Translational Research in Atrial Fibrillation
A Quest for Mechanistically Based Diagnosis and Therapy

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Atrial fibrillation (AF) is the most common arrhythmia seen in clinical practice and is associated with increased risk of stroke, heart failure, and death.[1] However, currently available treatments for AF are less than satisfactory. Many drugs have been tried with limited success.[2] However, the demonstration of AF triggers in the atrial sleeves of the pulmonary veins (PVs)[3] has led to a significant improvement in therapy. Today, PV isolation by means of radiofrequency (RF) ablation is a gold standard treatment for paroxysmal AF.[4] However, the success rate of RF ablation in the more prevalent and highly heterogeneous persistent and long-term persistent AF populations has been disappointing.[5] The reasons are probably multifactorial, but undoubtedly incomplete understanding of the mechanism(s) underlying this complex arrhythmia has contributed substantially to such a poor outcome.

Increased knowledge of cardiac arrhythmias in general has come from studies aimed at elucidating cardiac impulse formation and propagation at several levels of integration, from computer modeling, through electrophysiological experiments in cells, tissues and the whole animal, and the human patient.[6] In clinical electrophysiology (EP), progress has been driven, to a large extent, by available technology designed for the detection of electrical signals on the endocardial and epicardial surfaces of the heart. Although the surface ECG is the obvious first and compulsory starting point in the clinical diagnostic ladder, programmed cardiac stimulation, combined with intracardiac activation mapping, enables electrophysiological characterization and is the cornerstone for successful diagnosis and treatment of most types of arrhythmias.[7]

In contrast to what happens in most other arrhythmic problems, electrical impulses during AF often are highly irregular, changing in number, direction, and width on a beat-to-beat basis, with significant temporal variations in the timing and morphology of the intracardiac atrial electrograms. Consequently, time-domain analysis of the signals recorded by electrodes positioned concurrently at different locations in the atria reveals extremely complex spatiotemporal activation patterns that preclude meaningful interpretation. It is, therefore, fair to state that understanding the mechanisms of AF maintenance has been hampered in part by the inability to reproducibly quantify activation patterns during ongoing AF. Consequently, the demonstration in the late 1990s that the PVs are the most common site of triggers for AF led the clinical EP community to adopt an anatomic approach to the RF treatment of AF, involving the creation of circumferential lesions around the right and the left PV ostia,[8] which probably impacts not only the triggers, but also the substrate of AF. This was in great part responsible for reducing the interest in performing extensive mapping of the atria or immersing into the study of AF mechanisms.

Perhaps more important, the relatively poor outcomes of pharmacological AF therapy, together with the apparently greater effectiveness of RF ablation in controlling the arrhythmia, has contributed to significantly reduce the interest of clinical EPs for mechanistically based approaches to AF therapy. However, basic EPs have focused on scientific and technological innovations in biology, which have provided unprecedented opportunities for the discovery of genetic and molecular bases of both acquired and inheritable arrhythmogenic diseases, with the idea of searching for novel therapeutic targets.[9] Unfortunately, the unintended consequence of the above success stories was that clinical and basic EPs stopped speaking the same language. Probably, with few exceptions, basic and clinical EPs seem to be lost in translation and appear unaware of each other's work. In other words, the reduced communication between the 2 groups during the past 20 years has engendered a void separating on one side the world of the clinical electrophysiologist, whose primary immediate interest is the health and quality of life of his/her patient, and on the other, the world of the basic electrophysiologist whose novel scientific discoveries may take years to reach that patient.

During the past 20 years, and until recently, mechanistically oriented EP studies in the clinic have been relatively scarce.[10–13] Much of the insight into EP mechanisms and complex dynamics of wave propagation in AF have come from detailed investigation using numerical and animal models of AF,[14] where there is substantially greater flexibility to explore the AF process from the molecule to the organism

and where high spatiotemporal resolution of the fibrillatory dynamics can be achieved.[15]

Excellent reviews have appeared in the recent literature on the consensus among clinicians for treatment,[1] the epidemiology, and the genetic, molecular, and pathophysiological bases of AF and atrial remodeling[9,16–20] Therefore, our aim here is not to present an exhaustive discussion on fundamental AF mechanisms. Instead, this article focuses on attempts by clinicians and basic scientists to find potential AF diagnostic tools and treatments based on the pathophysiological mechanisms responsible for AF initiation and maintenance. By discussing the evidence, we hope to promote reflection and to encourage both clinical and basic EP communities to reacquaint with each other's work, find common ground, and collaborate toward improving knowledge and advance therapy of AF.

Finding Our Way in Translation

Translation of knowledge derived from the experimental setting to clinical practice plays a critical role in scientific advancement,[21,22] and the importance of building clinical research on the solid foundations provided by scientifically rigorous basic research has been long recognized. Basic research can advance clinical research and practice in several ways[22]: (1) it informs clinical research by building the foundation of biological knowledge; (2) it guides clinicians in their practice by indicating not only what direction to pursue but also what directions are possible; and (3) it contributes to the development and testing of medical devices, surgical procedures, and candidate drugs. In fact, most medical diagnostic tests and therapies in use today were initially developed and tried in animals. For example, the development, testing, and clinical application of angiotensin-converting enzyme inhibitors constitute a paradigmatic example of successful translational research from bench to bedside.[23] However, many results of animal experiments often never undergo clinical testing or eventually fail to be replicated when tested in rigorous human trials.[21,24] Francis Collins, Director of the National Institutes of Health, has commented that despite a dizzying rate of basic science discoveries, far too often promising diagnostic devices and treatments are not making it to market. In a systematic review of highly cited animal studies, only 37% were replicated in human randomized trials, and 45% remained untested.[21] According to an article published recently in *Nature*,[24] "…translational research is seen by many as a solution to this disparity that could ensure that many fundamental discoveries are effectively translated into benefits in medicine."

Diagnosis and treatment of AF is at a point at which it would greatly benefit from translational research. Despite many years of basic and clinical research, fundamental mechanisms governing AF initiation and maintenance, and the transition from paroxysmal to permanent forms of AF are far from being understood,[15] and we have not learned how to treat AF effectively. The inexorable progression from paroxysmal to persistent and permanent AF reflects the progressive electrophysiological and structural remodeling occurring in both atria,[25–28] making the sources of the arrhythmia more stable and long-lasting. A long-term follow-up study reported a 30-year cumulative probability risk of progression from paroxysmal/persistent to permanent AF of 29%, with most of the transitions occurring within the first 15 years after diagnosis.[29] Yet, it is unknown which factors contribute to that progression, let alone whether or not we have the appropriate tools to prevent it.

