Targeting Altered Calcium Physiology in the Heart: Translational Approaches to Excitation, Contraction, and Transcription

Calcium (Ca) is essential for excitation-contraction coupling. At the same time, Ca is of pivotal importance as a second messenger in cardiac signal transduction, where it regulates cardiac growth and function by activation of kinases and phosphatases, ultimately driving transcriptional responses and feeding back on Ca handling proteins, a phenomenon termed excitation-transcription coupling. Cardiac Ca homeostasis thus needs to be maintained via a delicate interplay of proteins to allow physiological function and adaptation, whereas disturbed Ca-handling and Ca-dependent signaling are hallmarks of heart failure. In this review, we will discuss the most recent mechanistic findings in Ca-handling and Ca-signaling proteins in the development of cardiac pathology with a focus on translational aspects.

Calcium in Cardiac Excitation-Contraction Coupling

Baseline membrane potentials of cardiac myocytes are maintained by a near equal relation of repolarizing and depolarizing currents (2). This imperfect balance, slightly favoring cationic influx, is especially realized in cells of the conduction system, where if channels allow resting myocytes to depolarize from their predominant potassium (K) potential. In hierarchical order, with the sinus nodal cells exhibiting the steepest spontaneous depolarization, followed by more remote myocytes of the AV nodal and Purkinje system, syncytial organization via gap junctions ensures a fast depolarization of the entire organ. Unlike skeletal muscle, where recruitment of motor units contributes to generated force, control of cardiac contraction relies on alternative mechanisms modulating excitation and contraction (3). As in other excitable cells, above-threshold depolarization is mediated by a transient increase in sodium (Na) conductivity of the cellular membrane via voltage-gated Na channels. However, the following long-lasting plateau phase of the cardiac action potential is largely maintained by L-type calcium (Ca) currents (as well as reduced inward flow of K) and unique to cardiac myocytes. L-type Ca currents are transmitted rapidly to inner compartments of the cell via transverse tubuli, which reach close proximity to the longitudinal system of the sarcoplasmic reticulum (SR). Thereby, the long-lasting Ca currents not only maintain the positive phase of the action potential but directly regulate contractile force. This control is via activation of Ca-sensitive channels of the SR or ryanodine receptors type 2 (RyR), named after their affinity to the plant alkaloid ryanodine. RyR cluster in close proximity to L-type Ca channels along the longitudinal system of the SR, governing the Ca storage compartment with the highest recruitable amount of Ca in cardiac myocytes. The RyR are chemically coupled to L-type Ca channels via Ca, and a relatively small amount of Ca entering the cell via L-type Ca channels (depending on species or stimulation rate) activates a limited number of adjacent RyRs. This leads to Ca release from the SR, and a subsequent marked increase in intracellular Ca concentration, and has been termed Ca-induced Ca release (11).

