

Regulatory Evolution

Alan Moses

Regulatory evolution vs. comparative genomics

- Evolutionary biologists care about the pufferfish!
- What makes the pufferfish special?



- What are the genetic differences that ensures that one genome makes a pufferfish and another genome makes a snapper?
- Regulatory evolution considers the evolutionary changes in gene activity (as opposed to protein function)

11 April 1975, Volume 188, Number 4184

SCIENCE

Evolution at Two Levels in Humans and Chimpanzees

Their macromolecules are so alike that regulatory mutations may account for their biological differences.

Mary-Claire King and A. C. Wilson

Soon after the expansion of molecular biology in the 1950's, it became evident that by comparing the proteins and nucleic acids of one species with those

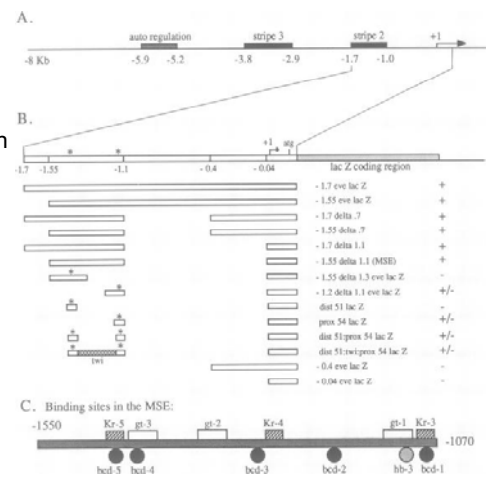
(*Pan troglodytes*) and humans (*Homo sapiens*). This pair of species is also unique because of the thoroughness with which they have been compared

evidence concerning the molecular basis of evolution at the organismal level. We suggest that evolutionary changes in anatomy and way of life are more often based on changes in the mechanisms controlling the expression of genes than on sequence changes in proteins. We therefore propose that regulatory mutations account for the major biological differences between humans and chimpanzees.

Similarity of Human and Chimpanzee Genes

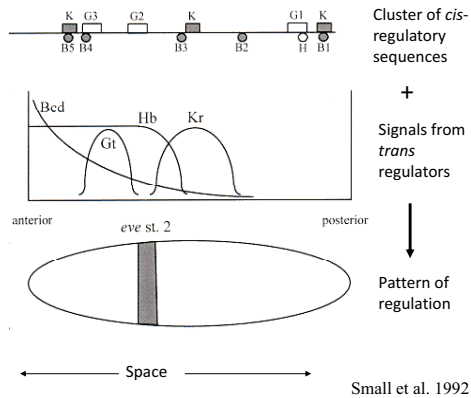
To compare human and chimpanzee genes, one compares either homologous proteins or nucleic acids. At the protein level, one way of measuring the degree of genetic similarity of two taxa is to determine the average number of amino acid differences between homologous

experiments begin to reveal the organization of regulatory information in the genome



Small et al. 1992

How is gene regulation encoded in the genome?



Enhancers (or *cis*-regulatory modules)

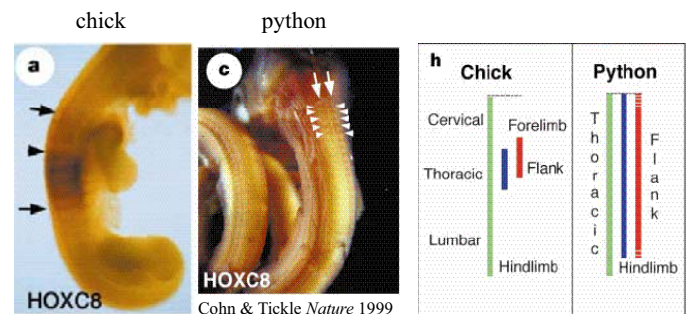
- 500bp-2000bp pieces of non-coding DNA carried in *cis* that specify patterns of gene activity
- Typically contain multiple binding sites for multiple *trans*-acting transcription factors that together specify the pattern
- These modules are often associated with clusters of conserved regions in non-coding DNA

Back to regulatory evolution

- Comparison of in situ hybridization patterns from related species shows striking correlations between morphological evolution and changes in gene expression
- 3 examples

Developmental basis of limblessness and axial patterning in snakes

Martin J. Cohn^{††} & Cheryl Tickle^{††}



Developmental basis for hind-limb loss in dolphins and origin of the cetacean bodyplan

