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Calcium signaling and transcription: elongation, DoGs, and eRNAs

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Abstract

The calcium ion (Ca^{2+}) is a key intracellular signaling molecule with far-reaching effects on many cellular processes. One of the most important such Ca^{2+} regulated processes is transcription. A body of literature describes the effect of Ca^{2+} signaling on transcription initiation as occurring mainly through activation of gene-specific transcription factors by Ca^{2+}-induced signaling cascades. However, the reach of Ca^{2+} extends far beyond the first step of transcription. In fact, Ca^{2+} can regulate all phases of transcription, with additional effects on transcription-associated events such as alternative splicing. Importantly, Ca^{2+} signaling mediates reduced transcription termination in response to certain stress conditions. This reduction allows readthrough transcription, generating a highly inducible and diverse class of downstream of gene containing transcripts (DoGs) that we have recently described.

Keywords

Ca^{2+} signaling; transcription regulation; readthrough transcription; DoGs

Introduction

In this review, we first provide a brief overview of Ca^{2+} signaling. Next, we describe how Ca^{2+} signaling affects the different aspects of transcription downstream of transcription initiation, as well as the generation of enhancer RNAs (eRNAs).

Ca^{2+} signaling – upstream of Ca^{2+} release

Ca^{2+}-signaling has previously been reviewed extensively (for example, see [1–5]). See Figure 1 for an overview. Effective Ca^{2+} signaling requires low cytoplasmic Ca^{2+} concentrations in resting cells, achieved by active Ca^{2+} removal. To this end, Ca^{2+} is pumped into the endoplasmic reticulum (ER) via sarcoendoplasmic reticular Ca^{2+} ATPase (SERCA) pumps, or pumped out of the cell via plasma membrane Ca^{2+} ATPase (PMCA) pumps. The resulting

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low cytoplasmic Ca^{2+} concentrations of ~100 nM enable rapid cytoplasmic entry once Ca^{2+} channels are activated by signaling [1.2].

Ca^{2+} can enter the cytoplasm from either the extracellular environment or intracellular stores. Ca^{2+} entry from the extracellular environment can occur through two types of plasma membrane Ca^{2+} channels: voltage-dependent Ca^{2+} channels (VDCCs) and receptor-operated (ligand-gated) channels (ROCs). The most important intracellular Ca^{2+} store is the ER; in the ER lumen, Ca^{2+} buffers bind Ca^{2+} with high capacity but low affinity, enabling efficient storage of Ca^{2+} that can be rapidly released upon signaling. The most prominent pathways for Ca^{2+} release from the ER rely on 1,4,5-inositol trisphosphate (IP3) receptors (IP3Rs) and ryanodine receptors (RyRs) [1–3].

IP3Rs are activated by IP3, which is generated at the plasma membrane by the action of Phospholipase C (PLC) on phosphatidylinositol 4,5 bisphosphate (PIP2). PLC transforms PIP2 into IP3 and diacylglycerol (DAG), another signaling molecule that activates a different set of downstream signaling pathways. Different PLC isoforms are activated by different membrane receptors, such as G-protein-coupled receptors and protein tyrosine kinase-linked receptors [1.2]. The IP3R functions as a homo- or heterotetramer. There are three different IP3R genes (IP3R1–3), which encode IP3R subtypes that have varying binding affinity for IP3; IP3R2 is the most sensitive, whereas both IP3R1 and 2 are significantly more sensitive than IP3R3. However, cell-specific variables such as IP3R isoform abundance also affect the relative importance of each receptor in an individual cell type [3].

RyRs are activated by an increase in cytosolic Ca^{2+} concentration into the low μM range [1]. Because Ca^{2+} is the primary RyR agonist, RyRs are mainly involved in propagating already initiated Ca^{2+} signaling [5]. RyRs exist in three different isoforms encoded by three different genes; they function as homotetramers [3].

Ca^{2+} signaling – downstream of Ca^{2+} release

Once Ca^{2+} is released into the cytosol, it quickly binds to various buffering and signaling molecules. Cytoplasmic Ca^{2+} buffering proteins bind released Ca^{2+} to prevent it from spreading far, thus keeping signaling local. Importantly for Ca^{2+} signaling, Ca^{2+} in the cytosol also binds a number of signaling molecules including calmodulin and protein kinase C (PKC), as described below.

Ca^{2+}-bound calmodulin binds and activates a large number of downstream effectors, such as Ca^{2+}/calmodulin-dependent protein kinases (CaMKs). CaMKs, most importantly CaMKIV, in turn phosphorylates and activates the transcription factor cyclic AMP (cAMP) response element-binding protein (CREB), which binds to cAMP response elements (CREs) in DNA to activate transcription [1. 2. 4]. Further, Ca^{2+}-bound calmodulin binds and activates the phosphatase calcineurin. Once activated, calcineurin dephosphorylates cytosolic nuclear factor of activated T cells (NFAT), allowing hypophosphorylated NFAT to display a previously buried nuclear localization signal; NFAT enters the nucleus and acts as a transcription factor [1].

Additionally, Ca^{2+}, once released into the cytoplasm, binds PKC. Association with positively charged Ca^{2+} allows PKC to relocalize to the negatively charged inside of the plasma membrane, where it becomes further activated by DAG and functions as a protein kinase. Once fully activated, PKC phosphorylates and regulates many substrates, including CREB [1.2].