RyR form multimeric complexes with a number of cytoplasmic and intraluminal proteins. This allows for integration of various signals, and numerous regulatory roles of these interactions have been suggested. Ca removal occurs via active transport back to the SR via the SR Ca ATPase (SERCA), which is subject to control by the protein phospholamban (PLB). A smaller amount of Ca (albeit the same amount that previously had entered the cell through L-type Ca channels) is removed toward the extracellular space via the Na/Ca exchanger (NCX). There is significant autoregulation of Ca fluxes (10), and a variety of post-translational modifications have been identified to modulate Ca-handling proteins. Protein kinase A (PKA) as well as Ca/calmodulin-dependent kinase II (CaMKII) are important kinases in this process, but other kinases as well as phosphatases are also being investigated. At the level of the RyR, phosphorylation influences the association of FKBP12.6, which was subsequently shown to be central to RyR modulation (33). In heart failure, disturbance of cardiac Ca cycling occurs at multiple levels and is closely connected to pathological performance (19), thus offering numerous sites for translational approaches to treat or prevent heart failure.
Three ryanodine receptor isoforms are known, but only type 2 is expressed at significant levels in the heart. Groundbreaking work from Marks’ laboratory and others has gradually changed the initial perception of this protein from that of a Ca-sensitive Ca pore toward a multi-protein-controlled Ca release unit (59). It has already been reported that PKA increases RyR sensitivity toward Ca and that dissociation of FKBP12.6 from RyR increased open probability of the channel, but a causative link between these observations had not been demonstrated. When RyR was purified by sucrose gradient centrifugation or precipitated with anti-RyR antibody, Marks’ group initially demonstrated the association of RyR with five proteins, namely PKA, (including its RII subunit), mAKAP (a known PKA anchoring protein), protein phosphatase 1 and 2A (PP1 and PP2A), as well as the 12.6-kDa FK506-binding protein (FKBP12.6) (35), advancing the concept of the RyR to represent a multi-protein complex. They demonstrated that RyR is PKA hyperphosphorylated at serine 2809 in heart failure, leading to the increased presence of subconductance states. In lipid bilayer experiments, PKA hyperphosphorylation led to similar subconductance states observed earlier on dissociation of FKBP with small molecules. Importantly, PKA, but not CaMKII, was able to abolish RyR-FKBP12.6 co-immunoprecipitation, and samples of heart failure patients exhibited higher PKA phosphorylation as well as reduced RyR-FKBP association. Therefore, they suggested a mechanism where FKBP12.6 represents a PKA-sensitive regulatory subunit of RyR. In a parallel study, Yano and co-workers (58) demonstrated that FK506 treatment (which is known to interfere with FKBP12.6 binding at the RyR) led to increased Ca efflux via RyR in isolated SR vesicles. Since FKBP12.6 was also found to be less abundant in SR vesicles of failing hearts, and these vesicles exhibited a Ca efflux similar to FK506 treated vesicles, FKBP12.6 depletion emerged as a novel mechanism of disturbed Ca homeostasis in heart failure (58).

Subsequent work has consequently focused on adding back FKBP12.6 to RyR to fix the observed defects. In isolated cardiac myocytes in vitro, Prestle et al. have demonstrated a decreased Ca leak and increased fractional shortening on adenoviral overexpression of FKBP12.6 (39). Coupled gating, meaning the concerted opening in systole and closed state of RyR clusters in lipid bilayers, could be restored by adding back recombinant FKBP12.6 to functionally uncoupled channels (34). In vivo, a transgenic mouse expressing a FKBP “sticky” mutant, which binds to RyR despite PKA-mediated phosphorylation, was reported to prevent loss of contractile performance following experimental myocardial infarction. Surprisingly, overexpression of wild-type FKBP12.6 was equally effective, and the authors suggested that high overexpression levels may compensate for PKA-mediated hyperphosphorylation (23).

Alternatively, and potentially more applicable from a pharmaceutical standpoint than adding additional FKBP, a small molecule, called JTV519, was reported to partially prevent pacing-induced heart failure in dogs, associated with reduced RyR hyperphosphorylation (56). This was associated with a restoration of the Ca leak and prevention of the FKBP12.6-RyR dissociation. Since JTV519 also preserved RyR conformation and prevented SR Ca leak after partial loss of FKBP12.6 from the complex, as determined with a conformation-sensitive fluorescence probe, the authors suggested that the drug might directly exert FKBP12.6-like stabilizing effects on the channel. Additional experiments strongly suggested that FKBP12.6 deficiency is a critical mediator of triggered arrhythmias and that JTV519 may be effective in correcting the increased Ca leak and arrhythmias observed in FKBP-deficient mice (28). Since JTV519 is known to exert effects on multiple ion channels, current work focuses on the development of more specific derivatives. It remains to be seen to what extend the beneficial aspects of JTV519 and future derivatives rely on the direct actions of FKBP rebinding to RyR and thus a novel mechanism. In addition, it is not known whether bringing back FKBP could really add benefit on-top of existing pharmacological interventions: Marks’ group has shown that α-adrenergic blockade, a clinically well proven strategy to treat heart failure and arrhythmia, could already completely normalize the stoichiometry of the RyR multi-protein complex with respect to the five proteins mentioned above and reverted the increased open probability of isolated channels (40). Likewise, low-dose propranolol treatment completely prevented SR Ca leak in a dog model of heart failure and conformational changes in RyR as well as RyR-FKBP12.6 stoichiometry (9), questioning potential on-top benefits of strategies to restore FKBP12.6-RyR binding. With all its significance to the field, many aspects of the FKBP12.6 theory have been questioned, including the effects of PKA phosphorylation on FKBP12.6 binding and RyR-mediated leak, changes, RyR phosphorylation, and FKBP12.6 binding in heart failure, and role of FKBP12.6 in coupled gating (1a, 5, 25, 29, 44, 53).

