# Editorial

# Dark Side of the Deep Heart

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Ischemic heart disease is the most common cause of death in Western countries.[1] It is consequence of a reduced cardiac blood supply leading to a decrease in oxygen and nutrients fundamental for the proper functioning of the myocardium.[1,2] The persistence of this condition induces fibrosis and scar deposition to replace the dead cardiac tissue.[2] This replacement damages pumping function and thus predisposes to arrhythmias.[3] Specific transcriptional programs finely regulate the cardiac remodeling.[2] Deciphering the deep transcriptional changes during ischemic heart disease might shed light on potential targets for early diagnosis and new drug development.

## See Article by Kaikkonen et al

Transcription permits to transform into RNA the coding and non-coding genetic information conserved into DNA. RNAs, in fact, are grouped into coding and non-coding transcripts (ncRNAs).[4,5] The former, commonly known as mRNAs, are commonly translated into proteins, whereas the latter carry out mainly regulatory functions at transcriptional, post-transcriptional, and epigenetic levels.[4,5] NcRNAs have been further classified, based on their length, into following types: small ncRNAs (<200 nucleotides) and long ncRNAs (lncRNAs; >200 nucleotides).[4,5] microRNAs (miRNAs), transfer RNAs, piwi-interacting RNAs, and endogenous short interfering RNAs are examples of the first class.[5] Instead, lncRNAs are generally categorized based on association to nearby protein-coding genes into following types: sense RNAs, antisense RNAs, intronic RNAs, intergenic RNAs, enhancer (eRNAs), and circular RNAs.[4]

## RNA Polymerase II Pausing

All these RNAs are mostly transcribed by RNA polymerase II (Pol II), a multiprotein complex of 550 kDa localized into the nucleus of eukaryotic cells.[6] Thanks to its prompt response to environmental signals, Pol II is able to immediately activate molecular mechanisms crucial for the response to ischemic insults involving every kind of the RNA species listed above. The rapidity of this activation is consequence of sudden activation of transcription factors. The latter not only induce de novo transcription but also are able to release Pol II pausing. This is a sort of transcriptional preactivation state characterized by recruitment of the enzyme at specific promoters to form preinitiation complexes, subsequent transcription initiation, and then pausing of the elongation ≈30 to 50 bp downstream of the transcription starting state.[7] Pausing is mediated by negative elongation factor (NELF) and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor (DSIF), 2 factors that binding Pol II reversibly halt elongation, possibly by interaction with the nascent RNA.[8] As soon as the release signal is activated by direct or indirect intervention of specific transcription factors, Pol II is ready and reactivates the productive elongation.[8] Specifically, positive transcription elongation factor b (P-TEFb) dissociates from the 7SK small nuclear ribonucleoprotein complex localized at promoters and phosphorylates NELF, DSIF and Pol II determining NELF inactivation, DSIF conversion into an elongation factor with consequent Pol II activation.[9] The study of this phenomenon and the identification of which class of RNAs is activated after ischemia will permit to have a clearer idea about the early molecular mechanisms important for myocardium repair and regeneration after the ischemic insult.

## Profiling Nascent RNA

Recently, with the beginning of the OMIC era, new methods have been developed to rapidly analyze the full profile of RNAs in physiological and pathological contexts, such as normal heart versus acute myocardial infarction. Among these techniques, the global nuclear run-on sequencing (GRO-Seq) is probably the best approach to analyze the profile of nascent RNAs.[10] In addition to this, GRO-Seq allows to identify the products of the elongation phase after activation of paused Pol-II.[9,10] This technique is based on the labeling of nascent transcripts with bromouridine and the concomitant cell treatment with sodium lauroyl sarcosinate (Sarkosyl), an inhibitor of DNA transcription. In this way, only Pol II already sat on specific promoters will give rise to bromouridine-labeled transcripts. The labeling is then used to immunoprecipitate RNA that, once eluted, may be converted into a DNA library suitable for deep sequencing.[10,11] The next-generation sequencing approach precisely determines the number of newly synthesized RNAs and gives a measure of the transcriptional activity in a given context.[10] GRO-Seq is extremely accurate and sensitive. The accuracy of GRO-Seq permits to point out Pol II pause release, elongation, and termination defects. Its sensitivity gives the possibility to reveal sense and antisense transcription, short-lived RNAs, and low-abundant lncRNAs, such as eRNAs.[10,11] Although the advantages of this technique are particularly appealing, GRO-Seq is still laborious and needs



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a huge amount of material to start with: at least $10^7$ cells for each condition.