Fortunately, the demonstration of AF triggers in the atrial sleeves of the PVs has led to a significant improvement in therapy.[3] Today, PV isolation by means of RF ablation is curative in ≈70% to 80% of patients with paroxysmal AF who are treated in the EP laboratory.[4] However, PV isolation is a relatively new procedure, and the very long-term outcomes are not known. In addition, AF ablation is reserved for the subset of patients with symptoms of paroxysmal or persistent AF. Furthermore, the financial burden of AF ablation therapy may be significant and is unlikely to be cost-effective for patients who have preserved quality of life despite their AF or for patients whose quality of life is not expected to improve substantially despite elimination of AF.[30] Therefore, there is much room for improvement, despite the great success. As will be further discussed later, here we and others see opportunities for translation toward the discovery of novel and effective therapies.[31] Interestingly, research into the fundamental understanding of some of the potential targets for therapy began ≈40 years ago, with the discovery of the cellular mechanisms of delayed afterdepolarization–induced triggered activity[32–34] and the mechanisms of rotor initiation and maintenance.[35]

Unfortunately, the success rate of RF ablation in the more prevalent and highly heterogeneous persistent and long-term persistent AF populations is ≈30% or less.[36] Furthermore, a slow but steady decline in arrhythmia-free survival after 5 years is noted, despite successful ablation treatment, especially among patients with persistent AF.[37] Perhaps our current incomplete understanding of the mechanisms involved in the maintenance and perpetuation of AF has prevented us from being able to generate more specific prevention and treatment of this dangerous and debilitating disease. It may be argued, however, that one important reason for such disappointing outcomes is the paucity of communication that has existed, and continues to exist, between the basic scientists and the clinicians during the past 20 years. But there is a glimmer of hope. Academic investigators on both sides of the aisle now have access to scientific resources and technology that should enable them to participate in translational research and collaborate in unprecedented ways. Advances have occurred in the basic understanding of the molecular mechanisms of AF-induced remodeling, including both electric and structural remodeling, on the one hand, and in the development of highly sophisticated magnetic resonance imaging and high-resolution electrode mapping technologies capable of identifying AF sources in the clinical EP laboratory, on the other hand. Such advances should help place basic and clinical investigators in a new position to partner together with industry and play a more powerful part in therapeutic development in both paroxysmal and persistent AF. In the following lines, we review the evolution of the principal ideas that have attempted to explain AF maintenance mechanisms, from the perspective of how translation of the results of experimental studies might influence future clinical practice.

AF Maintenance Mechanisms

The principal competing hypotheses attempting to explain AF mechanisms have been around >100 years, and their historical evolution has been recently reviewed.[15] From the beginning of the past century, 2 such hypotheses were proposed to explain AF maintenance mechanisms: single-source focus and circus movement reentry. According to these hypotheses, AF may be caused either by a rapidly discharging, spontaneously active atrial ectopic focus or by a single functional reentrant circuit. In either case, the activation wave fronts spawned at high frequency were thought to interact with the heterogeneous atrial muscle to give rise to what we know now as fibrillatory conduction. Although investigators at the time saw these 2 hypotheses as mutually exclusive, common to both hypotheses was the idea that AF was maintained by a localized source of atrial activity and required the continuous presence of the ectopic focus or, alternatively, the reentrant circuit. A third hypothesis, proposed subsequently by Moe et al,[38,39] postulated that AF depended on the sustained, but random propagation of multiple wavelets and that maintenance of the arrhythmia was self-sustaining and independent of the initiating event.

To our knowledge, not a single one of the above competing hypotheses has reached consensus among basic or clinical scientists. Over the years, arguments for or against each of the above schools of thought have been forcefully advanced by one or the other group, and the debate continues even today. Undoubtedly, the debate will continue for years to come. Below, we briefly review the pathophysiological nature of each of these mechanisms and their respective implications for potential therapy, as well as the treatment strategies that have been devised according to each conceptual hypothesis.

The Multiple Wavelet Hypothesis

The multiple wavelet hypothesis was the dominant mechanistic model of AF during the second half of the 20th century. In 1962, Moe postulated that AF was the result of randomly propagating multiple electrical wavelets, ever changing in number and direction, with local excitation limited by the heterogeneous distribution of refractory periods throughout the atria.[38,39] In Moe's model, the number of wavelets was a function of the mass of the tissue and inversely related to the product of the duration of refractory period and the conduction velocity (ie, the wavelength). The model predicted that a critical number of 23 to 40 wavelets was necessary for the maintenance of AF.[39] Any factor that increased the total number of wavelets (eg, wavelength shortening) would serve to perpetuate the arrhythmia, whereas, conversely, any factor that decreased the total number of wavelets would favor termination.[38] This hypothesis was later reinforced by high-resolution electrode mapping experiments in dogs.[40] In support of Moe's model,[39] Allessie et al[40] suggested that AF was maintained by 6 to 7 wavelets that propagated through relatively refractory tissue in which there was no fully excitable gap, enabling the coexistence of multiple simultaneous wavelets in a minimum amount of tissue (ie, critical mass). However, translation of the multiple wavelet hypothesis into human persistent AF diagnosis and therapy has been difficult and has not yet resulted in widely accepted strategies to effectively terminate AF. Nevertheless, the idea is widespread in the literature, although both experimental and clinical studies have raised important questions and argue against the applicability of the multiple wavelet hypothesis to explain sustained AF. Examples follow.

First, the surgical Maze procedure is able to successfully treat some cases of chronic AF, which has been attributed to a reduction in the tissue mass and thus the number of wavelets that the atria can sustain.[41] However, other factors may explain the success of this intervention, including PV isolation,[3] cardiac denervation,[42] and empirical elimination of sites responsible for AF maintenance.[43] Furthermore, several studies challenged the critical mass hypothesis by demonstrating that fibrillation can be sustained by tissues of very small size. Chen et al[44] showed that the minimal length that allows for the sustenance of reentry in the left atrium (LA) of the sheep heart is ≈4 mm. Gray et al[45] demonstrated theoretically that rodent hearts can accommodate 1 or 2 reentrant sources (rotors), whereas in larger hearts (eg, sheep, dogs, and humans), a greater number of rotors may coexist. Such a theoretical prediction has been borne out by the demonstration that in mammalian hearts from mouse to horse, the ventricular fibrillation cycle length scales with the fourth power of the body mass.[46] Finally, clinical studies have shown that AF can also be sustained by small portions of tissue, such as remnants of receptor atrial tissue after orthotopic heart transplantation (online-only Data Supplement Figure I),[47] which also argues against the idea of a critical mass for AF. Second, wavelength shortening would be expected to increase the number and instability of randomly propagating wavelets as a means to perpetuate the arrhythmia and increase its complexity. Instead, Schuessler et al[48] found in a canine model of acute AF that, with increasing concentrations of acetylcholine and below a critical level of refractory period (<95 ms), the rapid repetitive responses initially characterized by multiple reentrant circuits stabilized to a single circuit that became stable and dominated activation.[48]

