Navigating the Sea of Long Noncoding RNAs
ZFAS1, Friend or Foe?

Francisco J. Alvarado, Carmen R. Valdivia, Héctor H. Valdivia

With the completion of the human genome in 2003, it became evident what had been suspected for a time through isolated bits of data: a surprisingly small proportion of the genome—only ≈2%!—was actually translated into proteins (DNA→RNA→protein). The vast majority of the DNA had no protein correlate and was considered sterile, receiving the somewhat derisive term junk DNA. However, modern technology, such as next-generation sequencing, has revealed that almost the entire human genome is transcribed (DNA→RNA), meaning therefore that the majority of its nucleotides are associated with at least 1 transcript that does not code for a protein.[1] What function do these noncoding RNAs (ncRNAs) serve, then? Work from several fields has painstakingly assembled an emergent picture for ncRNAs in epigenetic processes. Generally speaking, ncRNAs are main actors in the regulation of gene expression, both at the translational and post-translational level. Noncoding RNAs, which are arbitrarily divided into short ncRNAs (<30 nucleotides) and long ncRNAs (lncRNA, >200 nucleotides), play a critical role in heterochromatin formation, histone modification, DNA methylation, and gene splicing and silencing; dysregulation of their interplay with DNA and its transcription machinery may lead to diseases, such as cancer, autism, and Alzheimer.[2] Thus, junk DNA, from which ncRNAs stem from, has turned out to be anything but junk, revealing amazing complexity and fruitful design.

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Although their expression levels are generally low, the number of known ncRNAs is already in the tens of thousands, evidently greater in magnitude than protein-coding mRNA. To be exact, a few lncRNAs have been wrongly annotated and do code for proteins, but differing from the polyvalent role of their parental lncRNAs, these proteins are known to date to circumscribe their function to modulation of other proteins. A good example is myoreguling,[3] a 46-amino acid micropeptide that regulates sarcoplasmic reticulum Ca^{2+} levels by inhibiting SERCA1 pump activity directly. However, the protein-coding capacity of lncRNAs is an exception to the rule, and most lncRNAs control a wide range of biological processes as transcribed RNA molecules,[4] by mechanisms that remain poorly understood.

In this issue of *Circulation Research*, Zhang et al[5] shed light on some of the molecular mechanisms underlying lncRNA regulation of biological processes. They provide evidence that Zinc-finger antisense 1 (*ZFAS1*), a lncRNA to the 5′ end of the protein-coding gene *ZNFX1*, is a major negative regulator of intracellular Ca^{2+} cycling in the heart, likely by repressing *SERCA2a* gene expression (and thus acting in a classical epigenetic process), but also via direct interaction with SERCA2a protein. A major novelty of this study, therefore, lies on unveiling the capacity of a lncRNA to regulate a target protein directly, via RNA–protein interactions. Although the case of *ZFAS1* direct interaction with SERCA2a was inferred by Zhang et al[5] and may require further experimental support, this mechanism if confirmed would add to the broad repertoire of strategies that lncRNAs use to modulate biological functions.

In addition to the novel concepts that Zhang et al[5] bring to the broader field of lncRNAs, their study uncovers a major role for *ZFAS1* on cardiac myocyte Ca^{2+} handling and its dramatic effect on myocardial infarction (MI). In a previous study by the same group,[6] *ZFAS1* levels in blood were found decreased in humans post-MI, and a mouse model of MI confirmed this finding, opening the possibility of using *ZFAS1* as a biomarker of MI. In addition, *ZFAS1* levels were increased in MI tissue,[6] and this observation led to the eternal dilemma that pervades most studies that find a molecule altered in disease, namely, is the molecule causal in the pathogenesis of the disease, or a simple bystander? Zhang et al[5] addressed here the relationship of *ZFAS1* to MI with elegant experiments. They found that MI induced a significant increase in the basal cytoplasmic and sarcoplasmic reticulum levels of *ZFAS1* and that knocking down endogenous *ZFAS1* lessened the contractile dysfunction brought about by MI. Conversely, artificially overexpressing *ZFAS1* in healthy mice recapitulated some of the cardiac dysfunction observed in MI mice. At the cardiomyocyte level, the deleterious effects of *ZFAS1* overexpression were observed as altered Ca^{2+} transients and intracellular Ca^{2+} overload, which likely contributed to the weakened contractility of whole hearts. The capacity of *ZFAS1* to elicit cellular, whole-heart, and intact animal phenomena characteristic of MI was persuasive evidence of its hierarchical importance in the cascade of events triggered by this pathological state. By Koch postulates, *ZFAS1* seems to be a causal agent in the pathogenesis of MI, not its byproduct.

