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COMMENTARY

Emerging roles of the single EF-hand Ca^{2+} sensor tescalcin in the regulation of gene expression, cell growth and differentiation

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ABSTRACT

Tescalcin (TESC, also known as calcineurin-homologous protein 3, CHP3) is a 24-kDa EF-hand Ca^{2+}-binding protein that has recently emerged as a regulator of cell differentiation and growth. The *TESC* gene has also been linked to human brain abnormalities, and high expression of tescalcin has been found in several cancers. The expression level of tescalcin changes dramatically during development and upon signal-induced cell differentiation. Recent studies have shown that tescalcin is not only subjected to up- or down-regulation, but also has an active role in pathways that drive cell growth and differentiation programs. At the molecular level, there is compelling experimental evidence showing that tescalcin can directly interact with and regulate the activities of the Na^+/H^+ exchanger NHE1, subunit 4 of the COP9 signalosome (CSN4) and protein kinase glycogen-synthase kinase 3 (GSK3). In hematopoetic precursor cells, tescalcin has been shown to couple activation of the extracellular signal-regulated kinase (ERK) cascade to the expression of transcription factors that control cell differentiation. The purpose of this Commentary is to summarize recent efforts that have served to characterize the biochemical, genetic and physiological attributes of tescalcin, and its unique role in the regulation of various cellular functions.

KEY WORDS: CHP3, COP9, GSK3, NHE1, Differentiation, Tescalcin

Introduction

The role of Ca^{2+} as a universal second messenger often depends on Ca^{2+}-binding proteins, which regulate the activities of enzymes, ion channels and other proteins that allow cells to respond to fluctuations in the concentration of free intracellular Ca^{2+}. The literature on Ca^{2+} sensing and signaling is rich, with many excellent reviews on the physiological role of Ca^{2+} and information on hundreds of Ca^{2+}-binding proteins (see e.g. Clapham, 2007; Zhou et al., 2013). This Commentary draws attention to the relatively unknown Ca^{2+}-sensor tescalcin, which was discovered as a gene (*TESC*) that is differentially expressed in the development of embryonic mouse testis (Perera et al., 2001). Subsequent investigations revealed broader expression patterns of *TESC*, and have illuminated its crucial role in several important cellular pathways. Tescalcin is an unusual Ca^{2+}-sensor in that it regulates relatively slow physiological processes such as cell differentiation. In contrast, most other Ca^{2+}-sensors are associated with rapid events, such as muscle contraction or neurotransmitter release (Clapham, 2007; Zhou et al., 2013). Thus, efforts to understand how tescalcin extends the duration of transient Ca^{2+} signals are expected to provide new insights into the graded signaling mechanisms that underlie development.

Tescalcin belongs to a large family of Ca^{2+}-binding proteins that contain the EF-hand, a structural motif annotated for the first time in 1973 in parvalbumin (Kretsinger and Nockolds, 1973; Mikhaylova et al., 2011; Zhou et al., 2013). The canonical EF-hand consists of a Ca^{2+}-binding loop that contains 12 highly conserved residues flanked by two helices, named as E and F, respectively. There are also four types of non-canonical EF-hands that have insertions, deletions or substitutions that fine-tune divalent cation coordination (Gifford et al., 2007; Lewit-Bentley and Réty, 2000). The superfamily of EF-hand proteins is divided into two groups. The first group has a Ca^{2+}-binding affinity of below 100 nM and does not undergo major Ca^{2+}-induced conformational changes; these Ca^{2+}-binding proteins are referred to as Ca^{2+} buffers or Ca^{2+} modulators (e.g. parvalbumin and calbindin). The second group is known as Ca^{2+}-sensors. These Ca^{2+}-sensing proteins have evolved to bind Ca^{2+} within the 10^{-5}–10^{-6} M range and typically exhibit Ca^{2+}-induced conformational changes that influence their interaction with target proteins (da Silva and Reinach, 1991; Bhattacharya et al., 2004). An archetypal Ca^{2+} sensor is calmodulin, which has four EF-hands and regulates a plethora of effectors (Clapham, 2007). Although Ca^{2+} sensors regulate the activities of ion channels and enzymes, Ca^{2+} buffers modulate the amplitude and kinetics of free Ca^{2+} transients and maintain Ca^{2+} homeostasis. For example, Ca^{2+} buffers keep the concentration of free Ca^{2+} below the levels that would induce cell death (Christakos and Liu, 2004; D'Orlando et al., 2001; Kook et al., 2014). Because the affinity of tescalcin for Ca^{2+} is in the micromolar range (Gutierrez-Ford et al., 2003), it is thought to be a Ca^{2+}-sensor.

In this article, we summarize the current insights into cellular functions of tescalcin, focusing on its unique biochemical properties and binding partners identified to date, namely, the Na^+/H^+ exchanger NHE1, glycogen synthase kinase 3 (GSK3) and the COP9 signalosome. We also discuss its potential physiological role and the potential association of *TESC* gene mutations with human pathologies.