Multiple other pathways are activated by Ca^{2+} signaling due to complex cross-talk mechanisms. Ca^{2+} activates calcium-sensitive adenylyl cyclases, which generate the signaling molecule cAMP. Ca^{2+} signaling can activate mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways [5]. For example, Ca^{2+}, once released, stimulates PLC activity to generate DAG. DAG generation can then activate PI3K and protein kinase D (PKD), thus initiating a separate set of signaling cascades [5.6].

Effect of Ca^{2+} signaling on transcription and co-transcriptional pre-mRNA processing

Transcription and co-transcriptional pre-mRNA processing have been reviewed extensively (for example, see [7–16]).

In short, transcription can be divided into transcription initiation, elongation, and termination, with promoter-proximal pausing and pause release as intermediate steps between initiation and productive elongation. Additionally, pre-mRNA splicing occurs largely co-transcriptionally and is therefore affected by transcriptional regulation. See Figure 2 for an overview of transcription and Figure 3 for an overview of how Ca^{2+} signaling regulates transcription.

Calcium signaling and transcript pause release

Transcription initiation is divided into two steps. First, a pre-initiation complex (PIC) forms on the core promoter, defined as the shortest DNA sequence (~50–100 nt) sufficient to initiate transcription [8]. The PIC contains RNA polymerase II (Pol II) and the general transcription factors. The PIC assembles on the core promoter through interaction with the mediator complex, which in turn contacts gene-specific transcription factors. Once the PIC is assembled, Pol II is released from the mediator complex and the core promoter sequence to start transcribing, concurrent with phosphorylation of the Pol II C-terminal domain (CTD) on serine-5 [7].

Recent studies show that on about a third of human genes, Pol II pauses after escaping the promoter. This pausing occurs within 100 nt of the transcription start site (TSS) in a process known as *promoter-proximal pausing*, which is induced by the association of Pol II with the factors DRB Sensitivity-Inducing Factor (DSIF) and Negative ELongation Factor (NELF). Promoter-proximal pausing is rate-limiting for transcription and transcriptional output can be regulated by varying the length of the pause [9.10].

The transition of Pol II from paused to productive elongation is mediated by Positive Transcription Elongation Factor-b (P-TEFb). P-TEFb phosphorylates the Pol II CTD at

Serine-2 residues, as well as DSIF and NELF, which reduces suppression of elongation mediated by negative transcription factors and further causes NELF release from the elongation complex, allowing Pol II to enter into productive elongation.

P-TEFb can be directly recruited to the elongation complex by positive transcription factors. Further, P-TEFb activity is inhibited by its binding to 7SK RNA and the HEXIM1 protein [9.10]. Phosphorylation of Threonine 186 (Thr-186) of the CDK9 subunit of P-TEFb is required both for P-TEFb activity as a positive elongation factor and for P-TEFb interaction with 7SK and HEXIM1 [14]. Phosphorylation of CDK9 at Thr-186 is affected by Ca^{2+} signaling in several ways. First, Chen and colleagues reported that the Ca^{2+}/calmodulin-activated phosphatase calcineurin is necessary but not sufficient to cause dephosphorylation of CDK9-Thr186 in HeLa cells [17]. The authors found that when calcineurin or calmodulin was inhibited by cyclosporine A or W-7, P-TEFb release from HEXIM1 and 7SK in response to UV- or hexamethylene bisacetamide (HMBA) treatment was prevented. They further observed that calcineurin affects Thr-186 phosphorylation by inducing a conformational change in the 7SK/HEXIM1/P-TEFb RNP, which in turn allows the phosphatase PP1α to access and dephosphorylate Thr-186, causing disassociation of the 7SK/HEXIM1/PTEFb complex. P-TEFb is then presumably re-phosphorylated, allowing it to function in pause release. In support for this model, the authors demonstrated that after calcineurin/PP1α-mediated dephosphorylation and release of P-TEFb from 7SK/HEXIM1, the released P-TEFb could act to enhance elongation in a luciferase reporter system [17].

Additionally, Ca^{2+} signaling may play a direct role in the phosphorylation of CDK9 at Thr-186. Resting CD4+ T-lymphocytes exhibit low levels of Thr-186 phosphorylation, whereas, after T-cell activation, Thr-186 is rapidly phosphorylated. Ramakrishnan and Rice found that inhibiting either CamK or calmodulin using the inhibitors KN-93 or W-7, respectively, reduced CDK9 Thr-186 phosphorylation in HeLa cells [18]. They further demonstrated the importance of Ca^{2+} signaling for Thr-186 phosphorylation in activated CD4+ T-cells by treating cells with Thapsigargin prior to activation. Thapsigargin prevents SERCA-mediated Ca^{2+} reuptake into the ER and therefore depletes ER Ca^{2+} stores, reducing the ER's ability to release Ca^{2+} in response to signaling. Thapsigargin pretreatment led to reduced CDK9 Thr-186 phosphorylation in response to T-cell activation. Further, calmodulin inhibition led to decreased PTEFb activity in promoting elongation, measured by a luciferase reporter assay. In a screen for kinases involved in CDK9 Thr-186 phosphorylation, Ramakrishnan and Rice identified CaMK1D, although the effect may be indirect [18].

