Published in final edited form as:

Prog Biophys Mol Biol. 2016 December ; 122(3): 165–186. doi:10.1016/j.pbiomolbio.2016.09.012.

Transmural Gradients in Ion Channel and Auxiliary Subunit Expression

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Abstract

Evolution has acted to shape the action potential in different regions of the heart in order to produce a maximally stable and efficient pump. This has been achieved by creating regional differences in ion channel expression levels within the heart as well as differences between equivalent cardiac tissues in different species. These region- and species-dependent differences in channel expression are established by regulatory evolution, evolution of the regulatory mechanisms that control channel expression levels. Ion channel auxiliary subunits are obvious targets for regulatory evolution, in order to change channel expression levels and/or modify channel function. This review focuses on the transmural gradients of ion channel expression in the heart and the role that regulation of auxiliary subunit expression plays in generating and shaping these gradients.

Keywords

ion channel; ion current; auxiliary subunit; transmural gradient

The level at which different ion channels are expressed in electrically excitable cells is tightly regulated by natural selection, just as the functional properties of the channels are subject to selection (Rosati et al., 2008; Rosati and McKinnon, 2009). Even modest changes in expression levels can have large effects on cellular electrophysiological function and/or the calcium metabolism of cardiac myocytes which, in turn, affects overall organ function (Rosati et al., 2008). Large differences in ion channel expression levels are observed, both between different regions of the heart (Barth et al., 2005; Gaborit et al., 2007; Marionneau et al., 2005; Rosati et al., 2006; Rosati et al., 2003; Szentadrassy et al., 2005) as well as between equivalent cardiac tissues in different species (Rosati et al., 2008). These species-

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and region-dependent differences in channel expression are established by regulatory evolution.

Regulatory evolution is a broad category that encompasses the evolution of all the various mechanisms that can affect expression of a given protein. Regulatory evolution establishes the baseline expression levels of the different ion channels in each differentiated compartment of the heart. Evolution of a given gene's promoter and of the various cis-regulatory modules that modulate the function of that promoter is known, more specifically, as cis-regulatory evolution. Baseline expression of ion channels in heart appears to be predominantly determined at the level of transcription (Abd Allah et al., 2012; Chandler et al., 2009; Gaborit et al., 2007; Marionneau et al., 2005; Rosati and McKinnon, 2004). Consequently, it is likely that cis-regulatory evolution will be a major factor determining tissue specific channel expression levels in the heart and there is experimental evidence to support this hypothesis (Yan et al., 2012). This does not preclude the possibility that virtually any aspect of the channel biosynthesis pathway, intracellular transport and signaling pathway regulation could also evolve to modify channel expression levels.

Ion channel auxiliary subunits can be important modifiers of all these processes and are obvious targets for regulatory evolution in order to change functional channel expression levels (Yan et al., 2012). Exactly which proteins qualify as bona fide channel auxiliary subunits eludes a simple definition. There are a plethora of proteins that can interact transiently with a given channel during its biosynthesis and transport within the cell (Vandenberg et al., 2012). Many of these are general purpose proteins, chaperones, co-chaperones, cytoskeletal proteins, etc., for which there is currently no evidence that they directly contribute to differential expression of channels within the heart. For the purposes of this review we rely on a functional definition, addressing only those proteins generally understood to be channel auxiliary subunits in the literature (Table 1). In general, these proteins are bound to the pore-forming subunit of the channel when it is located in the cell membrane, although they may first combine with the channel at much earlier stages of channel synthesis/transport.

Channel expression levels will tend to fall to the lowest levels consistent with the positive selection criteria acting on a given electrophysiological system during the course of evolution (Kim et al., 2015). This is because mutations generally erode regulatory function over time and regulatory function will only be maintained if there is some opposing positive selective pressure. For example, there will be strong positive selection for efficient action potential conduction and electrical stability, resulting in the maintenance of stable sodium channel expression levels over long periods of evolutionary time in a given species. Purifying selection will eliminate mutant alleles that reduce channel expression below this baseline level. Mutations in the human sodium channel promoter that reduce transcriptional function result in an increased risk of arrhythmia (Bezzina et al., 2006; Yang et al., 2008). Individuals carrying these mutations will have an increased mortality rate and, therefore, a reduced reproductive potential. Eventually these mutations will be eliminated from the population, albeit somewhat slowly.

Evolution has acted to shape the action potential in different regions of the heart in order to maximize electrical stability as well as to shape calcium influx (contraction timing and strength) to produce a maximally stable and efficient pump. In part, this has required establishing region specific patterns of ion channel expression, such as differential expression of channels within the ventricle wall. In the hearts of large mammals, the transient outward current (I_{to}) is expressed in a large transmural gradient across the ventricle walls. The relatively high expression of this channel in the epicardium and mid-myocardium of the ventricle wall can be interpreted in a similar way to the sodium channel there is positive selection for expression of the channel at relatively high levels in these tissues. Purifying selection maintains this expression level in the face of any mutations that might reduce or eliminate expression of this channel in these species. The relative absence of expression of the I_{to} in the endocardium can be explained in one of two ways. The simplest explanation is that there is no positive selection for I_{to} expression in the endocardium and the corresponding regulatory mechanisms have either eroded over time due to mutation or were never selected for in the first place. Alternatively, expression of the I_{to} channel in the endocardium could somehow reduce fitness, resulting in selection against high I_{to} expression levels in this tissue. In this case, competing constraints may exist, so that in addition to positive selection for I_{to} expression in the endocardium there is also negative selection, which dominates the overall fitness function for this particular tissue. It is difficult to distinguish between these two possibilities. The absence or reduced expression of a given current in a particular tissue compartment does not necessarily imply anything beyond the absence of positive selective pressure, since this is the default state, due to the cumulative effect of mutations on regulatory function (Kim et al., 2015). The constraints acting on the evolution of channel expression in the heart are complex and it can be difficult to make definitive conclusions about the necessity for particular channel expression levels in a specific species, although broad patterns can be discerned (Rosati et al., 2008).

Role of Auxiliary Subunits in Controlling Functional Channel Expression Levels

Most channel forming subunits are associated with auxiliary subunits (Table 1) (Abriel et al., 2015). These subunits have a diverse set of functions including facilitating transport from the ER to the Golgi or from the Golgi to the plasma membrane, modification of channel kinetic properties, connection to cytoskeletal elements, targeting to specific membrane compartments, connection to regulatory scaffolds and mediation of cell signaling pathways (Abbott, 2014; Trimmer, 1998; Vacher and Trimmer, 2011). Unlike the channel forming subunits, which are closely related evolutionarily, auxiliary subunits encompass a very diverse set of structures (Arikkath and Campbell, 2003; Brackenbury and Isom, 2011; Pongs and Schwarz, 2010), indicating broad evolutionary origins. Auxiliary subunits can affect channel expression in a similarly diverse fashion (Table 1).

Like other integral membrane proteins, the pore-forming subunit of voltage-gated ion channels is synthesized by ribosomes that become tethered to a translocator complex (the Sec61 translocon) located in the endoplasmic reticulum (ER) membrane (Cymer et al., 2015; Shao and Hegde, 2011). Typically the first transmembrane (TM) domain of the nascent

peptide functions as a targeting sequence (Fig. 1). This transmembrane sequence is recognized by the signal recognition particle (SRP), which in turn binds to the SRP receptor in the ER membrane. Following successful targeting to the ER membrane, the complex of ribosome and nascent peptide is transferred to the translocon. For most members of the voltage-gated channel superfamily the amino terminus of the protein is located intracellularly and the first membrane spanning domain functions as a start-transfer signal, causing the translocon to mediate the translocation of the peptide trailing this transmembrane sequence through the translocon pore. This displaces the intraluminal plug that normally gates the translocon channel. Transfer of the peptide continues until the translocon encounters a stop-transfer signal (typically the second membrane spanning domain) causing the translocon to stop transferring the peptide across the membrane, thereby allowing the peptide to accumulate on the cytoplasmic side. In general, alternating start and stop-transfer signals in the channel's peptide sequence will combine to allow the channel to assemble with the correct membrane topology. This topology signaling can include cues from regions of the polypeptide outside the transmembrane domain of the protein and more complex schemes may be required to ensure that proteins with non-canonical transmembrane domains, such as voltage sensors and channel pores, can achieve the correct topology (Cymer et al., 2015; Shao and Hegde, 2011).

In addition to having a trans-membrane pore for movement of the peptide across the membrane, the translocator complex is hinged and can open to allow the hydrophobic membrane spanning domains of the ion channel-forming protein to partition into the hydrophobic core of the membrane (Fig. 1). By this means, the channel-forming protein is integrated into the lipid bilayer of the membrane (Cymer et al., 2015; Shao and Hegde, 2011).

The transfer of the nascent channel peptide into the ER lumen and lipid bilayer occurs at the same time as the protein is being synthesized (cotranslational translocation). The amino terminal regions of the channel, therefore, become available for binding by cytosolic or membrane spanning accessory proteins even before synthesis of the main subunit is complete (Fig. 1). In principle, from this point forward ion channel auxiliary subunits could bind to the channel and modify channel expression by regulation any of the upstream steps in the synthesis/assembly/transport/degradation pathways (Fig. 2). In general, the pore-forming subunit will first undergo further maturation, aided by proteins associated with the translocon complex such as oligosaccharyl transferase as well as by ER resident membrane chaperones (such as calnexin) (Cymer et al., 2015; Green, 1999). Glycosylation of the channel peptide by oligosaccharyl transferase also occurs cotranslationally and can contribute to establishing the correct topology (Shao and Hegde, 2011).

For large integral membrane proteins, particularly proteins that have one or more non-canonical transmembrane sequences, such as the pore-forming helices of most ion channels (Cymer et al., 2015) or the S4 domain of voltage-gated ion channels (Freites et al., 2005), achieving the correct topology can be a very complex process (Shao and Hegde, 2011). This can require the pre-folding of membrane domains in the ribosome tunnel (Tu et al., 2014) or the formation of transmembrane helix pairs in the translocon pore (Zhang et al., 2007) in

order to shield charged and polar residues in the transmembrane domains before partitioning into the lipid bilayer through the lateral gate of the translocon.

In general, channel subunits must achieve the correct topology before they can assemble with other channel subunits and exit the ER. This is not a given. For large polytypic ion channels, the folding process can be slow (up to 2–3 hours) and inefficient, as few as 30% of newly synthesized α -subunits of the acetylcholine receptor are correctly assembled (Merlie and Lindstrom, 1983). Typically, the incorrectly folded membrane proteins are targeted for degradation by the ER quality-control machinery and the misfolded protein will retrotranslocated back into the cytosol to be degraded by proteasomes via the ER-associated degradation (ERAD) pathway (Feige and Hendershot, 2013) (Fig. 2). Some potassium channels can begin to dimerize cotranslationally, before the subunits are released from the ribosome (Liu et al., 2016; Lu et al., 2001; Phartiyal et al., 2007; Robinson and Deutsch, 2005; Tu et al., 2000). The amino terminal domains of these channels, such as the T1 tetramerization domain of Kv channels, make the assembly process considerably faster and presumably also more efficient (Lu et al., 2001).

Auxiliary subunits could potentially contribute to electrical heterogeneity within the ventricle via a wide variety of mechanisms (Abbott, 2014; Jackson and Nicoll, 2011; Trimmer, 1998; Vacher and Trimmer, 2011). These include:

1. Stabilize channel monomers, dimers or tetramers in the ER during channel assembly.
2. Facilitate assembly of subunits in the ER.
3. Facilitate transport of the assembled channel protein from the ER to the Golgi apparatus.
4. Facilitate transport of the assembled channel protein from the Golgi to the plasma membrane.
5. Modify the kinetics of the channel in the plasma membrane to produce more or fewer channels that respond to a given voltage.
6. Modify or mediate the responsiveness of the channel to signaling pathways.
7. Modify the rate of reuptake of channels from the cell membrane for degradation and/or recycling back to the cell membrane.