Structural features and metal-binding properties of tescalcin

Tescalcin contains only one canonical EF-hand motif in its C-terminal half (amino acids 113–142) (Fig. 1). It also has three additional putative helix-loop-helix motifs that have likely evolved from EF-hands, but because of substitutions of crucial residues and insertions, their similarity to the canonical EF-hand is too low to support chelation of Ca^{2+} (Gutierrez-Ford et al., 2003). Single EF-hand proteins are very rare (Day et al., 2002; Huang et al., 2009; Mochizuki et al., 1996), and in most proteins these domains are paired, which allows them to bind Ca^{2+} with higher affinity. Proteins with an odd number of EF-hand domains are known to dimerize, so that the non-paired EF-hands of the two subunits

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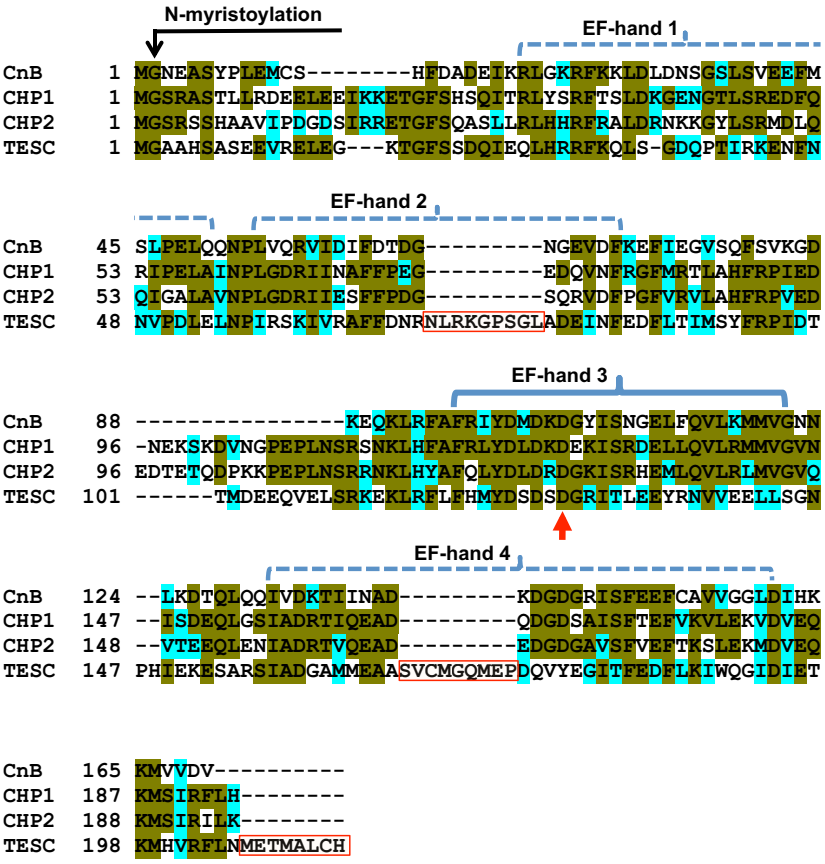

Fig. 1. Sequences of tescalcin and its close homologs. Shown here are the amino acid sequences human tescalcin (Genbank Accession Number AAL35615.1) with CHP1 (NP_009167), CHP2 (NP_071380.1) and calcineurin B (CnB, AAB08721.1). Sequence alignment was performed using ClustalW, with BoxShage 3.21 for presentation. Identical amino acids are shown in dark green; homologous residues are in teal. The four active EF-hand domains in CnB are denoted by blue brackets and are numbered. The solid bracket surrounding the canonical EF-hand 3 indicates that it is active in tescalcin. The red arrow indicates the position of the critical D123 residue necessary for Ca^{2+} binding; the D123A mutant has been used to study the role of Ca^{2+} in tescalcin function (see text). The dashed brackets show the EF-hands that are inactive in tescalcin. EF-hand 4 is active in CHP1 and CHP2. The unique amino acid 'inserts' found in tescalcin are highlighted with red frames; these interrupt the sequences in its homologous EF-hand domains 2 and 4. The N-myristoylation site, the G2 residue, is indicated by the arrow.

form a pair (Finn et al., 1992; Gifford et al., 2007; Shaw et al., 1994). Some proteins with single EF-hands do not chelate Ca^{2+}; their EF-hand functions instead as a protein–protein interaction domain. For example, the EF-hand of Nkd signaling proteins can bind to *Drosophila* and mouse Desheveled proteins, crucial participants of the Wnt pathway, in the absence of Ca^{2+} (Wharton et al., 2001).

The crucial aspartic acid in the canonical EF-hand of tescalcin, Asp123 is essential for chelating of Ca^{2+}, and its replacement by alanine (D123A) eliminates Ca^{2+} binding and Ca^{2+}-induced changes in tryptophan fluorescence (Gutierrez-Ford et al., 2003). According to UV circular dichroism spectrometry, the D123A mutation does not significantly disturb the overall secondary structure of the protein (Gutierrez-Ford et al., 2003). However, when the mutant is overexpressed in CHO-K1, HEK293 or HeLa cells, it exhibits considerably lower expression levels than wild type, suggesting that Ca^{2+} is essential for stabilizing the tescalcin fold. As discussed below, the D123A mutation has been instrumental in the functional studies of tescalcin (Kobayashi et al., 2015; Levay and Slepak, 2014; Zaun et al., 2012).

