



Review

Regulation of cardiac ion channels by transcription factors: Looking for new opportunities of druggable targets for the treatment of arrhythmias

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ABSTRACT

Cardiac electrical activity is governed by different ion channels that generate action potentials. Acquired or inherited abnormalities in the expression and/or function of ion channels usually result in electrophysiological changes that can cause cardiac arrhythmias. Transcription factors (TFs) control gene transcription by binding to specific DNA sequences adjacent to target genes. Linkage analysis, candidate-gene screening within families, and genome-wide association studies have linked rare and common genetic variants in the genes encoding TFs with genetically-determined cardiac arrhythmias. Besides its critical role in cardiac development, recent data demonstrated that they control cardiac electrical activity through the direct regulation of the expression and function of cardiac ion channels in adult hearts. This narrative review summarizes some studies showing functional data on regulation of the main human atrial and ventricular Na⁺, Ca²⁺, and K⁺ channels by cardiac TFs such as Pitx2c, Tbx20, Tbx5, Zfhx3, among others. The results have improved our understanding of the mechanisms regulating cardiac electrical activity and may open new avenues for therapeutic interventions in cardiac acquired or inherited arrhythmias through the identification of TFs as potential drug targets. Even though TFs have for a long time been considered as 'undruggable' targets, advances in structural biology have led to the identification of unique pockets in TFs amenable to be targeted with small-molecule drugs or peptides that are emerging as novel therapeutic drugs.

1. Introduction

Cardiac electrical activity is governed by a well-orchestrated interplay of different ion channels that provide inward (Na⁺ and Ca²⁺) and outward (K⁺) currents that transiently change the voltage across the cell membrane to initiate and shape action potentials (APs) [1–3]. Therefore, the number and type of channel molecules on the cardiomyocyte membrane determine cardiac excitability, impulse propagation, as well

as AP duration (APD) and refractoriness. For this reason, abnormalities in the expression and/or function of ion channels usually lead to electrophysiological alterations that can cause cardiac arrhythmias [1–3]. In agreement with this paradigm, pathogenic mutations in the genes encoding ion channel proteins increasing or decreasing their expression and/or function are linked to inherited arrhythmogenic syndromes such as the long- (LQTS) or short- (SQTS) QT-syndromes, the Brugada syndrome (BrS), or familial forms of atrial fibrillation (AF), among others

Abbreviations: ATBF1, AT-Binding Transcription Factor 1; ETV1, ETS (E twenty-six) variant transcription factor 1; Foxo1, Forkhead Box O1; Gata4-5, GATA Binding Protein 4-5; Hand1, Heart And Neural crest Derivatives expressed 1; HCN1-4, Hyperpolarization-activated cyclic nucleotide-gated channels 1-4; Hey2, Hes Related Family BHLH Transcription Factor With YRPW Motif 2; HSF1, heat shock factor 1; Irx1-6, Iroquois Homeobox 1-6; Klf12, Kruppel Like Factor 12; Mef2c, myocyte enhancer factor 2C; Msx1-2, Msh Homeobox Homolog 1-2; Nf1a, nuclear factor 1a; NF-κB, nuclear factor κB; Nkx2-5, NK2 Homeobox 5; Pitx2, Paired-Like Homeodomain Transcription Factor 2; Sp1, specificity protein 1; Tbx2, T-Box Transcription Factor 2; Tbx20, T-Box Transcription Factor 20; Tbx3, T-Box Transcription Factor 3; Tbx5, T-Box Transcription Factor 5; TASK-2, TWIK-Related Acid-Sensitive K⁺ 2; TCF, T-cell factor; TF, Transcription factor; Zfhx3, Zinc Finger Homeobox 3.

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[4–7]. This is why the term “cardiac channelopathies” was used for a long time as synonymous of those inherited arrhythmogenic syndromes caused by ion channel derangements [2]. However, growing evidence indicates that genetically-determined variations in proteins contributing to ion channel biology other than the channel subunits themselves can eventually underlie inherited arrhythmogenic syndromes [5,8–10]. The advance in the knowledge of physiopathological mechanisms and the advent of high throughput DNA sequencing methodologies have increased diagnosis and treatment opportunities for patients suffering these hereditary arrhythmias who had remained undiagnosed until then.

The expression of a particular ion channel begins with the transcription of the gene(s) encoding the channel protein(s) leading to the synthesis of mRNA that eventually results in the expression of the encoded protein(s). Thus, transcription is just the first step in the ion channel biosynthesis, a critical process that is highly regulated [11]. Sequence-specific transcription factors (TFs) control gene transcription by binding to discrete DNA sequences adjacent to target genes [12]. Evidence obtained by linkage analysis, candidate-gene screening within families, and genome-wide association studies (GWAS) in large populations has linked rare and common (polymorphisms) genetic variants in the genes encoding TFs with genetically-determined cardiac arrhythmias [13,14]. Given the critical role of TFs in cardiac development, this association was initially attributed to possible anatomical alterations produced by the variants during this process [14,15]. However, TF expression persists in the adult heart and recent data have demonstrated that TFs control cardiac electrical activity through the direct regulation of the expression and function of cardiac ion channels in adult hearts [13,16].

For a long time, it was considered that cardiac electrical activity mostly relied on ion channel proteins themselves and their intimately associated proteins (auxiliary subunits). Indeed as mentioned above, during the candidate gene approach era the term “Channelopathies” was used as a synonym for inherited cardiac arrhythmias caused by mutations/variants that almost exclusively affected gene encoding ion channel subunits. Studies were focused in the modulation of ion channel function (conductance, time- and voltage-dependent properties) by these mutations, as well as by other interventions and mechanisms including drugs, post-translational modifications (phosphorylation, glycosylation, ubiquitination, nitrosylation, etc), protein–protein interactions (regulatory subunits, other ion channels), etc. However, the processes responsible for regulating the number of ion channels expressed in the cardiomyocyte membrane were much less considered. Recent work has brought out that, beyond their critical role in development, TFs are important for cardiac electrical homeostasis in adult cardiomyocytes. Besides, the finding that dysfunction of the TFs regulation could be responsible of acquired- or inherited-arrhythmogenic syndromes further supports this contention and, thus, the term channelopathies as synonymous of inherited cardiac arrhythmias has fallen short. We aimed to bring together some of the information on this issue to provide a perspective of this important paradigm change in the cardiac cellular electrophysiology field. Therefore, in this narrative review, we summarize some of the studies, paying special attention to those that demonstrated a functional regulation of human atrial and ventricular voltage-gated ion channels by some of the major specific cardiac TFs. We focused in those studies that, in addition to analyzing gene expression, recorded ion currents and action potentials (APs) in cellular models and/or electrocardiograms (ECGs) *in vivo*. Elucidating the role of TFs has improved our understanding of the mechanisms regulating cardiac electrical activity. Furthermore, their involvement in signaling pathways responsible for cardiac arrhythmias may open new avenues for therapeutic interventions through the identification of novel drug targets potentially useful in these diseases, whose current treatment options are very limited [17,18].

2. Human cardiac action potential [1–3]

Fig. 1 depicts the main ion currents involved in the different phases of human atrial (Fig. 1A) and ventricular (Fig. 1B) AP. The upstroke of the AP (phase 0) generated by atrial and ventricular cardiomyocytes results from the activation of the fast inward Na^+ current (I_{Na}) (Na^+ -dependent APs). Initial rapid repolarization (phase 1) is caused by the rapid inactivation of the I_{Na} and the activation of K^+ currents, such as the transient outward (I_{toF}) and the atrial-selective ultrarapid component of the delayed rectifier K^+ (I_{Kur}) currents. The late component of the I_{Na} (I_{NaL}) can be considered as a residual I_{Na} carrying Na^+ ions over hundreds of milliseconds after the peak I_{Na} and although its amplitude is very small under normal conditions, it contributes to control of AP duration. Therefore, the presence of the I_{NaL} and the activation of the L-type Ca^{2+} current (I_{CaL}) slow the rate of repolarization, leading to the plateau phase (phase 2). Inward Ca^{2+} flux at this stage critically determines the link between electrical excitation of the myocyte and the contraction of the heart. Afterwards, repolarization is accelerated again (phase 3) by the activation of the rapid and slow components of the delayed rectifier K^+ current (I_{Kr} and I_{Ks}) and of the inward rectifier current (I_{K1}). I_{K1} critically determines the maintenance of a stable resting membrane potential (RMP) between two consecutive AP (phase 4) in atrial and ventricular cardiomyocytes. It is worth mentioning that, besides I_{K1} , some other voltage-independent inward rectifier currents, such as those activated by acetylcholine (I_{KACh}) or sensitive to adenosine triphosphate (I_{KATP}) also play a role in cardiac APs under some circumstances. Purkinje fiber cells generate Na^+ -dependent APs, but phase 4 is not isoelectric since RMP undergoes slow diastolic depolarization. The APs generated by automatic sinoatrial (SA), atrioventricular (AV) nodal cells also differ from those in the working myocardium. Phase 4 is not isoelectric as RMP progressively depolarizes mainly due to the absence of I_{K1} and the presence of a mixed Na^+ and K^+ current (pacemaker current or I_{p}) generated by Hyperpolarization-activated cyclic nucleotide-gated 1–4 (HCN1–4) channels that, in contrast to other voltage-gated channels, activate upon hyperpolarization [19]. Moreover, in these nodal cells the upstroke of phase 0 is much slower due to the activation of the I_{CaL} (i.e. these cells generate Ca^{2+} -dependent APs), which results in a slower propagation of the impulse in SA and AV nodes than in the working myocardium.

3. Cardiac ion channels

In this section, we briefly describe some properties of the main human cardiac ion currents responsible for atrial and ventricular APs. Table 1 summarizes them together with the corresponding channel subunits and encoding genes, as well as the main inherited primary arrhythmogenic syndromes associated to mutations in these genes. Conductance of voltage-gated ion channels changes in response to variations in the membrane potential. Most of these voltage-gated ion channels transit throughout at least 3 conformational states: closed, open or activated, and inactivated, and frequently, these states are divided in different substates. At very negative potentials, as those correspond to atrial or ventricular RMP, these channels remain closed and in response to membrane depolarization they open (activation) with time and voltage gating that differ depending on the channel type. Thereafter, most channels undergo a non-closed non-conductive state called inactivated (inactivation), which occurs when the flow of ions is blocked by a mechanism other than the closing of the channel pore. Under normal conditions, channels return from the inactivated to the closed state (reactivation) before a new opening can occur.