Auxiliary subunits could also modify the subunit composition of channels that assemble as tetramers or dimers by influencing the relative proportion of homomultimers versus heteromultimers that are assembled. Multiple channel subunits can be translated from the same mRNA molecule in the polyribosome complex resulting in a high local concentration of identical subunits. This will tend to favor the assembly of homo-dimers, especially for those channels that can begin dimerization before the subunits are released from the ribosome (Liu et al., 2016; Lu et al., 2001; Phartiyal et al., 2007; Robinson and Deutsch, 2005; Tu et al., 2000). Block of homo-dimerization by the binding of auxiliary subunits could facilitate the formation of heteromultimers.

Molecular Basis of Electrical Heterogeneity in the Ventricle

Electrical heterogeneity in the ventricles was first described using myocytes isolated from different regions of the rat ventricles (Watanabe et al., 1983). The authors proposed that the diversity in action potential duration and morphology that they observed in dissociated cells was due to regional differences in the magnitude of a transient outward current in the ventricle wall. Antzelevitch and colleagues (Litovsky and Antzelevitch, 1988) then showed, using voltage clamp, that the density of the transient outward potassium current (I_{to}) was larger in epicardial myocytes than in endocardial myocytes isolated from the canine left ventricular wall. They also showed that biophysical properties of the transient current differed between the two cell types, with the epicardial current recovering from inactivation much faster than the endocardial one (Litovsky and Antzelevitch, 1988). A similar gradient has been documented for this current in virtually every mammalian species expressing I_{to} , including human (Nabauer et al., 1996; Wettwer et al., 1994), canine (Litovsky and Antzelevitch, 1988; Rosati et al., 2003; Rosati et al., 2001), rabbit (Fedida and Giles, 1991), cat (Furukawa et al., 1990), rat (Ashamalla et al., 2001; Clark et al., 1993), mouse (Brunet et al., 2004) and ferret (Brahmajothi et al., 1999).

Subsequently it was shown that many other cardiac ionic currents also display transmural expression gradients in at least some species (summarized in Table 2, left ventricle only), thereby contributing to electrical heterogeneity within the left ventricle. Although transmural gradients can, in principle, be generated by many different mechanisms, including transcriptional, post-transcriptional or post-translational regulation as well as modulation by signaling pathways, transcriptional regulation appears to play the major role in determining regional differences in ion current expression patterns. Several large-scale ion channel gene expression surveys in the heart have shown a generally good correlation between gene expression levels and current expression for most of the channels and transporters analyzed (Abd Allah et al., 2012; Chandler et al., 2009; Gaborit et al., 2007; Soltysinska et al., 2009; Szentadrassy et al., 2005).

Cardiac electrophysiological heterogeneity appears to be established, at least in part, by the generation of distinct cardiac cell lineages during development (Boukens et al., 2009). Progenitor cells from different lineages give rise to the atria and ventricles, to the left and the right chambers and to the conduction system, providing a basis for the differences in gene expression patterns between myocytes in these regions (Mikawa and Fischman, 1996). Furthermore, ontogenesis of specific heart regions suggests mechanisms that may contribute to the heterogeneity of the myocytes found within some of these regions. For example, during the formation of the ventricular walls, midmyocardial tissue originates both from the endocardium (trabeculae) and from the epicardium (compact myocardium) (Evans et al., 2010; Moorman and Christoffels, 2003). This could contribute to cellular heterogeneity within this region (Boukens et al., 2009).

Although the early ontogenesis of the ventricular wall appears to provide the initial basis for the establishment of transmural gradients in channel and transporter gene expression in heart, the gradients observed in the adult are generally established during postnatal development (Abd Allah et al., 2012). Multiple transcription factors are differentially

expressed across the ventricular wall in adult heart, in keeping with the hypothesis that different, developmentally determined, gene regulatory programs are in place in these myocytes (Costantini et al., 2005; Rosati et al., 2006).

Elucidation of the molecular mechanisms underlying transmural gradients of ion channels requires an understanding of the molecular basis for each current. The scarcity of human cardiac tissue for research use has compelled researchers to use multiple different mammalian species as sources of cardiac tissue. This has, in turn, introduced considerable complexity into the field, since there is a high degree of diversity in cardiac ionic current expression between different species (Nerbonne and Kass, 2005; Rosati et al., 2008). This diversity is driven, in large part, by physiological constraints. In particular, there is an inverse dependency of both resting heart rate and inverse action potential duration on body weight (Fig. 4), which is required to maintain mean arterial blood pressure relatively constant across all mammals (Rosati et al., 2008; Westerhof and Elzinga, 1991). There are also more idiosyncratic changes, which may reflect evolutionary path dependencies, where a species sometimes loses expression of a given current that is broadly expressed in other species or, conversely, gains expression of a channel gene that is not generally expressed in other species.

While the molecular composition of the main inward currents, I_{Na} and $I_{Ca,L}$, remains relatively homogeneous across all mammals, the expression levels and molecular identity of potassium currents varies widely between species (Nerbonne and Kass, 2005; Rosati et al., 2008). This is to be expected, since the primary function of the two inward currents, action potential conduction and excitation-contraction coupling, respectively, remains relatively constant across species whereas large changes in the patterns of potassium channel gene expression between species are necessary to appropriately scale action potential duration with body weight (Fig. 4B) (Rosati et al., 2008).

Molecular Basis of I_{to} and its transmural gradient of expression

I_{to} Molecular Basis

Two components of I_{to} are generally recognized, $I_{to, fast}$ ($I_{to,f}$) and $I_{to, slow}$ ($I_{to,s}$). These native currents differ in their rates of inactivation and recovery from inactivation. There are marked species and regional differences in the expression of these two currents (reviewed in (Nerbonne and Kass, 2005)). Products from two different potassium channel gene subfamilies, the Kv4 subfamily (Kv4.2 (KCND2) and Kv4.3 (KCND3)) and the Kv1 subfamily (Kv1.4 (KCNA4)), are thought to underlie these currents. The general consensus is that the Kv4 channels underlie $I_{to,f}$, while Kv1.4 is the molecular correlate of $I_{to,s}$. In the mouse, where these two current components were first defined, this classification is well established through the use of reverse genetics and a thorough pharmacologic and biophysical characterization of the two currents (Barry et al., 1998; London et al., 1998; Xu et al., 1999). In other mammalian species, the $I_{to,s}$ label has been used more loosely to indicate a current component that has slower kinetics than $I_{to,f}$. The $I_{to,s}$ phenotype in these species is often different from that of the mouse $I_{to,s}$ and there have been inconsistencies between laboratories on the criteria used to define $I_{to,s}$ (Nerbonne and Kass, 2005; Patel and Campbell, 2005). At least in some species, including rabbit and canine, Kv4 channels may

contribute to 'I_{to,s}' and the slow inactivation and recovery from inactivation kinetics of the current in these species cannot be explained solely by a switch in the pore-forming subunits (Rosati et al., 2001; Wang et al., 1999). At present, the I_{to,s} channel, as a specific molecular identity, remains poorly defined in some species.

There is a considerable degree of diversity in I_{to} function and related gene expression in the species examined to date. In the mouse, I_{to,f} is encoded by Kv4.2/4.3 heteromultimers (Guo et al., 2002a), while Kv1.4 underlies I_{to,s}, which is expressed only in a subset of myocytes in the interventricular septum (Brunet et al., 2004; Guo et al., 1999). In the rat heart, I_{to,f} is predominant, while I_{to,s} expression is low and found primarily in endocardial myocytes (Huang et al., 2001; Wickenden et al., 1999). Expression of the pore-forming Kv4.2 and Kv4.3 subunit mRNAs and protein is relatively high, while the Kv1.4 gene is expressed at low levels in the adult rat heart (Barry et al., 1995; Dixon and McKinnon, 1994; Dixon et al., 1996; Xu et al., 1999). In this species, regional expression of Kv1.4 mRNA and protein correlates well with I_{to,s} expression in some regions (Wickenden et al., 1999) but not transmurally (Huang et al., 2001).

In the canine and human heart, the Kv4.2 gene is not expressed at significant levels and I_{to,f} is encoded by the Kv4.3 gene alone (Dixon et al., 1996). Recently, detection of a KCND2 (Kv4.2) gene mutation in a patient with J-wave syndrome led to the suggestion that the Kv4.2 subunit might also contribute to the generation of I_{to,f} in human heart (Perrin et al., 2014). The significance of this finding remains to be clarified since multiple investigators have shown that this gene is not expressed at significant levels in the human heart (Bertaso et al., 2002; Dixon et al., 1996; Gaborit et al., 2007; Isbrandt et al., 2000; Rosati et al., 2008; Zhu et al., 1999). The presence of I_{to,s} has also been described in endocardial myocytes from both canine and human ventricles, with recovery from inactivation being much slower for the transient outward current in endocardial myocytes than in epicardial cells (see for example (Nabauer et al., 1996; Yu et al., 2000), reviewed in (Nerbonne and Kass, 2005)). Endocardial I_{to,s} in the canine ventricle displays the same pharmacological properties as the epicardial current, namely sensitivity to block by flecainide, suggesting that the Kv4.3 subunit, and not Kv1.4, underlies the current in this species (Rosati et al., 2001). There is no corresponding pharmacological data on human endocardial I_{to,s}. Currently, there is a consensus that the Kv1.4 channel mediates I_{to,s} in human ventricle because of its biophysical properties (very slow recovery from inactivation), decreased functional Kv4.3 channel expression due to relative absence of KCHIP2 auxiliary subunit expression (see below), and the presence of Kv1.4 mRNA expression in this region (Nerbonne and Kass, 2005; Patel and Campbell, 2005).

The ferret ventricle expresses both Kv4.2 and Kv4.3 subunits, which are thought to underlie I_{to,f} in this species, while Kv1.4 is proposed to underlie I_{to,s} (Patel and Campbell, 2005). Presumably the Kv4.2/4.3 subunits form heteromultimers in the ferret heart, as seen in mouse, although this has not been directly demonstrated. I_{to,s} is primarily expressed in endocardial myocytes and expression of Kv1.4 protein, but not the mRNA, correlates with I_{to,s} expression in this species (Brahmajothi et al., 1999).

The molecular basis of I_{to} in the rabbit heart remains unclear. Several laboratories have described a transient outward current that has the biophysical properties of $I_{to,s}$ but did not find a corresponding $I_{to,f}$ current (Fedida and Giles, 1991; Wagner et al., 2009; Wang et al., 1999; Zhao et al., 2012). The Kv1.4 gene is expressed in the rabbit ventricles and atria, but so are the $I_{to,f}$ subunit genes, Kv4.2, Kv4.3 as well as the KChIP2 auxiliary subunit. In adult ventricle, these genes are expressed at comparable levels to Kv1.4 (Abd Allah et al., 2012; Rose et al., 2005); Rosati and McKinnon unpublished results). Furthermore, despite pharmacological and kinetic properties that resemble the Kv1.4 channel, the transient outward current in the rabbit atrium was inhibited by antisense oligonucleotides directed against Kv4.2 and Kv4.3, with the Kv4.3 antisense oligos having a larger inhibitory effect than Kv1.4 antisense oligos (Wang et al., 1999). Expression of Kv1.4 mRNA in the rabbit ventricle during development does not parallel the marked postnatal increase in I_{to} , whereas expression of all three $I_{to,f}$ subunit genes does match this increase (Abd Allah et al., 2012; Sanchez-Chapula et al., 1994). Similarly, the downregulation of I_{to} observed in rabbit ventricular myocytes in heart failure is paralleled by a decrease in expression of the Kv4.2 and KChIP2 proteins, while Kv1.4 protein levels remain unchanged (Rose et al., 2005). It is possible that Kv4/KChIP2 complexes, in addition to the Kv1.4, channel make a contribution to I_{to} in the ventricles of this species.