Tescalcin shares substantial sequence similarity with three other Ca^{2+}-binding proteins: calcineurin homologous protein 1 (CHP1; 34% identity, 54% similarity), calcineurin homologous protein 2 (CHP2; 29% identity, 48% similarity) and calcineurin B (CnB; 27% identity, 50% similarity). Therefore, sometimes tescalcin is referred to as CHP3 (Di Sole et al., 2012; Zaun et al., 2008). However, whereas tescalcin only has one functional EF-hand, CHP1 and CHP2 have two each and CnB has four (Naoe et al., 2005) (Fig. 1). Tescalcin contains three unique 'inserts' of eight to nine amino acids each that distinguish it from CHP1 and CHP2. Two of these inserts break up the sequences in the EF-hands two and four, apparently contributing to their inactivation. Deletion mutagenesis data suggest that the unique C-terminus extension of tescalcin might be necessary for interaction with its downstream effector CSN4, with which CHP1 and CHP2 do not interact (Levay and Slepak, 2014).

As determined by direct $^{45}Ca^{2+}$ binding and monitoring of Ca^{2+}-induced changes in the intrinsic tryptophan fluorescence, recombinant tescalcin binds Ca^{2+} with stoichiometry close to one Ca^{2+} ion per protein and a K_d of $\sim 0.8\times10^{-6}$ M (Gutierrez-Ford et al., 2003). Some EF-hand motifs are promiscuous and are able to bind other bivalent cations, such as Mg^{2+} and Zn^{2+} (Dizhoor et al., 2010; Donato, 1999). Experiments with recombinant tescalcin indicate that it can bind Mg^{2+}, but not Zn^{2+}, and might exist in a Mg^{2+}-associated form in resting cells, shifting to the Ca^{2+}-bound form during fluxes in Ca^{2+} (Gutierrez-Ford et al., 2003). Because the K_d of purified tescalcin for Ca^{2+} in the presence of Mg^{2+} is ~3 µM, Mg^{2+} exchange can only occur when (local) Ca^{2+} concentrations reach micromolar levels. It appears that in order to detect free Ca^{2+} in submicromolar concentrations, tescalcin must have higher affinity for Ca^{2+}. Indeed, the Ca^{2+} affinity of CHP1 and CHP2 is significantly higher (90 nM) (Pang et al., 2004). We speculate that one mechanism to increase the Ca^{2+} affinity of tescalcin could involve its dimerization and pairing of the EF-hands. Consistent with this idea, ~10% of *E.-coli*-expressed tescalcin elutes in fractions that correspond to the molecular mass of its dimer on a gel-filtration; however, the bulk of the protein runs as a monomer (Gutierrez-Ford et al., 2003). The peak that corresponds to the dimer is not observed in the presence of β-mercaptoethanol, indicating its dependence on a disulfide bond(s). Alternatively, the regions with low similarity to an EF-hand that are found in tescalcin might still contribute sites for chelating Ca^{2+}. It is possible that such

mechanisms can be engaged *in vivo*, and the affinity of tescalcin for Ca^{2+} is higher when it is bound to a protein partner, as is the case for CHP1 when it associates with the Na^{+}/H^{+} exchanger NHE1 (Pang et al., 2004).

Tescalcin has an N-myristoylation motif that is also present in CHP1 and CHP2. N-myristoylation of tescalcin has been demonstrated by incorporation of [^{3}H]myristate upon co-expression in *E. coli* with yeast N-myristoyl transferase-1 (Gutierrez-Ford et al., 2003). This suggests that tescalcin, similar to some other EF-hand proteins, such as recoverin (Ames et al., 1994; Ladant, 1995; Zozulya and Stryer, 1992), can undergo a Ca^{2+}-induced myristoyl switch, a conformational change that facilitates its interaction with membranes and effector proteins.

Binding partners and effectors of tescalcin

NHE1

The interaction of tescalcin with NHE1 was discovered in a yeast two-hybrid screen utilizing the C-terminal domain of NHE1 as the bait (Mailänder et al., 2001). The N-terminus of NHE1 contains 12 putative transmembrane domains that form an antiport ion channel, and its cytosolic C-domain is responsible for interacting with factors, such as CHP1, CHP2 and tescalcin (Ammar et al., 2006; Di Sole et al., 2012; Lin and Barber, 1996; Pang et al., 2004). Activation of NHE1 stimulates the efflux of protons from within the cell and causes a local rise in internal pH. In general, such pH increases are associated with increased cell proliferation and decreased apoptosis. However, considering that pH can influence signaling pathways by altering protein phosphorylation (Isom et al., 2013), prediction of the effect a change in pH has in a given biological system can be challenging.

