition of keratinocyte growth[186].
- Limitless replicative potential. Telomere erosion through successive cycles of replication normally leads to cellular senescence. To maintain their telomeres, cancer cells typically upregulate telomerase expression. The Ca^{2+}-binding protein S100A8 has been shown to mediate Ca^{2+}-induced inhibition of telomerase[187], suggesting that remodelling of Ca^{2+} signalling, in particular a reduced dependency on Ca^{2+} for cell-cycle progression, might be important in tumour cell immortality.
- Sustained angiogenesis. Tumours must acquire a blood supply to grow. Ca^{2+} is required for hypoxia-induced activation of hypoxia-inducible factor 1 (HIF1), the transcription factor that promotes expression of vascular endothelial growth factor (VEGF)[188] and for VEGF-dependent endothelial cell proliferation[189]. In addition, secretion of thrombospondin-1 (THBS1), an angiogenesis inhibitor, is controlled by Ca^{2+} entry through the TRPC4 (transient receptor potential ion channel 4) Ca^{2+} channel. Renal cell carcinomas exhibit a profound decrease in TRPC4 expression, impaired Ca^{2+} intake and diminished secretion of THBS1, thus enabling an angiogenic switch during carcinoma progression[190].
- Tissue invasion and metastasis. Intracellular Ca^{2+} signals appear to be important determinants of metastasis. T-type Ca^{2+} channel blockers inhibit Ca^{2+} spikes and cell motility and invasion in HT1080 fibrosarcoma cells[191]. The Ca^{2+} binding protein S100A13 is associated with a more aggressive invasive phenotype in lung cancer *in vitro*[192].

Ca^{2+} or inhibition of CaMKII blocks centrosome duplication in *Xenopus laevis* egg extracts independently of CDK2 (REF. 46). CP110, a centrosomal protein and CDK2 substrate, promotes centrosome duplication and inhibits centrosome separation, suggesting an important role in coordination of the centrosome cycle[47]. CP110 is found together with the Ca^{2+}-binding proteins CaM and centrin (CETN1) *in vivo*[48] and studies have revealed an intrinsic role for Ca^{2+}, CaM and CaM-binding proteins (including CaMKII and CP110) in control of the normal centrosome cycle[48]. To what extent this role for Ca^{2+} is deregulated or remodelled in cancer remains to be seen.

In summary, it is evident that Ca^{2+} is required for progression through G1 and entry into S phase. Ca^{2+} operates upstream of the cell-cycle machinery by regulating the expression, activity and/or location of the transcription factors that control expression of the G1 cyclins (FOS, JUN, MYC, CREB–ATF1 (activating transcription factor 1) and NFAT), but also acts more directly on the cyclins, CDKs and/or their small protein inhibitors to regulate the assembly and activation of CDK complexes (FIG. 3).

T-type Ca^{2+} channel
Voltage-operated Ca^{2+} channel that is activated at relatively negative membrane potential and exhibits a short-lasting (transient) opening.

Do tumour cells require Ca^{2+} for cell-cycle progression?
Conventional wisdom suggests that transformed or malignant cells exhibit a greatly relaxed requirement for Ca^{2+} during cell proliferation. This view stems from landmark studies from the early 1970s onwards, in which it was demonstrated that cellular transformation by SRC[49], KRAS[50], SV40 (REF. 51), adenovirus (AdV)[52] or human papillomavirus (HPV)[53] conferred the ability to proliferate at low extracellular Ca^{2+} levels, leading to the suggestion that loss of proliferative Ca^{2+} dependency was an indicator of tumorigenicity[7,54].

For a long time it was not obvious what properties Ras, SV40, HPV and AdV shared that could circumvent the cellular requirement for Ca^{2+}. However, it is now known that they all inactivate RB1, though they achieve this by quite different mechanisms: Ras, acting through extracellular regulated kinases 1 and 2 (ERK1 and ERK2; also known as mitogen-activated protein kinases 3 and 1 (MAPK3 and MAPK1)) and protein kinase B (PKB; also known as AKT1), promotes the activation of CDK4 and CDK2 and phosphorylation of RB1, whereas SV40, HPV or AdV all encode oncoproteins that sequester or degrade RB1. As Ca^{2+} and Ras are both required for phosphorylation of RB1 during the G1/S transition in normal cells, it has been suggested that the relaxed requirement for Ca^{2+} in tumour cell proliferation might simply reflect the fact that such cells have frequently lost RB1 function[8]. Certainly, cells that have lost RB1 are much less sensitive to inhibition of Ras[55] or the ERK1–ERK2 pathway[56]. Although this attractive hypothesis fits with the role of Ca^{2+} in RB1 inactivation[25], it needs to be tested with isogenic cell lines from wild-type, *Rb1*-null, *Cdkn1b*-null or *Cdkn2a* (which encodes p16)-null mice or investigated by determining whether overexpression of cyclin D1 or CDK4 can confer resistance to Ca^{2+} chelation during cell cycle re-entry.

Irrespective of this, the notion that tumour cells are independent of Ca^{2+} for proliferation is starting to be questioned. For example, the proliferation of the prostate cancer cell line LNCaP is acutely tuned to the expression of SERCA2 (sarcoplasmic reticulum Ca^{2+} ATPase 2, also known as ATP2A2) and the content of the ER Ca^{2+} store, $[Ca^{2+}]_{ER}$ (REF. 57). Moreover, inhibition of SERCA2 with thapsigargin inhibits proliferation. In addition, an increasing number of studies are demonstrating the requirement for Ca^{2+} influx for tumour cell proliferation. For example, proliferation of human U87 MG glioma and murine N1E-115 neuroblastoma cell lines is inhibited by the T-type Ca^{2+} channel blocker mibefradil and stimulated by retroviral overexpression of the α1H subunit of the channel[58,59]. Indeed a requirement for T-type Ca^{2+} channels for proliferation has been reported in breast, colorectal, gastric and prostate cancer cells[58], and TRPV6 expression was recently shown to be required for proliferation in a prostate cancer cell line[36]. There is not enough space here to list all such examples and readers are referred to recent reviews[58,60].

So, what explains the disparity between recent studies and those of the early 1970s? Notably, the more

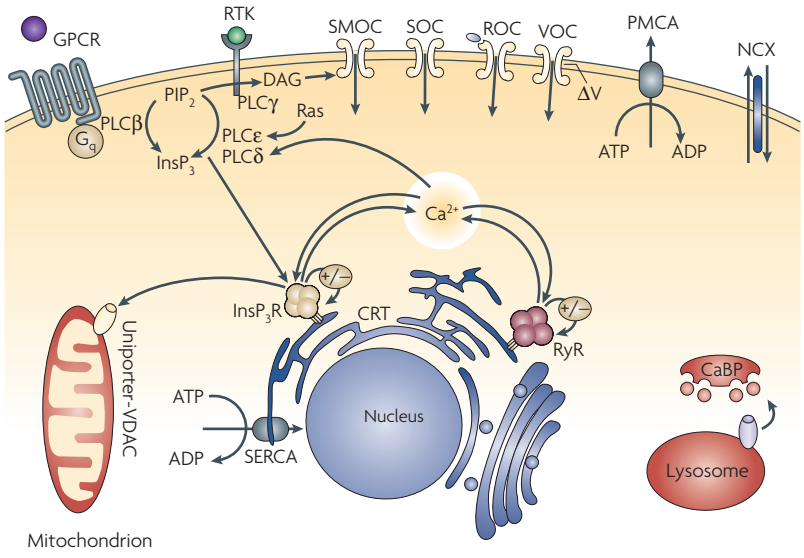

Figure 1 | **The Ca^{2+} signalsome.** In response to a change in their environment, intracellular Ca^{2+} levels increase and induce changes in cell physiology. Ca^{2+} signals are generated as a result of influx from the extracellular space through channels located at the plasma membrane (receptor-operated channels (ROCs), voltage-operated channels (VOCs), second-messenger-operated channels (SMOCs) and store-operated channels (SOCs)) or via release from intracellular stores, predominantly through inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) or ryanodine receptors (RyRs). Ca^{2+} channels and pumps are also functionally expressed in lysosomes and the Golgi. Ca^{2+} signals return to pre-stimulated levels through the concerted action of cytosolic Ca^{2+} buffer proteins (CaBPs), mitochondria, ATP-dependent pumps on the intracellular Ca^{2+} stores (SERCA) and plasma membrane (PMCA), as well as through the Na^+–Ca^{2+} exchanger (NCX). Ca^{2+} is stored within the endoplasmic reticulum bound to the low-affinity, high-capacity Ca^{2+} storage protein calreticulin (CRT). DAG, diacylglycerol; GPCR, G-protein coupled receptor; PIP_2, phosphatidylinositol bisphosphate; PLC, phospholipase C; RTK, receptor tyrosine kinase; VDAC, voltage-dependent anion channel.

recent work has been with *bona fide* human tumour cell lines, whereas much of the early work was on virally transformed fibroblasts. It is possible that tumour cells and transformed fibroblasts simply exhibit fundamental differences in cell physiology and that the acquisition of Ca^{2+} independency during the evolution of a tumour is not a trait that can be faithfully recapitulated by the simple heterologous expression of a single oncoprotein. In this sense, there is a case for systematically investigating which tumour types are indeed Ca^{2+}-dependent for proliferation using more sophisticated tools, human tumour cell lines and genetically modified mice that are now available.

Remodelling Ca^{2+} signalling in cancer

Ca^{2+}-dependent signalling mechanisms are frequently remodelled or deregulated in cancer cells. However, to date only mutations in *ATP2A2* (which results in changes to SERCA2 expression) have been described as occurring in or promoting cancer[61,62]. The paucity of studies reporting mutations in genes associated with the Ca^{2+} toolkit suggests that many of the changes that underpin remodelling of Ca^{2+} signalling reflect epigenetic changes in gene expression and/or post-translational changes in the properties of existing signalling components. This remodelling is a two-way process in which oncogene-dependent pathways can remodel Ca^{2+} signals and Ca^{2+} can refine oncogene-regulated signalling.

Oncogene-dependent remodelling of Ca^{2+} signalling. There are many reports describing changes in Ca^{2+} signalling in cells transformed by oncogenes such as *Ras* and *SRC*. The mechanisms underlying the amplification of Ca^{2+} mobilization in *Ras*-transformed cells remained elusive for many years[63–65], but the recent demonstration that PLCε binds Ras and is activated upon expression of Ras provides a direct link between Ras activation and generation of $InsP_3$ (REF. 66). SRC can also amplify $InsP_3$ and Ca^{2+} signalling by promoting the tyrosine phosphorylation of the Gq α-subunit, which increases its ability to stimulate PLC[67].

In addition to post-translational mechanisms, oncogenes cause striking changes in the expression of components of the Ca^{2+} toolkit. Expression of a *MYC* transgene can stimulate B-cell proliferation in part by decreasing expression of PMCA4b (also known as ATP2B4) Ca^{2+} efflux pump, resulting in more sustained increases in $[Ca^{2+}]_i$ and enhanced nuclear accumulation of NFATC1 (REF. 68). In other cases, such as SERCA2 (REF. 69), PMCA1 (also known as ATP2B1)[70] and the T-type channel CACNA1G[71], reduction in expression is due to silencing by promoter methylation. Microarray technologies have greatly enhanced our appreciation of the extent and variation of Ca^{2+} toolkit remodelling. For example, transformation by ERBB2, Ras, RAF, JUN, MYC or SV40 can all cause striking, but quite different, changes in expression of CaM, CaMK and various Ca^{2+} binding proteins[72–74]. Some of these changes 'make sense' or fit with our ideas of how Ca^{2+} is deregulated in tumours. For example, reduced expression of PMCA1 (REF. 70) or amplification of the type 2 $InsP_3R$[75] could both enhance growth factor-dependent increase in $[Ca^{2+}]_i$, whereas overexpression of CaMKII[72,74] could

Box 2 | **G1/S progression**

Progression of cells through G1 into S phase requires expression of the G1 cyclins (cyclins D & E), activation of the cyclin-dependent kinases (CDK4 and CDK2), phosphorylation and inactivation of the retinoblastoma protein (RB1) and the derepression and release of the E2F transcription factors[193]. CDKs are also subject to negative regulation by CDK inhibitor proteins such as p21 (a p53 target gene) and p27. The *de novo* expression of D-type cyclins and the destruction of p27 are mitogen-regulated events that are controlled by Ras-regulated signals[194,195]. Activation of the extracellular signal-regulated kinase (ERK1–ERK2) pathway can promote the expression of cyclin D[196], and protein kinase B (PKB) can stabilize the mature cyclin D1 protein[197]. Similarly, both ERK and PKB can reduce p27 levels, albeit by different mechanisms. Indeed, inhibition of the ERK1–ERK2 or PKB pathways causes a G1 arrest characterized by loss of cyclin D1 and accumulation of p27 and/or p21.