In a study published in the present issue of *Circulation: Cardiovascular Genetics*, Kaikkonen et al[12] report the results obtained from GRO-Seq in a large animal model of acute infarction. The authors adopted the GRO-Seq to determine the profiles of nascent RNAs associated to myocardial infarction, scanning the transcriptional activity occurring in nuclei derived from different areas of the ischemic heart at 1 day after ischemia.[12] By this approach, they found the induction of inflammatory markers (like tumor necrosis factor [TNF] and transforming growth factor β1 [TGFβ1]) and the downmodulation of genes involved in oxidative phosphorylation, cardiac muscle contraction, and peroxisome proliferator–activated receptor (PPAR) signaling. Interestingly, this study revealed for the first time the presence of a gradient of differentially regulated transcripts moving from the healthy myocardium to the hypoxic border zone up to the deep ischemic area.[12] Moreover, they took advantage from GRO-Seq to calculate the transcriptional pausing ratio. Specifically, they derived this information from the difference of Pol II density between the promoter region and the gene body, respectively, from 0 to 200 and from 200 bp to the end of the gene.[12] Indeed, they found an increase of pausing for repressed transcripts associated to mitochondrial function and muscle contraction and a decrease of pausing in transcribed transcripts involved in inflammation, cell motility, and necrosis, giving an evident view of the transcriptional dynamics ongoing in an ischemic heart.[12] Their experimental approach enriched our knowledge about the transcriptome remodeling occurring during myocardial ischemia exploiting the high resolution of GRO-Seq and indicated to focus the attention especially on ncRNA species. The authors were able to identify the primary miRNAs expressed into the ischemic heart after their expression changes after ischemia, pointing out for the first time the transcriptional regulation of miRNAs[12] in this condition. Although most of what they found was already described in the literature, they could detect also miRNAs, including let7a, miR-99a, miR-103b, miR-148b, and miR-365, not previously associated with myocardial infarction and useful as potential biomarkers. Regarding lncRNAs, Kaikkonen et al[12] found that 3503 cardiac intergenic RNAs overlapped between pigs and humans and confirmed the involvement of myocardial infarction-associated transcript (lncRNA: MIAT), metastasis lung adenocarcinoma transcript 1 (lncRNA: MALAT1), hypoxia-inducible factor 1A antisense RNA 2 (lncRNA: HIF1-AS2), and cyclin-dependent kinase inhibitor 2B antisense RNA1 (lncRNA: ANRIL) in cardiac ischemia.[12] Furthermore, they observed upregulation of GATA4 and GATA6 antisense lncRNAs and downregulation of the antisense KLF6 (Krüppel-like family of transcription factor 6).[12] Remarkably, the KLF family of transcription factors has been identified as the main enhancers' regulator. KLFs, in fact, are sensitive to ischemia and determine the differential expression of ≈1700 eRNAs.[12]

In conclusion, the present study put in the spotlight different classes of transcripts and their regulatory networks during myocardial ischemia. This is an elegant study that underlines the presence of a transcriptional gradient progressing from non-ischemic cardiac areas toward the most deep ischemic zone involving all kind of RNA species. Taking advantage from the high resolution of GRO-Seq, the authors were able to observe the transcriptional regulation of miRNAs and eRNAs. This approach significantly improved our understanding of the transcriptional dynamics associated to myocardial infarction, providing indications for the identification of novel therapeutic targets potentially useful to handle infarction and its associated complications.

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## Disclosures

None.

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