Modelling of Ago2 Binding using CLASH

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Argonaute proteins play a central role in the regulation of RNA stability and translation, via a targeting process mediated by small RNA 'driver' sequences that drive the protein to its targets. Ago2, the major mammalian Argonaute protein, is known to primarily associate with microRNAs, a family of small RNA 'driver' sequences, and identify its targets via a 'seed' mediated partial complementarity process. Despite a number of experimental and computational studies that have approached the question of Ago2 targeting, a clear experimental dataset of Ago2 'driver' - target interactions has not been available to date. We present the first Ago2 CLASH experiment, which produces thousands of Ago2 target sites supported by chimeric reads that include together fragments of the 'driver' and the target sequence. Using a novel analysis pipeline we report thousands of Ago2 target sites driven by microRNAs, but also a substantial number of Ago2 'drivers' derived from fragments of other small RNAs such as tRNAs, snoRNAs, rRNAs and others. We have produced machine learning based computational models that efficiently predict the binding potential for each of these 'driver' classes, and experimentally validate a number of interactions. We expand our knowledge of its 'driver' repertoire and potential function in development and disease. This research was funded by Grantová Agentura České Republiky, 19-10976Y Grant to P.A.