J. G. M. Thewissen¹, M. J. Cohn¹, L. S. Stevens², S. Bajpai³, J. Heyning⁴, and W. E. Horton, Jr.⁴

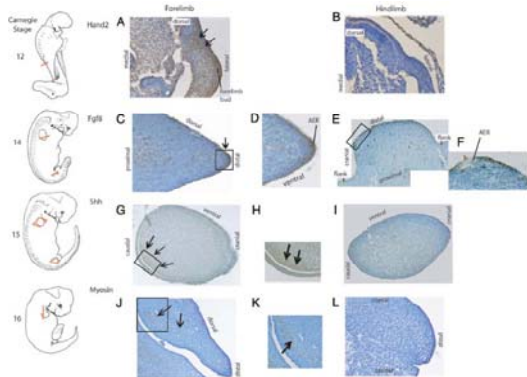


Fig. 3. Gene expression in *Stenella* embryos. Drawings of embryos at Carnegie Stages 12, 14, 15, and 16 are shown, and red lines indicate location of sections.

The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches

Arhat Abzhanov^{1,2}, Winston P. Kuo^{1,2,3}, Christine Hartmann⁴, B. Rosemary Grant⁵, Peter R. Grant⁵ & Clifford J. Tabin¹

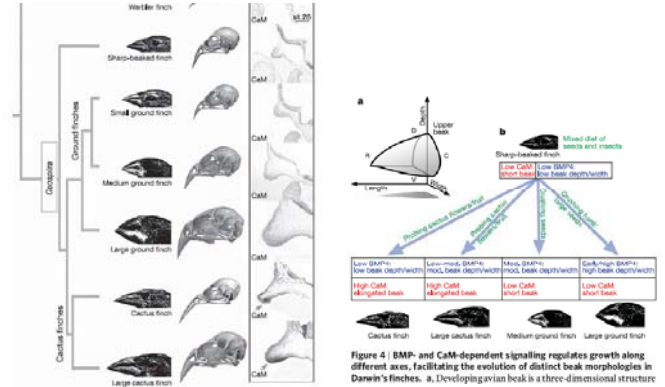


Figure 2 | Comparative analysis of CaM expression in finches.

Observation:

Changes in gene expression of key developmental regulators are associated with morphological differences between related organisms

In general, the changes in expression are of highly conserved regulatory proteins: Hox, Bmp, Shh, etc.

The big idea: *cis*-regulatory evolution

- Modular nature of regulatory information means that changes of *cis*-regulatory modules could alter only a specific part of the gene expression with few pleiotropic effects.
- Mutations in the protein of *trans*-acting factors would have not this property

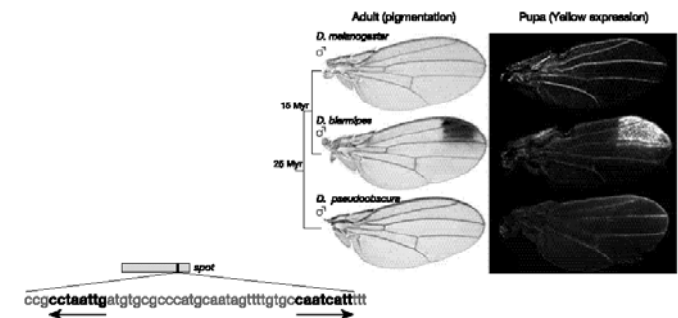
Can we put the pieces of the *cis*-regulatory evolution model together?

- Identify the specific changes in DNA that are responsible for the changes in gene expression
- These DNA differences are responsible for the evolutionary differences between species!
- 4 (+1) examples

articles

Chance caught on the wing: *cis*-regulatory evolution and the origin of pigment patterns in *Drosophila*

Nicolas Gompel^{1,2}, Benjamin Prud'homme^{1,2}, Patricia J. Wittkopp¹, Victoria A. Kassner² & Sean B. Carroll^{1,2}



LETTERS

Repeated morphological evolution through *cis*-regulatory changes in a pleiotropic gene

Benjamin Prud'homme^{1,2}, Nicolas Gompel^{1,2}, Antonia Rikaz¹, Victoria A. Kassner², Thomas M. Williams², Shu-Dan Yeh¹, John R. True¹ & Sean B. Carroll^{1,2}

