*Am J Physiol Gastrointest Liver Physiol* 294: G368–G371, 2008;
doi:10.1152/ajpgi.00530.2007.

# Do we need to revise the role of interstitial cells of Cajal in gastrointestinal motility?

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AS ITS NAME SUGGESTS, the *tunica muscularis* of the gastrointestinal tract is dominated by smooth muscle cells, which perform all the mechanical work required for digestion, absorption, and waste removal. The muscle layers also contain several other cell types, which, despite representing a much smaller percentage of the total cellular content, also contribute to gastrointestinal motility by regulating smooth muscle contractions. In this group belong interstitial cells of Cajal (named after Santiago Ramón y Cajal and commonly referred to as ICC), which represent ~5% of cells within the muscular coat. ICC are mesenchymal cells that have been described throughout the gastrointestinal tract of all vertebrates studied to date (15). They can be distinguished from other cell types on the basis of their light microscopic and ultrastructural morphology (16), gene expression pattern, and surface markers (2). Until the discovery of Kit, a type III receptor tyrosine kinase, as a light microscopic marker for ICC (10), investigators could only speculate on the function of these cells on the basis of less specific histochemical staining techniques and electron microscopy and by relying on relatively crude approaches to separate them from the rest of the tissues for physiological analyses. The identification of the interaction between Kit and stem cell factor (SCF or Kitl), its natural ligand, as the most specific target for genetic and pharmacological manipulation of ICC also paved the way for further, more mechanistic investigations. The first part of the "post-Kit era" culminated in the concept that functions previously attributed solely to smooth muscle cells and the extrinsic and intrinsic innervation of the gut may be performed, mediated, or aided by ICC (16). These include the generation and propagation of electrical slow waves underlying rhythmic contractile activity in the phasic parts of the gastrointestinal tract and mediation of communication between the smooth muscle and the autonomic (systemic and enteric) nerves. Later a role in mechanoreception was added (4), and the notion that changes in ICC populations likely play a role in the pathogenesis of various diseases also emerged (19). An exponential rise in interest and studies followed, which further enriched and refined these concepts and broadened the horizon by looking beyond the gut in search of ICC-like cells to explain functions shared by tubular, smooth muscle-lined organs. From these studies emerged a more integrative and nuanced view of the physiology and pathophysiology of gastrointestinal motility and of the role of ICC therein (5, 6, 13, 16). However, significant gaps in our knowledge remain, and it could be argued that filling those gaps and devising more rational therapeutic strategies for disorders involving ICC will require critical reevaluation of the existing data and the development and application of novel concepts and methodology to gastrointestinal motility research (13, 16).

In this issue of *American Journal of Physiology Gastrointestinal and Liver Physiology*, Dr. Sushil Sarna takes a critical look at the evidence supporting various roles of ICC in gastrointestinal motor functions and concludes that besides setting the membrane potential of smooth muscle cells by releasing the inhibitory gaseous neurotransmitter carbon monoxide, ICC play little, if any, physiological role (17). This concept is based on earlier views of the control of gastrointestinal motility that only assigned major roles to the smooth muscle and the autonomic (systemic and enteric) nervous system. In this paradigm, smooth muscle cells would produce electrical slow waves, perform mechanical work, and serve as the only relevant recipient and source of information needed for enteric reflexes and motor patterns.

Is such a dramatic return to an old paradigm really justified? In his review, Dr. Sarna points out data in the literature that he uses as an argument to refute the current concepts on the roles of ICC in slow-wave generation, mediation of neuromuscular neurotransmission, and mechanoreception. Reexamining concepts from a new aspect is always important for furthering scientific research, and this provocative review will certainly force many in the research community to reassess the literature. It is ultimately up to the informed reader to decide about the proper course of action in response to the issues raised. Are the new data strong enough to justify shutting down efforts in a particular direction? Is the alternative concept presented compelling enough to replace the one in question, or, rather, should we consider the highlighted inconsistencies as unsolved problems requiring that we "raise our game" and employ innovative approaches to get answers? The purpose of this editorial is to jump-start this process by examining Dr. Sarna's hypothesis and by discussing whether the right action in response to the raised issues is to abandon and ignore ICC and resuscitate old paradigms or, alternatively, to give serious consideration to the remaining inconsistencies and then attempt to resolve them by applying novel, state-of-the-art concepts and methods of the postgenomic era to gastrointestinal motility research.