Some species do not express a significant I_{to} current. The absence of I_{to} in the guinea pig heart has a straightforward explanation, I_{to} gene subunits are expressed at low levels in the hearts of this species compared to other mammals. In particular, Kv4.2 and KChIP2 mRNA are expressed at very low levels in guinea pig ventricles (Rosati et al., 2008; Yan et al., 2012). Oddly, in pig ventricular myocytes, which also have no discernible I_{to} , Kv4.2 and KChIP2 mRNA and Kv4.2 proteins, were found to be expressed at levels comparable with those of rat heart (Schultz et al., 2007). It is unclear why these channels, which were shown to localize in the cell membrane of pig ventricular myocytes, remain silent in this species.

I_{to} Auxiliary Subunits

When compared with the native $I_{to,f}$ (and neuronal I_A), Kv4 channels expressed in heterologous systems have a slower inactivation and recovery from inactivation rates (Fiset et al., 1997; Serodio et al., 1994; Serodio et al., 1996). This prompted a search for modifying factors or auxiliary subunits for these channels and several cytoplasmic and membrane spanning subunits have been identified, shown to bind to Kv4 channels and to contribute to the formation of native channels, both in cardiac myocytes and in neurons (Jerng and Pfaffinger, 2014; Niwa and Nerbonne, 2010) (see Table 1).

The first Kv4 accessory subunits were identified by Ken Rhodes and Jim Trimmer's groups using a yeast two-hybrid screen (An et al., 2000). The KChIP (K^+ Channel Interacting Proteins) genes encode relatively small proteins that belong to the neuronal Ca^{2+} sensor superfamily. These proteins bind to Kv4 subfamily channels but not to other voltage gated potassium channels. They dramatically increase current density and also increase the rate of recovery from inactivation for Kv4 channels, making the heterologously expressed Kv4 currents more closely resemble the native I_{to} and I_A currents found in myocytes and neurons. A total of four KChIP genes have been identified (An et al., 2000; Morohashi et al., 2002;

Rosati et al., 2001), of which only KChIP2 is expressed in heart (Rosati et al., 2001). KChIP2 binds to the cytosolic N-terminal domain of the Kv4 channels and promotes trafficking of the channels to the plasma membrane in a dose-dependent manner (Shibata et al., 2003). This interaction is thought to occur in the endoplasmic reticulum (Shibata et al., 2003). Interaction between KChIP2 and Kv4.2 seems to be necessary for stabilization of both proteins, since KChIP2 and Kv4.2 protein expression is dramatically decreased in Kv4.2 and KChIP2 null mice, respectively (Foeger et al., 2010; Guo et al., 2005).

Adenoviral-mediated overexpression of KChIP2 successfully induced the appearance of an $I_{to,f}$ current in E12 embryonic rat myocytes, in which Kv4.2 protein is already expressed (Kobayashi et al., 2003). Conversely, adenoviral-mediated overexpression of KChIP2 was unable to restore $I_{to,f}$ density in cultured adult mouse KChIP2(−/−) myocytes (Foeger et al., 2013) or to increase it in cultured adult canine endocardial myocytes (Rosati and McKinnon, unpublished). The apparent failure of KChIP2 to rescue expression of $I_{to,f}$ in adult cultured myocytes may reflect a technical issue, since transcription of multiple ion channel subunit genes, including Kv4.2, can be markedly down-regulated in these cells when placed in culture (Hershman and Levitan, 1998), Rosati and McKinnon, unpublished).

The dipeptidyl-aminopeptidase-like protein 6 (DPP6), originally identified in the brain, modulates the kinetics of A-type neuronal currents by accelerating inactivation and recovery from inactivation (Nadal et al., 2003). This subunit is expressed in human heart (Alders et al., 2009; Radicke et al., 2005) and has been implicated in familial idiopathic ventricular fibrillation (Alders et al., 2009). When expressed in heterologous systems, it modifies the biophysical properties of the Kv4.3/KChIP2 complex to more closely resemble those of the native $I_{to,f}$. Effects of DPP6 on Kv4.3 channel function include increasing current density by an increase in unitary conductance, a negative shift and acceleration of inactivation, and slower recovery from inactivation (Radicke et al., 2005). The contribution of this subunit to the native $I_{to,f}$ appears to be region- and species-specific. For example, DPP6 is not expressed in the rat heart (Radicke et al., 2005) and does not seem to contribute to native $I_{to,f}$ in the canine ventricle (Xiao et al., 2013). In contrast, DPP6 is expressed at high levels in canine Purkinje fibers and co-expression of DPP6 with Kv4.3 and NCS1 (neuronal calcium sensor 1; (Guo et al., 2002b)) in CHO cells recapitulates the pharmacological and kinetic properties of $I_{to,f}$ in these cells (Xiao et al., 2013). Recently, a related protein, DPP10, has been proposed to underlie a sustained component of I_{Kur} in human atrial myocytes by interacting with Kv4.3 (Turnow et al., 2015).

KCNE1 (MinK) and related peptides (KCNE2–5) are small auxiliary subunits with a single membrane-spanning domain. KCNE1 coassembles with the KCNQ1 channel to form native cardiac slow delayed rectifier (I_{Ks}) channels (Barhanin et al., 1996; Sanguinetti et al., 1996). KCNE2 (MiRP1) was identified as a potassium channel subunit gene associated with cardiac arrhythmia and proposed to coassemble with the alpha subunit KCNH2 (HERG) to form the native I_{Kr} channels, although this is still debated (Abbott et al., 1999; Anantharam and Abbott, 2005). Subsequently, other members of the KCNE gene family were shown to modulate the currents encoded by several ion channel genes (reviewed in McCrossan and Abbott, 2004). KCNE genes are expressed in the human heart and can modulate the biophysical properties of Kv4.3/KChIP2 channels in vitro (Radicke et al., 2006). In vivo,

genetic ablation of the KCNE2 gene in the mouse causes APD prolongation, reducing $I_{to,f}$ and $I_{K,slow}$ densities. In keeping with these effects, KCNE2 can be immunoprecipitated with both Kv4.2 and Kv1.5 subunits, suggesting a direct effect of this protein on the currents (Roepke et al., 2008). KCNE4 (MiRP3) subunits co-localize with the Kv4.2 subunit in the T-tubule system of rat ventricular myocytes and alter several biophysical properties of the Kv4.2/KChIP2 complex in vitro (Levy et al., 2010).

Conventional knockout of the Kv β 1 (KCNA1) gene in mouse results in decreased $I_{to,f}$ expression, possibly due to down-regulation of Kv4.3 protein expression (Aimond et al., 2005), as well as changes in the EKG and action potential duration that are consistent with an effect of Kv β 1 on $I_{to,f}$ expression (Tur et al., 2016). Other accessory subunits have been shown to alter $I_{to,f}$ current density and biophysical properties in vitro but specific functional roles for these subunits in vivo remains to be demonstrated (Deschenes and Tomaselli, 2002; Kuryshv et al., 2000).

I_{to} Transmural Gradient

The simplest mechanism that can account for the heterogeneity in I_{to} expression across the ventricle wall is differential transcription of the relevant pore-forming subunit gene(s). This appears to be the major mechanism by which the transmural expression gradient for $I_{to,f}$ is established in mouse and rat heart (Dixon and McKinnon, 1994; Dixon et al., 1996; Guo et al., 2002a). In these species, Kv4.2 mRNA and protein expression is significantly higher in the epicardium than in the endocardium (Table 2).

Alternatively, transcriptional regulation of an auxiliary subunit gene could create a gradient in current expression levels and/or channel properties. KChIP2 mRNA and protein are expressed in a large gradient across the ventricle wall of canine heart (Rosati et al., 2003; Rosati et al., 2001) and there is a strong correlation between KChIP2 mRNA, protein and $I_{to,f}$ current density across nine regions of the heart in this species (Fig. 5), suggesting that transcriptional regulation of the KChIP2 gene is the primary determinant of regional differences in $I_{to,f}$ expression in this species (Rosati et al., 2003). As with many other cardiac electrophysiological properties, there is considerable species-dependent variation, but one or the other, or a combination of both of these two mechanisms appears to account for the transmural gradient in $I_{to,f}$ expression in all species that express this current.

Kv4.2 gene expression parallels $I_{to,f}$ expression across the ventricle wall in mouse and rat ventricle, suggesting that this gradient in gene expression makes the largest quantitative contribution to the I_{to} gradient in these species (Costantini et al., 2005; Dixon et al., 1996; Goltz et al., 2007; Huang et al., 2001; Liu et al., 2015; Rosati et al., 2003; Rosati et al., 2001; Teutsch et al., 2007). In contrast, in most species (human, canine, rabbit, rat and mouse) it has been found that the Kv4.3 gene is expressed uniformly across the ventricle wall (Abd Allah et al., 2012; Ambrosi et al., 2013; Calloe et al., 2010; Dixon et al., 1996; Gaborit et al., 2007; Goltz et al., 2007; Huang et al., 2001; Liu et al., 2015; Radicke et al., 2006; Rosati et al., 2003; Rosati et al., 2001; Soltysinska et al., 2009). One exception is a report that Kv4.3 mRNA and protein are expressed in an epicardial to endocardial gradient in ferret (Brahmajothi et al., 1999).

KChIP2 mRNA is expressed in a steep transmural gradient across the left ventricle in human, canine, ferret and rabbit, in parallel with I_{to} expression in these species (Abd Allah et al., 2012; Ambrosi et al., 2013; Calloe et al., 2010; Gaborit et al., 2007; Patel et al., 2002; Radicke et al., 2006; Rosati et al., 2003; Rosati et al., 2001; Soltysinska et al., 2009; Zicha et al., 2004). There is correspondingly steep gradient in KChIP2 protein across the human and canine ventricles (Patel and Campbell, 2005; Rosati et al., 2003; Zicha et al., 2004). It is generally believed that this gradient in KChIP2 expression makes the primary contribution to the transmural expression gradient of I_{to} in these species.

In mouse left ventricular wall, either no transmural gradient in KChIP2 expression (Costantini et al., 2005; Rosati et al., 2003) or a small transmural gradient (1.2 or 1.6-fold higher in epicardium than in endocardium) (Liu et al., 2015; Teutsch et al., 2007) has been reported. In rat, two laboratories found a small gradient (1.3 or 1.5-fold) (Goltz et al., 2007; Stones et al., 2009) and one found no gradient (Rosati et al., 2001). The differential expression of KChIP2 may contribute to the gradient in I_{to} expression in these species although the larger gradient in Kv4.2 expression is likely to be the major determinant. In ferret (Brahmajothi et al., 1999; Patel et al., 2002) it has been reported that there are gradients in both KChIP2 and Kv4.2 gene expression, which presumably both contribute to the transmural gradient in $I_{to,f}$ expression in this species.

Similar to other mammalian species, I_{to} is expressed in a transmural gradient across the rabbit ventricle, with epicardium having a larger I_{to} than endocardium (Fedida and Giles, 1991; Sanchez-Chapula et al., 1994). KChIP2 mRNA is expressed in a transmural gradient in the ventricle wall (Abd Allah et al., 2012) and the pattern of KChIP2 expression in both ventricles is similar to that seen in canine heart (Rosati and McKinnon, unpublished data). Kv1.4, Kv4.2 and Kv4.3 mRNAs are expressed uniformly across the ventricle wall in the adult rabbit (Abd Allah et al., 2012).

As described above, it is likely that different cell lineages, established during development, are a primary mechanism by which the gradients in ion channel expression are established and maintained in the adult heart. In support of this hypothesis, it has been shown that there are transmural gradients in transcription factor expression in the ventricle wall of rat and canine heart (Rosati et al., 2006). Conventional knockout of the transcription factor *Irx5*, which is differentially expressed across the ventricle wall, results in loss of the I_{to} gradient, due to increased Kv4.2 expression in the endocardium of mouse heart (Costantini et al., 2005). *Irx5* is normally expressed at high levels in the endocardium. The authors of this study proposed that *Irx5* acts as a repressor of Kv4.2 gene promoter function. Curiously, knockout of both *Irx5* and *Irx3*, which is expressed in a similar gradient to *Irx5*, does not result in elimination of the transmural gradient in Kv4.2 expression (Gaborit et al., 2012). The transmural Kv4.2 gradient is also abolished in NFATc3 null mice, while the transmural gradient in *Irx5* expression is preserved in these same animals (Rossow et al., 2006). Taken together, these results suggest that the *Irx5* gradient is not sufficient to establish and/or maintain the Kv4.2 transmural gradient in mouse ventricle. Lower activity of the protein phosphatase calcineurin and the NFATc3 transcription factor in epicardium versus endocardium has also been proposed as the mechanism by which the transmural gradient in Kv4.2 expression is generated in the mouse heart (Rossow et al., 2006). Expression of the

Kv4.2 gene is down-regulated upon nuclear translocation of the NFATc3 protein (Rossow et al., 2006), however direct binding of NFATc3 to the Kv4.2 gene has not been demonstrated.

