HHS Public Access

Author manuscript

Circ Res. Author manuscript; available in PMC 2021 June 19.

Published in final edited form as:

Circ Res. 2020 June 19; 127(1): 51–72. doi:10.1161/CIRCRESAHA.120.316363.

The Molecular Basis of Atrial Fibrillation Pathophysiology and Therapy: A Translational Perspective

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Abstract

Atrial fibrillation (AF) is a highly prevalent arrhythmia, with substantial associated morbidity and mortality. There have been significant management advances over the past 2 decades, but the burden of the disease continues to increase and there is certainly plenty of room for improvement in treatment options. A potential key to therapeutic innovation is a better understanding of underlying fundamental mechanisms. This article reviews recent advances in understanding the molecular basis for AF, with a particular emphasis on relating these new insights to clinical translation opportunities. We first review the evidence relating basic electrophysiological mechanisms to the characteristics of clinical AF. We then discuss the molecular control of factors leading to some of the principal determinants, including abnormalities in impulse conduction (such as tissue fibrosis and other extra-cardiomyocyte alterations, connexin dysregulation and Na^+-channel dysfunction), electrical refractoriness and impulse generation. We then consider the molecular drivers of AF progression, including a range of Ca^{2+}-dependent intracellular processes, microRNA changes and inflammatory signaling. The concept of key interactome-related nodal points is then evaluated, dealing with systems like those associated with Ca^{2+}/calmodulin-dependent protein kinase-II, NLRP3 (NACHT, LRR and PYD domains-containing protein-3) and transcription-factors like TBX5 and PitX2c.We conclude with a critical discussion of therapeutic implications, knowledge gaps and future directions, dealing with such aspects as drug repurposing, biologics, multispecific drugs, the targeting of cardiomyocyte inflammatory signaling and potential considerations in intervening at the level of interactomes and gene-regulation. The area of molecular intervention for AF management presents exciting new opportunities, along with substantial challenges.

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Disclosures

Dobromir Dobrev is a member of Scientific Advisory Boards of OMEICOS Therapeutics, Acesion Pharma and Sanofi and received speaker's fees for educational lectures from Boston Scientific, Novartis and Bristol-Myers Squibb. His laboratory executed research contracts for OMEICOS. Stanley Nattel served on the Global Advisory Board of Amgen between 2015 and 2019 and is a consultant for Novint Technologies and LQT Therapeutics.

Keywords

atrial fibrillation; mechanisms; personalized therapy; translational research

Subject codes:

[106]; [130]; [132]

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and is associated with considerable morbidity and mortality.[1] Presently available therapies have many limitations, including limited efficacy and significant adverse-effect potential for antiarrhythmic drugs[1,2] and recurrences and potential complications for AF-ablation.[1] The progressive nature of the arrhythmic substrate is a major problem limiting the long-term success of both pharmacological and ablation therapies.[3] Preventive approaches targeting risk factors have shown promise,[4] but a better understanding of the mechanisms underlying AF and its progression is needed to improve both understanding and our ability to create and exploit novel therapeutic avenues.[2] The goal of the present narrative review is to provide a portrait of the molecular mechanisms underlying AF, with a particular focus on clinically-relevant mechanisms that are relevant both to understanding the clinical features of AF and to the development of novel treatments.

The area addressed is vast- a single Medline search with the term “molecular mechanisms atrial fibrillation” revealed 611 papers. We have therefore chosen to focus on clinically-relevant mechanisms with potential translational significance. We begin with an analysis of the available information from patient data (in vivo studies, analyses of atrial-tissue samples) and animal models regarding the mechanisms responsible for initial AF-occurrence. We then consider the molecular basis for some of the key macroscopic mechanisms like spontaneous atrial ectopic firing and the development of a reentry substrate. After examining the sources of initial AF-occurrence, we discuss the molecular mechanisms underlying the atrial remodeling resulting from AF that causes substrate progression once AF occurs. Following that, we detail the evolving concept of central interactome nexuses that are involved in producing patterns of molecular response leading to the arrhythmic substrate. We conclude with a discussion of the potential therapeutic relevance of understanding the molecular mechanisms, an analysis of the challenges to developing new therapeutic approaches targeting molecular processes and a consideration of potential future directions.

Electrophysiological Mechanisms Implicated in Initial AF Occurrence

AF is known to promote its own maintenance and to be progressive in nature;[3,5] a great deal has been written about the mechanisms and clinical importance of AF-induced remodeling.[6] However, before AF can become self-promoting, there must be mechanistic factors that determine its initial onset. In considering the molecular pathways leading to AF, it is important to have a global sense of the pathophysiological mechanisms leading to the occurrence of AF prior to its progression.

Studies in patients and patient tissue-samples provide insights into the pathophysiology of AF-occurrence, as summarized in Figure 1. Ectopic activity, particularly occurring in the pulmonary veins (PVs), is centrally involved in AF-onset.[7] A number of factors predispose the PVs to the generation of ectopic activity, including both ion-channel and structural features.[8,9] The canine PVs have smaller inward-rectifier K^+-current (I_{K1}) and L-type Ca^{2+}-current ($I_{Ca,L}$), as well as larger delayed-rectifier K^+-currents, compared to left-atrial cells.[8] These properties reduce action-potential (AP) duration (APD), making reentry more likely, and increase the likelihood of spontaneous ectopy due to delayed afterdepolarizations (DADs). Furthermore, the PVs have a unique anatomical structure, composed of branching fibers with limited lateral coupling and abrupt fiber-orientation change,[9] which also increases their ability to generate spontaneous activity and support reentry.[9,10] Finally, the PVs are situated very close to major cardiac autonomic ganglia, which strongly modulate their electric properties as well as those of other atrial regions and thereby contribute to determining AF-susceptibility.[11,12]

Clinical studies of electrophysiological indices and imaging analyses also provide insights into the mechanisms associated with AF-occurrence. Abnormalities are consistently noted in PVs of patients with paroxysmal AF (pAF), including smaller electrogram voltages, slowed conduction, shorter effective refractory periods and a greater vulnerability to AF-induction during programmed electrical stimulation, indicating a reentry-prone substrate.[10,13] Atrial fibrous-tissue content is increased in pAF patients and atrial scarring correlates with clinical outcomes.[14] Thus, a reentry-prone substrate clearly favors AF-occurrence. At the same time, spontaneous atrial ectopic activity appears to be an important trigger for reentry. Studies employing ambulatory monitoring reveal atrial premature complexes initiating arrhythmia for >95% of pAF episodes.[15]

Additional insights are provided by the analysis of gene-variants associated with AF and the cellular electrophysiology of pAF patients.[13] Short-QT syndromes due to gain-of-function K^+-channel mutations have been implicated in pAF, likely due to reentry facilitated by abbreviated refractoriness.[16–19] In addition to reentry facilitated by reduced refractoriness, gene-variants causing reentry-promoting conduction-abnormalities also cause AF. For example, gene-variants in the gap-junction connexin ion-channels impair conduction and are associated with AF.[20] Finally, atrial fibrosis, which causes conduction-abnormalities and creates a substrate for AF,[21] is present by voltage-mapping or delayed gadolinium-enhancement magnetic resonance imaging in pAF patients.[22]

There are also data pointing to mechanisms increasing spontaneous atrial activity in AF-onset. A variety of mutations that promote spontaneous Ca^{2+}-release events (SCaEs) during diastole and the formation of DAD-mediated ectopic activity have also been implicated in spontaneous AF-onset.[23–25] Long-QT syndrome has also been associated with spontaneous AF,[26] apparently by inducing early afterdepolarization (EAD)-mediated ectopic activity.[27] Abnormal atrial automaticity has been indirectly implicated in AF-associated ectopy via altered expression of HCN-channels and automaticity-modulating microRNAs in tissue-samples from AF-patients,[28] but direct evidence is lacking. Analyses of cellular electrophysiology in right-atrial samples from patients with pAF show no AF-induced remodeling of action potential (AP) or ion-current properties;[29] however, left-atrial inward-

rectifier K^+-current is upregulated,[30] potentially contributing to the stabilization of reentry. SCaEs and DADs associated with increased sarcoplasmic-reticulum (SR) Ca^{2+}-content, cardiac ryanodine-receptor channel type-2 (RyR2) expression and open-probability are present in right-atrial cardiomyocytes from pAF patients.[29]

Ca^{2+}-handling dysregulation can also promote beat-to-beat alternation in APD (alternans) that favors reentry.[31] In patients studied in the electrophysiology laboratory, APD alternans precedes pacing-induced AF, and is produced most readily in persistent-AF patients, less readily in pAF and least in sinus-rhythm controls.[31] In sheep, aging is associated with increased susceptibility to APD-alternans and persistence of AF.[32] In dogs, AF-induced remodeling delays recovery of the cellular Ca^{2+}-transient, which causes increased magnitude and spatial dispersion of susceptibility to Ca^{2+}- and repolarization-alternans.[33] Consequently, rapid pacing leads to spatially-discordant alternans in the presence of AF-induced remodeling, causing reentrant rotor formation and enhancing vulnerability to initiation and maintenance of AF.[33]

Clinical Perspectives

In summary, there is extensive evidence that AF-initiation involves atrial ectopic triggers and a reentry-prone substrate. The PVs play a central role as both ectopic sources and zones of reentry, with autonomic tone being a key regulator. Disturbances in conduction related to tissue fibrosis and/or connexin-abnormalities predispose to reentry, with abbreviated refractoriness being a potential contributor, particularly among individuals with gene-variants that accelerate atrial repolarization. Repolarization alternans is often a path to reentry. Novel therapeutic priorities might be the identification of tractable molecular targets at the level of ectopic-beat generation, mechanisms leading to conduction abnormalities and the early detection and targeted therapy of gene-based pathways.

