Analyzing molecular characteristics of small RNAs to assess the evolution of RNAi pathways

Aguiar, E.R.G.R.[1], Ferreira, F.V.[1,2], Olmo, R.P.[1], Vieira, K.P.O.[1], Paro, S.[3], Faria, I.J.S.[1], Drumond, B, P.[2], Abreu, V.A.C.[1], Meignin, C.[3,4], Sant'anna, M.R.V.[5], Gontijo, N. F.[5], Moreira, L.A.[6], Ferreira, R.S.[1], Kroon, E.G.[2], Imler, J.L.[3,4,7,] Marques, J.T.[1]

[1]Department of Biochemistry and Immunology, Instituto de Ciências Biológicas (ICB), Universidade Federal de Minas Gerais (UFMG), Brazil, [2]Department of Microbiology, ICB, UFMG, [3]CNRSUPR9022, Institut de Biologie Moléculaire et Cellulaire, [4]Faculté des Sciences de la Vie, Université de Strasbourg, [5]Department of Parasitology, ICB, UFMG, [6]Centro de Pesquisa Rene Rachou, Fundação Oswaldo Cruz (Fiocruz), [7]Institut d'Etudes Avancées de l'Université de Strasbourg (USIAS)

A large diversity of RNA interference (RNAi) mechanisms is found in most eukaryotes. RNAi mechanisms invariably involve the formation of an effector complex known as the RNA induced silencing complex (RISC) composed of an Argonaute protein associated with small non-coding RNAs. Despite studies based on phylogeny of Argonaute proteins addressing duplication and diversification, it is unclear how these events have impacted small RNA populations. Analysis of small RNAs is challenging since the use of sequence conservation is very limited. However, small RNAs have unique molecular characteristics that depend on the pathway from which they originated. Here, we compared small RNA populations of *Aedes aegypti* (Aae), *Drosophila melanogaster* (Dme) and *Lutzomyia longipalpis* (Llo), representing three branches of dipteran insects separated by ~200 million years, to investigate the impact of Argonaute evolution in small RNA products. Since Llo lacks information about any classes of small RNAs and in Aae they are poorly annotated, we performed de novo prediction of siRNAs, piRNAs and miRNAs classes using pattern-based analyses. We identified of 824 and 1,781 siRNA and piRNA clusters in Aae and 78 and 585 in Llo. Regarding miRNAs, we identified 206 miRNAs in Llo and 84 novel miRNAs in Aae. We compared the sequence and molecular characteristics of each class of small RNAs in Aae and Llo to the model organism Dme. We observed that miRNAs showed higher conservation, exhibiting similarity in sequence and molecular characteristics such as base enrichment, size profile and expression. siRNAs also presented high conservation, exhibiting similar base enrichment and size distribution. In contrast, piRNAs displayed the higher divergence. In Dme and Aae we observed U enrichment at position 1 of antisense and A enrichment at position 10 of sense small RNAs, which were not observed in Llo. Furthermore, we also noticed significant differences in the size distribution of piRNAs comparing the three insects. However, we observed 10-nt overlap between 5' end of reads in opposite strands, showed to be the most conserved piRNA feature among dipteran. Our results suggest that while miRNA and siRNA pathways are highly conserved displaying similar molecular features, piRNA pathway is the most divergent, showing discrepancy in size and base enrichment. These results showed correlation with divergence and expansion of Argonaute proteins in insects analyzed. Thus, studying small RNAs can directly contribute to evaluation of RNAi pathways regarding the impact of protein changes in small RNA products.

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