

## STATE-OF-THE-ART PAPERS

# Atrial Remodeling and Atrial Fibrillation

## Recent Advances and Translational Perspectives

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Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice. AF and its complications are responsible for important population morbidity and mortality. Presently available therapeutic approaches have limited efficacy and nontrivial potential to cause adverse effects. Thus, new mechanistic knowledge is essential for therapeutic innovation. Atrial arrhythmogenic remodeling, defined as any change in atrial structure or function that promotes atrial arrhythmias, is central to AF. Remodeling can be due to underlying cardiac conditions, systemic processes and conditions such as aging, or AF itself. Recent work has underlined the importance of remodeling in AF, provided new insights into basic mechanisms, and identified new biomarker/imaging approaches to follow remodeling processes. The importance of intracellular  $\text{Ca}^{2+}$  handling abnormalities has been highlighted, both for the induction of triggered ectopic activity and for the activation of  $\text{Ca}^{2+}$ -related cell signaling that mediates profibrillatory remodeling. The importance of microRNAs, which are a new class of small noncoding sequences that regulate gene expression, has emerged in both electrical and structural remodeling. Remodeling related to aging, cardiac disease, and AF itself is believed to underlie the progressive nature of the arrhythmia, which contributes to the complexities of long-term management. New tools that are being developed to quantify remodeling processes and monitor their progression include novel biomarkers, imaging modalities to quantify/localize fibrosis, and noninvasive monitoring/mapping to better characterize the burden of AF and identify arrhythmic sources. This report reviews recent advances in the understanding of the basic pathophysiology of atrial remodeling and potential therapeutic implications. (J Am Coll Cardiol 2014;63:2335–45) © 2014 by the American College of Cardiology Foundation

Atrial fibrillation (AF) is a major cause of cardiovascular morbidity and mortality (1). AF is a final common endpoint of atrial remodeling caused by a variety of cardiac diseases and conditions (2) and itself causes important remodeling that contributes to the progressive nature of the arrhythmia. A detailed review of the subject was published in 2008 (3). Since then, an enormous amount has been learned about atrial remodeling, its mechanisms, and its role in the AF disease process. Here, we will focus on progress since that report (over the past 6 years), particularly in terms of understanding the basic mechanisms and their clinically relevant applications.

### Overview of AF Pathophysiology and Contribution of Remodeling

There are 4 principal pathophysiological mechanisms contributing to AF (1–4): electrical remodeling, structural remodeling, autonomic nervous system changes, and  $\text{Ca}^{2+}$  handling abnormalities (red boxes in Fig. 1). Each of these can result from cardiac disease conditions (blue boxes in Fig. 1) and promote the development of AF; AF in turn causes AF-promoting abnormalities in each of these areas (red dashed arrows in Fig. 1). AF-induced atrial remodeling enhances the vulnerability of the heart to AF induction and maintenance; this auto-reinforcing property of AF is often referred to by the term “AF begets AF.” Focal ectopic firing (yellow boxes in Fig. 1) can maintain AF or trigger re-entrant AF through a re-entry-maintaining substrate (green boxes in Fig. 1) that has the appropriate conditions to allow re-entry to be induced and then sustained. Induction and maintenance of re-entry require a critical balance between refractory and conduction properties, as discussed in detail by Wakili et al. (2) and Nattel et al. (3). AF is by definition a highly irregular atrial rhythm, yet paradoxically it can be maintained by regularly firing sources (sometimes called “drivers”), whether rapidly firing ectopic foci or single rapidly rotating

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**Abbreviations  
and Acronyms**

- AF** = atrial fibrillation
- APD** = action potential duration
- AT-II** = angiotensin II
- CaMKII** = Ca<sup>2+</sup>/calmodulin kinase type 2
- CREM** = cyclic adenosine monophosphate response element modulator
- DAD** = delayed afterdepolarization
- ECM** = extracellular matrix
- ER** = endoplasmic reticulum
- ERK** = extracellular signal-regulated kinase
- HF** = heart failure
- I<sub>CaL</sub>** = L-type Ca<sup>2+</sup> current
- I<sub>K1</sub>** = inward rectifier background K<sup>+</sup> current
- I<sub>KACH</sub>** = acetylcholine-regulated K<sup>+</sup> current
- IP3** = inositol triphosphate
- LTCC** = L-type Ca<sup>2+</sup> channel
- miR or miRNA** = microRNA
- mRNA** = messenger RNA
- NCX** = Na<sup>+</sup>/Ca<sup>2+</sup> exchanger
- NFAT** = nuclear factor of activated T cells
- PLB** = phospholamban
- RyR2** = ryanodine receptor type 2
- SERCA2a** = sarcoplasmic reticulum Ca<sup>2+</sup>-adenosine triphosphatase
- SR** = sarcoplasmic reticulum
- TG** = transgenic
- TGF** = transforming growth factor
- TRPC3** = TRP canonical 3
- TRPM7** = TRP melastatin-related 7

re-entry circuits, by virtue of spatially heterogeneous “fibrillatory conduction” (5). The ways in which the 4 principal mechanisms (electrical remodeling, structural remodeling, autonomic nervous system changes, and Ca<sup>2+</sup> handling abnormalities) occur and promote AF are detailed in the following text.

**Electrical Remodeling**

Atrial electrophysiological properties are governed by ion channels, pumps, and exchangers, any of which can be altered by atrial remodeling. The principal components of electrical remodeling identified to date include decreased L-type Ca<sup>2+</sup> current (I<sub>CaL</sub>, carried by L-type Ca<sup>2+</sup> channels [LTCCs]), rectifier background K<sup>+</sup> current (I<sub>K1</sub>) and constitutive acetylcholine-regulated K<sup>+</sup> current (I<sub>KACH</sub>), and abnormal expression/distribution of the gap junction connexin hemichannels that connect cardiomyocytes electrically. Electrical remodeling creates a re-entry-prone substrate.