Molecular Determinants of AF-promoting Atrial Ectopy

Ca^{2+}-handling Abnormalities and DADs

Atrial cardiomyocyte excitation-contraction coupling is unique by virtue of a lack of a fully developed t-tubular system, although axial tubules are present in atrial cardiomyocytes of several species, including humans.[34–36] Upon cell-depolarization, Ca^{2+} influx via L-type Ca^{2+}-channels triggers a much larger Ca^{2+} release from neighboring SR sites via RyR2 channels. The resulting intracellular Ca^{2+} waves propagate to the cell center, activating non-junctional SR Ca^{2+} release sites, thereby causing a small delay in the rise of Ca^{2+} in the cell center. During diastole, SCaEs can activate the cardiac Na/Ca^{2+}-exchanger type-1 (NCX1), leading to a depolarizing transient-inward current, largely carried by I_{NCX}. Ectopic/triggered activity occurs when the resulting DAD is sufficiently large to overcome the electrotonic load and activate Na^+-channels, but *in silico* studies suggest that even sub-threshold DADs can have proarrhythmic effects by promoting dispersion of excitability with subsequent conduction block due to heterogenous voltage-dependent Na^+-channel inactivation (Figure 2).[37] Besides SCaEs, Ca^{2+} waves triggered by L-type Ca^{2+}-channels at the cell boundary might activate Ca^{2+}-overloaded SR sites at the cardiomyocyte center, leading to large triggered Ca^{2+} waves (TCWs) during the AP. TCWs may cause subcellular Ca^{2+} instability

with sub-threshold EADs increasing the dispersion of refractoriness[38] and can be observed in atrial cardiomyocytes from failing canine and human hearts.[39]

SCaEs are promoted by RyR2-dysfunction and SR Ca^{2+}-overload. The RyR2 is a large macromolecular complex and its dysfunction is often due to impaired interaction between stabilizing regulatory subunits and the pore-forming α-subunit, leading to increased open probability.[40] For example, loss of RyR2-associated calmodulin, the FK506-binding protein 12.6 or junctophilin-2 leads to RyR2-dysfunction and increased AF susceptibility in mouse and canine hearts.[24,41–44] RyR2 hyperphosphorylation by Ca^{2+}/calmodulin-dependent protein kinase-II (CaMKII) at Ser2814 and at Ser2808 or Ser2030 by protein kinase-A (PKA), along with RyR2 oxidation, also promote RyR2 dysfunction in human atrial samples and animal models.[45–50] Under physiological conditions, CaMKII is activated by binding of Ca^{2+}/calmodulin to its regulatory domain, which and leads to sustained, Ca^{2+}-independent activity due to autophosphorylation at Thr287, which accumulates between beats at faster heart rates.[51] Besides local CaMKII and PKA activity, RyR2 phosphorylation is controlled by the amount of RyR2-associated protein phosphatases type-1 (PP1) and type-2a. Accordingly, loss of subunits responsible for targeting PP1 to the RyR2 (e.g., spinophilin or PP1R3A, also known as R_{GL}) results in RyR2 dysfunction and increased AF susceptibility in mice[52,53] and PP1-complex expression, including the expression of PP1-subunits and of endogenous PP1 regulators, is altered in atria of AF patients.[54–56]

SR Ca^{2+}-overload is promoted by increased activity of the SR Ca^{2+}-ATPase-2a (SERCA2a), e.g., due to reduced expression or hyperphosphorylation of the inhibitory interacting proteins phospholamban or atrial-specific sarcolipin.[49] Furthermore, SR Ca^{2+}-load is increased by elevated intracellular Na^+-concentration. This occurs, for example in the presence of Na^+-K^+-ATPase inhibition by cardiac glycosides, which impair Ca^{2+}-extrusion via NCX1.[57] Increased Na^+-influx through non-inactivating Na^+-channels (persistent/late Na^+ current, $I_{Na,late}$) also promotes Ca^{2+}-handling abnormalities and AF inducibility in small-animal models[58,59]. CaMKII-dependent Ser571-hyperphosphorylation of the pore-forming subunit $Na_V1.5$ increases $I_{Na,late}$, potentially creating an AF-promoting positive feed-back loop.[59] Consistent with this idea, SR Ca^{2+}-overload and CaMKII hyperactivity can also cause TCWs and CaMKII-dependent dysregulation of $I_{Na,late}$, producing proarrhythmic activity in atrial cardiomyocytes from patients with sleep-disordered breathing,[60] who are at an increased risk for developing AF.[4] On the other hand, no significant $I_{Na,late}$ could be detected under physiological conditions in atrial cardiomyocytes from patients in sinus rhythm or with AF in a different study.[61] The potential role of $I_{Na,late}$ in AF pathophysiology needs further elaboration and validation.

Ca^{2+} handling abnormalities are a common finding in atrial cardiomyocytes of AF patients,[29,46,47,62,63] as well as in patients with risk factors and comorbidities promoting AF. For example, SR Ca^{2+}-load and the incidence of spontaneous I_{NCX} is increased in heart failure (HF) patients,[63] and patients with sleep-disordered breathing have increased CaMKII activity, DADs and EADs.[60] Although the exact molecular mechanisms differ between different forms of AF and different comorbidities, CaMKII activation appears to be a central element controlled by several upstream regulators. The autonomic nervous system plays a major role in AF-pathophysiology, with combined sympatho-vagal activation producing both

a substrate and triggers for AF initiation.[64] Sympathetic stimulation results in PKA-dependent phosphorylation of numerous Ca^{2+}-handling proteins through activation of β-adrenoceptors and cyclic adenosine monophosphate (cAMP) production.[64] The resultant increase in SR Ca^{2+}-load contributes to the positive inotropic effects of sympathetic stimulation and together with RyR2 hyperphosphorylation can promote proarrhythmic Ca^{2+}-handling abnormalities.[49,64] The elevated Ca^{2+}-levels and increased heart rate during sympathetic stimulation also sustains CaMKII activation.[51] Furthermore, cAMP production during β-adrenoceptor stimulation in mice can activate CaMKII via exchange-protein activated by cAMP[65] and cAMP/PKA-dependent phosphorylation of the inhibitor-1 protein that regulates PP1, preventing dephosphorylation of CaMKII-Thr287.[66]

Oxidative stress is common in a variety of cardiovascular diseases and has been associated with Ca^{2+}-handling abnormalities and increased risk of AF. In turn, rapid atrial activation during AF can further promote oxidative stress via CD44/NOX4 signaling.[67] Oxidative stress activates CaMKII through oxidation at Met281/282, which is increased in AF patients.[68] Oxidized CaMKII creates a substrate for TCWs and reentry in the atria of HF dogs[50] and has been implicated in AF promotion by the tyrosine kinase inhibitor ibrutinib used in cancer treatment.[69] Oxidative stress also activates the stress-response kinase c-Jun N-terminal kinase-2 (JNK2), which phosphorylates CaMKII, leading to increased CaMKII activity (Figure 2).[70] In addition, JNK2 activation upregulates CaMKII expression, further contributing to CaMKII-mediated arrhythmogenesis.[71] JNK2-dependent CaMKII activation has been implicated in several AF-promoting conditions, including advanced age[70,71] and binge-alcohol exposure.[72]

Atrial inflammatory signaling is increased in several conditions predisposing patients to AF and inflammatory markers such as interleukin (IL)-6, IL-1β and tumor necrosis factor-α correlate with AF progression.[73] Recent work has indicated that cardiomyocyte NACHT, LRR and PYD domains-containing protein-3 (NLRP3)-inflammasome signaling plays a causative role in AF development in genetically-targeted mouse-models.[74] Expression of NLRP3-inflammasome components (‘priming’) is increased in atrial samples of AF patients,[74] as well as in atrial samples from patients that subsequently develop post-operative AF,[75] or that have type-2 diabetes and an increased risk of AF.[76] Diabetic rabbits that show increased activity of the atrial NLRP3-infllammasome system, along with slowed heterogeneous conduction and increased atrial fibrosis and fibrotic markers, exhibit increased AF inducibility, with changes being reversed by the NLRP3-inflammasome inhibitor (and hypoglycemic agent) glyburide.[77] Assembly (‘triggering’) of the NLRP3-inflammasome complex consisting of NLRP3, apoptosis-associated speck-like protein containing a C-terminal caspase activation and recruitment domain (ASC) and pro-caspase-1 promotes auto-cleavage of pro-caspase-1. The resulting increase in active caspase-1 results in cleavage of inactive precursors of IL-1β and IL-18 to their active forms and of gasdermin-D, releasing the pore-forming N-terminal fragment that allows IL-1β and IL-18 to leave the cardiomyocyte to exert autocrine and paracrine effects.[73] Mice with cardiomyocyte-restricted constitutive activation of the NLRP3-inflammasome have increased atrial ectopy associated with Ca^{2+}-handling abnormalities due to increased RyR2 expression, as well as reentry-promoting APD abbreviation (due to increased atrial-selective ultra-rapid delayed-rectifier K^+-current, I_{Kur} and acetylcholine-activated inward-rectifier K^+-current, $I_{K,ACh}$) and

structural remodeling (hypertrophy and fibrosis).[74] Similarly, short-term (hours) exposure to IL-1β and tumor necrosis factor-α produces proarrhythmic Ca^{2+}-handling abnormalities in rodent cardiomyocytes,[78,79] highlighting the potential to trigger AF. CaMKII activation in response to pressure overload or chronic angiotensin-II (Ang-II) exposure activates NLRP3 in mice cardiomyocytes,[80,81] NLRP3-inflammasome-dependent activation of IL-18 upon acute β-adrenoceptor stimulation triggers pathological cardiac remodeling,[82] and oxidative stress drives NLRP3-inflammasome activation during high-glucose and ischemia/reperfusion injury in H9C2 rat cardiomyocytes.[83] While the recent evidence in favor of NLRP3-inflammasome signaling as a central proarrhythmic mediator of multiple pathophysiological signals in AF is intriguing, the relative contribution of these pathways to atrial ectopy and AF in humans remains to be conclusively established.

Early Afterdepolarizations

EADs are promoted by excessive APD prolongation, providing time for recovery of L-type Ca^{2+}-currents ($I_{Ca,L}$) from voltage- and Ca^{2+}-dependent inactivation during the plateau phase of the AP. The resulting increase in $I_{Ca,L}$ during the AP-plateau or phase-3 produces membrane depolarization and EAD-formation. EADs are a major arrhythmogenic mechanism in the ventricles, e.g., in the setting of (drug-induced) long-QT syndrome, where they promote ectopy, increased dispersion of repolarization and beat-to-beat repolarization alternans, all of which are proarrhythmic.[84,85] Long-QT syndrome can also promote AF.[86] Mice with long-QT syndrome type-3 due to a gain-of-function mutation in the Na^{+}-channel show excessive atrial APD-prolongation and EADs.[27,85,87] Similarly, acute CaMKII activation due to oxidative stress initiates AF through EAD-mediated triggered activity in old rats, which can be suppressed by inhibition of $I_{Na,late}$.[88] Nonetheless, there is little direct evidence for a major role of EADs in atrial arrhythmogenesis in patients with AF in the absence of repolarization-delaying mutations.

Abnormal Automaticity

The sinoatrial node (SAN) acts as the primary pacemaker via spontaneous AP generation (“automaticity”). In SAN cells, automaticity is controlled by a coupled-clock system involving hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and SR Ca^{2+}-leak activating depolarizing I_{NCX}.[89] On a background of low-level I_{K1} expression in SAN cells, these systems produce spontaneous diastolic depolarization leading to AP firing. The relevance of abnormal automaticity for atrial ectopy is unclear. The expression of HCN in the healthy atrium is limited, although an age-dependent increase has been observed in dogs.[90] Atrial HCN expression is also increased in end-stage failing human hearts compared to non-failing hearts,[91] as well as in AF-patients compared to sinus rhythm controls, possibly due to a reduction in inhibitory microRNA-1 and microRNA-133.[28] Normally, however, atrial I_{K1} appears to be sufficiently large to maintain a stable resting membrane potential that prevents substantial automaticity. Consistent with this notion, abnormal automaticity in canine PV sleeves can only be observed in the presence of I_{K1} inhibition.[92]

Clinical Perspectives

The mechanistic basis for spontaneous atrial ectopy is not fully established, but the weight of evidence points to DAD-related activity due to aberrant Ca^{2+}-release as the most likely

causative paradigm. Increased RyR2 open-probability as a result of CaMKII-mediated hyperphosphorylation is widely seen in studies of atrial samples from AF-patients and in animal models of AF. CaMKII-activation results from a variety of molecular pathways, including enhanced oxidative stress and inflammatory signaling, which could constitute novel therapeutic targets. EADs are also capable of mediating atrial ectopic firing, but to date the most convincing evidence is limited to contexts with clearly delayed atrial repolarization like Long-QT Syndromes.

