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# Patchy Interspecific Sequence Similarities Efficiently Identify Positive *cis*-Regulatory Elements in the Sea Urchin

Chiou-Hwa Yuh [a] … Eric H. Davidson [a1]

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## Abstract

We demonstrate that interspecific sequence conservation can provide a systematic guide to the identification of functional *cis*-regulatory elements within a large expanse of genomic DNA. The test was carried out on the *otx* gene of *Strongylocentrotus purpuratus*. This gene plays a major role in the gene regulatory network that underlies endomesoderm specification in the embryo. The *cis*-regulatory organization of the *otx* gene is expected to be complex, because the gene has three different start sites (X. Li, C.-K. Chuang, C.-A. Mao, L. M. Angerer, and W. H. Klein, 1997, *Dev. Biol.* 187, 253–266), and it is expressed in many different spatial domains of the embryo. BAC recombinants containing the *otx* gene were isolated from *Strongylocentrotus purpuratus* and *Lytechinus variegatus* libraries, and the ordered sequence of these BACs was obtained and annotated. Sixty kilobases of DNA flanking the gene, and included in the BAC sequence from both species, were scanned computationally for short conserved sequence elements. For this purpose, we used a newly constructed software package assembled in our laboratory, “FamilyRelations.” This tool allows detection of sequence similarities above a chosen criterion within sliding windows set at 20–50 bp. Seventeen partially conserved regions, most a few hundred base pairs long, were amplified from the *S. purpuratus* BAC DNA by PCR, inserted in an expression vector driving a CAT reporter, and tested for *cis*-regulatory activity by injection into fertilized *S. purpuratus* eggs. The regulatory activity of these constructs was assessed by whole-mount *in situ* hybridization (WMISH) using a probe against CAT mRNA. Of the 17 constructs, 11 constructs displayed spatially restricted regulatory activity, and 6 were inactive in this test. The domains within which the *cis*-regulatory constructs were expressed are approximately consistent with results from a WMISH study on *otx* expression in the embryo, in which we used probes specific for the mRNAs generated from each of the three transcription start sites. Four separate *cis*-regulatory elements that specifically produce endomesodermal expression were identified, as well as ubiquitously active elements, and ectoderm-specific elements. We confirm predictions from other work with respect to target sites for specific transcription factors within the elements that express in the endoderm.

## Keywords

*otx* gene; FamilyRelations; *cis*-regulatory sequence; computational genomics; sea urchin embryo

## REFERENCES


1 R.C. Angerer, E.H. Davidson, R.J. Britten
**Single copy DNA and structural gene sequence relationships among four sea urchin species**
Chromosoma, 56 (1976), pp. 213-226

2 S. Aparicio, A. Morrison, A. Gould, J. Gilthorpe, C. Chaudhuri, P. Rigby, R. Krumlauf, S. Brenner
**Detecting conserved regulatory elements with the model genome of the Japanese puffer fish *Fugu rubripes***
Proc. Natl. Acad. Sci. USA, 92 (1995), pp. 1684-1688

3 M.I. Arnone, L.D. Bogarad, A. Collazo, C.V. Kirchhamer, R.A. Cameron, J.P. Rast, A. Gregorians, E.H. Davidson
**Green fluorescent protein in the sea urchin: New experimental approaches to transcriptional regulatory analysis in embryos and larvae**
Development, 124 (1997), pp. 4649-4659

4 R.J. Britten
**Rates of DNA sequence evolution differ between taxonomic groups**
Science, 231 (1986), pp. 1393-1398

5 C.T. Brown, A.G. Rust, P.J.C. Clarke, Z. Pan, M.J. Schilstra, T. De Buysscher, G. Griffin, B.J. Wold, R.A. Cameron, E.H. Davidson, H. Bolouri
**New computational approaches for analysis of *cis*-regulatory networks**
Dev. Biol., 246 (2002), pp. 86-102
Article PDF (1MB)

6 R.A. Cameron, G. Mahairas, J.P. Rast, P. Martinez, T.R. Biondi, S. Swartzell, J.C. Wallace, A.J. Poustka, B.T. Livingston, G.A. Wray, C.A. Ettensohn, H. Lehrach, R.J. Britten, E.H. Davidson, L. Hood
**A sea urchin genome project: Sequence scan, virtual map, and additional resources**
Proc. Natl. Acad. Sci. USA, 97 (2000), pp. 9514-9518

7 J.L. Carr, C.S. Shashikant, W.J. Bailey, F.H. Ruddle
**Molecular evolution of *Hox* gene regulation: Cloning and transgenic analysis of the lamprey *HoxQ8* gene**
J. Exp. Zool., 280 (1998), pp. 73-85

8 C.-K. Chuang, A.H. Wikramanayake, C.-A. Mao, X. Li, W.H. Klein
**Transient appearance of *Strongylocentrotus purpuratus* Otx in micromere nuclei: Cytoplasmic retention of SpOtx possibly mediated through an α-actinin interaction**
Dev. Genet., 19 (1996), pp. 231-237