## *Which Cell has the Clock for Timing the Slow Waves?*

Manifestations of electrical slow waves can be simultaneously recorded from both smooth muscle cells and ICC (5, 8), but which of these two cell types times their periodicity? There is no controversy that ICC possess an electrical pacemaker mechanism that is robust and capable of producing large-amplitude oscillations even in isolation. However, according to the hypothesis advanced in Sarna's review, this clock is not the physiological source of slow waves and is

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replaced by another oscillator, presumably located in smooth muscle cells, which produces smaller, less regular, and more erratic activity but can still be detected in the absence of pacemaker ICC. To make these oscillations compatible with the rhythmic, large-amplitude, robust activity detectable in normal tissues, his hypothesis requires another factor that "stabilizes" them such as carbon monoxide, known to be generated by ICC (12), which would accomplish this task by hyperpolarizing the primary pacemaker away from the slow-wave reversal potential. However, this hypothesis is problematic. The experimental data do not support the notion that changes in the resting membrane potential could account for the loss (or loss of detectability) of slow waves in tissues depleted of ICC. First, the quoted value for the slow-wave reversal potential (about −40 mV) is too low for the tissues in which the effects of ICC depletion were studied. For example, depolarization of normal murine gastric antrum tissues with carbachol to at least −30 mV still permitted the resolution of clear slow waves (7). Second, flat lines, sporadic slow waves, or erratic spiking activity have been recorded at resting membrane potentials considerably less negative than −40 mV both in murine gastric tissues pharmacologically depleted of ICC and in the small intestine of *Sl*/*Sl*$^{d}$ (SCF mutant) mice and in the colon of *Ws*/*Ws* (Kit mutant) rats, which have profoundly reduced pacemaker ICC populations (1, 11, 14). A landmark study on the loss of slow waves in the small intestine of *W*/*W*$^{v}$ (Kit mutant) mice reported an average resting membrane potential of −57.4 ± 1.8 mV (20). Moreover, mice with genomic deletion of heme oxygenase-2, the major synthetic enzyme for carbon monoxide in ICC, still exhibited regular slow waves indistinguishable from wild-type mice, despite the loss of the smooth muscle membrane potential gradient (22). Thus the stabilization/destabilization hypothesis is not supported by the literature. In contrast, the concept that ICC are the dominant, primary pacemakers is supported by direct evidence such as the sequential activation of electrical slow waves and corresponding $Ca^{2+}$ signals in ICC and smooth muscle cells (5, 8). Also, the model proposed by Dr. Sarna replaces a simpler model (one pacemaker: the ICC) with a more complex one that involves one pacemaker (the smooth muscle cells) plus one stabilizer (the ICC), and the smooth muscle pacemaker would function in the presence of, but without any influence from, a coexisting robust electrical rhythm produced by the ICC. The model would still perform the same function as the one-pacemaker model but without any functional gain and with significantly higher energy expenditure. It is hard to accept the idea that without any evolutionary advantage such a mechanism would have survived from fish to man.