Third, the absence of a fully excitable gap should make the arrhythmia amenable for termination by pharmacological interventions that increase the refractory period (and thereby the wavelength). It should also limit the number of wavelets that may coexist simultaneously. Thus, several drugs capable of prolonging the atrial effective refractory period (ERP), which also increases the wavelength and the size of the reentry circuit during AF, have been used in the clinic with variable success.[49,50] However, the above prediction is incompatible with the antiarrhythmic effect of Na^+ channel blockade, which, by decreasing conduction velocity and thereby wavelength, should promote AF instead of terminating it, such as occurs in clinical practice.[51] Finally, because there is no anatomically determined circuit, there should be no theoretical possibility of interrupting the arrhythmia by disrupting the circuit through the application of localized lesions. However, several experimental and clinical studies have demonstrated that AF can terminate by limited RF applications directed to localized sources in the atria.[52–54]

In what appears to be a new variant of the multiple wavelet hypothesis, Allessie et al[55] recently put forth the idea that the electric dissociation of neighboring atrial muscle bundles is a key element in the electropathological substrate of

long-standing persistent AF in patients with structural heart disease. In addition, from experiments in the goat heart, the same investigators have suggested that, during AF, pronounced dissociation of electrical activity occurs between the epicardial layer and the endocardial bundle network, which they attributed to progressive uncoupling between the epicardial layer and the endocardial bundles.[56] This interesting idea might lead to identification of novel molecular targets for therapeutic agents and the design of more effective antifibrillatory drugs. However, it is unclear why such dissociation would occur in the absence of dissociation within any of the 2 layers. Furthermore, even if longitudinal dissociation occurs, unless otherwise demonstrated, the phenomena described by the Allessie group[55,56] are fully compatible with fibrillatory conduction maintained by a single high-frequency source, either focal or reentrant, generating waves from a remote location.

Single-Source Hypothesis

Experimental evidence showing that AF may be the result of a single localized electrical source, acting as a rapidly discharging ectopic focus, dates back to the beginning of the 20th century.[57] However, this observation fell largely in disfavor as a result of the convincing evidence provided at that time by the circus movement defenders, particularly Sir Thomas Lewis.[58] Nevertheless, Lewis recognized that "[atrial] fibrillation, like flutter, may also on occasion be terminated in the auricle by cold or pressure very locally applied".[58] In 1949, Scherf and Terranova[59] induced atrial tachycardia or fibrillation by applying aconitine crystals on the epicardial surface of the right atrial appendage of the dog. The arrhythmia could be terminated by cooling the area of injection, suggesting that the mechanism of AF was compatible with an ectopic focus.[59] More recently, Yamazaki et al[60] provided direct demonstration that reentrant ventricular tachycardia and ventricular fibrillation may be terminated by local cooling at or near the center of rotation. This argues against being able to distinguish between arrhythmogenic mechanisms based on changes in temperature alone.

Nevertheless, there is no question that although focalized atrial ectopy can result from microreentry, enhanced atrial automaticity (spontaneous diastolic depolarization) or triggered activity (afterdepolarizations) is an important mechanism involved in AF initiation.[61] The vast majority ($\approx$90%) of ectopic discharges that trigger AF localize in the PVs[3] and are thought to result from either early or delayed afterdepolarizations. Early afterdepolarizations occur when action potential duration is excessively prolonged, allowing Ca^{2+} channels to recover from inactivation. Ca^{2+} enters into the cell and initiates a new action potential upstroke.[62] Delayed afterdepolarizations may be the consequence of intracellular Ca^{2+} handling dysfunction, secondary to abnormal Ca^{2+} leak from the sarcoplasmic reticulum through the type-2 ryanodine receptors channels.[63] Clinical observation indicates that autonomic blockade with propranolol suppresses bursts of AF.[64] However, isoproterenol infusion, which facilitates calcium release from the sarcoplasmic reticulum and promotes delayed afterdepolarizations and triggered activity, has a high sensitivity and specificity for induction of AF in patients with persistent atrial fibrillation.[65] Figure SII (online-only Data Supplement) shows a possible mechanism of induction of triggered activity at the PV by the intravenous infusion of isoproterenol, as routinely done in the clinical EP laboratory in combination with rapid pacing.

Rotors and Fibrillatory Conduction

A significant deviation from the prevailing concepts used to explain AF maintenance was derived several years ago from the theory of wave propagation in excitable media[35] and from studies using voltage-sensitive probes and high-resolution video imaging. The increased understanding through mathematical modeling of the manner in which waves propagate in a wide variety of physical, chemical, and biological excitable media, together with the ability to record electrical wave propagation on the surface of isolated hearts with an unprecedented spatial and temporal resolution, lead to a better understanding of the dynamics of AF and of its nature.[44,45,66] Such an advanced technology has confirmed that the turbulent electrical activity seen in the electrogram recordings of the atria may in some cases be explained by a single or a small number of rapidly spinning rotors.[67,68] Rotor formation or its maintenance does not require a permanent anatomic obstacle; a transient functional heterogeneity that is able to generate a wavebreak suffices to generate a singularity around which the newly formed wave front with extreme curvature rotates (Figure 1).[69] These studies demonstrated that the self-organized functional reentrant sources (ie, the rotors) that maintain AF adopt the appearance of a pinwheel, which rotates rhythmically at high rates and sheds large numbers of spiral waves (online-only Data Supplement Figure IIIA). A rotor resembles a hurricane or a tornado, and its vortex-like activity is organized by a phase singularity near its center at which all phases (colors) of the excitation–recovery cycle converge (online-only Data Supplement Figure IIIB).[35,45] Accordingly, the spiral waves emitted by the rotor propagate through the heterogeneous cardiac muscle and interact with anatomic and functional obstacles, leading to wave front fragmentation and fibrillatory conduction.[70]

Wave Fractionation and CFAEs

Just as the advancement of clinical medicine requires insights from basic research (ie, forward translational research), clinical work also helps clarify unresolved biological behaviors. This notion is known as backward translational research.[71,72] In fact, several proposals for basic research stemming from the AF Guidelines have been recently proposed.[72] Although these proposals are yet to be resolved, in the following lines we describe several examples of backward translational research that provided significant insights into the mechanisms of AF.