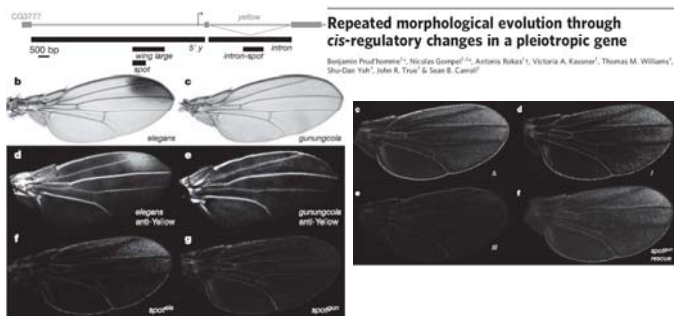


Figure 2 | Changes in the yellow spot *cis*-regulatory DNA underlie the loss of the pigmentation spot in *D. gunungcola*.

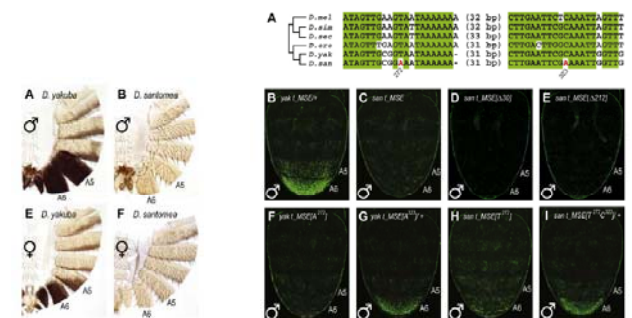
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TCTTTTCTATGGCTTAACAGATTAAAGAATCTCTTGTGACATTACAAGTCTTTT  e1e
TCTTTTCTATGGCTTAACAGATTAAAGAATCTCTTGTGACATTACAAGTCTTTT  gun
TTAGTTAGATCTCTTAACAGTCTTTTGTACACATCTGTAATTTCAAATAACACA  e1e
TTAGTTAGATCTCTTAACAGTCTTTTGTACACATCTGTAATTTCAAATAACACA  gun
TTATGGCAATACATAAATATATAAAAAAATATATATATATATATATATATATAT  e1e
TTATGGCAATACATAAATATATAAAAAAATATATATATATATATATATATATAT  gun
TTAATTTGAAACACAATTATATAGTGAACATCTGACATGACTCTAGTAGTAACT  e1e
TTAATTTGAAACACAATTATATAGTGAACATCTGACATGACTCTAGTAGTAACT  gun

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The Evolution of Gene Regulation Underlies a Morphological Difference between Two *Drosophila* Sister Species

Sangyun Jeong,¹ Mark Rebeiz,¹ Peter Andolfatto,² Thomas Werner,¹ John True,³ and Sean B. Carroll^{1,4}



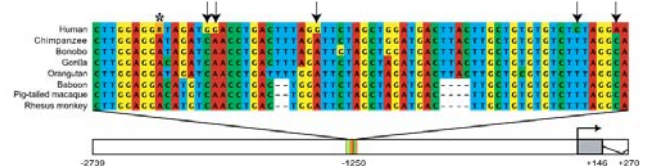
All of these cases connect changes in pigmentation between species to specific changes in regulatory DNA

- None of them show evidence for natural selection having caused the changes between species
- Very few convincing cases of natural selection acting on regulatory sequences

Ancient and Recent Positive Selection Transformed Opioid *cis*-Regulation in Humans

Matthew V. Rockman^{1,2a}, Matthew W. Hahn^{1,2b}, Nicole Soranzo³, Fritz Zimprich⁴, David B. Goldstein^{1,3,5}, Gregory A. Wray^{1,5}

¹ Department of Biology, Duke University, Durham, North Carolina, United States of America, ² Center for Population Biology, University of California, Davis, California, United States of America, ³ Department of Biology, University College London, United Kingdom, ⁴ Department of Clinical Neurology, Medical University of Vienna, Vienna, Austria, ⁵ Institute for Genome Sciences and Policy, Duke University, Durham, North Carolina, United States of America



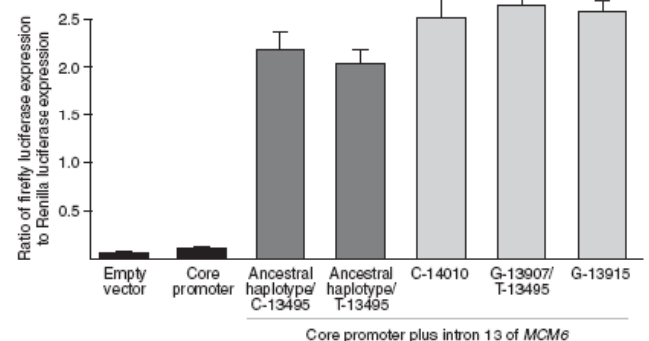
For thousands of years, people have used opiates to alter consciousness and ameliorate pain. Our data indicate that the evolution of our species involved changes in the inducibility of an endogenous opioid precursor, and that these changes were driven by positive natural selection.