Molecular Control of Factors Leading to Conduction Abnormalities

Conduction abnormalities are a ubiquitous feature in patients with AF-substrates,[93,94] presumably by increasing susceptibility to reentry initiation and maintenance. The most important determinants of conduction are structural integrity of atrial tissue (importantly disturbed by fibrosis), effective cell-to-cell coupling (principally determined by connexin hemichannels in intercalated disks) and the integrity of the rapid phase-0 Na^+-current (I_{Na}) which provides the electrical energy for conduction.

Molecular Determinants of Structural Remodeling

Atrial structural remodeling, prominently including tissue fibrosis, is associated with conduction abnormalities that create a substrate for AF-maintenance.[21] Atrial fibrosis is a common feature in AF-patients and the extent of fibrosis is a predictor of AF-recurrence after catheter ablation.[95] A vast body of research has established a wide range of molecular mechanisms controlling atrial fibrosis.[96] The principal determinants are illustrated in Figure 3. A variety of cell-membrane receptor systems cause fibroblasts to proliferate and differentiate into profibrotic collagen-secreting myofibroblasts. These include receptors for Ang-II, platelet-derived growth factor, connective-tissue growth factor, and transforming growth-factor β. The receptor systems are coupled to second-messenger systems, which activate transcription-factors (TFs) that modify the mRNA-transcription of proteins like procollagen, fibronectin, matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases that govern extracellular-matrix remodeling and lead to fibrosis. Reactive oxygen species (ROS) are generated by reduced nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase coupled to type-1 Ang-II receptors and transforming growth-factor β-receptors and play a prominent role in AF.[97] ROS are important stimulants to tissue-fibrosis, acting via downstream systems like mitogen-activated protein kinases[98] and NLRP3-inflammasome formation and profibrotic inflammatory signaling.[74,99,100] All of these molecular systems eventually lead to increased synthesis of procollagen α- and β-chains in the endoplasmic reticulum. These trimerize to form procollagen molecules, which are secreted into the extracellular space where the N-terminal and C-terminal ends are removed by PNPase and PCPase enzymes, respectively, to produce mature collagen molecules. Self-assemble generates collagen myofibrils that are crosslinked by lysine-oxidase enzymes to form mature collagen. Fibroblasts also contain membrane ion-channels that mediate (receptor- and store-operated transient-receptor potential [TRP] channels) or regulate (I_{K1} and Ca^{2+}-dependent K^+-channels) transmembrane Ca^{2+}-entry, which acts as a key signal for fibroblast-activation.[101–103]

Besides atrial fibrosis, there are additional changes in extracellular-tissue properties that have been implicated in AF-promotion. For example, in one study the presence of atrial amyloidosis correlated better with AF-occurrence in patients than that of fibrosis.[104] Human atrial adipose tissue secretome induced rat atrial-tissue fibrosis in organo-culture,[105] and AF is associated with fibrosis in subepicardial fatty infiltrates in humans and sheep.[106] The importance of the extracellular-tissue changes associated with AF are reflected in the fact that they are incorporated in 3 of the 4 classes of atrial cardiomyopathy of the EHRAS classification system.[107]

While the molecular determinants of atrial fibrosis have been extensively investigated, the links between clinical conditions leading to AF and extracellular-matrix remodeling are poorly understood. Atrial stretch, for example due to valvular heart disease, HF or diastolic dysfunction, is commonly assumed to lead to atrial fibrosis. However, the underlying molecular mechanisms are poorly studied, particularly at the atrial level, and therapeutic manipulation has proven quite challenging.[108] Cardiomyocyte NLRP3-inflammasome activation appears to be a common feature in patients prone to AF and to lead to atrial fibrosis,[74,75,99,100] but the factors causing NLRP3-inflammasome activation in the atria are poorly understood. Clearly, much more work needs to be done in this area.

Molecular Determinants of Connexin Dysfunction

Connexins are ion-channel proteins that form hemichannels specialized to ensure cell-to-cell communication at the intercalated disks.[109] Connexins have a large single-channel conductance and allow cardiac tissue to function as an electrically-continuous syncytium. The principal atrial connexins are connexin-43 (Cx43) and connexin-40 (Cx40), encoded by *GJA1* and *GJA5* respectively. While Cx43 is expressed ubiquitously in cardiac tissues, Cx40 is expressed in the atria, but not ventricles. Connexins are located primarily in “connexons”, 6-hemichannel complexes generally localized to gap junctions in intercalated disks connecting cells at their cell-to-cell junctions. Each connexon connects to a complementary connexon from the next cell, with the hemichannels coupling to form cell-to-cell channels. Abnormalities in connexin number, function or localization impair electrical propagation and can lead to initiation and/or stabilization of reentry circuits that support AF (Figure 1). Figure 4 illustrates the molecular determinants of connexin function that, when disturbed, may lead to AF. *GJA5* polymorphisms are well-recognized to be associated with AF in man, affecting conduction via reduced expression,[110] reduced single-channel open probability,[20] and impaired channel formation or trafficking.[111] The expression of AF-associated mutated channels in mice slows atrial conduction and increases AF-vulnerability.[20,112] Atrial tissues from pAF patients show reduced Cx40 expression and increased heterogeneity of Cx40-distribution, while persistent (chronic)-AF (cAF) patients show severe reductions of Cx40-immunostaining.[113] Goats with AF-induced atrial remodeling show markedly-increased Cx40-distribution heterogeneity.[114] Connexin downregulation and/or relocalization from cell-ends to lateral margins might play a role in AF-promoting conduction abnormalities due to obstructive sleep apnea,[115] acute inflammation[116], aging[117] and chronic kidney disease.[99] Assessing the relative importance of connexin changes in AF may be difficult when connexin-changes occur along with other aspects of atrial remodeling. In at least one context, that of HF, connexin alterations occur (dephosphorylation, increased Cx40/Cx43

protein-ratios, lateralization), but appear to be of much less functional significance than concomitant fibrosis-development.[118] An additional layer of complexity is added by the observation that reduced Cx43-expression can enhance the fibrotic response by increasing fibroblast activity.[119]

Phosphorylation regulates connexin channel function, membrane expression and cellular localization in complex ways. Cx43 has at least 21 discrete phosphorylation sites, which are phosphorylated specifically by a variety of kinases including PKA, protein kinase C (PKC), CaMKII and mitogen-activated protein kinases.[120] Phosphorylation can increase or decrease gap-junctional conductance, alter connexin transport to the cell membrane, affect connexin degradation in the proteasome and affect gap-junction assembly, resulting in a potentially wide range of functional effects.[120] In addition, phosphorylation of transcription factors like c-Jun (AP-1) affects connexin production by entering the nucleus and regulating transcription to decrease *GJA1* mRNA-production.[117] Intracellular ROS production alters connexin function, either directly or via the activation of kinases like CaMKII or JNK.[120]

Connexin-dysfunction in AF has a number of potential therapeutic implications. Cx43 gene-therapy has been used effectively to suppress AF-progression in a porcine model,[121,122] but the barriers to applying cardiac gene-therapy in man are substantial. An antiarrhythmic peptide has been developed that enhances gap-junction conductivity. Detailed study in experimental AF-models of atrial-tachycardia, HF and ischemic atrial remodeling shows improved conduction in all, but antiarrhythmic efficacy limited to the acute ischemic paradigm.[123]

Changes in Na^+-channel Function

I_{Na} is another important determinant of cardiac conduction-properties. Experimental atrial-tachycardia remodeling reduces atrial I_{Na} and slows conduction over a time-frame of weeks.[124] Studies of AF-associated I_{Na}-changes in man have provided varying results, with early studies showing only a depolarizing voltage-shift in voltage-dependence[125] and more recent work pointing to a decrease in peak-I_{Na} and increased $I_{Na,late}$.[126] The latter effect was associated with increased susceptibility to Ca^{2+}-related ectopic firing that could be suppressed by the $I_{Na,late}$-blocker ranolazine.[126] Finally, CaMKII-regulation of I_{Na} appears to be an upstream-event to AF-related Ca^{2+}-handling abnormalities and associated ectopic activity[59] and CaMKII-dependent dysregulation of $I_{Na,late}$ results in proarrhythmic activity in atrial cardiomyocytes from patients with sleep-disordered breathing,[60], who are prone to AF. Overall, I_{Na}-changes appear to have functional significance in AF, but are much less studied than other molecular components of the system.

Clinical Perspectives

Conduction abnormalities are common in AF and play an important role in AF-maintaining reentry. Structural remodeling is a central motif, with tissue-fibrosis being a common finding but also potential contributions from other extra-cardiomyocyte components like adipose tissue and amyloid-proteins. Many AF-associated molecular signaling systems like those associated with the renin-angiotensin-aldosterone axis, platelet-derived growth factors, connective-tissue growth factor, and transforming growth-factor β, as well as heart-disease

risk factors, promote the development of atrial fibrosis. There is evidence that oxidative stress and proinflammatory signaling may be significant contributors. Cell-to-cell conduction depends on connexin-proteins, which are susceptible to genetic abnormalities and remodeling by heart disease leading to AF-promoting conduction abnormalities. The prevention of structural remodeling is potentially a very interesting therapeutic approach, but the ubiquitousness of many of the mechanisms makes safe and specific targeting a challenge.

Molecular Drivers of AF Progression

Progression of the AF-supporting substrate is recognized to be an important factor leading to therapeutic resistance and failure.[3,127] Progression of the AF-substrate can be caused by remodeling associated with the arrhythmia or through the effects of predisposing heart disease and/or risk factors.[3,127,128] Risk-factor control helps to prevent AF-progression;[4,128] while it is likely that effective management of heart-disease can also prevent progression of the AF-substrate, this is difficult to address and to our knowledge there is no confirmatory evidence to date from well-controlled randomized trials.

Here we will focus on the AF-associated molecular factors that lead to progression of the AF-maintaining reentrant substrate. Figure 5 illustrates some of the key pathways shown to be involved in this process, which involve electrophysiological, Ca^{2+}-handling and structural remodeling.