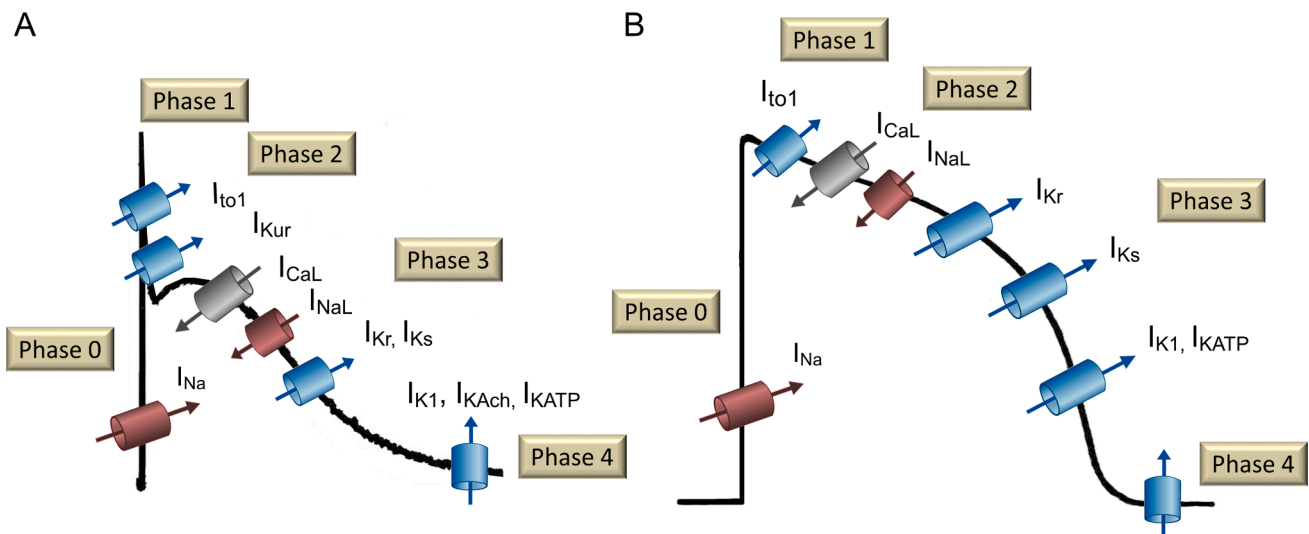


Fig. 1. Schematic diagram showing the main ion currents involved in the different phases of human atrial (A) and ventricular (B) APs.

3.1. Na^+ channels

Cardiac I_{Na} is generated by channels composed of pore forming Nav1.5 α -subunits encoded by the *SCN5A* gene [20]. The Nav1.5 subunit exhibits 4 homologous domains, DI-DIV, and each domain is formed by six transmembrane segments (S1 to S6). The first four segments (S1–S4) comprise the voltage sensor domain whereas the S5 and S6 segments and the pore loop (P-loop) between them form the pore module [21]. The N- and C-terminal domains are intracellular and both regulate channel expression and function [22,23]. Nav β 1– β 4 subunits interact with Nav1.5 and modulate its cell surface expression and gating. Moreover, a plethora of proteins including regulatory and scaffolding proteins or enzymes, contribute to form the macromolecular complex of the cardiac Na^+ channel (i.e. channelosome) [24]. Na^+ channels generate a rapid activating and inactivating inward current that determines cardiac excitability and conduction velocity in cells generating Na^+ -dependent APs. Nav1.5 subunits are the receptors of class I anti-arrhythmic drugs (AADs), which are classified according to the kinetics of unbinding from the channel in drugs with fast (class IB; e.g. lidocaine), intermediate (class IA; e.g. quinidine), or slow (class IC; e.g. flecainide and propafenone) unbinding kinetics [17,25,26]. Most of loss-of-function *SCN5A* mutations reduce the membrane expression of Nav1.5 channels and the I_{Na} density, and are the most frequent cause of BrS by affecting the fine balance between inward and outward currents at the end of phase 1 of action potentials generated by epicardial ventricular cardiomyocytes [7]. Other inherited primary arrhythmogenic syndromes such as sick sinus syndrome, progressive cardiac conduction disease, and some forms of sudden infant death syndrome are also associated to loss-of-function mutations in *SCN5A* [27,28]. Conversely, *SCN5A* gain-of-function mutations impair Na^+ channel inactivation, prolong the APD and underlie the LQTS type 3 [4]. Mutations in the genes encoding Nav β subunits and other proteins within the Na^+ channelosome can also lead to different inherited arrhythmogenic syndromes [4,5,7].

3.2. L-type Ca^{2+} channels

Cardiac I_{CaL} is generated by Ca^{2+} channels composed by the association of the pore-forming subunit Cav1.2 (or $\alpha_{1\text{C}}$) encoded by the *CACNA1C* gene, and several accessory subunits $\alpha_2\text{-}\delta$ and β [29]. The general structure of Cav1.2 is similar to that of Nav1.5 channels, with 4 homologous domains with each domain composed of 6 transmembrane segments (S1–S6). L-type Ca^{2+} channels are found in all cardiac cell types being critical for the excitation–contraction coupling in the working

myocardium and automaticity and impulse conduction in SA and AV nodes. They are the target of Ca^{2+} channel blockers such as dihydropyridines and class IV AADs (e.g. diltiazem and verapamil) [17]. Loss-of-function mutations in the *CACNA1C* may cause BrS [30], whereas gain-of-function mutations underlie the Timothy Syndrome or LQTS type 8 [31]. Besides their role in inherited arrhythmogenic syndromes, alterations in L-type Ca^{2+} channels are also critical in the pathophysiology of AF, which is the most frequent arrhythmia. One important hallmark of AF is the appearance of progressive atrial electrical alterations (i.e. atrial remodeling) mostly characterized by the shortening of atrial APD and refractoriness that favors arrhythmia recurrence and maintenance [32]. Several ion channels are misregulated in myocytes from patients with persistent forms of the arrhythmia, although the reduced expression of L-type Ca^{2+} channels is one of the most consistent finding [33,34].

3.3. K^+ channels

Several voltage-gated K^+ channels are expressed in cardiac cells, where they shape the different phases of AP repolarization [35,36]. They are composed of tetramers of α -subunits, with each α -subunit being equivalent to one of the four domains of Na^+ and Ca^{2+} channels, and multiple β -subunits. The S1–S4 segments of each α -subunit form the voltage sensor domain, while the S5 and S6 segments and the P-loop establish the pore domain, which includes the K^+ selectivity filter signature GYG [37].

In human cardiomyocytes, the α -subunit of the channels underlying the I_{toF} is mainly composed by Kv4.3 channels encoded by the *KCND3* gene. Multiple accessory subunits including KChIP (*KCNIP2*) and MiRP2 (*KCNE3*) regulate the expression and function of the α -subunit. The I_{toF} is a rapidly activating and inactivating K^+ current mainly responsible for the initial repolarization (phase 1), especially in atrial and ventricular epicardial cells. Gain-of-function mutations in *KCND3* or *KCNE3* have been associated to some forms of BrS [7,38,39]. A slower component of the transient outward current (I_{toS}) has been identified in a subset of human ventricular subendocardial myocytes carried by Kv1.4 channels [40].

The I_{Kur} is a rapidly activating, but slowly inactivating K^+ current generated by channels composed by Kv1.5 α -subunits encoded by the *KCNA5* gene. Functional Kv1.5 channels are expressed in the atria but not in the ventricles, despite the fact that *KCNA5* mRNA is detected in human ventricles [41]. This fact led to the proposal that Kv1.5 channels were a potential pharmacological target for the treatment of supraventricular arrhythmias [42,43].

Table 1

Main Na^+ , Ca^{2+} , and K^+ currents involved in human atrial and ventricular action potentials together with their respective channel subunits, encoding genes and associated inherited primary arrhythmogenic syndromes.

ION CURRENT	CHANNEL SUBUNIT	GENE	ASSOCIATED INHERITED PAS	REFERENCES*
I_{Na}	α	Nav1.5	SCN5A	BrS1, ERS, Familial AF, LQT3, IVF, PCCD, SIDS, SSS
	β	Nav β 1	SCN1B	BrS5, Familial AF, PCCD
		Nav β 2	SCN2B	BrS14, Familial AF
		Nav β 3	SCN3B	BrS7, IVF, SIDS
		Nav β 4	SCN4B	LQT10, SIDS
I_{CaL}	α	Cav1.2	CACNA1C	BrS3, J Wave Syndrome, LQT8 (Timothy syndrome), SQT4
	β	$\alpha_2\delta$	CACNA2D1	BrS9
		β	CACNAB2	BrS4, SQT5
I_{tof}	α	Kv4.3	KCND3	BrS10, Familial AF, SIDS
		Kv4.2	KCND2	
	β	KChIP2	KCNIP2	
		MiRP2	KCNE3	BrS6, Familial AF
I_{Kur}	α	Kv1.5	KCNA5	Familial AF
	β	Kv β 1	KCNAB1	
I_{Kr}	α	Kv11.1 (hERG)	KCNH2	BrS8, LQT2, SQT1,
	β	MiRP1	KCNE2	Familial AF, LQT6
		MiRP2	KCNE3	Familial AF
I_{Ks}	α	Kv7.1	KCNQ1	Familial AF, JLN1, LQT1, SQT2
	β	minK	KCNE1	Familial AF, JLN2, LQT5
I_{K1}	α	Kir2.1	KCNJ2	CPVT3, Familial AF, LQT7 (Andersen-Tawil Syndrome), SQT3
		Kir2.2	KCNJ12	
		Kir2.3	KCNJ4	
	α	Kir6.1	KCNJ8	BrS8, ERS
I_{KATP}		Kir6.2	KCNJ11	
	β	SUR2A/SUR2B	ABCC9	BrS13, ERS, Familial AF
I_{KACH}	α	Kir3.1	KCNJ3	
		Kir3.4	KCNJ5	LQT13

The quoted articles are generally those in which the first mutation associated with each inherited arrhythmogenic syndrome was identified.