The structural basis and functional role of the NHE1–tescalcin interaction has been confirmed and investigated by different laboratories (Li et al., 2003, 2004; Zaun et al., 2008). According to the detailed studies of Zaun et al., the amino acid residues of NHE1 that are necessary for tescalcin binding are also crucial for binding of CHP1 and CHP2, and their mutation has a profound effect on the $^{22}Na^{+}/H^{+}$ exchange activity. Co-expression of tescalcin with NHE1 increased the abundance and surface stability of NHE1 in AP-1 cells (Zaun et al., 2008). The authors conclude that tescalcin stabilizes the mature (i.e. glycosylated) form of NHE1 on the cell surface, increasing its half-life by approximately threefold. A subsequent study by the same group showed that both Ca^{2+} and N-myristoylation of tescalcin were involved in regulation of NHE1 expression and its ion exchange activity (Zaun et al., 2012). Specifically, it was shown that protein with a D123A mutation in conjunction with a G2A mutation, which prevents N-myristoylation and membrane localization, still interacts with the ion exchanger NHE1, but reduces its cell surface half-life and transport velocity. Thus, although Ca^{2+} is not essential for the interaction between tescalcin and NHE1, the Ca^{2+}-bound form of tescalcin clearly regulates NHE1 turnover and activity (Zaun et al., 2012). Overall, these studies support the notion that tescalcin undergoes a Ca^{2+}–myristoyl switch, which plays a role in the upregulation of NHE1 activity (Zaun et al., 2012).

It is worth noting that some discrepancies exist with regard to the described tescalcin–NHE1 interactions, suggesting that the relationship between tescalcin and NHE1 might be nuanced. To date, all studies have implicated the C-terminus of NHE1 in the interaction with tescalcin, but have identified different regions to be of functional importance, for example, either juxtamembrane sites (Zaun et al., 2008) or the distant C-tail positions (Li et al., 2003). The functional effects of the tescalcin–NHE1 interaction are also being debated, with the former report showing stimulation of NHE1 by tescalcin, whereas the latter demonstrated its suppression. It is also worth noting that whereas co-expression of tescalcin with NHE1 in CHO cells results in a redistribution of tescalcin to the plasma membrane (Zaun et al., 2008), in cardiac myocytes, tescalcin does not colocalize with NHE1 (Kobayashi et al., 2015). However, under the same conditions, NHE1 colocalizes with CHP1 in both HEK293 cells and myocytes, indicating that NHE1 is available for the interaction with both proteins (Kobayashi et al., 2015). It is, therefore, possible that the interaction of NHE1 with tescalcin is either weaker than that with CHP1 in specific cellular environments, or it might depend on thus far unidentified regulatory mechanisms.

Most experiments on NHE1–tescalcin binding have been performed by using recombinant proteins. However, recent evidence is suggestive of an association between endogenous tescalcin and NHE1 (Man et al., 2014). Here, the knockdown of tescalcin in acute myeloid leukemia (AML) cell lines resulted in a reduction of pH by ∼0.15 units. Given that pharmacological blockade of NHE1 eliminated the effect of tescalcin knockdown, the authors attributed this reduction in pH to the loss of interaction between tescalcin and NHE1. These results are consistent with the tescalcin-mediated activation of NHE1 that has been reported previously in transfected cell models (Zaun et al., 2008).

COP9 signalosome

Another yeast two-hybrid screen using tescalcin as bait identified subunit 4 of the COP9 signalosome (CSN4, encoded by *COPS4*) as a binding partner of tescalcin (Levay and Slepak, 2014). The COP9 signalosome (CSN) is an evolutionary conserved complex composed of eight subunits (CSN1–CSN8, encoded by *GPS1* and *COPS2–COPS8*) that has substantial similarity to the 26S proteasome. CSN was first discovered as a negative regulator of photomorphogenesis in *Arabidopsis thaliana*, and to date, many studies have established its essential role in the normal development of all eukaryotes. The CSN is a modulator of the cell cycle, DNA repair, MAPK and steroid hormone signaling, axonal guidance and other vital cellular processes (Kato and Yoneda-Kato, 2009; Wei et al., 2008). At the mechanistic level, this broad range of biological functions involves protein phosphorylation, gene transcription, and protein degradation through cullin–RING–E3 ubiquitin ligases (Kato and Yoneda-Kato, 2009; Wei et al., 2008). More than 50 proteins have been shown to interact with CSN subunits, but there are only two binding partners of CSN4: torsinA (also known as torsin-1A) (Granata et al., 2011), a poorly understood ATPase known for its localization in endoplasmic reticulum and association with movement disorders (Rose et al., 2015) and tescalcin. Tescalcin specifically binds to CSN4 through its proteasome-Cop9-eIF3 (PCI) domain in a Ca^{2+}-dependent manner; this was shown by using purified recombinant proteins in a yeast two-hybrid system and a luciferase reporter assay in transfected HEK293 cells (Levay and Slepak, 2014). CHP1 and CHP2 do not interact with the COP9 signalosome in the same assays, which distinguishes tescalcin from its closest homologs. According to deletion mutagenesis, the interaction with CSN4 requires the unique C-terminus of tescalcin (Levay and Slepak, 2014). Tescalcin can also interact with CSN5 (also known as Jab1; Wei et al., 2008), although this interaction is weaker than that with CNS4, whereas it does not interact with any of the other CSN subunits. Importantly, we have shown that knockdown of tescalcin in human erythroleukemia HEL cells increases CSN activity. This increase correlates with a negative effect on the ability of these cells to

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undergo induced differentiation and results in a change in the expression levels of p53 and the c-Jun transcription factors, which control a multitude of genes involved in cell cycle and differentiation (Levay and Slepak, 2014).