Ca^{2+} and release of transcription elongation blocks

Once Pol II has been released from pausing and becomes committed to productive elongation, the Pol II complex is generally stable, as demonstrated by the fact that it can continue transcribing for hundreds of kilobases [9]. However, several studies have reported Ca^{2+} regulated blocks to elongation after of pause-release. The best-studied instance occurs in the fos gene close to the exon1/intron1 border, as mapped by nuclear run-on assays and *in vitro* transcription assays in nuclear extracts [19.20]. The role of Ca^{2+} signaling in overcoming this block has been demonstrated in several systems. Elongation of fos

transcripts is induced by treatment with a Ca^{2+} ionophore, which enables extracellular Ca^{2+} to cross the plasma membrane and thus activate Ca^{2+} signaling. Conversely, fos induction is prevented either by using cell culture media lacking Ca^{2+} or by pretreating cells with the Ca^{2+} chelator EGTA [20–24].

Another Ca^{2+}-regulated transcription elongation block has been identified in exon 1 of rat MAP Kinase Phosphatase-1 (MKP-1), again mapped by nuclear run-on assays [25]. In rat pituitary GH4C1 cells treated with thyrotropin-releasing hormone (TRH), MKP-1 is rapidly induced upon Ca^{2+} -mediated release of this block to elongation. Ca^{2+}-dependency was shown by pretreatment with EGTA prior to TRH stimulation, which caused reduced MKP-1 induction [25]. The distance between the TSSs and the sites of the fos or MKP-1 transcription elongation block – estimated at 300–400 nt downstream of the 5′ end of both transcripts – is not compatible with promoter-proximal pausing, which occurs within 100 nt of the TSS. Therefore, these situations might represent a different kind of transcription elongation control, but the nature of the block and the Ca^{2+}-regulated pathways involved remain to be investigated.

Ca^{2+} transcription elongation rate, and alternative splicing

The nascent RNA generated through elongation is further processed by splicing: introns are removed and exons are joined together. Nearly all human genes are subject to alternative splicing, where alternative exons are included or excluded, or alternative splice sites generate longer or shorter exon variants. Recently, it has become evident that a large proportion of splicing occurs co-transcriptionally. Interestingly, Pol II elongation rate can affect splice site choice. A slower Pol II elongation rate promotes recognition of weaker splice sites [10.13.15.16]. In chromatin, DNA is wrapped around histone proteins to form nucleosomes, and nucleosomes need to be partially disassembled to allow ongoing transcription. The Pol II elongation rate is affected by nucleosome density and chromatin structure, which in turn correlate with chromatin and histone modifications. Histone acetylation is known to relax chromatin structure, increasing the Pol II elongation rate [26.27].

Ca^{2+} signaling has repeatedly been implicated in regulating histone modification. For example, Ca^{2+} signaling induced by depolarization of rat hippocampal cells has been coupled to histone 2B acetylation through a mechanism requiring CamK activity [28]. Further, ROCs activated by NMDA (NMDA receptors) cause Ca^{2+} influx, which ultimately leads to histone H3 acetylation through activation of the MAPK ERK [29.30].

Although the effects of these Ca^{2+}-induced modifications on alternative splicing have not been explored in rat hippocampus, similar studies in mouse cells have connected Ca^{2+}-induced chromatin modifications and alternative splicing. Depolarization of the mouse neuroblastoma cell line N2 causes Ca^{2+} influx through VDCCs and induces skipping of NCAM exon 18 [31]. This exon skipping event is dependent on increased histone H3 and H4 acetylation. Specifically, H3K9ac was increased in a region of the NCAM gene encompassing the skipped exon and was accompanied by increased chromatin accessibility, suggesting less rigid chromatin structure in response to Ca^{2+} signaling [31]. An additional

study found that depolarization of mouse cardiomyocytes induced histone H3 and H4 hyperacetylation, resulting in splicing changes in a number of genes [32]. Histone H3 and H4 acetylation was increased in the two alternatively spliced genes selected by the authors for detailed study, and this hyperacetylation was associated with increased Pol II elongation rate. Further, the authors observed decreased association of type II histone deacetylases (HDACs) – which act to remove histone acetyl modifications – and these genes after depolarization. Because phosphorylation of class II HDACs leads to their nuclear exclusion (reviewed in [33]), the authors hypothesized that a Ca^{2+}-dependent kinase was involved in regulating class II HDAC localization in response to Ca^{2+}-signaling. Indeed, they observed reduced histone acetylation in response to the combined inhibition of both CamKIIδ and PKD1, either by using inhibitors or through siRNA-mediated knockdown [32].

Ca^{2+} and transcription termination versus DoG production

The process of transcription ends with *transcription termination*. Transcription termination requires elongation through a cleavage and polyadenylation (polyA) signal (most commonly AAUAAA). Shortly after the polyA sequence, the nascent RNA is cleaved by the process of cleavage and polyadenylation. Non-templated A residues are added to the 3′ end of what will become the mRNA, while the 5′ end of the downstream RNA that is still attached to, and being extended by, Pol II remains unprotected. Actual transcription termination – the dissociation of Pol II from the template DNA and from the RNA – occurs within nucleotides to kilobases downstream of the polyA signal. Two models for the mechanism of transcription termination have been proposed: the allosteric model and the torpedo model. In the allosteric model, transcription through the polyA signal causes a conformation change in Pol II, which later leads to dissociation from the template DNA. In the alternative torpedo model, the unprotected 5′ end of the downstream transcript generated by the cleavage of the nascent RNA serves as an entry point for an exonuclease, which degrades the nascent transcript and causes Pol II dissociation from the template DNA [11.12]. Two recent conflicting studies have provided new support for each model [34.35]; however, as these models are not mutually exclusive, the actual mechanism for termination is likely a combination of the two. In addition to the transcription of the polyA signal and the process of cleavage and polyadenylation, Pol II pausing near the end of genes may also favor transcription termination [11.12].