In 2004, Nademanee et al[43] proposed that areas with complex fractionated atrial electrograms (CFAEs) were critical sites for perpetuation of AF, and their elimination with RF ablation was associated with a high probability of sinus rhythm maintenance. Recent studies have targeted CFAEs at a range of atrial sites as a means to increase AF ablation success after circumferential PV isolation.[1,53] However, clinical results have been controversial at best, calling into question the role of CFAEs in AF maintenance, especially in patients with paroxysmal AF.
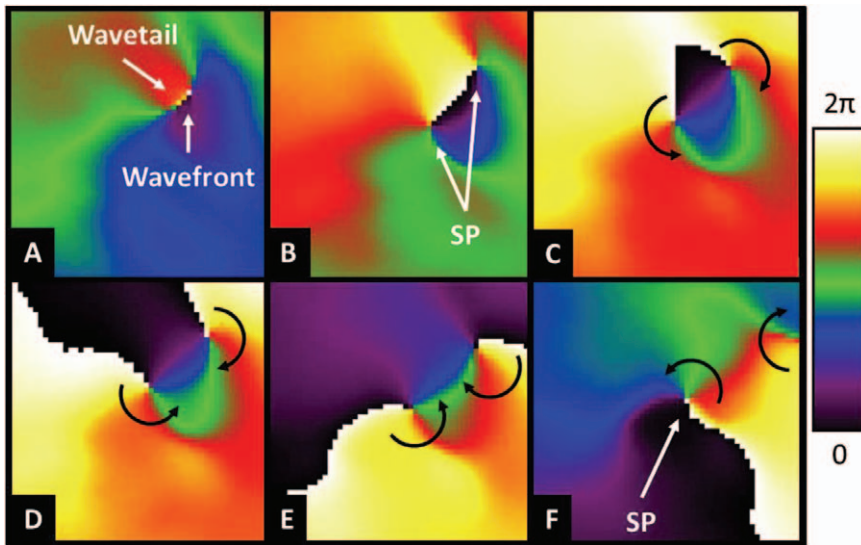
Figure 1. Wavebreak and singularity point (SP) formation. **A**, The front of a wave (green) moves rapidly to the neighborhood of the refractory tail of a slowly dissipating wave tail of a preceding wave. **B**, When the wave front reaches the wave tail, it attaches to it and divides into 2 daughter waves that propagate forward as they encounter excitable tissue. **C** to **F**, Under appropriate conditions of reduced excitability, the 2 wave fronts detach from the wave tail forming an SP at their respective broken tips. At each SP, the curvature of the wave front is extreme, which forces the rest of the wave front to curl around it and spin. In this case, 2 counter revolving rotors would be formed.

Although some fractionated signals might represent critical zones related to AF maintenance (ie, high-frequency sources driving AF), others might be passive and unrelated to the primary arrhythmia mechanism (ie, wave front collision or overlapping).[1,10,73] Still others appear to be located in areas surrounding the autonomic ganglionated plexi.[74,75] In fact, the pathophysiological relationship between high-frequency PV discharges and CFAEs on the posterior left atrial wall is far from being understood. Experimental and clinical studies have shown that fractionated signals need not be critical for AF maintenance. Kalifa et al[76] found that the periphery of high-frequency AF drivers is the area at which most fractionation occurs. That is the area where the phenomenon of fibrillatory conduction is most manifest. Rotor meandering might also underlie, at least in part, the electrogram fractionation at close proximity of the source.[73] This might explain the success of some CFAE ablation procedures that by chance produce an anatomic obstacle around the highest dominant frequency (DF) site. However, other studies suggest that CFAEs might be unrelated to the primary arrhythmia mechanism and simply represent transient pivoting, wave front collision, or wave fractionation.[10,13,77] The latter has been confirmed by a recent study with typical bench-to-bedside design showing that, in paroxysmal AF, electrogram fractionation at the posterior left atrial wall is a reflection of fibrillatory conduction and a consequence of the dynamic interaction between high-frequency reentrant sources and the atrial anatomy (online-only Data Supplement Figure IV).[78,79]

Validation of Experiments Using Optical Mapping

Not surprisingly, most experimentally derived information on mechanisms of cardiac fibrillation has come from large animal models.[14] In general, fibrillation in small animals such as mice or rats is difficult to initiate or maintain for relatively long periods of time. Nevertheless, as discussed above, there might be a strong similarity in the underlying mechanisms of fibrillation in most, if not all, mammalian species,[46] which may be of considerable fundamental and practical

significance and provides some validation for the transition of data derived from experimental studies to arrhythmias in humans, including AF. We summarize some of these results in the next section.

Left-to-Right Atrial Frequency Gradients

Several methods have been used to analyze and compare AF signals. On the one hand, time-domain analysis is routinely used in the clinical EP laboratory to record the electrical signals of the atria during AF. However, a few catheter-based electrodes positioned at different locations in the atria commonly reveal extremely complex spatiotemporal activation patterns that often preclude straightforward interpretation. On the other hand, analyses in the frequency domain, while imperfect,[66] enable reliable quantification of the local frequency of activation, which reveals a high degree of spatiotemporal organization.[80] Using these signal processing methods, experimental studies in the isolated sheep heart have demonstrated that the highest frequency rotors that maintain acute cholinergic AF are usually localized in the LA. Propagation of the waves that emanate from such rotors undergo spatially distributed intermittent block processes that give rise to fibrillatory conduction and result in a left-to-right gradient of excitation frequencies.[71,73,79,80] More recently, a significant number of studies have characterized the spatial distribution of DFs during AF in patients, confirming the existence of a similar hierarchical organization in the rate of atrial activation in both patients with paroxysmal and chronic AF.[52,54,81,82] Simultaneous time- and frequency-domain analyses have demonstrated a high correlation between the location of maximal DF areas and the origin of incoming waves, which led investigators to infer that during organized AF episodes the posterior wall of the LA is passively activated from high-frequency sources located at the PV–LA junction.[7,79] As a natural consequence of these studies, real-time spectral mapping techniques have been used to identify and ablate high-frequency sites in patients with AF,[54] and recent work has demonstrated that an unequal distribution of inward rectifier[28] and other K^{+} currents[27] underlies this

spatial distribution of frequencies, which may represent new therapeutic targets.

An important limitation of using DF mapping in human patients is the low number of intracardiac electrodes that can be used simultaneously in the clinical EP laboratory, which makes it hard to localize the highest DF sources.[81] Optical mapping techniques, on the other hand, enable high-resolution mapping and relatively easy localization of the highest frequency sources, as well as the determination of the frequency gradients and their quantification. In fact, cardiac optical mapping has evolved to become a powerful tool that can provide new mechanistic insights into the most complex atrial and ventricular arrhythmias.[83] In the ventricles, its submillimeter spatial resolution enabled the demonstration that ventricular fibrillation in isolated human hearts is associated with wavebreaks and singularity point formation and is maintained by high-frequency rotors and fibrillatory conduction.[84] With the development of new infrared voltage-sensitive dyes,[85] optical mapping based on sophisticated catheter-based endoscopic mapping protocols (online-only Data Supplement Figure V) may someday be used in the clinical EP laboratory to localize AF sources in the human heart.[86] However, until that day comes, catheter electrode mapping will have to suffice. As discussed below, important advances in this area have created new enthusiasm for the possibility of using mechanistic approaches to effectively terminate AF based on novel RF ablation strategies.