GSK3

A recent comprehensive study showed that tescalcin inhibits GSK3 (Kobayashi et al., 2015), a Ser/Thr protein kinase involved in a multitude of cellular functions that range from proliferation and apoptosis to migration and metabolism (see e.g. Doble and Woodgett, 2003; Rayasam et al., 2009). The original observation of Kobayashi et al. was that adenovirus-mediated knockdown of tescalcin resulted in hypertrophy of cultured neonatal ventricular myocytes (NRVMs) (Kobayashi et al., 2015). Here, the extent of cell enlargement was comparable to that caused by phenylephrine or IGF-1, and was accompanied by upregulation of atrial natriuretic peptide (ANP), a factor secreted by cardiac myocytes and one of the markers of pathologic heart hypertrophy and other conditions (Song et al., 2015). The magnitude of this effect demonstrated the importance of tescalcin in regulation of NRVM size and gene expression.

With regard to the molecular mechanisms underlying tescalcin function, Kobayashi et al. observed an approximately threefold increase in the phosphorylation of GSK3α and GSK3β at Ser9 upon knockdown of tescalcin. In contrast, overexpression of tescalcin in NRVMs inhibited stimulus-induced hypertrophy and blocked both basal and insulin-induced GSK3 phosphorylation. Some of these mechanisms were recapitulated in HEK293 cells, where expression of GFP-fused tescalcin reduced insulin-induced phosphorylation of GSK3 isoforms. This effect was selective for tescalcin, as under the same conditions, CHP1 did not influence GSK3 phosphorylation. Importantly, neither tescalcin nor CHP1 affect the phosphorylation of AKT, one of the regulators of GSK3. Given that AKT-mediated phosphorylation at Ser9 inhibits GSK3 activity, these results showed that tescalcin is a selective inhibitor of GSK3. Moreover, in a convincing series of co-immunoprecipitation and other biochemical assays on NRVMs and co-transfected HEK293 cells, Kobayashi et al. also demonstrated that tescalcin can form complexes with either GSK3 or AKT. These results suggest that the effect of tescalcin on GSK3 phosphorylation is mediated by its direct binding to AKT and/or GSK3; however, participation of another protein(s) in the tescalcin–GSK3 interaction cannot be ruled out at this point. By using the Ca^{2+}-binding-defective D123A tescalcin mutant, Kobayashi et al. showed that Ca^{2+} is not required for its interactions with these kinases and/or its inhibition of GSK3 activity (Kobayashi et al., 2015).

Taken together, these findings clearly show that tescalcin can bind to and influence the activities of NHE1, the CSN and AKT and/or GSK3 (Fig. 2). Tescalcin has also been found to block the activation of calceneurin B by calmodulin *in vitro* (Gutierrez-Ford et al., 2003), as well as to bind to G protein-coupled receptor kinase 2 in yeast two-hybrid experiments (J. Benovic, personal communication). However, these latter findings have not yet been confirmed by additional work. Regarding the selectivity of tescalcin–effector interactions and the role of Ca^{2+}, studies have shown that tescalcin is the only member of the CHP family capable of binding to GSK3 and CSN4, whereas NHE1 can also bind to CHP1 and CHP2. Ca^{2+} is important for the interaction of tescalcin with CSN4 and NHE1, but not with GSK3.

It is not unusual for a Ca^{2+} sensor to interact with several effectors, for example, calmodulin is known to regulate dozens of proteins, typically through distinct α-helical motifs present in its targets. Additional experiments are needed to understand the molecular basis of the interaction between tescalcin and its target proteins, which are all structurally and functionally unrelated. It is reasonable to assume that the binding sites for tescalcin in CSN4 (or CSN5) and AKT and/or GSK3 are different from those in NHE1, as the latter can distinguish between tescalcin and CHP1 or CHP2. Considering the affinity of tescalcin for Ca^{2+}, we can also speculate that at very high Ca^{2+} concentrations, tescalcin will bind to its target as a monomer, whereas its dimeric form, which presumably has higher affinity for Ca^{2+}, might interact with additional effectors. It is also possible that the Ca^{2+}-free EF-hand of tescalcin could function as a protein docking module in the interaction with GSK3, similar to that of Nkd proteins that regulate Wnt pathway (Wharton et al., 2001).

The tescalcin targets also have different subcellular localization. NHE1 is localized at the plasma membrane, whereas GSK3 and CSN can be found in the cytoplasm and the nucleus. However, as we discuss below, tescalcin is found throughout the cell, supporting the possibility that these interactions occur *in situ*. Moreover, although its effectors are expressed ubiquitously, the expression of tescalcin is tightly regulated, suggesting that its ability to regulate