Convergent adaptation of human lactase persistence in Africa and Europe

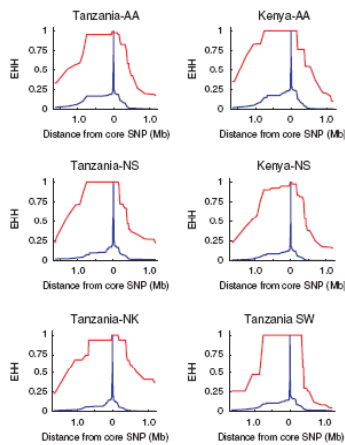
Sarah A Tishkoff^{1,2}, Floyd A Reed^{1,2}, Alessia Ranciaro^{1,2}, Benjamin F Voight³, Courtney C Babbitt⁴, Jesse S Silverman⁴, Kweli Powell¹, Holly M Mortensen¹, Jibril B Hirbo¹, Maha Osman⁵, Muntaser Ibrahim⁵, Sabah A Omar⁶, Godfrey Lema⁷, Thomas B Nyambo⁷, Jilur Ghorri⁸, Suzannah Bumpstead⁹, Jonathan K Pritchard³, Gregory A Wray⁴ & Panos Deloukas⁸

samples⁹ and reporter gene assays driven by the *LCT* promoter *in vitro*^{10–12}, suggest that the C/T-13910 SNP regulates *LCT* transcription in Europeans.

Here, we examine genotype-phenotype associations in 470 East Africans, and we identify three previously undescribed variants associated with the lactase persistence trait, each of which arose independently from the European T-13910 allele and resulted in



enhanced transcriptional activity in *LCT* promoter-driven reporter gene assays. We demonstrate that the most common variant in



variants have one of the strongest genetic signatures of natural selection yet reported in humans.

Evolution of regulatory sequences

- Should be easy to find more examples: just look for known enhancers that have many sequence differences !
- Evolution of regulatory DNA turns out to be complicated...

Evolutionary Dynamics of the Enhancer Region of *even-skipped* in *Drosophila*

Michael Z. Ludwig and Martin Kreitman
Department of Ecology and Evolution, University of Chicago

Binding sites seem to have surprising diversity...

Functional analysis of *eve* stripe 2 enhancer evolution in *Drosophila*: rules governing conservation and change

Michael Z. Ludwig^{1,2,*}, Nipam H. Patel^{2,3} and Martin Kreitman¹

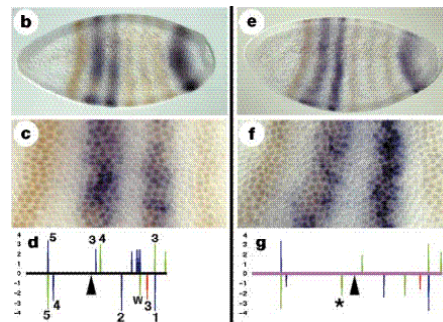
But expression patterns don't change...

Evidence for stabilizing selection in a eukaryotic enhancer element

Michael Z. Ludwig^{*}, Casey Bergman^{*}, Nipam H. Patel[†] & Martin Kreitman^{*}

Compensatory changes preserve function

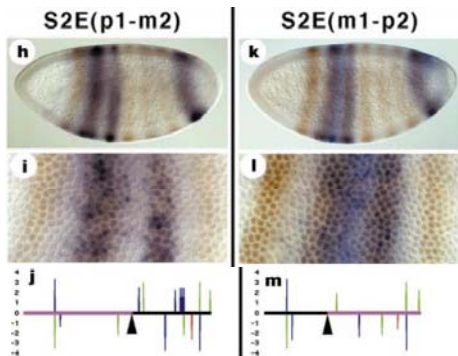
eve stripe 2 enhancer



Binding site changes that don't seem to change function

Ludwig et al. *Nature* 2000

Compensatory changes preserve function



Chimeric enhancers don't function properly

More studies of transcription factor binding site evolution

- Dermitzakis & Clark studied human & mouse binding sites in promoters

Evolution of Transcription Factor Binding Sites in Mammalian Gene Regulatory Regions: Conservation and Turnover