Gaborit and colleagues (2007, 2010) have reported that Kv1.4 mRNA is more abundantly expressed in human endocardium than the epicardium and that Kv1.4 protein levels match those of the cognate mRNA. Szabo et al. (2005) did not find differential expression of the Kv1.4 protein in epicardium compared to mid-myocardium and Ambrosi et al. (2013) did not see any transmural difference for Kv1.4 mRNA expression in humans.

Regional variation in the auxiliary subunit DPP6 has also been described. DPP6 mRNA expression is higher in the midmyocardium compared to epicardium and endocardium in the canine left ventricle (Calloe et al., 2010), suggesting that it plays little or no role in establishing the I_{to} gradient in this species.

As shown in Table 2, there are discrepancies in the literature regarding transmural expression of some ion channel subunits. At least in part, this reflects technical difficulties associated with these experiments. When the signal is large, such as the gradient in KChIP2 mRNA expression across the human and canine ventricles, consistent results are achieved by multiple different laboratories, using a variety of techniques (Ambrosi et al., 2013; Gaborit et al., 2007; Gaborit et al., 2010; Rosati et al., 2001; Soltysinska et al., 2009; Szabo et al., 2005; Zicha et al., 2004). If the transmural gradient for a given ion channel gene or current is small, however, it can be more difficult to obtain consistent measurements. Differences in how the ventricle wall was dissected, whether it was split into equal thirds or whether relatively fine slices were taken from the endocardial and epicardial surfaces can also produce discrepancies in the measurement of transmural gradients. In principle, narrow surface slices should produce larger regional expression differences. In addition, it can be difficult to obtain a reasonable number of human tissue samples of similar quality, making it harder to achieve adequate statistical power.

Transmural heterogeneity in $I_{Ca,L}$ expression

The L-type calcium current is activated during the cardiac action potential and mediates a calcium influx that triggers calcium release from the sarcoplasmic reticulum triggering sarcomere contraction (excitation-contraction coupling) (Bers, 2002). Electrically, activation of this current contributes to the plateau phase of the ventricular action potential. Beta-adrenergic stimulation of the L-type calcium channels causes a current increase that underlies the inotropic effect of norepinephrine on the heart (reviewed in (Weiss et al., 2013)). The L-type calcium channels are macromolecular complexes comprising a principal, pore forming subunit (α) and three types of auxiliary subunits, termed β , $\alpha_2\delta$ and γ (Catterall, 2000). Each of these auxiliary subunits can be encoded by several related genes (CACNB1-4, CACNA2D1-4, CACNG1-8, respectively), of which only a subset are expressed in heart. They modify the biophysical properties of the α subunit, affect channel trafficking and determine cellular localization (Table 1) (Catterall, 2000; Simms and Zamponi, 2012). The molecular basis of $I_{Ca,L}$ is relatively homogeneous in the ventricles, with the bulk of it mediated by the Cav1.2 α subunit encoded by the CACN1C gene with low or undetectable expression of other alpha subunits (Rosati et al., 2007). Cav1.3

expression is relatively higher in Purkinje fibers, the atrioventricular and sinoatrial node and functional contribution of this subunit to the native $I_{Ca,L}$ has been described for both nodes (Gaborit et al., 2007; Platzer et al., 2000; Rosati et al., 2007; Zhang et al., 2011; Zhang et al., 2002). All four Cav β subunits are expressed in heart, although mouse knockout and quantitative expression data suggest that the $\beta 1$, $\beta 2$ and $\beta 3$ subunits are the most important (Haase et al., 1996; Hullin et al., 2003; Weissgerber et al., 2006). Genetic ablation of the $\alpha 2\delta$ -1 gene (CACNA2D1) in the mouse causes defects in myocardial contractility because of a reduced $I_{Ca,L}$ density (Fuller-Bicer et al., 2009). The $\alpha 2\delta$ -3 and $\alpha 2\delta$ -4 subunits are also expressed in the human heart but a functional contribution of $\alpha 2\delta$ -3 and $\alpha 2\delta$ -4 to the cardiac $I_{Ca,L}$ has not been demonstrated (Gong et al., 2001; Qin et al., 2002). Several γ subunits are expressed in heart and have been shown to interact with the Cav1.2 channel in vitro (Yang et al., 2011) but a direct demonstration of their contribution to the native current has not yet been described.

$I_{Ca,L}$ Transmural Gradient

A majority of reports, in several different species, suggest that the L-type Calcium current is not expressed in a transmural gradient across the ventricle wall (Table 2). In mouse, expression of $I_{Ca,L}$ in epicardial and endocardial myocytes was found to be homogeneous in the Balb/c and Swiss strains (Dilly et al., 2006; Shi et al., 2007). In contrast, two reports have described a small transmural gradient in C57/B16 mice, with the current being 1.2–1.4 fold higher in epicardial than endocardial myocytes (Wang et al., 2008; Xu et al., 2011). Regional- and gender-specific differences in ion current and channel subunit expression have been shown to be strain dependent in mouse (Brouillette et al., 2005; Brunet et al., 2004), which may account for this discrepancy.

In rat, several laboratories have reported the absence of a transmural gradient in $I_{Ca,L}$ expression (Bryant et al., 1999; Clark et al., 1993; Volk et al., 1999) although one paper described a small increase in $I_{Ca,L}$ density in epicardial compared to endocardial myocytes (Kim et al., 2010). In this case, differences in the animal strain cannot explain the discrepancies between the studies, since both Kim et al (2010) and Bryant et al (1999) used Wistar rats.

$I_{Ca,L}$ current density has been reported to be uniform in canine, guinea pig and cat ventricle wall (Banyasz et al., 2003; Bryant et al., 1997; Cordeiro et al., 2004; Kimura et al., 1991; Li et al., 2002; Xiao et al., 2006). A small, gender-specific transmural gradient has been reported in rabbit ventricles, where $I_{Ca,L}$ density was found to be 1.3-fold larger in the epicardium, compared to the endocardium in female but not male hearts (Pham et al., 2002). A second laboratory found a small (1.2-fold) epicardium-to-endocardium gradient in $I_{Ca,L}$, but the authors did not specify the sex of the animals used (Li et al., 2004). At odds with these results, Cav1.2 mRNA expression was found to be 1.8 fold higher in the endocardium of male rabbit left ventricle (females not tested (Abd Allah et al., 2012)).

The expression of $I_{Ca,L}$ across human ventricular wall has not been analyzed to date. Several molecular surveys of ion channel gene expression in the human heart did not find either a transmural gradient or gender-specific differences in expression of Cav1.2 mRNA (Ambrosi et al., 2013; Soltysinska et al., 2009; Szabo et al., 2005). By itself, this is not definitive, since

multiple reports suggest that expression of $I_{Ca,L}$ in the ventricle is complex and not dependent solely on transcriptional regulation of the Cav1.2 gene (Rosati et al., 2008; Rosati et al., 2011; Schroder et al., 1998; Sims et al., 2008).

A lower $I_{Ca,L}$ density was observed in left ventricular myocytes from conventional KChIP2 knockout mice compared to control animals (Thomsen et al., 2009) and it was proposed that the KChIP2 auxiliary subunit can modulate expression of $I_{Ca,L}$. A suggested mechanism was a direct interaction of KChIP2 with the N-terminus of the $Ca_v1.2$ channel since there was no effect of the knockout on either $Ca_v1.2$ mRNA or protein expression or on trafficking of the channel to the plasma membrane. It was hypothesized that KChIP2 may increase the open probability of the $Ca_v1.2$ channels (Thomsen et al., 2009). This hypothesis is in apparent conflict with the observation of a large transmural gradient in KChIP2 mRNA and protein expression in canine heart (Table 2). If the KChIP2 subunit increased the calcium channel open probability in these species, then a large transmural gradient in $I_{Ca,L}$ would be expected, with current density being much larger in epicardium than endocardium. Several laboratories have described the absence of a transmural gradient in $I_{Ca,L}$ in the canine ventricle (Banyasz et al., 2003; Cordeiro et al., 2004; Li et al., 2002; Xiao et al., 2006). Wang and Cohen (Wang and Cohen, 2003) reported a larger $I_{Ca,L}$ in the endocardium, which is opposite to that expected if KChIP2 increased the P_o of the L-type calcium channels in the canine heart. This apparent discrepancy between species has not been fully resolved although it should be noted that interpretation of the effects of conventional knockouts can be complex due to the potentially disruptive effect of the knockout on cardiac development.

Transmural Gradient in I_{Na} Density

The fast cardiac sodium current ($I_{Na,fast}$ or simply I_{Na}) is responsible for the upstroke of the action potential in atrial and ventricular myocytes (Abriel, 2010). The bulk of the I_{Na} channel found in the ventricles are encoded by the SCN5A (Nav1.5) gene channel (Nerbonne and Kass, 2005). Lower levels of SCN1A, SCN2A, SCN3A, SCN4A and SCN8A expression have also been reported in heart (Dhar Malhotra et al., 2001; Kaufmann et al., 2013; Krause et al., 2015; Maier et al., 2002; Rogart et al., 1989; Sills et al., 1989; Westenbroek et al., 2013; Zimmer et al., 2002) and shown to contribute approximately 23% of the total functional sodium channels in mouse ventricular myocytes (Westenbroek et al., 2013) and 27% in human atrial myocytes, based on TTX sensitivity (Kaufmann et al., 2013).

Several auxiliary subunits and other interacting proteins contribute to the native cardiac sodium channel complex and modulate I_{Na} function and/or current density (Table 1). In particular, all four sodium channel beta subunits, SCN1-4B are expressed in the (human) heart and associate with Nav1.5 (Dhar Malhotra et al., 2001; Fahmi et al., 2001; Qu et al., 1995; Yu et al., 2003). Co-expression of SCN1-3B altered the biophysical properties and current density of the Nav1.5 channel in heterologous systems, whereas SCN4B had no apparent effect (summarized in Table 1). All the SCNB subunit genes are associated with cardiac genetic disorders such as Brugada syndrome, atrial fibrillation, Long QT Syndrome and conduction abnormalities, highlighting their functional importance in vivo (Abriel, 2010).

I_{Na} Transmural Gradient

In most species, I_{Na} expression across the ventricle wall appears to be determined predominantly at the level of transcription of the SCN5A gene. Variation in I_{Na} expression across the ventricle wall has been reported in three species. A higher I_{Na} density or channel availability has been described in left endocardial myocytes compared to left epicardial myocytes in the rat (Ashamalla et al., 2001) and mouse (Remme et al., 2009). In canine ventricle, there is no difference between epicardial and endocardial I_{Na} current density or SCN5A mRNA expression levels (Cordeiro et al., 2008; Rosati et al., 2006). There is, however, still some regional heterogeneity, with I_{Na} density, as well as SCN5A mRNA and protein expression, being higher in the midmyocardium than either the epicardium or endocardium (Barajas-Martinez et al., 2009; Szabo et al., 2005).