In view of the ample data suggesting a central role of FKBP12.6 in the pathophysiology of altered Ca cycling in heart failure, it is puzzling that a FKBP12.6 knockout mouse does not exhibit heart failure. Currently, two independently created models exist in two different genetic backgrounds, which differ in their phenotype. The first model, created by Xin et al. (54) targeting exon 3 in SV129 mice exhibits no macroscopic abnormalities in female mice, yet male mice exhibit a slight concentric hypertrophy due to increased septal wall thickness. The interpretation is complicated by hypertension in male mice, which could theoretically have provoked such abnormalities independently of a direct effect on cardiomyocyte Ca cycling. Indeed,
since female mice are normotonic, yet, like male mice, exhibit increased and disturbed Ca release (Ca spark duration and amplitude as well as excitation-contraction coupling gain is increased), it is possible that systemic rather than cardiomyocyte effects cause this hypertrophic phenotype. A second, independently created model in a DBA genetic background targeted exons 3 and 4 and did not exhibit hypertension or hypertrophy. In this model, a thorough analysis of Ca-release events led to the finding that loss of FKBP12.6 led to defective gating, promoting delayed after-depolarizations (DAD) after strenuous exercise combined with phenylephrine treatment. Indeed, these mice were reported to develop ventricular arrhythmias, caused by exercise-induced sudden cardiac death, whereas wild-type mice remained unaffected. Lack of FKBP led to increased PKA sensitivity in these animals and reflected the phenotype of catecholaminergic polymorphic ventricular tachycardia (CPVT), a potentially lethal disease. The authors could further show that three known mutations in RyR, known to be associated with CPVT in humans, are also associated with both an increased Ca leak as well as a reduced FKBP12.6 affinity of RyR. One mutant was further examined and demonstrated to be functionally normalized in vitro by adding a sticky mutant of FKBP (D37S), adding another potential clinical application of restoring FKBP-RyR stoichiometry (50). Liu and colleagues (30) provided the first convincing evidence in vivo that CPVT are indeed caused by mutations in RyR by creating a knock-in mouse of the RyR R4497C mutation. These mice exhibit the typical ECG pattern of bidirectional tachycardias when challenged with epinephrine plus caffeine or treadmill exercise. This mutation was reported to exhibit increased leak and has less FKBP12.6 affinity when expressed as recombinant RyR in vitro. Therefore, in addition to the two FKBP12.6 knockout mice, it could also be interpreted as a third genetic model of murine FKBP depletion at the RyR. However, contrasting data showing lack of efficacy of K201 (JTV519 from a different manufacturer) and lack of FKBP dissociation in SR vesicles from RyR4497C mice (30) or recombinantly expressed RyR4497C (12) reflect the current controversy on the importance of RyR-FKBP interaction in CPVT. In addition, it was recently shown that, also from the luminal side a mutation in calsequerin, an important Ca storage protein associated with RyR in the SR may lead to CPVT (46), suggesting a role for abnormal intrastore Ca signaling (27).