**Down-regulation of I<sub>CaL</sub>.** Ca<sup>2+</sup> handling in atrial cardiomyocytes is shown schematically in Figure 2. For more details, see reviews by Wakili et al. (2) and Nattel and Dobrev (4). Ca<sup>2+</sup> enters through LTCCs during AP depolarization, triggering a secondary release of large amounts of Ca<sup>2+</sup> from the sarcoplasmic reticulum (SR) Ca<sup>2+</sup> stores via SR Ca<sup>2+</sup> release

channels called ryanodine receptors (RyR2s). This Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release induces myofibril movement/cell contraction. SR Ca<sup>2+</sup> stores are governed by the balance between Ca<sup>2+</sup> release from the SR into the cytosol via RyR2 channels and Ca<sup>2+</sup> uptake into the SR via the SR Ca<sup>2+</sup>-adenosine triphosphatase (SERCA2a). Under basal conditions, SERCA2a function is limited by the inhibitory subunit phospholamban (PLB). Most Ca<sup>2+</sup> in the SR is bound to the buffer calsequestrin. In situations of adrenergic stimulation or cellular Ca<sup>2+</sup> loading, Ca<sup>2+</sup>/calmodulin kinase type 2 (CaMKII) and protein kinase A become

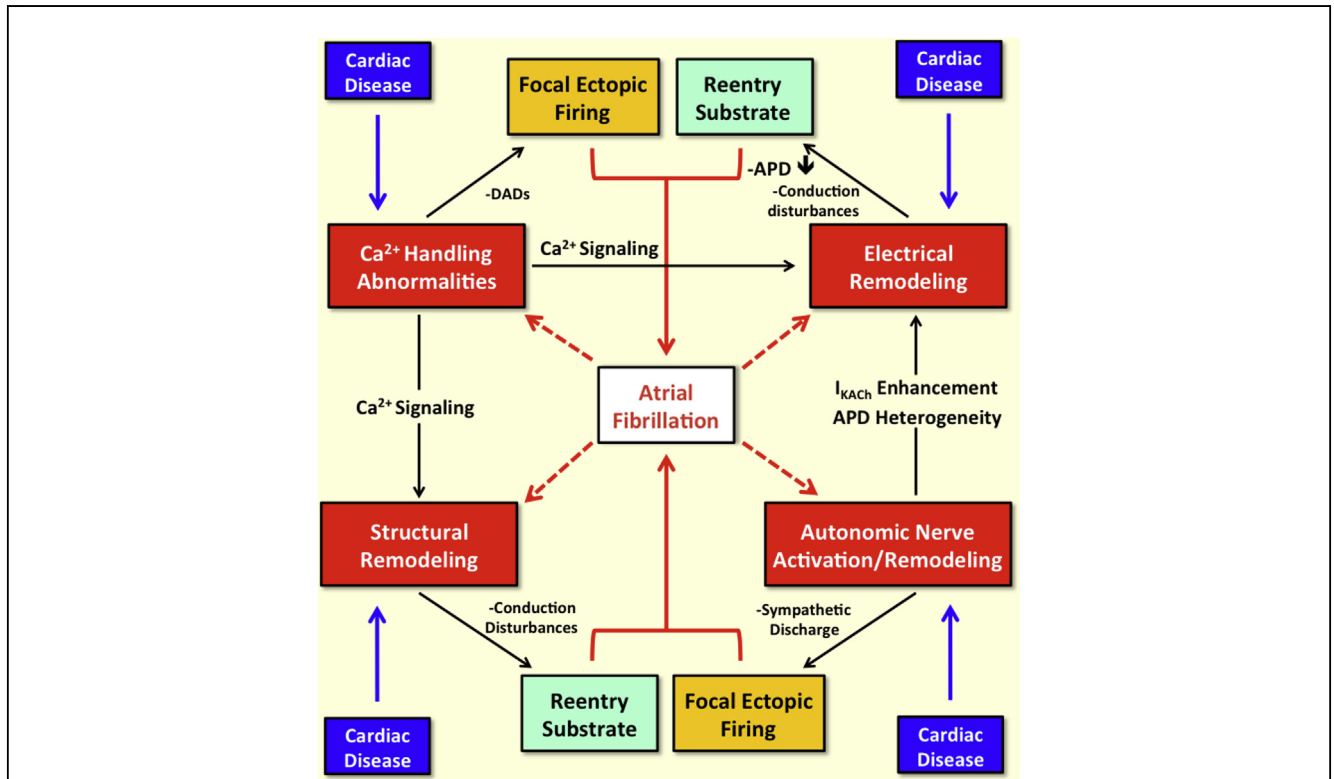
activated, phosphorylating RyR2s (increasing their open probability) and PLB (causing it to dissociate from and disinhibit SERCA2a). While this system is adaptive under conditions of acute stress-related increases in demand for cardiac work, sustained Ca<sup>2+</sup> loading and CaMKII activation cause abnormal diastolic RyR2 Ca<sup>2+</sup> releases. The released Ca<sup>2+</sup> is handled via transmembrane extrusion through the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX), which carries an inward current that causes phase 4 membrane depolarizations known as delayed afterdepolarizations (DADs).

During AF, the high atrial rate causes accumulation of intracellular Ca<sup>2+</sup>, engaging homeostatic defense mechanisms against chronic Ca<sup>2+</sup> overload. The Ca<sup>2+</sup>-dependent calcineurin/nuclear factor of activated T cells (NFAT) system is then activated. NFAT translocates into the nucleus and suppresses transcription of the gene encoding Cav1.2 LTCCs (*CACNA1C*), decreasing I<sub>CaL</sub> (Fig. 3) (2,6). Reduced I<sub>CaL</sub> decreases the inward Ca<sup>2+</sup> current, maintaining the AP plateau, shortening the AP duration (APD), and thereby promoting re-entry.