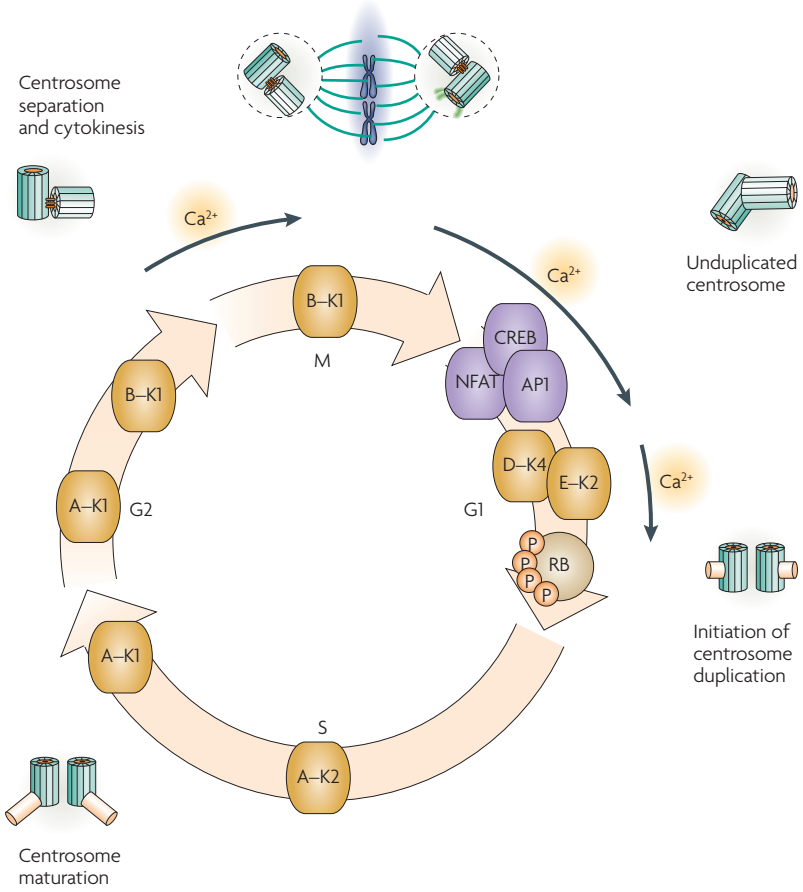

Figure 2 | **Ca^{2+} and the cell cycle.** Ca^{2+} signalling is required at various key stages of the cell cycle (shown in yellow). An early burst of Ca^{2+} signalling is required early in G1 as cells re-enter the cell cycle for activation and/or expression of transcription factors of the AP1 (FOS and JUN), cAMP-responsive element binding protein (CREB) and nuclear factor of activated T-cells (NFAT) families. These factors coordinate the expression of cell-cycle regulators, notably the D-type cyclins, which are required for activation of cyclin D–CDK4 (cyclin-dependent kinase 4) complexes (D–K4). Ca^{2+} is also required for correct assembly and activation of D–K4 and E–K2 complexes later in G1 to ensure phosphorylation and inactivation of retinoblastoma (RB) and entry into S phase. Ca^{2+} oscillations at the G1/S and G2/M transitions are thought to be important for the centrosome cycle. Ca^{2+} acts in concert with calmodulin (CaM), CaM kinase II and CP110, a centrosomal protein and CDK2 substrate, to initiate centrosome duplication at the G1/S transition. CP110 also inhibits centrosome separation allowing temporal coordination of the centrosome cycle by Ca^{2+}.

enhance the coupling of Ca^{2+} signals to the G1 CDKs. The significance of other changes is less clear. For example, the S100 proteins are frequently deregulated in cancer and some are excellent biomarkers and prognostic indicators[76], but only in a few cases has a functional significance been proposed. For example, S100A2 is downregulated in some tumours by CpG methylation and its forced re-expression inhibits cell motility, suggesting it has a role in suppressing metastases[77]. By contrast, S100A4 expression is increased in many cancers and this might relate to its ability to bind to and inhibit wild-type p53 (REF. 76). Other than these examples, the functions of S100 proteins appear to be many and varied and readers are referred to a recent review[76].

S100 proteins
A family of EF-hand-containing Ca^{2+} binding proteins.

CpG island
A DNA region of >500 base pairs that has a high CpG density and is usually unmethylated. CpG islands are found upstream of many mammalian genes; methylation leads to transcriptional silencing.

Ca^{2+}-dependent remodelling of oncogene signalling. There is an emerging role for Ca^{2+} in influencing the Ras pathway[8,78]. Activation of Ras is controlled by guanine nucleotide exchange factors (GEFs), which promote the release of GDP, allowing GTP to bind[79], and GTPase-activating proteins (GAPs), which catalyse the hydrolysis of GTP to GDP[80]. The recruitment of SOS (a Ras GEF) to receptor tyrosine kinases (RTKs) stimulates Ras, whereas recruitment of p120GAP (also known as RASA1) inactivates Ras (FIG. 4a). There is a growing appreciation of the importance of Ca^{2+}-regulated Ras GEFs and GAPs. The Ca^{2+}-regulated Ras GEFs include Ras guanine nucleotide-releasing factors 1 and 2 (RASGRF1 and RASGRF2) and Ras guanyl-releasing proteins 1 and 2 (RASGRP1 and RASGRP2)[78]. Among the GAP1 proteins, CAPRI (also known as RASA4) and RASAL1 are recruited to the plasma membrane to inactivate RAS in a Ca^{2+}-dependent fashion[81,82]. Remarkably, they respond to qualitatively different Ca^{2+} signals; CAPRI senses the amplitude of the Ca^{2+} signal, whereas RASAL responds to the frequency of Ca^{2+} oscillations[83] (FIG. 4b). Thus, CAPRI and RASAL translate discrete Ca^{2+} signals into changes in the kinetics and amplitude of RAS activation.

An activated Ras oncogene is expressed in 20–30% of human tumours[84] but neurofibromin 1, the gene mutated in neurofibromatosis type 1 (NF1), is the only Ras GAP that has been defined as a tumour suppressor gene[85], although rare nonsense mutations in *RASA1* had been reported[86]. However, recent studies have also suggested that CAPRI and RASAL might be tumour suppressor genes[87,88]. The case is most compelling for RASAL. First, suppression of RASAL increased fibroblast transformation in an RNA interference screen[89]. More importantly, RASAL has now been shown to be downregulated in human tumours by epigenetic silencing through CpG island methylation[90] (FIG. 4b). RASAL silencing was observed in both cell lines and tumour tissue from multiple tumour types including nasopharyngeal, oesophageal, hepatocellular and breast carcinoma; tumour types noted for their low incidence of Ras mutations. Thus, silencing of the Ca^{2+}-regulated Ras GAPs is an alternative mechanism for Ras activation in certain tumours.

Ca^{2+} may influence cell-cycle progression by modulating the activity of pathways downstream of Ras. A moderate level of ERK1 or ERK2 activation appears to be required to promote cell-cycle progression, whereas excessive, sustained activation of ERK1 or ERK2 can promote cell-cycle arrest or senescence[91–93]. Ca^{2+} can remodel or fine-tune the ERK1 and ERK2 pathway in a number of ways: CaM appears to negatively regulate the pathway and might help to set a threshold of ERK activity suitable for proliferation[94,95], and Ca^{2+}-dependent upregulation of dual-specificity phosphatases (DUSPs, which inactivate MAPKs and stress-activated protein kinases (SAPKs)) might control the magnitude and duration of ERK activation[96].

Ca^{2+}-dependent effects on oncogene function are not confined to signalling proteins. Prostate cancer cells overexpress NFATC1, resulting in the strong Ca^{2+}- and calcineurin-dependent upregulation of MYC and

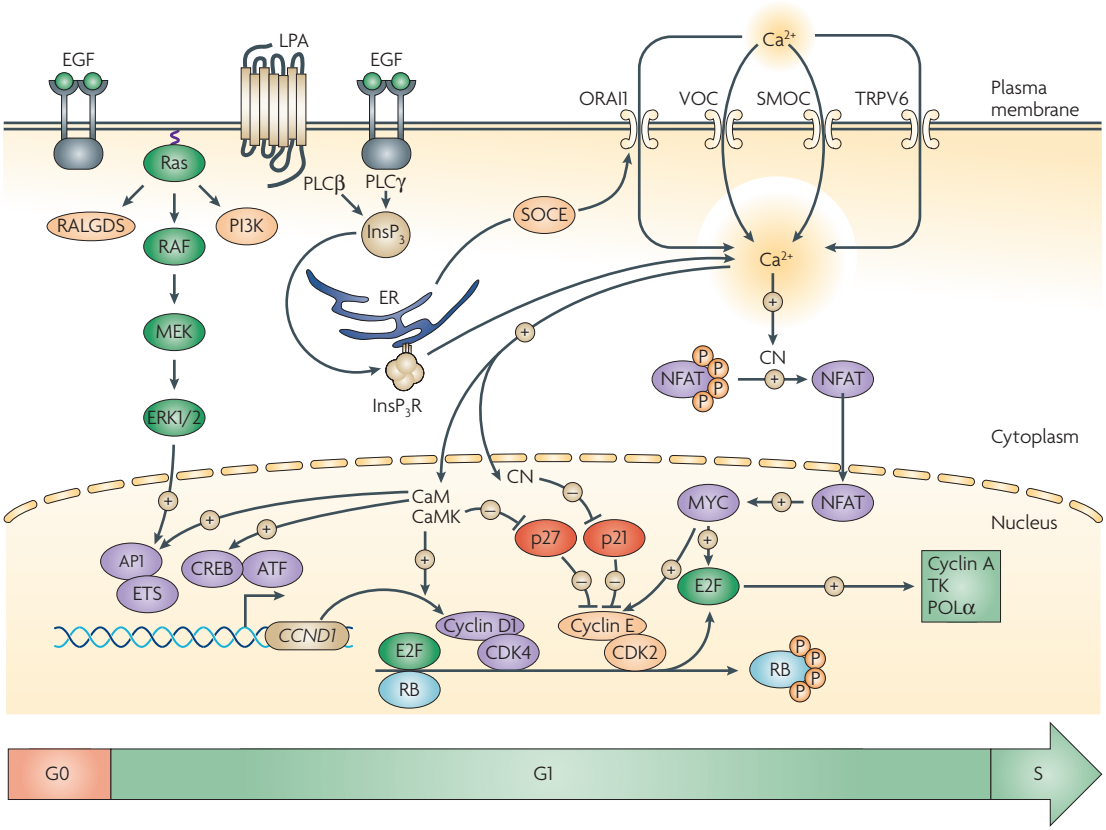

Figure 3 | **Ca^{2+}-dependent signalling pathways controlling the G1/S transition.** Progression through G1 and into S phase requires activation of the cyclin-dependent kinases CDK4 and CDK2, which phosphorylate retinoblastoma 1 (RB1), thereby de-repressing and releasing the E2F transcription factors. Activation of the CDKs requires expression of their cognate cyclins, which is regulated by growth factor-dependent signalling pathways, most notably those controlled by the Ras GTPases. Growth factors binding to receptor tyrosine kinases (RTKs; for example, epidermal growth factor receptor (EGFR)) or G-protein coupled receptors (GPCRs; for example, lysophosphatidic acid (LPA) receptor) can activate Ras and Ras effectors (for example, RAF, phosphatidylinositol 3-kinase (PI3K) and Ral guanine nucleotide dissociation stimulator (RALGDS)). The RAF–MEK–ERK1 (extracellular signal-regulated kinase 1)–ERK2 pathway activates AP1 and ETS transcription factors, driving expression of cyclin D1 and activation of CDK4. Subsequent E2F-dependent expression of cyclin E activates CDK2. Ca^{2+} is also required for CDK activation and G1/S transition. Plasma membrane receptors activate phospholipase C (PLCβ by GPCRs; PLCγ by RTKs) to promote the generation of inositol-1,4,5-trisphosphate ($InsP_3$) and release of Ca^{2+} from the endoplasmic reticulum (ER) into the cytosol. Ca^{2+} entry across the plasma membrane is also required for cell proliferation and might enter by store-operated capacitative Ca^{2+} entry (SOCE) through the ORAI1 channel, through voltage-operated channels (VOCs), second messenger-operated Ca^{2+} channels (SMOCs; for example, TRPC6) or the constitutively active TRPV6, the expression of which is enhanced in certain cancers. The increase in $[Ca^{2+}]_i$ promotes the activation of Ca^{2+}-dependent signalling enzymes such as calmodulin kinase (CaMK) and the Ca^{2+}-dependent phosphatase PP2B or calcineurin (CN). Ca^{2+}–CaMK is required for expression of cyclin D1 and might act by regulating the expression or activity of transcription factors such as FOS, JUN and cAMP-responsive element binding protein (CREB) or by enhancing the translation of *CCND1* mRNA. Calcineurin promotes the de-phosphorylation and nuclear entry of NFATC1 (nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1); in this way Ca^{2+} mobilization can be linked to expression of MYC, cyclin E and E2F. In addition, CaMK and calcineurin are required for repression of the CDK inhibitor proteins $p27^{KIP1}$ and $p21^{CIP1}$ as their expression increases upon treatment of cells with CaMK or calcineurin inhibitors. Stimulation or inhibition of activity or expression are denoted by + and –. POLα, DNA polymerase α.

enhanced malignant potential[41]. Ca^{2+} is also involved in the stabilization of JUN as calcineurin promotes the dephosphorylation of JUN at Ser243, a site normally involved in JUN degradation[97]. In addition, JUN was identified in a functional genomics screen for genes that could bypass the block in cell proliferation that is induced by Ca^{2+} channel blockers such as nifedipine[98]. The cysteine–glutamate exchanger-encoding gene (*xCT;* also known as *SLC7A11*), which carries out a rate-limiting step in glutathione synthesis, was identified in the same screen and cells overexpressing *xCT* exhibited increased expression of JUN (a redox-regulated transcription factor) and AP1 transcriptional activity following growth factor stimulation. These results emphasize the close link between Ca^{2+} homeostasis and redox balance (see below) and suggest that these processes cooperate to regulate AP1 during the cell cycle. Given the frequent deregulation of Ca^{2+} and glutathione[99] in tumours, it might be worth exploring this link further in tumour cell lines.

AP1
A transcription factor that is composed of JUN, FOS, MAF and ATF proteins in various combinations.