Even the most fervent supporters of ICC cannot deny that many tissues and organs depleted of slow wave-producing ICC still display electrical oscillations capable of inducing mechanically productive contractions occurring at frequencies very close to those elicited by normal slow waves. Although this activity is smaller, less regular, and quite erratic, it is biologically significant since it keeps *Sl*/*Sl*$^{d}$ and *W*/*W*$^{v}$ mice alive (6, 11). However, this rhythm in its most robust form may be a compensatory mechanism that only occurs when ICC are lost in development. Indeed, in vivo depletion of ICC in newborn BALBc mice by repeated injections of a neutralizing anti-Kit antibody caused a more severe disruption of gastrointestinal motility than seen in *W*/*W*$^{v}$ mice (10). Nonneutralizing and other control antibodies had no effect, indicating that the results were not due to nonspecific antibody effects as suggested in the review (10). The nature and source of this rhythm is also unclear. The author considers these events "destabilized" slow waves. However, there is a large body of evidence indicating that they are distinct from normal slow waves by virtue of their high sensitivity to L-type $Ca^{2+}$ channel blockade (see Ref. 11). For the same reason, they could be considered a manifestation of the spikes (action potentials) that occur on the plateaus of some slow waves under excitatory neural control (as depicted in Fig. 2 of the review). It is also interesting that, in many ICC-deficient tissues, these spikes are entrained by a pacemaker mechanism, which becomes more robust in response to distention (6). This pacemaker activity may originate from smooth muscle cells or residual ICC such as those that occur in the region of the deep muscular plexus (6) or, in larger animals, in intramuscular septa (8). However, no electrical oscillator mechanism has been described in smooth muscle cells. Perhaps, rather than abandoning ICC as pacemakers for electrical slow waves, a more productive approach would be to identify the source of this residual rhythmicity, describe the subcellular oscillator that drives it, and study its contribution to rhythmic contractions in health and disease, where it may gain particular importance. This task may require embracing novel technology such as selective harvesting of various cell types for large-scale molecular analyses, testing the hypotheses derived from them by gene knockout and knockdown studies, and, ultimately, by physiological and advanced imaging techniques at the whole-animal level.

### *On the Utility of Kit and SCF Mutant Rodents and the Natural History of Gastrointestinal Dysmotilities*

The concepts that ICC mediate nitrergic inhibitory and cholinergic excitatory neuromuscular neurotransmission were largely based on experiments in gastric muscles of *W*/*W*$^{v}$ mice and were not reexamined in the review. However, subsequent studies in other organs, in other mutant strains, or by utilizing different (e.g., in vivo) approaches have produced negative or at least less clear-cut results. Purinergic inhibition and noncholinergic (peptidergic) excitation are also relatively preserved in the congenital absence of intramuscular ICC (1, 21). These observations have prompted investigators to reconsider the concept that intramuscular ICC may be solely responsible for mediating neuromuscular neurotransmission and to acknowledge the possibility of direct parallel innervation of smooth muscle cells (1, 21). These issues were pointed out in the review, but do they justify the dismissal of a substantial body of evidence supporting a role for ICC and declaring that they cannot play a role in mediating neural inputs to the smooth muscle? To a great extent, arguments both in favor of and against the role of ICC were generated in mutant rodents, primarily *W*/*W*$^{v}$ and *Sl*/*Sl*$^{d}$ mice and *Ws*/*Ws* rats. Therefore, it is important to recognize not only the utility of these constitutive knockouts but also the fact that, like any other model system, they are not without problems and potential confounding factors that must be carefully considered when interpreting experimental results (9). First, remote effects from a loss-of-function-type change (e.g., loss of ICC) may affect cells that express neither the affected gene nor another gene required for the action of the affected gene (e.g., Kit in the case of a SCF

mutant). Such remote effects are invoked several times in the review and are attributed to some unexplained effect of the mutation in Kit or its ligand directly on cells that do not express the Kit receptor, an explanation which does not have parallels in the scientific literature. Rather, remote effects are more likely to be due to the dropout of ICC or other Kit-expressing cells, the primary target of these mutations. In fact, the evidences proffered (lack of hyperpolarization of the smooth muscle in response to sodium nitroprusside and altered response to neostigmine in $W/W^v$ fundus and antrum, respectively) are better interpreted by the loss of intramuscular ICC and their role in neuromuscular neurotransmission. Remote effects can actually be useful in identifying unexpected functions. The best example is the impaired development of vagal intramuscular arrays in Kit and SCF mutant mice lacking intramuscular ICC in the stomach, which not only provided the first evidence for an involvement of ICC in afferent responses to stretch, but also raised the possibility that ICC may be able to release neurotrophic factors to attract developing axons (4). Unfortunately, this line of evidence was not discussed in the review. Another well-known problem with constitutive knockouts or mutants is that their phenotype may be affected by developmental compensation (9). This may not only impact electrical pacemaking but also neuromuscular neurotransmission in Kit/SCF mutant rodents. For example, in the absence of intramuscular ICC, the sensitivity of the smooth muscle to neurotransmitters may increase and lead to an overestimation of the relative significance of direct innervation when ICC are present. Indeed, a supersensitive phenotype has been demonstrated at the molecular level in $W/W^v$ mice (18). Assumption of some ICC functions by other cells such as intramuscular fibroblast-like cells may be another mechanism of developmental compensation (3). The development of better, state-of-the-art techniques for genetic manipulation (inducible knockouts and knockdown approaches targeting Kit, SCF, and other relevant genes) (9) will help us address these issues, a likely better strategy than simply dismissing the presently available experimental evidence because of inconsistencies.