Transmural gradients in SCN5A (Nav1.5) mRNA and/or protein have been described in both rat and mouse, as well as in human and guinea pig hearts (Gaborit et al., 2007; Gaborit et al., 2010; Osadchii et al., 2011; Remme et al., 2009; Rosati et al., 2006; Soltysinska et al., 2009; Szabo et al., 2005). In the rabbit ventricle, SCN5A mRNA is expressed at higher levels in the endocardium than in the epicardium of neonatal heart, but the gradient is lost during subsequent post-natal development (Abd Allah et al., 2012). It has been reported that there is no transmural gradient in SCN5A or SCN1B mRNA expression in the sheep heart, while the SCN3B gene was more abundantly expressed in endocardium compared to epicardium (Fahmi et al., 2001). SCN1B auxiliary subunit mRNA is also more abundant in human endocardium compared to the epicardium, potentially contributing to transmural heterogeneity of I_{Na} (Gaborit et al., 2007).

$I_{Na,late}$ Transmural Gradient

The small, sustained sodium current, $I_{Na,late}$ (Antzelevitch et al., 2014; Horvath and Bers, 2014), is also expressed heterogeneously in the ventricle wall of some species. This current, although much smaller than the peak I_{Na} (less than 0.1%, (Antzelevitch et al., 2014)), can have significant effects on the cardiac action potential and plays an important role in many pathologies such as arrhythmias and heart failure (Antzelevitch et al., 2014; Horvath and Bers, 2014). $I_{Na,late}$ has been shown to contribute to transmural electrical heterogeneity in the canine ventricle, with larger current densities observed in the mid-myocardium compared to the epicardium and endocardium (Zygmunt et al., 2001). Conversely, in the guinea pig ventricle, $I_{Na,late}$ density is smaller in mid-myocardium compared to epicardium and endocardium (Main et al., 1998; Sakmann et al., 2000), while no transmural heterogeneity in $I_{Na,late}$ density was observed in rabbit ventricle (Guo et al., 2014).

Regional differences in $I_{Na,late}$ expression could be due to several different mechanisms. The sustained sodium current is thought to be produced, in large part, by Nav1.5 channels that reopen during the prolonged depolarization of the cardiac action potential plateau (Antzelevitch et al., 2014). It was recently proposed that sodium channel pore-forming subunits other than the SCN5A subunit can also contribute to this current (Biet et al., 2012; Mishra et al., 2015). In particular, about 44% of $I_{Na,late}$ in the canine ventricles is highly TTX sensitive and therefore can be ascribed to sodium channels other than SCN5A (Biet et

al., 2012). These non-SCN5A channels also contribute to alterations in $I_{Na,late}$ expression in a canine model of heart failure (Mishra et al., 2015).

Post transcriptional silencing of SCN1B and SCN2B using virally delivered siRNAs differentially affects $I_{Na,late}$ density but not the peak I_{Na} in the canine heart (Mishra et al., 2011), suggesting that transcriptional regulation of these two auxiliary subunits could also contribute to the regulation of the $I_{Na,late}$ current. As mentioned above, SCN1B mRNA is more abundantly expressed in endocardium compared to epicardium in human ventricles (Gaborit et al., 2007). Unfortunately, there is no comparable data in human midmyocardium or in the canine heart.

Transmural gradients in delayed rectifier potassium currents

Cardiac delayed rectifier currents activate relatively slowly with depolarization and mediate the phase 3 repolarization of the action potential (Charpentier et al., 2010). They are the most important repolarizing currents in the heart of large mammalian species, including humans (Charpentier et al., 2010). Multiple types of delayed rectifier potassium currents (I_K) have been described, depending on the species and cardiac region examined, each endowed with different biophysical and pharmacological properties.

In the guinea pig ventricle, two components of the delayed rectifier current were described and named 'rapid' (I_{Kr}) and 'slow' (I_{Ks}), based on their activation kinetics (Sanguinetti and Jurkiewicz, 1991). These currents had quite distinct biophysical and pharmacological properties indicating a different molecular basis. Subsequently, I_{Kr} and I_{Ks} currents were described in the rabbit, canine and human hearts (Li et al., 1996; Liu and Antzelevitch, 1995; Salata et al., 1996). The physiological importance of these two currents in the human heart was highlighted by the discovery of genetic mutations in the underlying genes that cause a series of inherited arrhythmias (Charpentier et al., 2010). In mouse and rat, several different delayed rectifier current components have been identified, based on biophysical and pharmacological features (Himmel et al., 1999; Xu et al., 1996; Xu et al., 1999).

I_{Ks}

Following the discovery that expression of a single membrane spanning domain protein (MinK, also known as KCNE1) in *Xenopus* oocytes gave rise to a current that resembled I_{Ks} (Takumi et al., 1988), it was hypothesized that KCNE1 encoded I_{Ks} (Freeman and Kass, 1993). It was subsequently shown that a complex of the KCNQ1 pore-forming subunit with KCNE1 underlies I_{Ks} (Barhanin et al., 1996; Sanguinetti et al., 1996). The initial result in *Xenopus* oocytes is explained by the fact that the KCNE1 subunit enhances a native current in the oocytes encoded by a *Xenopus* KCNQ1 gene (Sanguinetti et al., 1996). Several KCNE family subunit genes have been identified, all of which can co-assemble with KCNQ1 and modulate its properties (summarized in Table 1). Based on gene expression data and biophysical properties, it was proposed that the KCNE1, KCNE2 and KCNE3 subunits might all contribute to the native I_{Ks} complex in the human heart (Bendahhou et al., 2005). This hypothesis has not yet been fully tested and the specific contribution of KCNE subunits other than KCNE1 to the native I_{Ks} remains unclear (Crump and Abbott, 2014).

I_{Ks} is very small or undetectable in the rat and mouse ventricles (Drici et al., 1998; Lees-Miller et al., 2003; Pond et al., 2000; Wang et al., 1996). Although KCNE1 and KCNQ1 mRNAs are expressed in the mouse heart, KCNE1 gene expression is dramatically down-regulated during post-natal development (1 to 8 weeks) to very low levels in the adult (Drici et al., 1998). In contrast, mRNA expression for the delayed rectifier alpha subunit KCNQ1 remains unaltered during this period. A conventional knockout of the KCNQ1 gene in mouse does produce some EKG abnormalities but no alterations in cellular electrophysiological function were observed in isolated cardiomyocytes, suggesting that the EKG effects could be due to I_{Ks} disruption in other tissues (Casimiro et al., 2001).

I_{Ks} Transmural Gradient

Transmural heterogeneity in I_{Ks} expression across the ventricle has been documented in several mammalian species, although there are some discrepancies in the results from different laboratories that remain unresolved (Table 2). In the guinea pig, where this current was first described, one study did not report any significant transmural gradient (Main et al., 1998) whereas a second study found higher I_{Ks} density in epicardium and mid-myocardium compared to endocardium (Bryant et al., 1998).

Discordant results have also been reported for the canine heart. Four laboratories reported a higher I_{Ks} density in epicardium and endocardium compared to mid-myocardium (Gintant, 1995; Li et al., 2002; Liu and Antzelevitch, 1995; Szabo et al., 2005; Xiao et al., 2006). In contrast, one laboratory failed to find any significant difference in I_{Ks} expression between the three regions (Obreztkhikova et al., 2006). Szabo et al (2005) found higher levels of KCNQ1 protein in epicardium compared to mid-myocardium and the opposite result for the KCNE1 protein. In contrast, Obreztkhikova et al. (2006) described opposing transmural gradients in KCNQ1 (Epi>Endo) and KCNE1 (Endo>Epi) mRNA expression in canine heart, while they found no difference in the expression of the corresponding subunit proteins in the same tissues.

I_{Ks} current density has been measured in the human ventricle (Li et al., 1996; Virag et al., 2001) but its distribution across the ventricular wall has not been analyzed. Expression data for the KCNQ1 and the KCNE1 genes in the human heart are conflicting. Two groups found higher expression for KCNQ1 mRNA or protein in epicardium compared to mid-myocardium and endocardium (Gaborit et al., 2007; Szabo et al., 2005). Two other laboratories found no transmural differences (Ambrosi et al., 2013; Soltysinska et al., 2009). Gaborit et al. (2010) reported higher KCNQ1 mRNA expression in endocardium compared to epicardium in the hearts of females but not males. Two groups reported that KCNE1 expression was higher in mid-myocardium versus epicardium and endocardium in human heart (Soltysinska et al., 2009; Szabo et al., 2005) but other laboratories did not find a difference (Ambrosi et al., 2013; Gaborit et al., 2007; Gaborit et al., 2010).

Pereon and colleagues suggested that the transmural heterogeneity in I_{Ks} density depends on differential expression of an N-terminal truncated KCNQ1 splice variant that exerts a dominant negative effect on the full-length KCNQ1 protein (Pereon et al., 2000). While they found that expression of the total KCNQ1 mRNA was homogeneous in epicardium, mid-myocardium and endocardium in the human ventricles, the expression levels of the short

KCNQ1 isoform mRNA was about 1.3-fold higher in mid-myocardium, than in epicardium and endocardium, and therefore correlates negatively with the I_{Ks} density (Pereon et al, 2000). A recent study confirmed these results at the protein level in the canine left ventricle (Lee et al, 2013). The conflicting KCNQ1 mRNA and protein expression results in canine and human could reflect variable detection of different isoforms.

In the rabbit, there is a consensus between the electrophysiological and molecular data, obtained by different laboratories. In particular, I_{Ks} density is higher in epicardium than endocardium (Xu et al., 2001) and there is a concordant gradient in KCNQ1 mRNA, while KCNE1 mRNA is not significantly different (Abd Allah et al., 2012).

I_{Kr}

The pore-forming subunit for I_{Kr} was initially identified when heterologous expression of the KCNH2 (HERG) protein, yielded a current with similar properties to I_{Kr} (Sanguinetti et al., 1995). The existence of an auxiliary subunit for the KCNH2 channel was postulated because, while the peculiar biophysical properties of I_{Kr} are recapitulated by KCNH2 channels alone, certain pharmacological characteristics of these channels differed from those of the native I_{Kr} (Sanguinetti et al., 1995). Subunits of the KCNE gene family were later shown to interact with KCNH2 and proposed to form the native I_{Kr} channel complex. In particular, KCNE2 (MiRP1) coassembles with the KCNH2 channels to yield currents that closely resemble I_{Kr} and mutations in this gene are associated with cardiac arrhythmias (Abbott et al., 1999). KCNE1 (MinK) can also be coimmunoprecipitated with KCNH2 in cardiac myocytes and antisense oligonucleotides against KCNE1 decrease I_{Kr} in an atrial tumor cell line (McDonald et al., 1997; Yang et al., 1995). Indeed several KCNE subunit genes are mutated in human cardiac genetic disorders, although the dependence of these phenotypes on disruption of I_{Kr} or I_{Ks} function is unclear (Abbott and Goldstein, 2002). As a consequence, molecular identification of auxiliary subunits for I_{Kr} is controversial and still debated (Abbott et al., 2007). To further complicate dissection of the native I_{Kr} channel composition, it has been shown that co-expression of KCNQ1 with KCNH2 in a mammalian expression system increased KCNH2 current density and deactivation rate. The increased current density was presumably due to an increased KCNH2 membrane localization and the two subunits could be co-immunoprecipitated in native cardiac tissue, as well as in vitro (Ehrlich et al., 2004). Finally, it has been proposed that an amino-terminal KCNH2 splice variant can co-assemble with the full length KCNH2 protein to yield currents more closely resembling the native I_{Kr} , through the formation of heteromeric I_{Kr} channels in mouse, rat, canine and human (Jones et al., 2004; London et al., 1997).

The physiological significance of these findings in rodents is unclear since, similarly to I_{Ks} , I_{Kr} is poorly expressed in the rodent heart. In addition, the biophysical properties of both these currents limit their potential contribution to action potential repolarization in these species, which have rapidly repolarizing ventricular action potentials (Pond et al., 2000; Wang et al., 1996). KCNH2 mRNA is relatively abundant in rat ventricle (Wymore et al., 1997) and the reason for low current levels in this species remains unresolved.

