Control of notochord gene expression by Brachyury: lessons from a sea squirt

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The notochord

- defining chordate feature
- precursor to the backbone
- source of key signals for floor plate, somites, endoderm development
- development of liver, pancreas, heart, blood vessels, …
- establishment of left-right asymmetry
Vertebrae formation and notochord regression

From Smith et al., *Disease Models and Mechanisms*, 2013
Pikaia gracilens and the chordate ancestry

Cambrian Explosion
The Chordate phylum

Vertebrates

Urochordates

Ascidians

Larvaceans

Salps

Cephalochordates

amphioxus

Vertebral column

Notochord
Life cycle of the ascidian *Ciona intestinalis*

- Compact, sequenced genome
- Biomolecular tools
- Ease of transgenesis

1 cm

15 hrs.

24 hrs.

~ 6 weeks
Main steps of *Ciona* notochord formation

Evolutionarily conserved molecular markers of notochord development
Brachyury is necessary for notochord development in all chordates.
**Brachyury** encodes a sequence-specific DNA-binding protein

FoxA2 is necessary for node and notochord formation in mouse

FoxA2 encodes a sequence-specific DNA-binding protein
Ciona Brachyury (Ci-Bra) is notochord-specific

Corbo et al., Development 1997

Mouse

Wilson et al., Development 1993

Chick

Kispert et al., Dev. Biol. 1995
As its vertebrate counterparts, *Ci-FoxA2* is expressed in multiple tissues.

Di Gregorio et al., *Dev. Biol.* 2001
Subtractive screen for Ci-Bra target genes

54 notochord genes identified

Takahashi et al., 1999
Oda-Ishii and Di Gregorio, 2007; Kugler et al., 2008;
Dunn and Di Gregorio, 2009.
Experimental approach: systematic identification of notochord cis-regulatory modules (CRMs)
Control of gene expression by Ci-Bra

- Synergy with Ci-FoxA2
- Individual or Cooperative binding sites
- Relay
Control of gene expression by Ci-Bra

- Synergy with Ci-FoxA2
- Individual or Cooperative binding sites
- Relay
A novel notochord gene, *Ci-tune*

Passamanecck et al., *Development*, 2009
The *Ci-tune* notochord CRM is controlled synergistically by Brachyury and FoxA2.
Control of gene expression by Ci-Bra

- Synergy with Ci-FoxA2

- Individual or Cooperative binding sites

- Relay
Position-biased identification of CRMs neighboring Ci-Bra targets
Sequence-unbiased identification of minimal elements required for notochord activity

~2 kb

~100-200 bp

mutation analysis

LacZ
Mutation of an individual TGGCAC site abolishes notochord activity of the \textit{Ci-Noto1} CRM
Mutations of related Ci-Bra sites abolish notochord activity of the *Ci-Noto9* and *Ci-Noto4* CRMs
The *leprecan* notochord CRM contains two Ci-Bra binding sites that function cooperatively.

\[
\text{TATCAC} \quad \text{TTACAC}
\]
The *Ci-thrombospondin3* notochord CRM requires three cooperative *Ci-Bra* sites.
Control of gene expression by Ci-Bra

- Synergy with Ci-FoxA2
- Individual or Cooperative binding sites
- Relay
The ATP citrate lyase notochord CRM
Development of a specific Ci-Bra antibody
Chromatin Immunoprecipitation (ChIP) assays
110-cell gastrula neural plate early tailbud mid-tb

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P

- Bra
- pk
- thbs3
- FCol1
- Noto5
- ERM
- Noto1
- Noto8
- Noto4
- Noto9
- ACL
- β4GalT

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A working model for the temporal control of notochord gene expression by Ci-Bra
The search for transcriptional intermediaries of Ci-Bra
Novel transcription factors expressed in the *Ciona* notochord

Jose’-Edwards et al., *Dev. Dyn.* 2011
Jose'-Edwards et al., *Dev. Dyn.* 2011
Brachyury

Jose’-Edwards et al., submitted
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CONCLUSIONS

-In *Ciona*, Brachyury controls its direct transcriptional targets through two mechanisms: small clusters of cooperative binding sites or individual binding sites.

-cooperative and individual binding sites control early-onset and middle-onset targets, respectively.

-Ci-Bra controls its late-onset targets indirectly, through a relay mechanism, using transcriptional intermediaries.
Is Brachyury required for the expression of other notochord transcription factors?

Jose’-Edwards et al., Dev. Dyn. 2011
Regional notochord morphogenesis in the mouse embryo

From Yamanaka et al., *Dev. Cell*, 2007
Cross-section of the developing backbone

- Neural arch
- Centrum
- Basal process
- Spinal cord
- Notochordal sheath
- NOTOCHORD

Image: Cross-section of the developing backbone with labeled anatomical structures.
The *Ci-Noto5* notochord CRM also requires two cooperative *Ci-Bra* sites.
The *Fibrillar Collagen* notochord CRM also relies upon two cooperative Ci-Bra binding sites.