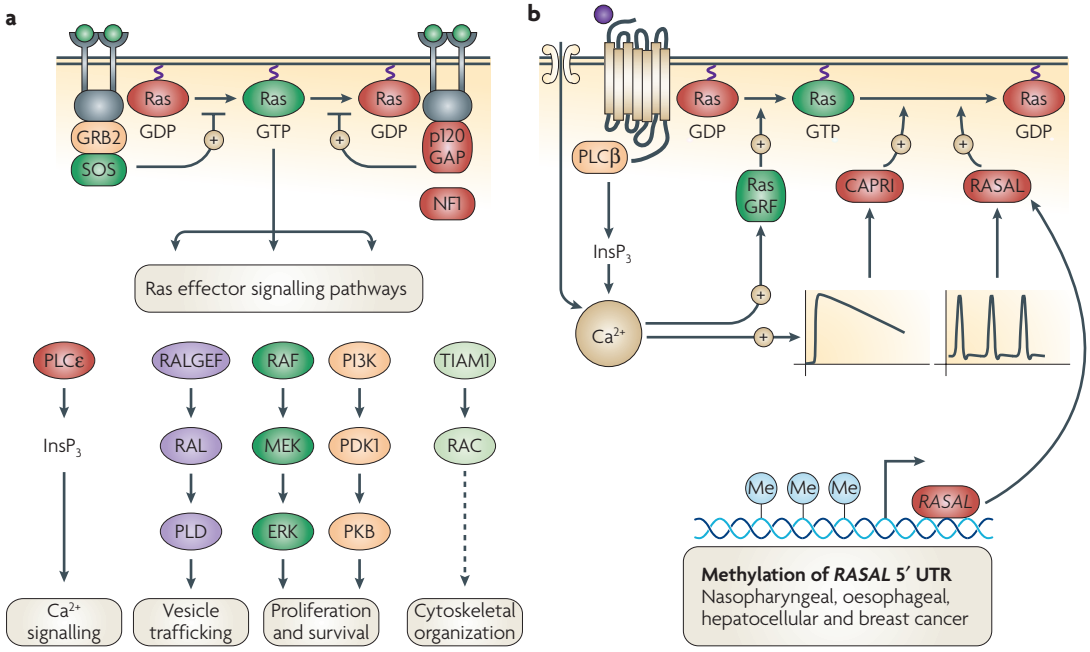

Figure 4 | **Ca^{2+}-dependent activation and inactivation of Ras. a** | The canonical receptor tyrosine kinase (RTK) paradigm for signal-regulated activation and inactivation of Ras. Activation of growth factor RTKs (for example, epidermal growth factor receptor (EGFR)) results in auto-phosphorylation of the receptor, providing binding sites for the recruitment of the adaptor protein GRB2 (growth factor receptor-bound protein 2) and SOS1, a Ras guanine nucleotide exchange factor (GEF), which promotes dissociation of GDP from Ras, allowing binding of GTP. In this active GTP-liganded state Ras activates downstream signalling effectors such as RAF, phosphatidyl inositol 3-kinase (PI3K), RALGEF, phospholipase Cε (PLCε) and T-cell lymphoma invasion and metastasis 1 (TIAM1) to transduce its cellular effects. Inactivation of Ras can also be achieved by RTK-based signalling; the recruitment of p120 GAP to activated receptors allows for hydrolysis of GTP and inactivation of Ras. GTP hydrolysis and inactivation of Ras is also promoted by neurofibromin (NF1) GAP, a tumour suppressor gene in Von Recklinghausen type I neurofibromatosis. **b** | Mobilization of intracellular Ca^{2+} can also regulate the activation status of Ras. A family of Ca^{2+}-regulated Ras GEFs includes the Ras guanine nucleotide-releasing factors (GRFs) and Ras guanyl releasing proteins (GRPs) and can be activated in response to Ca^{2+} mobilization arising from activation of G-protein-coupled receptors (GPCRs), ion channels and antigen receptors. In addition, CAPRI and Ras protein activator-like 1 (RASAL) are two Ca^{2+}-regulated Ras GAPs. They are both recruited to the plasma membrane in a Ca^{2+}-dependent fashion to inactivate Ras, but respond to different types of Ca^{2+} signals. CAPRI senses the amplitude or magnitude of the Ca^{2+} signal (amplitude modulation) whereas RASAL translocates on and off the plasma membrane in response to repetitive Ca^{2+} oscillations (frequency modulation). Cell-culture studies suggest that both CAPRI and RASAL can behave as tumour suppressor genes and RASAL has recently been shown to be silenced by methylation (represented by 'Me') in certain tumour types that are noted for their low frequency of Ras mutations. Thus, epigenetic inactivation of RASAL might represent a novel, non-canonical pathway for Ras activation in tumours. ERK, extracellular signal-regulated kinase; $InsP_3$, inositol 1,4,5-trisphosphate; PDK1, 3-phosphoinositide-dependent protein kinase 1.

Ca^{2+} signalling and cell death

Cancer cells acquire their increased capacity to survive in the face of death-inducing stimuli or conditions[2,100] by commandeering pro-survival signalling pathways (BOX 3) and anti-apoptotic proteins (such as the anti-apoptotic BCL2 family members, BOX 4) to suppress or neutralize death signals[101,102]. Evidence generated over the past 10 years has demonstrated the importance of Ca^{2+} in the activation and execution of cell death[103]. Indeed, $[Ca^{2+}]_i$ increases have been observed during apoptotic cell death[104–107] and have been shown to be required for apoptosis to take place[104,106,108].

The ER–mitochondria Ca^{2+} flux. The ER and mitochondria are the principal locations for signalling cell fate choices and are crucial nodes at which intracellular Ca^{2+} fluxes are governed. Indeed, despite controlling many processes essential for life, Ca^{2+} arising from the ER can be a potent death-inducing signal[109–111] (FIG. 5a). For example, cells in which $InsP_3R$ expression has been ablated or reduced exhibit significantly less apoptosis[112,113]. Moreover, reduction in basal $InsP_3$ levels also prevents apoptosis[104]. A direct link between $InsP_3R$ activity and the induction of cell death is provided by the enhanced Ca^{2+} flux and apoptosis resulting from cytochrome *c* binding to the $InsP_3R$ or its cleavage by caspases[107,114,115]. Ryanodine receptors exhibit a capacity to generate apoptotic Ca^{2+} signals[116] similar to that of $InsP_3Rs$.

A proximal target of Ca^{2+} signals arising from the ER is the mitochondrial network[117–121]. Several observations underline the significance of the role of this ER–mitochondrial Ca^{2+} flux in stimulating apoptosis. First, low Ca^{2+} within the ER store decreases the apoptotic effect of ceramide[110] and underlies the lack of sensitivity

Caspases
A family of cysteine-dependent proteases, evolutionarily conserved from *Caenorhabditis elegans*, which are involved in the initiation and execution of cell death pathways.

Box 3 | **Survival signalling pathways in cancer cells**

Foremost among the survival pathways activated in cancers is the phosphatidyl inositol 3-kinase (PI3K)–PKB (protein kinase B) axis[10]. PI3K catalyses the formation of phosphatidylinositol-3,4,5-trisphosphate (PIP_3)[198], which recruits phosphoinositide-dependent kinase 1 (PDK1) and PKB through their pleckstrin homology (PH) domains to the plasma membrane[199,200]. PKB is then activated by phosphorylation catalysed by PDK1 and the recently described complex comprising mammalian target of rapamycin (mTOR, also known as FRAP1) and RICTOR (rapamycin-insensitive companion of mTOR)[201–203]. PKB exerts its pro-survival role through the phosphorylation of numerous downstream targets[204,205], including BCL2 proteins (for example, BAD)[206], the tuberous sclerosis 1 and 2 (TSC1 and TSC2) regulators of the mTOR pathway[207] and transcription factors (such as forkhead box O3 (FOXO3)[208]. In cancer, the PI3K–PKB pathway is engaged as a result of activating mutations in receptors (epidermal growth factor receptor (EGFR)), Ras, the PI3K catalytic subunit (PI3KCA) and PKB, or inactivating mutations or deletion of the PTEN tumour suppressor, which serves as a PIP_3 phosphatase. All of these lesions can lead to increased PIP_3 levels and/or PKB activity[209]. In addition, PDK1 can also phosphorylate and activate p70S6K, a protein kinase that phosphorylates the S6 ribosomal protein, thereby regulating protein translation[210].

to apoptotic stimuli in BAX/BAK knockout cells[111]. Second, $InsP_3$ application enhances the death-inducing effects of ceramide[122]. Third, metabolism of basal $InsP_3$ reduces the pro-apoptotic effect of reactive oxygen species (ROS; for example, O_2^- and H_2O_2)[104]. Fourth, $InsP_3Rs$ have been localized to sites of ER–mitochondrial interaction (mitochondrial associated ER membranes) where their activity is regulated by chaperoning through the activity of SIGMA receptors and GRP75 (also known as HSPA9)[123,124]. At mitochondrial associated ER membranes, $InsP_3Rs$ are juxtaposed to the Ca^{2+}-conducting voltage-dependent anion channel of the outer mitochondrial membrane, VDAC1 (REF. 123). Overexpression of VDAC1 increases mitochondrial Ca^{2+} accumulation and cell death[125]. It is likely that chaperones at this 'ER–mitochondrial synapse' can act as gatekeepers to regulate flux of Ca^{2+} to the mitochondria. This ability of mitochondria to acutely sense Ca^{2+} release from the ER might allow them to act as cellular sentinels of ER-mediated apoptotic signals.

The ability of Ca^{2+} flux into the mitochondria to stimulate both respiration and cell death is paradoxical. As increased ROS generated by the Ca^{2+}-accelerated respiratory chain promotes death, it is likely that regulation of the rate of electron transport or ROS production is crucial for avoiding cell death and tumorigenesis[126]. Mitochondrially localized p66Shc (also known as SHC1) has been proposed to be the mitochondrial source of ROS, accepting electrons from cytochrome *c*[127], and is regulated in a Ca^{2+}-dependent manner by PKC[128]. Within mitochondria, ROS damages DNA, facilitates Ca^{2+}-induced permeability transition pore opening, inhibits respiration and peroxidates cardiolipin, causing it to dissociate from cytochrome *c*, which then exits the mitochondria to activate the intrinsic apoptotic pathway[126]. ROS have many other cellular targets, including cellular membranes, genomic DNA, ion channels and kinase cascades. For example, ROS-dependent inactivation of protein phosphatases amplifies signalling by receptor tyrosine kinases (RTKs), including PLCγ activation and $InsP_3$ production[129]. ROS promotes mobilization of Ca^{2+} from intracellular Ca^{2+} release channels[130,131] and allows Ca^{2+} entry by activating the melastatin subfamily of TRP channels[132]. The phosphoinositide 3-kinase (PI3K)–PKB survival pathway is targeted in both positive and negative ways by ROS. ROS can induce cleavage and inactivation of PKB[133], yet under certain acute conditions, they can enhance PKB activity through oxidative inactivation of the phosphatidylinositol (3,4,5)-trisphosphate phosphatase PTEN[134].

ER stress-associated cell death and Ca^{2+}. ER stress, as a result of chronic depletion of Ca^{2+} from the ER, is also a signal for cell death[135,136]. Calnexin, an integral membrane protein chaperone of the ER, is important in transducing this signal by creating a scaffold for B-cell receptor-associated protein 31 (BAP31, also known as BCAP31) cleavage by caspase 8 (REF. 137). The BAP31 cleavage product, BAP20, subsequently causes Ca^{2+} release from the ER, which is then taken up by mitochondria, sensitizing them to apoptotic stimuli[135,138]. ER stress also induces cell death by activating the SAPKs. SAPKs are stimulated following the oligomerization of IRE1 (also known as ER to nucleus signalling 2 (ERN2)), which induces the formation of a complex involving TNF receptor-associated factor 2 (TRAF2) and ASK1 (also known as MAP3K5), a MAP3K that can activate JUN N-terminal kinase (JNK, also known as MAPK8) and p38 (also known as MAPK14)[139]. The ASK1 pathway is also activated in a CaMKII-dependent manner[140].

Other Ca^{2+}-dependent cell-death pathways. Despite many of the initiating events occurring at intracellular membranes, increases in $[Ca^{2+}]_i$ can also activate death effectors in the cytosol, including calpains, which are potent amplifiers and initiators of death signalling[141–143]. Calpains can engage apoptotic pathways by processing and activating caspases[141–143]. Additionally, calpain-mediated proteolysis of BCL2 decreases its ability to protect cells from death and may promote mitochondrial permeabilization and cytochrome *c* release[144]. Deregulated Ca^{2+} mobilization can also engage directly with the BCL2 protein family (BOX 4), most notably through the dephosphorylation of the BCL2 homology 3 (BH3)-only protein BAD by calcineurin, resulting in its dissociation from 14-3-3, translocation to the mitochondria and cell death[145].

Ca^{2+}-dependent apoptosis can also proceed through activation of apoptosis-linked gene 2 (ALG2, also known as programmed cell death 6 (PDCD6)). This penta-EF hand-containing protein, which binds Ca^{2+} within the normal physiological range, was discovered in a screen for proteins that could affect apoptosis induction. The precise functions of ALG2 are unclear, but it is established that coordination of Ca^{2+} causes ALG2 to translocate within cells, and alters its interaction with a number of target proteins including ASK1 and the receptor CD95 (also known as FAS). Although it was originally identified as an inducer of apoptosis, ALG2 has also been shown to have an anti-apoptotic function, depending on the prevailing cellular conditions. Interestingly, ALG2 expression is upregulated in a variety of tumour cells[146].

BAX/BAK knockout cells
Cells that do not express the pro-apoptotic proteins BAX and BAK are resistant to many cell death stimuli.

Reactive oxygen species
Highly unstable oxygen-containing chemical entities (such as O_2^- and H_2O_2) that have both a role in pathology and cell signalling.