I_{Kr} Transmural Gradient

Expression of I_{Kr} in the ventricle wall has been found to be relatively homogeneous, suggesting that this current makes little or no contribution to dispersion of repolarization in ventricle. In canine heart, multiple laboratories have found that I_{Kr} density is unchanged across the left ventricle wall (Gintant, 1995; Li et al., 2002; Liu and Antzelevitch, 1995; Szabo et al., 2005; Xiao et al., 2006). In keeping with these results, no difference in KCNH2 mRNA or protein was found in comparisons of epicardium and mid-myocardium (Szabo et al., 2005). One laboratory reported that I_{Kr} density was 2.2-fold higher in epicardium than in the endocardium of canine heart (Obreztkhikova et al., 2006) and also reported a similar gradient in KCNH2 protein expression, with a smaller gradient in mRNA expression.

No transmural gradient in I_{Kr} density was detected in rabbit ventricle (Xu et al., 2001), although KCNH2 mRNA expression was found to be higher in the epicardium compared to the endocardium (Abd Allah et al., 2012). In the guinea pig, I_{Kr} current density was approximately 1.3-fold higher in epicardium than mid-myocardium and 2-fold higher than endocardium (Main et al., 1998).

Although I_{Kr} has been measured in human ventricular myocytes (Iost et al., 1998; Li et al., 1996), its transmural distribution has not been described. Molecular data on transmural expression of KCNH2 and the putative I_{Kr} auxiliary subunits KCNE1 and KCNE2 are available in human heart, with most authors reporting no difference in KCNH2 expression between epicardium and endocardium (Ambrosi et al., 2013; Gaborit et al., 2007; Gaborit et al., 2010; Holzem et al., 2016; Soltysinska et al., 2009). As mentioned above, some authors did not find any difference KCNE1 expression across the ventricle wall (Ambrosi et al., 2013; Gaborit et al., 2007; Gaborit et al., 2010) whereas others found expression to be higher in mid-myocardium compared to epicardium and endocardium (Soltysinska et al., 2009; Szabo et al., 2005). No difference in transmural expression of KCNE2 mRNA or protein was observed in human heart (Gaborit et al., 2007; Szabo et al., 2005).

$I_{Kslow,1}$ and $I_{Kslow,2}$

The $I_{Kslow,1}$ and $I_{Kslow,2}$ delayed rectifier currents are present in the ventricles of rodent hearts and are encoded by the Kv1.5 and Kv2.1 subunit genes, respectively (Nerbonne and Kass, 2005). They have not been described in the ventricular myocytes of large mammals, but a similar current has been described in the human and canine atria, known as the 'ultra-rapid delayed rectifier' current (I_{Kur}). This current is encoded by the Kv1.5 (KCNA5) subunit gene and contributes to action potential repolarization in atrial myocytes. Loss-of-function mutations in this gene cause atrial fibrillation in humans (Olson et al., 2006).

Kv2.1 (KCNB1) mRNA (Ordog et al., 2006) and protein (Kaab et al., 1998; Van Wagoner et al., 1997) have been detected in human and canine ventricle, but at relatively low abundance compared to the rat ventricle (Dixon et al., 1996). Human mutations in KCNB1 cause several forms of epilepsy but no cardiac phenotype (Saito et al., 2015; Thiffault et al., 2015; Torkamani et al., 2014), suggesting that this gene does not play a prominent role in human cardiac function.

Very few data exist on the transmural distribution of $I_{K_{slow,1}}$ and $I_{K_{slow,2}}$. Brunet and colleagues (2004) measured $I_{K_{slow}}$ across the murine left ventricle and found higher current density in epicardium compared to endocardium. The two components, $I_{K_{slow,1}}$ and $I_{K_{slow,2}}$, were not distinguished in this analysis. Kv1.5 and Kv2.1 protein expression was homogeneous in the heart regions that were analyzed (apex, base, atria, ventricles and septum (Brunet et al., 2004)). An absence of transmural gradients for Kv1.5 and Kv2.1 mRNA or protein expression has been described in canine, human, mouse, rat and rabbit (Abd Allah et al., 2012; Calloe et al., 2010; Costantini et al., 2005; Dixon and McKinnon, 1994; Gaborit et al., 2007; Huang et al., 2001; Rossow et al., 2006).

Transmural gradient in inward rectifier current (I_{K1}) density

The ‘classic’ inward rectifier potassium current (I_{K1}) is only active at potentials negative to -40 mV and is the major membrane conductance during diastole, contributing to the setting of the resting potential. During the action potential plateau, these channels are largely blocked but they rapidly become unblocked during repolarization, contributing to the terminal ‘regenerative’ phase of repolarization of the cardiac action potential (Shimoni et al., 1992), when the I_{Kr} channels are mostly closed (Gintant, 2000; Hancox et al., 1998; Rocchetti et al., 2001).

Three of the strong inward rectifier potassium channel genes (Kir2.1, Kir2.2 and Kir2.3) are expressed in the heart, with Kir 2.1 generally being the most abundant in the mammalian species that have been tested (Dhamoon et al., 2004; Gaborit et al., 2007; Wang et al., 1998; Zaritsky et al., 2001). The Kir2.2 and Kir2.3 genes may, however, also make a significant contribution to I_{K1} (Liu et al., 2001) although their relative importance is species- and region dependent (Cordeiro et al., 2015; Dhamoon et al., 2004). To further complicate the molecular identity of I_{K1} , the different subunits can combine to form heteromultimeric channels (Dhamoon et al., 2004; Zobel et al., 2003).

In the mouse ventricles, both Kir2.1 and Kir2.2 contribute to I_{K1} , as shown by conventional gene knockout. In particular, knockout of Kir2.1 almost abolished I_{K1} , while Kir2.2 knockout produced an approximate 50% decrease in I_{K1} density, indicating that the former gene plays the major role in encoding I_{K1} in this species (Zaritsky et al., 2001).

Kir2.1 is the most abundantly expressed strongly inward rectifying channel gene in the human heart, although Kir2.2 and Kir2.3 are also expressed (Wang et al., 1998). Human mutations in the Kir2.1 gene cause Andersen-Tahil syndrome, a complex disorder whose patients present a long QT interval and predisposition to ventricular arrhythmias (Tristani-Firouzi and Etheridge, 2010).

The Kir2.1 subunit is the major I_{K1} gene in the guinea pig and sheep atria, with Kir2.3 also being expressed at significant levels. Kir2.1/2.3 heteromeric channels are thought to contribute to I_{K1} in the ventricles of both these species (Dhamoon et al., 2004). In rabbit ventricular myocytes, Kir2.3 is not expressed and adenoviral delivery of Kir2.1 and 2.2 dominant negative constructs equally inhibited I_{K1} (Zobel et al., 2003).

I_{K1} Transmural Gradient

In most mammalian hearts, I_{K1} is evenly distributed across the ventricle wall. This is the case in rat (Casis et al., 1998; Volk et al., 2001), guinea pig (Main et al., 1998), canine (Li et al., 2002; Liu et al., 1993; Szabo et al., 2005; Xiao et al., 2006) and human heart (Li et al., 1998). In concurrence with this result, Kir2.1 protein expression was found to be homogeneous between epicardium and mid-myocardium in canine and human left ventricle (Szabo et al., 2005) and there was no transmural difference in mRNA expression for Kir2.1 (Ambrosi et al., 2013; Soltysinska et al., 2009) or Kir2.2 and Kir2.3 (Ambrosi et al., 2013) mRNA in the human left ventricle. In contrast, Gaborit et al (2007) found a gradient in gene expression for both Kir2.1 and 2.2 across the human right ventricle. In this report the gradients are in opposite direction, with Kir2.1 mRNA more abundant in endocardium compared to epicardium whereas Kir2.2 mRNA was more abundant in epicardium. This finding would also be consistent with the absence of transmural gradient in the I_{K1} current density across the human (right) ventricle (Li et al., 1998), with the Kir2.1 gradient being counterbalanced by the opposing gradient in Kir2.2.

At variance with the other mammalian species, I_{K1} density was found to be higher in endocardium compared to epicardium in the cat left ventricle (Furukawa et al., 1992) but no molecular analysis on I_{K1} subunit genes has been performed in this species. A small transmural gradient in I_{K1} , with higher I_{K1} density in epicardium than in endocardium, was reported in the left ventricular wall of rabbit heart (Li et al., 2008), which is consistent with a gradient of Kir2.1 mRNA expression in the same species (Abd Allah et al., 2012).

Conclusion

In general, transmural gradients in native ion current expression in the heart are determined by differential regulation of the pore-forming subunit genes. The one clear exception to this trend is the regulation of I_{to} in large mammals by transcriptional regulation of the auxiliary subunit KChIP2 gene. Given the large number of auxiliary subunits associated with different ion channels expressed in heart and the broad effects that these subunits can have on channel expression this is a somewhat surprising outcome. One potential explanation for this result revolves around the key role that pleiotropy can play in constraining the evolution of ion channel expression (Rosati and McKinnon, 2009; Stern, 2000). Many auxiliary subunits are thought to interact with multiple different cardiac ion channels (Table 1). As a consequence, changes in the expression levels of these auxiliary subunits would be expected to have pleiotropic effects, modifying the expression of multiple different currents. This would, in principle, make it more difficult for gradients of differential expression in these subunits to evolve in the heart. Pleiotropic effects are a strong constraint on regulatory evolution in many biological systems (Rosati and McKinnon, 2009; Stern, 2000). This hypothesis does, however, suggest that evolution of KChIP2 expression levels was relatively unconstrained in the ventricle of large mammals, which suggests that KChIP2 subunits may play a very limited role in the regulation of currents other than I_{to} in the hearts of these species.

Acknowledgments

The project was supported by a VA Merit Review Grant, a Carol M. Baldwin Award and the Institute of Molecular Cardiology. The authors wish to acknowledge all the colleagues who contributed to the advancement of this field and apologize beforehand to any colleagues whose work we may have inadvertently omitted in this review.

Abbreviations

Epi	epicardium
Mid	mid-myocardium
Endo	endocardium

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Figure 1.

Channel forming subunits have hydrophobic transmembrane (TM) domains of 20–25 residues in length that form membrane spanning α -helices in the fully assembled protein (marked with red and green in the figure). The first of these TM domains acts as a targeting sequence to target the nascent peptide to the translocon in the ER membrane. This sequence is recognized by the signal recognition particle (SRP), which targets the entire complex to the ER membrane by binding to the SRP receptor. The ribosome and nascent peptide then attach directly to the translocon. The first TM domain is recognized as a start-transfer sequence by the translocon. This initiates movement of the downstream peptide through the translocon pore into the ER lumen. During subsequent translation of the protein the TM domains each signal to the translocon to start or stop transfer of the peptide across the ER membrane. The translocon has a lateral gate that can open to allow lateral transfer of each of the hydrophobic TM domains into the lipid bilayer. Auxiliary subunits can, in principle, begin binding to the nascent channel peptide before synthesis of the pore-forming subunit is complete.

Figure 2.

Intracellular pathways for the transport of ion channels to the membrane, recycling of channels and degradation of channels. The arrows indicate the main pathways taken by ion channels between different membrane compartments. The exception is the quality control and ER-associated degradation (ERAD) pathway, which degrades proteins that are misfolded in the ER. In this case, proteins are retrotranslocated into the cytosol where they are degraded by proteasomes. The many antero- and retrograde transport pathways that are required for recycling of compartment components in order to maintain compartment identity are not indicated in this figure.

Figure 3.

Ontogenesis of the cardiac ventricular wall, adapted from (Misra and Garg, 2013). (a,b) The heart tube in a 3-week old embryo consists of an inner endothelial cell lining surrounded by a myocardial cell layer, separated by an acellular gelatinous extracellular matrix, called the cardiac jelly. (c,d) At 4 weeks of gestation, the heart undergoes looping and prominent trabeculae develop in the ventral portion, which will give rise to the ventricles. (e) During weeks 5 to 8, epicardial cells invade and contribute to the developing coronary vessels. The trabecular myocardium further develops and undergoes compaction (disappearance of intratrabecular cavities). (f,g) The compacted myocardial layer thickens during the later stages of gestation to form the mature ventricular wall.

Figure 4.