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Zhang et al[5] also uncovered at least some of the molecular mechanisms underlying *ZFAS1* detrimental effect on cardiac contractility. Apparently, a major role of *ZFAS1* is to limit Ca^{2+} uptake by the sarcoplasmic reticulum, which it exerts by (1) using a discrete functional domain (ZFAS1-FD) conserved among species to bind to SERCA2a protein and inhibit its activity, and (2) repressing SERCA2a-encoding *ATP2A2* gene expression. This dual-prong approach disrupts Ca^{2+} homeostasis and leads to intracellular Ca^{2+} overload, in turn negatively affecting myofilament proteins, gene expression, Ca^{2+} entry and extrusion, and presumably many other processes controlled by Ca^{2+}-signaling mechanisms. Zhang et al,[5] however, do not hypothesize on a potential mechanism that may instruct cardiomyocytes to increase *ZFAS1* levels after the ischemic insult. All they find is that NFATc2 (nuclear factor of activated T-lymphocytes c2) is activated after MI, which then increases *ZFAS1* expression (Figure). In a recent study, Wu et al[7] found that *ZFAS1* promotes cardiomyocyte apoptosis by acting as a competing endogenous RNA to miR-150 and increasing C-reactive protein expression. Given the wide spectrum of processes used by lncRNAs, it would not be surprising that *ZFAS1* may affect cardiac function by modulating intracellular Ca^{2+} and apoptotic cell death, 2 different but potentially interdependent pathways.

Zhang et al[5] derive from their work some important implications for our consideration. First, they tout *ZFAS1* as independent predictor of acute MI, but is it realistic to use *ZFAS1* as a biomarker of disease severity in MI and perhaps other cardiac diseases, or as prognostic tool or risk factor? This is to date a hopeful wish, given that existent biomarkers for MI, such as creatine kinase MB and cardiac troponin I, perform suboptimally in the clinical arena[8]; nonetheless, the fact that blood levels of *ZFAS1* decrease significantly after MI[6] makes it a tantalizing possibility. Second, Zhang et al[5] observed that downregulation of *ZFAS1* levels in heart not only prevented but also reversed major deleterious consequences of MI, thus advancing the notion that anti-*ZFAS1* maneuvers may be effective therapies for certain cardiomyopathies. But their major ground for this rationale is their finding that inhibiting *ZFAS1* in the heart improves *SERCA2a* function, a template that has failed to benefit patients with heart failure in a large-scale clinical trial.[9] If history serves any purpose here, it might, therefore, seem speculative to propose that a complex