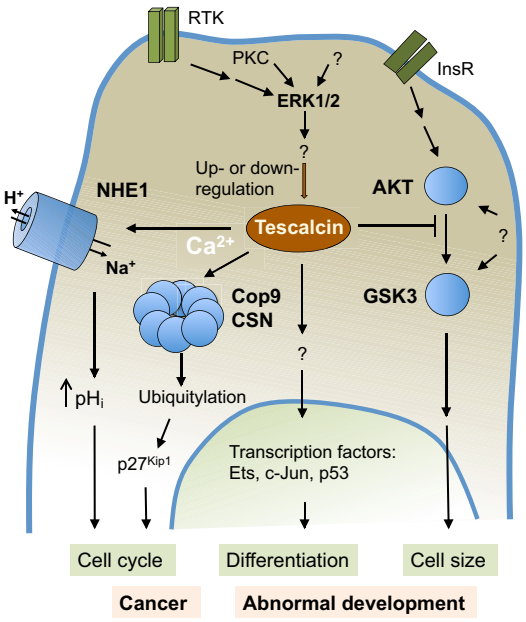

Fig. 2. Protein–protein interactions and biological activities of tescalcin. Tescalcin modulates the activities of NHE1, the Cop9 signalosome and the AKT–GSK3 axis. It interacts directly with NHE1, increasing its stability and the rate of exchange of Na^+ for H^+ ions, which leads to elevation of intracellular pH (pH_i). Tescalcin has also been shown to interact with the CSN4 (and CSN5) subunit of the signalosome which affects many processes including degradation of proteins involved in cell cycle. Finally, tescalcin interacts with the protein kinases AKT and GSK3, which have multiple substrates and a number of known mechanisms of activation, e.g. through insulin receptor (InsR), which has been shown to activate GSK3 in a tescalcin-dependent manner (see text). Each of these pathways has many downstream effectors, including cell-type-specific transcription factors. The level of tescalcin expression is regulated by ERK1/2 (brown arrow) through an unknown mechanism; it can be up- or down-regulated depending on the cell type. ERK1/2 can be activated by PKC, receptor tyrosine kinases (RTKs) and other mechanisms. The interaction of tescalcin with NHE1 and CSN4, but not that with AKT and/or GSK3, requires Ca^{2+}. Depending on the cell type, the physiological effects include cell cycle arrest and differentiation, or growth. Furthermore, genetic evidence has implicated the tescalcin gene (*TESC*) in cancer and developmental abnormalities (see text).

the pathways described above is limited to certain cell types and stages of development.

Subcellular localization and tissue distribution of tescalcin

According to fractionations of mouse heart or HeLa cells, endogenous tescalcin partitions to soluble, membrane and nuclear, but not to cytoskeletal, fractions (Gutierrez-Ford et al., 2003). In addition, immunofluorescence microscopy of tescalcin that had been overexpressed in PC12, HeLa or CHO-K1 cells detected it throughout the cell, but concentrated in the perinuclear region. Here, mutations that prevent Ca^{2+} binding or N-myristoylation, or manipulations of cellular Ca^{2+} with BAPTA-AM or ionomycin did not alter the localization of tescalcin (Gutierrez-Ford et al., 2003). Furthermore, in the myelogenous leukemia cell line K562, we found stably expressed tescalcin in the nucleus (Levay and Slepak, 2007). In a clone of CHO cells that lack NHE1, Myc-tagged tescalcin is distributed evenly throughout the cytoplasm, with an increase in its concentration in and around the nucleus (Zaun et al., 2008). However, when these cells are transfected with NHE1, the bulk of tescalcin redistributes to the plasma membrane. More recent work addressing endogenous tescalcin in cardiac myocytes has shown that it is spread uniformly throughout the cytoplasm and nucleus (Kobayashi et al., 2015). With respect to its cellular localization, tescalcin is similar to CHP1, which has been shown to translocate between the nucleus, cytoplasm and plasma membrane (Jiménez-Vidal et al., 2010).

The expression levels of tescalcin vary considerably between tissues, and in what appears to be unique for tescalcin compared with CHP1 and CHP2, changes dramatically during development. CHP1 is broadly expressed and the most conserved member of the family across many species. CHP2 is found in normal intestinal epithelia, but is preferentially expressed in tumor tissues, for example in human liver cancer specimens (Inoue et al., 2003; Li et al., 2008). Moreover, overexpression of CHP2 increases the oncogenic potential of stably transfected HEK293 cells following their inoculation into nude mice (Li et al., 2008). In contrast to CHP1, CHP2 and tescalcin are only found among vertebrates (Di Sole et al., 2012).

In mouse embryos, the highest concentration of tescalcin mRNA is observed in the testis, hence the name of the protein (Perera et al., 2001). However, in adult mouse, protein levels are highest in the stomach, heart and brain, with only a minor expression in testis (Gutierrez-Ford et al., 2003). Human tescalcin has a localization pattern that is similar to mice, with low mRNA levels in adult testes and abundant expression in the adult heart. In addition, the protein levels in the adult rat heart (ventricles and atria) are considerably higher than in the brain (Kobayashi et al., 2015). Interestingly, tescalcin is not present in skeletal or smooth muscle at all (Gutierrez-Ford et al., 2003), suggesting that is has a cardiac-myocyte-specific function or that it could be involved in the differentiation of muscle cells. Tescalcin mRNA has also been found in human pancreas, bone marrow, salivary and adrenal glands, and placenta (Gutierrez-Ford et al., 2003). Furthermore, antibodies against tescalcin have detected the endogenous protein in a number of tumor cell lines, pointing to a potential link to cancer (Fan et al., 2015; Gutierrez-Ford et al., 2003; Kang et al., 2014; Levay and Slepak, 2010, 2014).