AF: Atrial Fibrillation; BrS: Brugada Syndrome; CPVT: catecholaminergic polymorphic ventricular tachycardia; ERS: Early Repolarization Syndrome; I_{CaL} : L-type Ca^{2+} current; I_{K1} : inward rectifier current; I_{KACH} : acetylcholine-activated inward-rectifying K^+ current; I_{KATP} : ATP-sensitive inward-rectifying K^+ current; I_{Kr} : rapid component of the delayed rectifier K^+ current; I_{Ks} : slow component of the delayed rectifier K^+ current; I_{Kur} : ultrarapid component of the delayed rectifier K^+ current; I_{Na} : fast inward Na^+ current; IST: Inappropriate Sinus Tachycardia; I_{tof} : transient outward K^+ current; IVF: Idiopathic Ventricular Fibrillation; JLN: Jervell and Lange-Nielsen Syndrome; LQT: Long QT Syndrome; PAS: Primary Arrhythmogenic Syndrome; PCCD: Progressive Cardiac Conduction Disease; SIDS: Sudden Infant Death Syndrome; SQT: Short QT Syndrome; SSS: Sick Sinus Syndrome.

The I_{Kr} plays a critical role in human ventricular repolarization [35,36]. It is generated by channels whose α -subunit is formed by hERG/Kv11.1 proteins encoded by the *KCNH2* gene. The minK-related peptide 1 (MiRP1) encoded by *KCNE2* is considered the main auxiliary β -subunit [35], although association of hERG and MiRP1 does not fully recapitulate native I_{Kr} properties [44]. Additionally, hERG may interact with several other MiRPs subunits and interacting partners, such as Kv7.1 channel or the K^+ channel regulator KCR1, which suggests that the actual composition of ion channels underlying the I_{Kr} is largely unknown [45]. I_{Kr} biophysical properties have some peculiarities that determine its critical function. It exhibits a very rapid voltage-dependent inactivation that leads to strong inward rectification at positive potentials. Moreover, the fast recovery from inactivation as AP repolarization progresses results in a large outward current after the plateau phase that critically contributes to accelerating the process. Therefore, I_{Kr} plays a major role in determining cardiac APD and refractoriness. In rat hearts, I_{Kr} density is greater in atria than in ventricles, while in humans, hERG channels are more expressed in the ventricles [46]. I_{Kr} is the target of class III AADs of the methanesulfonanilide group (e.g. dofetilide, ibutilide, and D-sotalol) that prolong APD [17,47,48]. Moreover, other drugs, with diverse chemical structures and not primarily used to treat cardiac arrhythmias, inhibit I_{Kr} , slow ventricular repolarization, lengthen the QT interval (acquired LQTS) and can induce a rapid polymorphic ventricular tachycardia called *Torsades de Pointes* (TdP) [47,48].

The I_{Ks} is a slowly activating and deactivating outward K^+ current generated by homotetramers of Kv7.1 α -subunits (encoded by *KCNQ1*) coassembled with minK auxiliary proteins (encoded by *KCNE1*) [47,49]. This current is expressed in human atrial and ventricular cardiomyocytes where it is involved in the control of repolarization, especially at high beating frequencies and/or after β -adrenergic stimulation,

since the slow kinetics of activation and deactivation promotes accumulation of channels in their open state under these conditions [34]. It has been shown that I_{Ks} density is increased in atrial myocytes enzymatically isolated from chronic AF (CAF) patients, contributing to the shortening of atrial APD [50]. I_{Ks} channels are selectively blocked by several non-therapeutic compounds including Chromanol 293B and HMR-1556 [49] and by other drugs such as the Class I AAD propafenone [51].

Mutations in the genes encoding I_{Ks} - and I_{Kr} -channel subunits are responsible of more than 80 % of autosomal dominant LQTS. Indeed, loss-of-function mutations in *KCNQ1* and *KCNE1* cause LQTS type 1 and 5, respectively, while mutations in *KCNH2* and *KCNE2* cause LQTS type 2 and 6, respectively. Mutations in genes encoding proteins that regulate these channels, such as the A-kinase anchoring proteins (AKAP9) have also been related with LQTS [5]. Conversely, gain-of-function mutations in these genes may lead to the short QT syndrome [6].

Cardiac I_{K1} stabilizes RMP close to the reversal potential of K^+ and shapes the final repolarization phase of the AP both in atrial and ventricular myocytes [52]. It exhibits a strong inward rectification that allows the flux of K^+ ions over a narrow range of membrane potentials. In fact, upon depolarization K^+ efflux stops immediately, and remains so throughout the plateau phase, resuming at potentials negative to -20 mV. I_{K1} is generated by channels composed of homomeric or heteromeric tetramers of Kir2.1/2.2/2.3 encoded by the *KCNJ2*, *KCNJ12*, and *KCNJ4* genes, respectively [52]. In human cardiomyocytes, Kir2.x channels are distributed differentially between atria and ventricles in such a way that Kir2.3 channel expression is larger in atria than in ventricles, whereas Kir2.1 channels seem to predominate in the ventricles [53]. Kir2.x subunits only contain two transmembrane segments (named M1 and M2) analogous to S5 and S6 transmembrane domains of

voltage-gated channels, but lack the voltage-sensor domain. The N- and C-terminal domains are cytoplasmic and associate to make up the so-called “cytoplasmic pore” that is an extension of the transmembrane pore and of the same length. I_{K1} inward rectification is caused by the voltage-dependent block of the channel pore produced by cytosolic polyamines (spermine, spermidine, putrescine) and Mg^{2+} [52,54]. Kir2.x channels are also regulated by phosphatidyl inositol-bisphosphate (PIP2) that promotes channel activity by binding to critical residues at N- and C-termini [54]. Therapeutic concentrations of AADs such as flecainide and propafenone increase human ventricular I_{K1} , an effect that might contribute to their proarrhythmic effects [55,56]. It was demonstrated that these and other drugs meet the structural requirements (pharmacophore) that allow them to bind to Cys311 that is located at the C-terminal domain and is present in Kir2.1 channels but not in Kir2.2/2.3. Drug-binding to this residue decreases the affinity of the channel for polyamines and, thus, reduces current rectification [55,56]. Interestingly, at supratherapeutic concentrations, propafenone blocks Kir2.x channels by an unprecedented mechanism. Indeed, propafenone molecules in their cationic form incorporate into the cytoplasmic domain of the channel, reducing the net negative charge sensed by K^+ ions and polyamines. This decrease, in turn, promotes the appearance of subconductance levels and the reduction of PIP2 affinity of the channels [57]. In patients with CAF, an AF-induced increase of I_{K1} contributes to the APD shortening and is critical for arrhythmia stabilization and maintenance [34,58]. Loss-of-function mutations in *KCNJ2* prolong ventricular APD and cause Andersen Syndrome (LQTS type 7), while gain-of-function mutations cause ventricular APD shortening and may account for SQTs type 3 [36].

4. Regulation of cardiac ion channels by transcription factors

As mentioned, TFs are DNA-binding proteins that promote or repress transcription of the target genes playing a critical physiological role by ensuring the expression of genes in the right amount, in the right cell, and at the right time [12]. To this end, they interact with specific sequences of DNA located in regions close to (promoters) or distal to genes (enhancers) through their DNA-binding domains (e.g. T-box, homeodomain, GATA-binding domain, etc), whose sequence and length is variable depending on each particular TF family. Adjacent to the DNA-binding domain, TFs exhibit activation and/or repression domains that allow them to either favor or impair the transactivation of the target genes.

However, TFs only bind to a small fraction of the multiple elements throughout the genome in which their consensus DNA sequence is found. Chromatin immunoprecipitation experiments had shown that in each particular cell type there are ≈ 100 bp DNA sequences in which different TF-binding sites may overlap. The binding of TF to one of these regulatory sequences (enhancers) may modulate the interaction of other TFs in such a way that conditions the net response. The binding of different TFs to the enhancers can be produced by different modes. In the “cooperative mode”, the binding of one TF promotes the cooperative binding of other TFs to multiple enhancers [59] similarly to what occurs when a drug binds to a receptor with multiple binding sites. In the “billboard” mode, there may not be interaction between all binding sites and not all sites need to be occupied for transcriptional activity to occur [60]. Therefore, depending on the degree of occupation, the degree of activity of the enhancer will vary between inactive-empty or completely active-completely occupied. This model becomes relevant when heterozygous mutations in the genes encoding TFs are considered, a situation in which the availability of a TF will be reduced to 50 %. TF-binding to any DNA sequence can be modulated by protein or RNA cofactors, by the position of the nucleosome (the binding of a TF can change the nucleosome position that, in turn, would affect the binding of other TFs), and by the physical properties of the DNA strand and of the surrounding chromatin [61–63]. Although cofactors do not bind DNA directly, they regulate gene expression by interacting with TFs [62].

Furthermore, TF can interact with other TFs in different modes including: 1) a physical interaction. 2) By the presence of binding sites for some TFs in the promoter and/or activator regions of genes encoding other TFs. In this way, the binding of one TF can regulate the expression of the other and modulate its transcriptional activity [61,62]. 3) Interaction between TFs at the activation sites. Therefore, TF-TF interactions, co-activators, and/or co-repressors create networks that regulate mammalian development and adult tissue homeostasis [64]. These complex TF-interactions are frequently affected in pathological condition leading to unexpected consequences.