We have recently discovered a link between Ca^{2+} signaling and transcription termination that affects more than 10% of human genes [36]. Our interest in Ca^{2+}-mediated regulation of transcription termination originated from our studies of a putative ncRNA associated with bad-prognosis neuroblastoma. We found that this ncRNA was induced ~30-fold in response to osmotic stress in SK-N-BE(2)C neuroblastoma cells as well as in several other human cell lines. Using RNA-Seq and bioinformatic analyses, we determined that the ncRNA was part of a transcript that appeared to be generated by transcriptional readthrough from the upstream protein-coding gene. By extending our analyses to encompass the whole transcriptome, we found that osmotic stress widely impacts transcription downstream of genes. We coined the term DoGs for these downstream of gene containing transcripts. We found that DoGs are generated downstream of more than 10% of human protein-coding genes. Their prevalence, in combination with the length of each individual DoG (often >45

kb), suggested that DoGs could explain a significant portion of intergenic transcripts. Further study confirmed this hypothesis: DoGs can account for as much as 20% of intergenic transcription in human cells.

DoGs appeared to be generated by transcriptional readthrough. However, alternatively, DoG transcription could be the result of initiation at TSSs overlapping or just downstream of the upstream DoG-associated protein-coding transcript. To distinguish between these two possibilities, we applied a high-throughput method of sequencing and mapping 5′-capped RNAs (Cap Seq) that we had recently developed [37]. Using this approach, we demonstrated that DoGs do not initiate at downstream, stress-inducible TSSs, but indeed result from transcriptional readthrough. This point was further confirmed by 1) using biotinylated probes targeting upstream transcripts combined with pull-down on streptavidin beads and detection of both the upstream transcript and DoG by qRT-PCR, and 2) by inhibiting transcription through the upstream gene using catalytically inactive CRISPR/Cas9 [38], which also prevented DoG generation. Together, these data demonstrated that DoG production depends on transcription of the upstream gene, and that the upstream transcript and the transcribed DoG region reside in the same molecule [36].

We found that DoGs are induced at the level of transcription after stress, as demonstrated by an increase in DoGs using the uridine analogue 5-ethynyl uridine to label newly synthesized RNA. After incorporation and cell lysis, 5-ethynyl uridine can be coupled to biotin, allowing the labeled RNA to be isolated on streptavidin beads. We observed full induction of selected DoGs already after 25 minutes of labeling, while transcript levels from the upstream genes that generate DoGs were unchanged. DoG half-lives of ~1 hour were unaffected by KCl treatment, as demonstrated by an actinomycin D chase. The fact that DoG upstream transcripts are not increased after stress indicates that DoG induction is due to reduced transcription termination of the upstream transcripts, not increased initiation of the upstream gene [36].

Further, we found Ca^{2+} signaling to be necessary for DoG induction; specifically, the release of intracellular Ca^{2+}. This conclusion rests on pretreating cells with BAPTAAM, a membrane-permeable Ca^{2+} chelator that chelates intracellular as well as extracellular Ca^{2+}, or with EGTA, a Ca^{2+} chelator that does not penetrate cell membranes. We found that BAPTA-AM prevented DoG induction by osmotic stress, whereas pretreating cells with EGTA did not affect the increase in DoG levels. We thus excluded extracellular Ca^{2+} as the agent for DoG-inducing Ca^{2+} signaling, indicating that the effect is due instead to release of intracellular Ca^{2+} [36].

As mentioned above, intracellular Ca^{2+} is mainly released from the ER through IP3Rs or RyRs. We investigated the effect of IP3R signaling on DoG induction by pretreating cells with the IP3R inhibitor 2-ABP. We found that in the presence of 2-ABP, DoG induction by KCl was abolished, indicating that DoG induction is mediated by Ca^{2+} release from the ER by the IP3R. To confirm this conclusion, we knocked down the three IP3R isoforms using siRNA and found that knocking down IP3R1 prevented DoG induction in response to osmotic stress. Next, we investigated downstream Ca^{2+}-dependent pathways required for DoG induction and found that the combined inhibition of both CamKII and PKC/PKD using

the inhibitors KN-93 and Gö6976 prevented DoG induction in response to stress [36]. See Figure 4 for an overview of the role of Ca^{2+} signaling in DoG induction.

Outstanding questions with regard to Ca^{2+}-regulated DoG induction concern the mechanism through which osmotic stress induces IP3R-mediated Ca^{2+}-release, as well as the nature of the actual perturbation of transcription termination. Several studies report increased cytosolic Ca^{2+} and IP3 release after osmotic stress [39] or heat shock [40]. Surprisingly, pretreatment of human neuroblastoma SK-N-BE(2)C cells with U73122, which is generally considered to be an inhibitor of receptor-mediated PLC activation, did not prevent DoG induction in response to osmotic stress (our unpublished results). This result suggests that receptor-activated IP3 generation does not increase in response to osmotic stress in SK-N-BE(2)C cells. However, U73122 effects on PLC activation may be complex [41], raising the possibility that U73122 may not prevent all PLC activation. For example, osmotic stress might activate PLCs by a mechanism that is not dependent on agonist binding to a membrane receptor. It is unclear how U73122 would affect such atypical PLC activation.