**Up-regulation of I<sub>K1</sub>.** I<sub>K1</sub>, the principal background cardiac inward rectifier current, determines the resting potential and terminal phase 3 repolarization and is composed primarily of Kir2.1 subunits. Inward rectifier currents such as I<sub>K1</sub> are a particularly important determinant of AF-maintaining re-entry (7); I<sub>K1</sub> is up-regulated in AF (2). Another important inward-rectifier current, I<sub>KACH</sub>, mediates the effects of acetylcholine and underlies the marked ability of vagal activation to promote AF by causing spatially heterogeneous increases in inward rectifier current and reductions in APD (8). AF suppresses agonist-induced I<sub>KACH</sub> but enhances a constitutive form (I<sub>KACHc</sub>), promoting maintenance of AF (9,10). Activation of I<sub>KACHc</sub> is induced by altered protein kinase C regulation of I<sub>KACH</sub> function resulting from AF-induced atrial tachycardia and Ca<sup>2+</sup> loading (10).

**Up-regulation of the small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel.** Small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels, encoded by the genes *KCNN1/KCNN2/KCNN3*, are activated by increased levels of intracellular Ca<sup>2+</sup>. Single nucleotide polymorphisms of *KCNN3* are associated with the prevalence of AF (11). Recent work suggests that rapid atrial activation, as seen in AF, may up-regulate small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel expression, which contributes to AF maintenance and susceptibility by abbreviating APD (12). In addition, noncardiomyocyte small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels could play a role in promotion of AF, for example, in fibroblasts (via fibrosis), smooth muscle cells (cardiac vascular changes and/or hypertension), or neurons (altered autonomic regulation).

**Gap junction remodeling.** Gap junction ion channels such as connexin 40 and connexin 43 mediate cardiomyocyte-to-cardiomyocyte electrical coupling. Connexin 43, which is encoded by *GJA1*, is expressed in all



**Figure 1** Major Components of Atrial Remodeling Underlying the Pathophysiology of AF

The principal AF-promoting mechanisms resulting from AF-inducing remodeling are shown in red boxes. Focal ectopic firing is usually due to DADs that reach threshold and cause spontaneous AP generation. A susceptible reentry substrate requires abbreviated refractoriness (which depends primarily on APD) and/or conduction abnormalities. These mechanisms can be the result of AF-promoting cardiac diseases but can also result from the consequences of AF itself. AF = atrial fibrillation; APD = action potential duration; DADs = delayed afterdepolarizations; *I<sub>KACH</sub>* = acetylcholine-regulated K<sup>+</sup> current.

working cardiac tissue; connexin 40, which is encoded by *GJA5*, is expressed mainly in atria and the conduction system (13). Alterations in gap junction function contribute significantly to AF-induced remodeling (14). Missense somatic mutations in *GJA5* underlie cases of idiopathic AF (15), and *GJA5* promoter sequence variants are associated with AF vulnerability (16).

### Structural Remodeling

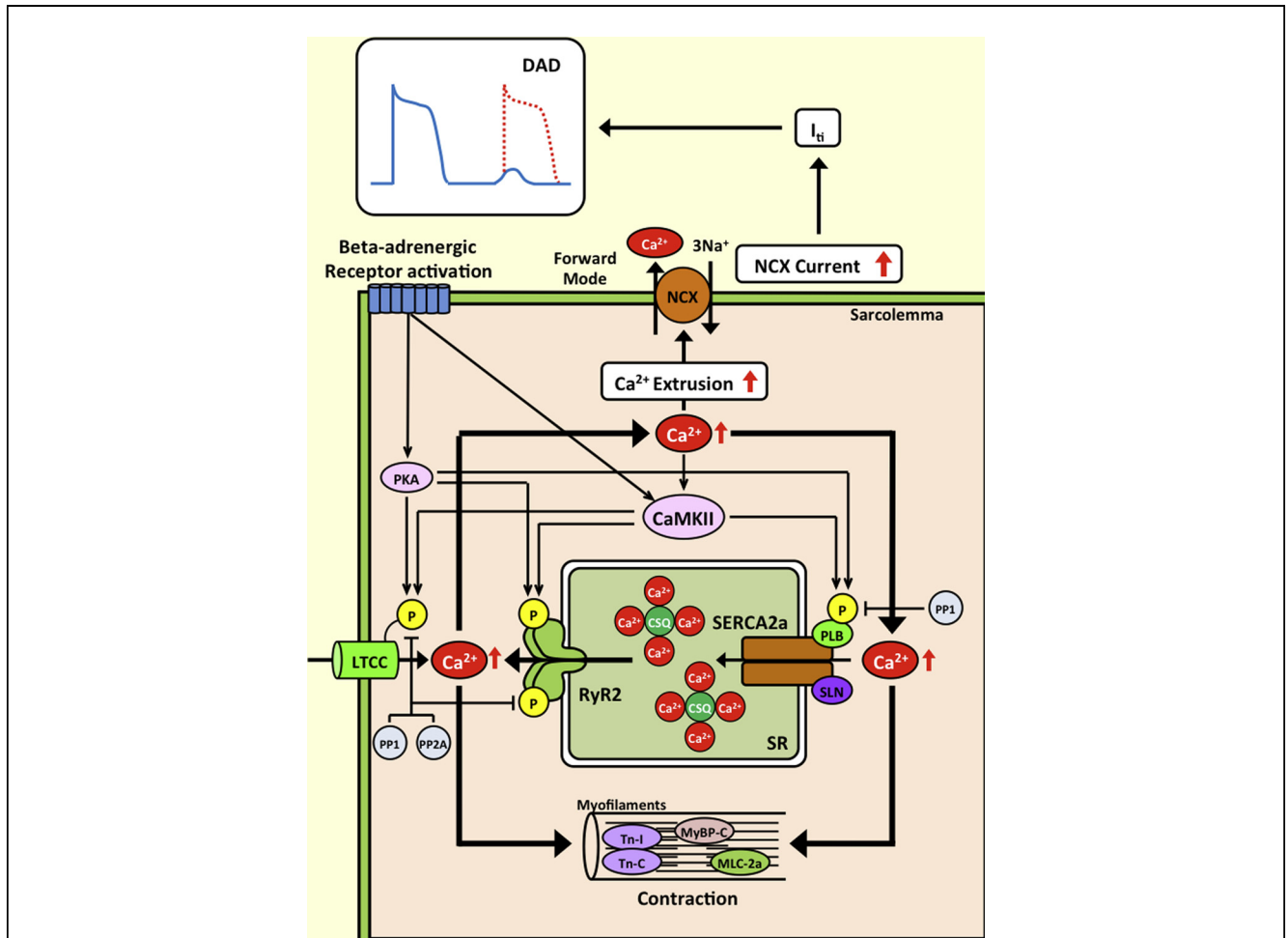
Structural remodeling is characterized by atrial enlargement and tissue fibrosis. Under some functional conditions, atrial dimension is a key determinant of the persistence of AF-maintaining reentry (17). Fibrosis promotes AF by interrupting fiber bundle continuity and causing local conduction disturbances (18). In addition, fibroblast-cardiomyocyte interactions may cause arrhythmogenic changes in cardiomyocyte bioelectricity (19). Atrial fibrosis appears to be a common endpoint of a wide range of AF-promoting conditions and may predict recurrences (20). Furthermore, AF appears to promote atrial fibrosis (21), which contributes importantly to therapeutic resistance in patients with long-standing arrhythmia (22).