Collectively, these models suggest that FKBP12.6 is not required to prevent heart failure under baseline conditions but is necessary to maintain proper RyR gating with relevance for susceptibility toward catecholaminergic arrhythmia. Additionally, Marks group reported that JTV519, while being effective in FKBP12.6–/– mice, did not change the open probability of RyR in FKBP12.6+/- mice, suggesting that the drug’s action on RyR require FKBP12.6. However, whether adding back FKBP12.6 either via overexpression or small molecules represents a future tool to treat CPVT mutations regardless of their position in the large RyR protein remains to be elucidated (13). Another approach in modulating the RyR function focuses on conformational changes. Subdomains of the giant RyR protein, which also represent areas of clustered mutations found in arrhythmic patients, were identified to particular associate either loosely or tightly depending on, and likely supporting, the closed or open conformations of the whole channel. This observation (reviewed in Ref. 24) may offer a readout or represent a direct target for development of new therapeutics in arrhythmia and heart failure, since these conditions were demonstrated to exhibit altered interdomain interaction. Indeed, Yano et al. demonstrated correction of interdoamin interaction, associated with reduced Ca leak using the small molecule antioxidant (Edaravone) (57). Changes in interdomain interaction may underly several of the previously suggested mechanisms altering RyR function and may represent another autoregulatory mechanism disturbed in pathological RyR Ca release (13).

Other than PKA-dependent regulation of RyR, CaMKII-dependent phosphorylation and hence CaMKII regulation of RyR were shown recently. In a transgenic mouse model overexpressing the cytosolic isoform of CaMKII (CaMKIIΔC), a similar Ca leak from the SR was found (32). This leak was associated with cardiac hypertrophy and heart failure. However, by overexpressing CaMKIIΔC by adenovirus-mediated overexpression in isolated myocytes, it was shown that, even after 24 h, significant Ca leak from the SR occurs (26). This CaMKII-dependent SR Ca leak was confirmed by other groups (1, 6, 16, 51) but was not associated with FKBP dissociation from the SR (26, 51). Recently, it was even shown that severe ventricular arrhythmias can be elicited in CaMKIIΔC transgenic mice, which may be a consequence of increased SR Ca leak. However, this may be complicated by the fact that CaMKII not only phosphorylates RyR but also L-type Ca channels and even cardiac Na channels (49). At present, there is an ongoing debate on the relative importance of PKA vs. CaMK in the regulation of RyR.

**Consequences of Altered RyR Function and Ca Leak**

The concept of an increased SR Ca leak in heart failure and arrhythmias, independent of whether it is PKA- or CaMKII-dependent, is based on observations of increased RyR open probability in isolated RyR preparations, increased Ca loss from SR vesicles isolated from failing hearts, increased Ca spark frequency, and number of Ca waves. A diastolic Ca leak could be the common mechanism behind several unfavorable alterations in failing myocytes, including diminished...
systolic performance due to reduced Ca concentrations in systole, increased diastolic stiffness due to diastolic activation of myofilaments, increased ATP consumption due to futile Ca cycling via SERCA, and last but not least altered signal transduction due to activation of Ca sensitive kinases and phosphatases (21). Yet, although the above-mentioned experiments demonstrate significant leak of Ca from the SR, it is too early to claim Ca leak as the fundamental mechanism directly promoting heart failure. To date, no mutation in RyR is known to cause heart failure. Perhaps more importantly, RyR mutations, experimentally shown to provoke increased open probability, are not associated with heart failure in humans at all but are associated solely with CPVT. In addition, FKBP12.6 knockout mice were demonstrated to exhibit significant Ca leak via RyR in vitro but do not exhibit signs of heart failure (28). One possible explanation is that increased open probability of any cause is rapidly corrected via autoregulatory mechanisms (8). For instance, a SR Ca load-leak relationship was recently demonstrated (42). Likewise, Venetucci and colleagues showed that the accompanying reduction of SR load in experimentally increased RyR open probability rapidly counterbalances increased Ca leak (48). They also provided an explanation for the catecholamine dependence in CPVT, in that catecholaminergic stimulation of SERCA via PLB, possibly in concert with PKA-mediated phosphorylation of the RyR, leads to a rapid loading of the SR, allowing sudden superthreshold Ca release leading to delayed afterdepolarisations (DAD) and CPVT. Thus, although the concept of increased SR Ca leak is attractive, its role as the underlying cause of heart failure awaits further confirmation. Fixing the leak of RyR therefore bears the risk of insufficiently addressing the whole picture of SR dysfunction and Ca perturbation in heart failure but remains a promising strategy to translate the latest research findings.