Several cardiac-enriched highly conserved TFs act during the fetal period in an orchestrated form to control cardiogenesis. The main cardiac-specific TFs are (among others) some T-box family members (e.g. Tbx5, Tbx3, Tbx18, Tbx20), homeodomain-containing transcription factors (e.g. Pitx2c, Nkx2-5, Irx1-6) or members of the GATA family (Gata4-6) [11,65–68]. In the heart, for instance, it has been demonstrated the physical interaction of Tbx5 with Gata4 or 6, Tbx20, Mef2c or Nkx2-5 [69]. Tbx5 can establish networks with Msx1 and Msx2 [70], two homeodomain TFs that can also interact with Tbx2 and Tbx3 favoring their repressor effects [71]. Importantly, the absence of one of these TFs may lead to the inappropriate expression of “non-cardiac” genes activated by the other [72]. As an example, Tbx2 and Tbx3 are selectively expressed in the cardiac conduction tissue where they repress the transcription of atrial and ventricular muscle cell-specific genes by interacting with and inhibiting the protranscriptional activity of Nkx2-5 [73,74].

In accordance with this critical role during cardiogenesis, rare mutations in the genes encoding these TFs are usually associated with congenital cardiac defects [11,65–67,75]. As mentioned above, recent evidence, especially that obtained in GWAS performed in large cohorts of control individuals and cases in the context of AF [76], suggested an association of TFs with the control of cardiac electrical activity and susceptibility to arrhythmias [13,14]. These GWAS identified several common variants in genes encoding TFs such as *PITX2*, *TBX5*, *ZFHX3*, *PRRX1*, *NKX2-5*, or *HAND2* that increase the susceptibility to AF [13]. Moreover, variants in genes encoding TFs have been also associated to parameters such as PR, QRS, or QT intervals of the electrocardiogram (ECG) [77,78]. Initially, due to the absence of functional analyses, the effects of these variants were attributed to putative structural alterations caused by disruptions during cardiac development that would indirectly affect electrical activity [15]. In the following subsections we review some recent evidences describing the role of TFs in controlling expression and/or function of ion channel proteins or of associated proteins in the adult myocardium.

4.1. *Pitx2c* is involved in atrial fibrillation pathophysiology

PITX2 encodes the pituitary homeobox family of TFs that is critical for a correct embryonic morphogenesis and include Pitx2a, Pitx2b, and Pitx2c. Pitx2a and Pitx2b share the same promoter, while Pitx2c (the isoform that is predominantly expressed in the embryonic and adult hearts) uses an alternative promoter upstream of exon 4 [79]. In humans, it has been reported a fourth isoform, named Pitx2d, which functions as a dominant negative protein [67]. It seems that Pitx2c is a major determinant of the left/right asymmetry during cardiac development [79]. However, for some time its role in adult cardiac tissue was a matter of debate. A seminal study published in 2007 [15] described a GWAS conducted in AF cases and controls from independent European and Han Chinese populations and found some single nucleotide polymorphisms (SNPs) on chromosome 4q25 that strongly correlated with AF risk. These variants were located in an intergenic non-coding region, with *PITX2* being the closest gene [15]. More recently, this association was replicated in multiple cohorts of similar or distinct ethnic backgrounds [13,80]. At that time the mechanism underlying this relationship was unknown, although it was suggested that changes in the development of myocardial sleeves of the pulmonary veins and in the

genetic program of the SA node [79]. Later experimental studies suggested a role of Pitx2c in adult atria and showed that Pitx2c deficiency increased arrhythmogenesis in mouse models [67,81–85]. In this context, Chinchilla and coworkers demonstrated that conditional cardiac *Pitx2*-deficient mice displayed remarkable electrical alterations including AV node block and absence of P waves [81]. These ECG alterations were accompanied by a depolarized RMP and a smaller AP amplitude compared with control mice that were attributed to a misregulation of microRNA-1 (miR-1) and *Kcnj2* expression [81]. Molecular analyses of the left atrial appendages from adult atrial Pitx2-deficient mice demonstrated that the expression of multiple genes was altered including AF-related genes identified in GWAS (e.g. *Zfx3* and *Kcnn3*) or genes encoding Ca^{2+} channel (*Cacna1c*) and Ca^{2+} -handling proteins such as Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase 2 [SERCA2 (*Atp2a2*)], calsequestrin (*Casq2*), and phospholamban (*Pln*). Electrophysiological analyses demonstrated a significant decrease in the I_{CaL} density in atrial myocytes isolated from atrial-specific Pitx2 mutants compared with Sham mice [83]. In a different mouse Pitx2c knockout model, flecainide was reported to suppress atrial arrhythmias more effectively when Pitx2c expression was reduced. These authors attributed their findings to a reduction of the expression of both mRNA and protein of the TWIK-related acid-sensitive K^+ channel 2 (TASK-2) [86]. In the last few years, some data regarding Pitx2c regulation of human cardiac electrical activity have been provided. Atrial myocytes from patients carrying the rs13143308T *PITX2* variant exhibited increased SERCA2 protein expression, sarcoplasmic reticulum Ca^{2+} load, and RyR2 phosphorylation; changes that may be the cause of triggered activity in these patients [87]. More recently, two mutations in the homedomain of Pitx2c (Gln103His and Glu124Lys) were identified in a patient with recurrent AF. The expression of the mutated forms in HL-1 cells, revealed that they impaired endogenous expression of proteins involved in Ca^{2+} -handling and altered Ca^{2+} accumulation at the sarcoplasmic reticulum, markedly affecting the beat-to-beat stability upon elevation of the stimulation frequency [88]. Our group explored the hypothesis of Pitx2c could participate in the events involved in the electrical remodeling associated with CAF in humans. To this end, we conducted RT-qPCR experiments in enzymatically isolated cardiomyocytes from atrial appendage samples obtained from patients in sinus rhythm (SR) and patients with CAF (lasting for at least 6 months at the time of surgery) [89]. The use of single myocytes allowed us to circumvent the presence of non-myocyte cells (e.g. endothelial cells and fibroblasts) in whole atrial samples that had led to contradictory results in previous studies [81,90]. We showed that cardiac *PITX2* mRNA levels were doubled in CAF than in SR myocytes [89]. Interestingly, *PITX2* mRNA expression was positively and negatively correlated, respectively, with the densities of I_{Ks} and I_{CaL} , which as mentioned are critically involved in the AF-induced electrical remodeling [33,50]. In HL-1 cells, Pitx2c expression remarkably affected the I_{Ks} , increasing maximal and tail current density, accelerating activation kinetics and shifting voltage dependence of activation to more negative potentials [89]. Luciferase assays demonstrated that Pitx2c expression exerted a pro-transcriptional effect over human *KCNQ1* and *KCNE1* gene promoters by binding specifically to its DNA-binding consensus sequence (TAATCC). As a result, HL-1 cells expressing Pitx2c exhibited significantly larger expressions of *KCNQ1* and *KCNE1* mRNA and Kv7.1 and minK proteins. Pitx2c expression, however, did not modify I_{Kr} density or its voltage- and time-dependent properties. On the other hand, Pitx2c markedly decreased I_{CaL} density, shifted inactivation curves to more depolarized potentials and slowed recovery from inactivation. Unexpectedly, Pitx2c did not inhibit the current by regulating the transcription of the genes encoding the Ca^{2+} channel, pointing to a post-transcriptional mechanism. We showed that Pitx2c increased atrial natriuretic peptide (ANP) production by HL-1 cells [89] and that the Pitx2c-induced reduction of the I_{CaL} was suppressed by the incubation of Pitx2c-expressing HL-1 cells with the ANP type A receptor antagonist A71915. Therefore, our proposal was that Pitx2c promotes the expression of the *NPPA* gene, which

encodes ANP, and that the ANP increase was responsible for the I_{CaL} inhibition. Therefore, Pitx2c regulates human atrial I_{Ks} and I_{CaL} and contributes to the most important changes that characterize the AF-induced electrical remodeling [89], which converted this TF in a master regulator of “cardiac channelopathy” in this arrhythmia [91].

4.2. *Tbx20* selectively regulates human hERG channels and the I_{Kr}

Tbx20 plays a fundamental role in cardiac development by regulating multiple transcriptional networks [92]. Rare *TBX20* mutations are associated with congenital heart defects, including defects in chamber growth and septation, and valvulogenesis. Furthermore, common variants in *TBX20* were associated with dilated cardiomyopathy [92]. This TF has a 180-aminoacid DNA-binding domain characteristic of the T-box family of TFs that allows its binding to the DNA-consensus sequence (AGGTGTG) located in the promoter and/or enhancer regions of the target genes. The net effect on gene expression is also determined by transactivation and transrepressor regions located just after the T-box [66]. It had been demonstrated that *Tbx20* deletion in adult mouse myocardium led to electrical alterations including slow heart rate, changes in AV node conduction, and altered ventricular depolarization and repolarization, as well as tachyarrhythmias and bradyarrhythmias [93]. Furthermore, these *Tbx20*-deficient mice showed a significant downregulation of the expression of genes encoding subunits of multiple K^+ and Ca^{2+} channels [93]. GWAS studies had demonstrated that SNPs within *TBX20* were associated with ECG parameters such as the QRS duration [94]. All these results suggested that *Tbx20* could regulate human cardiac electrical activity. However, the effects of *Tbx20* on human cardiac ion channels had not been shown yet. Our group demonstrated that this TF controls human ventricular repolarization by modulating the I_{Kr} [95]. *Tbx20* enhanced human *KCNH2* gene minimal promoter activity and increased hERG channel protein expression in HL-1 cells. Accordingly, *Tbx20* increased the density of I_{Kr} recorded in these cells and the opposite result was observed when the expression of endogenous *Tbx20* was silenced by means of short hairpin RNA (shRNA). The *Tbx20*-increasing I_{Kr} effect was replicated in cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) where, consequently, *Tbx20* shortened APD. In contrast to that expected from the results yielded by mouse models [93], *Tbx20* did not modify the expression or activity of any other K^+ , Na^+ or Ca^{2+} channel involved in human ventricular APs [67]. Interestingly, our results demonstrated that *Tbx20* increased *KCNE1* transcription and, consequently, the expression of minK [95]. Therefore, since minK increases Kv7.1 conductance [96,97], a *Tbx20*-induced I_{Ks} augmentation would have been expected. But, it has to be considered that minK can also act as an endocytic chaperone promoting membrane Kv7.1–minK complexes internalization [98], which would result in the decrease of the I_{Ks} density. Thus, these two opposite actions would explain why the *Tbx20*-induced minK increase did not lead to a parallel augmentation of the I_{Ks} density. In addition, we identified a rare missense mutation in *TBX20* in several members of a Spanish family of African ancestry suffering LQTS and with 3 cases of sudden cardiac death [95]. The variant (p. R311C), located in the transactivation region of *Tbx20*, produced a loss-of-function phenotype as demonstrated by the functional analysis. Indeed, p.R311C *Tbx20* did not enhance human *KCNH2* promoter activity and did not increase but, surprisingly, decreased hERG channel expression and I_{Kr} density. This result was explained by the analysis of the transcriptional effects over *KCNH2* minimal promoter by the combination of wild-type (WT) and p.R311C that simulated the heterozygous condition of the probands. In the presence of the mutated form, the protranscriptional effect of WT *Tbx20* over *KCNH2* was significantly reduced, suggesting that p.R311C *Tbx20* did not enhance *KCNH2* expression and impaired the effect of the WT form. Therefore, using a pharmacological concept, we proposed that the mutated *Tbx20* binds to its binding site at the promoter but, since it lacks protranscriptional activity, it behaves as a “competitive antagonist” of the WT form.