Box 4 | **The BCL2 family**

The anti-apoptotic action of members of the BCL2 family of proteins is important in oncogenic transformation[211,212]. The BCL2 family are divided into three classes: the anti-apoptotic BCL2 family members that include BCL2, BCL-X_L, BCL-W, BCL2A1 and myeloid cell leukaemia 1 (MCL1), the multi-domain pro-apoptotic proteins BCL2-associated X protein (BAX) and BCL2-antagonist/killer (BAK), and the 'BH3 (BCL2 homology 3)-only proteins', such as BIM (also known as BCL2L11), BH3-interacting domain death agonist (BID), BCL2-antagonist of cell death (BAD), NOXA (also known as PMAIP1) and PUMA (also known as BCL2 binding component 3 (BBC3))[101]. In viable cells, BAX and BAK are restrained by their binding to pro-survival BCL2 proteins. Two models for the role of BH3-only proteins have been proposed. The first 'passive model' involves the BH3-only proteins binding to BCL2 proteins, releasing BAX and BAK to promote cell death. The second, 'active model' suggests certain BH3-only proteins can also bind and activate BAX and BAK following their dissociation from BCL2 proteins[213–215]. The different BH3-only proteins respond to distinct forms of cellular stress and are subject to regulation at both the transcriptional and post-translational level[145,213,215,216]. The principal targets of BAX and BAK are the endoplasmic reticulum and mitochondria, which they permeabilize causing the release of pro-apoptotic proteins and ions[147]. Apoptosis is also initiated by extrinsic stimuli, which engage death receptors on the plasma membrane[100,217]. As a result, caspase 8 is activated, promoting activation of downstream executioner caspases[100]. This death receptor-initiated apoptotic pathway can also converge on the intrinsic mitochondrial apoptotic pathway through caspase 8 cleavage of BID to tBID, which then inserts into mitochondrial membranes causing release of mitochondrial pro-apoptotic factors[217].

BCL2 might also protect cells from death by modulating redox conditions, although the mechanism by which this is achieved is unclear[218]. This effect of BCL2 on cellular redox is consistent with the need of the cancer cell to control the generation of deleterious reactive oxygen species (ROS; for example, O_2^- and H_2O_2). Indeed, antioxidant enzymes such as glutathione S-transferase and thioredoxin are frequently upregulated in transformed cells[99]. Through these mechanisms cancer cells can perhaps harness the beneficial aspects of ROS and avoid their death-inducing qualities.

Remodelling Ca^{2+} signalling for survival

A tumour cell must harness the Ca^{2+} signalling machinery to promote proliferation yet protect itself from apoptosis. Owing to their principal roles in the control of cell death and Ca^{2+} signalling, the ER and mitochondria are at the frontline of this battle during oncogenic transformation, and are thus sites where significant remodelling of Ca^{2+} signalling apparatus occurs to limit death-inducing Ca^{2+} signals during cancer (FIG. 5b).

Regulation of Ca^{2+} flux by the BCL2 proteins. Virtually all cancer cells exhibit an increase in the expression of anti-apoptotic members of the BCL2 family of proteins, or decreased expression of the pro-apoptotic BH3-only proteins or BAX or BAK[147]. It has become apparent that the anti-apoptotic proteins BCL2 and BCL-X_L (also known as BCL2-like 1 (BCL2L1)) can inhibit apoptosis by modulating intracellular Ca^{2+} signals[109,111,148–151]. BCL2 diminishes the magnitude of Ca^{2+} fluxes emanating from the ER by either binding to and inhibiting $InsP_3Rs$ or by decreasing Ca^{2+} levels in the ER lumen[149–151]. Reduced ER Ca^{2+} levels and Ca^{2+} signals have also been reported in apoptosis-resistant *Bax*- and *Bak*-knockout mouse embryonic fibroblasts[111]. Interestingly, increasing ER Ca^{2+} levels in these cells by ectopic expression of SERCA2 rescues their sensitivity to death stimuli, demonstrating the significance of BCL2 proteins in regulating the ER–mitochondrial Ca^{2+} gateway and cell death[111]. BCL2 has also been reported to reduce ER Ca^{2+} by inhibition of SERCA2[152]. A consequence of chronically reduced ER Ca^{2+} levels mediated by BCL2 is a reduction in SOCE, which is downregulated as a result of its sustained activation[151,153]. Not all the effects of BCL2 on Ca^{2+} signalling are at the level of the ER; it appears to decrease the sensitivity of the mitochondrial uptake process as well as increasing their capacity to accumulate more Ca^{2+} (REFS 148,154). Thus, BCL2 can prevent Ca^{2+} uptake into the mitochondria from sources other than the mitochondria. Under certain conditions, both BCL2 and BCL-X_L also appear to enhance physiological Ca^{2+} signals, including Ca^{2+} oscillations, thus promoting cell proliferation and survival[155,156]. However, it remains to be determined how these pleiotropic proteins are able to control Ca^{2+} signals in such a way.

Ca^{2+} flux from the ER is also reduced by other mechanisms that serve to diminish ER Ca^{2+} levels. For example, some cancers exhibit reduced SERCA2 expression, either as a result of mutation or promoter methylation[61,69]. This oncogenic effect is recapitulated in *Serca1*$^{+/-}$ mice, which have an increased propensity to develop spontaneous tumours[157]. Squamous cell tumours also arise in mice as a result of deletion of one allele of the Golgi Ca^{2+}/Mn^{3+} pump *Spca1* (also known as *Atp2c1*)[158]. In humans, however, loss of one functional copy of this gene causes Hailey–Hailey disease, a skin disorder characterized by recurrent vesicles and erosions in the flexural areas[159]. The importance of Ca^{2+} pumps in cancer is described in greater detail elsewhere[60].

Regulation of $InsP_3R$ by PKB. The $InsP_3R$ is also regulated by the pro-survival PKB pathway[104]. The consensus site for phosphorylation by PKB has been identified at the carboxyl terminus of all three mammalian $InsP_3R$ isoforms and is conserved from mammals to flies[104,160]. This site is phosphorylated by PKB *in vitro* and in a PKB-dependent manner in cells following growth factor stimulation as well as under normal growth conditions. This phosphorylation event decreases $InsP_3$-stimulated Ca^{2+} release from the ER and so diminishes flux of Ca^{2+} to the mitochondria following stimulation with pro-apoptotic agonists, thereby reducing apoptosis[104]. Both LnCaP prostate cancer and U87 glioblastoma cell lines, which have deletions in *PTEN* and augmented PKB activity[161,162], exhibit increased PKB-dependent

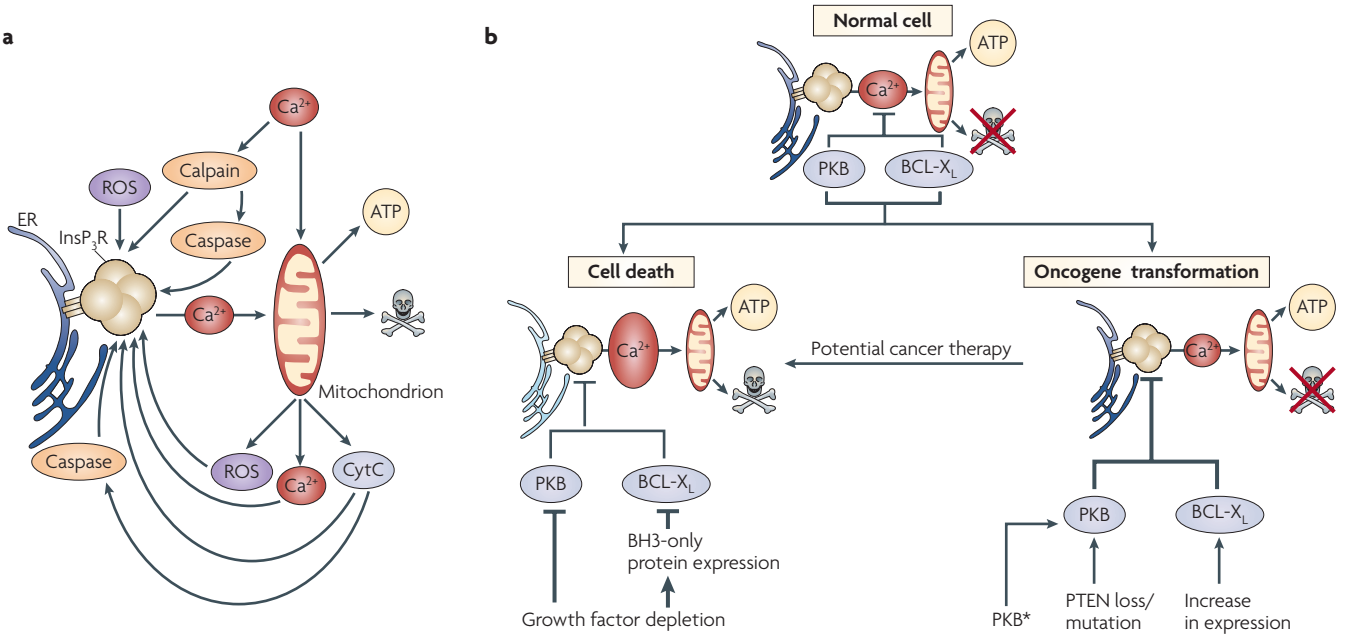

Figure 5 | **Apoptotic signals that induce endoplasmic reticulum (ER)–mitochondrial Ca^{2+} flux and their remodelling during cancer. a** | The transfer of Ca^{2+} from the ER to the mitochondria is a potent signal for death. A conduit for this Ca^{2+} transfer from the ER is the inositol 1,4,5-trisphosphate receptor ($InsP_3R$). $InsP_3Rs$ are sensitized by phosphorylation (cell division cycle 2 (CDC2)–cyclin B), reactive oxygen species (ROS) and Ca^{2+}. $InsP_3Rs$ are deregulated by caspase, calpain cleavage and/or binding of cytochrome *c* (CytC). As mitochondria release many of these $InsP_3R$ regulatory factors, a feed-forward loop is set up to amplify death signalling. **b** | Suppression of $InsP_3R$–mitochondrial Ca^{2+} flux during cancer. In naturally dividing cells experiencing normal levels of growth factor stimulation, $InsP_3Rs$ are tonically inhibited as a result of protein kinase B (PKB) phosphorylation and/or binding of BCL-X_L. Reduction of growth factors or cell stress causes decreased PKB activity and the induction of BH3 (BCL2 homology 3)-only proteins. As a result, $InsP_3Rs$ are no longer phosphorylated by PKB and the interaction with BCL-X_L is lost. Under these conditions, Ca^{2+} flux to the mitochondria is enhanced and cell death ensues. During cancer, BCL-X_L expression and/or PKB expression and/or activity are increased, resulting in greater inhibition of $InsP_3R$ activity and mitochondrial Ca^{2+} accumulation. Thus, cell death is prevented and oncogenesis progresses. Reversing the remodelling that occurs during cancer to suppress Ca^{2+} flux to the mitochondria might be a therapeutic opportunity. Indeed, this might be achieved with BH3 mimetics or PKB inhibitors.

phosphorylation of $InsP_3Rs$[104,160]. Indeed, as well as the increased PKB-dependent phosphorylation of $InsP_3Rs$, U87 cells exhibit decreased flux of Ca^{2+} from the ER to the mitochondria and decreased apoptosis compared with their isogenic derivatives in which PTEN is re-expressed[104] (FIG. 5b). These data suggest that this functional interaction between PKB and $InsP_3Rs$ is retained in tumour cells, endowing them with a significant survival advantage by limiting Ca^{2+}-dependent death signalling.

Remodelling of metabolic pathways can mitigate the cytotoxicity of Ca^{2+}. Normal cells produce most of their ATP from glucose through mitochondrial oxidative phosphorylation (Ox Phos), whereas tumour cells remodel their metabolome to use glycolysis with reduced Ox Phos, a phenomenon known as the Warburg effect[163]. The driver for this might be the increased glycolytic flux in proliferating cells coupled with the hypoxic environment of the tumour. The glycolytic shift might arise from somatic mutations in respiratory chain components or, more frequently, oncogene-dependent reprogramming of key metabolic enzymes. For example, loss of p53 and concomitant downregulation of synthesis of cytochrome *c* oxidase 2 (SCO2) impairs assembly of the COXII cytochrome *c* oxidase complex[155]. The glycolytic shift as a result of MYC-induced transformation might reflect direct transcriptional activation of lactate dehydrogenase by MYC, whereas cancers in which the PKB pathway is hyperactive exhibit changes in expression of glycolytic enzymes[164,165].

Given the decreased efficiency of ATP generation per glucose molecule (2 molecules for glycolysis versus 24 for Ox Phos), the shift to glycolysis must provide distinct advantages and these have been well documented elsewhere[164]. In the context of Ca^{2+} signalling and cell death a reduction in mitochondrial respiration is highly desirable during tumour progression. On the one hand, physiological levels of Ca^{2+} can stimulate substrate oxidation and phosphorylation in the mitochondria and this could be enhanced in tumour cells exhibiting increased Ca^{2+} signalling. However, mitochondrial ROS production arising from this facilitates Ca^{2+}-induced permeability transition pore opening, driving cytochrome *c* release, activation of the apoptosome and caspase-dependent cell death. In addition, ROS can promote further Ca^{2+} mobilization including Ca^{2+} entry (see above). Thus, a consequence of the glycolytic shift and reduction in ROS

production is a lower driving force for mitochondrial Ca^{2+} accumulation, resulting in reduced sensitivity to death-inducing signals. Furthermore, this mitigation of Ca^{2+}-induced toxicity mechanisms might allow the pro-survival effects of Ca^{2+} to be manifest, leading to enhanced tumour cell survival. For example, glioblastoma cells selectively upregulate Ca^{2+}-permeable AMPA receptor isoforms[166,167] and use glutamate-stimulated Ca^{2+} entry to activate PKB in a PI3K-independent manner. CaMK might be responsible for this PI3K-independent Ca^{2+}-induced activation of PKB[168].

Therapeutic opportunities

In this article, we have reviewed the role of Ca^{2+} as a key mediator of cell proliferation and an arbitrator of cell survival or death. To what extent can therapeutic strategies exploit these Ca^{2+}-regulated processes? Control of cancer cell proliferation by inhibitors of plasma membrane Ca^{2+} channels has received much attention and remains a potential strategy[60,166,167,169,170]. Inhibition of CaMKs could also prove to be a viable strategy for some tumour types. In MCF-7 breast cancer cells, pharmacological or molecular genetic inhibition of CaMKI inhibited growth, causing a G1 arrest[171], and inhibition of CaMKII inhibited the growth of osteosarcoma cells[172]. Inhibition of CaMK-dependent survival signalling might also prove to be effective in combination with certain other therapies. For example, the discovery that ROS could activate certain CaMK isoforms[173] led to the demonstration that CaMK inhibition augmented tumour cell death in response to cancer therapies that increase ROS (such as doxorubicin, ionizing radiation or photodynamic therapy)[174]. These studies indicate that while CaMK inhibition alone may be anti-proliferative, it might be more effective in tumour cell killing as an adjunct to other therapies.