A recent survey of the Allen Brain Atlas examined gene expression profiles of the entire EF-hand superfamily (Girard et al., 2015). There is no expression of CHP2 and no data for CHP1, but tescalcin mRNA has been found to be present in many regions, including the main olfactory bulb, basal ganglia and amygdala. Strong expression of tescalcin is also found in the hippocampus, which is particularly interesting in light of the genetic linkage of the *TESC* gene with human central nervous system (CNS) abnormalities (see below). Obviously, detailed analysis of the dissected regions are needed to validate the Brain Atlas data and to further dissect the physiological roles of tescalcin and how they are linked to human disorders.

The role of tescalcin in cell differentiation and proliferation

Induced *in vitro* differentiation of the hematopoetic cell lines HL60 and K562 results in a dramatic change in tescalcin expression, which occurs at either the transcription or post-transcriptional level (Levay and Slepak, 2007, 2010). Experimental manipulation of tescalcin expression highlights its role in the pathways that control differentiation in these cell lines, as well as in primary megakaryocytes. For instance, tescalcin overexpression in K562 cells leads to spontaneous polyploidization, a reduction in growth rate and the appearance of megakaryocytic markers such as GPIIb integrin. At the same time, knockdown of tescalcin by RNA interference significantly slows down differentiation that is induced by factors such as phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C (PKC) (Levay and Slepak, 2007). However, a later study that generated a tescalcin knockout in mice did not detect changes in the amount of megakaryocytes and platelets (Ukarapong et al., 2012). To explain the conflicting data, the authors speculate that CHP1 and/or CHP2 could compensate for the lack of tescalcin, or that their genetic knockout was incomplete and allowed for the expression of a partial protein (Ukarapong et al., 2012). Another possible explanation is that Ukarapong et al. might have only analyzed the adult mice. Thus, any effects of tescalcin knockout during development or in response to stress such as blood loss, which restores the megakaryocyte population, might have been missed. In fact, tescalcin knockdown does not completely block megakaryocytic differentiation, but instead delays it considerably (Levay and Slepak, 2007).

The exact mechanism of how tescalcin affects the differentiation of hematopoetic precursors has not been determined. Tescalcin does not have a DNA-binding domain and is not a transcription factor itself. What is known is that human K562 or HEL cells can be induced to differentiate along the megakaryocytic lineage through a cascade that involves activation of PKC, ERK1 and ERK2 (ERK1/2, also known as MAPK3 and MAPK1) and upregulation of Fli-1, Ets-1 and Ets-2 factors (Levay and Slepak, 2007). Activation of this pathway drives the expression of megakaryocyte-specific genes and coincides with a several-fold increase in tescalcin level. Importantly, after *TESC* knockdown, the transcription factors Ets-1 and Fli-1 can no longer be detected at either the protein or mRNA level, even in PMA-treated cells. This indicates that tescalcin acts upstream of the Ets transcription factors. Pharmacological blockade of ERK1/2 or PKC prevents upregulation of tescalcin, indicating that tescalcin acts downstream of ERK1/2 (Fig. 2). Although the molecular mechanism of tescalcin-mediated upregulation of Ets transcription factors has not been elucidated, it is possible that GSK3 and/or the COP9 signalosome play a role in this process. Given that the expression of other transcription factors, such as GATA1 or MafB, remains unchanged, it is clear that this mechanism is selective toward particular transcription factors, and does not influence gene transcription in general (Levay and Slepak, 2007).

Ets factors do not only participate in hematopoiesis (Ciau-Uitz et al., 2013) but also have a role in normal development and pathology of cardiovascular and endocrine systems (Dittmer, 2015; Gutierrez-Hartmann et al., 2007; Meadows et al., 2011); this might,

at least in part, explain the *TESC*-associated conditions affecting the CNS and other organs (see below). Tescalcin is also involved in the differentiation and maturation of granulocytes, a process that is also mediated by ERK1/2 signaling (Levay and Slepak, 2010). It is important to note that although tescalcin is upregulated in some differentiation pathways (e.g. in granulocytes or in the developing heart), it is downregulated in others (macrophages, testes) (Gutierrez-Ford et al., 2003; Kobayashi et al., 2015; Levay and Slepak, 2010). Consistent with its role in differentiation, recent studies have implicated tescalcin in some developmental defects and cancer, as discussed in the following sections.

The *TESC* gene in human abnormalities affecting the CNS

The *TESC* gene is localized in 12th human chromosome and contains eight exons. Genome-wide association analysis has revealed a significant linkage between the single-nucleotide polymorphism (SNP) rs7294919 on chromosome 12q24 and reduced hippocampal volume. This SNP appears to regulate the transcriptional activity of the *TESC* gene (Stein et al., 2012). This work was extended by Dannlowski et al. who used different imaging methods and confirmed the association of the *TESC* gene SNPs with the reduction of volume in the gray matter of hippocampal formation, an area of relatively high tescalcin expression (Dannlowski et al., 2015). The authors also demonstrated an interaction of tescalcin with the rs2299403 variant of the *RELN* gene, encoding reelin, through an unknown molecular mechanism. Reelin, a secreted glycoprotein involved in neuronal migration and signaling, has been shown to affect higher cognitive processes and to play a role in schizophrenia and bipolar disorder (Guidotti et al., 2016).

