generate chromosomal alterations with this same tool would greatly enhance zebrafish genetics. This study demonstrates that the HSP70 promoter can be used to inducibly control expression of an EGFP-Cre fusion protein. The EGFP-Cre fusion protein is capable of promoting recombination between lox sites in injected plasmids or in stably inherited transgenes as early as 2 h post-heat shock induction. Finally, the levels of Cre expression achieved in a transgenic fish line carrying the HSP70-EGFP-cre transgene are compatible with viability, and both male and female transgenic fish are fertile subsequent to induction of EGFP-Cre expression. Hence, our data suggest that Cre-mediated recombination is a viable means of manipulating gene expression in zebrafish.

doi:10.1016/j.ydbio.2006.04.141

121
Regulation of Cadherin6B expression by the transcriptional repressor Slug during epithelial-to-mesenchymal transitions in the avian neural crest

Edward G. Coles, Lisa A. Taneyhill, Marianne Bronner-Fraser
California Institute of Technology, Division of Biology, Pasadena, CA, USA

The neural crest is a population of motile cells that migrates away from the dorsal neural tube during neurulation. The migration of neural crest cells relies in part upon the activity of Slug, a protein that plays a key role in regulating the epithelial-to-mesenchymal transition (EMT) that characterizes neural crest cell detachment from the dorsal neural tube. Slug is a member of the Snail superfamily of transcriptional repressors and functions as an important regulator of the neural crest through its ability to modulate the activity of other genes whose expression is important during neural crest development. We have applied the technique of morpholino antisense oligonucleotide (MO) knock-down to examine effects of a decrease in Slug on potential downstream target gene expression in avian embryos at various time periods by quantitative PCR. Treatment with the Slug MO results in the upregulation of many dorsal neural tube genes, including the rapid (30-min) upregulation of cadherin6B, a cell adhesion molecule known to be downregulated during EMT. Genomic sequence analysis of the cadherin6B locus reveals 6 potential Slug binding motifs (E boxes) upstream and downstream of the cadherin6B initiation codon. We present evidence to suggest that the Slug protein binds to these motifs both in vitro and in vivo and represses synthesis of the cadherin6B transcript. Our data are the first to mechanistically describe the regulation of a downstream target of Slug in the context of avian neural crest migration and EMT.

doi:10.1016/j.ydbio.2006.04.142

122
Developmental and stressor-dependent regulation of corticotropin-releasing factor genes of the South African clawed frog, *Xenopus laevis*

Meng Yao, Robert J. Denver
University of Michigan, Ann Arbor, MI, USA

Amphibians exhibit extreme plasticity in the timing of metamorphosis, and the stress neuropeptide corticotropin-releasing factor (CRF) influences this timing by controlling the activity of the thyroid and interrenal axes. To understand developmental and stressor-induced regulation of the CRF genes in *Xenopus laevis*, we analyzed the functionality of predicted *cis* regulatory elements. Exposure of juvenile frogs to shaking stress caused a rapid increase (by 30 min) of phosphorylated cAMP-response element (CRE) binding protein (pCREB) and a robust increase in CRF in the preoptic nucleus. Comparative genomic analysis revealed significant conservation among regulatory regions of vertebrate CRF genes, including a conserved CRE in the core promoters. CREB bound to the frog CRE in vitro and in vitro transfection assays showed that it was required for forskolin-induced activation of the CRF core promoter. We transfected tadpole brain with CRF promoter-luciferase constructs using electroporation-mediated gene transfer. Shaking stress caused activation of the wild type CRF promoter but not the CRE-mutated derivative in a developmental stage-dependent manner. This is the first demonstration of the in vivo functionality of the proximal CRE in a vertebrate CRF gene. Our data show that the basic regulatory elements of the CRF gene responsible for stressor-induced expression arose early in vertebrate evolution and have been maintained through strong stabilizing selection (supported by NSF grant IBN0235401 to R.J.D.).

doi:10.1016/j.ydbio.2006.04.143

123
Regulation of SoxB1 genes in neural induction in *Xenopus laevis*

C. Rogers [1], T.C. Archer [1], T. Grammer [2], E. Silva Casey [1]
[1] *Georgetown Univ., Washington, DC, USA*
[2] *UC Berkeley, CA, USA*