As to the exact mechanism by which the IP3R signaling affects the process of transcription termination, there are a number of possibilities. We found that knockdown of CPSF73, the catalytic subunit of the cleavage and polyadenylation complex, leads to modest DoG induction (about 4-fold) [36], suggesting that the cleavage and polyadenylation complex may be targeted by Ca^{2+} signaling to reduce transcription termination. Further, joint signaling of the PKD and CamKII pathways have been found to enhance Pol II elongation through reducing the nuclear localization of class II HDACs (see the discussion on Ca^{2+} and alternative splicing above) [32], which may reduce the amount of Pol II pausing near the end of genes, thus preventing termination. However, it is unclear whether an indirect effect on chromatin modification would occur quickly enough to explain rapid DoG induction after stress. Additionally, our preliminary data do not suggest any changes in HDAC localization after osmotic stress in our system. Finally, it is possible that other, perhaps as-of-yet unidentified, factors involved in transcription termination are affected by Ca^{2+} signaling. Further studies are required to elucidate IP3R activation and effect on termination in response to osmotic stress.

DoG Function

To pursue the putative function(s) of DoGs, we used the Ca^{2+} dependency of DoG transcription as a tool to prevent global DoG induction in response to stress. DoG localization on chromatin – demonstrated by cellular fractionation assays and RNA FISH – suggests that DoGs may function in a chromatin stress response. Additionally, the diversity among DoGs suggests that their putative function(s) is/are sequence independent. Because hyperosmotic stress – the type of stress used in [36] – forces water to leave the cell, nuclear shrinkage and chromatin condensation result. Recently, ncRNAs have been implicated in reinforcing the nuclear scaffold and maintaining euchromatin [42]. Thus, we hypothesized that DoGs may similarly reinforce the nuclear scaffold after stress. After exposure of SK-N-BE(2)C neuroblastoma cells to osmotic stress in the presence of the IP3R inhibitor 2-ABP, which prevents DoG induction (see above), nuclear phenotypes associated with osmotic stress were aggravated. These preliminary indications that DoGs are involved in reinforcing

the nuclear scaffold after stress suggest the exciting possibility of sequence-independent, structural roles for this new large class of ncRNAs [36].

Interestingly, we found induction of selected DoGs also in response to heat shock [36]. Similar transcriptional readthrough has been observed in response to viral stress induced by HSV-1 infection [43] and in renal carcinoma [44]. Together these observations suggest that DoG induction is not limited to osmotic stress but may be a feature of a more general nuclear stress response. Although cytoplasmic responses to proteotoxic stresses such as heat shock and osmotic stress have been studied extensively, less is known about how the nucleus copes with such stress. Evidence from yeast suggests that proteotoxic stress responses are connected to pathways protecting DNA and RNA integrity (reviewed in [45]). Perhaps DoGs represent such a mechanism through which the nucleus protects its DNA content and RNA output from the deleterious effects of stress.

Ca^{2+} signaling and other types of non-coding transcripts

In recent years, a wealth of non-coding RNAs (ncRNAs) has been discovered (reviewed in [46.47]). Considering the abundance of types of ncRNA species, it is perhaps not surprising that other Ca^{2+}-regulated ncRNAs apart from DoGs have been identified. An important class of ncRNAs, namely eRNAs was identified through Ca^{2+}-mediated enhancer activation. Kim and co-authors [48] induced Ca^{2+} signaling by KCl-mediated depolarization of mouse primary neurons in culture, which leads to influx of Ca^{2+} through VDCCs. They observed that a large fraction (~40%) of activity-induced enhancers were transcribed bi-directionally, generating short (<2 kb) transcripts, referred to as eRNAs. Moreover, eRNA induction by Ca^{2+} signaling correlated with induction of neighboring genes [48]. Further studies have confirmed that at least a fraction of eRNAs contribute to enhancer function, potentially by facilitating chromatin looping to enable contacts between enhancers and promoters (reviewed in [49]).

Conclusions and outlook

As our understanding of transcriptional regulation deepens, it is becoming increasingly clear that all steps of the transcription cycle are subject to tight regulation. Such regulation affects the transcriptional output of a given gene, but may also lead to the generation of alternative, often non-coding, transcripts. Clearly, regulation of any and all stages of transcription has the potential to significantly affect cellular processes. Because Ca^{2+} signaling is such a conserved and important regulatory circuit, it is not surprising that it influences so many aspects of transcription. Additionally, dysregulated Ca^{2+} signaling is associated with a number of human diseases, for instance heart disease and neurological pathologies [2]. Further research will identify the molecular targets of Ca^{2+} signaling that execute the various effects of Ca^{2+} on transcription and will aid our understanding of how these effects can be manipulated to improve treatment of Ca^{2+} signaling-related disease.