Emmanouil T. Dermitzakis and Andrew G. Clark

Department of Biology, Institute of Molecular Evolutionary Genetics, Pennsylvania State University

- I studied binding sites in yeast promoters

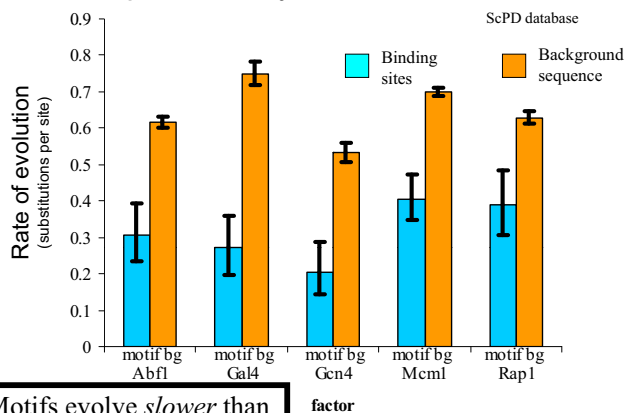
Research article

Open Access

Position specific variation in the rate of evolution in transcription factor binding sites

Alan M Moses¹, Derek Y Chiang², Manolis Kellis^{3,5}, Eric S Lander^{4,5} and Michael B Eisen^{*1,2,6}

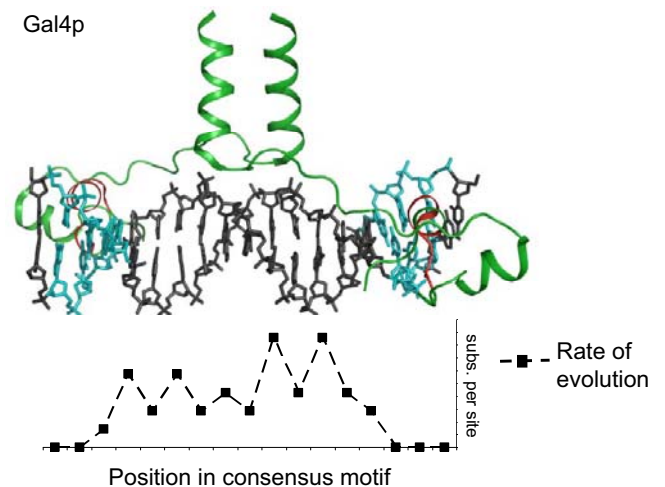
Experimentally characterized sites



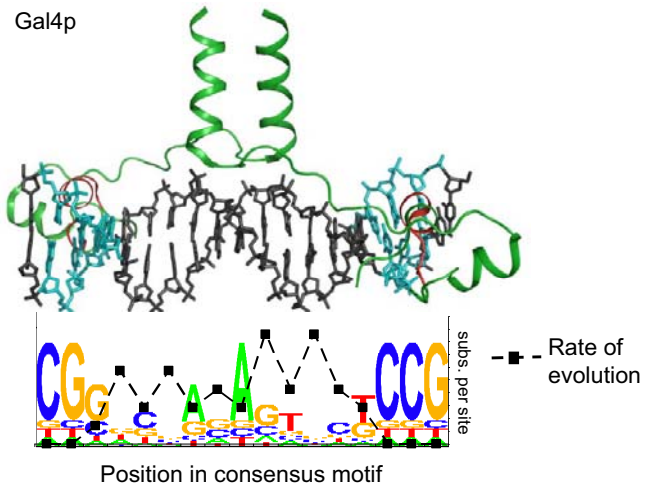
Motifs evolve *slower* than the surrounding sequence

