intergenic regions. Specifically, we found that *cis*-elements conferring endogenous Bmp2 expression in osteoblasts, tooth buds, hair follicle placodes, mammary glands, kidney, and interdigital mesenchyme are all within a distant 3′ intergenic region. Although the Bmp2 gene resides in large genomic region devoid of other genes, numerous "islands" of conserved noncoding sequences exist across this region, consistent with a widespread distribution of distant Bmp2 *cis*-regulatory sequences. Further tests using BAC transgenes that carry engineered deletions across the 3′ region allowed us to parse this region into separate regulatory sub-domains. Our data suggest that critical regulatory sequences controlling Bmp2 expression in osteoblasts are separate from those controlling expression in other tissues and are located greater than 130 kilobases from the Bmp2 promoter. To recapitulate these data in an in vitro system, we have transfected Bmp2 BACs into pre-osteoblastic MC3T3-E1 cells. These experiments also suggest that distant 3′ sequences are required for normal up-regulation of Bmp2 during osteoblast differentiation.

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Exploring the role of long-range evolutionarily conserved regions (ECRs) flanking Bmp4

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Bmp4 is a critical developmental gene that has been suggested to play a role in human disease, however, little is known about the transcriptional regulation of Bmp4. Studies have indicated the Bmp4 proximal promoter is unable to recapitulate all endogenous expression patterns in mouse and fish, and our results suggest that highly conserved, noncoding sequences exist at long distances from the mouse Bmp4 promoter. To test our hypothesis that Bmp4 maintains long-range ECRs, present in fish and mammals, which are required for tissue-specific expression of Bmp4, we have utilized several experimental approaches. To map regulatory elements within a 398 kb genomic segment containing Bmp4, transgenic mice derived from two overlapping BAC reporter transgenes have been generated and analyzed for reporter expression. Our findings indicate that several tissue-specific enhancers reside >28 kb 5′ and 3′ to the mouse Bmp4 transcription unit. To test the sufficiency of ECRs to direct tissue-specific expression in fish, each ECR was coinjected with a GFP reporter construct in zebrafish embryos. These experiments suggest that 2/3 ECRs reliably function as tissue-specific enhancers in fish. We are currently testing ECR minigenes in mouse. In addition, we are testing the requirement of each ECR to direct tissue-specific expression of Bmp4 in mouse by deleting each ECR from the reporter BAC transgenes and testing deletion BACs for reporter activity in vivo. In summary, these experiments provide information regarding the poorly understood regulatory landscape of Bmp4 and will determine if ECRs present in both fish and mouse are required for Bmp4 expression.

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The Hand1 transcription factor functions as a homodimer during mouse development

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Basic helix–loop–helix (bHLH) transcription factors are thought to function as heterodimers with E factors. In studying the function of the Hand1 bHLH factor in the placenta, we found that E factor expression is undetectable in trophoblast giant cells (TGC). We hypothesized that Hand1 can function as homodimer. We generated a construct encoding a tethered homodimer (TH) in which tandem Hand1 proteins were linked by a linker peptide. In transfected Cos7 cells, the Hand1TH protein was detectable at the expected molecular weight. Expression of Hand1TH in transfected trophoblast stem cells promoted TGC differentiation, similar to the effects of Hand1 monomer. Whereas the effect of Hand1 monomer can be competed by other bHLH factors, the Hand1TH was resistant implying that the homodimer is stable. To further investigate the role of Hand1TH, we generated Hand1TH/+ knock-in mice. Through breeding Hand1TH/+ with Hand1−/+ mice, we observed that all Hand1TH/+ and many of the Hand1TH/− pups are viable, fertile and apparently normal. However, through breeding Hand1TH/+ with Hand1TH/− mice, we found that Hand1TH/TH embryos die in utero, though not until E12.5, and ~2/3 of the Hand1TH/− embryos die but not until E14.5. While Hand1−/− embryos die by E8.5 and show defects in TGC, yolk sac and heart, Hand1TH/TH and Hand1TH/− mutants do not show any of the null phenotypes at E8.5. The results indicate that the Hand1TH allele can rescue the early lethal phenotypes in Hand1−/− embryos and Hand1 can act exclusively as a homodimer to regulate various developmental processes.

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Conserved regulation and role of Pitx2 in situs-specific morphogenesis of visceral organs

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Pitx2 is expressed in developing visceral organs on the left side and is implicated in left–right (L–R) asymmetric organogenesis. The asymmetric *Pitx2* expression is controlled by an