Ca^{2+}-handling Changes and AF-progression

The rapid rate of atrial-cardiomyocyte firing in AF leads to Ca^{2+}-loading in canine atrial cardiomyocytes.[129] The atrial arrhythmia associated with AF, even in the absence of any alterations in cardiac function, contributes to progression of the AF-substrate due to both electrophysiological and structural remodeling in dogs.[130] Differences in cardiomyocyte Ca^{2+}-handling properties between pAF and cAF patients,[29,47] as well as animal models with atrial tachycardia-related remodeling[131,132] also point towards progressive changes in atrial Ca^{2+}-handling due to AF itself. Ca^{2+}-transient amplitude is increased in pAF, but decreased in cAF and animal models of atrial tachycardia-related remodeling, at least when studied at slow pacing rates ex vivo, potentially reflecting a protective mechanism against Ca^{2+}-overload induced cell death at fast rates.[127] This reduction in Ca^{2+}-transient amplitude contributes to prothrombotic atrial hypocontractility.[131] Consistent with its role as frequency sensor, CaMKII autophosphorylation and CaMKII-dependent RyR2 phosphorylation are elevated in cAF but not pAF. In addition to the rapid activation rate itself, variability in beating rates contributes to AF-related Ca^{2+}-handling remodeling, with irregular 3-Hz pacing producing more Ca^{2+}-handling abnormalities and CaMKII activation than regular 3-Hz pacing in neonatal rat cardiomyocytes.[133] Despite the reduction in Ca^{2+}-transient amplitude in cAF, elevated diastolic Ca^{2+}-levels persist due to increased CaMKII-dependent SR Ca^{2+}-leak, particularly during tachycardia, and may trigger proarrhythmic TCWs, SCaEs and drive Ca^{2+}-dependent structural remodeling, thus contributing to AF maintenance.

Electrophysiological Changes that Promote Reentry

Canine atrial cardiomyocytes adapt to Ca^{2+}-loading by engaging intracellular pathways that reduce cell-$[Ca^{2+}]_i$ directly via reduced Ca^{2+}-influx through decreased $I_{Ca,L}$ and indirectly via abbreviated APD (since a large portion of Ca^{2+}-entry occurs during the AP-plateau).[134] Rate-induced Ca^{2+}-loading causes enhanced binding of Ca^{2+} to calmodulin, which activates the phosphatase calcineurin. Calcineurin then dephosphorylates the nuclear factor of activated T-cells (NFAT), which translocates into the nucleus and inhibits production of *CACNA1C* mRNA, decreasing the message for the $I_{Ca,L}$ α-subunit, decreasing its protein and ion-transport function.[134] In parallel, NFAT suppresses the production of microRNA (miR)-26 by binding to and negatively regulating sites upstream to the transcriptional start site in human and mouse atrium.[135] One of the binding targets of the miR-26 seed site is *KCNJ2*, the gene encoding the I_{K1} channel. Reduced miR-26 expression caused by AF removes miR-26-induced destabilization of the *KCNJ2* message and inhibition of its translation.[135] Inward-rectifier current functional expression is enhanced by this mechanism, as well as by increased constitutive acetylcholine-dependent current ($I_{K,ACh}$) in human and canine models,[136,137] leading to acceleration of repolarization and resting-membrane-potential hyperpolarization, both of which stabilize AF-maintaining rotors and promote AF-maintenance.[138] Recent work also shows that NLRP3 inflammasome activity is increased via increases in both triggering and priming in patients with persistent AF, and that NLRP3 activation increases the gene expression of the channels subunits underlying the atrial-selective currents I_{Kur} and $I_{K,ACh}$.[74] The molecular basis for AF-associated NLRP3-activation, on one hand, and the mechanisms by which NLRP3 regulates I_{Kur} and $I_{K,ACh}$, on the other, remain to be established. In addition to the pathways illustrated in Figure 5, some other molecular candidate mechanisms have been reported to contribute to APD-shortening in AF. These include miR-328 dysregulation of $I_{Ca,L}$,[139] protein-kinase isoform switches that upregulate the constitutive activity of $I_{K,ACh}$,[140] and upregulation of 2-pore and Ca^{+}-dependent K^{+}-channels.[141,142]

AF-induced Structural Changes that Promote Reentry

There is now clear evidence that AF induces atrial structural remodeling, particularly fibrosis, some of which can be attributed to poor control of the ventricular rate, but a component of which is clearly due to the atrial tachyarrhythmia *per se*.[130] Rapid cardiomyocyte firing leads to fibroblast activation via a diffusible substance in HL-1 atrial-derived cardiomyocytes,[143] which appears to be ROS-derived from cardiomyocyte NADPH oxidase stimulated by Ca^{2+}-loading.[144] The mechanisms through which ROS diffusing from cardiomyocytes to fibroblasts causes their activation are unknown. ROS are known to activate NLRP3-inflammasomes in other systems[145] and NLRP3-inflammasome activation is known to cause atrial fibrosis.[74] Whether this system is in fact operative in atrial fibroblasts in AF remains to be defined. As in cardiomyocytes, fibroblast miR-26 is downregulated by AF in a canine model,[101] possibly by ROS. There is evidence that TRP channels, both TRPC3 in canine, goat and human models[101] and TRPM7 in human AF,[146] are upregulated in AF-fibroblasts and enhance fibroblast Ca^{2+}-entry; TRPC3 is under the control of miR-26 and its upregulation is related to miR-26 downregulation.[101] Once again similar to the system in cardiomyocytes, fibroblast I_{K1} is upregulated in response to miR-26 decreases in canine AF.[102] Fibroblast I_{K1}-upregulation increases the driving force for Ca^{2+}-

entry, activating fibroblasts by enhancing fibroblast-stimulating Ca^{2+}-influxes through store-operated channels.[102] Ca^{2+}-loading appears to be the proximal signal for many of the AF-induced remodeling processes that cause progression of the arrhythmic substrate.[147] Interestingly, an increased intracellular Ca^{2+}-load resulting from leaky RyR2 also appears to promote progression of the AF-substrate via CaMKII-activation.[148]

Clinical Perspectives

Progression of the AF-substrate is a major clinical problem. Aggressive early reversal of AF and sinus-rhythm maintenance might be a successful strategy, but this is as yet unproven. There is much evidence favoring vigorous control of risk factors in preventing progression and possibly even reversing the AF-substrate. Key components identified in substrate progression include Ca^{2+}-loading of both cardiomyocytes and fibroblasts, oxidative stress and low-grade local inflammation. Further research into underlying molecular mechanisms and specific therapeutic targeting approaches has the potential to provide new and helpful treatments.

Interactomes relevant for AF

Nodal Points

Targeting nodal intersection points in the signaling pathways underlying atrial remodeling might constitute a promising strategy to prevent or halt AF-progression. AF-related Ca^{2+}-overload and Ca^{2+}-handling abnormalities contribute to the triggers and substrates that maintain AF.[147] CaMKII is a central nodal point integrating electrical, Ca^{2+}-handling and structural atrial remodeling (Figure 6).[49–51,59,70,88,131,149–151] CaMKII senses and integrates signals via receptor systems leading to oxidative stress and ROS generation.[50,67,68]

Oxidative stress is another nodal point in AF-promoting remodeling.[152] Oxidative stress and mitochondrial dysfunction are caused by numerous AF risk factors and amplified by Ca^{2+} overload-inducing atrial tachycardia.[153–155] Besides promoting ectopic activity through Ca^{2+}-handling abnormalities, oxidative stress promotes reentry via ion-channel and gap-junction remodeling, as well as pro-inflammatory and pro-fibrotic signaling (e.g., via nuclear factor κB).[152–154] Oxidative stress-related remodeling also increases thrombogenesis[156] and causes microvascular flow abnormalities in pig ventricles, coupling electrical consequences to potential thromboembolic and cardiac-functional complications of AF.[157] NLRP3-mediated caspase-1 derived IL-1β also causes atrial electrical, Ca^{2+}-handling and structural remodeling in mouse and rabbit atria,[73,74,77,158] representing another therapeutically tractable nodal point of AF-related remodeling. Chronic IL-1β increases promote CaMKII-autophosphorylation at Thr287 and oxidation at Met281/282, and increase Ca^{2+}-spark frequency and APD in rodents.[78,79,159] These findings support a model in which chronic cardiomyocyte-derived IL-1β creates a self-amplifying feed-forward loop of NLRP3-inflammasome activation at the expense of CaMKII-dependent electrical, Ca^{2+}-handling and structural abnormalities, mechanistically linking IL-1β- and CaMKII-signaling pathways with NLRP3-inflammasome activation (Figure 6). Novel strategies to inhibit CaMKII-, oxidative and/or NLRP3-inflammasome-related signaling might provide new therapeutic approaches for AF prevention and management. The development of novel,

multispecific drug approaches provides a potentially innovative way to specifically target signaling systems of interest.[160]

Gene-regulatory Networks

Many gene-regulatory systems contribute to AF-related atrial remodeling in canine models and AF-patients.[161,162] Epigenetic and transcriptional networks underlying AF are reviewed in another review article of this compendium.[163] Here, we provide for completeness a short summary of key findings, which are schematically depicted in Figure 7.

Increased nuclear Ca^{2+}-load during AF enhances nuclear CaMKII-phosphorylation and associated HDAC4 export in dogs,[164] which is expected to upregulate TFs like Nkx2–5, GATA4 and MEF2c. TFs such as PITX2c and TBX5 are recognized contributors to AF susceptibility via gene-regulatory networks (Figure 7).[165–168] TBX5-deficient mice show DAD- and EAD-mediated triggered activity, spontaneous diastolic depolarizations, prolonged APD and slowed atrial conduction, providing both arrhythmogenic triggers and a reentry-maintaining substrate.[166] Reduced PITX2c rescues the arrhythmic phenotype.[166] Conversely, low PITX2c levels in human atria are associated with Ca^{2+}-handling abnormalities and cellular triggered activity,[169] resembling those present in atrial cardiomyocytes of pAF patients,[29] suggesting that low levels of either TBX5 or PITX2c could both lead to AF. GATA4 represses the transcription of SERCA2a and RyR2, while reductions in GATA4 eliminate the proarrhythmic phenotype of TBX5-deficient mice.[170] ETV1, a TF negatively regulating TBX5, is upregulated in atria of cAF patients.[171] Ang-II-driven increases in ETV1 in mice are associated with TBX5 reductions and AF development, mechanistically linking increased Ang-II signaling, abnormal gene-regulatory networks and AF.[171] NLRP3 also acts as a transcriptional regulator in T-helper type-2 cells.[172] Atrial mRNA levels of RyR2, Kv1.5 (I_{Kur}), Kir3.1/3.4 ($I_{K,ACh}$), collagen-1a, and galectin-3 are all upregulated in mice with cardiomyocyte-restricted constitutive NLRP3 activation.[74] Thus, NLRP3 may act as a transcription factor in cardiomyocytes, a concept to be investigated in future work. Finally, miRs control the expression of proteins involved in altered Ca^{2+}-handling, APD-changes, and conduction slowing (reviewed in[173,174]), in some cases likely by modulating TF-expression and gene-regulatory networks.