Another group has described a patient with symptoms of developmental delay and other anomalies related to hemizygous deletion at the 12q telomere, which encompasses at least 52 annotated genes. Given that most similar patients had male genitalia abnormalities, the authors suggested that the *TESC* gene was crucial for the defective development (Al-Zahrani et al., 2011). *TESC* mutations are also present among the genes associated with intellectual disability in a cohort of psychiatric patients in the Dagestan region of southern Russia (Bulayeva et al., 2015).

Considering the role of tescalcin in cell differentiation and the regulation of Ets transcription factors (Levay and Slepak, 2007), the link between *TESC* mutations and brain development could be potentially explained by disrupted expression of multiple genes that are regulated by tescalcin-mediated pathway(s). Obviously, GSK3 and NHE1 are also involved in multiple fundamentally important pathways, and any aberration in the interaction between tescalcin and these factors is likely to also have effects in the CNS.

Tescalcin and cancer

Alterations in the expression and copy number of the *TESC* gene have also been linked to radiation-induced papillary thyroid carcinoma (PTC) in pediatric patients that have been exposed to radioactive fallout from the Chernobyl disaster (Stein et al., 2010). In particular, the authors reported increases in the copy numbers of the subtelomeric and telomeric regions of chromosome 12q. By comparing gene expression between sporadic and radiation-induced PTC samples, they identified over 100 genes that are uniquely expressed in the tumors originating from Chernobyl, with the *TESC* gene being one of the genes that showed the highest increase in expression.

Other groups around the world have also recently implicated tescalcin in different cancers. For example, one study has shown that the *TESC* gene is expressed in human colorectal cancer (CRC) tissues, but not in normal mucosa and premalignant lesions (Kang et al., 2014). Here, the level of *TESC* expression in cancer tissues correlated with the pathological characteristics of patients with CRC. According to this study, patients with high *TESC* expression are more likely to have a shorter survival period than patients with low expression. As there are currently no efficient prognostic factors for CRC, the expression levels of tescalcin in the tumor could represent a diagnostic marker. In addition, the authors also reported significantly higher levels of tescalcin in the serum of CRC patients compared to control individuals (Kang et al., 2014). However, in our opinion, these results should be viewed with caution because it is unclear how tescalcin, as an intracellular protein of low abundance, could be detected in serum.

As mentioned above, a link between AML pathogenesis and the interaction of tescalcin with NHE1 has been found in AML MOLM-13 and MV4-11 cell lines, where tescalcin knockdown leads to intracellular acidosis and apoptosis (Man et al., 2014). Furthermore, *TESC* is one of the genes that is upregulated when these leukemia cells develop resistance to sorafenib, a tyrosine kinase inhibitor that has been approved for treatment of some types of cancer but is unable to fully inhibit subsequent leukemia progression. The authors found that pharmacological inhibition of NHE1 by a derivative of the known diuretic amiloride results in cell acidification and apoptosis, and repression of resistance of AML to sorafenib. The authors conclude that upregulation of tescalcin causes soranfenib resistance through an increase in NHE1 activity and subsequent rise in the intracellular pH, which favors cell survival and growth (Man et al., 2014).

Another recent study has observed an upregulation of tescalcin in melanoma and colon tumors, as well as in gastric and colorectal tumor cell lines (Fan et al., 2015). In the course of their studies, the authors identified an interesting epigenetic mechanism underlying the increase of *TESC* expression. They found that binding of the long non-coding RNA ROR to the *TESC* promoter prevents access of the histone methyltransferase G9A (also known as EHMT2), thereby counteracting histone H3K9-methylation-mediated gene repression (Fan et al., 2015).

Taken together with the insights in its role in cell differentiation, the rapidly growing evidence shows that aberrant expression of tescalcin plays a role in many types of cancer, which identifies it as a potential oncotarget.

Conclusions

Tescalcin is an EF-hand Ca^{2+}-binding protein with tissue-specific and developmentally regulated expression. At the molecular level, tescalcin interacts with NHE1, GSK3 and the COP9 signalosome (Fig. 2). It has also been shown to link activation of ERK1/2 with Ets and a few other transcription factors through an as-yet-unknown mechanism. Thus, not unlike its distant and much more renowned EF-hand relative calmodulin, it appears to have the ability to regulate diverse protein targets. In contrast to calmodulin, tescalcin is involved in the regulation of relatively slow cellular processes, such as cell differentiation and proliferation. Consistent with this notion, human genetics and gene expression profiling studies have uncovered correlations between abnormal tescalcin expression and brain developmental deficiencies and cancer. However, we have only just begun unraveling the molecular mechanisms underlying these abnormalities. As with other new gene products, this understanding will require research at the molecular, cellular and whole organism levels. More information on protein–protein interactions of tescalcin, the role of Ca^{2+} and its unique structural

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elements will be necessary to elucidate the contribution of this protein in signaling networks. Precise characterization of the nature of *TESC* mutations will also shed more light on the mechanisms of tescalcin involvement in human pathology.

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