The SoxB1 genes *Sox2* and *Sox3* are expressed in the presumptive neural ectoderm and developing nervous system of *Xenopus laevis*. While *Sox3* is expressed prior to neural induction, *Sox2* is expressed at the onset and is required for neural induction in chick. Overexpression of both leads to an expanded neural plate in *Xenopus*. In animal ectodermal explants, ectoderm forms neural tissue in the absence of BMP signaling, and both *Sox2* and *Sox3* are expressed in response to BMP inhibition. The spatio-temporal expression and their response to BMP make *Sox2* and *Sox3* strong candidates for primary targets of BMP repression required

for neural formation. By studying the regulation of *Sox2* and *Sox3*, we hope to identify the molecular mechanisms that drive the expression of early neural genes. Animal cap assays were performed in the presence of the protein synthesis inhibitor cycloheximide to determine if de novo protein synthesis is required for the repression or expression of *Sox2* and *Sox3*. Dominant negative Xfz8, TCF3 and FGF receptor were overexpressed to expose the roles of FGF and Wnt in early neural induction. Transient reporter assays were used to assess the level of activity of the 1.5 kb *Sox3* upstream regulatory region. Analysis of transgenic embryos was used to define a *cis*-element in *Sox3* required for repression, and computational analysis was used to identify candidate regulatory proteins in that element. The role of these candidate proteins *Sox3* regulation is being tested. The results from these experiments along with current research suggest that *Sox2* and *Sox3* have distinct modes of regulation, though they have very similar expression patterns.

doi:10.1016/j.ydbio.2006.04.144

124

The POZ-ZF transcription factor xZnf131: Roles in *Xenopus* neurogenesis and p120ctn/Kaiso signaling?

Juliet M. Daniel [1], Jae-Il Park [2], Nickett S. Donaldson [1], Hong Ji [2], Pierre D. McCrea [2]
[1] *McMaster University, Hamilton, Ontario, USA*
[2] *Univ. Texas MD Anderson Cancer Center, Houston, TX, USA*

The BTB/POZ zinc finger (POZ-ZF) family of transcription factors plays diverse roles in development and cancer. These proteins have a highly conserved, amino-terminal protein–protein interaction POZ domain and a DNA-binding, zinc finger domain at their carboxy-terminus. The POZ domain mediates homo- and heterodimerization with other POZ-ZF proteins and recruits histone deacetylase corepressor complexes. We study two POZ-ZF proteins: the dual-specificity transcription repressor Kaiso that interacts with the beta-catenin-like protein p120-catenin ($p120^{ctn}$) and Znf131, an uncharacterized POZ-ZF protein that heterodimerizes with Kaiso and also appears to interact with $p120^{ctn}$. Recently, in *Xenopus*, $p120^{ctn}$ and Kaiso were linked to the canonical and non-canonical Wnt signaling pathways via their regulation of known Wnt gene targets. Like Kaiso, Znf131 transcripts appear enriched in the developing central nervous system and adult brain, and preliminary morpholino knock-down experiments in *Xenopus* revealed central nervous tissue defects. Our hypothesis is that Znf131 is required in vertebrate embryogenesis especially during neural development. We are currently using morpholino knock-down and over-expression approaches to assess the developmental roles of *Xenopus* Znf131 in more detail. We will also determine whether Znf131, like Kaiso, participates in regulating canonical (Wnt/beta-catenin/TCF) Wnt target genes and whether $p120^{ctn}$ displaces Znf131 from gene promoters to cause the transcriptional activation of developmentally important genes.

doi:10.1016/j.ydbio.2006.04.145

125

Cloning and functional characterization of GAD67 upstream regulatory regions in *Xenopus laevis*

Conor W. Sipe [1], David Solomon [2], Margaret S. Saha [1]
[1] *College of William and Mary, Williamsburg, VA, USA*
[2] *Georgetown University School of Medicine, Washington, D.C., USA*

The adoption of a correct neurotransmitter phenotype by neurons during development is essential for proper functioning of the adult nervous system. The molecular mechanisms controlling the establishment of a final neurotransmitter phenotype are key issues in the field. Glutamic acid decarboxylase (GAD) is the enzyme responsible for synthesizing GABA, the major inhibitory neurotransmitter present in the central nervous system, and is widely used as a marker for GABAergic neurons. To elucidate the molecular basis of GAD67 expression and analyze the regulatory elements required for its proper expression during neural development, we have cloned and functionally characterized the gene's upstream regulatory regions in *Xenopus laevis*. Analyses of transgenic embryos containing truncated upstream regions reveal a number of discrete elements required for GAD67 expression in specific regions of the nervous system. A short region located proximal to the transcriptional start site is sufficient to drive GFP expression in the retina in a pattern identical to endogenous GAD67. In contrast, the regulatory elements required for proper GAD67 expression in the anterior central nervous system are located further upstream. The identification of the regulatory elements required to reproduce the complete endogenous GAD67 expression pattern will allow real-time monitoring of the GABAergic phenotype in transgenic embryos following exposure to extracellular effector molecules or pharmacological treatment with drugs.

doi:10.1016/j.ydbio.2006.04.146

126

Regulation of retinal homeobox gene transcription: Implication of involvement of forkhead transcription factors

Holly E. Moose [1], Lisa E. Kelly [2], Reyna I. Martinez [2], Heithem M. El-Hodiri [2]
[1] *Integrated Biomedical Sciences Program, The Ohio State University, Columbus, OH, USA*
[2] *Center for Molecular and Human Genetics, Children's Research Institute, Columbus, OH, USA*

The retinal homeobox (Rx) gene product is essential for proper eye development. *Rx* is expressed in the retinal