Furthermore, through this mechanism of action, p.R311C Tbx20 prolonged the duration of APs recorded in hiPSC-CMs, producing a greater prolongation at slow than at fast driving frequencies. Thus, p.R311C Tbx20 variant would cause or at least contribute to the expressivity of the LQTS phenotype together with other variants identified in the family and suggested that *TBX20* may be considered a LQTS-modifying gene [95].

4.3. *Tbx5* regulates human Nav1.5 channels and controls peak and late I_{Na}

Tbx5 is another member of the T-box family of TFs encoded by the *TBX5* gene. Human Tbx5 is a 518-aminoacid protein comprising a 180 T-box region and transactivation and transrepressor domains [74,99]. It plays key roles in cardiac and forelimb development [66] and, accordingly, loss-of-function mutations in *TBX5* cause Holt–Oram syndrome mostly characterized by defects of the cardiac septa and conduction system and of anterior forelimbs [100,101]. During early cardiac development in animal models, Tbx5 activates transcription of genes encoding proteins involved in cardiac myocyte maturation and septation [69,100,102]. However, during later cardiac development, Tbx5 regulates patterning of the cardiac conduction system and mature cardiomyocyte function [103]. Tbx5 is abundantly expressed in adult cardiac cells and *Tbx5* gene deletion in the specialized conduction system slowed conduction velocity and caused ventricular arrhythmias and sudden death, as a consequence of a reduced expression of cardiac Nav1.5 channels [104]. Indeed, it had been described that in adult mice Tbx5 increased Nav1.5 expression by binding at least to two *Scn5a* enhancers [104–106]. In adult cardiomyocytes from humans, the expression of Tbx5 had been shown by immunohistochemical and WB analyses [107,108]. Several studies including GWAS had associated Tbx5 with cardiac conduction velocity and arrhythmias in humans with structurally normal hearts [103,109] and with the modulation of the P-wave, PR interval, and QRS durations of the ECG [110–112]. Despite this evidence that pointed to the existence of a regulatory role of Tbx5 on Nav1.5 channels, this fact had not yet been functionally demonstrated in human cardiac cells. Thus, we performed a detailed study encompassing luciferase assays, WB and electrophysiological experiments conducted in HL-1 cells, hiPSC-CMs, and cardiomyocytes from mice *trans*-expressing human Tbx5 that led us to demonstrate that this TF regulates the expression and function of human cardiac Na^+ channels [113]. A co-expression analysis of RNA-seq and proteomic data from human cardiac samples showed a positive correlation between Tbx5 and Nav1.5 mRNA and protein expressions [113]. Moreover, Tbx5 exerted a pro-transcriptional effect over human *SCN5A* promoter that in hiPSC-CM led to an increase of the peak I_{Na} and, consequently, of the AP amplitude. Strikingly, Tbx5 hyperpolarized the RMP in these cells. Previous results showed that the expression of Nav1.5 and Kir2.1 channels are reciprocally and positively modulated, i.e. an augmented expression of Nav1.5 channels is accompanied by an increase in the expression of Kir2.1 channels and, *vice versa* [22,114–117]. Furthermore, Tbx5 enhanced the transcriptional activity of human *KCNJ2* promoter. This pro-transcriptional effect together with the positive reciprocal modulation between Kir2.1 and Nav1.5 channels resulted in an increased I_{K1} density in hiPSC-CMs [113]. Since I_{K1} controls the RMP, the Kir2.1 expression increase would explain the Tbx5-induced hyperpolarization of the RMP. The regulatory role of Tbx5 over human Nav1.5 channels was further confirmed by the fact that a loss-of-function *TBX5* variant (p.F206L) identified in two siblings diagnosed with BrS with structurally normal hearts, decreased I_{Na} . p.F206L Tbx5 was unable to transactivate the *SCN5A* minimal promoter despite maintaining its ability to bind it, thus, preventing the pro-transcriptional effect of the WT form (“competitive antagonist”). Therefore, p.F206L markedly reduced I_{Na} density in all the cellular models tested; effects that in p.F206L Tbx5 *trans*-expressing mice resulted into a remarkable QRS widening that was exacerbated in the presence of flecainide (a potent I_{Na} inhibitor) [113].

It has been described that Nav1.5, Calcium/calmodulin-dependent protein kinase II (CaMKII), and β_{IV} -spectrin form a ternary complex in such a way that β_{IV} -spectrin allows Nav1.5 channels to be phosphorylated by CaMKII favoring an increase in the magnitude of the persistent component of the current (I_{NaL}) [118]. In our study, we observed that native Tbx5 exhibited a repressor effect over the promoters of human *CAMK2D* and *SPTBN4* genes encoding CaMKII and β_{IV} -spectrin proteins [113]. As a consequence, Tbx5 reduced I_{NaL} and shortened APD measured at 50 and 90 % of repolarization in hiPSC-CM. The functional study of another *TBX5* variant (p.D111Y) identified in a family affected of LQTS demonstrated that this mutant failed to repress *CAMK2D* and *SPTBN4* promoter activities yielding an increase of the expression of CaMKII δ and β_{IV} -spectrin proteins. As a consequence of the effects at the transcriptional level, the p.D111Y variant augmented I_{NaL} and prolonged APD in ventricular myocytes, as well as the QT and QTc intervals in p.D111Y Tbx5 *trans*-expressing mice. Interestingly, the QT/QTc prolonging effects were suppressed by ranolazine, a selective I_{NaL} inhibitor [113].

The majority of the heterozygous *TBX5* loss-of-function mutations that had been described lead to the Holt–Oram Syndrome [119]. However, the carriers of the p.F206L and p.D111Y *TBX5* variants identified in our study exhibited structurally normal hearts with no anatomical malformations [113]. Tbx5 interacts with Nkx2-5 and Gata4 to synergistically promote cardiomyocyte differentiation and development of the conduction system [103,104,109,119]. It has been proposed that the lower the synergistic interaction between mutated Tbx5 and Nkx2-5 the more severe the cardiac malformation [120]. Amino acids D111 and F206 are located outside the Tbx5 N-terminal domain (residues 1–90), which is important for the synergistic activation of NPPA through establishing interactions with its C-terminal domain or Nkx2-5 [122]. Apparently, during cardiogenesis both p.D111Y and p.F206L Tbx5 variants can synergistically interact with Gata4 and Nkx2-5. Indeed, our results demonstrated that both variants may bind to and transactivate the *NPPA* gene, which suggests that both of them retain the core functionality of WT Tbx5. Therefore, p.D111Y and p.F206L represented a new type of *TBX5* variants that selectively modify cardiac electrophysiological properties [113,121]. All these results demonstrated that Tbx5 regulates cardiac electrical activity in adult human hearts, and that variants compromising this Tbx5 function produce selective electrical defects consistent with human inherited syndromes such as LQTS and BrS [121].

4.4. A *Pitx2*-*Tbx5* transcriptional network is involved in the control of atrial electrical activity

Recent data have shown that a transcriptional network involving Tbx5 and *Pitx2* regulates atrial electrical activity and would have a relevant role in the context of AF [122]. *Tbx5* deletion in mouse altered an atrial gene regulatory network, favouring the appearance of primary, spontaneous, and sustained AF [122]. Adult atrial cardiomyocytes from Tbx5-deficient mice displayed AP abnormalities, including triggered activity (early and delayed afterdepolarizations) [122]. These effects induced by Tbx5 removal seem to be associated with a decreased SERCA2-mediated Ca^{2+} uptake, which was counteracted by increased *trans*-membrane Ca^{2+} fluxes driven by the I_{CaL} and Na^+/Ca^{2+} exchanger [123]. In hiPSC-CM, it was identified a regulatory element at the *PITX2* gene that was modulated by a *Tbx5*-binding site [122]. Therefore, these two TF form a transcriptional network, in such a way that in the mouse myocardium Tbx5 activated *Pitx2*, whereas Tbx5 and *Pitx2* produce opposite effects on the expression of ion channel genes such as *Scn5a* (Nav1.5), *Gja1* (Cx43), and Ca^{2+} cycling genes such as *Ryr2* (ryanodine receptor 2) and *Atp2a2* (SERCA2) [122]. The functional consequences of this network in the mouse model were that a reduced *Tbx5* dose caused by adult-specific haploinsufficiency diminished target gene expression, myocardial automaticity, and AF inducibility. Interestingly, all these effects were rescued by *Pitx2* haploinsufficiency. Overall, these findings

pointed to the existence of a transcriptional network critical for atrial rhythm control driven by Tbx5 and modulated by Pitx2c, supporting the genetic implication of multiple AF-risk loci identified throughout GWAS studies [13,122,124].