Despite some debate about the relative contribution of Ca leaking from the SR via RyR, it is generally accepted that diastolic Ca levels are increased in heart failure. This was demonstrated to result at least in part from a reduced expression of SERCA and upregulation of NCX. In the early days of molecular medicine, these changes in gene expression of Ca handling proteins were the first to be clearly disease dependent (20, 38) and were since confirmed multiple times. Normalizing SERCA expression has therefore been on the agenda for a long time, and to date a significant number of animal studies have been conducted. Important novel aspects are based on technical advancements such vectors for gene delivery or make use of the evolving RNAi technology to knockdown PLB. Based on previous work suggesting that continuous stimulation of SERCA using a pseudophosphorylated (hence inactive) form of PLB should result in upregulated SERCA activity with beneficial effects, an interesting model was published by Hoshijima’s group (22), who delivered pseudophosphorylated PLB mutant (S16E) to the BIO14.6 Syrian hamster using an adeno-associated virus. Using this AAV, chronic expression of the mutant was achieved, and important phenotypic hallmarks of heart failure could be corrected (22). Since the Bio14.6 hamsters underlying cause is a large deletion in the 5’ end of the delta-sarcoglycan gene, one important message from this study is that correcting abnormalities in Ca cycling, such as that due to altered expression of Ca handling proteins can correct for functionally remote causes of heart failure, such as alterations in genes of structural proteins. Despite additional reports of phenotypic rescue by crossing PLB knockout mice into diseased mice, it is questionable whether interventions targeting SERCA may serve as a general tool to correct heart failure. Kranias group has crossed PLB knockout mice with either Go_q overexpression mice or mutant myosin binding protein C mice, yet normalizing Ca cycling in vitro they did not reach a rescue of the heart failure phenotype in vivo (43). In addition, human mutations encoding a premature stop codon (with no detectable PLB protein), were reported to cause hypertrophy in the heterozygous state and severe dilative cardiomyopathy in homozygous individuals, suggesting that simple rescue strategies bear a significant risk of failure (18). Another PLB mutation (deletion of arginine in position 14) was identified in a family suffering from severe heart failure and subsequently shown to encode a super-inhibitory PLB (17). Thus manipulations of SERCA activity seem to require exact titration and probably timing, complicating their potential use as targets in gene therapeutic trials. This has also been described in an in vitro study, where exaggerated levels of SERCA overexpression led to enhanced cycling of Ca yet reduced systolic activation of myofilaments, possibly due to overly fast reuptake of Ca into the SR (47). Thus future gene therapy approaches in humans will need to take into account promoter control strategies, in addition to the demand of directed insertion and a high level of tissue specificity. Unlike in cancer, where the success of gene therapy is hampered by the demand of near complete transfection efficacy and the enormous selection pressure of quickly replicating transformed cells, there may be a brighter future for gene therapy in the heart. Although still far from the

“Fixing the leak of RyR therefore bears the risk of insufficiently addressing the whole picture of SR dysfunction and Ca perturbation in heart failure but remains a promising strategy to translate the latest research findings.”
REVIEWS

