ESC European Society of Cardiology

Cardiovascular Research (2020) **116**, 1240–1241
doi:10.1093/cvr/cvaa020

EDITORIAL

CircRNAs in the heart: bricks in Brunelleschi's Dome

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Online publish-ahead-of-print 7 February 2020

This editorial refers to 'Circular RNA circRNA_000203 aggravates cardiac hypertrophy via suppressing miR26b-5p and miR-140-3p binding to Gata4' by H. Li *et al.*, pp. 1323–1334.

There are monuments that are so beautiful that they are universally admired and celebrated, generation after generation. Brunelleschi's Dome in Florence, the masterpiece of the Italian Renaissance, is rightfully one of those. When it was designed in the 13th century, it was the largest dome in the world. Nowadays, it still holds the record as the largest brick dome ever built. This architectural masterpiece has a unique history that starts in 1418, when the Opera del Duomo announced a public competition for the design of a dome to complete the monumental church of Santa Maria del Fiore, of which the construction had begun a century before under the leadership of the architect and sculptor Arnolfo di Cambio. The big challenge left unsolved by the architect was to engineer a structure able to cover a circular hole of 54.8 m in diameter and placed on top of the 60-m high roof above the high altar of the cathedral. No indications were given in the sketches of Arnolfo on how to accomplish such an unprecedented architectural challenge. The competition held in 1418 was indeed meant to solve this problem. Not 'whether' to make the dome, but 'how' to do it. Of the 17 architects who approached the contest, 41-year-old Filippo Brunelleschi was the one with the winning idea. A self-supporting dome, built without ribs and capable of bearing its own weight at every stage of the construction. Brunelleschi's innovative solution to make this ambitious project possible was that of weaving regular courses of herringbone brickwork, little known before his time, into the texture of the cupola: rather than arranging the bricks in parallel rows, as was the custom, he placed them diagonally, with the end of each brick against the side of the adjoining one. Four million of these bricks, positioned in perfect balance with one another, constituted Brunelleschi's Dome, a miraculous piece of architecture of unprecedented beauty that still rises from the terra-cotta sea of Florence's roof tiles, magnificent yet weightless.

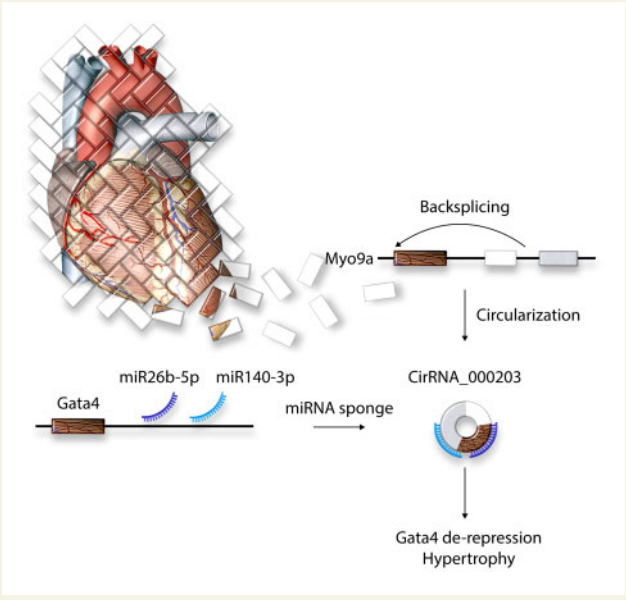

Figure 1 Biogenesis of circRNAs. CircRNA formation via backsplicing.

CircRNAs in the pathophysiology of the heart

The first findings regarding circRNAs date back to more than 40 years ago, when covalently closed circular RNAs were identified in plant viroids.[1] Since then, examples of this peculiar class of 'scrambled' non-coding RNAs have been found in several other life forms, from archaea and yeasts to mammals, including humans.[2,3] After their serendipitous discovery, however, circRNAs remained neglected by the scientific community for quite some time, regarded as the results of rare and abnormal splicing events. Only recently, aided by the development of high-throughput RNA sequencing and bioinformatic analysis, circRNAs have been 'rediscovered' and interest in their function has kept growing ever since.[4]

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CircRNAs belong to a peculiar class of RNA molecules different from others for their structure, characterized by 3′ and 5′ ends covalently joined together in a closed loop; most commonly, this organization arises from back-splicing events between exons of protein-coding genes, where a 5′ splice donor joins an upstream 3′ splice acceptor (*Figure 1*).[5]

Thousands of genes encode for circRNAs and not to a negligible extent. In humans, at least 10% of all transcripts are circular and, for some genes, circular RNA isoforms can be even more abundant than their linear counterparts.[4,6] So far, most of the studies on circRNAs have been conducted on the central nervous system (CNS), since CNS appears to show the highest abundance of circRNAs, but they have been also found to be produced and functional in other organs, as the heart.