### Autonomic Nervous System Changes

Autonomic nervous system control regulates atrial bioelectricity and contributes to the initiation and maintenance of AF. Adrenergic activation increases *I<sub>CaL</sub>*, RyR2 open probability, and SR Ca<sup>2+</sup> load (Fig. 2) via phosphorylation by CaMKII and protein kinase A. The risk of DADs is consequently enhanced, and adrenergic activation may play a critical role in AF due to formation of ectopic activity in the context of various remodeling-induced paradigms (23,24). Autonomic hyperinnervation is a consequence of AF-related remodeling and contributes to the vulnerable AF substrate (25).

### Ca<sup>2+</sup> Handling Abnormalities

The most significant direct atrial profibrillatory consequence of Ca<sup>2+</sup> handling abnormalities is the induction of DAD-related spontaneous atrial ectopic activity. Patients with long-standing persistent AF have an increased risk of arrhythmogenic DADs/triggered activity (26). They show enhanced CaMKII activity, with associated hyperphosphorylation and increased open probability of RyR2, causing increased CaMKII-dependent SR Ca<sup>2+</sup> leak (26).



**Figure 2 Cellular Ca<sup>2+</sup> Handling and Mechanisms Underlying DADs**

Key structures governing Ca<sup>2+</sup> handling include the LTCC, through which Ca<sup>2+</sup> enters the cell; cardiac SR, which is the principal subcellular Ca<sup>2+</sup> storage organelle; RyR2, through which Ca<sup>2+</sup> leaves the SR to produce Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release; SERCA2a, the principal SR Ca<sup>2+</sup> uptake mechanism that maintains SR Ca<sup>2+</sup> load; and NCX, which removes Ca<sup>2+</sup> from the cytosol by exchanging it for extracellular Na<sup>+</sup> across the cell membrane. Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release during systole causes cardiomyocyte contraction. The Ca<sup>2+</sup> released during systole is removed from the cytosol during diastole by SERCA2a and NCX, allowing cell relaxation. Diastolic Ca<sup>2+</sup> release from the SR is abnormal and transpires with disturbed RyR2 function and/or SR Ca<sup>2+</sup> overload; when it occurs, the released Ca<sup>2+</sup> is handled by NCX, producing an inward NCX current that causes a DAD. For more detailed discussion, see the text. CaMKII = Ca<sup>2+</sup>/calmodulin-dependent protein kinase II; CSQ = calsequestrin; LTCC = L-type Ca<sup>2+</sup> channel; MLC-2a = myosin light chain 2a; MyBP-C = myosin binding protein C; NCX = Na<sup>+</sup>/Ca<sup>2+</sup> exchanger; P = phosphate; PKA = protein kinase A; PLB = phospholamban; PP1 = protein phosphatase 1; PP2A = protein phosphatase 2A; RyR2 = ryanodine receptor type 2; SERCA2a = sarcoplasmic reticulum Ca<sup>2+</sup>-adenosine triphosphatase; SLN = sarcolipin; SR = sarcoplasmic reticulum; Tn-I = troponin I; Tn-C = troponin C; other abbreviations as in Figure 1.

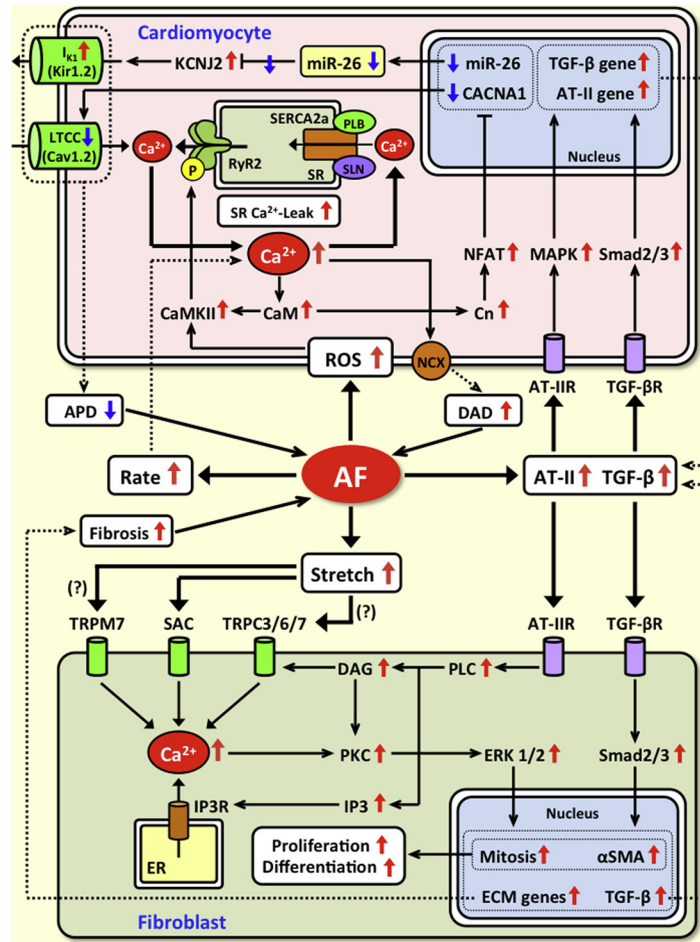
In addition, NCX is up-regulated, increasing the size of DAD-generating inward currents for any given amount of aberrant Ca<sup>2+</sup> release. These abnormalities appear to be caused by AF-induced remodeling, with CaMKII activation resulting from Ca<sup>2+</sup> loading due to sustained very rapid atrial activation. While long-standing persistent AF is likely maintained by complex multiple circuit reentry (2-5,27), ectopic activity may contribute by reinitiating AF should it terminate spontaneously or via medical intervention. Recent work points to a pre-disposition to DADs in patients with paroxysmal AF that likely plays a more primary role in arrhythmogenesis (28). The principal underlying mechanisms include increased SR Ca<sup>2+</sup> load due to PLB hyperphosphorylation and RyR2