Moses et al. *BMC Evol. Biol.* 2003

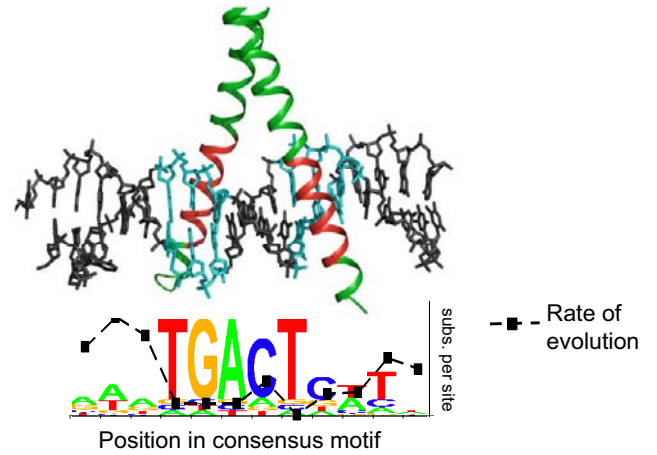
Gal4p



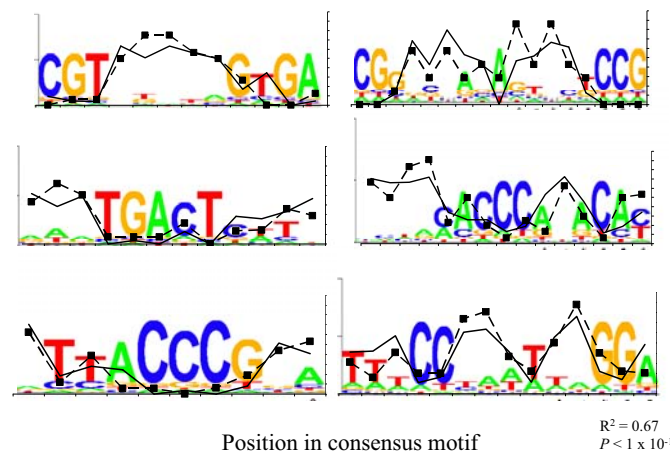
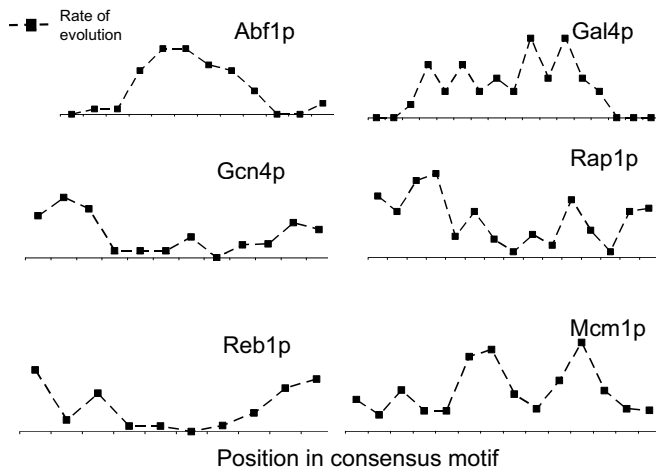
Gal4p



Gcn4p



Each motif has its own pattern of evolution



$R^2 = 0.67$
 $P < 1 \times 10^{-10}$

Systematic analyses of binding site evolution

- Combine large sets of binding sites from chip-chip with genomic alignments of non-coding regions

OPEN ACCESS Freely available online

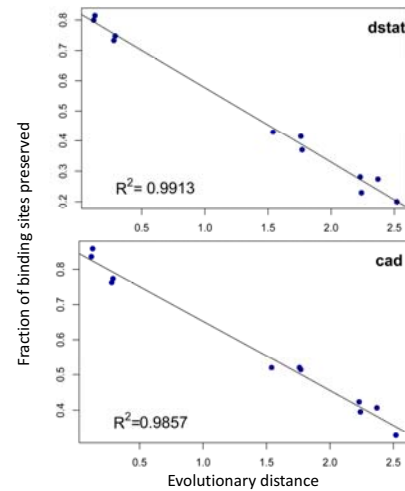
PLOS COMPUTATIONAL BIOLOGY

Large-Scale Turnover of Functional Transcription Factor Binding Sites in *Drosophila*

Alan M. Moses¹, Daniel A. Pollard¹, David A. Nix², Venky N. Iyer³, Xiao-Yong Li², Mark D. Biggin², Michael B. Eisen^{1,2,3,4*}

Divergence of Transcription Factor Binding Sites Across Related Yeast Species

Anthony R. Borneman,^{2*} Tara A. Gianoulis,² Zhengdong D. Zhang,² Haiyuan Yu,² Joel Rozowsky,² Michael R. Seringhaus,² Lu Yong Wang,⁴ Mark Gerstein,^{2,3,4} Michael Snyder^{2,3,4}