Na/Ca Exchange: Old and New Aspects in Heart Failure and Arrhythmia

Probably one of the earliest approaches to the treatment of heart failure were cardiac glycosides, albeit translational science occurred reversely from a therapeutic approach toward the mechanism. The drugs, long known for their positively inotropic effect, were identified to inhibit Na/K pump followed by increased Na load of the myocyte and subsequent Na extrusion through NCX, leading to increased Ca concentration in the cytosol, and represented the only effective heart failure therapy for a long time. Intracellular Ca accumulation clearly leads to positive inotropy, but triggered arrhythmias or negative chronotropy up to conduction block occur if the narrow therapeutic range is exceeded. Despite these disadvantages, glycosides still play an important role in the daily clinical management of heart failure. Regulation of NCX by remote mechanisms is only partially understood. Recently, sorcin, a Ca-binding protein, also known to associate with cardiac RyR and to reduce open probability, was found to inhibit NCX currents following overexpression in vitro (41).

Ca in Excitation Transcription Coupling

Disturbances in Ca handling, such as increased diastolic Ca, loss of compartmentalization of local Ca signals, or continuously increased systolic release, have been suggested to activate a number of signaling proteins, which could serve as indirect targets of translational strategies. Promising candidates mediating excitation transcription handling are the phosphatase calcineurin, CaMKII, and protein kinase C (PKC), all being directly activated by Ca. An important question remains as to how myocytes distinguish between Ca signaling for excitation contraction coupling and excitation transcription coupling. One possible explanation is that these proteins are prebound to Ca channels (either sarcotomal L-type channels or RyR of the SR) and dissociate from these channels when activated by Ca. On the other side, it is possible that these proteins are activated very locally (e.g., in the dyadic cleft where Ca rises very high) or directly in the nucleus (e.g., by Ca release though InsP3 receptors with little impact on global [Ca] where these proteins can easily initiate the hypertrophic transcriptional pathways (see below), thereby being largely independent of the Ca transients associated with excitation-contraction coupling (31).

Activated calcineurin, also known as protein phosphatase 2B, is a serin threonin phosphatase that was demonstrated to provoke a robust hypertrophic response and heart failure on overexpression in transgenic mice (36). Following this report from almost a decade ago, calcineurin inhibitors were suggested to treat conditions leading to heart failure such as hypertrophic cardiomyopathy (45). Systemic application of calcineurin inhibitors did not reach the clinic for the treatment of heart failure but proved efficient as potent immunosuppressors resulting from calcineurin/NFAT inhibition in lymphocytes. Nevertheless, research in calcineurin signaling in the heart is continuously extending, and a substantial support of calcineurin as an essential component for hypertrophic signaling has evolved. Importantly, calcineurin gene-targeted mice were reported resistant to hypertrophic remodeling following aortic banding or infusion of isoprotorenol (4) and were reported to rescue genetic models of murine heart failure. NFAT transcription factors are dephosphorylated by calcineurin, allowing nuclear localization and activation of a pro-hypertrophic gene program. Calcineurin/NFAT represents an important Ca-dependant Ca effector pathway, but calcineurin activity as well as NFAT phosphorylation and localization are also controlled by multiple proteins not directly affected by cardiac Ca. Thus targeting these associated proteins could be the key to blunting Ca-dependant stimulation of pathological growth. Like calcineurin, CaMKII is activated on binding of Ca/calmodulin (although with an ~200-fold higher $K_d$ compared with calcineurin). CaMK signaling induces cardiac hypertrophy and activates transcriptional pathways, such as via Mef2 and HDAC (37). The source of Ca activating CaMK was traditionally perceived to originate from RyR. However, local CaMK activation, which is independent from RyR-induced Ca transients, exists with relevance to hypertrophy. Accordingly, Ca release through inositol 1,4,5-trisphosphate (IP$_3$) receptors was recently demonstrated to regulate activation of nuclear export of histone deacetylase (HDAC) (52). This is of particular interest, since HDACs were clearly demonstrated to limit cardiac-exaggerated hypertrophy in gene-targeting studies. Although overall cytosolic Ca levels remained unchanged, IP$_3$ receptors in the nuclear envelope caused a local Ca release, leading to nuclear HDAC export and hypertrophy. IP$_3$ receptor knockout mice did not exhibit HDAC export, thus demonstrating that local Ca release via IP$_3$ receptors is the causative event to permit a hypertrophic response. CaMK is one HDAC kinase and was demonstrated to control IP$_3$ receptor-mediated HDAC export. Increasing Ca transient amplitude did not affect the latter mechanism, suggesting that disturbed Ca handling deserves attention also at sites remote from the classical SR-centered view. A promising hypothesis unifying several reports on a role of nuclear CaMKII involvement in