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Abbreviations

cAMP	cyclic AMP
CaMKs	Ca^{2+}/calmodulin-dependent protein kinases
CREs	cAMP response elements
CREB	CRE-binding protein
CTD	C-terminal domain
DAG	diacylglycerol
DoG	downstream of gene containing transcript
DSIF	DRB Sensitivity-Inducing Factor
ER	endoplasmic reticulum
eRNAs	enhancer RNAs
HDACs	histone deacetylases
HMBA	hexamethylene bisacetamide
IP3	1,4,5-inositol trisphosphate
IP3R	IP3 receptors
MAPK	mitogen-activated protein kinase
MKP-1	MAP Kinase Phosphatase-1
ncRNAs	non-coding RNAs
NELF	Negative ELongation Factor
NFAT	nuclear factor of activated T cells
PI3K	phosphoinositide 3-kinase
PIP2	phosphatidylinositol 4,5 bisphosphate
PLC	Phospholipase C
PKC	protein kinase C
PMCA	plasma membrane Ca^{2+} ATPase pumps
Pol II	RNA polymerase II
polyA signal	polyadenylation signal

P-TEFb	Positive Transcription Elongation Factor-b
ROC	receptor-operated channel
RyRs	ryanodine receptors
SERCA	sarcoendoplasmic reticular Ca^{2+} ATPase pumps
Thr-186	Threonine 186
TSS	transcription start site
VDCC	voltage-dependent Ca^{2+} channel

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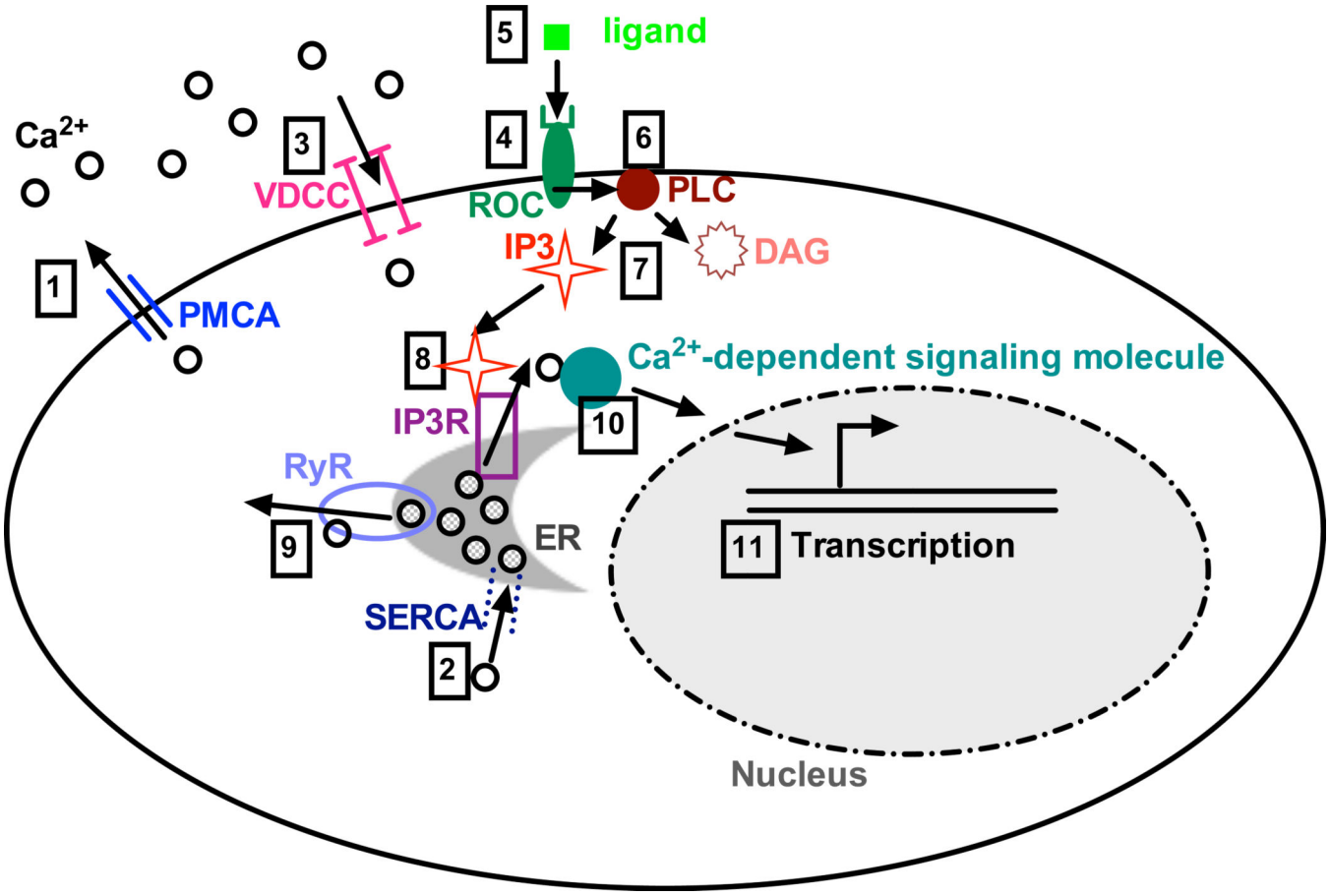

Figure 1. Overview of Ca^{2+} signaling

Ca^{2+} is removed from the cytoplasm by export to the cell exterior by PMCA pumps (1) and by import to the ER by SERCA pumps (2). Signaling induces Ca^{2+} influx through VDCCs (3) and/or activation of ROCs (4). Ligand binding to ROCs (5) causes activation of PLCs (6), which generate the signaling molecules DAG and IP3 (7). IP3 then activates the IP3R at the ER, leading to Ca^{2+} release from the ER (8). The ER also contains RyRs that release Ca^{2+}, with the main RyR ligand being Ca^{2+}. Therefore RyRs effectively amplify Ca^{2+} signaling (9). Once released, Ca^{2+} binds to and activates downstream signaling molecules (10), ultimately affecting transcription (11). See text for details, abbreviations, and references.