abnormalities, including increased expression and enhanced open probability without RyR2 hyperphosphorylation (28). These abnormalities may be due to underlying heart disease or genetic pre-disposition but are not secondary to AF because patients who provided atrial tissue samples had been in sinus rhythm for a median of 10 to 20 days before surgery.

### Signaling Systems Involved in Atrial Remodeling

In the past few years, there have been substantial advances in understanding the signaling systems that cause atrial remodeling. Such work is important if more effective preventive measures are to be developed.





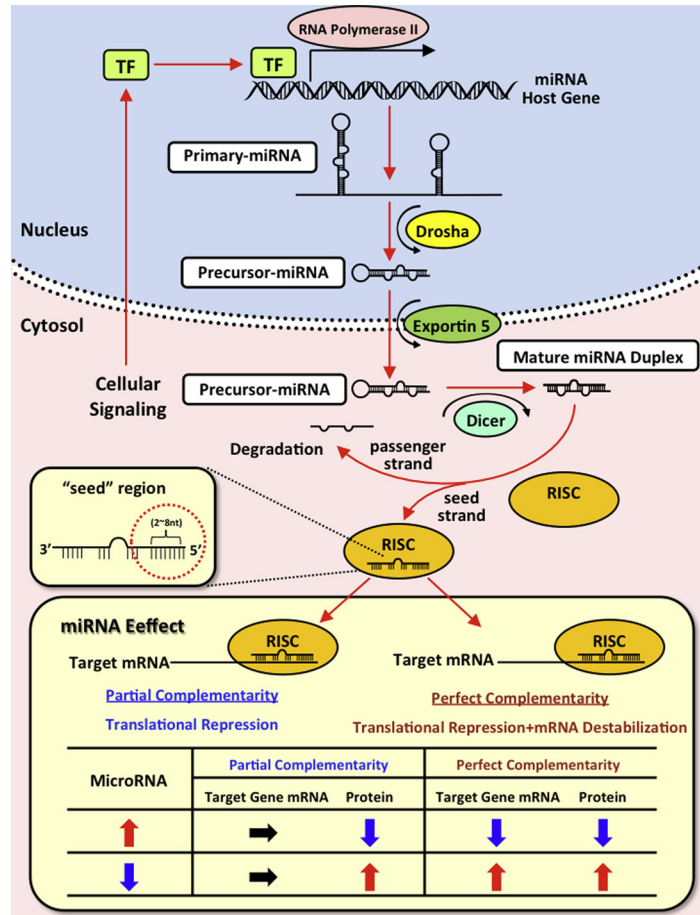
**Figure 3**  $Ca^{2+}$ -Related Cell Signaling and Atrial Remodeling in AF

Remodeling in a cardiomyocyte is represented at the **top** and a fibroblast at the **bottom**. AF engages  $Ca^{2+}$ -dependent signaling via increased rate and reactive oxygen species (ROS) generation in cardiomyocytes, cell stretch in fibroblasts, and receptor-activating molecules (AT-II and TGF- $\beta$ ) in both. AT-II = angiotensin II; AT-IIR = angiotensin II receptor; CaM = calmodulin; Cn = calcineurin; DAG = diacylglycerol; ECM = extracellular matrix; ER = endoplasmic reticulum; ERK 1/2 = extracellular signal-regulating kinase 1/2; IP3 = inositol triphosphate; IP3R = inositol triphosphate receptor; MAPK = mitogen-activated protein kinase; miR = microRNA; NFAT = nuclear factor of activated T cells; PKC = protein kinase C; PLC = phospholipase C; SAC = stretch activated channel;  $\alpha$ SMA =  $\alpha$ -smooth muscle actin; TGF $\beta$  = transforming growth factor- $\beta$ ; TGF $\beta$ R = transforming growth factor  $\beta$  receptor; TRPM7 = TRP melastatin-related 7; other abbreviations as in Figures 1 and 2.

**$Ca^{2+}$  signaling and electrical remodeling.** Recent work has indicated a central role for  $Ca^{2+}$  signaling in atrial remodeling (4). Figure 3 shows the molecular pathways that induce AF-related remodeling in cardiomyocytes and fibroblasts, highlighting the role of intracellular  $Ca^{2+}$ . In addition to down-regulating the transcription of Cav1.2 subunits and down-regulating  $I_{CaL}$ , NFAT nuclear translocation resulting from AF also down-regulates the production of a microRNA, miR-26 (29). Mir-26 targets *KCNJ2*, the gene that encodes Kir2.1 subunits, and down-regulation of miR-26 causes up-regulation of  $I_{K1}$  that strongly promotes persistence of AF (29).