4.5. *Zfhx3* regulates Nav1.5 channels and the I_{Na} by a complex mechanism

Zfhx3 or AT motif binding factor (ATBF1) is a homeobox TF encoded by the *ZFHX3* gene [125,126]. It is a large protein (404 kDa) that contains 4 homeodomains and 23 zinc finger motifs. *Zfhx3* is widely distributed being involved in the regulation of neuronal and myogenic differentiation [127,128], although it also functions as a tumor suppressor in several cancers [129]. In cardiac cells, *Zfhx3* establishes transcriptional networks together with other key TFs such as Pitx2c, Tbx5 and Nkx2-5 [130]. GWAS have associated variants in non-coding regions of the *ZFHX3* gene with AF [131,132], and more recently, with sick sinus syndrome [133], although the functional link was unknown. There are data showing that *Zfhx3* silencing in HL-1 cells increased Kv1.4, Kv1.5, Kir3.4 [10] and Kir6.x + SUR2 [11] channel expression, which leads to an augmented I_{Tos} , I_{Kur} , I_{KACH} and I_{KATP} , as well as to the shortening of the APD [10]. In the same cell line, *Zfhx3* knockdown increased the ryanodine receptor (*Ryr2*), and *SERCA2a* (*Atp2a2*) mRNA expression pointing to a role of this TF in regulating intracellular Ca^{2+} handling [10]. Very recently, we showed that *Zfhx3* is expressed in human atrial and ventricular samples [134]. Expression of human *Zfhx3* in HL-1 cardiomyocytes remarkably diminished peak I_{Na} , while silencing of the endogenous *Zfhx3* expression produced the opposite result. Expression or silencing did not alter current voltage-dependent or kinetic properties or the I_{NaL} , suggesting that the *Zfhx3* was acting through the regulation of the expression of channel proteins, rather than gating. We also showed that *Zfhx3* decreased human *SCN5A* and *TBX5* transcriptional activity, leading to a reduction of mRNA and protein levels of both Nav1.5 and Tbx5. In contrast, the factor augmented the expression of Nedd4-2, an ubiquitin-protein ligase that promotes Nav1.5 proteasomal degradation [135]. Therefore, *Zfhx3* inhibited I_{Na} as a result of a triple mechanism mediated by the direct repressor effect over the *SCN5A* promoter, the reduction of Tbx5-augmenting effects on the I_{Na} , and the increased Nav1.5 protein degradation consequence of a higher expression of Nedd4-2. Besides *TBX5*, *Zfhx3* also reduced the luminescence activity generated by the human *PITX2* and *NKX25* minimal promoters, suggesting that *Zfhx3*, Pitx2c, Tbx5, and Nkx2-5 TFs establish a complex transcriptional network that regulates Nav1.5 channel expression, and, in turn, cardiac

excitability and conduction velocity [134]. Therefore, it can be suggested that putative changes in *Zfhx3* levels produced by the variants associated to AF or sick sinus syndrome in GWAS could act by disrupting the equilibrium within the transcriptional networks that would eventually affect cardiac excitability. In this study, we also functionally analyzed the consequences on the I_{Na} of three *ZFHX3* variants found in unrelated probands diagnosed with familial AF and BrS. Surprisingly, the three variants reduced the I_{Na} magnitude to the same extent than the WT form, suggesting that a differential effect on the I_{Na} was not the cause of the respective syndromes [134].

4.6. Other transcription factors

In this section we are summarizing those data functionally demonstrating the effects of other TFs in the regulation of cardiac electrical activity. Additionally, other reports described the effects of TFs just on the level of expression of mRNA and/or proteins participating in the generation and conduction of cardiac APs. The latter are summarized in Table 2.

Many SNPs [rs593479 and rs577676 (1q24 in *PRRX1*), rs1906617 (4q25 near *PITX2*), rs6490029 (12q24 in *CUX2*), rs10507248 (12q24 in *TBX5*), rs8180252 (4q34.1 in *HAND2*), and rs12932445 (16q22 in *ZFHX3*)] in genes encoding TFs were confirmed as being associated with AF in different populations [136–139]. More recently it has been described that alterations in transcriptional networks formed by Pitx2c, Tbx5, Prrx1, and *Zfhx3* TFs may alter cardiomyocyte function through an effect on ionic currents that, in turn, could increase AF risk [13]. Also the group of directed by Dr. Moskowitz demonstrates in an elegant work that Tbx5 and Gata4 interact to control the atrial rhythm in genetically modified mice [140]. The molecular mechanism of the Tbx5/Gata4 interaction comprised normalization of Ca^{2+} fluxes and expression of *Atp2a2* and *Ryr2* [140].

Regarding transcriptional networks, in embryonic stem cell-derived cardiomyocytes Pitx2c was able to dynamically upregulate some cardiac-enriched TFs including Isl1, Mef2c and Gata4 [141]. Furthermore, there is a synergistic transactivation between Isl1 and Tbx5 as well as Gata4. Isl1 also exerts a pro-transcriptional effect over the genes encoding two other TFs: Mef2c and Nkx2-5. On the other hand, development and function of pacemaker cardiomyocytes of the SA node depends on the network of Tbx3, Isl1, and Shox2 TFs [142]. These results illustrate the complexity of the transcriptional networks described in the atrial myocardium, and explains why loss-of-function variants of a particular TFs can produced familial AF [143].

Furthermore, Hand1 and Nfia have been associated with QRS

Table 2
Regulation of cardiac ion channels by some TFs not detailed in the text.

TF	GENE	PROTEIN	CURRENT	AP characteristics	EFFECT	REFERENCE
CUX2				APD80	0	[248]
Gata4/5	<i>SCN5A</i>	Nav1.5	I_{Na}		+	[147]
Gata4	<i>KCNE1</i>	minK	I_{Ks}		+	[149]
HAND1	<i>GJA1</i>	Connexin 43			–	[249]
NF-κB	<i>SCN5A</i>	Nav1.5	I_{Na}			[250]
Nkx2-5	<i>GJA1</i>	Connexin 43			+	[251]
	<i>GJA5</i>	Connexin 40			+	[251,252]
	<i>HCN4</i>	HCN4	I_f		–	[253]
Mef2c	<i>SCN5A</i>	Nav1.5	I_{Na}		+	[165]
	<i>HCN4</i>	HCN4	I_f		+	[165]
Msx1	<i>GJA1</i>	Connexin 43			–	[71]
Nr2f2 (COUP-TF2)	<i>CACNA1G</i> , <i>CACNA1C</i>	Cav3.1, Cav1.2	I_{CaL}			[254]
Shox2	<i>HCN4</i>	HCN4	I_f	APA, APD	–	[255,256]
	<i>GJC1</i>	Connexin 45				
Snail	<i>SCN5A</i>	Nav1.5	I_{Na}		–	[257]

0: no effect; +: increase; -: decrease.

APA: action potential amplitude; APD: action potential duration; I_{CaL} : L-type Ca^{2+} current; I_f : funny or pacemaker current; I_{Ks} : slow component of the delayed rectifier K^+ current; I_{Na} : fast inward Na^+ current.

interval duration in GWAS conducted in African [144] and Hispanic/Latino populations [145]. Indeed, more than 20 loci are significantly associated with QRS duration including loci in or near genes encoding Tbx5, Tbx3, Tbx20, Nfia, and Klf12 TFs [94]. Tbx5 and Pitx2c have also been associated, together with other genetic loci, with the P-wave duration [139]. Six loci near *NKX25*, *SOX5*, *MEIS1*, and *TBX5-TBX3* genes are associated with the PR interval duration [112,146].

Gata4 and Gata5 bind to the minimal promoter and intron 1 of the *SCN5A* gene [147] and synergistically activated the *SCN5A* promoter and increase *SCN5A* transcript levels in the human heart (Table 2). Gata4 decreased the expression of Cav1.2 channels thus reducing the I_{CaL} density in transfected rat neonatal cardiomyocytes, while it did not modify the expression of Cav3.2 channels [148]. Gata4 also exerted a protranscriptional effect over *KCNE1*, which as mentioned encodes minK [149].

In transfected rat neonatal cardiomyocytes, Nkx2-5 increased the expression of Cav3.2 channels while it significantly decreased that of Cav2.1 and Cav1.3, thus inhibiting I_{CaL} [148]. Conditional deletion of Nkx2-5 after birth, decreased the expression of RyR2 and Nav1.5 channels and, thus, the I_{Na} density in mouse cardiomyocytes, indicating that Nkx2-5 function is critical beyond the cardiogenesis [150]. Further studies confirmed the regulatory role of Nkx2-5 over the expression of *Scn5a* and *Cacna1c* in homo- and heterozygous knockout mice [151]. These results also demonstrated that Nkx2-5 exerted a protranscriptional effect over *Kcnh2* gene, thus increasing I_{Kr} density [151]. Nkx2-5 directly binds to an Isl1 enhancer and repressed Isl1 transcriptional activity. Isl1, in turn, promoted the expression of nodal lineage markers and repressed the ventricular program of differentiating myocytes increasing the expression of HCN4 channels [152].

The ETV1 TF is highly expressed in mouse atria and ventricular conduction system (VCS), where it regulates expression of Nkx2-5, connexin-40, and Nav1.5 [153]. Interestingly, atrial expression of ETV1 was upregulated in CAF patients [154]. In mouse cardiac myocytes ETV1 regulated the transcription of several genes *Kcnh2*, *Kcnk3* and *Tbx5*, identifying the role of ETV1 in the atrial remodeling associated with atrial arrhythmias [154].