The causative role of the putative interactions summarized in Figure 7 in AF pathophysiology remains unproven and for some genes both a decrease and an increase in function relate to AF.[167,168] Contemporary approaches using large datasets, extensive genetic information and computational analyses offer new opportunities to identify novel risk variants and mechanisms for AF. A recent study highlighted novel systems and candidate-genes mediating effects on atrial electrophysiology, Ca^{2+}-signaling and structure.[175] Pathway and functional enrichment analyses highlighted pathways related to cardiac development; experiments in rabbits with left-atrial enlargement identified the role of a molecular switch from adult to fetal myosin heavy chain, causing contractile and functional heterogeneity that might predispose to AF.[175]

Clinical Perspectives

A number of molecular paradigms come up repeatedly as central nexuses in various types of AF-substrate development. Examples include CaMKII-activation, oxidative stress and inflammatory signaling, particularly related to the NLRP3-inflammasome. These might represent nodal points worth prioritizing as potential therapeutic targets. Several key gene-regulatory networks in AF have also been identified and subjected to intensive study, as discussed in detail elsewhere in this Compendium. The computational exploration of large genetic datasets coupled to clinical databases has the potential to reveal previously unsuspected novel mechanisms and targets.

Therapeutic Implications, Knowledge Gaps and Future Directions

Despite significant advances in AF-management with respect to antithrombotic therapy and catheter ablation, there remains an important need for improved treatment options.[174,176] In particular, catheter ablation has limited efficacy in persistent AF (particularly when long-standing), cannot readily be applied to the millions of symptomatic AF patients worldwide and does not target the underlying AF-promoting atrial cardiomyopathy.[107] Risk factor management may indirectly target the AF-promoting atrial cardiomyopathy and can reduce AF burden while increasing the success of rhythm control therapy,[4] but intense risk-factor management is challenging to apply and is limited to specific subgroups of AF patients.

Recent advances in our understanding of AF pathophysiology and the identification of nodal players involved in AF-promoting atrial remodeling highlights potential opportunities for new therapeutic approaches. To be practical, any such approach must include absence of non-cardiac toxicity and lack of proarrhythmic liability, feasibility of chronic administration and the absence of negative influences on key homeostatic processes. The latter aspect may be particularly challenging for the key nodal regulators described above because of their key functions in numerous cell types and organ systems. For example, although CaMKII inhibition could be a promising anti-AF strategy, systemic inhibition of CaMKII may impair memory or fertility.[177,178] The development of inhibitors that specifically target complex pathways via protein-protein interactions has been proposed for a long time, but this strategy is recently inching closer to practical clinical application.[179] This may prove to be a feasible approach to targeting key nodal regulators while leaving their actions on other essential processes intact.[180] Biologics have greatly improved the opportunities for specific targeting and are being developed to block inflammatory signaling, which plays a major role in AF.[181] They may also prove to be of value in targeting other nodal actors. The major challenges here would be to design a delivery system that can produce stable expression and target it specifically to the atria,[182] or at least the heart. Finally, multispecific drugs that combine targeting and effector moieties are increasingly being developed for the therapy of cancer and dyslipidemia, transplant-rejection prevention and management of hemophilia.[160] Such compounds might allow for the development of cardiac-specific, possibly even atrial-specific, targeting of key molecular pathways.

Several other elements need to be considered when developing new AF therapies. Since AF is not immediately life-threatening, successful therapies need to be relatively conservative and safe, favoring small molecules or biologicals over complex genetic approaches.

Furthermore, market realities including the size of clinical trials required to demonstrate improved clinical outcomes with a new therapeutic approach challenge the willingness to pursue new concepts. The repurposing of existing drugs might help to overcome these limitations. For example, low-dose colchicine significantly reduced the risk of ischemic cardiovascular events after myocardial infarction, possibly (at least partly) through inhibition of the NLRP3-inflammasome.[183] Thus, targeting inflammation with low-dose colchicine or other already approved agents might be a viable approach to test the inflammatory hypothesis of AF in general and the putative involvement of the NLRP3 inflammasome in particular. Failure of resolution of inflammation is an emerging concept to explain persisting inflammation that underlies chronic illnesses and a variety of compounds have been identified that promote inflammation resolution. Resolvins are a group of non-toxic omega-3 fatty acid derivatives that promote inflammation-resolution, and one of these (resolvin D1) has shown efficacy in an animal model of AF associated with right heart disease.[184] A related substance, icosapent ethyl, has been demonstrated to reduce cardiovascular risk in hypertriglyceridemic patients,[185] and might be interesting to study in AF.

Thus, translational research has produced several promising avenues for improved AF therapy. Although it is at present not clear if any of these targets will ultimately be successful in clinical practice, one success story has the capacity to dramatically change AF management and spark substantial additional innovations. Further work is needed to clarify the details of AF-controlling molecular mechanisms and to develop practical approaches to intervening clinically to prevent the occurrence and progression of this important arrhythmia. Our discussion in this section is intended to illustrate the translational potential of the molecular mechanisms we discuss. For a more detailed discussion of challenges and trends in therapeutic development for AF, we refer readers to 2 detailed review articles in this Compendium.[186,187]

Conclusions

Our understanding of the molecular pathophysiology of AF has increased considerably over the past 10–15 years. Effective translation of this knowledge into practical approaches to taming the arrhythmia has the potential to lead to important advances in patient management, but major challenges in fully understanding and actively applying it need to be overcome for this potential to be realized.

Sources of Funding

The authors' work is supported by the Netherlands Organization for Scientific Research (ZonMW Veni 91616057 to J.H.), the National Institutes of Health (R01-HL131517, R01-HL136389, and R01-HL089598 to D.D.), the German Research Foundation (DFG, Do 769/4-1 to D.D.), the Canadian Institutes of Health Research (Foundation Grant 148401 to S.N.) and the Heart and Stroke Foundation of Canada (16-12708 to S.N.).

Non-standard Abbreviations and Acronyms

AF	Atrial fibrillation
Ang-II	Angiotensin-II

AP	Action potential
APD	Action potential duration
cAF	Persistent (chronic) atrial fibrillation
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase-II
cAMP	Cyclic adenosine monophosphate
Cx	Connexin
DAD	Delayed afterdepolarization
EAD	Early afterdepolarization
HCN channel	Hyperpolarization-activated cyclic nucleotide-gated channel
HDAC	Histone deacetylases
HF	Heart failure
$\mathbf{I_{Ca,L}}$	L-type Ca^{2+}-current
$\mathbf{I_{K1}}$	Basal inward-rectifier K^+-current
$\mathbf{I_{K,ACh}}$	Acetylcholine-activated inward-rectifier K^+-current
$\mathbf{I_{Kur}}$	Ultra-rapid delayed-rectifier K^+-current
IL	Interleukin
$\mathbf{I_{Na}}$	Fast Na^+-current
$\mathbf{I_{Na,late}}$	Persistent/late Na^+-current
miR	MicroRNA
NADPH	Nicotinamide adenine dinucleotide phosphate
NCX1	Na^+-Ca^{2+}-exchanger type-1
NFAT	Nuclear factor of activated T-cells
NLRP3	NACHT, LRR and PYD domains-containing protein-3
pAF	Paroxysmal AF
PKA	Protein kinase-A
PP1	Protein phosphatase type-1
PVs	Pulmonary veins
RyR2	Ryanodine receptor channel type-2

ROS	Reactive oxygen species
SAN	Sinoatrial node
SCaEs	Spontaneous Ca^{2+}-release events
SERCA2a	SR Ca^{2+}-ATPase type-2a
SR	Sarcoplasmic reticulum
TCW	Triggered Ca^{2+} waves
TFs	Transcription factors
TRP	Transient receptor potential

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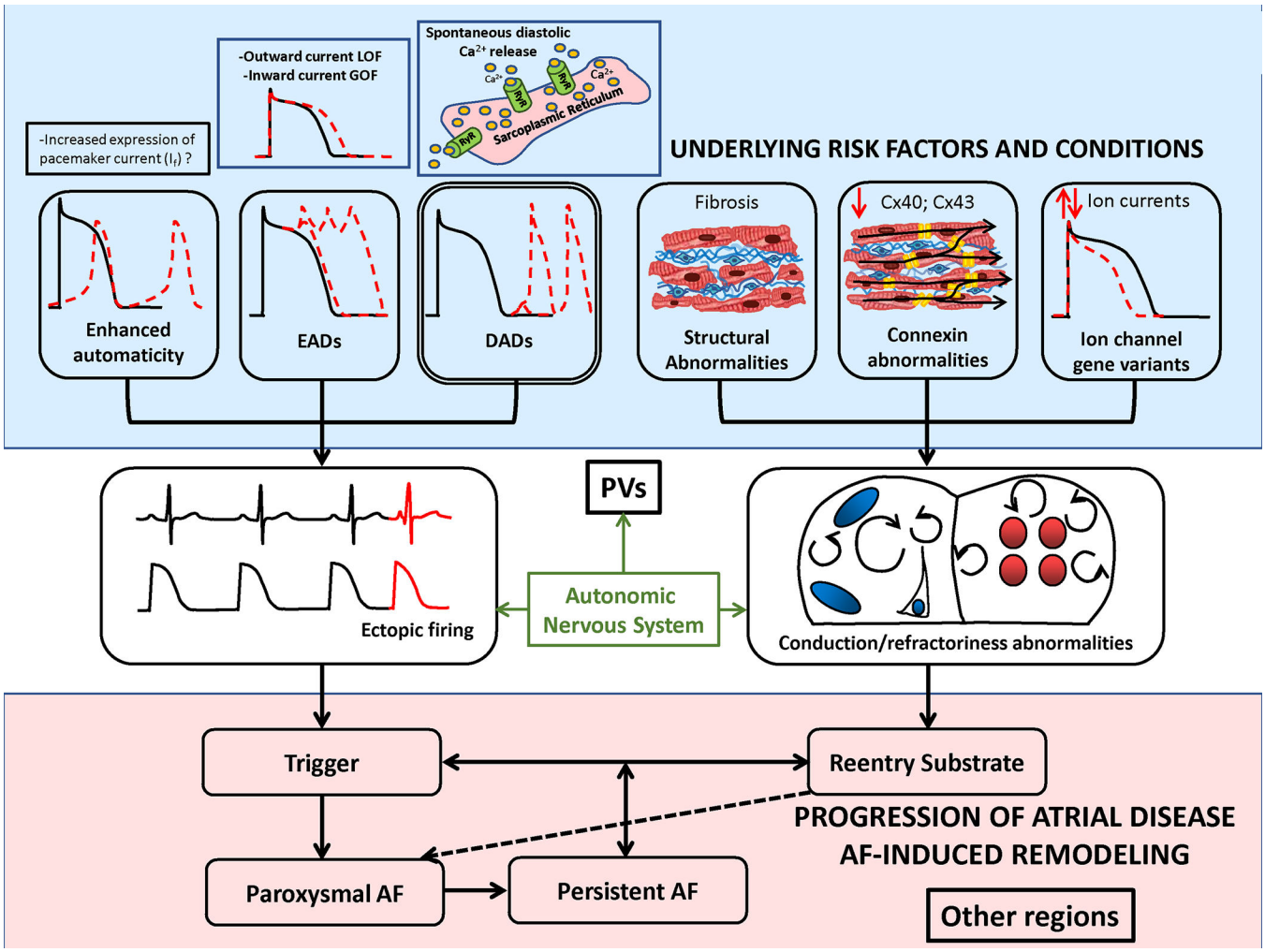

Figure 1. Overview of mechanisms linked to AF-occurrence.

