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 evolutional 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

[1] Friedman JR and Kaestner KH, The Foxa family of transcription factors in development and metabolism. (2006) Cell Mol Life Sci, **63** 2317-2328.

[2] Oliveri P, Walton KD, Davidson EH, and McClay DR, Repression of mesodermal fate by foxa, a key endoderm regulator of the sea urchin embryo. (2006) Development, **133** 4173-4181.

[3] Ben-Tabou de-Leon S and Davidson EH, Gene regulation: gene control network in development. (2007) Annu Rev Biophys Biomol **36**, 191-212.

[4] Ben-Tabou de-Leon S and Davidson EH, Modeling the dynamics of transcriptional gene regulatory networks for animal development. (2009) Dev Biol, **325**, 317-328.

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