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The PhD thesis review - Andrea Cappannini

„*Development of MODOMICS and NACDDB databases and their application to studying the impact of RNA modifications on structure*”

The doctoral dissertation of Mr. Andrea Cappannini, submitted for review, was completed at the International Institute of Molecular and Cell Biology in Warsaw under the supervision of Professor Janusz Bujnicki.

Dissertation topic

The dissertation investigates the role of post-transcriptional RNA modifications in shaping RNA structure and function across all domains of life. These chemical modifications affect RNA folding, stability, and molecular interactions, thereby influencing translation. To address gaps in understanding their distribution, conservation, and structural impact, the work involved the development and expansion of specialized databases. The MODOMICS database was significantly enhanced to provide structural context for RNA modifications, while a new resource, NACDDB, was created to compile and analyze circular dichroism spectra of both natural and synthetic nucleic acids. Detailed analyses focused particularly on the functionally critical noncoding RNAs, tRNA and rRNA, revealing evolutionary conservation of key modifications, context-dependent structural roles, and effects on RNA stability. Together, these findings provide new insights into the structural consequences of RNA modifications and establish valuable resources for future integrative studies.

Candidate’s knowledge

Mr. Cappannini demonstrates strong expertise in RNA structural biology and bioinformatics. The introduction provides a broad and well-organized compendium of knowledge on RNA structural research, RNA modifications, and the experimental and computational approaches available for their investigation. The thesis offers an important and original perspective on how RNA molecules and their functional roles should be perceived, moving beyond the prevalent static view derived from transcriptomic “snapshots.” This static perception, which overlooks the fundamental role of structure in determining RNA function, remains a limitation for many experts in the field. In contrast, Mr. Cappannini highlights the dynamic and adaptable nature of RNA structures, effectively integrating this perspective with the study of stability and modifications. His work convincingly demonstrates that RNA structures are not fixed entities and that the epitranscriptome introduces an additional regulatory layer, fine-tuning RNA conformations to enable highly specialized and context-dependent functions. Importantly, this dynamic perspective also reflects the evolutionary trajectory of RNA, illustrating how structural flexibility and chemical modifications have contributed to the adaptation of RNA-mediated regulation in parallel with the increasing complexity of living organisms over time.

Candidate's scientific independence

The dissertation demonstrates a high level of methodological sophistication, both in the creation of complex databases for extensive structural data and in their analysis to investigate the evolution and impact of RNA modifications across the three domains of life: Bacteria, Archaea, and Eukaryota. Mr. Cappannini has shown full competence in conducting independent research, solving complex scientific problems, and critically evaluating results. He played a leading role in the development of the databases MODOMICS and NACDDB, and made important contributions to other initiatives, including Sci-ModoM, a resource for integrating epitranscriptomic data from high-throughput sequencing, and DEGRONOPEDIA, a web server for proteome-wide inspection of degrons. Notably, despite the demanding nature of his doctoral research, Mr. Cappannini also contributed to additional projects beyond his PhD, such as studies on stem cell-like tumor growth. In total, he co-authored 10 scientific publications, including two as first or co-first author, with three of them arising from work outside the doctoral project.

Original contributions

The dissertation provides an original and comprehensive perspective on structural RNA biology, with particular emphasis on the functionally critical noncoding RNAs, tRNA and rRNA, which are essential for translation. A central concept explored in this work is dual selection, acting both on the chemical identity of RNA modifications and on their structural location within tRNA and rRNA molecules. Selection occurs at the chemical and functional level, determining the type of modification (e.g., t^6A, m^1A, 2′-O-methylation), while the positional and structural level dictates molecular stability, interactions with the ribosome, and decoding efficiency. The thesis demonstrates the existence of both core and lineage-specific modifications. Core modifications are under strong structural and functional selection, being indispensable for ribosome stability and function. Whereas, lineage-specific modifications are subject to fine-tuning selection, adapting ribosome performance to species- or tissue-specific translational requirements.