Role of High-Frequency Sites in Human AF Maintenance

Using a canine chronic AF model, Morillo et al[87] demonstrated that AF cycle lengths were consistently shorter in the LA than in the right atrium (RA). The shortest cycle lengths were measured in the area of the posterior left atrial wall near the PV–LA junctions. This suggested that AF in that model was maintained by high-frequency drivers in the posterior LA that gave rise to consistent left-to-right frequency gradient.[69] Furthermore, cryoablation of this area significantly prolonged atrial cycle length and successfully restored sinus rhythm in most dogs, confirming the crucial role of localized sites with high activation frequency. The observations of Morillo et al[87] were amply confirmed and expanded upon by large numbers of experiments in the sheep heart.[67,70] Confirmation of a hierarchical organization of AF frequencies in humans, however, came later, with seminal work of Haïssaguerre et al[3] demonstrating that ectopic foci in the PVs were the most common triggers, that they were capable of initiating and even maintaining AF, and that they could be eliminated by treatment with RF ablation.

Recently, we used a combination of real-time DF mapping and RF ablation to determine the safety and long-term outcome of targeting maximal DF sites in patients.[54] We demonstrated that real-time spectral analysis of AF was safe and that it enabled identification and elimination of sources responsible for AF maintenance (Figure 2). Most important, we showed that targeting such sources followed by circumferential PV isolation resulted in long-term sinus rhythm

maintenance in 75% of paroxysmal and 50% of persistent AF patients.[54]

As discussed above, most experimentally derived information on the existence of rotors or drivers as a mechanism of cardiac fibrillation has come from large animal models using high-resolution video imaging.[14] This is not surprising and not only caused by conceptual differences but also by substantial differences of mapping techniques used in the clinic as well as in various experimental laboratories. Not only is the number of electrodes highly variable, but the electrodes may be unipolar or bipolar, the mapped area may be too small, and while some investigators use high-resolution cameras others use photodiode arrays.[83] The type of voltage-sensitive dyes and the software specifically designed to look for AF sources also vary. Undoubtedly, all of the above have contributed to the inconsistent detection of rotors in both animal models and humans. Therefore, perhaps the most remarkable development in the field of AF therapy since the discovery of the PV triggers has been the recent direct demonstration by Narayan et al[88] that AF in humans may result of long-standing rotors with fibrillatory conduction to the surrounding atrium. They used a novel computational mapping approach via 2 basket catheters, one in the RA and the other in the LA, each with 64 electrodes, to reveal AF rotors in the left or right atrium. Most important, brief RF ablation at or near the center of rotation alone acutely terminated AF,[88] which supported the conclusion that rotors are the primary drivers of AF, at least in some patients. The results further suggest that heterogeneity in the atrium in the form of spatially distributed refractory period gradients constitutes a likely arrhythmogenic substrate in which rotors remain relatively stable as a result of such gradients, and that waves emanating at high frequency result in the turbulent electrical activation which manifests as fibrillatory conduction. Although the results of Narayan et al[88] need to be confirmed by other laboratories, they are nevertheless an exciting mechanistically-based approach to AF therapy, which derives from translation of basic science knowledge into clinical practice.

Conclusions

There is an urgent need for enhanced communication between basic scientists and clinical electrophysiologists. Clearly, both communities are interested in preventing the expansion of the AF epidemic by improving outcomes in AF therapy. Together, both communities, in collaboration with industry, should endeavor to identify strategies to pursue the translation of their discoveries into clinical practice. Although RF ablation has been relatively successful in improving the quality of life of some patients with AF, it is clear that current ablation strategies are insufficient for controlling the arrhythmia in the majority of patients. Mechanistically based approaches to AF therapy offer a potentially exciting alternative, but this requires greater communication and collaboration. We submit that generating insights into AF mechanisms through novel theory and experimentation will require the use of relevant numerical and biological models, where translation of basic science concepts into clinical practice should be the critical final step. Translational EP will be essential in our future
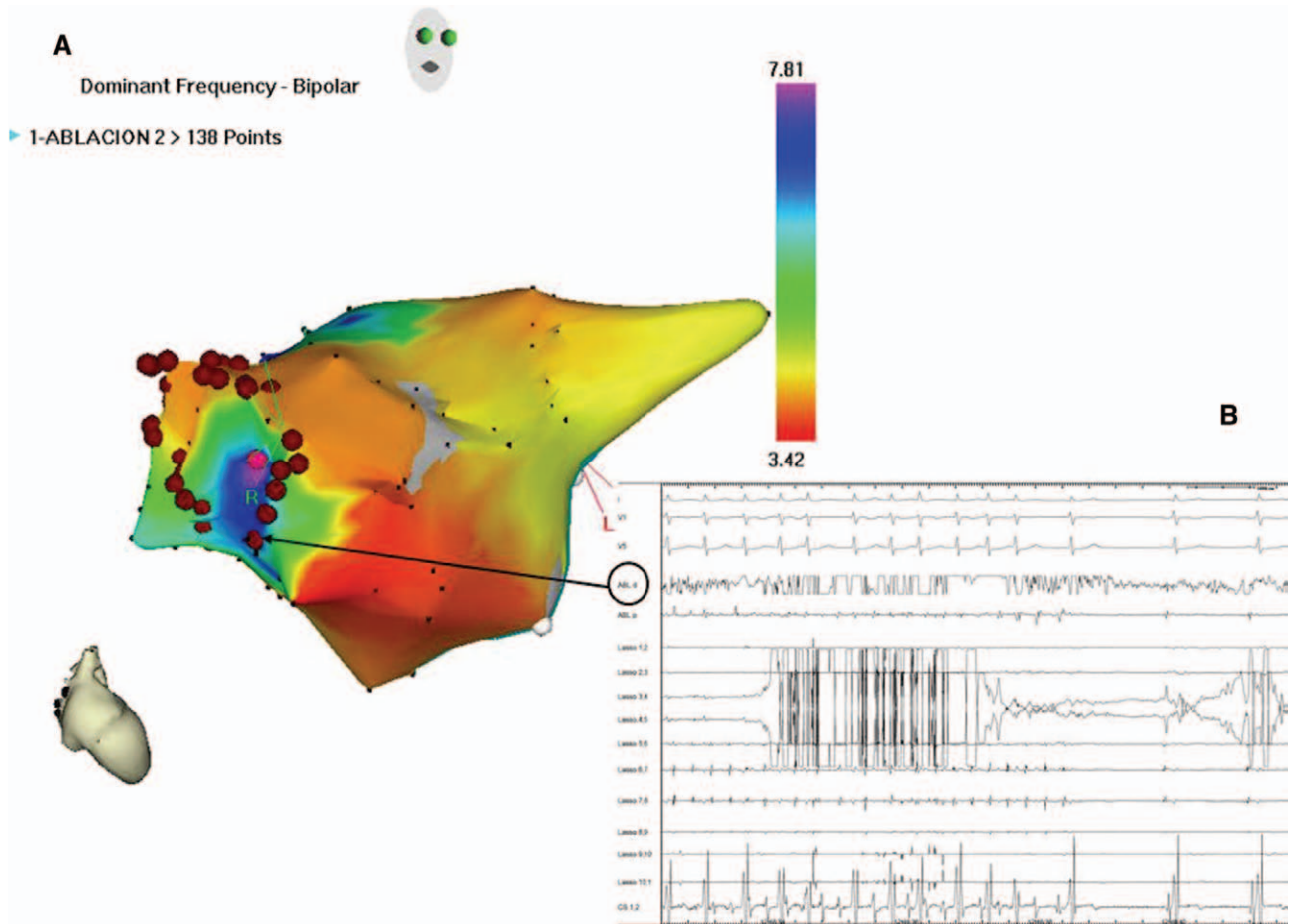
Figure 2. **A**, Real-time atrial dominant frequency (DF) map (right anterior view; CARTO system) in a paroxysmal atrial fibrillation (AF) patient. Purple, High DF site on right superior pulmonary vein (PV) antrum. Red dots, Circumferential ablation line. **B**, Surface ECG leads and intracardiac lasso catheter electrograms within the right superior pulmonary vein (RSPV); coronary sinus and ablation catheter (**black arrow**) during radiofrequency delivery, with sinus rhythm conversion, before isolation of the RSPV, confirming the reentrant nature of AF, incompatible with the multiple wavelet theory (unpublished).