Scaling of heart rate and action potential duration with body weight in mammals. A. Relationship between resting heart rate and body weight for terrestrial mammals. Data were fitted with the allometric equation $y = a \cdot M^b$, where: a is constant, M is the body weight, and b is the scaling coefficient ($b = -0.25 \pm 0.02$). Black squares represent, from left to right: Mouse, Chinese Hamster, Syrian Hamster, Rat, Marmoset, Guinea Pig, Ferret, Rabbit, Cat, Dog, Goat, Sheep, Human, Horse and Cow. bpm, Beats per minute. B. Relationship between the inverse ventricular action potential duration ($1/APD$) and body weight for terrestrial mammals (scaling coefficient $b = -0.22 \pm 0.02$). Ventricular action potential duration was estimated from uncorrected QT intervals obtained from electrocardiogram studies using conscious resting animals, where available. Data adapted from (Rosati et al., 2008).

Figure 5.

KChIP2 gene expression across the canine ventricles. A. RNase protection analysis of KChIP2 mRNA expression in the canine ventricles. The upper band is the KChIP2-specific band and the lower band is the internal control cyclophilin. B. Histogram of KChIP2 mRNA expression. Data are means of 2–4 determinations and error bars are s.e.m. C. Correlation of KChIP2 mRNA expression with I_{to} current density ($R^2 = 0.94$). From: Rosati et al, 2003.

Table 1

Cardiac Ion Channel Auxiliary Subunit Function

Subunit (Gene Name)	Native Current	Principal Subunit	Effects on Channel	Established Mechanisms of action	Involved channel sites/domains	References
KChIP2 (KCNP2)	Ito,f	Kv4.2, Kv4.3	Affects protein folding, increases current density	Slows channel degradation, induces proper folding, masks hydrophobic domains that may interact and cause ER retention	Kv4 N-terminus (first-20AAs), C-terminus	(An et al., 2000; Bähring et al., 2001; Han et al., 2006; Shibata et al., 2003)
	ICa,L	Cav1.2	Increases current density	Increases P_0 due to masking of a Cav1.2 N- terminus inhibitory domain (NTI)	Ca _v 1.2 NTI domain	(Thomsen et al., 2009)
DPP6	IKur, IKslow,1	Kv1.5	Decreases current density	Inhibits forward trafficking from ER		(Li et al., 2005)
	Ito,f	Kv4.3	Increases current density, alters biophysical properties	Increases unitary conductance, faster and more negative inactivation, slower recovery from inactivation		(Radicke et al., 2005; Xiao et al., 2013)
DPP10		Kv4.2	Increases current density, alters biophysical properties	Masks ER retention motifs, increases unitary conductance, increases inactivation rate, decreases activation rate, left-shift in voltage-dependence of both activation and inactivation	S4 and pore domains of Kv4.2	(Dougherty and Covarrubias, 2006; Kaulin et al., 2009; Nadal et al., 2003)
	Ito,f	Kv4.3	Alters biophysical properties, increases current density	Left-shift in voltage-dependence of both activation and inactivation, accelerates inactivation and recovery from inactivation	S1-S2 domains	(Cotella et al., 2010; Cotella et al., 2012; Ren et al., 2005; Turnow et al., 2015)
Kvβ1 (KCNP1.2, 1.3)	IKur, IKslow,1	Kv1.5	Alters biophysical properties	N-type inactivation, left shift in voltage- dependence of activation, slows deactivation; regulates PKA and PKC modulation	N-terminal tetramerization domain for binding; A501, T480 in the channel pore for inactivation	(Decher et al., 2008; England et al., 1995a; England et al., 1995b; Kwak et al., 1999; Majumder et al., 1995; Williams et al., 2002)
	Ito,f	Kv4.3	Increases current density			(Aimond et al., 2005)
Kvβ2 (KCNP2)	IKslow,2	Kv2.1	Decreases current density			(Aimond et al., 2005)
	IKur	Kv1.5	Alters biophysical properties	Left shift in V-dependent activation and inactivation, slows inactivation rate	N-terminal tetramerization domain for binding	(Uebele et al., 1996)
MinK (KCNE1)	IKs	KCNQ1	Increases current density, alters biophysical properties	Increases single-channel conductance, right-shift in voltage dependence of activation, decreases activation and deactivation rates, blocks inactivation	numerous (see review)	Reviewed in (Abbott, 2016)
	IKr	HERG	Increases current density			Reviewed in (Abbott, 2016)
	Ito	Kv4.3	Alters biophysical properties	Increases activation rate, decreases inactivation rate, left shift in voltage dependence of inactivation, decreases rate		(Radicke et al., 2006)

Subunit (Gene Name)	Native Current	Principal Subunit	Effects on Channel	Established Mechanisms of action	Involved channel sites/domains	References
MiRP1 (KCNE2)	IK _{slow,1}	Kv1.5	Promotes protein maturation and targeting to intercalated discs	of recovery from inactivation		(Roepke et al., 2008)
	Ito,f	Kv4.2	Increases current density, alters biophysical properties	Decreases fast inactivation rate		(Roepke et al., 2008)
	IKs	KCNQ1	Decreases current density			(Wu et al., 2006)
	IKr	HERG	Decreases current density, alters biophysical properties	Decreases single channel conductance, accelerates deactivation, positive shift in V _{1/2}		Reviewed in (Anantharam and Abbott, 2005)
	If	HCN4, HCN2	Increases current density, alters biophysical properties	Increases single channel conductance		(Brandt et al., 2009; Decher et al., 2003; Qu et al., 2004)
MiRP2 (KCNE3)	IKs	KCNQ1	Alters biophysical properties	Affects voltage sensing	S4 domain of KCNQ1	Reviewed in (Abbott, 2016)
	IKr	HERG	Alters biophysical properties			(Schroeder et al., 2000)
	Ito	Kv4.3	Alters biophysical properties			(Delpon et al., 2008)
MiRP3 (KCNE4)	IKs	KCNQ1	Alters biophysical properties			(Bendahhou et al., 2005; Manderfield and George, 2008)
	Ito	Kv4.2	Alters biophysical properties	Several effects on kinetics and V dependence		(Levy et al., 2010)
	Ito	Kv4.3	Alters biophysical properties	Several effects on kinetics and V dependence		(Radicke et al., 2006)
KCNE5	IKs	KCNQ1	Alters biophysical properties			(Bendahhou et al., 2005)
Navβ1 (SCN1B)	INa	SCN5A	Increases current density, alters biophysical properties	Several effects on kinetics and V dependence	loop between S5 and S6 of DI and DIV of Nav1.5	(Mishra et al., 2011), reviewed in (Abriel, 2010)
Navβ2 (SCN2B)	INa	SCN5A	Alters biophysical properties	Decreases INa,late density, accelerates its decay kinetics		(Mishra et al., 2011), reviewed in (Abriel, 2010)
Navβ3 (SCN3B)	INa	SCN5A	Affects membrane localization, trafficking, alters biophysical properties	Right shift in V-dependent inactivation		(Fahmi et al., 2001; Hu et al., 2009), reviewed in (Abriel, 2010)

Subunit (Gene Name)	Native Current	Principal Subunit	Effects on Channel	Established Mechanisms of action	Involved channel sites/domains	References
Navβ4 (SCN4B)	INa	SCN5A	No apparent effects on channel V- dependence or kinetics			(Medeiros-Domingo et al., 2007; Yu et al., 2003)
Cavβ1 (CACNB1)	ICa,L	Cav1.2	Affects trafficking, alters biophysical properties	Masks ER retention signals on the I-II loop of Cav. Prevents a-subunit entry into ERAD complex. Increases current density. Decreases P _o .	surface expression: AID (a interaction domain), I-II loop; V-dep effects due to Cavb N- term, or its SH3-HOOK-GK domains	Reviewed in (Buraei and Yang, 2013), (Altier et al., 2011; Colecraft et al., 2002)
Cavβ2 (CACNB2)	ICa,L	Cav1.2	Affects trafficking, stabilization, alters biophysical properties	Masks ER retention signals on the I-II loop of Cav. Biophysical effects depend on splice variant used. Increases current density.	surface expression: AID (a interaction domain), I-II loop; V-dep effects due to Cavb N- term, or its SH3-HOOK-GK domains; prevents proteosomal degradation.	Reviewed in (Buraei and Yang, 2013), (Chien et al., 1995; Colecraft et al., 2002; Hullin et al., 2003; Link et al., 2009; Takahashi et al., 2003; Yamada et al., 2001)
Cavβ3 (CACNB3)	ICa,L	Cav1.2	Affects trafficking, alters biophysical properties	Masks ER retention signals on the I-II loop of Cav	surface expression: AID (a interaction domain), I-II loop; V-dep effects due to Cavb N- term, or its SH3-HOOK-GK domains	Reviewed in (Buraei and Yang, 2013), (Colecraft et al., 2002; Murakami et al., 2003)
Cavβ4 (CACNB4)	ICa,L	Cav1.2	Affects trafficking, alters biophysical properties	Masks ER retention signals on the I-II loop of Cav	surface expression: AID (a interaction domain), I-II loop; V-dep effects due to Cavb N- term, or its SH3-HOOK-GK domains	Reviewed in (Buraei and Yang, 2013), (Altier et al., 2011; Colecraft et al., 2002)
Cavα2δ1 (CACNA2D1)	ICa,L	Cav1.2	Affects trafficking, alters biophysical properties	Affects gating and voltage-dependence	δ sub is resp for functional effects, α2 for membrane localization	(Felix et al., 1997; Fuller-Bicer et al., 2009),
Cavγ4 (CACNG4)	ICa,L	Cav1.2	Alters biophysical properties	Modulates voltage-dependent activation and inactivation ^{**}		(Yang et al., 2011)
Cavγ6 (CACNG6)	ICa,L	Cav1.2	Alters biophysical properties	Modulates voltage-dependent activation and inactivation ^{**}		(Yang et al., 2011)
Cavγ7 (CACNG7)	ICa,L	Cav1.2	Alters biophysical properties	Modulates voltage-dependent activation and inactivation ^{**}		(Yang et al., 2011)
Cavγ8 (CACNG8)	ICa,L	Cav1.2	Alters biophysical properties	Modulates voltage-dependent activation and inactivation ^{**}		(Yang et al., 2011)
Calmodulin (CALM1)	ICa,L	Cav1.2	Alters biophysical properties	Mediates calcium-dependent inactivation or facilitation	IQ domains of Cav1.2	(Peterson et al., 1999)

* contrasting results

** the detailed effects depend on the channel subunit combinations tested

Table 2

Transmural Gradient of Cardiac Ion Currents and Ion Channel Transcripts and Proteins