Binding site turnover is proportional to species divergence time

Kim et al. *PLoS Genetics* 2009

Binding site turnover

- Major current challenge in regulatory evolution field
- Hard to distinguish 'neutral' binding site changes that preserve function (stabilizing selection) from those that lead to species specific differences in expression

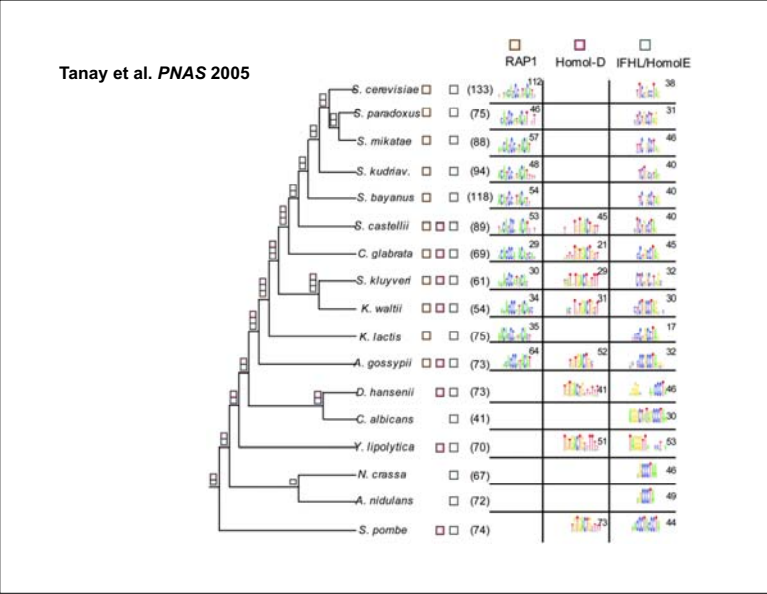
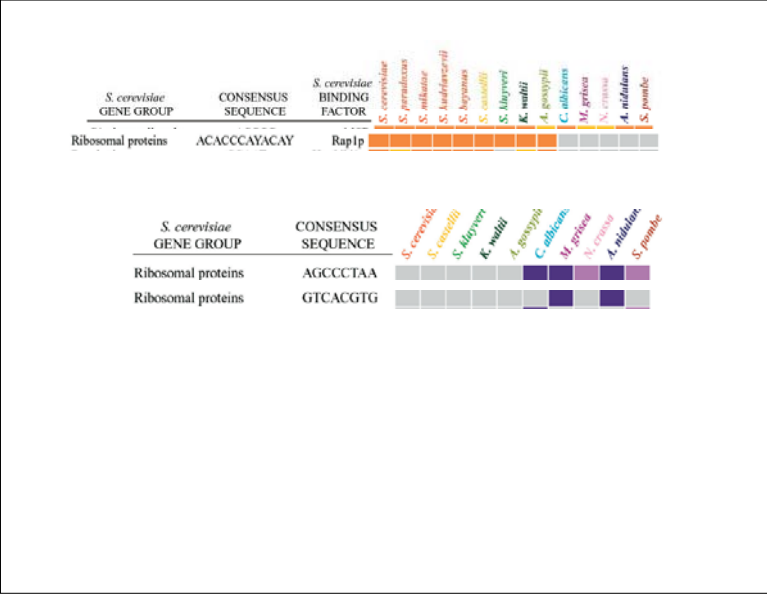
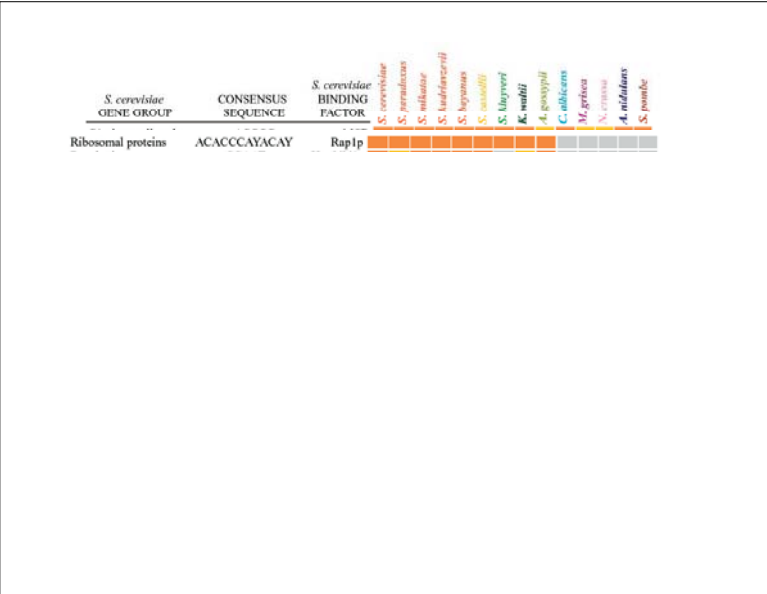
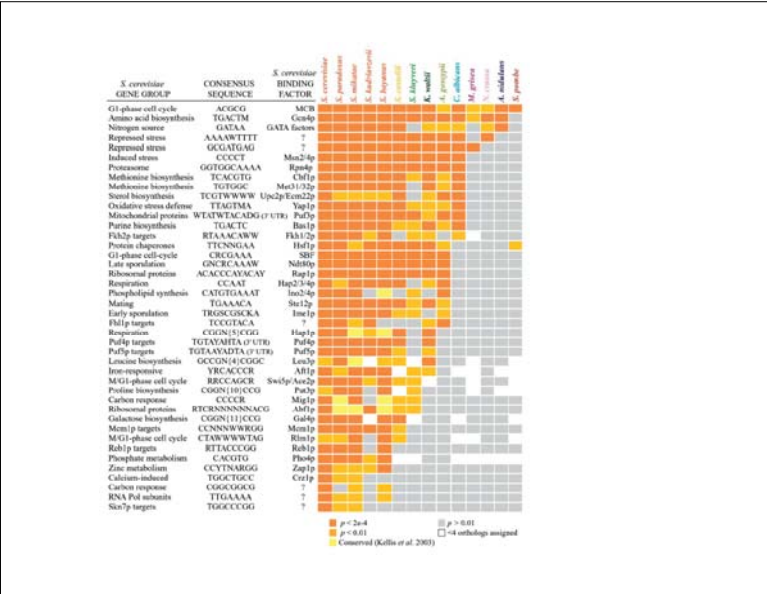
Systematic studies reveal changes in regulatory networks