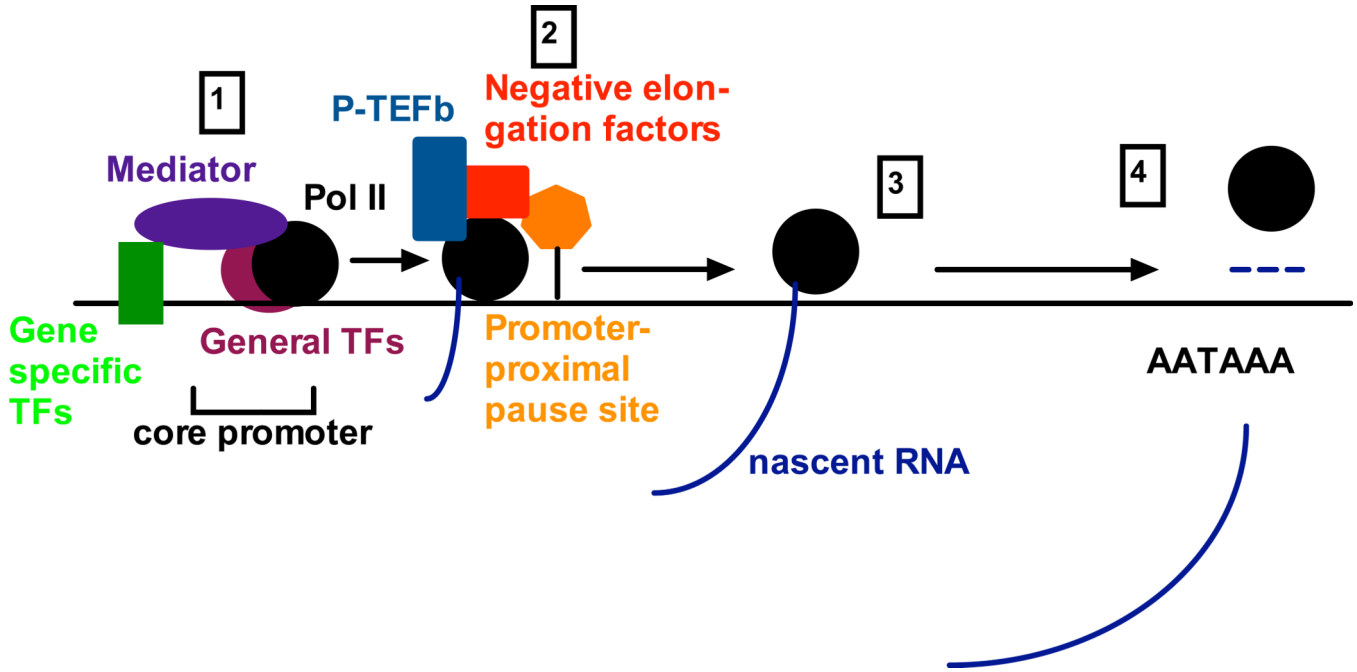

Figure 2. Overview of transcription

Transcription is initiated when the PIC, consisting of Pol II and general transcription factors (TFs), is recruited to the core promoter by gene-specific transcription factors through interaction with the mediator complex (1). After initiating transcription of many genes, Pol II pauses within 100 nts of the core promoter – *promoter proximal pausing* – and is released through P-TEFb-mediated phosphorylation of the CTD of Pol II and of negative elongation factors (2). Once released, Pol II continues with productive elongation (3) until RNA synthesis undergoes the process of cleavage and adenylation and transcription termination. This process generates the 3′ end of the mRNA, and Pol II dissociates from the template DNA and from the RNA transcribed downstream of the cleavage and polyadenylation site in a mechanism that either precedes or is preceded by degradation of the downstream RNA (the allosteric or torpedo model, respectively) (4). See text for details, abbreviations, and references.