CaMKII is activated in AF because of the persistent  $Ca^{2+}$  load resulting from high atrial rates as well as AF-induced reactive oxygen species formation (30). CaMKII regulates

the activities of key  $Ca^{2+}$ -handling proteins such as LTCC, RyR2, and PLB (Fig. 2) (4), promoting DADs/triggered activity. CaMKII phosphorylation also activates downstream effectors of remodeling such as class II histone deacetylase, which plays an important role in atrial remodeling (31), and nuclear factor  $\kappa$ B, which modulates proinflammatory genes associated with AF (32). Angiotensin II (AT-II) and other profibrotic mediators can enhance production of diacylglycerol, which activates protein kinase C. Conventional protein kinase C is stimulated by an increase in levels of intracellular  $Ca^{2+}$  and induces downstream signaling cascades such as mitogen-activated protein kinases and nuclear factor  $\kappa$ B, controlling diverse cell functions such as fibroblast activation, cardiomyocyte hypertrophy, and inflammation (Fig. 3) (33), which can affect global atrial electrical function.



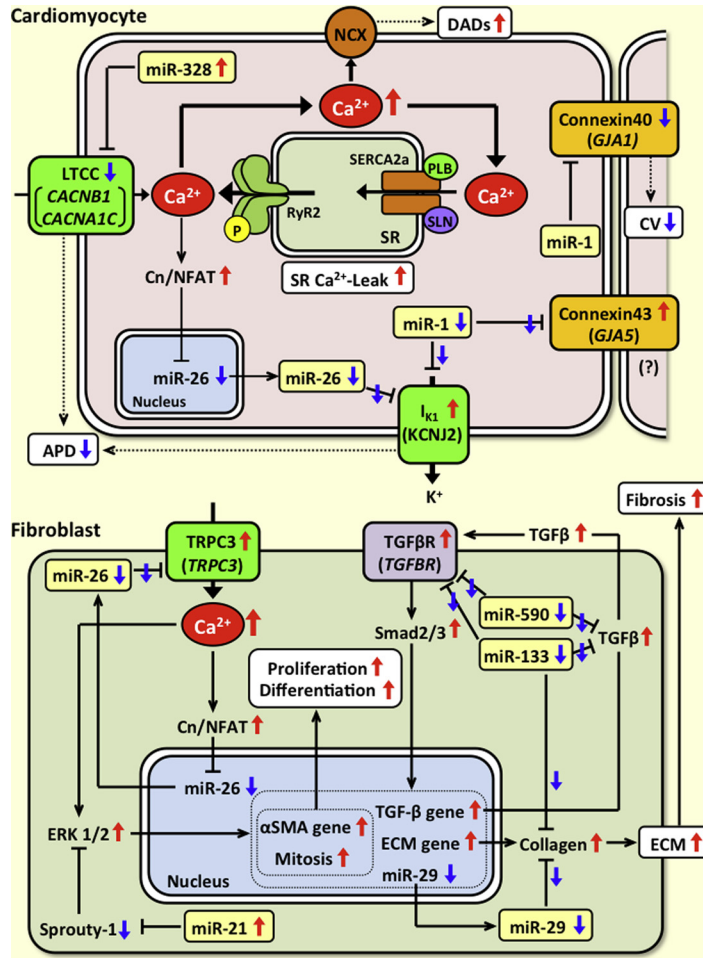
**Figure 4** Biogenesis and Actions of miRNA

Schematic representation of microRNA (miRNA) biogenesis and action. miRNAs produce their effects via single-stranded short nucleotide sequences (“seed strands”) that bind with high affinity via “seed sequences” to complementary sequences on the noncoding portion of messenger RNAs (mRNAs). This binding interferes with ribosomal translation of protein from the target mRNA, reducing its protein expression. In addition, under conditions of very high complementarity, the target mRNA is destabilized, reducing mRNA expression. RISC = RNA-induced silencing complex; TF = transcription factor.

**Ca<sup>2+</sup> signaling and structural remodeling.** Fibroblasts are key players in cardiac structural remodeling (19). Key fibroblast Ca<sup>2+</sup> handling processes and their role in atrial remodeling are represented in Figure 3. Nonexcitable cells such as fibroblasts lack voltage-gated Ca<sup>2+</sup> channels. Instead, they possess 2 primary mechanisms of intracellular Ca<sup>2+</sup> regulation, inositol triphosphate (IP3)-mediated Ca<sup>2+</sup> release from the endoplasmic reticulum (ER) (Ca<sup>2+</sup> storage organelle in nonexcitable cells, analogous to the SR in cardiomyocytes) and Ca<sup>2+</sup> entry via voltage-independent Ca<sup>2+</sup>-permeable sarcolemmal channels such as stretch-activated channels, receptor-operated channels, and Ca<sup>2+</sup> store depletion-operated channels (19,32). Stretch-activated channels, receptor-operated channels, and Ca<sup>2+</sup> store depletion-operated channels are functional classifications; the details of channel structure and their role still remain unclear.

Profibrotic mediators modulate ER Ca<sup>2+</sup> release and Ca<sup>2+</sup> entry into fibroblasts. For example, receptor binding of AT-II activates phospholipase C, cleaving phosphatidylinositol-4,5-bisphosphate into diacylglycerol and IP3. IP3 diffuses to the ER and binds to IP3 receptors, inducing ER Ca<sup>2+</sup> release. Diacylglycerol can directly activate specific Ca<sup>2+</sup>-permeable transient receptor-potential TRP channels (TRPC3/6/7), causing Ca<sup>2+</sup> entry (32). Increased cytosolic Ca<sup>2+</sup> levels (whether because of increased Ca<sup>2+</sup> entry across the cell membrane or increased release from the ER) cause fibroblast proliferation and differentiation into myofibroblasts, promoting fibrosis (34).