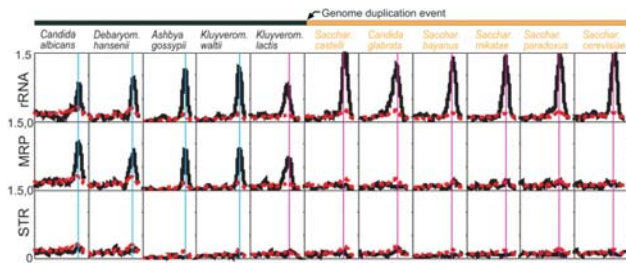
- Regulatory networks controlling genes with highly conserved functions also change over evolution
- E.g., ribosome

Open access, freely available online PLOS BIOLOGY

Conservation and Evolution of *Cis*-Regulatory Systems in Ascomycete Fungi

Audrey P. Gasch^{1,2*}, Alan M. Moses², Derek Y. Chiang³, Hunter B. Fraser³, Mark Berardini⁴, Michael B. Eisen^{1,3*}





Ihmels et al. *Science* 2005

PERSPECTIVE

Evolution of Eukaryotic Transcription Circuits

Brian B. Tuch,^{1,2} Hao Li,³ Alexander D. Johnson^{1,2,*}

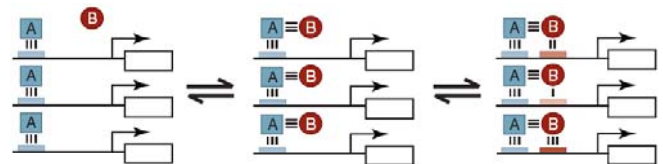


Fig. 2. A plausible pathway to the concurrent rewiring of a large set of genes. In this scenario an interaction is acquired between TRs A and B, after which interactions between B and DNA are optimized gene-by-gene. Rewiring in this manner could avoid fitness barriers imposed by initially changing regulation one gene at a time.

Conclusions

- Regulatory changes underlie evolutionary differences in function
- Both cis-regulatory regions and trans-regulatory factors can change over evolution without leading to differences in function