Cardiac Ion Current	Current or Gene	Mammalian Species		References
		Gradient	No Gradient	
Ito,f	Current	Human, Canine, Cat, Ferret, Rat, Mouse		(Ashamalla et al., 2001; Brahmajothi et al., 1999; Brunet et al., 2004; Bryant et al., 1999; Clark et al., 1993; Costantini et al., 2005; Furukawa et al., 1990; Litovsky and Antzelevitch, 1988; Nabauer et al., 1996; Rosati et al., 2003; Rosati et al., 2001; Rossow et al., 2006; Wettwer et al., 1994)
	Kv4.3 mRNA	Ferret	Human ^a , Canine ^a , Rat ^b , Rabbit, Mouse	(Abd Allah et al., 2012; Ambrosi et al., 2013; Brahmajothi et al., 1999; Calloe et al., 2010; Costantini et al., 2005; Dixon et al., 1996; Gaborit et al., 2007; Goltz et al., 2007; Huang et al., 2001; Liu et al., 2015; Radicke et al., 2006; Rosati et al., 2003; Rosati et al., 2001; Rossow et al., 2006; Soltysinska et al., 2009)
	Kv4.3 protein	Ferret	Human ^a , Rat, Mouse	(Brahmajothi et al., 1999; Costantini et al., 2005; Gaborit et al., 2007; Rossow et al., 2006; Su et al., 2012)
	Kv4.2 mRNA	Ferret, Rat, Mouse	Rabbit	(Abd Allah et al., 2012; Costantini et al., 2005; Dixon and McKinnon, 1994; Dixon et al., 1996; Goltz et al., 2007; Huang et al., 2001; Liu et al., 2015; Rosati et al., 2003; Rosati et al., 2001; Rossow et al., 2006; Teutsch et al., 2007)
	Kv4.2 protein	Ferret, Rat, Mouse		(Brahmajothi et al., 1999; Costantini et al., 2005; Gaborit et al., 2007; Stones et al., 2009)
	KChIP2 mRNA	Human, Canine, Rabbit, Ferret, Rat [*]	Rat [*] , Mouse ^d	(Abd Allah et al., 2012; Ambrosi et al., 2013; Calloe et al., 2010; Costantini et al., 2005; Gaborit et al., 2007; Gaborit et al., 2010; Goltz et al., 2007; Guo et al., 2002a; Liu et al., 2015; Patel et al., 2002; Radicke et al., 2006; Rosati et al., 2003; Soltysinska et al., 2009; Stones et al., 2009; Szabo et al., 2005; Teutsch et al., 2007; Zicha et al., 2004)
	KChIP2 protein	Human, Canine, Ferret,		(Calloe et al., 2010; Gaborit et al., 2007; Gaborit et al., 2010; Patel et al., 2002; Rosati et al., 2003; Soltysinska et al., 2009; Szabo et al., 2005)
	DPP6 mRNA		Canine ^e	(Calloe et al., 2010)
	DPP10 mRNA		Canine	(Calloe et al., 2010)
Ito,s	Current	Rabbit, Ferret (Endo>Epi)	Rat	(Brahmajothi et al., 1999; Fedida and Giles, 1991; Huang et al., 2001)
	Kv1.4 mRNA	Ferret (Endo>Epi), Rabbit (neonatal, Endo>Epi), Human ^f (Endo>Epi)	Canine, Rabbit (adult), Rat	(Abd Allah et al., 2012; Brahmajothi et al., 1999; Calloe et al., 2010; Dixon and McKinnon, 1994; Gaborit et al., 2007; Gaborit et al., 2010; Huang et al., 2001; Rosati et al., 2003)
	Kv1.4 protein	Ferret (Endo>Epi),		(Brahmajothi et al., 1999; Gaborit et al., 2010; Huang et al., 2001)
ICa,L	Current	Rabbit (females), Mouse [*]	Canine ^g , Feline, Rabbit (males), Guinea Pig, Rat ^h , Mouse [*]	(Banyasz et al., 2003; Bryant et al., 1997; Bryant et al., 1999; Clark et al., 1993; Cordeiro et al., 2004; Dilly et al., 2006; Kimura et al., 1991; Li et al., 2002; Li et al., 2004; Main et al., 1998; Pham et al., 2002; Shi et al., 2007; Volk et al., 1999; Wang et al., 2008; Xiao et al., 2006; Xu et al., 2011)
	CACNA1C mRNA	Rabbit (males)	Human ⁱ	(Abd Allah et al., 2012; Ambrosi et al., 2013; Soltysinska et al., 2009; Szabo et al., 2005)
	CACNA1C protein		Human (Epi=Mid), Canine (Epi=Mid), Mouse	(Szabo et al., 2005; Wang et al., 2008; Xu et al., 2011)

Cardiac Ion Current	Current or Gene	Mammalian Species		References
		Gradient	No Gradient	
	CACNA2D1 mRNA	Human (Endo>Epi)		(Gaborit et al., 2007)
INa	Current	Canine (Mid>Epi=Endo), Rat (Endo>Epi) Mouse ^J		(Ashamalla et al., 2001; Barajas-Martinez et al., 2009; Cordeiro et al., 2008; Remme et al., 2009)
	SCN5a mRNA	Human (Endo>Epi), Canine (Mid>Epi=Endo), Rabbit (neonate, Endo>Epi), Rat (Endo>Epi), Mouse (Endo>Epi)	Sheep, Rabbit (adult)	(Abd Allah et al., 2012; Barajas-Martinez et al., 2009; Cordeiro et al., 2008; Fahmi et al., 2001; Gaborit et al., 2007; Gaborit et al., 2010; Osadchii et al., 2011; Remme et al., 2009; Rosati et al., 2006; Soltysinska et al., 2009; Szabo et al., 2005)
	SCN5a protein	Human (Endo>Epi), Canine (Mid>Epi=Endo), Guinea Pig (Endo>Epi), Mouse (Endo>Epi)		(Barajas-Martinez et al., 2009; Gaborit et al., 2010; Osadchii et al., 2011; Remme et al., 2009; Soltysinska et al., 2009; Szabo et al., 2005)
	SCNB1 mRNA	Human (Endo>Epi)	Sheep	(Fahmi et al., 2001; Gaborit et al., 2007)
	SCNB3 mRNA	Sheep (Endo>Epi)		(Fahmi et al., 2001)
INa,late	Current	Canine (Mid>Epi=Endo), G.Pig ^K (Endo=Epi>Mid)	Rabbit	(Guo et al., 2014; Main et al., 1998; Sakmann et al., 2000; Zygmunt et al., 2001)
IKs	Current	Canine ^I (Mid<Epi=Endo), Rabbit, G.Pig (Epi=Mid>Endo) *	Guinea Pig *	(Bryant et al., 1999; Gintant, 1995; Li et al., 2002; Liu and Antzelevitch, 1995; Main et al., 1998; Szabo et al., 2005; Xiao et al., 2006; Xu et al., 2001)
	KCNQ1 mRNA	Canine, Rabbit	Human ^m	(Abd Allah et al., 2012; Ambrosi et al., 2013; Gaborit et al., 2007; Obrezhtchikova et al., 2006; Soltysinska et al., 2009; Szabo et al., 2005)
	KCNQ1 protein	Human (Epi>Mid), Canine (Epi>Mid)	Canine	(Obrezhtchikova et al., 2006; Szabo et al., 2005)
	KCNE1 mRNA	Canine ⁿ (Endo/Mid>Epi)	Human ^o , Rabbit	(Abd Allah et al., 2012; Ambrosi et al., 2013; Gaborit et al., 2007; Gaborit et al., 2010; Obrezhtchikova et al., 2006; Radicke et al., 2006; Soltysinska et al., 2009; Szabo et al., 2005)
	KCNE1 protein	Canine (Mid> Epi=Endo)		(Obrezhtchikova et al., 2006; Szabo et al., 2005)
	KCNE2 mRNA		Human, Canine	(Calloe et al., 2010; Gaborit et al., 2007; Radicke et al., 2006; Szabo et al., 2005)
	KCNE2 protein		Human, Canine	(Szabo et al., 2005)
	KCNE3 mRNA		Human, Canine	(Calloe et al., 2010; Radicke et al., 2006)
	KCNE4 mRNA		Human, Canine	(Calloe et al., 2010; Radicke et al., 2006)
	KCNE5 mRNA		Human, Canine ^p	(Calloe et al., 2010; Radicke et al., 2006)
IKr	Current	Guinea Pig (Epi>Mid=Endo),	Canine ^q , Rabbit	(Gintant, 1995; Li et al., 2002; Liu and Antzelevitch, 1995; Main et al., 1998; Szabo et al., 2005; Xiao et al., 2006; Xu et al., 2001)
	KCNH2 mRNA	Canine *, Rabbit, Ferret	Canine *, Human	(Abd Allah et al., 2012; Ambrosi et al., 2013; Brahmajothi et al., 1999; Gaborit et al., 2007; Gaborit et al., 2010; Holzem et al., 2016; Soltysinska et al., 2009; Szabo et al., 2005)
	KCNH2 protein	Human (Epi>Mid), Canine (Epi>Endo), Ferret	Human(Epi=Endo), Canine (Epi=Mid)	(Brahmajothi et al., 1997; Holzem et al., 2016; Obrezhtchikova et al., 2006; Szabo et al., 2005)
	KCNE2 mRNA		Human, Canine	(Calloe et al., 2010; Gaborit et al., 2007; Radicke et al., 2006; Szabo et al., 2005)
	KCNE2 protein		Human, Canine	(Szabo et al., 2005)

Cardiac Ion Current	Current or Gene	Mammalian Species		References
		Gradient	No Gradient	
IK _s slow	Current	Mouse		(Brunet et al., 2004)
	Kv1.5 mRNA	Human (Endo>Epi)	Canine, Rabbit, Rat, Mouse	(Abd Allah et al., 2012; Calloe et al., 2010; Costantini et al., 2005; Dixon and McKinnon, 1994; Gaborit et al., 2007; Huang et al., 2001; Rossow et al., 2006)
	Kv1.5 protein		Human, Rat, Mouse	(Costantini et al., 2005; Gaborit et al., 2007; Huang et al., 2001)
	Kv2.1 mRNA		Rat	(Huang et al., 2001)
	Kv2.1 protein		Rat	(Huang et al., 2001)
IK ₁	Current	Cat (Endo>Epi), Rabbit	Human, Canine, Guinea Pig, Rat,	(Brahmajothi et al., 1999; Bryant et al., 1999; Casis et al., 1998; Furukawa et al., 1992; Li et al., 1998; Li et al., 2002; Li et al., 2008; Liu et al., 1993; Main et al., 1998; Szabo et al., 2005; Volk et al., 2001; Xiao et al., 2006)
	Kir2.1 mRNA	Rabbit	Human ^f , Canine, Mouse	(Abd Allah et al., 2012; Ambrosi et al., 2013; Soltysinska et al., 2009; Szabo et al., 2005; Teutsch et al., 2007)
	Kir2.1 protein		Human, Canine	(Szabo et al., 2005)
	Kir2.2 mRNA	Human ^s		(Ambrosi et al., 2013; Gaborit et al., 2007)
	Kir2.3 mRNA	Human		(Ambrosi et al., 2013)

Abbreviations: Epi, epicardium, Mid, mid-myocardium, Endo, endocardium The table lists Epi>Endo expression gradients, unless noted otherwise

* Contrasting results, not enough reports were available to form a consensus Other discordant results and notes are listed in the footnotes:

^a(Zicha et al., 2004) reported an Epi>Endo gradient for Kv4.3 mRNA and protein; (Szabo et al., 2005) reported an Epi>Mid gradient for the Kv4.3 protein

^b(Rosati et al., 2001) found an Epi>Endo gradient in Kv4.3 mRNA

^d(Liu et al., 2015) and (Teutsch et al., 2007) reported a very small (1.6 fold) Epi>Endo gradient for KCHIP2 (mRNA only) in this species

^e(Calloe et al., 2010) report higher DPP6 mRNA levels in mid but no difference between Epi and Endo

^f(Szabo et al., 2005) and (Ambrosi et al., 2013) did not find any gradient Epi vs Mid or Epi vs Endo in Kv1.4 protein and mRNA, respectively

^g(Wang and Cohen, 2003) reported an Endo>Epi gradient in ICa_L

^h(Kim et al., 2010) found Epi>Endo ICa_L gradient in the rat heart

ⁱ(Gaborit et al., 2007) found an Epi>Endo gradient in Ca_v1.2 mRNA

^jINa_f measured as upstroke velocity, not in V-clamp

^k(Sakmann et al., 2000) report INa_{late} density Endo=Epi, while Main et al, 1998 found Endo>Epi using a less direct measure

^l(Obreztkhikova et al., 2006) reported no gradient in IK_s

^m(Gaborit et al., 2007) reported an Epi>Endo gradient in KCNQ1 mRNA; (Gaborit et al., 2010) found Endo>Epi in female LV only

ⁿ(Calloe et al., 2010) did not find a transmural gradient in KCNE1 expression across the canine ventricle

^o(Soltysinska et al., 2009) also report no difference between Epi and Endo in KCNE1 mRNA but found a higher expression level in Mid

^pExtremely low expression levels

^q(Obreztkhikova et al., 2006) found a gradient Epi>Endo in IK_r

^r(Gaborit et al., 2007) reported a gradient Endo>Epi in Kir2.1 expression only in the right ventricle

^s(Gaborit et al., 2007), right ventricle only

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