9 E.H. Davidson
Genomic Regulatory Systems. Development and Evolution, Academic Press, San Diego (2001)

10 E.H. Davidson, J.P. Rast, P. Oliveri, A. Ransick, C. Calestani, C.-H. Yuh, T. Minokawa, G. Amore, V. Hinman, C. Arenas-Mena, O. Otim, C.T. Brown, C.B. Livi, P.Y. Lee, R. Revilla, M.J. Schilstra, P.J.C. Clarkes, A.G. Rust, Z. Pan, M.I. Arnone, L. Rowen, R.A. Cameron, D.R. McClay, L. Hood, H. Bolouri
**A provisional regulatory gene network for specification of endomesoderm in the sea urchin embryo**
Dev. Biol., 246 (2002), pp. 162-190
Article PDF (1MB)

11 C.V. Kirchhamer, E.H. Davidson
**Spatial and temporal information processing in the sea urchin embryo: Modular and intramodular organization of the *CyIIIa* gene *cis*-regulatory system**
Development, 122 (1996), pp. 333-348

12 C.V. Kirchhamer, C.-H. Yuh, E.H. Davidson
**Modular *cis*-regulatory organization of developmentally expressed genes: Two genes transcribed territorially in the sea urchin embryo, and additional examples**
Proc. Natl. Acad. Sci. USA, 93 (1996), pp. 9322-9328

13 X. Li, C.-K. Chuang, C.-A. Mao, L.M. Angerer, W.H. Klein
**Two Otx proteins generated from multiple transcripts of a single gene in *Strongylocentrotus purpuratus***
Dev. Biol., 187 (1997), pp. 253-266
Article PDF (786KB)

14 D.L. Livant, B.R. Hough-Evans, J.G. Moore, R.J. Britten, E.H. Davidson
**Differential stability of expression of similarly specified endogenous and exogenous genes in the sea urchin embryo**
Development, 113 (1991), pp. 385-398

15 G.G. Loots, R.M. Locksley, C.M. Blankespoor, Z.E. Wang, W. Miller, E.M. Rubin, K.A. Frazer
**Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons**
Science, 288 (2000), pp. 136-140

16 M. Manzanares, H. Wada, N. Itasaki, P.A. Trainor, R. Krumlauf, P.W.H. Holland
**Conservation and elaboration of *Hox* gene regulation during evolution of the vertebrate head**
Nature, 408 (2000), pp. 854-857

17 Y. Nishizaki, K. Shimazu, H. Kondoh, H. Sasaki
**Identification of essential sequence motifs in the node/notochord enhancer of *Foxa2* (*Hnf3β*) gene that are conserved across vertebrate species**
Mech. Dev., 102 (2001), pp. 57-66
Article PDF (871KB)

18 S. Nonchev, M. Maconochie, C. Vesque, S. Aparicio, L. Ariza-McNaughton, M. Mansanares, K. Maruthainar, A. Kuroiwa, S. Brenner, P. Charnay, R. Krumlauf
**The conserved role of *Krox-20* in directing *Hox* gene expression during vertebrate hindbrain segmentation**
Proc. Natl. Acad. Sci. USA, 93 (1996), pp. 9339-9345

19 A. Ransick, J.P. Rast, T. Minokawa, C. Calestani, E.H. Davidson
**New early zygotic regulators of endomesoderm specification in sea urchin embryos discovered by differential array hybridization**
Dev. Biol., 246 (2002), pp. 132-147
Article PDF (250KB)

20 S.W. Ruffins, C.A. Ettensohn
**A fate map of the vegetal plate of the sea urchin (*Lytechinus variegatus*) mesenchyme blastula**
Development, 122 (1996), pp. 253-263

21 R. Sivasankaran, M.A. Vigano, B. Müller, M. Affolter, K. Basler
**Direct transcriptional control of the Dpp target *omb* by the DNA binding protein Brinker**
EMBO J., 19 (2000), pp. 6162-6172

22 M.S. Springer, E.H. Davidson, R.J. Britten
**Retroviral-like element in a marine invertebrate**
Proc. Natl. Acad. Sci. USA, 88 (1991), pp. 8401-8404

23 C.-H. Yuh, E.H. Davidson
**Modular *cis*-regulatory organization of *Endo16*, a gut-specific gene of the sea urchin embryo**
Development, 122 (1996), pp. 1069-1082

24 C.-H. Yuh, J.G. Moore, E.H. Davidson
**Quantitative functional interrelations within the *cis*-regulatory system of the *S. purpuratus Endo16* gene**
Development, 122 (1996), pp. 4045-4056

25 C.-H. Yuh, H. Bolouri, E.H. Davidson
**Genomic *cis*-regulatory logic: Functional analysis and computational model of a sea urchin gene control system**
Science, 279 (1998), pp. 1896-1902

26 C.-H. Yuh, H. Bolouri, E.H. Davidson
***cis*-Regulatory logic in the *endo16* gene: Switching from a specification to a differentiation mode of control**
Development, 128 (2001), pp. 617-628

1 To whom correspondence should be addressed. Fax: (626) 793-3047. E-mail: davidson@caltech.edu.