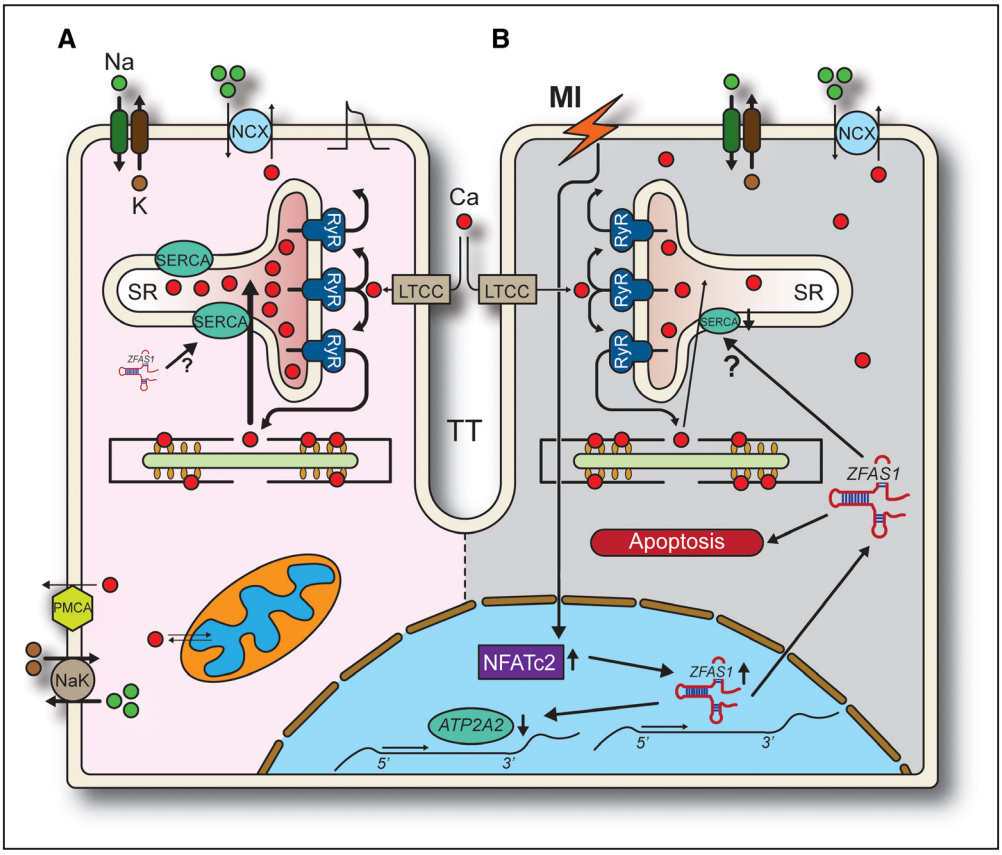

Figure. Proposed mechanisms underlying zinc-finger antisense 1 (ZFAS1) modulation of cardiac function in normal and myocardial infarction (MI)–induced states. A, Normal excitation–contraction coupling. ZFAS1 is expressed at low levels, and while it may interact with SERCA, excitation–contraction coupling proceeds typically: action potentials trigger an inward Ca^{2+} current through L-type Ca^{2+} channels (LTCC), activating ryanodine receptors (RyR). RyRs release Ca^{2+} from the sarcoplasmic reticulum (SR) producing contraction of the myofilaments. Relaxation occurs when SERCA2a recaptures Ca^{2+} into the SR and NCX (sodium–calcium exchanger) extrudes it from the cell. **B**, The ischemia and tissue hypoxia produced by MI promote upregulation of *ZFAS1* through unknown mechanisms that involve NFATc2. *ZFAS1* then decreases expression of SERCA2a-encoding *ATP2A2* gene. Although *ZFAS1* also interacts with SERCA2a, it is unclear whether this entails direct regulation of pump activity (?). Ultimately, reduced SERCA2a expression and function contributes to lower SR Ca^{2+} content, increases cytosolic Ca^{2+}, and ends in contractile dysfunction. Increased *ZFAS1* also induces apoptosis through yet unidentified mechanisms,[7] which may also contribute to overall cardiac dysfunction after MI. NaK indicates Na^+/K^+ pump; and PMCA, plasma membrane Ca^{2+} ATPase.

condition, such as MI, where many and apparently independent cellular processes and pathways converge in dramatically deranged functional and structural end points, may be stopped or even reversed by improving the activity of a single molecule (SERCA2a). Soberly, Zhang et al[5] acknowledge limitations by pointing to the fact that SERCA2a depression was lower than cardiac deterioration after MI, implying additional intervening factors in *ZFAS1* pathway to trigger overt cardiac dysfunction. Again, this would be of little surprise considering the broad array of biological processes affected by lncRNAs in general[2] and *ZFAS1* in particular.[10] *ZFAS1* has been previously cast as a foe because of its implication in several human cancers,[10] but if evolutionary pressure has not weed it out yet from our genome is because it must serve a desirable function, meaning that is also a friend. Indeed, suppression of *ZFAS1* expression seems to promote cell proliferation in breast cancer.[11] The challenge, therefore, for cardiac and cancer therapies, will be to find the right balance between suppression and preservation of *ZFAS1* function, avoiding its undesirable activity while maintaining its most indispensable services.