Other approaches exploit the interface between Ca^{2+} signalling and apoptosis. Inhibition of the mitochondrial Na^+–Ca^{2+} exchanger by the benzothiazepin CGP-37157, to cause mitochondrial Ca^{2+} overload, did not induce cell death on its own but increased cell death 25-fold when used in combination with TNF-related apoptosis-inducing ligand (TRAIL, also known as TNFSF10)[175]. The BH3-mimetic ABT-737 binds to BCL2, BCL-X_L and BCL-W (also known as BCL2L2) with sub-nanomolar affinity, thereby inhibiting them[176]. Its efficacy as a single agent is limited, but combination with stressors that induce ER Ca^{2+} release or promote ER store loading might exploit the link between BCL2, BCL-X_L and $InsP_3R$[109] by promoting Ca^{2+}-dependent cell death. Similarly, PKB-dependent phosphorylation of $InsP_3R$[104] might be exploited by combining ER stressors that induce Ca^{2+} release with newly emerging PI3K inhibitors[177] (FIG. 5b).

The single biggest impediment to the success of such approaches is still a lack of understanding of which key Ca^{2+} channels or enzymes to inhibit in specific tumour types. For example, the Ca^{2+} channel blockers that are being considered for cancer therapy (such as mibefradil) were developed for other indications or have been identified in screens for inhibition of proliferation in normal cells and then applied to cancer cells. This means that, although there is already a rich pharmacology to tap into, there is no reason to believe these drugs are targeting Ca^{2+} channels or Ca^{2+}-regulated enzymes that are actually required for tumour maintenance because the identification of these drugs was not predicated on such considerations.

The 'oncogene addiction' hypothesis[178] suggests that among the array of genetic changes accumulated during tumour progression, tumour cells evolve an unusual dependence upon certain key mutations to maintain their malignant state. For example, the fact that deletion of activated KRAS in tumour cells renders them non-tumorigenic[179] or that restoration of wild-type p53 causes tumour regression[180,181] tells us that inhibition of the KRAS pathway and restoration of p53 function are attractive therapeutic strategies. This addiction or acquired dependency upon particular oncogenes and/or pathways often reflects a loss of pathway redundancy in the tumour cell compared with normal cells, providing a therapeutic window. We need to know precisely which components of the Ca^{2+} toolkit are upregulated or downregulated in which tumour types; which of these are 'drivers', which we might want to inhibit, rather than 'passengers', and which are validated as rate-limiting for tumour growth or survival (that is, have the tumours acquired an addiction to these Ca^{2+} signalling components). This information will allow development of assays to inhibit the appropriate targets, and it is this strategy that has led to the recent success stories in cancer therapy. For example, notwithstanding acquired resistance, imatinib was discovered and has proved successful because BCR–ABL is a driver mutation to which chronic myeloid leukaemia cells are addicted[182].

Closing remarks

Cellular transformation is supported on the one hand by Ca^{2+}-stimulated proliferation yet limited on the other by Ca^{2+}-dependent cell death. This might seem paradoxical but the examples of MYC and E2F, which also have effects on cell life and death, show that such contradictions are emerging as the norm and drive vital remodelling of tumour cell physiology. Indeed, the Ca^{2+} signalling proteome might be remodelled in cancer to sustain the malignant phenotype. For example, a relatively under-appreciated consequence of the Warburg glycolytic shift will be to reduce Ca^{2+}-dependent ROS production and resultant toxicity, allowing the pro-survival and pro-proliferative effects of Ca^{2+} signalling to be manifest. The extent to which transformed cells become addicted to this sort of remodelling remains to be fully appreciated. However, this is an important consideration as it will determine which components are prime targets for therapeutic intervention. In this sense, there are a variety of challenges ahead that require a coordinated approach. The use of ever more sophisticated Ca^{2+} imaging techniques should provide a more thorough spatial resolution of Ca^{2+} signalling in tumour cells. This can guide the expression of targeted Ca^{2+}-binding proteins, such as calbindin[183,184], to specific organelles to buffer discrete subcellular Ca^{2+} signals, and should tell us precisely which Ca^{2+} signals in which

cellular locales are required to support transformation. In parallel, application of the latest array, sequencing and proteomics technologies should define which components of the Ca^{2+} toolkit are remodelled in which tumour types. This can be done for primary tumour tissue but also for cell lines so that results can be correlated with the Ca^{2+} imaging and Ca^{2+} buffering studies above. To date, most efforts in this area have been piecemeal. What is required is a coordinated and focused analysis across large sample sizes for individual tumour types (primary tissue and cell lines) that will define the extent of Ca^{2+} toolkit remodelling, its importance to tumour cell viability and identification of the key drivers for transformation.

The question remains, however: how can cancer cells increase Ca^{2+} cycling to drive cell proliferation and avoid Ca^{2+}-dependent cell death? One attractive possibility is that Ca^{2+} influx drives cell proliferation and Ca^{2+} flux from the ER promotes cell death. The increased expression of plasma membrane Ca^{2+} channels and suppression of Ca^{2+} release channels by pro-survival pathways might support this idea. Moreover, mitochondria, which are central to cell death, do not accumulate Ca^{2+} that enters across the plasma membrane as efficiently as Ca^{2+} that is released from the ER. The uptake of Ca^{2+} by mitochondria may be further diminished by changes in their physiology occurring as a result of transformation.

1. Berridge, M. J., Lipp, P. & Bootman, M. D. The versatility and universality of calcium signalling. *Nature Rev. Mol. Cell Biol.* **1**, 11–21 (2000).
This reference is a good starting point to gain an understanding of the fundamentals of Ca^{2+} signalling. It describes the 'toolkit' and how specificity is encoded by Ca^{2+} signals.
2. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
3. Futreal, P. A., Wooster, R. & Stratton, M. R. Somatic mutations in human cancer: insights from resequencing the protein kinase gene family. *Cold Spring Harb. Symp. Quant. Biol.* **70**, 43–49 (2005).
4. De Koninck, P. & Schulman, H. Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations. *Science* **279**, 227–230 (1998).
This is one of the first papers to describe CaMK acting as a molecular machine to decode Ca^{2+} oscillations.
5. Dolmetsch, R. Excitation-transcription coupling: signaling by ion channels to the nucleus. *Sci. STKE* **2003**, PE4 (2003).
6. Dolmetsch, R. E., Xu, K. & Lewis, R. S. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* **392**, 933–936 (1998).
This was a seminal paper that described how information was conveyed by the frequency of Ca^{2+} oscillations and that Ca^{2+} oscillations were more efficient than sustained Ca^{2+} increases in promoting gene transcription.
7. Whitfield, J. F. Calcium signals and cancer. *Crit. Rev. Oncog.* **3**, 55–90 (1992).
8. Cook, S. J. & Lockyer, P. J. Recent advances in Ca^{2+}-dependent Ras regulation and cell proliferation. *Cell Calcium* **39**, 101–112 (2006).
9. Sjoblom, T. *et al.* The consensus coding sequences of human breast and colorectal cancers. *Science* **314**, 268–274 (2006).
10. Wood, L. D. *et al.* The genomic landscapes of human breast and colorectal cancers. *Science* **318**, 1108–1113 (2007).
11. Berridge, M. J., Bootman, M. D. & Roderick, H. L. Calcium signalling: dynamics, homeostasis and remodelling. *Nature Rev. Mol. Cell Biol.* **4**, 517–529 (2003).
This paper proposes the hypothesis that the Ca^{2+} signalling apparatus is remodelled during development and disease to change its own signalling capacity to suit the needs of the cell. In this way, a long-term consequence of a Ca^{2+} signal may be to remodel how it is generated.
12. Rizzuto, R. & Pozzan, T. Microdomains of intracellular Ca^{2+}: molecular determinants and functional consequences. *Physiol. Rev.* **86**, 369–408 (2006).
13. Pozzan, T., Rizzuto, R., Volpe, P. & Meldolesi, J. Molecular and cellular Physiol.ogy of intracellular calcium stores. *Physiol. Rev.* **74**, 595–636 (1994).
A good review of intracellular Ca^{2+} stores and organelles that have a role in Ca^{2+} homeostasis.
14. Missiaen, L. *et al.* Calcium release from the Golgi apparatus and the endoplasmic reticulum in HeLa cells stably expressing targeted aequorin to these compartments. *Cell Calcium* **36**, 479–487 (2004).
15. Churchill, G. C. *et al.* NAADP mobilizes Ca^{2+} from reserve granules, lysosome-related organelles, in sea urchin eggs. *Cell* **111**, 703–708 (2002).
16. Clapham, D. E. Calcium signaling. *Cell* **131**, 1047–1058 (2007).
A comprehensive contemporary review of Ca^{2+} signalling, which discusses the biophysics of the proteins involved in encoding and decoding Ca^{2+} signals.
17. Rhee, S. G. Regulation of phosphoinositide-specific phospholipase C. *Annu. Rev. Biochem.* **70**, 281–312 (2001).
18. Foskett, J. K., White, C., Cheung, K. H. & Mak, D. O. Inositol trisphosphate receptor Ca^{2+} release channels. *Physiol. Rev.* **87**, 593–658 (2007).
19. Lewis, R. S. The molecular choreography of a store-operated calcium channel. *Nature* **446**, 284–287 (2007).
A recent review that brings together the recent research and discovery of the molecular players that underlie store-operated Ca^{2+} entry.
20. Means, A. R. Regulatory cascades involving calmodulin-dependent protein kinases. *Mol. Endocrinol.* **14**, 4–13 (2000).
21. Klee, C. B., Ren, H. & Wang, X. Regulation of the calmodulin-stimulated protein phosphatase, calcineurin. *J. Biol. Chem.* **273**, 13367–13370 (1998).
22. Zhang, T. *et al.* CaMKIIδ isoforms differentially affect calcium handling but similarly regulate HDAC/MEF2 transcriptional responses. *J. Biol. Chem.* **282**, 35078–35087 (2007).
23. Pande, G., Kumar, N. A. & Manogaran, P. S. Flow cytometric study of changes in the intracellular free calcium during the cell cycle. *Cytometry* **24**, 55–63 (1996).
24. Kahl, C. R. & Means, A. R. Regulation of cell cycle progression by calcium/calmodulin-dependent pathways. *Endocr. Rev.* **24**, 719–736 (2003).
25. Takuwa, N., Zhou, W., Kumada, M. & Takuwa, Y. Ca^{2+}-dependent stimulation of retinoblastoma gene product phosphorylation and p34cdc2 kinase activation in serum-stimulated human fibroblasts. *J. Biol. Chem.* **268**, 138–145 (1993).
26. Rasmussen, C. D. & Means, A. R. Calmodulin is required for cell-cycle progression during G1 and mitosis. *EMBO J.* **8**, 73–82 (1989).
References 25 and 26 were two of the first to show by pharmacological and molecular genetic approaches that Ca^{2+}- and CaM-dependent signalling is required for RB phosphorylation and cell-cycle progression through G1 and mitosis.
27. Tombes, R. M., Grant, S., Westin, E. H. & Krystal, G. G1 cell cycle arrest and apoptosis are induced in NIH 3T3 cells by KN-93, an inhibitor of CaMK-II (the multifunctional Ca^{2+}/CaM kinase). *Cell Growth Differ.* **6**, 1063–1070 (1995).
28. Morris, T. A., DeLorenzo, R. J. & Tombes, R. M. CaMK-II inhibition reduces cyclin D1 levels and enhances the association of p27kip1 with Cdk2 to cause G1 arrest in NIH 3T3 cells. *Exp Cell Res.* **240**, 218–227 (1998).
29. Kahl, C. R. & Means, A. R. Regulation of cyclin D1/Cdk4 complexes by calcium/calmodulin-dependent protein kinase I. *J. Biol. Chem.* **279**, 15411–15419 (2004).
30. Khanna, A. K. & Hosenpud, J. D. Cyclosporine induces the expression of the cyclin inhibitor p21. *Transplantation* **67**, 1262–1268 (1999).
31. Tomono, M., Toyoshima, K., Ito, M., Amano, H. & Kiss, Z. Inhibitors of calcineurin block expression of cyclins A and E induced by fibroblast growth factor in Swiss 3T3 fibroblasts. *Arch. Biochem. Biophys.* **353**, 374–378 (1998).
32. Kahl, C. R. & Means, A. R. Calcineurin regulates cyclin D1 accumulation in growth-stimulated fibroblasts. *Mol. Biol. Cell* **15**, 1833–1842 (2004).
33. Schneider, G. *et al.* Cyclosporine inhibits growth through the activating transcription factor/cAMP-responsive element-binding protein binding site in the cyclin D1 promoter. *J. Biol. Chem.* **277**, 43599–43607 (2002).
References 27–33 define the role of CaMK and calcineurin in controlling expression and activation of the G1 CDKs.
34. Hogan, P. G., Chen, L., Nardone, J. & Rao, A. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev.* **17**, 2205–2232 (2003).
35. Gwack, Y., Feske, S., Srikanth, S., Hogan, P. G. & Rao, A. Signalling to transcription: store-operated Ca^{2+} entry and NFAT activation in lymphocytes. *Cell Calcium* **42**, 145–156 (2007).
36. Lehen'kyi, V., Flourakis, M., Skryma, R. & Prevarskaya, N. TRPV6 channel controls prostate cancer cell proliferation via Ca^{2+}/NFAT-dependent pathways. *Oncogene* **26**, 7380–7385 (2007).
37. Thebault, S. *et al.* Differential role of transient receptor potential channels in Ca^{2+} entry and proliferation of prostate cancer epithelial cells. *Cancer Res.* **66**, 2038–2047 (2006).
38. Fixemer, T., Wissenbach, U., Flockerzi, V. & Bonkhoff, H. Expression of the Ca^{2+}-selective cation channel TRPV6 in human prostate cancer: a novel prognostic marker for tumor progression. *Oncogene* **22**, 7858–7861 (2003).
39. van Abel, M., Hoenderop, J. G. & Bindels, R. J. The epithelial calcium channels TRPV5 and TRPV6: regulation and implications for disease. *Naunyn Schmiedebergs Arch. Pharmacol.* **371**, 295–306 (2005).
40. Neal, J. W. & Clipstone, N. A. A constitutively active NFATc1 mutant induces a transformed phenotype in 3T3-L1 fibroblasts. *J. Biol. Chem.* **278**, 17246–17254 (2003).
41. Buchholz, M. *et al.* Overexpression of c-myc in pancreatic cancer caused by ectopic activation of NFATc1 and the Ca^{2+}/calcineurin signaling pathway. *EMBO J.* **25**, 3714–3724 (2006).
42. Ma, Z. *et al.* Interaction between ROCK II and nucleophosmin/B23 in the regulation of centrosome duplication. *Mol. Cell Biol.* **26**, 9016–9034 (2006).
43. Fisk, H. A., Mattison, C. P. & Winey, M. Human Mps1 protein kinase is required for centrosome duplication and normal mitotic progression. *Proc. Natl Acad. Sci. USA* **100**, 14875–14880 (2003).
44. Fukasawa, K. Oncogenes and tumour suppressors take on centrosomes. *Nature Rev. Cancer* **7**, 911–924 (2007).
45. Flory, M. R., Moser, M. J., Monnat, R. J., Jr. & Davis, T. N. Identification of a human centrosomal calmodulin-binding protein that shares homology with pericentrin. *Proc. Natl Acad. Sci. USA* **97**, 5919–5923 (2000).
46. Matsumoto, Y. & Maller, J. L. Calcium, calmodulin, and CaMKII requirement for initiation of centrosome duplication in Xenopus egg extracts. *Science* **295**, 499–502 (2002).
An important study demonstrating the requirement for Ca^{2+}, CaM and CaMKII in the initiation of centrosome duplication.