TRP channels are important Ca<sup>2+</sup> entry pathways in fibroblasts. TRP melastatin-related 7 (TRPM7) channels are up-regulated in fibroblasts isolated from the right atrial tissues of patients with long-standing persistent AF compared with sinus rhythm controls (35). In vitro TRPM7



**Figure 5** miRNAs and Atrial Remodeling in AF

Schematic representation of established miRNA signaling consequences in cardiomyocytes (**top**) and fibroblasts (**bottom**). miRNAs act by down-regulating the expression of target genes. Thus, miRNA down-regulation increases the expression of the target gene, whereas miRNA up-regulation suppresses the target gene. Cn/NFAT = calcineurin/nuclear factor of activated T cells; CV = conduction velocity; other abbreviations as in Figures 1 to 3.

knockdown decreases fibroblast Ca<sup>2+</sup> entry and prevents fibroblast differentiation caused by transforming growth factor (TGF)-β (35). TGF-β is produced by both fibroblasts and cardiomyocytes and is a key mediator of atrial fibrosis induced by a wide range of stimuli, including AT-II (36). AT-II stimulation increases TGF-β gene/protein synthesis in both cardiomyocytes and fibroblasts. Autocrine and paracrine TGF-β/AT-II networks act to amplify profibrotic reactions (Fig. 3) (36,37). TGF-β activates Smad2/3 signaling to potentiate fibroblast activation (36,37). Rapid pacing of atrial cardiomyocytes induces paracrine AT-II signaling, which causes adjacent atrial fibroblasts to secrete TGF-β, likely contributing to fibrosis due to long-standing AF (38). Thus, the ability of TRPM7 knockdown to suppress TGF-β signaling points to a central role of fibroblast Ca<sup>2+</sup> in mediating profibrotic responses. Atrial TRP canonical 3 (TRPC3) channels also mediate

fibroblast Ca<sup>2+</sup> entry; TRPC3-mediated Ca<sup>2+</sup> entry enhances phosphorylation of extracellular signal-regulated kinase (ERK), activating fibroblasts (34). TRPC3 expression is up-regulated in patients with long-standing persistent AF and animal models of AF (34). Inhibition of TRPC3 suppresses fibroblast proliferation and myofibroblast differentiation in vitro, and in vivo TRPC3 blockade in dogs with 1-week electrically maintained AF prevents AF-induced fibroblast activation and suppresses AF promotion (34).

Recent evidence points to a key role of cell Ca<sup>2+</sup> in atrial structural remodeling. Mice overexpressing a repressor variant of the cyclic adenosine monophosphate response element modulator (CREM) develop age-dependent atrial arrhythmias, presenting atrial ectopy at a young age (3 months) followed by spontaneous-onset AF (39). CREM-transgenic (TG) mice manifest atrial dilation,

fibrosis, and conduction slowing, along with increased SR  $\text{Ca}^{2+}$  leak (40). The  $\text{Ca}^{2+}$  leak appears to be caused by CaMKII hyperphosphorylation of RyR2, because inhibition of CaMKII hyperphosphorylation by organic blockers or by genetic block with a nonphosphorylatable RyR2 mutation suppresses  $\text{Ca}^{2+}$  leak (40). Long-term prevention of RyR2  $\text{Ca}^{2+}$  leak by crossing CREM-TG mice with RyR2-S2814A-TG (CaMKII nonphosphorylatable) mice delays atrial ectopy, prevents atrial structural remodeling, and eliminates spontaneous development of AF (40). These results suggest that accumulation of intracellular  $\text{Ca}^{2+}$  may cause not only DADs/triggered activity but also long-term atrial structural remodeling. These observations may provide novel insights into mechanisms underlying the pattern of AF progression seen in many patients.

### MicroRNAs and AF

A rapidly emerging area in the pathophysiology of AF is the role of microRNAs (miRNAs or miRs), which are small noncoding RNAs (~22 nucleotides) that negatively regulate target genes. Cardiac miRNA expression profiles change in response to pathological conditions and are believed to play a pathophysiological role in many.

**Fundamentals of miRNAs.** Figure 4 summarizes the factors governing the production and effects of miRNAs. RNA polymerase II mediates transcription of primary miRNAs (100 to 1,000 base pairs) under the control of standard transcription factors. Primary miRNAs are cleaved by the enzyme Drosha into precursor miRNAs (~70 nucleotides with short stem-loop structure), which are exported into the cytoplasm by Exportin 5. Dicer trims the stem loops from precursor miRNAs, producing double-stranded mature miRNA duplexes. The miRNA duplex then dissociates into a “passenger” strand and a “seed” strand, which is incorporated into the RNA-induced silencing complex. The miRNA/RNA-induced silencing complex binds to the 3′ untranslated region of target messenger RNAs (mRNAs) and blocks ribosomal processing/translation of the target protein (reducing protein expression, the principal miRNA effect). When complementarity between the miRNA seed region and the target mRNA is particularly high, mRNA stability is reduced (also reducing mRNA expression).

**miRNAs involved in atrial remodeling.** miRNAs contribute to a wide range of atrial remodeling processes (Fig. 5). Evidence for the participation of specific miRNAs is presented in the following text.

**miR-1.** miR-1 is abundantly expressed in cardiomyocytes (but not fibroblasts). In myocardial infarction, miR-1 is up-regulated, suppressing the expression of *KCNJ2* (encoding  $\text{I}_{\text{K1}}$ ) and *GJA1* (encoding connexin 43) (41). There is evidence that down-regulation of miR-1 may contribute to up-regulation of  $\text{I}_{\text{K1}}$  in AF (42). MiR-1 targets protein phosphatases, and up-regulation of miR-1 in heart failure (HF) may underlie  $\text{Ca}^{2+}$ -dependent arrhythmic activity (43).

**miR-21.** miR-21 targets Sprouty 1, a negative regulator of fibroblast ERK signaling (44). Rats with post-myocardial infarction HF show AF-promoting fibrotic remodeling with up-regulation of miR-21, decreased expression of Sprouty 1, and increased ERK phosphorylation; miR-21 knockdown suppresses left atrial fibrosis and promotion of AF (45). Up-regulation of MiR-21 and down-regulation of Sprouty 1 are seen in left atrial tissues from patients with AF, miR-21 is up-regulated in murine AF models, and miR-21 knockdown suppresses atrial fibrosis in mice with myocardial infarction (46).