Global Ca where these proteins can easily initiate the release through InsP3 receptors with little impact on Ca rises very high) or directly in the nucleus (e.g., by Ca release though InsP3 receptors with little impact on global [Ca]$_i$ where these proteins can easily initiate the hypertrophic transcriptional pathways (see below), thereby being largely independent of the Ca transients associated with excitation-contraction coupling (31).
hypertrophy, IP3 receptor association with CaMKII and endothelin-mediated regulation of IP3 was suggested by Wu’s group (52). An endothelin-1 pathway leading to InsP3 production and local nuclear envelope Ca release via IP3 receptors was demonstrated to activate nuclear CaMKII, which triggers HDAC5 phosphorylation and nuclear export derepressing transcription. This study also highlights the importance of compartmentalized Ca signals, as this Ca-dependent pathway was insulated from changes in global beat-to-beat Ca transients. As exemplified in this study, Ca can act as a powerful and specific second messenger between membrane proteins and the transcriptional machinery. Therefore, coding of Ca signals such as waveform, frequency, persistence, and localization was studied intensively, and a focus on nuclear Ca codes will add insight into Ca in transcripational regulation (52, 55). These codes deserve further attention, since a translational approach targeting these promising aspects of Ca coding directly is not yet in sight. The most prominent translational interventions currently focus on HDAC inhibitors.

**L-Type Ca Channels: New Molecular Insights**

Non-vasoselective Ca channel blockers are not indicated as routine treatment for heart failure. Due to negative inotropic effects, they may worsen outcome in patients with heart failure. Is there a role for targeting disturbed Ca signaling at the level of L-type channels? Exciting work, mainly conducted in neurons, offers a new concept on excitation transcription coupling related to Ca,1.2 L-type voltage-gated Ca channels. Although earlier work in excitable cells, including cardiac myocytes, reported partial proteolytic cleavage of the 1.2Ca, channel (7, 14), the group of Gomez-Ospina demonstrated that the cleaved fragment COOH-terminal fragment translocates to the nucleus in neurons and cardiac myocytes and acts as a transcription factor (15). The fragment forms a complex with another nuclear protein and regulates the transcription of a variety of genes and was hence christened Ca channel-associated transcription regulator (CCAT). Although most of the work reported was conducted in neurons, the presence of CCAT in cardiac myocytes and nuclear translocation of artificially expressed YFP-tagged CCAT in myocytes suggests this mechanism will become the subject of intensive study in cardiac myocytes as well. Since both nuclear localization and regulation of transcription by CCAT are regulated by intracellular Ca, it seems that excitation transcription coupling also occurs directly at the level of L-type channels. It will be interesting to see whether the two components of L-type activity (i.e., Ca influx and CCAT-mediated transcriptional events) can be separated to study their role in development of heart failure. ■

Dr. Seidlter and Prof. Hasenfuß acknowledge funding by the Deutsche Forschungsgemeinschaft (DFG) Grant HA1233/7-3. Dr. Maier is funded by the DFG through an Emmy-Noether Grant (MA 1982/1-5) and a DFG Klinische Forschergruppe Grant (MA 1982/2-1).

**References**