BIOCHEMISTRY

Unraveling the lncRNA mystery

A method to study the domain architecture of long noncoding RNAs provides insights into their biological functions.

Thousands of long noncoding RNAs (lncRNAs) are now known, thanks to sequencing efforts, but their biological roles remain a mystery. One way to study a molecule's function is to find out what other molecules it 'talks' to. In 2011, Howard Chang's lab at the Stanford University School of Medicine reported a method called ChIRP, or chromatin isolation by RNA purification, a genome-wide, RNA-centric approach to identify RNA-chromatin interactions.

To obtain deeper insights into lncRNA function, however, one must study them on the domain level. Chang and colleagues recently reported a variation on ChIRP, called dChIRP (domain-specific ChIRP), which dissects the functions of individual domains of a lncRNA within its natural environment, the cell.

Their dChIRP approach involves first designing biotinylated, antisense oligonucleotide pools that target specific domains (defined by functional evidence or simply by dividing up the lncRNA by length) of a lncRNA. Next, whole cells are cross-linked to preserve lncRNA interactions. The chromatin fraction is sonicated to fragment the lncRNA into domain-sized lengths. The pooled, biotinylated oligonucleotides are then added to the divided chromatin samples and allowed to hybridize. Finally, the lncRNA regions of interest are purified along with any binding partners, on magnetic streptavidin beads. Each purification is then subjected to different analyses: immunoblotting, reverse-transcription quantitative PCR, and quantitive PCR or sequencing to identify RNA-protein, RNA-RNA and RNA-chromatin interactions, respectively.

Chang's team applied dChIRP to roX1, a lncRNA found in flies that is essential for doubling gene expression from the single X chromosome in males, known as dosage compensation. Their analysis revealed the functional domain architecture of roX1, suggesting 'three-fingered hand' structure with three distinct domains extending from a 'palm'. The team found that these finger domains interact with chromatin and the male-specific lethal riboprotein complex and behave as independent functional RNA subunits, a result they confirmed by showing that individual finger domains could rescue roX deficiency in male flies. No doubt the approach will be useful for dissecting the domain functions of other mysterious lncRNAs.

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RESEARCH PAPERS

Quinn, J.J. *et al.* Revealing long noncoding RNA architecture and functions using domain-specific chromatin isolation by RNA purification. *Nat. Biotechnol.* doi:10.1038/nbt.2943 (6 July 2014).