AF AF-triggers result from focal ectopic firing. Ectopic activity is most clearly linked to spontaneous diastolic Ca^{2+}-release from the sarcoplasmic reticulum Ca^{2+}-stores via leaky ryanodine-receptor (RyR2) Ca^{2+}-release channels. Early afterdepolarizations (EADs) due to loss-of-function (LOF) outward-current mutations or gain-of-function (GOF) inward-current mutations have also been linked to spontaneous ectopy. Enhanced automaticity, for example due to pacemaker current expression, is another possible cause of ectopic activitty, but has not been definitively demonstrated. AF-persistence is linked to AF-maintaining reeentry that requires both trigger and a vulnerable reentrant substrate. The latter can be caused by abbreviated refractoriness (e.g. due to a GOF K^+-channel mutation or to enhanced vagal tone) or by conduction abnormalities due to tissue fibrosis, connexin (Cx) dysfunction or LOF Na^+-channel mutations. Ectopic firing typically originates from the pulmonary veins (PVs), but the PVs are also a priviledged site for reentry susceptibility.

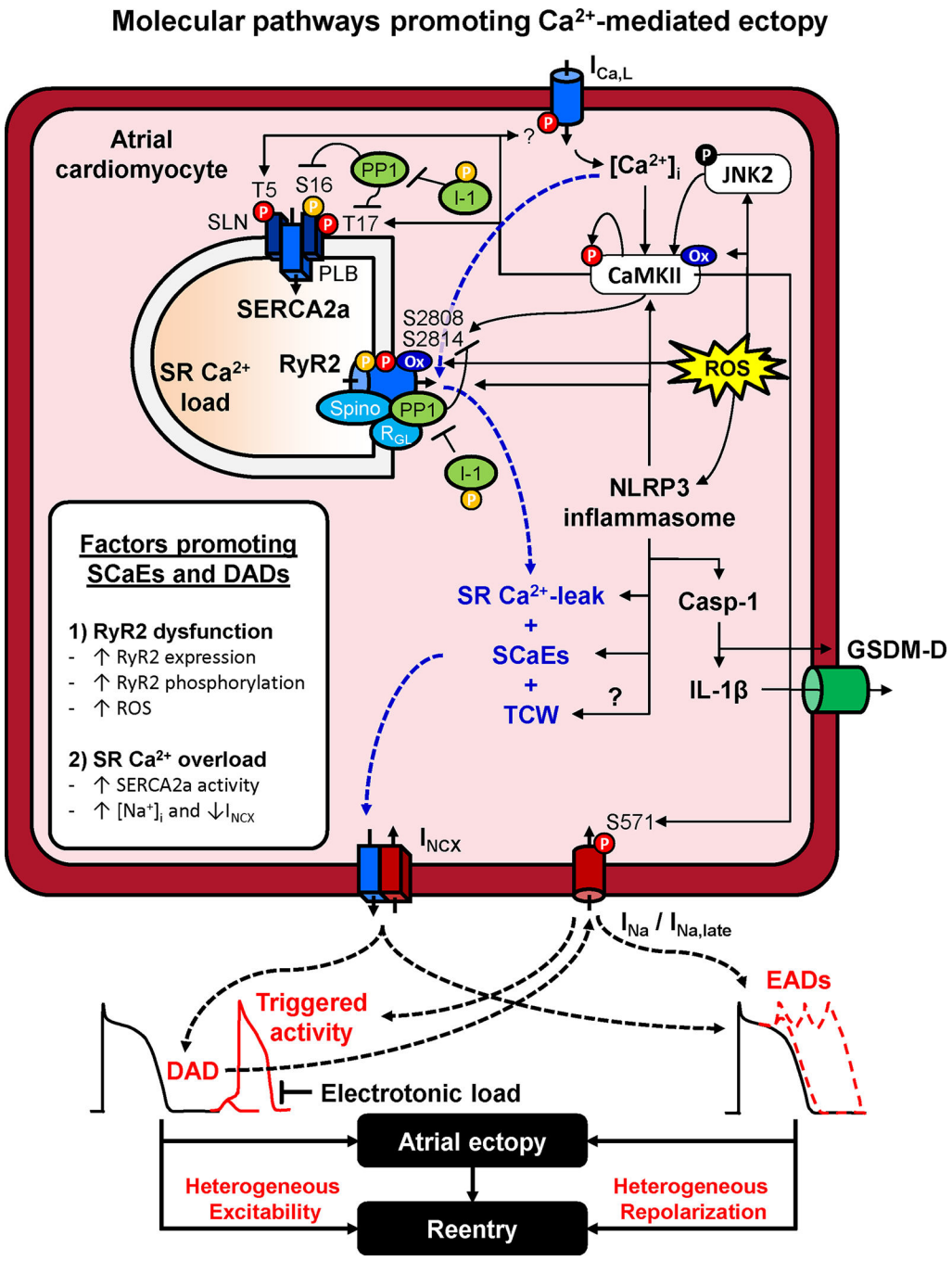

Figure 2. Atrial ectopy. Molecular pathways promoting Ca^{2+}-mediated ectopy.

Increased sarcoplasmic reticulum (SR) Ca^{2+} leak and spontaneous SR Ca^{2+}-release events (SCaEs) primarily result from dysfunction of the cardiac ryanodine receptor type-2 (RyR2) channel or SR Ca^{2+}-overload. RyR2 dysfunction is promoted by increased RyR2 expression, hyperphosphorylation (e.g., due to increased Ca^{2+}/calmodulin-dependent protein kinase-II, CaMKII, activity or improper targeting of protein phosphatase-1, PP1), or RyR2 oxidation due to increased reactive oxygen species (ROS). ROS mediated NLRP3 inflammasome activation amplifies the Ca^{2+}-handling abnormalities and activates caspase-1 (Casp-1) which

increases interleukin (IL)-1β generation and the formation of gasdermin-D-derived plasmamembrane-pores, allowing the release of IL-1β out of the cell, spreading inflammatory signaling. SR Ca^{2+}-overload is promoted by increased activity of the SR Ca^{2+}-ATPase-2a (SERCA2a) or elevated intracellular Na^{+}, reducing Ca^{2+}-extrusion via the Na^{+}/Ca^{2+} exchanger type-1 (NCX1). SR Ca^{2+} overload also promotes L-type Ca^{2+}-current ($I_{Ca,L}$)-dependent triggered Ca^{2+} waves (TCW). SCaEs and TCW activate a transient-inward current mediated by NCX (I_{NCX}) resulting in DADs or EADs, depending on their timing relative to the atrial action potential. DADs and EADs can promoted atrial ectopy, as well as reentry through increased heterogeneity of excitability and repolarization. **Abbreviations:** GSDM-D, N-terminal Gasdermin-D fragment; I-1, inhibitor-1 of PP1; JNK2, c-Jun N-terminal kinases-2; NLRP3, NACHT, LRR and PYD domains-containing protein 3; PLB, phospholamban; SLN, sarcolipin.

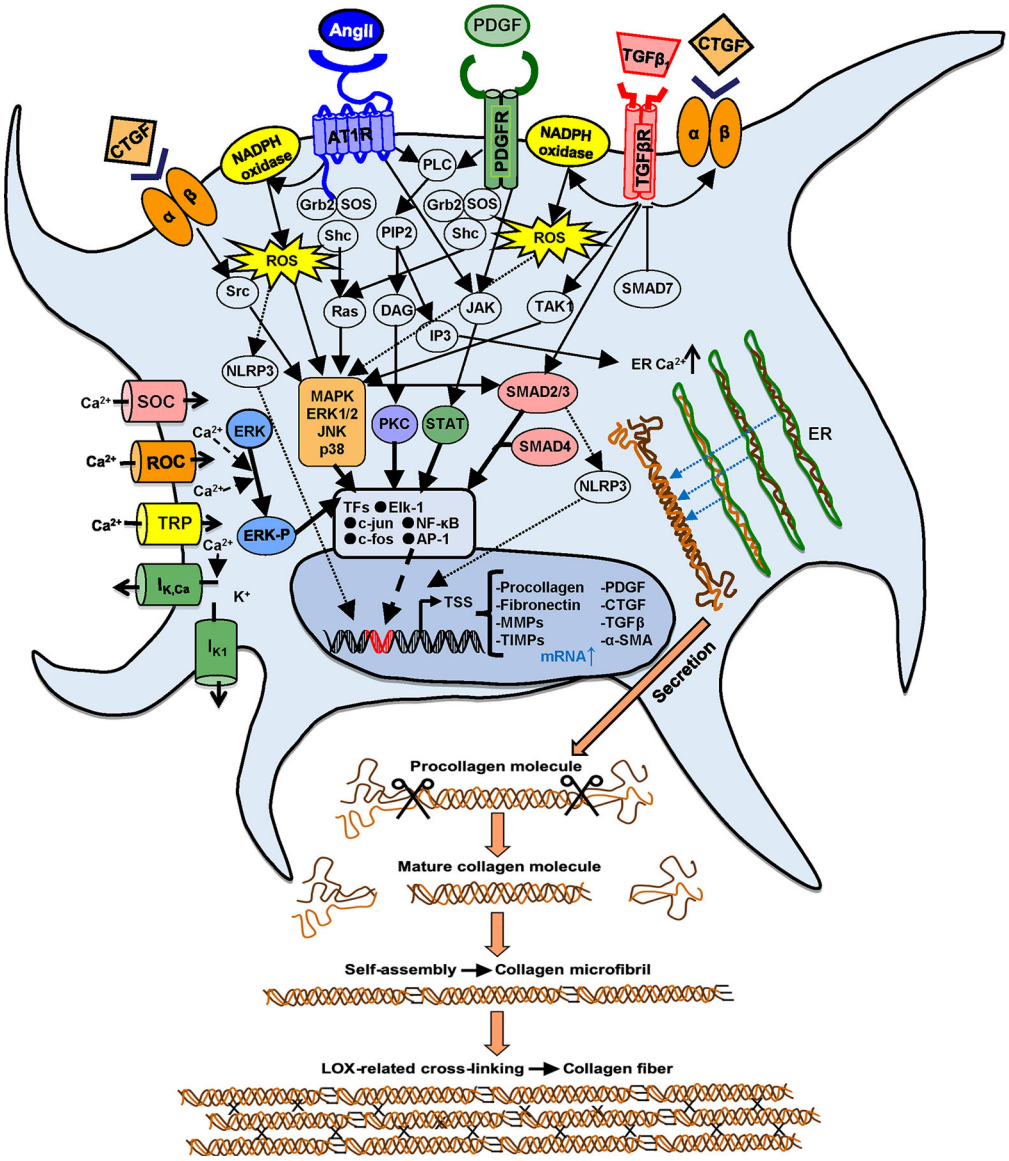

Figure 3. Molecular determinants of tissue fibrosis.

The main pathways governing profibrotic signaling. Extracellular profibrotic signaling molecules like angiotensin-II (Ang-II), transforming growth factor-β1 (TGFβ), platelet-derived growth-factor (PDGF) and connective- tissue growth-factor (CTGF) activate membrane receptors coupled to downstream signaling which leads to enhanced gene-transcription to increase extracellular matrix (ECM) production. Fibroblast ion-channels control Ca^{2+}-entry to regulate fibroblast activation. For additional discussion, see text.