In the cardiovascular field, studying circRNAs and their role in the pathophysiology of the heart has become increasingly engaging: an impressive report from the group of Li *et al.*[7] establishes the involvement of circular RNA_000203 in cardiac hypertrophy and provides in-depth description of its mechanism of function. They found circRNA_000203, the circular RNA transcribed from the protein-coding gene *Myo9a* (*Unconventional Myosin-9a*), to be significantly up-regulated in both *in vivo* and *in vitro* models of angiotensin-II (Ang-II) induced cardiac hypertrophy. Their main hypothesis was that this RNA molecule exerts its function by sequestering two miRNAs, miR-26b-5p, and miR-140-3p, and impeding their binding to their specific target *Gata4*, with hypertrophy being the final outcome.[8] Briefly, the authors demonstrated that miR-26b-5p can interact with two sites and miR-140-3p with one site in the sequence of circRNA_000203 and that these two miRNAs can specifically bind the 3′-UTRs of *Gata4*. By suppressing these miRNAs, circRNA_000203 would ultimately aggravate the pro-hypertrophic response to Ang-II treatment, otherwise counterbalanced by miR-26b-5p and miR-140-3p. The authors have also indicated NF-κB as a possible initiator of circRNA_000203 transcription, since a genomic sequence analysis detected three potential NF-κB recognition elements in the promoter region of *Myo9a*, of which one was experimentally confirmed to participate in the Ang-II-induced *Myo9a* transcription. Additionally, pre-treatment of neonatal mouse ventricular cardiomyocytes with NF-κB inhibitors upon Ang-II administration prevented circRNA_000203 and *Myo9a* mRNAs expression. These data, therefore, provide a possible mechanism of action of circRNA_000203, which would exacerbate cardiac hypertrophy via suppressing miR-26b-5p and miR-140-3p and indirectly increasing *Gata4* expression levels.[7]

In the present report, one particular mode of action for circRNA_000203, that of miRNAs 'sponge', is proposed. The relevance of this as an actual mechanism of action of circular RNAs, despite supporting empirical evidences exist,[9] is not above controversy. In fact, multiple studies have raised the attention on the importance of verifying whether the stoichiometry between circRNAs and their miRNAs targets supports the hypothesis of circRNAs titrating miRNAs away from their canonical targets *in vivo*.[10,11] Nonetheless, the current report occupies a relevant place in the trail of recent publications acknowledging the non-marginal involvement of circRNAs in the pathophysiology of the heart. Additionally, in this study, the authors dared to investigate in depth the pathway in which circRNA_000203 is possibly involved, despite the hurdles of tackling such mechanistical questions when directed towards a class of ncRNAs still so poorly characterized.

As a candidate of the competition announced by the Opera del Duomo in 1418, Filippo Brunelleschi was just a short, homely, and hot-tempered goldsmith, who twice got restrained and almost ejected from the assembly because he was regarded as 'a buffoon and a babbler'. Brunelleschi's life was, however, that of an unconventionally brilliant man. As a boy, he mastered drawing and painting, wood carving, and sculpture in silver and bronze. Later, he studied optics and tinkered endlessly with wheels, gears, weights, and motion, building a number of ingenious clocks. He also spent several years in Rome, measuring and sketching the ancient Roman monuments, feeling nurtured by their architectural secrets. Nonetheless, during this competition Brunelleschi needed to prove himself. To do so, he built a scale model to showcase his vision, a 3-m high cupola that he presented to the oversees of the contest. It was its mysterious design to pique their imagination and convince them of the genius hiding in that moody goldsmith, who had ultimately gifted Florence of a dome of unequalled beauty. As the scale dome realized by Brunelleschi, this report can be regarded as the representation of one in the landscape of countless and tightly interconnected pathways that are seemingly in play in the heart. The work from Li *et al.* unveils that unconventional 'bricks', circRNAs, are part of the repertoire of building blocks that make up the delicate structure of the myocardium, and make another small but valuable step in the direction of a more comprehensive understanding of the function of that intriguing, endlessly beating organ.

Conflict of interest: none declared.

Funding

F.D.M. is supported by ERA-CVD JTC2016 EXPERT. P.A.d.C.M. is supported by a Dutch Heart Foundation grant (NHS2015T066) and a Foundation for Science and Technology of Portugal (FCT) grant (PTDC/BIM-MEC/4578/2014).

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