attempts to improve patient care and develop new, safe, and more successful therapies.

Disclosures

Dr Atienza, received a research grants from St Jude Medical and is on the Advisory Board of Medtronic, Inc. Dr Jalife receives a research grant from Gilead, Inc; he is on the Scientific Advisory Board of Topera, Inc (stock options) and the Scientific Advisory Board of Rhythm Solutions, Inc (stock options). R.P.M has no conflicts to report.

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Translational Research in Atrial Fibrillation: A Quest for Mechanistically Based Diagnosis and Therapy

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Translational Research in Atrial Fibrillation:

A Quest for Mechanistically Based Diagnosis and Therapy

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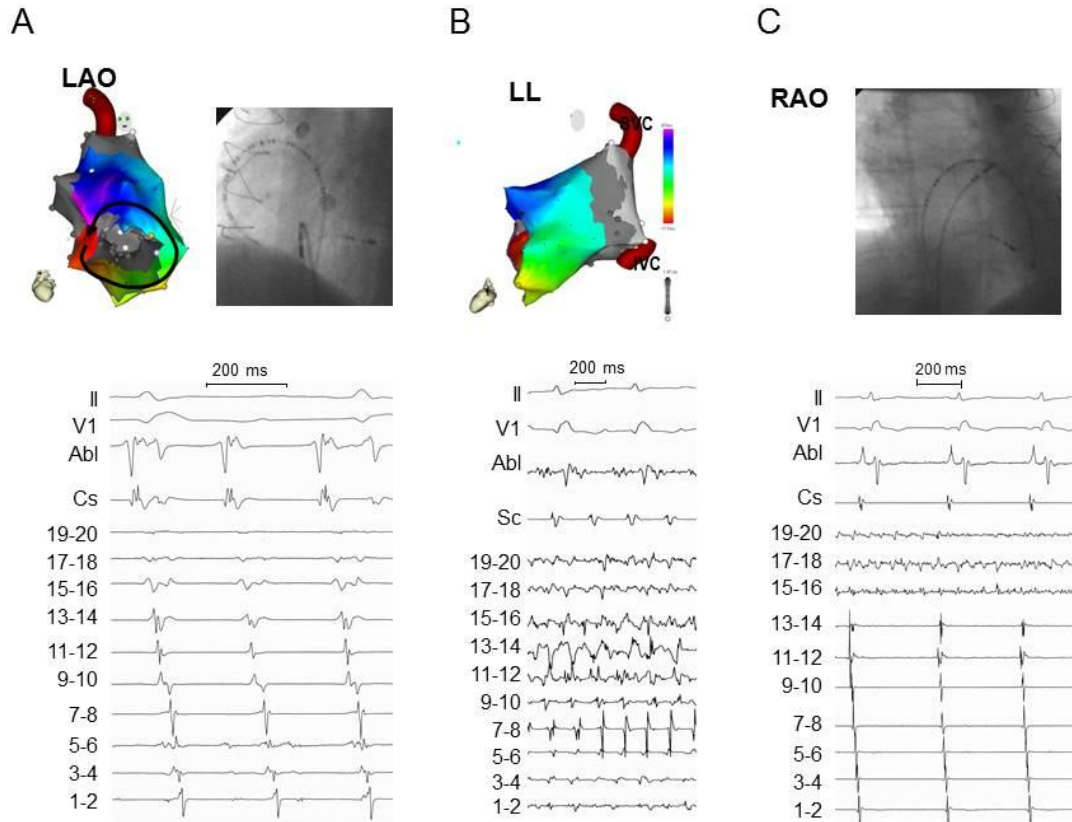
Figure S1. Typical atrial flutter in a transplanted heart, with atrial fibrillation in the remnant atrial tissue of the recipient atrium. Panel A. Surface electrocardiogram shows typical atrial flutter in leads II and V1, and the sequence of intracardiac electrograms recorded along the tricuspid annulus of the right donor atrium (corresponding to the LAO fluoroscopic view above). The LAO electroanatomic activation map shows counterclock wavefront propagation around a reentrant circuit bounded anteriorly by the tricuspid annulus and posteriorly by extensive areas of scar in the connection with the recipient atrium. Dark gray represents zones of low voltage (<0.1 mV) that correspond to the connecting orifice scar and light gray area corresponds to the recipient atrium. Panel B shows typical atrial flutter recordings in surface electrocardiogram and CS and ablation catheter positioned at the cavotricuspid isthmus, while intracardiac electrograms recorded from the multipolar catheter positioned at the recipient atrium (light gray area in LL projection) is recording atrial fibrillation. Panel C, RAO fluoroscopic projection after sinus rhythm conversion at the donor atrium following cavo-tricuspid isthmus ablation, with sinus rhythm activation recorded in bipoles located at the donor´s atrium, while the recipient atrium remained in atrial fibrillation (15-20). LAO: left anterior oblique; LL: left lateral; RAO: right anterior oblique. (Modified from ref. [1] with permission).