The findings of the dissertation introduce two important concepts that have the potential to advance our understanding of RNA structure and translation regulation. First, evolutionary pressures may act not only on primary RNA sequences but also on RNA structural features, with post-transcriptional chemical modifications providing subtle yet functionally meaningful adjustments that fine-tune RNA activity. Second, the regulation of translation is considerably more complex than previously appreciated, with important implications for human health and disease.

Discussion points

I do not have significant concerns about the analyses presented in the dissertation. However, I would like to raise several points for discussion with the candidate:

1. The thesis concludes that positional constraints on RNA modifications are largely shaped by convergent selective pressures on RNA structure and modification type. An alternative explanation is the idea of indirect conservation. In this view, conserved modification patterns may arise not because particular positions are directly under selection, but because the enzymes that install them are themselves highly conserved. Since a modification can only occur if the corresponding RNA modifying protein exists, the presence and evolution of these enzymes are prerequisites for modification fixation. How do you see the relative roles of direct selection on modification positions versus constraints imposed by the modifying enzymes themselves? Could the nonrandom distribution of modifications be more a consequence of biochemical feasibility than adaptive pressure, and how might multifunctional enzymes such as the KEOPS complex influence this picture?
2. A chemical modification does not change the primary DNA or RNA sequence, but it introduces a chemical mark that can affect structure, function, and fitness. At the same time, genetic variants can create or disrupt modification motifs, such as DRACH motifs for m^6A. How do you see the role of population genomics in studying these dynamics, and in what ways could natural genetic variation help us understand the evolutionary and functional significance of RNA modifications?
3. Despite their essential function, rRNAs are often excluded from sequencing experiments. Recent studies (Rothschild et al., *Cell Genomics* 2024, PMID: 39111318) report thousands of rRNA subtypes with distinct structures that show differential expression across tissues and between healthy and pathological states. This diversity is likely to increase further as cell-type specific resolution improves. To what extent do you think we will be able to detect and systematically catalogue these structural variants, and what challenges or opportunities do you foresee in doing so?
4. Your dissertation emphasizes the dynamic and adaptable nature of RNA structures. Computational predictions often produce a single static model. How do you think these models can be reconciled with the dynamic view of RNA, especially considering that chemical modifications can subtly influence stability and conformational ensembles?
5. MODOMICS and NACDDB provide comprehensive resources for RNA structural and modification data. How could integration of these databases with predictive frameworks, such as AlphaGenome-like models trained on RNA structural and epitranscriptomic data, help to prioritize modifications that are most likely to be functionally relevant?

Final evaluation

Mr. Cappannini's dissertation is an impressive and highly significant work that demonstrates exceptional scientific independence, methodological sophistication, and deep expertise in RNA structural biology and bioinformatics. The analyses, databases, and novel insights presented substantially advance our understanding of the role of RNA modifications in shaping RNA structure, function, and evolution. While minor editorial or punctuation errors may be present, I do not consider them relevant, as they do not affect the scientific content or the high quality of the research. Overall, this thesis represents a major contribution to the field and reflects a remarkable level of scholarly achievement.

In view of the above, I, the undersigned, hereby state that the doctoral dissertation of Andrea Cappannini meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science (c.t., Journal of Laws of 2024, item 1571, as amended). I hereby recommend to the Doctoral Committee of the International Institute of Molecular and Cell Biology in Warsaw to admit Andrea Cappannini to the subsequent stages of the procedure for the conferment of the doctoral degree in the field of natural sciences, in the discipline of biological sciences.

Given the originality of the approach, the large amount of work carried out, and the clear scientific importance of the study, I strongly recommend that this doctoral dissertation be awarded distinction.

Poznań, 6 October 2025

Barbara Uszczyńska-Ratajczak

Podpisane elektronicznie przez Barbara Uszczyńska-Ratajczak (Certyfikat kwalifikowany) w dniu 2025-10-06.