Abbreviations: bb, integrin receptor oblast activation; Ang-II, angiotensin- II; AP, activator-protein; AT1R, angiotensin-II type-1 receptor; CTGF, connective-tissue growth- factor; ER, endoplasmic reticulum; ERK1/2, extracellular signal-related kinase-1/2; ERK-P, phosphorylated extracellular signal-related kinase; Grb2, growth-factor receptor binding-protein 2; I_{K1}, inward-rectifier K^+-channel; IP3, inositol 1,4,5-trisphosphate; JAK, Janus kinase; JNK, c-jun N-terminal kinase; LOX, lysyl oxidase; MAPK, mitogen-activated

protein kinase; MMP, matrix metalloproteinase; NADPH, nicotine adenine dinucleotide-phosphate; NF-κB, nuclear factor-kappa B; NLRP3, NACHT-, LRR- and PYD domains-containing protein 3; PKC, protein-kinase C; PDGF, platelet-derived growth factor; PDGFR, PDGF-receptor; PIP_2, phosphatidylinositol bisphosphate; PLC, phospholipase-C; ROC, receptor-operated channel; ROS, reactive-oxygen species; Shc, src homologous and collagen protein; SMAD, sma- and mad-related proteins; SOC, store-operated channel; SOS, son of sevenless protein; Src, sarcoma proto-oncogene tyrosine kinase; STAT, signal transducers and activators of transcription; TAK1, TGFTGF TAK1, ed kinase-1; TF, transcription factor; TGFβR, transforming growth factor β receptor; TIMP, tissue inhibitor of matrix metalloproteinase; TRP, transient receptor potential; TSS, transcriptional start site.

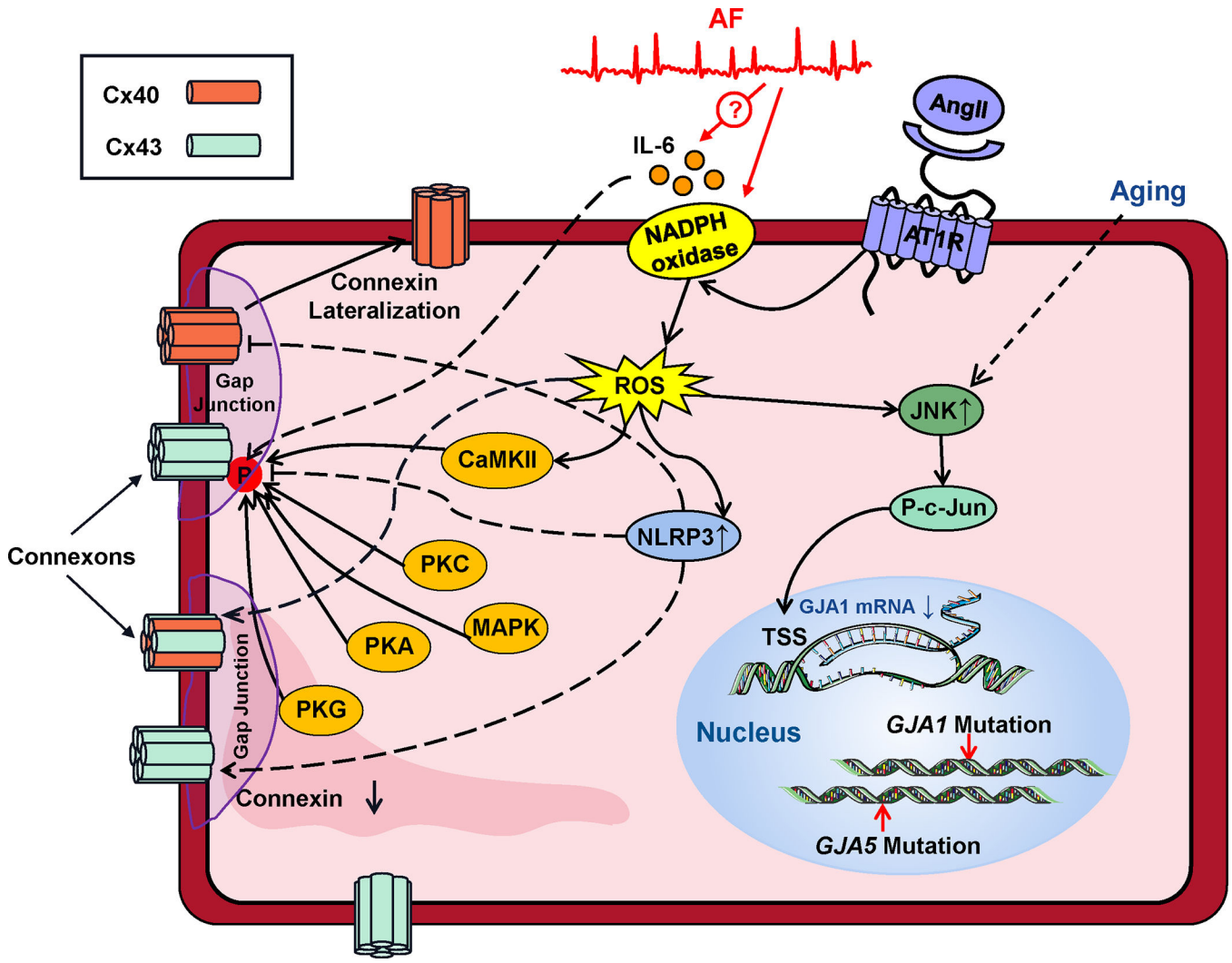

Figure 4. Connexin dysregulation in AF.

Dysfunction of the connexins that ensure cell-to-cell coupling in gap junctions results from connexin (Cx) downregulation or lateralization to transverse cell-borders. The moecular mechanisms governing these changes are illustrated. Abbreviations: Ang-II, angiotensin-II, AT1R, angiotensin-receptor type-1; CaMKII, Ca^{2+}-calmodulin dependent kinase type-II; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein-kinase; P, phosphate; PKA, PKC, PKG, protein-kinases A, C, G respectively; ROS, reactive-oxygen species.

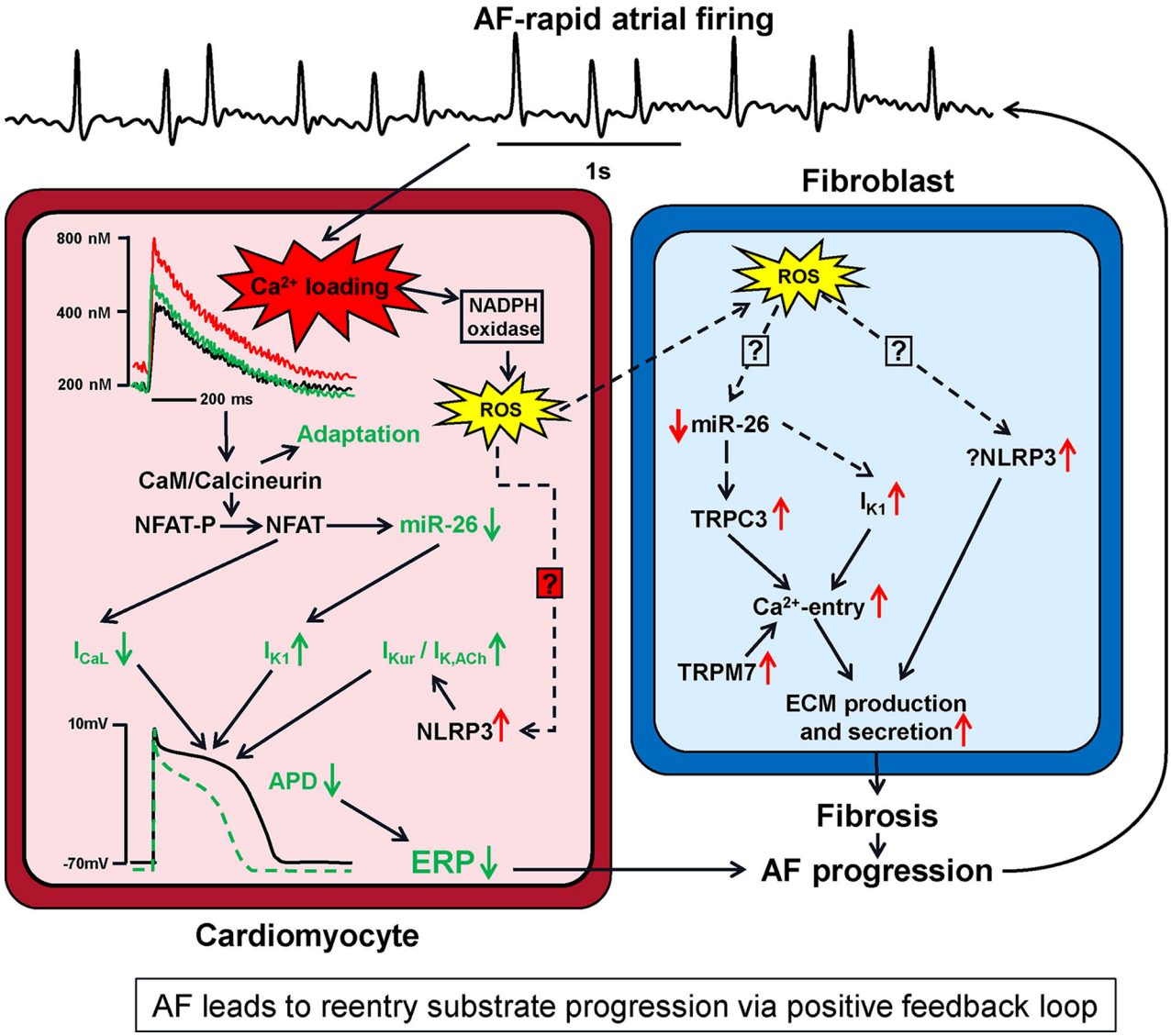

Figure 5. Molecular pathways involved in AF-progression.

The rapid atrial firing in AF leads to cellular Ca^{2+}-loading, which engages compensatory mechanisms (shown in green) that attenuate Ca^{2+}-loading at the price of action-potential duration (APD) abbreviation that favors reentry. Ca^{2+}-loading is a proximal signal to this and other processes resulting ultimately in APD and refractoriness-abbreviation in cardiomyocytes, along with enhanced collagen-production in fibroblasts, to cause progression of the reentry substrate and greater resistance of AF to therapy. A positive feedback loop results, wherein AF causes changes that increase AF-vulnerability and perpetuate the events that cause progression. Abbreviations: ECM, extracellular matrix; ERP, effective refractory period; $I_{Ca,L}$, L-type Ca^{2+}-current; I_{K1}, inward-rectifier K^{+}-current; miR, microRNA; NLRP3, NACHT-, LRR- and PYD domains-containing protein 3; TRPC3, transient-receptor channel potential current canonical type-3; TRPM7, transient-receptor channel potential current melastatin type-7; ROS, reactive-oxygen species.