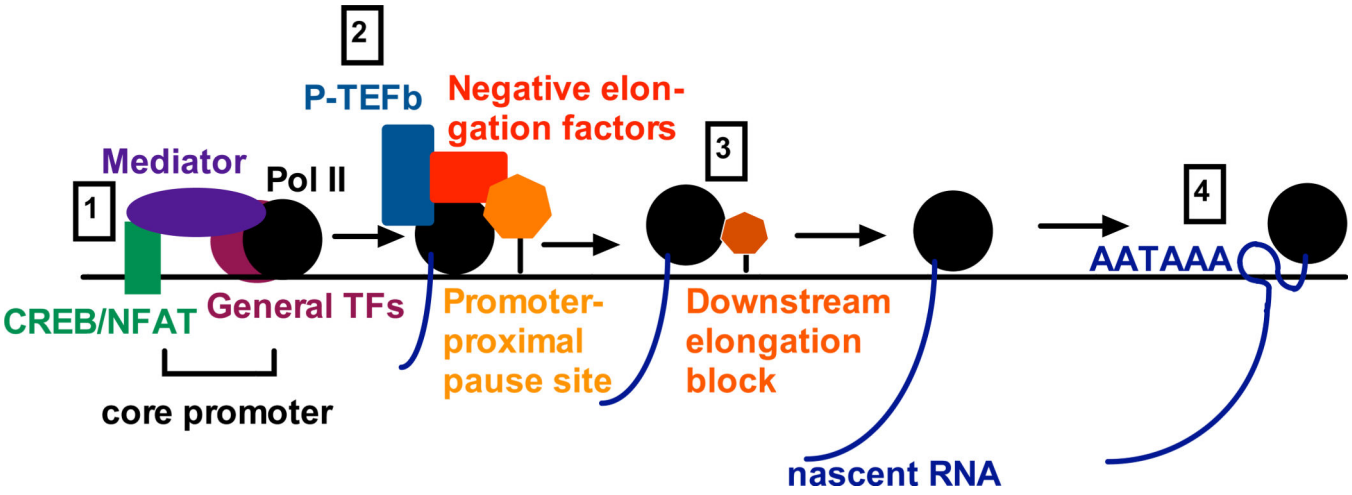

Figure 3. Aspects of transcription affected by Ca^{2+} signaling

Ca^{2+} signaling regulates gene-specific transcription factors (TFs) such as CREB and NFAT, which activate transcriptional initiation (1). Further, Ca^{2+} signaling regulates phosphorylation of the PTEF-b subunit CDK9) on Thr-186, affecting PTEF-b activity in pause release (2). Ca^{2+} can also regulate release from downstream elongation blocks (3) and inhibit transcription termination (4), thus allowing DoG transcription. See text for details, abbreviations, and references.

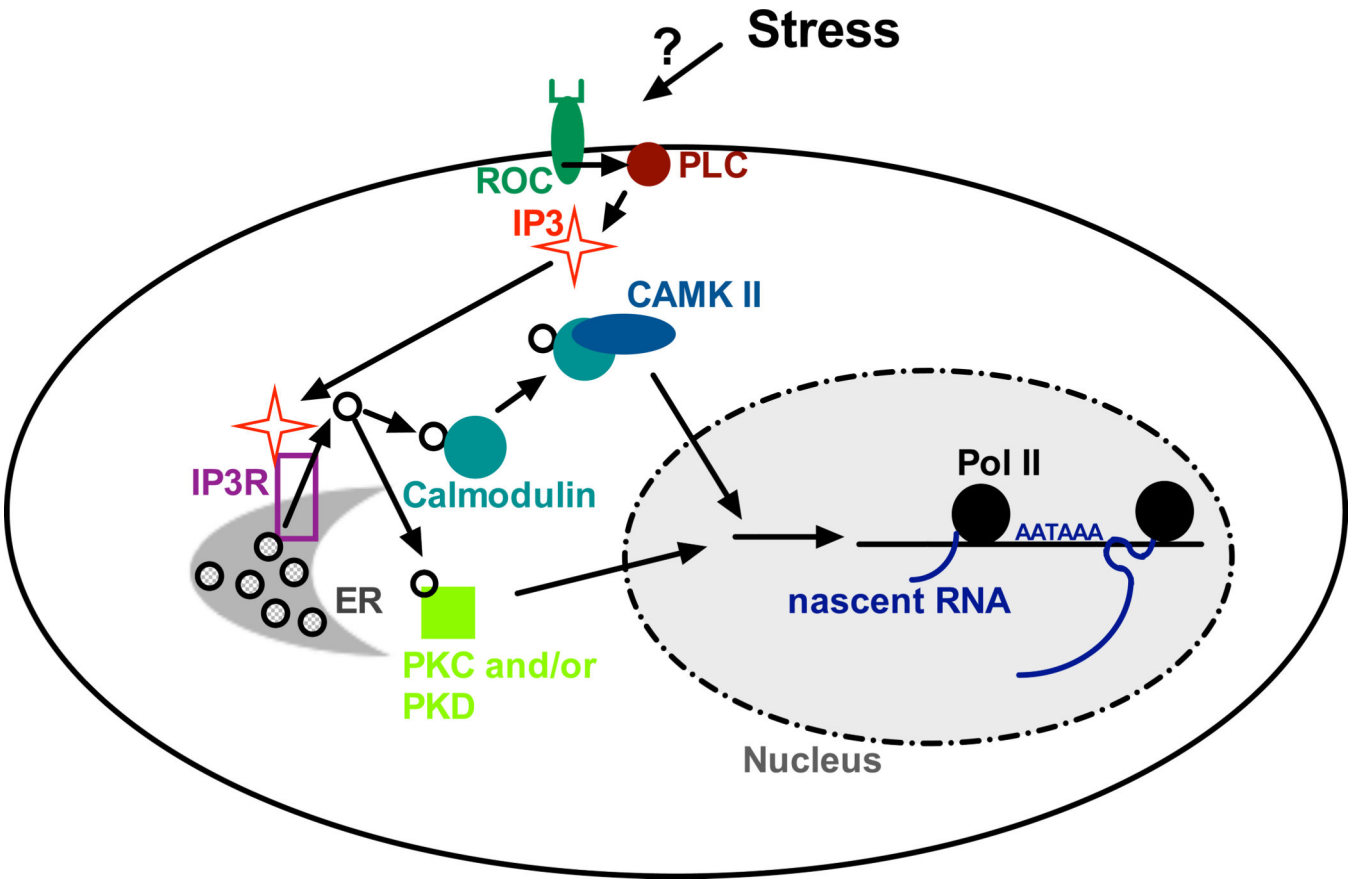

Figure 4. Ca^{2+} signaling results in DoG transcription

IP3/IP3R signaling causes Ca^{2+} release from the ER, activating downstream CAMKII and PKC/PKD pathways, ultimately decreasing transcription termination. The mechanism remains to be elucidated. See text for details, abbreviations, and references.