The information processing at the foxa node of the sea urchin gene regulatory network

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The transcription factor foxa plays a central and evolutionary ancient role in the endoderm specification. We utilize the advanced state of the sea urchin endomesoderm gene regulatory network to study the cis-regulatory device that controls foxa expression. We find that no fewer than five cis-regulatory modules interact with each other and switch their dominance in controlling foxa expression in different spatial domains and at different times. Our mutation and perturbation analysis revealed the inputs to each of the modules. The complex and dynamic expression of foxa is regulated by a combination of repressors, permissive switches and localized activators. We applied a kinetic model to solve a critical question regarding foxa dynamic regulation. We were therefore able to decipher the specific genomic regulatory code controlling a key gene and also to gain insights into the general design principles of the regulatory genome.

Keywords — quantitative information processing, cis-regulatory analysis, gene regulatory networks, developmental biology.

I. BACKGROUND

The transcription factor foxa is expressed in the developing endoderm of many bilaterians and also in cnidarians, indicating strong conservation of its function and regulation [1]. In the sea urchin embryo foxa is essential for the gut formation and for exclusion of mesodermal fate in the endoderm [2, 3]. The early expression pattern of foxa is very broad in the endomesoderm progenitor field, however in about 12 hours it resolves to specific endodermal sub-domain [2]. We combined quantitative experimental methodologies with kinetic modeling to study the cis-regulatory device that controls foxa expression.

II. RESULTS

We used Spfoxa BAC GFP and RFP knock-in constructs spanning 150 Kb of the foxa locus to identify the cis-regulatory modules controlling foxa expression. There are five cis-regulatory modules that together control foxa expression. The most upstream module is located 25Kb upstream of foxa exon and is necessary for the correct control of foxa expression level through time. Another module is located 12 Kb upstream of foxa and is critical for the correct spatial expression by repressing the expression in the ectoderm and in the mesoderm. Three other modules contribute to the activation level of foxa. We conducted a detailed mutation and perturbation analysis to study the inputs to each of the modules. We identified the ectoderm and mesoderm repressor and the early and late activators of foxa. The binding sites of these inputs are located at different cis-regulatory modules, which indicates that the modules interact with each other to produce the correct spatial pattern and expression level. A critical question that came out of our study was the lingering effect of foxa transient activators on foxa expression level. We applied a kinetic model [4] to study the effect of a transient activator on the output of its downstream gene. The kinetic model explains how transient inputs affect foxa level hours after the input genes had turned off in the foxa expressing cells.

III. CONCLUSIONS

The regulation of foxa expression is processed by five distinct cis-regulatory modules spanning about 33Kb of foxa genomic locus. A combination of repressors, permissive switched and localized activators give rise to the dynamic expression pattern of foxa. The temporal resolution of our quantitative experimental methodologies together with kinetic modeling, open the gate to a deeper understanding of the complex mechanisms that underlie gene regulation.

REFERENCES


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