Biology and pathobiology of cardiac connexins: From cell to bedside

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This paper, presented on the occasion of the 1st Annual Douglas Zipes Lecture at the 2005 Scientific Sessions of the Heart Rhythm Society, briefly reviews current knowledge on the role of gap junctions in normal cardiac electrophysiology and the contributions of gap junction remodeling in the pathogenesis of malignant ventricular arrhythmias and sudden cardiac death. It highlights recent advances and new research directions in gap junction biology.

KEYWORDS Douglas Zipes Lecture; Gap junctions; Connexins; Cell–cell communication; Conduction

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Introduction

Being selected to present the Douglas Zipes Lecture at the 2005 Scientific Sessions of the Heart Rhythm Society and to author this paper is a great honor. Because this lecture was the first to honor Dr. Zipes, it is appropriate to spend a few moments highlighting his contributions to academic medicine in general and cardiac electrophysiology in particular.

The 20th century was the century of academic medicine, an era in which medical schools and academic health centers developed and matured into the principal institutions driving advances in knowledge and improving the health and well-being of people worldwide. During this century of ascent, the tripartite missions of academic medicine were defined and nurtured: patient care, research to advance knowledge, and training of future generations of physicians and life scientists. Throughout this development, special individuals who, by virtue of their prodigious intellect, uncommon vision, relentless energy, and enormous generosity of spirit, have defined the very essence of academic medicine. Douglas Zipes is one of these exceptional leaders, not only in electrophysiology and cardiology but in all of academic medicine.

Throughout his extraordinary career, Dr. Zipes has literally pioneered a field of medicine that has had immeasurable impact on patients. Perhaps more than any single individual, he has defined clinical electrophysiology and has made fundamental, seminal contributions to our understanding of arrhythmia mechanisms and sudden death. Along with his close friend and colleague, José Jalife, he produced the definitive textbook in the field, *Cardiac Electrophysiology: From Cell to Bedside,* now in its fourth edition. He founded and nurtured not one, but two, journals, which have become major sources for the dissemination of new knowledge in electrophysiology. He has fulfilled prominent national leadership roles as President of the North American Society of Pacing and Electrophysiology (now the Heart Rhythm Society), President of the American College of Cardiology, President of the Association of American Cardiologists. and President of the Cardiac Electrophysiology Society. Along the way, Dr. Zipes has trained innumerable students and fellows and has touched the lives of countless colleagues in so many positive ways. It is entirely fitting, therefore, that the Heart Rhythm Society recognize Dr. Zipes with the Douglas P. Zipes Lectureship, which will serve as enduring recognition of his remarkable contributions to our field.

Cell–cell communication in the heart

In this lecture, I would like to briefly highlight selected aspects of intercellular electrical coupling at gap junctions

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in the heart and comment on exciting new research directions in this field. Intercellular communication via gap junctions is a fundamental biologic process by which cells communicate with one another. Nearly all cells in all metazoan organisms are connected to neighboring cells by *gap junctions,* aggregates of intercellular channels that span the extracellular space to create aqueous pores that directly connect the cytoplasmic compartments of neighboring cells. Interestingly, however, vertebrates and invertebrates use two distinct types of proteins to form gap junction channels. Vertebrates make gap junctions from *connexins,* whereas invertebrates make intercellular communicating junctions having strikingly similar properties from a completely unrelated family of proteins referred to as *innexins* (*in*vertebrate co*nnexins*).[1] Furthermore, innexin orthologues have been detected in vertebrates, leading to the discovery of yet another family of cell–cell communication proteins termed *pannexins.*[2,3] These proteins are expressed in the central nervous system, where they may participate in synchronized, oscillatory activity in neuronal ensembles.[3]

Gap junction channels traditionally have been thought of as relatively nonselective pores that allow passage of ions and small molecules up to ~1 kDa molecular weight. Recently, however, it has been convincingly demonstrated that small peptides can traverse gap junction channels,[4] thus expanding our understanding of the range of chemical signals that may spread through gap junctions within solid organs. In the heart, gap junctions fulfill a major function in electrical coupling. Because cardiac muscle is not a true electrical syncytium, electrical activation requires intercellular transfer of current at gap junctions and, because current can only flow from one cell to another through gap junctions, it follows that the number, size, and spatial distribution of gap junctions are important determinants of impulse propagation. Moreover, alterations in gap junction number or distribution may lead to conduction disturbances that contribute to arrhythmogenesis.