The homeobox *Irx5* TF is specifically expressed in the developing endocardium lining the ventricular and atrial working myocardium [155]. In mice lacking *Irx5*, the I_{toF} density was increased in endocardial myocardium, resulting in augmented susceptibility to arrhythmias [156]. Homozygous loss-of-function *IRX5* mutations have been associated with a rare inherited developmental disease called Hamamy syndrome that is characterized by widened QRS intervals of the ECG. It has been shown that hiPSC-CMs derived from two Hamamy syndrome patients carrying different mutations exhibited an altered cardiac gene expression program, including a decreased Nav1.5 expression [157]. *Irx5* interacted with Gata4, in such a way that *Irx5* potentiated Gata4-induction of *SCN5A* expression [157]. On the other hand, *Irx3* (another TF of the Iroquois-related homeobox family), Nkx2-5 and Tbx5 form a transcriptional complex involved in the development of the VCS by regulating the expression of gap junction connexin 40 (Cx40, encoded by *GJA5*) and Nav1.5. Indeed, *Irx3* silencing in mice led to a QRS prolongation and notched R' wave of the ECG [158]. In this context, two novel *IRX3* mutations associated with idiopathic ventricular fibrillation have been identified [159]. It has been also shown that *Irx3* and *Irx5* display a complex interaction since both TFs had a redundant protranscriptional function over *SCN5A* and activated the expression Nav1.5 channels, while *Irx3* activity can be repressed by *Irx5* [160]. In embryonic mouse heart Tbx2 acts as a repressor of differentiation of ventricular tissue, decreasing the expression of both *Scn5a* and *Gja1* [161]. It seems possible that these repressor effects remain in the adult right ventricular outflow tract (RVOT), being responsible for its low conduction velocity [161]. In embryonic mouse heart Tbx3 also suppressed the expression of the genes encoding connexins (Cx40 and Cx43) and channels (Nav1.5 and inwardly rectifier K^+ channels) necessary for fast conduction in ventricular myocardium [162]. The latter effect was

produced by the Tbx3-induced regulation of 2 enhancers in the *Scn5a/Scn10a* locus [106]. Tbx3, Tbx18, another member of the Tbox family of TFs, and Shox2 upregulated the expression of *HCN1*, *HCN2* [163], and *HCN4* genes encoding the pacemaker channels underlying I_f [164]. This explains why these factors promote the differentiation of cardiac cells into a pacemaker-like phenotype [164]. Mef2c also promoted the expression of HCN4 channels [165] (Table 2). It is interesting to see the redundancy of TFs that enhance the expression of the channels generating the I_f current.

A GWAS conducted in 312 individuals with BrS and 1,115 controls identified a SNP located at 6q22.31 near the *HEY2* gene [166] and the relationship of *HEY2* variants with inherited cardiac arrhythmias was later described in other cohorts [167,168]. Adult heterozygous *Hey2*-knockout mice (*Hey2*^{+/-}) exhibited an increased conduction velocity in the RVOT measured in isolated hearts. Accordingly AP maximum depolarization velocity (V_{max}) was augmented in *Hey2*^{+/-} cardiomyocytes isolated from the RVOT region, suggesting an increased I_{Na} density [166]. In the same mouse model, *Hey2* knockdown reduced I_{toF} in myocytes from the subepicardium what diminished the transmural subendocardium-to-subepicardium differences in AP characteristics [169]. Contradictory results were published regarding regulation of *SCN5A* and *Hey2*. Indeed, it has been described that V_{max} and *SCN5A*, *KCNJ2*, and *CACNA1C* expression were markedly reduced, while the appearance of delayed afterdepolarizations was increased in neonatal cardiomyocytes from *Hey2*-knockout mice [170].

In HL-1 cells it was described that FoxO1 reduced Nav1.5 expression by repressing the *Scn5a* promoter activity [171]. Moreover, FoxO1 deletion increased Nav1.5 and Navβ3 mRNA expression and I_{Na} density, which accelerated AP depolarization velocity in mouse ventricular cardiomyocytes [172]. Accordingly, ECG recordings in cardiac FoxO1 knock-out mice showed shortened QRS duration and increased P waves [172]. FoxO1 also regulates the expression of K_{ATP} channel subunits (Kir6.1 and SUR2B) and the I_{KATP} [173].

Creb activated the *KChIP2* promoter activity, while Creb knockdown suppressed I_{toF} and the phase 1-notch in monophasic APs [174]. Accordingly, inhibition of Creb effects by overexpression of the *Crem-IbΔC-X* reduced I_{toF} , prolonged APD, and led to the appearance of early afterdepolarizations in isolated ventricular myocytes [175]. In addition, mouse cardiomyocyte-specific silencing of Creb reduced I_{CaL} and increased I_{Na} densities as a consequence of a decreased expression of *Cacna1c* (Cav1.2) mRNA and upregulation of *Scn8a* (Nav1.6) mRNA, respectively. Strikingly, *Scn5a* mRNA levels remained unaltered in these myocytes [176]. Atrial myocytes isolated from the same mouse model exhibited decreased I_{toF} , I_{K1} , and I_{KACH} and prolonged APD, and ECG recordings in these transgenic mice revealed a higher risk of spontaneous AF than WT animals [177]. Activation of a CaMKII/Creb signaling upregulated *KCNN2* promoter activity and increased small-conductance, Ca^{2+} -activated K^+ (SK2) channel expression and apamin-sensitive SK current amplitude [178].

NF-κB bound to the *SCN5A* promoter region [179] and reduced Nav1.5 expression and I_{Na} density in H9c2 myocytes [180]. On the other hand, NF-κB repressed transcription of the *Cacna1c* gene in mice and decreased I_{CaL} , I_{toF} and I_{Kur} densities, and shortened APD [181–183]. Moreover, it has been described that NF-κB reduced the expression and function of hERG [184] and Kir2.1 [185] channels, whereas increased the expression of K_{ATP} channel subunits and the amplitude of I_{KATP} [186].

β-catenin interacts with TFs of the TCF family (mainly TCF-4 or more recently named as TCF7L2) to transcriptionally regulate *Scn5a* expression [187–189]. β-catenin/TCF-4 inhibited the *Scn5a* promoter activity and decreased Nav1.5 mRNA, I_{Na} density and the amplitude of cardiac APs [188], and prolonged the QRS interval [190].

HSF1 increased the expression of Kv1.5 channel proteins and I_{Kur} [191,192] and rescued the membrane expression of hERG channels carrying a double mutation (p.G572S and an in-frame insertion p.D1037_V1038insGD) associated with LQT2 [193].

Sp1 transactivated the genes encoding HCN2 and HCN4 channels that generate the pacemaker current I_f [194]. Similarly, Sp1 transactivated *KCNQ1* and *KCNH2* genes encoding Kv7.1 and hERG channels, respectively, and increased I_{Ks} and I_{Kr} due to the presence of multiple Sp1 sites in the core promoter regions of these genes [89,195–197]. MED12 is one of the 4 proteins that compose the kinase submodule that controls the mediator complex, which enables regulatory signals to be communicated from DNA-bound TFs to the RNA polymerase II. Cardiac specific deletion of MED12 altered the expression of Ca^{2+} -handling genes and disrupted cardiac electrical activity in the mouse heart [198]. These authors also described that MED12 interacted with Mef2c in cardiomyocytes and overexpression of both proteins increased expression of Ca^{2+} -handling genes in cardiomyocytes [198].

Finally, Msx1 and Msx2 proteins, together with Tbx2 and Tbx3, repress Cx43 promoter activity [71]. Binding of Msx1 to the Cx43 promoter in close proximity to a T-box binding site is responsible for this effect (Table 2) [71].

5. Possible therapeutic implications

The evidence mentioned above supports that several TFs participate in the mechanisms underlying the control of cardiac electrical activity by regulating ion channel expression and/or function (Figs. 2 and 3). Derangement of this regulatory role appears to participate in the pathophysiology of cardiac arrhythmias through acquired (AF-induced electrical remodeling) and genetically-determined (e.g. BrS, LQTS) mechanisms, suggesting that TFs can be proposed as potential drug targets. It has been surmised that TFs exhibit a huge capacity for highly

specific disease modulation, more than other signalling proteins such as GPCRs or kinases [199]. On this basis, a modulator of a dysregulated TF would have a robust efficacy with a small toxicity, since it is expected to target exclusively the transcriptional programs driven by the TF of interest, without the collateral damage caused by actions over other off-target proteins unrelated to the disease [199]. In addition, since individual TFs regulate a limited set of gene targets depending on their DNA-binding specificity, compensatory resistance mechanisms commonly observed with other pharmacological interventions are less likely [199]. Despite these advantages, TFs act via protein–protein and/or protein–DNA interactions that involve extensive surface areas difficult to disrupt using traditional approaches and, thus, TFs have for a long time been considered as ‘undruggable’ targets [200]. However, recent advances in structural biology have led to decipher unique pockets in TFs to which small molecule drugs or peptides can be directed [199,201,202]. As a general rule, these compounds may affect TF function by at least four mechanisms including the inhibition of protein–protein dimerization, protein–DNA recognition, or cofactor recruitment, as well as the enhancement of protein degradation [199,201,202]. There are several examples of TF modulating compounds that are emerging as novel therapeutic drugs currently evaluated in the treatment of several diseases including cancer [199,202]. Apart from being drug targets, another possible therapeutical perspective of TFs could be through their exogenous administration to correct dysregulated gene expression in pathophysiological conditions where a function augments or declines due to upregulation or downregulation of a target protein [203]. In this context, TF usefulness would rely on a successful delivery into the cells and on the genes whose expression can