**miR-26.** miR-26 targets the *KCNJ2* gene encoding  $\text{Kir}2.1$  ( $\text{I}_{\text{K1}}$ ). miR-26 knockdown causes up-regulation of  $\text{I}_{\text{K1}}$  and promotion of AF in mice (29). The host gene encoding miR-26 has multiple NFAT binding sites. MiR-26 expression decreases in patients with AF and canine AF models; therefore, NFAT-dependent down-regulation of miR-26 likely underlies up-regulation of  $\text{I}_{\text{K1}}$  in AF (29).

There is also evidence that MiR-26 contributes to atrial structural remodeling. As detailed in the preceding text, AF-associated TRPC3 up-regulation is an important fibroblast activator. miR-26 targets the gene encoding TRPC3 channels, and miR-26 knockdown increases TRPC3 expression in canine atrial fibroblasts, mimicking the effects of AF (34). NFAT nuclear translocation is observed in left atrial fibroblasts of dogs with AF. Pharmacological blockade of NFAT in canine atrial fibroblasts increases miR-26 expression and decreases levels of TRPC3 protein, confirming NFAT/miR-26 regulation of TRPC3 (34).

**miR-29.** TGF- $\beta$  negatively regulates miR-29 expression, and miR-29 targets extracellular matrix (ECM) genes such as collagen 1 and fibrillin 1 (47). Expression of miR-29 decreases in the left atria of dogs with HF, which develop an AF-maintaining fibrotic substrate, whereas expression of miR-29-targeted ECM genes (collagen 1 and collagen 3) increases (48). miR-29 knockdown in mice, to mimic the HF-induced decrease in miR-29, increases atrial collagen production. Furthermore, both plasma and atrial tissue miR-29 levels decrease in patients with AF, suggesting potential value as a biomarker (48).

**miR-133 and miR-590.** TGF- $\beta$  and TGF- $\beta$  receptor are targets of miR-133 and miR-590 (49). Prolonged administration of nicotine to dogs, mimicking sustained tobacco smoking, induces atrial profibrillatory fibrotic remodeling (49). Nicotine decreases miR-133 and miR-590 expression in atrial fibroblasts and up-regulates TGF- $\beta$  and TGF- $\beta$  receptor, increasing collagen production (49). miR-133 also targets collagen 1, and its down-regulation directly increases ECM production in cardiac fibroblasts (50).

**miR-328.** miR-328 targets the genes encoding LTCC subunit genes *CACNA1C* (encoding LTCC  $\alpha$  subunits) and *CACNB1* (encoding  $\beta 1$  subunits) (51). Atrial miR-328 is up-regulated in dogs with AF. Cardiac-specific miR-328-overexpressing mice are vulnerable to AF, and miR-328 knockdown restores Cav1.2 and Cav $\beta 1$  expression and reduces AF vulnerability (51).



## Translational Implications

Since the first description of the role of fibrotic structural remodeling in AF 15 years ago (52), there has been increasing recognition of the importance of atrial fibrosis in the pathophysiology of AF. Modern imaging methods have been developed to detect, localize, and quantify atrial fibrosis (53), which has been found to be correlated with outcomes such as stroke (54) and recurrence of AF. Biomarkers are becoming increasingly valuable in monitoring cardiac disease activity and therapeutic responses, with a variety of interesting candidates in AF (55). ECM-related proteins are measurable in the blood, and their plasma concentrations have been found to correlate with the risk of AF, suggesting potential value as biomarkers (55–57). Imaging approaches are being combined with highly sophisticated mathematical models of cardiac bioelectricity to gain insights into mechanisms by which fibrosis promotes AF in humans, with the hope of eventual individual-patient, mechanism-based therapeutics (58). In vivo activation mapping methods may allow targeted therapy of AF-specific mechanisms (59,60) and may eventually permit relationships to be established between atrial substrates and mechanisms initiating and/or maintaining AF.

Recognition of the role and mechanisms of remodeling in AF led to the notion of targeting underlying substrates to provide more specific approaches (61). Although this attractive goal is still far from unrealized, great progress has been made. “Upstream therapy” is a term that describes the targeting of remodeling-induced substrate development. Recent detailed reviews indicate that some efforts at upstream therapy have shown promise but that clear clinical indications remain to be confirmed and defined (62,63). To provide effective preventive therapy, it will be necessary to identify at-risk patients early enough to prevent the development of AF substrates before they become fixed and irreversible and to have available safe and effective therapeutic interventions (64). An improved understanding of key molecular pathways leading to AF substrates should help in defining targets for therapeutic intervention.

Besides targeting pathways leading to remodeling, better appreciation of the resulting arrhythmogenic mechanisms may also help in improving therapeutic targeting. Our increasing recognition of the importance of  $Ca^{2+}$  mishandling in AF has led to the development of interventions targeting specific  $Ca^{2+}$  handling components such as CaMKII and RyR2 leak (4). Molecular therapeutics with cell- and gene-based therapies are becoming conceivable, and proof of principle has been achieved with connexin gene transfer in a pig model of electrical pacing-induced AF (14,65) and miR-21 knockdown in a rat model of fibrosis-related AF post-myocardial infarction (42). Autonomic remodeling has been targeted by low-level vagal nerve stimulation in a canine model of paroxysmal AF (66), and autonomic ganglion ablation shows some clinical promise (67).

Recognition of the importance of atrial remodeling has led to the notion of intervening early and aggressively in patients with AF in an attempt to forestall remodeling and attendant complications (68). The ultimate goal is to provide safe and effective, ideally personalized, therapies for AF. Although we are certainly not yet at the threshold of achieving this goal, advances in knowledge, technologies, and analytical approaches make effective personalized therapy a realistic goal for the near future (69).

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**Key Words:** calcium handling ■ cardiac arrhythmias ■ electrophysiology ■ heart disease ■ pharmacology.

 **APPENDIX**

**For a list of abbreviations, please see the online version of this article.**