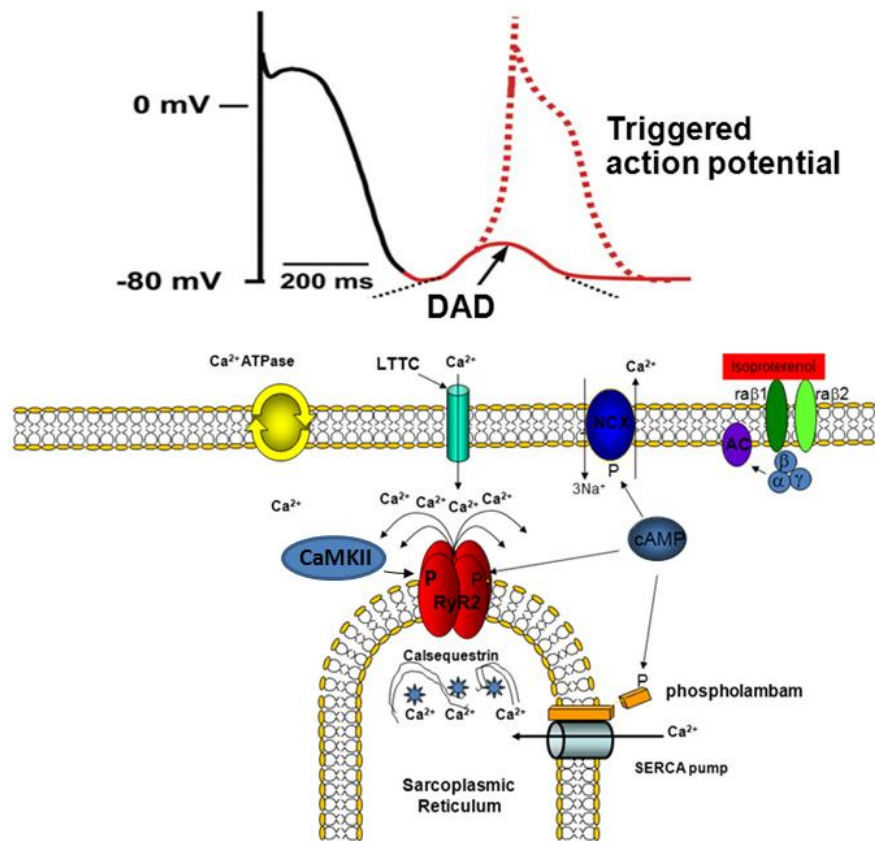
Figure S2. Hypothetical mechanism of induction of triggered activity at the PV induced by intravenous infusion of isoproterenol in the clinical EP lab. Beta-adrenergic stimulation induces cAMP mediated phosphorylation of RyR2 and other calcium regulatory proteins. This, together with rapid pacing promotes the leakage of calcium from the SR, but the precise mechanism is still under debate.[2] Ca^{2+} leak might be a consequence of an increase in luminal SR Ca^{2+} concentration, a reduced SR Ca^{2+} binding to calsequestrin or hyperphosphorylation of RyR2, which would increase its sensitivity to cytosolic Ca^{2+},[3] thus reducing the threshold SR Ca^{2+} content at which SR Ca^{2+} leak occurs. Ca^{2+} leak results in calcium overload during diastole and also activates CaMKII leading in return to RyR2 phosporylation.[4] The calcium is recycled to the SR by the SERCA pump or extruded from the cell by the sodium-calcium exchanger (NCX). NCX transports three Na^+ ions into the cardiomyocyte in exchange for a single Ca^{2+} ion moving out. Therefore, the exchange across the membrane is electrogenic, which creates a net depolarizing current called 'transient inward current' or I_{ti} leading to a DAD, which if of sufficient amplitude may generate triggered activity and premature impulse propagation, ultimately leading to wavebreak, reentry and sustained AF. Voigt et al recently demonstrated that this mechanisms in human right-atrial myocytes was mediated by hyperphosphorylation of RyR2 induced by the Ca-calmodulin dependent kynase-II (CaMKII).[4] Therefore, while not universally accepted, the general consensus appears to be that CaMKII, and not protein kinase A (PKA), increases calcium leak.[2] Consequently, it is likely that the proarrhythmic activity of CaMKII mediates DAD initiation. As discussed very recently by Dobrev et al this and other recent discoveries provide a robust mechanistic rationale for the development of new anti-AF drugs[5]
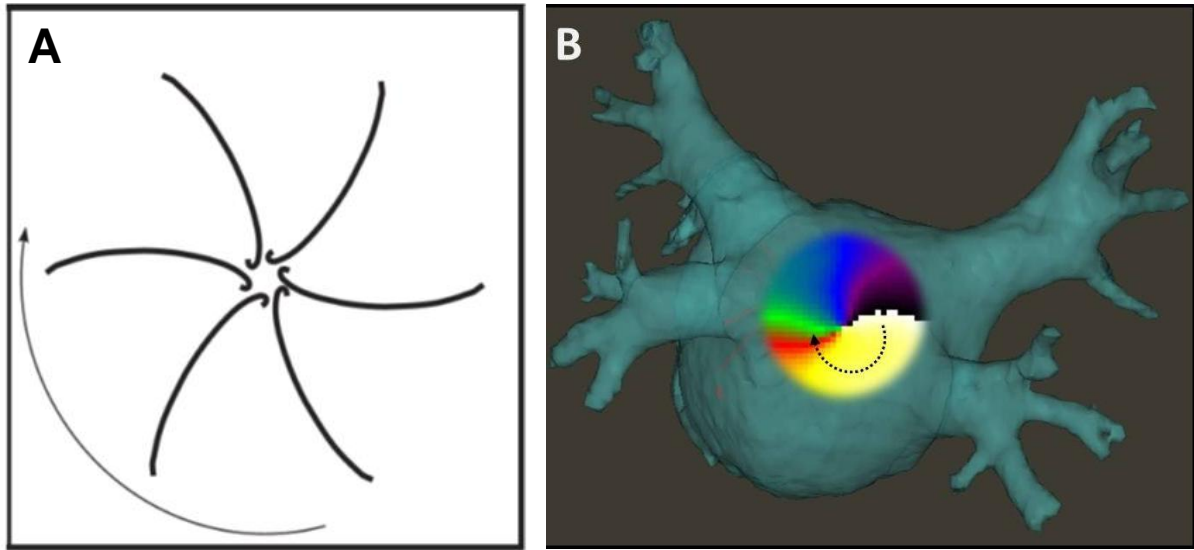