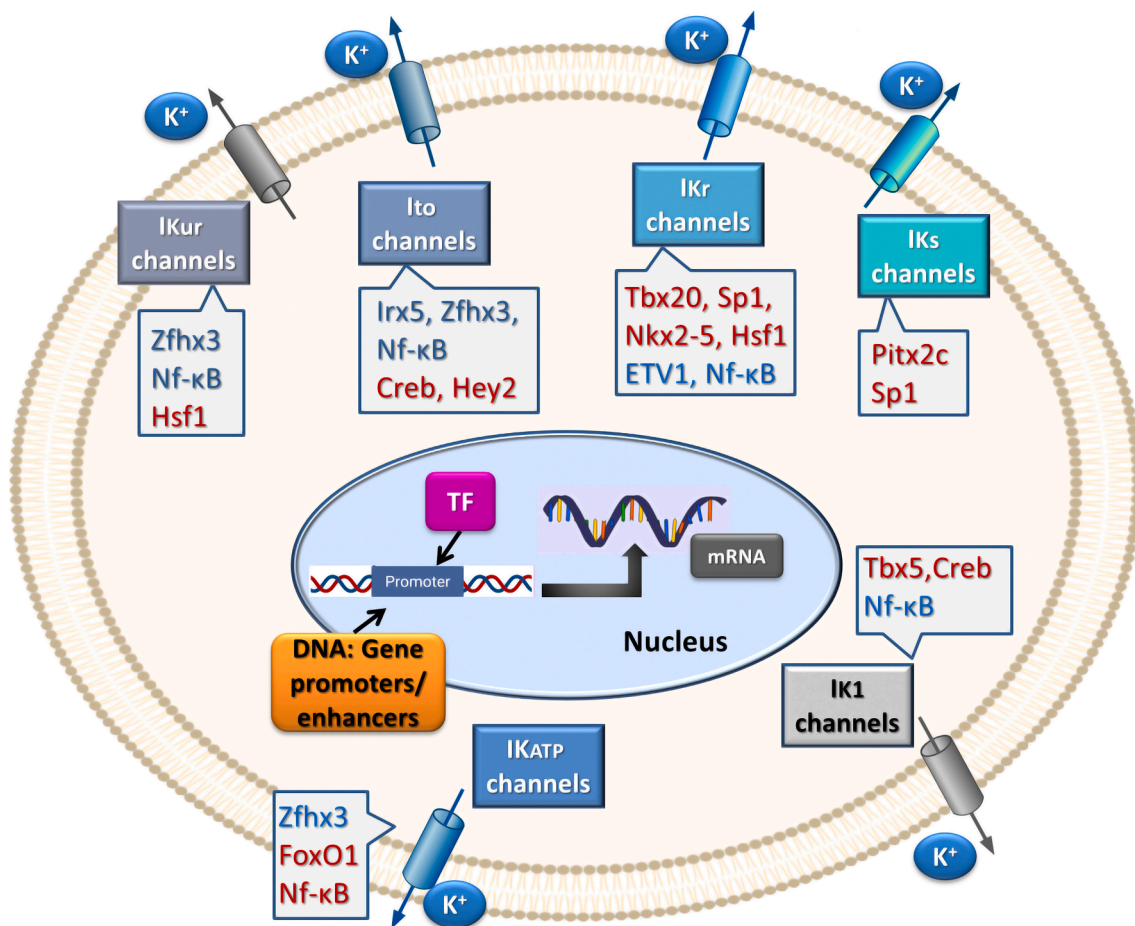


Fig. 2. Overview of the regulation of cardiac K⁺ channels by TFs. TFs interact with specific DNA sequences located in regions close to (promoters) or distal to (enhancers) genes through their DNA-binding domains. In human adult cardiomyocytes, TFs may enhance (in red) or repress (in blue) the expression of sarcolemmal K⁺ channel subunits. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

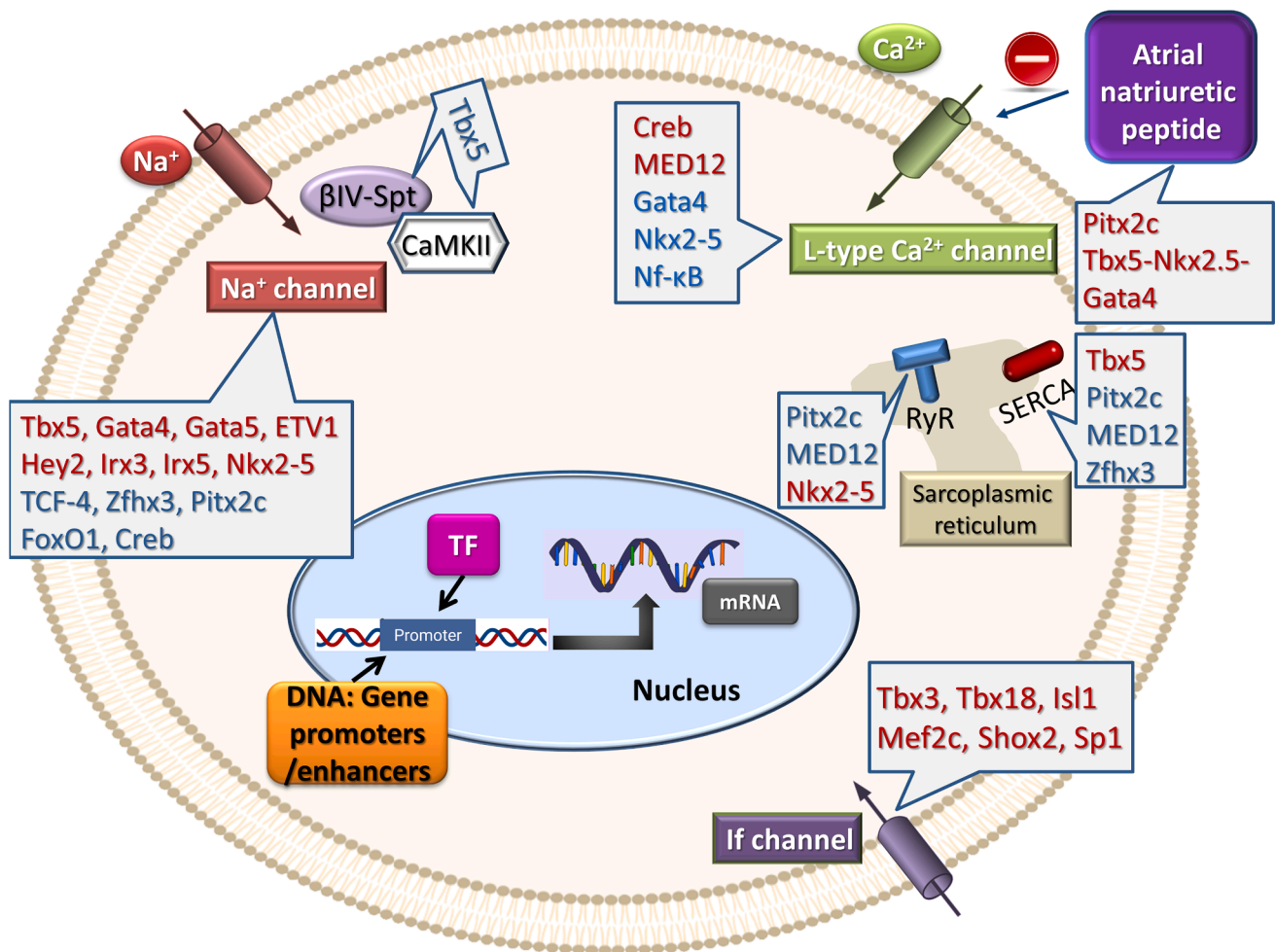


Fig. 3. Schematic representation of the regulation of cardiac Na⁺, Ca²⁺ and I_f channels and of intracellular Ca²⁺ handling by TFs. TFs interact with specific DNA sequences located in regions close to (promoters) or distal to (enhancers) genes through their DNA-binding domains. In human adult cardiomyocytes, TFs may enhance (in red) or repress (in blue) the expression of sarcolemmal ion channel subunits, ion channel associated proteins (enzymes, structural and scaffolding proteins, etc), ionic pumps, and intracellular Ca²⁺ handling proteins to modulate Na⁺, mixed Na⁺/K⁺, and Ca²⁺ currents. β IV-Spt: β IV-spectrin; CaMKII: Calcium calmodulin-dependent kinase II; RYR: Ryanodine Receptor; SERCA: sarcoplasmic reticulum Ca²⁺ ATPase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

be modulated [203]. An example of this kind of use is the application of a zinc-finger protein-TF complex to increase the expression of the vascular endothelial growth factor (VEGF) stimulating microvasculature regeneration in animal models of diabetic neuropathy [203,204]. Exogenous TFs can be also part of theranostic devices, which are multifunctional systems designed for specific and personalized disease management [203]. They are composed of a single biocompatible and biodegradable nanoparticle that can simultaneously diagnose, deliver therapy, and monitor the response to therapy [205]. A good example of a TF-based theranostic device has been developed as a therapeutic option for diseases associated with hyperuricemia (e.g. gout) [206]. Novel TF-related therapeutically relevant opportunities in the context of personalized medicine could be provided by cell therapy or genome editing [207,208]. Using combination of TFs, differentiated cells can be reprogrammed into embryonic stem cells that can be differentiated to almost any cell type, which represents a key step for cell therapy development [203]. On the other hand, the enormous advance of genome editing techniques is mainly based on the use of engineered TFs with several key advantages over natural TFs. Engineered TFs are composed of a DNA-binding domain and a regulatory domain (also named as activation domain of effector domain). The DNA-binding domain is able to target specific DNA sequences, while the effector domain can activate or repress gene expression by regulating

components of the transcriptional machinery or altering the epigenetic state [148]. Since each element or domain in a TF uniquely defines the DNA nucleotide that is recognized, by combining these elements TFs can be engineered to interact with an arbitrary DNA sequence. This principle guides genome editing tools such as transcription activator-like effectors (TALEs), zinc-finger TFs, and the most recent catalytically dead derivatives of CRISPR-Cas9 [203]. Furthermore, applying classical pharmacological concepts, the finding that some mutated forms of TFs such as Tbx20 and Tbx5 can behave as competitive antagonists [95,113] could be exploited to design engineered TFs to reduce the function of upregulated TFs.

6. Concluding statements

The number and type of ion channels on the cardiomyocyte membrane critically determine cardiac excitability and impulse propagation and, thus, alterations of ion channel expression and/or function result in electrophysiological changes that may be responsible of cardiac arrhythmias. Recent evidence has demonstrated that some specific TFs regulate ion channels in human adult atrial and ventricular cardiomyocytes and that derangement of this regulation contribute to electrical alterations associated to acquired and inherited arrhythmias. Deciphering the role of TFs in this context allows a deeper insight into

the mechanisms regulating cardiac electrical activity and opens new avenues for therapeutical interventions through the identification of TFs as potential drug targets.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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