47. Chen, Z., Indjeian, V. B., McManus, M., Wang, L. & Dynlacht, B. D. CP110, a cell cycle-dependent CDK substrate, regulates centrosome duplication in human cells. *Dev. Cell* **3**, 339–350 (2002).
48. Tsang, W. Y. *et al.* CP110 cooperates with two calcium-binding proteins to regulate cytokinesis and genome stability. *Mol. Biol. Cell* **17**, 3423–3434 (2006).
49. Balk, S. D. Calcium as a regulator of the proliferation of normal, but not of transformed, chicken fibroblasts in a plasma-containing medium. *Proc. Natl Acad. Sci. USA* **68**, 271–275 (1971).
A landmark study, one of the earliest demonstrating that cell transformation by oncogenes (in this case SRC) reduces the requirement for Ca^{2+} for proliferation. This and subsequent studies (50–55) led to the widely held notion that malignant transformation obviates the requirement for Ca^{2+} in cell proliferation.
50. Boynton, A. L. & Whitfield, J. F. Different calcium requirements for proliferation of conditionally and unconditionally tumorigenic mouse cells. *Proc. Natl Acad. Sci. USA* **73**, 1651–1654 (1976).
51. Boynton, A. L., Whitfield, J. F., Isaacs, R. J. & Tremblay, R. The control of human WI-38 cell proliferation by extracellular calcium and its elimination by SV-40 virus-induced proliferative transformation. *J. Cell Physiol.* **92**, 241–247 (1977).
52. Fisher, P. B. & Weinstein, I. B. Enhancement of cell proliferation in low calcium medium by tumor promoters. *Carcinogenesis* **2**, 89–95 (1981).
53. Pei, X. F., Sherman, L., Sun, Y. H. & Schlegel, R. HPV-16 E7 protein bypasses keratinocyte growth inhibition by serum and calcium. *Carcinogenesis* **19**, 1481–1486 (1998).
54. Swierenga, S. H., Whitfield, J. F. & Karasaki, S. Loss of proliferative calcium dependence: simple *in vitro* indicator of tumorigenicity. *Proc. Natl Acad. Sci. USA* **75**, 6069–6072 (1978).
55. Mittnacht, S., Paterson, H., Olson, M. F. & Marshall, C. J. Ras signalling is required for inactivation of the tumour suppressor pRb cell-cycle control protein. *Curr. Biol.* **7**, 219–221 (1997).
56. D'Abaco, G. M., Hooper, S., Paterson, H. & Marshall, C. J. Loss of Rb overrides the requirement for ERK activity for cell proliferation. *J. Cell Sci* **115**, 4607–4616 (2002).
57. Legrand, G. *et al.* Ca^{2+} pools and cell growth. Evidence for sarcoendoplasmic Ca^{2+}-ATPases 2B involvement in human prostate cancer cell growth control. *J. Biol. Chem.* **276**, 47608–47614 (2001).
A notable demonstration that proliferation of some tumour cell lines remains intimately linked to the size of the ER Ca^{2+} store.
58. Panner, A. & Wurster, R. D. T-type calcium channels and tumor proliferation. *Cell Calcium* **40**, 253–259 (2006).
59. Panner, A. *et al.* Variation of T-type calcium channel protein expression affects cell division of cultured tumor cells. *Cell Calcium* **37**, 105–119 (2005).
60. Monteith, G. R., McAndrew, D., Faddy, H. M. & Roberts-Thomson, S. J. Calcium and cancer: targeting Ca^{2+} transport. *Nature Rev. Cancer* **7**, 519–530 (2007).
61. Korosec, B., Glavac, D., Rott, T. & Ravnik-Glavac, M. Alterations in the ATP2A2 gene in correlation with colon and lung cancer. *Cancer Genet. Cytogenet.* **171**, 105–111 (2006).
62. Prasad, V. *et al.* Haploinsufficiency of Atp2a2, encoding the sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoform 2 Ca^{2+} pump, predisposes mice to squamous cell tumors via a novel mode of cancer susceptibility. *Cancer Res.* **65**, 8655–8661 (2005).
References 61 and 62 demonstrate that the SERCA Ca^{2+} pump, *ATP2A2*, which is subject to germline mutation in colon and lung cancer, is a haploinsufficient tumour suppressor gene in mice.
63. Wakelam, M. J. *et al.* Normal p21N-ras couples bombesin and other growth factor receptors to inositol phosphate production. *Nature* **323**, 173–176 (1986).
64. Lang, F. *et al.* Bradykinin-induced oscillations of cell membrane potential in cells expressing the Ha-ras oncogene. *J. Biol. Chem.* **266**, 4938–4942 (1991).
65. Hashii, M., Nozawa, Y. & Higashida, H. Bradykinin-induced cytosolic Ca^{2+} oscillations and inositol tetrakisphosphate-induced Ca^{2+} influx in voltage-clamped ras-transformed NIH/3T3 fibroblasts. *J. Biol. Chem.* **268**, 19403–19410 (1993).
66. Bunney, T. D. & Katan, M. Phospholipase C ε: linking second messengers and small GTPases. *Trends Cell Biol.* **16**, 640–648 (2006).
67. Liu, W. W., Mattingly, R. R. & Garrison, J. C. Transformation of Rat-1 fibroblasts with the v-src oncogene increases the tyrosine phosphorylation state and activity of the α subunit of Gq/G11. *Proc. Natl Acad. Sci. USA* **93**, 8258–8263 (1996).
68. Habib, T. *et al.* Myc stimulates B lymphocyte differentiation and amplifies calcium signaling. *J. Cell Biol.* **179**, 717–731 (2007).
69. Endo, Y. *et al.* Sarcoendoplasmic reticulum Ca^{2+} ATPase type 2 downregulated in human oral squamous cell carcinoma. *Int. J. Cancer* **110**, 225–231 (2004).
70. Saito, K. *et al.* Plasma membrane Ca^{2+} ATPase isoform 1 down-regulated in human oral cancer. *Oncol. Rep.* **15**, 49–55 (2006).
71. Toyota, M., Ho, C., Ohe-Toyota, M., Baylin, S. B. & Issa, J. P. Inactivation of CACNA1G, a T-type calcium channel gene, by aberrant methylation of its 5′ CpG island in human tumors. *Cancer Res.* **59**, 4535–4541 (1999).
72. Zuber, J. *et al.* A genome-wide survey of RAS transformation targets. *Nature Genet.* **24**, 144–152 (2000).
73. Schulze, A., Lehmann, K., Jefferies, H. B., McMahon, M. & Downward, J. Analysis of the transcriptional program induced by Raf in epithelial cells. *Genes Dev.* **15**, 981–994 (2001).
74. Desai, K. V. *et al.* Initiating oncogenic event determines gene-expression patterns of human breast cancer models. *Proc. Natl Acad. Sci. USA* **99**, 6967–6972 (2002).
75. Heighway, J., Betticher, D. C., Hoban, P. R., Altermatt, H. J. & Cowen, R. Coamplification in tumors of *KRAS2*, type 2 inositol 1, 4, 5 triphosphate receptor gene, and a novel human gene, *KRAG*. *Genomics* **35**, 207–214 (1996).
76. Emberley, E. D., Murphy, L. C. & Watson, P. H. S100 proteins and their influence on pro-survival pathways in cancer. *Biochem. Cell Biol.* **82**, 508–515 (2004).
77. Nagy, N. *et al.* S100A2, a putative tumor suppressor gene, regulates *in vitro* squamous cell carcinoma migration. *Lab. Invest.* **81**, 599–612 (2001).
78. Cullen, P. J. & Lockyer, P. J. Integration of calcium and Ras signalling. *Nature Rev. Mol. Cell Biol.* **3**, 339–348 (2002).
79. Downward, J. Control of ras activation. *Cancer Surv.* **27**, 87–100 (1996).
80. Donovan, S., Shannon, K. M. & Bollag, G. GTPase activating proteins: critical regulators of intracellular signaling. *Biochim. Biophys. Acta* **1602**, 23–45 (2002).
81. Lockyer, P. J., Kupzig, S. & Cullen, P. J. CAPRI regulates Ca^{2+}-dependent inactivation of the Ras-MAPK pathway. *Curr. Biol.* **11**, 981–986 (2001).
82. Walker, S. A. *et al.* Identification of a Ras GTPase-activating protein regulated by receptor-mediated Ca^{2+} oscillations. *EMBO J.* **23**, 1749–1760 (2004).
83. Liu, Q. *et al.* CAPRI and RASAL impose different modes of information processing on Ras due to contrasting temporal filtering of Ca^{2+}. *J. Cell Biol.* **170**, 183–190 (2005).
Demonstration that CAPRI and RASAL, two Ca^{2+}-regulated Ras GAPS, respond to qualitatively different Ca^{2+} signals to inactivate Ras.
84. Bos, J. L. *ras* oncogenes in human cancer: a review. *Cancer Res.* **49**, 4682–4689 (1989).
85. Cichowski, K. & Jacks, T. *NF1* tumor suppressor gene function: narrowing the GAP. *Cell* **104**, 593–604 (2001).
86. Friedman, E., Gejman, P. V., Martin, G. A. & McCormick, F. Nonsense mutations in the C-terminal SH2 region of the GTPase activating protein (GAP) gene in human tumours. *Nature Genet.* **5**, 242–247 (1993).
87. Westbrook, T. F. *et al.* A genetic screen for candidate tumor suppressors identifies REST. *Cell* **121**, 837–848 (2005).
88. Kratz, C. P. *et al.* Candidate gene isolation and comparative analysis of a commonly deleted segment of 7q22 implicated in myeloid malignancies. *Genomics* **77**, 171–180 (2001).
89. Kolfschoten, I. G. *et al.* A genetic screen identifies PITX1 as a suppressor of RAS activity and tumorigenicity. *Cell* **121**, 849–858 (2005).
90. Jin, H. *et al.* Epigenetic silencing of a Ca^{2+}-regulated Ras GTPase-activating protein RASAL defines a new mechanism of Ras activation in human cancers. *Proc. Natl Acad. Sci. USA* **104**, 12353–12358 (2007).
Demonstration that RASAL expression is silenced by CpG island methylation in certain tumour types, providing an alternative mechanism for Ras activation in cancer.
91. Woods, D. *et al.* Raf-induced proliferation or cell cycle arrest is determined by the level of Raf activity with arrest mediated by p21Cip1. *Mol. Cell Biol.* **17**, 5598–5611 (1997).
92. Marshall, C. J. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80**, 179–185 (1995).
93. Balmanno, K. & Cook, S. J. Sustained MAP kinase activation is required for the expression of cyclin D1, $p21^{Cip1}$ and a subset of AP-1 proteins in CCL39 cells. *Oncogene* **18**, 3085–3097 (1999).
94. Bosch, M., Gil, J., Bachs, O. & Agell, N. Calmodulin inhibitor W13 induces sustained activation of ERK2 and expression of $p21^{cip1}$. *J. Biol. Chem.* **273**, 22145–22150 (1998).
95. Agell, N., Bachs, O., Rocamora, N. & Villalonga, P. Modulation of the Ras/Raf/MEK/ERK pathway by Ca^{2+}, and calmodulin. *Cell Signal.* **14**, 649–654 (2002).
96. Cook, S. J., Beltman, J., Cadwallader, K. A., McMahon, M. & McCormick, F. Regulation of mitogen-activated protein kinase phosphatase-1 expression by extracellular signal-related kinase-dependent and Ca^{2+}-dependent signal pathways in Rat-1 cells. *J. Biol. Chem.* **272**, 13309–13319 (1997).
97. Huang, C. *et al.* Calcineurin-mediated dephosphorylation of c-Jun Ser-243 is required for c-Jun protein stability and cell transformation. *Oncogene* 22 Oct 2007 (doi: 10.1038/sj.onc.1210888).
98. Lastro, M., Kourtidis, A., Farley, K. & Conklin, D. S. xCT expression reduces the early cell cycle requirement for calcium signaling. *Cell Signal.* **20**, 390–399 (2008).
99. Townsend, D. M., Findlay, V. L. & Tew, K. D. Glutathione S-transferases as regulators of kinase pathways and anticancer drug targets. *Methods Enzymol.* **401**, 287–307 (2005).
100. Green, D. R. & Evan, G. I. A matter of life and death. *Cancer Cell* **1**, 19–30 (2002).
101. Cory, S. & Adams, J. M. The Bcl2 family: regulators of the cellular life-or-death switch. *Nature Rev. Cancer* **2**, 647–656 (2002).
A comprehensive, at the time of print, resource on the Bcl2 family members and their functions.
102. Edinger, A. L. & Thompson, C. B. Death by design: apoptosis, necrosis and autophagy. *Curr. Opin. Cell Biol.* **16**, 663–669 (2004).
A good resource for information on the cell death process.
103. Orrenius, S., Zhivotovsky, B. & Nicotera, P. Regulation of cell death: the calcium-apoptosis link. *Nature Rev. Mol. Cell Biol.* **4**, 552–565 (2003).
An excellent review that describes the mechanisms by which Ca^{2+} can induce cell death.
104. Szado, T. *et al.* Phosphorylation of inositol 1, 4, 5-trisphosphate receptors by protein kinase B/Akt inhibits Ca^{2+} release and apoptosis. *Proc. Natl Acad. Sci. USA* 4 Feb 2008 (doi:10.1073/pnas.0711324105).
105. Miyamoto, S., Howes, A. L., Adams, J. W., Dorn, G. W. 2nd & Brown, J. H. Ca^{2+} dysregulation induces mitochondrial depolarization and apoptosis: role of Na^{+}/Ca^{2+} exchanger and AKT. *J. Biol. Chem.* **280**, 38505–38512 (2005).
106. Gerasimenko, J. V. *et al.* Menadione-induced apoptosis: roles of cytosolic Ca^{2+} elevations and the mitochondrial permeability transition pore. *J. Cell Sci* **115**, 485–497 (2002).
107. Boehning, D. *et al.* Cytochrome c binds to inositol (1, 4, 5) trisphosphate receptors, amplifying calcium-dependent apoptosis. *Nature Cell Biol.* **5**, 1051–1061 (2003).
108. Dowd, D. R., MacDonald, P. N., Komm, B. S., Haussler, M. R. & Miesfeld, R. L. Stable expression of the calbindin-D28K complementary DNA interferes with the apoptotic pathway in lymphocytes. *Mol. Endocrinol.* **6**, 1843–1848 (1992).
109. Rong, Y. & Distelhorst, C. W. Bcl-2 protein family members: versatile regulators of calcium signaling in cell survival and apoptosis. *Annu Rev. Physiol.* **70**, 73–91 (2008).
A balanced view of the mechanisms by which BCL2 and Ca^{2+} signalling interacts to control cell death and survival.
110. Pinton, P. *et al.* The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the