Figure S3. The behavior of a rotor. A. Once formed, the rotor adopts the shape of a pinwheel that spins at high speed and sheds multiple spiraling wavelets in its periphery. B. Montage indicating how a clockwise spinning rotor on the posterior left atrial wall of a human heart would be seen when recorded optically at high spatio-temporal resolution.
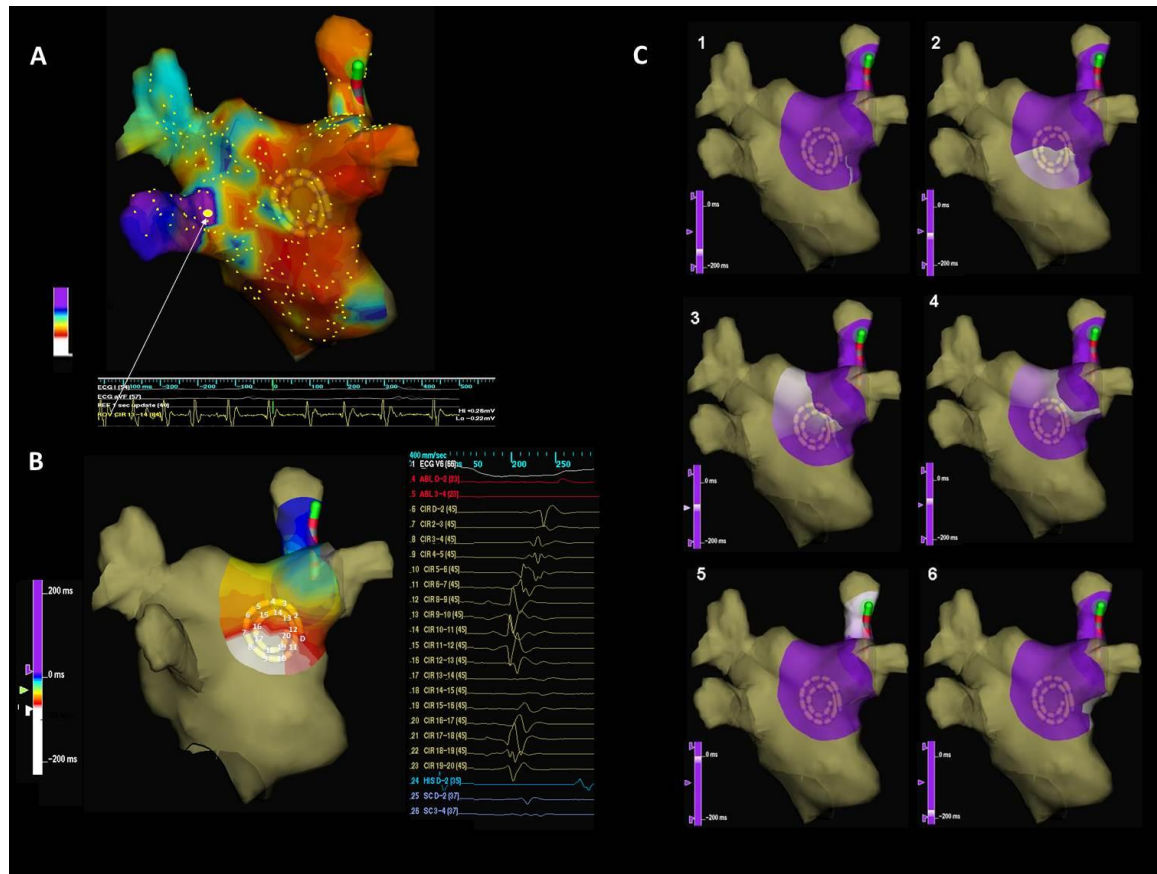
Figure S4. A: Left atrial dominant frequency (DF) map (posterior view) with highest DF site (10.8 Hz) at the left inferior pulmonary vein antrum (white arrow). B: Posterior left atrial wall (PLAW) activation map and corresponding intracardiac recordings during organized phase before fragmentation shows an incoming wave pattern of activation progressing from closest to the highest DF site at the left inferior pulmonary vein (left, white) to the right (purple-blue). C: Snapshots of wave propagation at the PLAW during transitions to fragmentation. Sequence 1 to 6: purple, unactivated regions; white, advancing activation. Re-entrant circuit with a clockwise propagation around a pivoting point located to the right edge of the septo-pulmonary bundle. (Modifed from Ref. 82 by permission of the publisher).
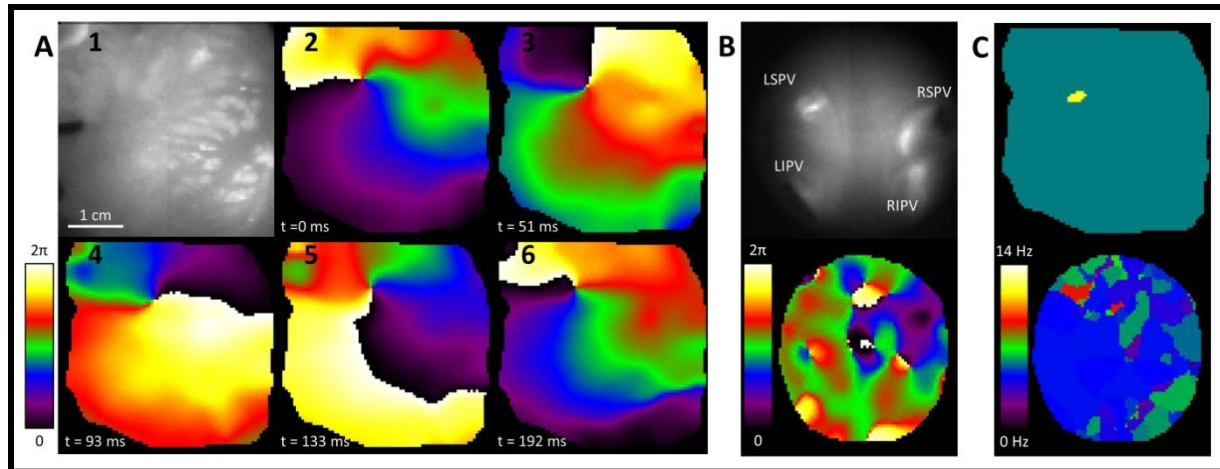
Figure S5. A. Spiral wave on the left atrial free wall of an isolated sheep heart during sustained AF induced by burst pacing in the presence of stretch (11 cm H_2O); A1: Snapshot of the area visualized by the CCD camera. The tip of the pacing lead is visualized on the left side of the image. A2 through A6: Snapshots of a phase movie revealing the presence of a stable spiral wave rotating clockwise. B. The posterior left atrium mapped simultaneously using a rigid borescope (upper panel) demonstrates fibrillatory conduction in the posterior wall of the left atrium (lower panel). C: Dominant frequency (DF) maps of the left atrial free wall (upper panel) and the posterior left atrium (lower panel) showing the presence of a high DF area at the center of the core (upper panel, DF = 11.9 Hz) and lower DFs at the periphery of the spiral wave (5.5 Hz). The DF in posterior left atrium (lower panel) is heterogeneous, with a maximum of 9 Hz and resulting of the fibrillatory conduction.

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