When interpreting the impact of a mutation on gastrointestinal motor functions, we should also consider the fact that the manifestations of many of the motility diseases are not nearly as dramatic as in other organs, for example, certain major cardiac arrhythmias. Many gastrointestinal dysmotilities do not significantly affect survival or cause dramatic symptoms, and their significance is in their impact on quality of life (13). It follows that the effects of relevant mutations in rodents could be easily missed without rigorous in vivo studies. For example, daily food intake is not reduced in $Sl/Sl^d$ mice, but meal size is reduced and meal frequency increased (4), a pattern consistent with impaired accommodation arising from a combined effect of reduced neurotransmission and mechanoreception. Relatively few studies have investigated in vivo gastrointestinal functions in these rodents, and thus their gastrointestinal phenotype may yet hold surprises.

### *On the Value of Expecting the Unexpected*

When considering a proposed function for a cell or other entity, we frequently examine whether its features are consistent with the perceived requirements of that particular function. Such comparisons are important since they establish how well the characteristics of the target of our investigations measure up to an established reference or "idea" in the philosophical sense. Although such idealistic arguments can and should be used in determining function of particular cells such as ICC, we also need to be careful to withdraw them when not supported by available experimental evidence. A good example is the argument that ICC cannot actively propagate the electrical oscillations underlying rhythmic contractions because the frequency of the slow waves generated by their networks would be phase locked over substantial areas of their networks, due to strong coupling among ICC, and that this would not be compatible with nonpropagating (segmenting) contractions. The experimental evidence argues against this idea. Propagation distance and direction have been found to be variable in myenteric ICC networks imaged in situ, and the activation of the underlying smooth muscle bundles was also variable (8). These findings are, in fact, compatible with the proposed function. But even when an idealistic argument seems valid, it should not detract from looking for answers beyond what is available as evidence at a given time. For example, as Sarna points out, the morphology of the myenteric ICC networks certainly seems incompatible with the anisotropic nature of slow-wave propagation. However, it has been shown that the answer to this problem can be found outside the realm of the primary pacemakers. In an elegant set of experiments, Hirst and Edwards (5) demonstrated that the rapid circumferential propagation of gastric slow waves is facilitated by the intramuscular ICC running parallel to the smooth muscle cells. It is approaches such as these that advance, explore, and rigorously test new ideas that will be needed to solve the logistical problem of how intramuscular ICC could mediate the effects of neural input to a sufficiently large number of smooth muscle cells. Since ICC are already suspected to have a secretory phenotype (16), could they fulfill the role of an integrator receiving excitatory and inhibitory inputs and translating them for smooth muscle cells by releasing mediators into the interstitium (i.e., by volume transmission), integrating past concepts with current evidence?

In summary, a growing body of evidence suggests that normal gastrointestinal motility depends on interactions among several cell types occurring within the smooth muscle layers. The bulk of evidence suggests that ICC, just like other regulatory cell types, perform specialized functions and should retain their place among the key players. Rather than reverting to concepts that were developed before we had tools to study ICC, a likely more fruitful approach is to incorporate our present knowledge into an integrated model of gastrointestinal motility. Unsolved issues remain and will undoubtedly continue to arise from such an integration of complex knowledge. Finding solutions to these problems will likely require looking beyond currently accepted models and approaches and applying novel, state-of-the-art concepts and methods of the postgenomic era to gastrointestinal motility research. New data will certainly force us to continuously revise and refine the roles of ICC but, most likely, not to ignore them.

**GRANTS**

Work in the author's laboratory is supported by Grant DK-58185 from National Institutes of Health.

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