In ultrathin tissue sections viewed by transmission electron microscopy, gap junctions appear as discrete structures in the sarcolemmas of adjacent cells in which the outer lipid layers are closely opposed, creating a characteristic pentilaminar appearance (Figure 1). Freeze-fracture preparations demonstrate that gap junctions consist of closely packed arrays of intramembranous particles, each a single intercellular channel (Figure 1). Mammalian cardiac myocytes are among the most highly coupled cells in nature, presumably reflecting the evolution of extensive low-resistance intercellular connections to ensure efficient, safe electrical activation of the heart.

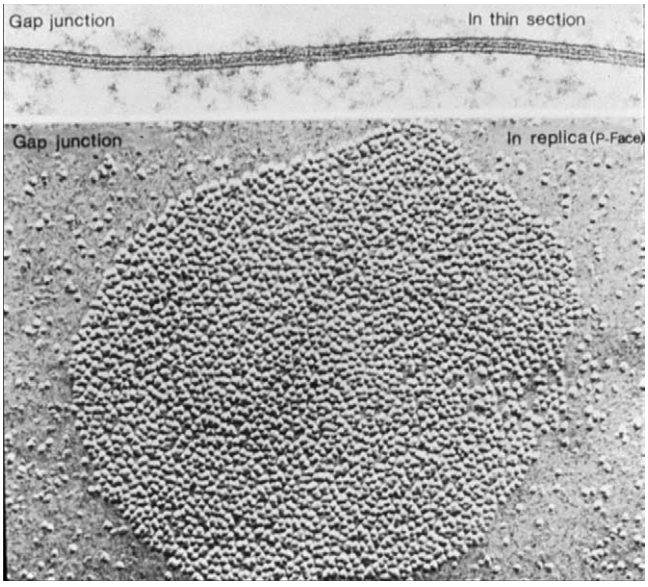

Figure 1 Gap junctions viewed by transmission electron microscopy in ultrathin tissue sections **(top)** and by freeze-fracture to split membranes **(bottom).** In tissue sections, a gap junction displays a pentilaminar structure caused by the close approximation of the outer lipid bilayers of the cell membranes of neighboring cells. Freeze-fracture reveals closely packed, intramembranous particles, each of which is a single intercellular channel.

Diversity of cardiac connexins and tissue-specific patterns of cardiac myocyte connections

The modern era of cardiac connexin biology began in 1987 with the cloning of connexin43 (Cx43), the major cardiac gap junction protein.[5] With the advent of genomic sequencing, we now know that the human genome contains 21 different connexin genes.[6] It also has become clear that, with few exceptions, individual cells express multiple connexins. At least five connexins (Cx43, Cx40, Cx45, Cx31.9 and Cx37) are expressed in whole-heart tissues, which contain cardiac myocytes, vascular and interstitial cells, and other cells types such as adipocytes and mesothelium. Four of these connexins are expressed by cardiac myocytes, but different regions of the heart express different amounts and combinations of the cardiac connexins.[7] For example, atrial myocytes express abundant amounts of Cx43 and Cx40 but only a very limited amount of Cx45. In contrast, ventricular myocytes express large amounts of Cx43 but only trace levels of Cx45 and no detectable Cx40. Specific tissues of the cardiac conduction system, such as the sinus and atrioventricular (AV) nodes and the ventricular bundle branches, exhibit variable connexin phenotypes. Cx40 and Cx45 are the major coupling proteins in different regions of the conduction system, whereas Cx43 is expressed less abundantly or is absent. Recently, Cx30.2 expression has been convincingly demonstrated in the murine cardiac conduction system,[8] and it appears that the human homolog Cx31.9 also is expressed in the human heart.[9]

Another important observation that has emerged during nearly two decades of research on cardiac gap junctions is the fact that cardiac myocytes in different cardiac tissues are interconnected by gap junctions in varying numbers and three-dimensional patterns that are consistent with the conduction properties of the tissue.[7] For example, a typical canine ventricular myocyte is connected, on average, to 11.3 other myocytes in compact ventricular muscle (Figure 2). These connections