molecular mechanism of Bcl-2 action. *EMBO J.* **20**, 2690–2701 (2001).
111. Scorrano, L. *et al.* BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis. *Science* **300**, 135–139 (2003).
A seminal paper describing the mechanism by which BCL2 family members regulate cell death.
112. Sugawara, H., Kurosaki, M., Takata, M. & Kurosaki, T. Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1, 4, 5-trisphosphate receptors in signal transduction through the B-cell antigen receptor. *EMBO J.* **16**, 3078–3088 (1997).
One of the first papers to definitively describe the significant contribution of Ca^{2+} release through $InsP_3Rs$ in cell death processes.
113. Jayaraman, T. & Marks, A. R. T cells deficient in inositol 1, 4, 5-trisphosphate receptor are resistant to apoptosis. *Mol. Cell Biol.* **17**, 3005–3012 (1997).
114. Hirota, J., Furuichi, T. & Mikoshiba, K. Inositol 1, 4, 5-trisphosphate receptor type 1 is a substrate for caspase-3 and is cleaved during apoptosis in a caspase-3-dependent manner. *J. Biol. Chem.* **274**, 34433–34437 (1999).
115. Assefa, Z. *et al.* Caspase-3-induced truncation of type 1 inositol trisphosphate receptor accelerates apoptotic cell death and induces inositol trisphosphate-independent calcium release during apoptosis. *J. Biol. Chem.* **279**, 43227–43236 (2004).
116. Hajnoczky, G., Csordas, G., Madesh, M. & Pacher, P. Control of apoptosis by IP_3 and ryanodine receptor driven calcium signals. *Cell Calcium* **28**, 349–363 (2000).
117. Csordas, G. *et al.* Structural and functional features and significance of the physical linkage between ER and mitochondria. *J. Cell Biol.* **174**, 915–921 (2006).
118. Rizzuto, R., Duchen, M. R. & Pozzan, T. Flirting in little space: the ER/mitochondria Ca^{2+} liaison. *Sci. STKE* **2004**, re1 (2004).
119. Rizzuto, R., Brini, M., Murgia, M. & Pozzan, T. Microdomains with high Ca^{2+} close to IP3-sensitive channels that are sensed by neighboring mitochondria. *Science* **262**, 744–747 (1993).
A groundbreaking paper that elegantly described Ca^{2+} flux in a microdomain between the ER and mitochondria.
120. Hanson, C. J., Bootman, M. D. & Roderick, H. L. Cell signalling: IP_3 receptors channel calcium into cell death. *Curr. Biol.* **14**, R933–935 (2004).
121. Csordas, G., Thomas, A. P. & Hajnoczky, G. Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. *EMBO J.* **18**, 96–108 (1999).
122. Szalai, G., Krishnamurthy, R. & Hajnoczky, G. Apoptosis driven by IP_3-linked mitochondrial calcium signals. *EMBO J.* **18**, 6349–6361 (1999).
An elegant characterization of stimulus-dependent $InsP_3$-induced Ca^{2+} flux between the ER and mitochondria promoting apoptosis.
123. Szabadkai, G. *et al.* Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels. *J. Cell Biol.* **175**, 901–911 (2006).
124. Hayashi, T. & Su, T. P. Sigma-1 receptor chaperones at the ER–mitochondrion interface regulate Ca^{2+} signaling and cell survival. *Cell* **131**, 596–610 (2007).
125. Rapizzi, E. *et al.* Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca^{2+} microdomains to mitochondria. *J. Cell Biol.* **159**, 613–624 (2002).
126. Ott, M., Gogvadze, V., Orrenius, S. & Zhivotovsky, B. Mitochondria, oxidative stress and cell death. *Apoptosis* **12**, 913–922 (2007).
127. Giorgio, M. *et al.* Electron transfer between cytochrome *c* and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* **122**, 221–233 (2005).
128. Pinton, P. *et al.* Protein kinase C β and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. *Science* **315**, 659–663 (2007).
129. Hong, J. H. *et al.* Critical role of phospholipase Cγ1 in the generation of H_2O_2-evoked $[Ca^{2+}]_i$ oscillations in cultured rat cortical astrocytes. *J. Biol. Chem.* **281**, 13057–13067 (2006).
130. Madesh, M. *et al.* Selective role for superoxide in $InsP_3$ receptor-mediated mitochondrial dysfunction and endothelial apoptosis. *J. Cell Biol.* **170**, 1079–1090 (2005).
131. Camello-Almaraz, C., Gomez-Pinilla, P. J., Pozo, M. J. & Camello, P. J. Mitochondrial reactive oxygen species and Ca^{2+} signaling. *Am J. Physiol. Cell Physiol.* **291**, C1082–1088 (2006).
132. Aarts, M. *et al.* A key role for TRPM7 channels in anoxic neuronal death. *Cell* **115**, 863–877 (2003).
133. Martin, D., Salinas, M., Fujita, N., Tsuruo, T. & Cuadrado, A. Ceramide and reactive oxygen species generated by H_2O_2 induce caspase-3-independent degradation of Akt/protein kinase B. *J. Biol. Chem.* **277**, 42943–42952 (2002).
134. Leslie, N. R. *et al.* Redox regulation of PI 3-kinase signalling via inactivation of PTEN. *EMBO J.* **22**, 5501–5510 (2003).
135. Breckenridge, D. G., Germain, M., Mathai, J. P., Nguyen, M. & Shore, G. C. Regulation of apoptosis by endoplasmic reticulum pathways. *Oncogene* **22**, 8608–8618 (2003).
136. Moenner, M., Pluquet, O., Bouchecareilh, M. & Chevet, E. Integrated endoplasmic reticulum stress responses in cancer. *Cancer Res.* **67**, 10631–10634 (2007).
137. Delom, F., Fessart, D. & Chevet, E. Regulation of calnexin sub-cellular localization modulates endoplasmic reticulum stress-induced apoptosis in MCF-7 cells. *Apoptosis* **12**, 293–305 (2007).
138. Breckenridge, D. G., Stojanovic, M., Marcellus, R. C. & Shore, G. C. Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome *c* release to the cytosol. *J. Cell Biol.* **160**, 1115–1127 (2003).
139. Nishitoh, H. *et al.* ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev* **16**, 1345–1355 (2002).
140. Takeda, K. *et al.* Involvement of ASK1 in Ca^{2+}-induced p38 MAP kinase activation. *EMBO Rep.* **5**, 161–166 (2004).
141. Gomez-Vicente, V., Donovan, M. & Cotter, T. G. Multiple death pathways in retina-derived 661W cells following growth factor deprivation: crosstalk between caspases and calpains. *Cell Death Differ.* **12**, 796–804 (2005).
142. Nakagawa, T. & Yuan, J. Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis. *J. Cell Biol.* **150**, 887–894 (2000).
143. Nicotera, P. & Bano, D. The enemy at the gates. Ca^{2+} entry through TRPM7 channels and anoxic neuronal death. *Cell* **115**, 768–770 (2003).
144. Gil-Parrado, S. *et al.* Ionomycin-activated calpain triggers apoptosis. A probable role for Bcl-2 family members. *J. Biol. Chem.* **277**, 27217–27226 (2002).
145. Wang, H. G. *et al.* Ca^{2+}-induced apoptosis through calcineurin dephosphorylation of BAD. *Science* **284**, 339–343 (1999).
An important paper demonstrating the potentially toxic effects of Ca^{2+} and calcineurin: promotion of apoptosis through activation of BAD.
146. la Cour, J. M. *et al.* Up-regulation of ALG-2 in hepatomas and lung cancer tissue. *Am J. Pathol* **163**, 81–89 (2003).
147. Danial, N. N. & Korsmeyer, S. J. Cell death: critical control points. *Cell* **116**, 205–219 (2004).
148. Hanson, C. J., Bootman, M. D., Distelhorst, C. W., Wojcikiewicz, R. J. & Roderick, H. L. Bcl-2 associates with inositol 1, 4, 5-trisphosphate receptors, reduces inositol 1, 4, 5-trisphosphate- evoked calcium release from the endoplasmic reticulum and inhibits mitochondrial calcium uptake, but does not alter calcium store content. *Cell Calcium* (in the press).
149. Foyouzi-Youssefi, R. *et al.* Bcl-2 decreases the free Ca^{2+} concentration within the endoplasmic reticulum. *Proc. Natl Acad. Sci. USA* **97**, 5723–5728 (2000).
150. Chen, R. *et al.* Bcl-2 functionally interacts with inositol 1, 4, 5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1, 4, 5-trisphosphate. *J. Cell Biol.* **166**, 193–203 (2004).
151. Pinton, P. *et al.* Reduced loading of intracellular Ca^{2+} stores and downregulation of capacitative Ca^{2+} influx in Bcl-2-overexpressing cells. *J. Cell Biol.* **148**, 857–862 (2000).
One of the first papers to describe that BCL2 inhibited apoptosis induced by decreased ER Ca^{2+} content.
152. Dremina, E. S. *et al.* Anti-apoptotic protein Bcl-2 interacts with and destabilizes the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). *Biochem J.* **383**, 361–370 (2004).
153. Vanden Abeele, F. *et al.* Bcl-2-dependent modulation of Ca^{2+} homeostasis and store-operated channels in prostate cancer cells. *Cancer Cell* **1**, 169–179 (2002).
154. Murphy, A. N., Bredesen, D. E., Cortopassi, G., Wang, E. & Fiskum, G. Bcl-2 potentiates the maximal calcium uptake capacity of neural cell mitochondria. *Proc. Natl Acad. Sci. USA* **93**, 9893–9898 (1996).
155. Zhong, F., Davis, M. C., McColl, K. S. & Distelhorst, C. W. Bcl-2 differentially regulates Ca^{2+} signals according to the strength of T cell receptor activation. *J. Cell Biol.* **172**, 127–137 (2006).
156. White, C. *et al.* The endoplasmic reticulum gateway to apoptosis by Bcl-X_L modulation of the $InsP_3R$. *Nature Cell Biol.* **7**, 1021–1028 (2005).
157. Liu, L. H., Boivin, G. P., Prasad, V., Periasamy, M. & Shull, G. E. Squamous cell tumors in mice heterozygous for a null allele of Atp2a2, encoding the sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoform 2 Ca^{2+} pump. *J. Biol. Chem.* **276**, 26737–26740 (2001).
158. Okunade, G. W. *et al.* Loss of the Atp2c1 secretory pathway Ca^{2+}-ATPase (SPCA1) in mice causes Golgi stress, apoptosis, and midgestational death in homozygous embryos and squamous cell tumors in adult heterozygotes. *J. Biol. Chem.* **282**, 26517–26527 (2007).
159. Missiaen, L. *et al.* SPCA1 pumps and Hailey–Hailey disease. *Biochem. Biophys. Res. Commun.* **322**, 1204–1213 (2004).
160. Khan, M. T., Wagner, L. 2nd, Yule, D. I., Bhanumathy, C. & Joseph, S. K. Akt kinase phosphorylation of inositol 1, 4, 5-trisphosphate receptors. *J. Biol. Chem.* **281**, 3731–3737 (2006).
161. Cheney, I. W. *et al.* Suppression of tumorigenicity of glioblastoma cells by adenovirus-mediated *MMAC1/PTEN* gene transfer. *Cancer Res.* **58**, 2331–2334 (1998).
162. Li, D. M. & Sun, H. PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cycle arrest in human glioblastoma cells. *Proc. Natl Acad. Sci. USA* **95**, 15406–15411 (1998).
163. Meier, P. & Vousden, K. H. Lucifer's labyrinth — ten years of path finding in cell death. *Mol. Cell* **28**, 746–754 (2007).
A contemporary review of pathways involved in controlling cell death.
164. Deberardinis, R. J., Lum, J. J., Hatzivassiliou, G. & Thompson, C. B. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab.* **7**, 11–20 (2008).
165. Racker, E., Resnick, R. J. & Feldman, R. Glycolysis and methylaminoisobutyrate uptake in rat-1 cells transfected with ras or myc oncogenes. *Proc. Natl Acad. Sci. USA* **82**, 3535–3538 (1985).
166. Ishiuchi, S. *et al.* Ca^{2+}-permeable AMPA receptors regulate growth of human glioblastoma via Akt activation. *J. Neurosci.* **27**, 7987–8001 (2007).
167. Ishiuchi, S. *et al.* Blockage of Ca^{2+}-permeable AMPA receptors suppresses migration and induces apoptosis in human glioblastoma cells. *Nature Med.* **8**, 971–978 (2002).
168. Yano, S., Tokumitsu, H. & Soderling, T. R. Calcium promotes cell survival through CaM-K kinase activation of the protein-kinase-B pathway. *Nature* **396**, 584–587 (1998).
169. Gray, L. S. & Macdonald, T. L. The pharmacology and regulation of T type calcium channels: new opportunities for unique therapeutics for cancer. *Cell Calcium* **40**, 115–120 (2006).
170. Enfissi, A., Prigent, S., Colosetti, P. & Capiod, T. The blocking of capacitative calcium entry by 2-aminoethyl diphenylborate (2-APB) and carboxyamidotriazole (CAI) inhibits proliferation in Hep G2 and Huh-7 human hepatoma cells. *Cell Calcium* **36**, 459–467 (2004).
171. Rodriguez-Mora, O. G., LaHair, M. M., McCubrey, J. A. & Franklin, R. A. Calcium/calmodulin-dependent kinase I and calcium/calmodulin-dependent kinase kinase participate in the control of cell cycle progression in MCF-7 human breast cancer cells. *Cancer Res.* **65**, 5408–5416 (2005).
172. Yuan, K., Chung, L. W., Siegal, G. P. & Zayzafoon, M. α-CaMKII controls the growth of human osteosarcoma by regulating cell cycle progression. *Lab. Invest.* **87**, 938–950 (2007).
References 171 and 172 demonstrate that CaMKI or CaMKII inhibition can reduce tumour cell proliferation.
173. Howe, C. J., Lahair, M. M., McCubrey, J. A. & Franklin, R. A. Redox regulation of the calcium/calmodulin-dependent protein kinases. *J. Biol. Chem.* **279**, 44573–44581 (2004).
174. Rodriguez-Mora, O. G. *et al.* Inhibition of the CaM-kinases augments cell death in response to oxygen radicals and oxygen radical inducing cancer therapies in MCF-7 human breast cancer cells. *Cancer Biol. Ther.* **5**, 1022–1030 (2006).