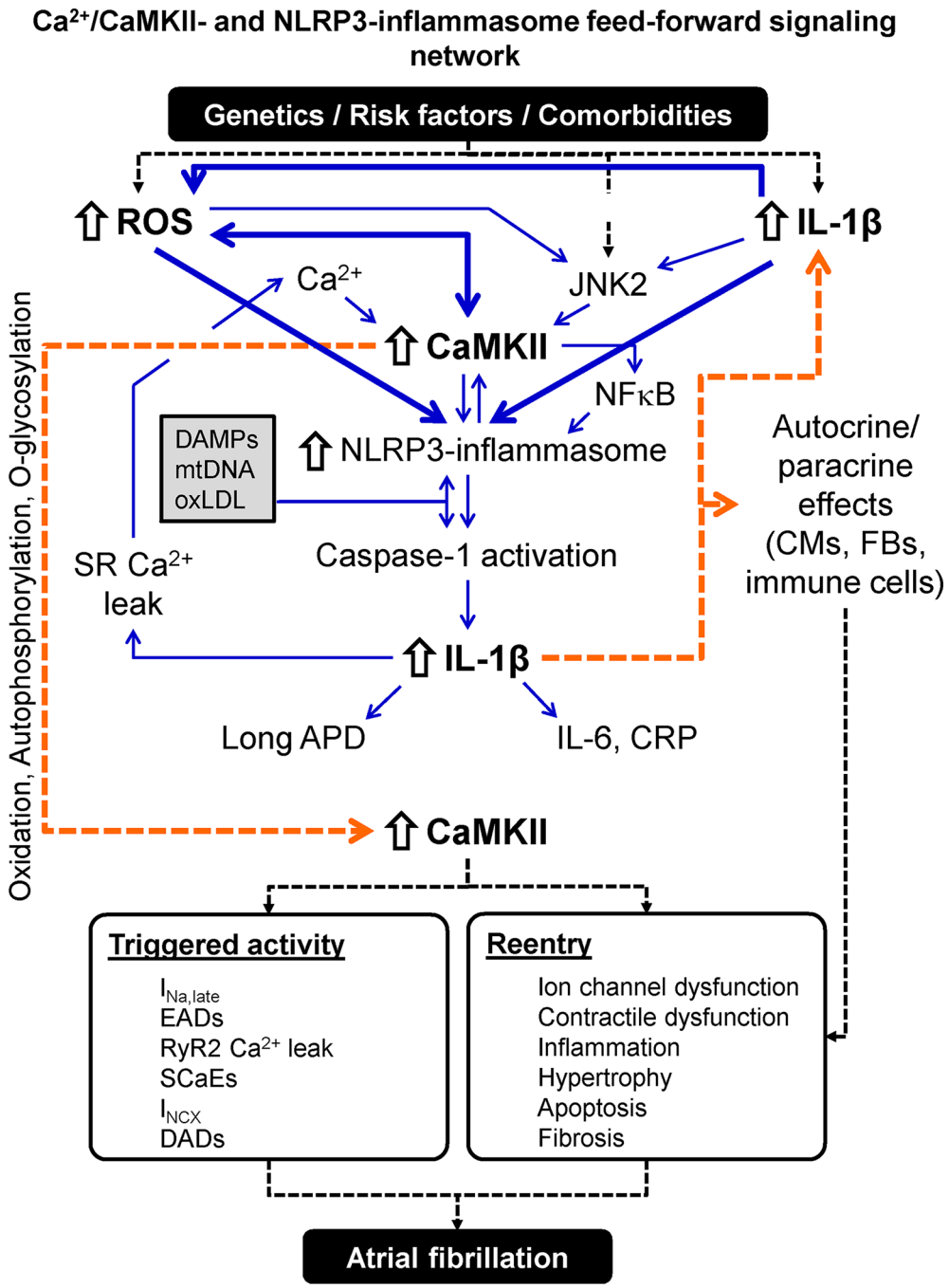

Figure 6. Atrial fibrillation-promoting Ca^{2+}/calmodulin-dependent protein kinase-II (CaMKII)- and NACHT, LRR and PYD domains-containing protein 3 (NLRP3)-inflammasome feed-forward signaling network.

Risk factors and comorbidities create an environment in which danger-associated molecular patterns (DAMPs), mitochondrial DNA (mtDNA) and oxLDL activates the atrial NLRP3 inflammasome. Cardiac-restricted increases in reactive oxygen species (ROS) production and c-Jun N-terminal kinase-2 (JNK2) activity further stimulate the NLRP3 inflammasome via CaMKII-dependent and -independent pathways. The resulting stimulation of caspase-1 maturates interleukin (IL)-1β, which leaves the cell, thereby spreading the inflammatory

signaling and increasing the synthesis of IL-6 and C-reactive protein (CRP). IL-1β amplifies the NLRP3 inflammatory signaling and promotes sarcoplasmic reticulum (SR) Ca^{2+} leak and action potential duration (APD) changes in cardiomyocytes (CMs), creating a feedforward signaling network. IL-1β also exerts paracrine effects on cardiac fibroblasts (CFs) and immune cells causing hypertrophy, apoptosis and fibrosis. Activation and perpetuation of this feedforward Ca^{2+}/CaMKII/NLRP3-inflammasome signaling network promotes triggered activity and reentry and increases AF susceptibility. **Abbreviations:** DADs, delayed afterdepolarizations; EADs, early afterdepolarizations; INa,late, Persistent/late Na^+ current; I_{NCX}, Na^+-Ca^{2+}-exchanger current; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; RyR2, ryanodine receptor type-2; SCaEs, spontaneous SR Ca^{2+}-release events.

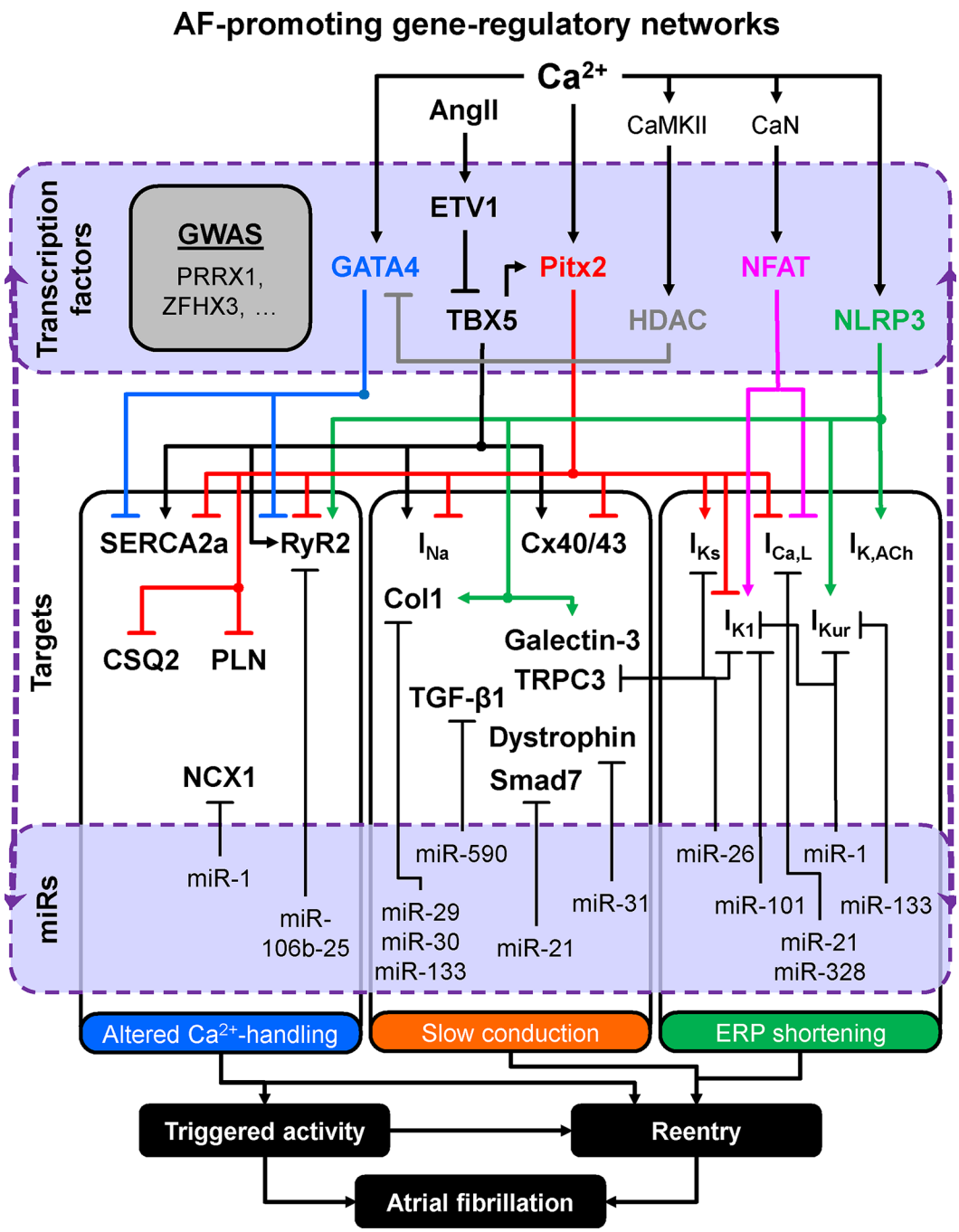

Figure 7. Atrial fibrillation-promoting gene-regulatory networks.

Multiple gene-regulatory networks interact to fine-tune the expression levels of key proteins shaping the effective refractory period (ERP), enabling proper impulse conduction and governing Ca^{2+}-handling processes. Transcriptional alterations in the level of key regulator genes disrupt the network balance, increasing the likelihood of ERP, conduction and Ca^{2+}-handling abnormalities, along with superimposed post-transcriptional changes due to abnormal microRNA (miR) function, leading to the formation of AF triggers and substrates. **Abbreviations:** Ang-II, angiotensin-II; CaMKII, Ca^{2+}/calmodulin-dependent protein

kinase-II; CaN, calcineurin; Col1, collagen-1; CSQ2, calsequestrin-2; Cx40/43, connexin 40/43; ETV1, ETS translocation variant 1 transcription factor; HDAC, histone deacetylase; $I_{Ca,L}$, L-type Ca^{2+}-current; I_{K1}, inward-rectifier K^+-current; $I_{K,ACh}$, acetylcholine-activated inward-rectifier K^+-current; I_{Ks}, slow delayed-rectifier K^+-current; I_{Kur}, ultra-rapid delayed-rectifier K^+-current; I_{Na}, Na^+-current; NCX1, Na^+-Ca^{2+}-exchanger type-1; NFAT, nuclear factor of activated T-cells; NLRP3, NACHT, LRR and PYD domains-containing protein-3; PITX2, paired Like Homeodomain 2; PLN, phopsholamban; RyR2, ryanodine receptor channel type-2; SERCA2a, SR Ca^{2+}-ATPase type-2a; Smad7, mothers against decapentaplegic homolog 7; TBX5, T-box transcription factor 5; TGF-β1, transforming growth factor β1; TRPC3 transient-receptor potential channel canonical type-3.