The increasing evidence for the role of lncRNAs in pathophysiological processes cast them as potential therapeutic targets not only in cardiac disease but also beyond. Still, significant hurdles lie ahead as the field of lncRNAs, as vast as it is, is still in infancy and holds many unknowns.

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Disclosures

None.

References

1. Birney E, Stamatoyannopoulos JA, Dutta A, et al; ENCODE Project Consortium; NISC Comparative Sequencing Program; Baylor College of Medicine Human Genome Sequencing Center; Washington University Genome Sequencing Center; Broad Institute; Children's Hospital Oakland Research Institute. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature*. 2007;447:799–816. doi: 10.1038/nature05874.
2. Maass PG, Luft FC, Bähring S. Long non-coding RNA in health and disease. *J Mol Med (Berl)*. 2014;92:337–346. doi: 10.1007/s00109-014-1131-8.
3. Nelson BR, Makarewich CA, Anderson DM, Winders BR, Troupes CD, Wu F, Reese AL, McAnally JR, Chen X, Kavalali ET, Cannon SC, Houser SR, Bassel-Duby R, Olson EN. A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. *Science*. 2016;351:271–275. doi: 10.1126/science.aad4076.
4. Boon RA, Jaé N, Holdt L, Dimmeler S. Long noncoding RNAs: from clinical genetics to therapeutic targets? *J Am Coll Cardiol*. 2016;67:1214–1226. doi: 10.1016/j.jacc.2015.12.051.
5. Zhang Y, Jiao L, Sun L, et al. LncRNA *ZFAS1* as a SERCA2a inhibitor to cause intracellular Ca^{2+} overload and contractile dysfunction in a mouse model of myocardial infarction. *Circ Res*. 2018;122:1354–1368. doi: 10.1161/CIRCRESAHA.117.312117.
6. Zhang Y, Sun L, Xuan L, et al. Reciprocal changes of circulating long non-coding RNAs ZFAS1 and CDR1AS predict acute myocardial infarction. *Sci Rep*. 2016;6:22384. doi: 10.1038/srep22384.
7. Wu T, Wu D, Wu Q, Zou B, Huang X, Cheng X, Wu Y, Hong K, Li P, Yang R, Li Y, Cheng Y. Knockdown of long non-coding RNA-ZFAS1 protects cardiomyocytes against acute myocardial infarction via anti-apoptosis by regulating miR-150/CRP. *J Cell Biochem*. 2017;118:3281–3289. doi: 10.1002/jcb.25979.
8. Jaffe AS, Babuin L, Apple FS. Biomarkers in acute cardiac disease: the present and the future. *J Am Coll Cardiol*. 2006;48:1–11. doi: 10.1016/j.jacc.2006.02.056.
9. Hulot JS, Salem JE, Redheuil A, et al; AGENT-HF Investigators. Effect of intracoronary administration of AAV1/SERCA2a on ventricular remodelling in patients with advanced systolic heart failure: results from the AGENT-HF randomized phase 2 trial. *Eur J Heart Fail*. 2017;19:1534–1541. doi: 10.1002/ejhf.826.
10. Lan T, Lan X, Li G, Zheng Z, Zhang M, Qin F. Prognostic role of long noncoding RNA ZFAS1 in cancer patients: a systematic review and meta-analysis. *Oncotarget*. 2017;8:100490–100498. doi: 10.18632/oncotarget.19162.
11. Fan S, Fan C, Liu N, Huang K, Fang X, Wang K. Downregulation of the long non-coding RNA ZFAS1 is associated with cell proliferation, migration and invasion in breast cancer. *Mol Med Rep*. 2018;17:6405–6412. doi: 10.3892/mmr.2018.8707.

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