175. Kaddour-Djebbar, I. *et al.* Therapeutic advantage of combining calcium channel blockers and TRAIL in prostate cancer. *Mol. Cancer Ther.* **5**, 1958–1966 (2006).
176. Oltersdorf, T. *et al.* An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* **435**, 677–681 (2005).
177. Raynaud, F. I. *et al.* Pharmacologic characterization of a potent inhibitor of class I phosphatidylinositide 3-kinases. *Cancer Res.* **67**, 5840–5850 (2007).
178. Weinstein, I. B. Addiction to oncogenes — the Achilles heal of cancer. *Science* **297**, 63–64 (2002).
179. Shirasawa, S., Furuse, M., Yokoyama, N. & Sasazuki, T. Altered growth of human colon cancer cell lines disrupted at activated Ki-ras. *Science* **260**, 85–88 (1993).
180. Xue, W. *et al.* Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **445**, 656–660 (2007).
181. Ventura, A. *et al.* Restoration of p53 function leads to tumour regression in vivo. *Nature* **445**, 661–665 (2007).
182. Quintas-Cardama, A., Kantarjian, H. & Cortes, J. Flying under the radar: the new wave of BCR–ABL inhibitors. *Nature Rev. Drug Discov.* **6**, 834–848 (2007).
183. Hanson, C. J., Bootman, M. D., Distelhorst, C. W., Maraldi, T. & Roderick, H. L. The cellular concentration of Bcl-2 determines its pro- or anti-apoptotic effect. *Cell Calcium* 22 Jan 2008 (doi:10.1016/j.ceca.2007.11.014).
184. D'Orlando, C., Celio, M. R. & Schwaller, B. Calretinin and calbindin D-28k, but not parvalbumin protect against glutamate-induced delayed excitotoxicity in transfected N18-RE 105 neuroblastoma-retina hybrid cells. *Brain Res.* **945**, 181–190 (2002).
185. Walker, D., Sun, T., MacNeil, S. & Smallwood, R. Modeling the effect of exogenous calcium on keratinocyte and HaCat cell proliferation and differentiation using an agent-based computational paradigm. *Tissue Eng.* **12**, 2301–2309 (2006).
186. Sakaguchi, M. *et al.* S100C/A11 is a key mediator of Ca^{2+}-induced growth inhibition of human epidermal keratinocytes. *J. Cell Biol.* **163**, 825–835 (2003).
187. Rosenberger, S., Thorey, I. S., Werner, S. & Boukamp, P. A novel regulator of telomerase. S100A8 mediates differentiation-dependent and calcium-induced inhibition of telomerase activity in the human epidermal keratinocyte line HaCaT. *J. Biol. Chem.* **282**, 6126–6135 (2007).
188. Mottet, D. *et al.* Role of ERK and calcium in the hypoxia-induced activation of HIF-1. *J. Cell Physiol.* **194**, 30–44 (2003).
189. McLaughlin, A. P. & De Vries, G. W. Role of PLCγ and Ca^{2+} in VEGF- and FGF-induced choroidal endothelial cell proliferation. *Am J. Physiol. Cell Physiol.* **281**, C1448–1456 (2001).
190. Veliceasa, D. *et al.* Transient potential receptor channel 4 controls thrombospondin-1 secretion and angiogenesis in renal cell carcinoma. *FEBS J.* **274**, 6365–6377 (2007).
191. Huang, J. B., Kindzelskii, A. L., Clark, A. J. & Petty, H. R. Identification of channels promoting calcium spikes and waves in HT1080 tumor cells: their apparent roles in cell motility and invasion. *Cancer Res.* **64**, 2482–2489 (2004).
192. Pierce, A. *et al.* Identification of a novel, functional role for S100A13 in invasive lung cancer cell lines. *Eur. J. Cancer* **44**, 151–159 (2008).
193. Ortega, S., Malumbres, M. & Barbacid, M. Cyclin D-dependent kinases, INK4 inhibitors and cancer. *Biochim. Biophys. Acta* **1602**, 73–87 (2002).
194. Liu, J. J. *et al.* Ras transformation results in an elevated level of cyclin D1 and acceleration of G1 progression in NIH 3T3 cells. *Mol. Cell Biol.* **15**, 3654–3663 (1995).
195. Gille, H. & Downward, J. Multiple ras effector pathways contribute to G_1 cell cycle progression. *J. Biol. Chem.* **274**, 22033–22040 (1999).
196. Albanese, C. *et al.* Transforming p21ras mutants and c-Ets-2 activate the cyclin D1 promoter through distinguishable regions. *J. Biol. Chem.* **270**, 23589–23597 (1995).
197. Diehl, J. A., Cheng, M., Roussel, M. F. & Sherr, C. J. Glycogen synthase kinase-3β regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev* **12**, 3499–3511 (1998).
198. Cantley, L. C. The phosphoinositide 3-kinase pathway. *Science* **296**, 1655–1657 (2002).
199. Hanada, M., Feng, J. & Hemmings, B. A. Structure, regulation and function of PKB/AKT — a major therapeutic target. *Biochim Biophys Acta* **1697**, 3–16 (2004).
200. Alessi, D. R. & Cohen, P. Mechanism of activation and function of protein kinase B. *Curr. Opin. Genet. Dev.* **8**, 55–62 (1998).
201. Alessi, D. R. *et al.* Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bα. *Curr. Biol.* **7**, 261–269 (1997).
202. Sarbassov, D. D., Guertin, D. A., Ali, S. M. & Sabatini, D. M. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **307**, 1098–1101 (2005).
203. Stephens, L. *et al.* Protein kinase B kinases that mediate phosphatidylinositol 3, 4, 5-trisphosphate-dependent activation of protein kinase B. *Science* **279**, 710–714 (1998).
204. Brunet, A. *et al.* Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **96**, 857–868 (1999).
205. Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M. & Hemmings, B. A. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **378**, 785–789 (1995).
206. Datta, S. R. *et al.* Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* **91**, 231–241 (1997).
207. Inoki, K., Li, Y., Zhu, T., Wu, J. & Guan, K. L. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature Cell Biol.* **4**, 648–657 (2002).
208. Brunet, A., Datta, S. R. & Greenberg, M. E. Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. *Curr. Opin. Neurobiol.* **11**, 297–305 (2001).
209. Luo, J., Manning, B. D. & Cantley, L. C. Targeting the PI3K–Akt pathway in human cancer: rationale and promise. *Cancer Cell* **4**, 257–262 (2003).
210. Williams, M. R. *et al.* The role of 3-phosphoinositide-dependent protein kinase 1 in activating AGC kinases defined in embryonic stem cells. *Curr. Biol.* **10**, 439–448 (2000).
211. Tsujimoto, Y., Cossman, J., Jaffe, E. & Croce, C. M. Involvement of the bcl-2 gene in human follicular lymphoma. *Science* **228**, 1440–1443 (1985).
212. Rinkenberger, J. L. & Korsmeyer, S. J. Errors of homeostasis and deregulated apoptosis. *Curr. Opin. Genet. Dev.* **7**, 589–596 (1997).
213. Willis, S. N. & Adams, J. M. Life in the balance: how BH3-only proteins induce apoptosis. *Curr. Opin. Cell Biol.* **17**, 617–625 (2005).
214. Bensaad, K. & Vousden, K. H. p53: new roles in metabolism. *Trends Cell Biol.* **17**, 286–291 (2007).
215. Puthalakath, H. & Strasser, A. Keeping killers on a tight leash: transcriptional and post-translational control of the pro-apoptotic activity of BH3-only proteins. *Cell Death Differ.* **9**, 505–512 (2002).
216. Ley, R., Ewings, K. E., Hadfield, K. & Cook, S. J. Regulatory phosphorylation of Bim: sorting out the ERK from the JNK. *Cell Death Differ.* **12**, 1008–1014 (2005).
217. Li, H., Zhu, H., Xu, C. J. & Yuan, J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* **94**, 491–501 (1998).
218. Hockenbery, D. M., Oltvai, Z. N., Yin, X. M., Milliman, C. L. & Korsmeyer, S. J. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* **75**, 241–251 (1993).

Acknowledgements

The authors would like to acknowledge M. Bootman (Babraham Institute) for insightful discussions and critical reading of the manuscript. Work in the authors' research groups is funded by the Babraham Institute, Association for International Cancer Research, AstraZeneca, the Biotechnology and Biological Sciences Research Council, the British Heart Foundation and the Royal Society.

DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
AKT1 | ATF1 | ATP2A2 | ATP2B1 | ATP2C1 | BAD | BAK | BAX | BBC3 | BCAP31 | BCL2 | BCL2A1 | BCL2L1 | BCL2L11 | BCL2L2 | BID | calnexin | calreticulin | caspase 8 | CCND1 | CCND2 | CCNE1 | CDK2 | CDK4 | *CDKN1A* | *CDKN1B* | *CDKN2A* | CETN1 | CP110 | CREB1 | ERBB2 | ERN2 | FAS | *FOS* | FOXO3 | FRAP1 | GRB2 | HIF1A | HSPA5 | HSPA9 | *JUN* | KRAS | MAPK1 | MAPK14 | MAPK3 | MAP3K5 | MAPK8 | MCL1 | *MYC* | neurofibromin 1 | NFATC1 | ORAI1 | p53 | PDCD6 | PMAIP1 | RALGDS | RASA1 | RASA4 | RASGRF1 | RASGRF2 | RASGRP1 | RASGRP2 | RB1 | RICTOR | ROCK2 | S100A11 | S100A13 | S100A2 | S100A8 | SCO2 | SHC1 | *SLC7A11* | SLC8A1 | SOS1 | SRC | THBS1 | TIAM1 | TNFSF10 | TRAF2 | TRPC4 | TRPC6 | TRPV6 | TSC1 | TSC2 | VDAC1

National Cancer Institute: http://www.cancer.gov/
breast cancer | colorectal cancer | gastric cancer | glioblastoma | glioma | hepatocellular carcinoma | lung cancer | nasopharyngeal cancer | neuroblastoma | oesophageal cancer | osteosarcoma | prostate cancer | renal cell carcinomas

National Cancer Institute Drug Dictionary:
http://www.cancer.gov/drugdictionary/
ceramide | cyclosporin A | doxorubicin | imatinib

FURTHER INFORMATION

H. L. Roderick's homepage:
http://www.phar.cam.ac.uk/ri/roderick.html
H. L. Roderick and S. J. Cook's homepage:
http://www.babraham.ac.uk/signalling/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF