de Jong, Danielle (2005) ***Characterisation of homologs of nervous system patterning genes in the staghorn coral, Acropora millepora (Cnidaria; Anthozoa; Scleractinia).*** **PhD thesis, James Cook University.**

Chapter 6 - General Conclusions

As detailed anatomical descriptions of coral development are still some way off, there is no morphological framework to aim the interpretation of expression patterns. Indeed, even comparing expression patterns of what should be orthologous genes within the Cnidaria is not a trivial task; for example, while *ind/Gsh* orthologs (*cnox2/anthox2* genes) are restricted along the O/A axis in all cnidarians investigated, in some expression is biased towards the oral end, in others to the aboral end (Schummer *et al.*, 1992; Shenk *et al.*, 1993a; Shenk *et al.*, 1993b; Cartwright *et al.*, 1999; Gauchat *et al.*, 2000; Hayward *et al.*, 2001; Yanze *et al.*, 2001). It is also difficult to interpret the very different expression patterns of *ems*-related genes seen in *Acropora* and *Hydractinia*; in *Acropora, ems* is expressed in a subset of presumed neurons (i.e. in the ectoderm) that is spatially restricted along the O/A axis (Hislop, 2003), while in *Hydractinia*, the corresponding gene is expressed in the endodermal digestive cells of the hypostome (Mokady *et al.*, 1998). This thesis describes the isolation and preliminary characterisation of 6 novel homeobox genes (*vnd1Am, vnd2Am, vnd3Am, msx3Am, cnox1Am* and *barhAm*) from *Acropora*, as well as extending what is known about 3 other coral homeobox genes (*cnox-2Am, PaxBam* and *PaxCam*). From the results presented, it is clear that members of most of the homeobox gene families known from higher animals are represented in cnidarians, and there are some intriguing hints that aspects of gene function may be conserved.

In general, cnidarian genes show surprising levels of similarity with their vertebrate (rather than their invertebrate) counterparts. A preliminary EST project revealed that 10% of those genes with clear database matches corresponded to mammalian genes that have been lost from the model invertebrates *Drosophila* and *Caenorhabditis* (Kortschak *et al.*, 2003). Moreover, those *Acropora* sequences that correspond to genes present across the Metazoa resembled much more closely human sequences than any *Drosophila* or *Caenorhabditis* sequences (Kortschak *et al.*, 2003). The picture that is emerging is that cnidarian genomes are much more complex at the level of gene complements than was previously thought.

An additional aspect of the genetic complexity of cnidarians is that many of the genes likely to play key roles in development have been independently duplicated (Hayward *et al.*, unpublished; Go *et al.*, unpublished; Ball *et al.*, 2004 and Hislop *et al.*, DGE in press; Samuel *et al.*, 2001). Analysis of a larger EST dataset using the INPARANOID software implies that at least 5% of *Acropora* genes have been independently duplicated (Technau *et al.*, submitted). In addition, more data is required before it can be ascertained if duplication is shared amongst all cnidarians; the duplication of the *nanos* genes is common to both Hydra and *Acropora* (Mochizuki *et al.*, 2000), (Go *et al*, unpublished), while in other cases (for example *Acropora Dmbx1/2*) it would seem that the duplication is anthozoan specific (Hislop *et al*, DGE in press). In any case, it is clear that cnidarians in general possess a greater genetic complexity than was previously thought; best estimates suggest that the anthozoans *Acropora* and *Nematostella* have more genes than the model invertebrates *Drosophila* (~14,000 genes) and *Caenorhabditis* (~17,000 genes) and that their gene numbers are in the same range as vertebrates (Technau *et al.*, submitted). Gene duplications clearly contribute to this complexity, but their significance in cnidarian evolution is unknown.

The identification of members of the *vnd* and *msx* families (described in Chapter three) demonstrates that *Acropora* possesses at least one ortholog of each of the genes of the *vnd/ind/msh* cascade. Each of the newly isolated genes is expressed in a spatially restricted manner along the O/A axis. Together with data for *cnox-2Am*, the expression patterns of these genes are consistent with the hypothesis of a conserved repressive cascade also functioning in the Cnidaria. However, unlike the situation in higher animals, the expression of *vnd* and *msx* is not restricted to the nervous system; expression in putative neurons is only seen with *cnox-2Am*. Assigning possible functions to the *barhAm* and *cnox1Am* genes identified in this project has also been difficult. Generally speaking, *Bar* genes are important in specifying the cells (including neurons) surrounding the eye, craniofacial features and in the CNS. The *Chlorohydra Bar* gene (*cnox3*) is expressed in the head region, suggesting a role in head regeneration, and this is consistent with an ancestral role in the specification of anterior embryonic structures (Schummer *et al.*, 1992). *Acropora barhAm* is also expressed in the oral region, but is highly restricted both in its spatial and temporal expression pattern. In addition, the transcript is not restricted to specific cell types, complicating the interpretation of the expression pattern.

The work described in this thesis clearly indicates how informative anthozoan cnidarians can be with respect to the evolution of function in gene families. Genes that are clearly related to those known to play key roles in specification of both A/P and D/V axes in the Bilateria are present in the 'radially-symmetric' cnidarians. A number of these A/P and D/V patterning genes are differentially expressed along the single overt axis of the anthozoan planula, but a few, such as *Dpp*, are expressed in what seems to define a second axis. Clearly we are still some way off understanding how cnidarians use these genes to specify their body plans, but the work described in this thesis represents a substantial contribution towards this goal. Ultimately, the information gained from work on cnidarians will be a major contributing factor to our understanding of the evolution of body plans, as cnidarian research becomes an essential component of the growing field of evo-devo.

References

Adachi, J. and M. Hasegawa (1996). MOLPHY, Programs for Molecular Phylogenetics Based on Maximum Likelihood.

Adachi, Y., B. Hauck, J. Clements, H. Kawauchi, M. Kurusu, Y. Totani, Y. Y. Kang, T. Eggert, U. Walldorf, K. Furukubo-Tokunaga and P. Callaerts (2003). Conserved cis-regulatory modules mediate complex neural expression patterns of the eyeless gene in the Drosophila brain. *Mech Dev* **120**(10): 1113-26.

Adams, K. L., R. Cronn, R. Percifield and J. F. Wendel (2003). Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proc Natl Acad Sci U S A* **100**(8): 4649-54.

Adamska, M., A. Wolff, M. Kruesler, J. Wittbrodt, T. Braun and E. Bober (2001). Five Nkx5 genes show differential expression patterns in anlagen of sensory organs in medaka: insight into the evolution of the gene family. *Dev Genes Evol* **211**: 338 - 349.

Akam, M. (2000). Arthropods: developmental diversity within a (super) phylum. *Proc Natl Acad Sci U S A* **97**(9): 4438-41.

Allen, T., Y. Zhu, T. Hawley and R. Hawley (2000). Tale homeoproteins as HOX11-interacting partners in T-cell leukemia. *Leuk Lymphoma* **39**: 241- 256.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman (1990). Basic local alignment search tool. *J Mol Biol* **215**(3): 403-10.

Anctil, M. (2000). Evidence for gonadotropin-releasing hormone-like peptides in a cnidarian nervous system. *Gen Comp Endocrinol* **119**(3): 317-28.

Andermann, P. and E. S. Weinberg (2001). Expression of zTlxA, a Hox11-like gene, in early differentiating embryonic neurons and cranial sensory ganglia of the zebrafish embryo. *Dev Dyn* **222**(4): 595-610.

Aniello, F., A. Locascio, M. G. Villani, A. Di Gregorio, L. Fucci and M. Branno (1999). Identification and developmental expression of Ci-msxb: a novel homologue of Drosophila msh gene in Ciona intestinalis. *Mech Dev* **88**(1): 123-6.

Arber, S., B. Han, M. Mendelsohn, M. Smith, T. M. Jessell and S. Sockanathan (1999). Requirement for the homeobox gene Hb9 in the consolidation of motor neuron identity. *Neuron* **23**(4): 659-74.

Arendt, D. and K. Nubler-Jung (1999). Comparison of early nerve cord development in insects and vertebrates. *Development* **126**: 2309 - 2325.

Artavanis-Tsakonas, S., M. D. Rand and R. J. Lake (1999). Notch Signalling: Cell Fate Control and Signal Integration in Development. *Science* **284**: 770 - 776.

Arthur, W. (2004). The effect of development on the direction of evolution: toward a twenty-first century consensus. *Evol Dev* **6**(4): 282-8.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl (1996). Current Protocols in Molecular Biology. New York, John Wiley and Sons, Inc.

Averof, M. and N. H. Patel (1997). Crustacean appendage evolution associated with changes in Hox gene expression. *Nature* **388**: 682 - 686.

Bagni, C., S. Bray, J. A. Gogos, F. C. Kafatos and T. Hsu (2002). The Drosophila zinc finger transcription factor CF2 is a myogenic marker downstream of MEF2 during muscle development. *Mech Dev* **117**(1-2): 265-8.

Baguna, J. and J. Garcia-Fernandez (2003). Evo-Devo: the long and winding road. *Int J Dev Biol* **47**(7-8): 705-13.

Bailey, J. A., Z. Gu, R. A. Clark, K. Reinert, R. V. Samonte, S. Schwartz, M. D. Adams, E. W. Myers, P. W. Li and E. E. Eichler (2002). Recent segmental duplications in the human genome. *Science* **297**(5583): 1003-7.

Bailey, W. J., J. Kim, G. P. Wagner and F. H. Ruddle (1997). Phylogenetic reconstruction of vertebrate Hox cluster duplications. *Mol Biol Evol* **14**(8): 843-53.

Baker, N. E. (2001). Master regulatory genes; telling them what to do. *BioEssays* **23**(9): 763-6.

Baker, N. E. and S. Y. Yu (2001). The EGF receptor defines domains of cell cycle progression and survival to regulate cell number in the developing Drosophila eye. *Cell* **104**(5): 699-708.

Balczarek, K. A., Z. C. Lai and S. Kumar (1997). Evolution of functional diversification of the paired box (Pax) DNA-binding domains. *Mol Biol Evol* **14**(8): 829-42.

Ball, E. E., D. C. Hayward, J. S. Reece-Hoyes, N. R. Hislop, G. Samuel, R. Saint, P. L. Harrison and D. J. Miller (2002). Coral Development: From classical embryology to molecular control. *Int J Dev Biol* **46**: 671 - 678.

Ball, E. E., D. C. Hayward, R. Saint and D. J. Miller (2004). A simple plan - cnidarians and the origins of developmental mechanisms. *Nat Rev Genet* **5**(8): 567-77.

Barker, K. (1998). At the bench: A laboratory navigator. New York, Cold Spring Harbour Laboratory Press.

Barlow, A. J., J. P. Bogardi, R. Ladher and P. H. Francis-West (1999). Expression of chick Barx-1 and its differential regulation by FGF-8 and BMP signaling in the maxillary primordia. *Dev Dyn* **214**(4): 291-302.

Barolo, S., T. Stone, A. G. Bang and J. W. Posakony (2002). Default repression and Notch signaling: Hairless acts as an adaptor to recruit the corepressors Groucho and dCtBP to Suppressor of Hairless. *Genes Dev* **16**(15): 1964-76.

Baulcombe, D. C. (1999). Gene silencing: RNA makes RNA makes no protein. *Curr Biol* **9**(16): R599-601.

Beklemishev, V. N. (1969). Principles of Comparative Anatomy of Invertebrates. Chicago, University of Chicago Press.

Belfort, M. and P. S. Perlman (1995). Mechanisms of intron mobility. *J Biol Chem* **270**(51): 30237-40.

Bellomonte, D., M. Di Bernardo, R. Russo, G. Caronia and G. Spinelli (1998). Highly restricted expression at the ectoderm-endoderm boundary of PIHbox 9, a sea urchin homeobox gene related to the human HB9 gene. *Mech Dev* **74**(1-2): 185-8.

Bender, W., P. Spierer and D. S. Hogness (1983). Chromosomal walking and jumping to isolate DNA from the Ace and rosy loci and the bithorax complex in Drosophila melanogaster. *J Mol Biol* **168**(1): 17-33.

Bergman, C. M., B. D. Pfeiffer, D. E. Rincon-Limas, R. A. Hoskins, A. Gnirke, C. J. Mungall, A. M. Wang, B. Kronmiller, J. Pacleb, S. Park, M. Stapleton, K. Wan, R. A. George, P. J. de Jong, J. Botas, G. M. Rubin and S. E. Celniker (2002). Assessing the impact of comparative genomic sequence data on the functional annotation of the Drosophila genome. *Genome Biol* **3**(12): RESEARCH0086.

Berman, B. P., B. D. Pfeiffer, T. R. Laverty, S. L. Salzberg, G. M. Rubin, M. B. Eisen and S. E. Celniker (2004). Computational identification of developmental enhancers: conservation and function of transcription factor binding-site clusters in Drosophila melanogaster and Drosophila pseudoobscura. *Genome Biol* **5**(9): R61.

Blin-Wakkach, C., F. Lezot, S. Ghoul-Mazgar, D. Hotton, S. Monteiro, C. Teillaud, L. Pibouin, S. Orestes-Cardoso, P. Papagerakis, M. Macdougall, B. Robert and A. Berdal (2001). Endogenous Msx1 antisense transcript: in vivo and in vitro evidences, structure, and potential involvement in skeleton development in mammals. *Proc Natl Acad Sci U S A* **98**(13): 7336-41.

Bode, H. R. (2001). The Role of Hox genes in axial patterning in *Hydra. Hydra. Am. Zool.* **41**: 621 - 628.

Bonini, N. M. and M. E. Fortini (1999). Surviving Drosophila eye development: integrating cell death with differentiation during formation of a neural structure. *BioEssays* **21**(12): 991-1003.

Bopp, D., M. Burri, S. Baumgartner, G. Frigerio and M. Noll (1986). Conservation of a large protein domain in the segmentation gene paired and in functionally related genes of Drosophila. *Cell* **47**(6): 1033-40.

Borod, E. R. and U. Heberlein (1998). Mutual regulation of decapentaplegic and hedgehog during the initiation of differentiation in the Drosophila retina. *Dev Biol* **197**(2): 187-97.

Bourque, G. and P. A. Pevzner (2002). Genome-scale evolution: reconstructing gene orders in the ancestral species. *Genome Res* **12**(1): 26-36.

Bowles, J., G. Schepers and P. Koopman (2000). Phylogeny of the SOX family of Developmental Transcription Factors Based on Sequence and Structural Indicators. *Developmental Biology* **227**: 239-255.

Brake, R. L., P. K. Chatterjee, U. R. Kees and P. M. Watt (2004). The functional mapping of long-range transcription control elements of the HOX11 proto-oncogene. *Biochem Biophys Res Commun* **313**(2): 327-35.

Brand, A. H. and N. Perrimon (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**: 401 - 415.

Brennan, C. A., M. Ashburner and K. Moses (1998). Ecdysone pathway is required for furrow progression in the developing Drosophila eye. *Development* **125**(14): 2653-64.

Bridge, D., C. W. Cunningham, R. DeSalle and L. W. Buss (1995). Class-level relationships in the phylum Cnidaria: molecular and morphological evidence. *Mol Biol Evol* **12**(4): 679-89.

Bridge, D., C. W. Cunningham, B. Schierwater, R. DeSalle and L. W. Buss (1992). Class-level relationships in the phylum Cnidaria: evidence from mitochondrial genome structure. *Proc Natl Acad Sci U S A* **89**(18): 8750-3.

Bridge, D. M., N. A. Stover and R. E. Steele (2000). Expression of a novel receptor tyrosine kinase gene and a paired-like homeobox gene provides evidence of differences in patterning at the oral and aboral ends of hydra. *Dev Biol* **220**(2): 253-62.

Briscoe, J., L. Sussel, P. Serup, H. Hartigan-O'Connor, T. Jessell, J. Rubenstein and J. Ericson (1999). Homeobox gene *Nkx2.2* and Specification of Neuronal Identity by Graded Sonic Hedgehog Signalling. *Nature* **398**: 622 - 627.

Brodbeck, S. and C. Englert (2004). Genetic determination of nephrogenesis: the Pax/Eya/Six gene network. *Pediatr Nephrol* **19**(3): 249-55.

Broihier, H. T. and J. B. Skeath (2002). Drosophila homeodomain protein dHb9 directs neuronal fate via crossrepressive and cell-nonautonomous mechanisms. *Neuron* **35**(1): 39-50.

Brooke, N., J. Garcia - Fernandez and P. Holland (1998). The ParaHox Gene Cluster is an Evolutionary Sister of the Hox Gene Cluster. *Nature* **392**: 920 - 922.

Brosius, J. (1999). RNAs from all categories generate retrosequences that may be exapted as novel genes or regulatory elements. *Gene* **238**(1): 115-34.

Broun, M., S. Sokol and H. R. Bode (1999). Cngsc, a homologue of goosecoid, participates in the patterning of the head, and is expressed in the organizer region of Hydra. *Development* **126**(23): 5245-54.

Brusca, R. C. and G. J. Brusca (1990). Invertebrates. Sunderland, Sinauer Associates: 246 - 247.

Buescher, M. and W. Chia (1997). Mutations in lottchen cause cell fate transformations in both neuroblast and glioblast lineages in the *Drosophila* embryonic central nervous system. *Development* **124**: 673 - 681.

Buescher, M., F. S. Hing and W. Chia (2002). Formation of neuroblasts in the embryonic central nervous system of Drosophila melanogaster is controlled by SoxNeuro. *Development* **129**(18): 4193-203.

Bulfone, A., E. Menguzzato, V. Broccoli, A. Marchitiello, C. Gattuso, M. Mariani, G. Consalez, S. Martinez, A. Ballabio and S. Banfi (2000). Barhl1, a gene belonging to a new subfamily of mammalian homeobox genes, is expressed in migrating neurons of the CNS. *Human Molecular Genetics* **9**(9): 1443-1452.

Burglin, T. R. (1994). A comprehensive classification of homeobox genes. Guidebook to the Homeobox Genes. D. Duboule. Oxford, Oxford University Press: 27 - 71.

Burke, A. C., C. E. Nelson, B. A. Morgan and C. Tabin (1995). Hox genes and the evolution of vertebrate axial morphology. *Development* **121**: 333 - 346.

Callaerts, P., G. Halder and W. J. Gehring (1997). PAX-6 in development and evolution. *Annu Rev Neurosci* **20**: 483-532.

Canaple, L., M. Decoville, M. Leng and D. Locker (1997). The *Drosophila DSP1* gene encoding an HMG 1-like protein: genomic organization, evolutionary conservation and expression. *Gene* **184**: 285-290.

Carroll, S. B. (2001). Chance and necessity: the evolution of morphological complexity and diversity. *Nature* **409**(6823): 1102-9.

Cartwright, P., J. Bowsher and L. W. Buss (1999). Expression of a Hox gene, Cnox-2, and the division of labor in a colonial hydroid. *Proc Natl Acad Sci U S A* **96**: 2183 - 2186.

Castro, L. F. and P. W. Holland (2003). Chromosomal mapping of ANTP class homeobox genes in amphioxus: piecing together ancestral genomes. *Evol Dev* **5**(5): 459-65.

Catmull, J., D. C. Hayward, N. E. McIntyre, J. S. Reece-Hoyes, R. Mastro, P. Callaerts, E. E. Ball and D. J. Miller (1998). Pax-6 origins--implications from the structure of two coral pax genes. *Dev Genes Evol* **208**(6): 352-6.

Catron, K. M., H. Wang, G. Hu, M. M. Shen and C. Abate-Shen (1996). Comparison of MSX-1 and MSX-2 suggests a molecular basis for functional redundancy. *Mech Dev* **55**(2): 185-99.

Catron, K. M., H. Zhang, S. C. Marshall, J. A. Inostroza, J. M. Wilson and C. Abate (1995). Transcriptional repression by Msx-1 does not require homeodomain DNA-binding sites. *Mol Cell Biol* **15**(2): 861-71.

Cavallo, R., D. Rubenstein and M. Peifer (1997). Armadillo and dTCF: a marriage made in the nucleus. *Curr Opin Genet Dev* **7**(4): 459-66.

Cavodeassi, F., R. Diez Del Corral, S. Campuzano and M. Dominguez (1999). Compartments and organising boundaries in the Drosophila eye: the role of the homeodomain Iroquois proteins. *Development* **126**(22): 4933-42.

Chen, C. and R. Schwartz (1995). Identification of Novel DNA Binding Targets and Regulatory Domains of a Murine *Tinman* Homeodomain Factor, *nkx-2.5*. *The Journal of Biological Chemistry* **270**(26): 15628 - 15633.

Chen, C. N., Y. C. Chiang, T. H. Ho, B. A. Schaal and T. Y. Chiang (2004). Coalescent processes and relaxation of selective constraints leading to contrasting genetic diversity at paralogs AtHVA22d and AtHVA22e in Arabidopsis thaliana. *Mol Phylogenet Evol* **32**(2): 616-26.

Cheng, S. and T. Mak (1993). Molecular characterisation of three murine HOX11-related homeobox genes, *Tlx-1, -2* and *-3* and restricted expreesion of *Tlx-1* during embryogenesis. *Dev Growth Differ* **35**: 655 - 663.

Cheyette, B. N., P. J. Green, K. Martin, H. Garren, V. Hartenstein and S. L. Zipursky (1994). The Drosophila sine oculis locus encodes a homeodomain-containing protein required for the development of the entire visual system. *Neuron* **12**(5): 977-96.

Chia, F. S. and R. Koss (1979). Fine Structural Studies of the Nervous System and the Apical Organ in the Planula Larva of the sea anenome *Anthopleura elegantissima*. *Journal of Morphology* **160**.

Chu, H., C. Parras, K. White and F. Jimenez (1998). Formation and Specification of Ventral neuroblasts is controlled by *vnd* in *Drosophila* neurogenesis. *Genes and Development* **12**: 3613 - 3624.

Clackson, T., D. Gussow and P. Jones (1991). General Applications of PCR to Gene Cloning and Manipulation. PCR - A Practical Approach. M. Mc Person, Quirke, P., and Taylor, G.R. Oxford, Oxford University Press: 207 - 210.

Collins, A. G. (2002). Phylogeny of Medusozoa and the Evolution of cnidarian life cycles. *J Evol Biol* **15**: 418 - 432.

Conery, J. S. and M. Lynch (2001). Nucleotide substitutions and the evolution of duplicate genes. *Pac Symp Biocomput*: 167-78.

Conway Morris, S. (2000). The Cambrian "explosion": slow-fuse or megatonnage? *Proc Natl Acad Sci U S A* **97**(9): 4426-9.

Cook, C. E., E. Jimenez, M. Akam and E. Salo (2004). The Hox gene complement of acoel flatworms, a basal bilaterian clade. *Evol Dev* **6**(3): 154-63.

Cornell, R. and T. Von Ohlen (2000). *Vnd/nkx, ind/gsh*, and *msh/msx*: conserved regulators of dorsoventral neural patterning? *Current Opinion in Neurobiology* **10**: 63 - 71.

Coutinho, C. C., R. N. Fonseca, J. J. Mansure and R. Borojevic (2003). Early steps in the evolution of multicellularity: deep structural and functional homologies among homeobox genes in sponges and higher metazoans. *Mech Dev* **120**(4): 429-40.

Cowden, J. and M. Levine (2003). Ventral dominance governs sequential patterns of gene expression across the dorsal-ventral axis of the neuroectoderm in the Drosophila embryo. *Dev Biol* **262**(2): 335-49.

Czerny, T. and M. Busslinger (1995). DNA-Binding and Transactivation Properties of Pax-6: Three Amino Acids in the Paired Domain Are Responsible for the Different Sequence Recognition of Pax-6 and BSAP (Pax-5). *Molecular and Cellular Biology* **15**(5): 2858 - 2871.

Czerny, T., G. Halder, U. Kloter, A. Souabni, W. J. Gehring and M. Busslinger (1999). twin of eyeless, a second Pax-6 gene of Drosophila, acts upstream of eyeless in the control of eye development. *Mol Cell* **3**(3): 297-307.

Czerny, T., G. Schaffner and M. Busslinger (1993). DNA sequence recognition by Pax proteins: bipartite structure of the paired domain and its binding site. *Genes Dev* **7**(10): 2048-61.

Dailey, L. and C. Basilico (2001). Coevolution of HMG Domains and Homeodomains and the Generation of Transcriptional Regulation by Sox/POU Complexes. *Journal of Cellular Physiology* **186**: 315-328.

D'Alessio, M. and M. Frasch (1996). msh may play a conserved role in dorsoventral patterning of the neuroectoderm and mesoderm. *Mech Dev* **58**: 217 - 231.

Damante, G., D. Fabbro, L. Pellizzari, D. Civitareale, S. Guazzi, M. Polycarpou-Schwartz, S. Cauci, F. Quadrifoglio, S. Formisano and R. Di Lauro (1994). Sequence-specific DNA recognition by the thryroid transcription factor-1 homeodomain. *Nucleic Acids Res* **22**: 3075 - 3083.

Damante, G., L. Pellizzari, G. Esposito, F. Fogolari, P. Viglino, D. Fabbro, G. Tell, S. Formisano and R. Di Lauro (1996). A molecular code dictates sequence-specific DNA recognition by homeodomains. *Embo J* **15**(18): 4992-5000.

Darwin, C. (1859). On the origin of species by means of natural selection. London, Murray.

David, C. N. and R. D. Campbell (1972). Cell cycle kinetics and development of Hydra attenuata. I. Epithelial cells. *J Cell Sci* **11**(2): 557-68.

Davidson, D. (1995). The Function and Evolution of Msx Genes: pointers and paradoxes. *Trends Genet* **11**: 405 - 411.

Davidson, E. (1991). Spatial Mechanisms of gene regulation in metazoan embryos. *Development* **113**: 1 - 26.

de Rosa, R., J. K. Grenier, T. Andreeva, C. E. Cook, A. Adoutte, M. Akam, S. B. Carroll and G. Balavoine (1999). Hox genes in brachiopods and priapulids and protostome evolution. *Nature* **399**(6738): 772-6.

de Souza, S. J., M. Long, R. J. Klein, S. Roy, S. Lin and W. Gilbert (1998). Toward a resolution of the introns early/late debate: only phase zero introns are correlated with the structure of ancient proteins. *Proc Natl Acad Sci U S A* **95**(9): 5094-9.

Dear, T., I. Sanchez-Garcia and T. Rabbitts (1993). The *HOX11* gene encodes a DNA-binding nuclear transcription factor belonging to a distinct family of homeobox genes. *Proc. Natl. Acad. Sci. USA* **90**: 4431 - 4435.

Degnan, B. M., S. M. Degnan, A. Giusti and D. E. Morse (1995). A hox/hom homeobox gene in sponges. *Gene* **155**: 175 - 177.

Dent, C., M. Smith and D. Latchman (1998). The DNA Mobility Shift Assay. Transcription Factors, A Practical Approach. D. Latchman. London, The Practical Approach Series: 1 - 13.

Deschet, K., F. Bourrat, D. Chourrout and J. J.S. (1998). Expression Domains of the medaka (*Oryzias latipes*) Ol-Gsh 1 gene are reminiscent of those of clustered and orphan homeobox genes. *Dev Genes Evol* **208**: 235 - 244.

DiBello, P. R., D. A. Withers, C. A. Bayer, J. W. Fristrom and G. M. Guild (1991). The Drosophila Broad-Complex encodes a family of related proteins containing zinc fingers. *Genetics* **129**(2): 385-97.

Dominguez, M. and J. F. de Celis (1998). A dorsal/ventral boundary established by Notch controls growth and polarity in the Drosophila eye. *Nature* **396**(6708): 276-8.

Dominguez, M., D. Ferres-Marco, F. J. Gutierrez-Avino, S. A. Speicher and M. Beneyto (2004). Growth and specification of the eye are controlled independently by Eyegone and Eyeless in Drosophila melanogaster. *Nat Genet* **36**(1): 31-9.

Dominguez, M. and E. Hafen (1997). Hedgehog directly controls initiation and propagation of retinal differentiation in the Drosophila eye. *Genes Dev* **11**(23): 3254-64.

Domon, C. and A. Steinmetz (1994). Exon shuffling in anther-specific genes from sunflower. *Mol Gen Genet* **244**(3): 312-7.

Dorfler, P. and M. Busslinger (1996). C-terminal activating and inhibitory domains determine the transactivation potential of BSAP (Pax-5), Pax-2 and Pax-8. *Embo J* **15**(8): 1971-82.

D'Ovidio, R., A. Raiola, C. Capodicasa, A. Devoto, D. Pontiggia, S. Roberti, R. Galletti, E. Conti, D. O'Sullivan and G. D. Lorenzo (2004). Characterization of the Complex Locus of Bean Encoding Polygalacturonase-Inhibiting Proteins Reveals Subfunctionalization for Defense against Fungi and Insects. *Plant Physiol.*

Drier, E. A. and R. Steward (1997). The Dorsoventral signal transduction pathway and the Rel-like transcription factors in *Drosophila. Semin. Cancer Biol* **8**: 83 - 92.

Dube, I. D., S. Kamel-Reid, C. C. Yuan, M. Lu, X. Wu, G. Corpus, S. C. Raimondi, W. M. Crist, A. J. Carroll, J. Minowada and et al. (1991). A novel human homeobox gene lies at the chromosome 10 breakpoint in lymphoid neoplasias with chromosomal translocation t(10;14). *Blood* **78**(11): 2996-3003.

Duboule, D. (1994). Guidebook to the Homeobox Genes. Oxford, Oxford University Press.

Duboule, D. (1998). Vertebrate hox gene regulation: clustering and/or colinearity? *Curr Opin Genet Dev* **8**(5): 514-8.

Dykhuizen, D. and D. L. Hartl (1980). Selective neutrality of 6PGD allozymes in E. coli and the effects of genetic background. *Genetics* **96**(4): 801-17.

Eberhard, D. and M. Busslinger (1999). The partial homeodomain of the transcription factor Pax-5 (BSAP) is an interaction motif for the retinoblastoma and TATA-binding proteins. *Cancer Res* **59**(7 Suppl): 1716s-1724s; discussion 1724s-1725s.

Eberhard, D., G. Jimenez, B. Heavey and M. Busslinger (2000). Transcriptional repression by Pax5 (BSAP) through interaction with corepressors of the Groucho family. *Embo J* **19**(10): 2292-303.

Edelman, D. B., R. Meech and F. S. Jones (2000). The homeodomain protein Barx2 contains activator and repressor domains and interacts with members of the CREB family. *J Biol Chem* **275**(28): 21737-45.

Eichler, E. E. (2001). Recent duplication, domain accretion and the dynamic mutation of the human genome. *Trends Genet* **17**(11): 661-9.

Emery, I. F., V. Bedian and G. M. Guild (1994). Differential expression of Broad-Complex transcription factors may forecast tissue-specific developmental fates during Drosophila metamorphosis. *Development* **120**(11): 3275-87.

Endo, T., A. Fedoroc, S. de Souza and W. Gilbert (2002). Do Introns Favor or Avoid Regions of Amino Acid Conservation? *Mol Biol Evol* **19**(4): 521 - 525.

Epstein, J., J. Cai, T. Glaser, L. Jepeal and R. Maas (1994). Identification of a Pax paired domain recognition sequence and evidence for DNA-dependent conformational changes. *J Biol Chem* **269**(11): 8355-61.

Ericson, J., J. Muhr, M. Placzek, T. Lints, T. M. Jessell and T. Edlund (1995). Sonic Hedgehog induces differentiation of ventral forebrain neurons: A common signal for ventral patterning within the neural tube. *Cell* **81**: 747 - 756.

Ericson, J., Rashbass, P., Schedl, A., Brenner-Morton, S., Kawakami, A., van Heyningen, V., Jessell, T.M., and Briscoe, J. (1997). Pax6 Controls Progenitor Cell Identity and Neuronal Fate in Response to Graded Shh Signalling. *Cell* **90**: 169 - 180.

Evangelisti, A. M. and A. Wagner (2004). Molecular evolution in the yeast transcriptional regulation network. *J Exp Zoolog Part B Mol Dev Evol* **302**(4): 392-411.

Evans, S. (1999). Vertebrate tinman homologues and cardiac differentiation. *Cell and Developmental Biology* **10**: 73 - 83.

Faiella, A., M. D'Esposito, M. Rambaldi, D. Acampora, S. Balsofiore, A. Stornaiuolo, A. Mallamaci, E. Migliaccio, M. Gulisano and A. Simeone (1991). Isolation and mapping of *EVX1*, a human homeobox gene homologous to *even-skipped*, localised at the 5' end of HOX1 locus on chromosome 7. *Nucleic Acids Res* **19**: 6541 - 6545.

Fautin, D. G. and R. N. Mariscal (1991). Cnidaria: Anthozoa. Microscopic Anatomy of Invertebrates. F. W. Harrison, and Westfall, J.A. New York, Wiley-Liss, Inc.: 267 - 358.

Fernandes, M., H. Xiao and J. T. Lis (1995). Binding of heat shock factor to and transcriptional activation of heat shock genes in Drosophila. *Nucleic Acids Res* **23**(23): 4799 - 4804.

Ferrando, A. A., D. S. Neuberg, R. K. Dodge, E. Paietta, R. A. Larson, P. H. Wiernik, J. M. Rowe, M. A. Caligiuri, C. D. Bloomfield and A. T. Look (2004). Prognostic importance of TLX1 (HOX11) oncogene expression in adults with T-cell acute lymphoblastic leukaemia. *Lancet* **363**(9408): 535-6.

Ferrier, D. E., N. M. Brooke, G. Panopoulou and P. W. Holland (2001). The Mnx homeobox gene class defined by HB9, MNR2 and amphioxus AmphiMnx. *Dev Genes Evol* **211**(2): 103-7.

Ferrier, D. E. and P. W. Holland (2001). Ancient origin of the Hox gene cluster. *Nat Rev Genet* **2**(1): 33-8.

Ferrier, D. E. and P. W. Holland (2002). Ciona intestinalis ParaHox genes: evolution of Hox/ParaHox cluster integrity, developmental mode, and temporal colinearity. *Mol Phylogenet Evol* **24**(3): 412-7.

Ferrier, D. E. and C. Minguillon (2003). Evolution of the Hox/ParaHox gene clusters. *Int J Dev Biol* **47**(7-8): 605-11.

Field, K. G., G. J. Olsen, D. J. Lane, S. J. Giovannoni, M. T. Ghiselin, E. C. Raff, N. R. Pace and R. A. Raff (1988). Molecular phylogeny of the animal kingdom. *Science* **239**(4841 Pt 1): 748-53.

Finnerty, J., and Martindale, M (1999). Ancient Origins of Axial Patterning Genes: Hox Genes and ParaHox Genes in the Cnidaria. *Evolution and Development* **1**(1): 16 - 23.

Finnerty, J. and M. Martindale (1997). Homeoboxes in sea anemones (Cnidaria:Anthozoa): a PCR-based survey of *Nematostella vectensis* and *Metridium senile*. *Biol. Bull.* **193**: 62 - 76.

Finnerty, J., R., Pang, K., Burton, P., Paulson, D., and Martindale, M.P. (2004). Origins of Bilateral Symmetry: Hox and dpp expression in a sea anenome. *Science* **304**: 1335 - 1337.

Finnerty, J. R. (1998). Homeoboxes in sea anenomes and other nonbilaterian animals: Implications for the evolution of the hox cluster and the zootype. *Curr. Top. Dev. Biol.* **40**: 211 - 254.

Finnerty, J. R. (2003). The origins of axial patterning in the metazoa: how old is bilateral symmetry? *Int J Dev Biol* **47**(7-8): 523-9.

Finnerty, J. R., V. A. Master, S. Irvine, M. J. Kourakis, S. Warriner and M. Q. Martindale (1996). Homeobox Genes in the Ctenophora: identification of paired-type and Hox homologues in the atentaculate ctenophore, Beroe ovata. *Mol. Mar. Biol. Biotechnol.* **5**: 249 - 258.

Finnerty, J. R., D. Paulson, P. Burton, K. Pang and M. Q. Martindale (2003). Early evolution of a homeobox gene: the parahox gene Gsx in the Cnidaria and the Bilateria. *Evol Dev* **5**(4): 331-45.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver and C. C. Mello (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* **391**(6669): 806-11.

Fisher, D. A. and H. R. Bode (1989). Nucleotide sequence of an actin-encoding gene from *Hydra attenuata*: strucutral characteristics and evolutionary implications. *Gene* **84**: 55 - 64.

Force, A., M. Lynch, F. Pickett, A. Amores, Y. Yan and J. Postlethwait (1999). Preservation of Duplicate Genes by Complementary Degenerative Mutations. *Genetics* **151**: 1531 - 1545.

Fortier, E. and J. Belote (2000). Temperature-Dependent Gene Silencing by an Expressed Inverted Repeat in *Drosophila. Genesis* **26**: 240 - 244.

Freeman, G. (1981a). The Cleavage initiation site establishes the posterior pole of the hydrozoan embryo. *Roux's Arch. Dev. Biol* **190**: 123 - 125.

Freeman, G. (1981b). The role of polarity in the development of the hydrozoan planula larva. *Roux's Arch. Dev. Biol* **190**: 168 - 184.

Friedman, R. and A. L. Hughes (2001a). Gene duplication and the structure of eukaryotic genomes. *Genome Res* **11**(3): 373-81.

Friedman, R. and A. L. Hughes (2001b). Pattern and timing of gene duplication in animal genomes. *Genome Res* **11**(11): 1842-7.

Frigerio, G., M. Burri, D. Bopp, S. Baumgartner and M. Noll (1986). Structure of the segmentation gene paired and the Drosophila PRD gene set as part of a gene network. *Cell* **47**(5): 735-46.

Galliot, B. (2000). Conserved and divergent genes in apex and axis development of cnidarians. *Current Opinion in Genetics and Development* **10**: 629-637.

Galliot, B., C. de Vargas and D. Miller (1999). Evolution of homeobox genes: Q50 Paired-like genes founded the Paired class. *Dev Genes Evol* **209**(3): 186-97.

Ganfornina, M. D. and D. Sanchez (1999). Generation of evolutionary novelty by functional shift. *BioEssays* **21**(5): 432-9.

Garcia-Fernandez, J. (2004). Hox, ParaHox, ProtoHox: facts and guesses. *Heredity.*

Gauchat, D., S. Kreger, T. Holstein and B. Galliot (1998). prdl-a, a gene marker for hydra apical differentiation related to triploblastic paired-like head-specific genes. *Development* **125**(9): 1637-45.

Gauchat, D., F. Mazet, C. Berney, M. Schummer, S. Kreger, J. Pawlowski and B. Galliot (2000). Evolution of Antp-class genes and differential expression of *Hydra* Hox/paraHox genes in anterior patterning. *Proc. Natl. Acad. Sci. USA* **97**(9): 4493 - 4498.

Gehring, W. J. and K. Ikeo (1999). Pax 6: mastering eye morphogenesis and eye evolution. *Trends Genet* **15**(9): 371-7.

Gogos, J. A., T. Hsu, J. Bolton and F. C. Kafatos (1992). Sequence discrimination by alternatively spliced isoforms of a DNA binding zinc finger domain. *Science* **257**(5078): 1951-5.

Goodman, M., G. W. Moore and G. Matsuda (1975). Darwinian evolution in the genealogy of haemoglobin. *Nature* **253**(5493): 603-8.

Grad, Y. H., F. P. Roth, M. S. Halfon and G. M. Church (2004). Prediction of similarly-acting cis-regulatory modules by subsequence profiling and comparative genomics in D. melanogaster and D. pseudoobscura. *Bioinformatics*.

Grasso, L. C., D. C. Hayward, J. W. Trueman, K. M. Hardie, P. A. Janssens and E. E. Ball (2001). The evolution of nuclear receptors: evidence from the coral Acropora. *Mol Phylogenet Evol* **21**(1): 93-102.

Greenberg, M. (1959). Ancestors, embryos and symmetry. *Syst. Zool* **8**: 212 - 221.

Grens, A., Gee, L, Fisher, D and Bode, H (1996). *CnNK-2*, an NK-2 Homeobox Gene, Has a Role in Patterning the Basal End of the Axis in Hydra. *Developmental Biology* **180**: 473 - 488.

Grens, A., E. Mason, J. L. Marsh and H. R. Bode (1995). Evolutionary Conservation of a cell fate specification gene: the Hydra achaete-scute homolog has proneural activity. *Development* **121**(12): 4027 - 4035.

Griffin, C., D. A. Kleinjan, B. Doe and V. van Heyningen (2002). New 3' elements control Pax6 expression in the developing pretectum, neural retina and olfactory region. *Mech Dev* **112**(1-2): 89-100.

Grimmelikhuijzen, C. J. (1985). Antisera to the sequence Arg-Phe-amide visualise neuronal centralisation in hydroid polyps. *Cell Tissue Res* **241**: 171 - 182.

Grimmelikhuijzen, C. J. and D. Graff (1986). Isolation of pyroGlu-Gly-Arg-Phe-NH2 (Antho-RFamide), a neuropeptide from sea anemones. *Proc Natl Acad Sci U S A* **83**(24): 9817-21.

Grimmelikhuijzen, C. J., I. Leviev and K. Carstensen (1996). Peptides in the nervous systems of cnidarians: structure, function, and biosynthesis. *Int Rev Cytol* **167**: 37-89.

Grimmelikhuijzen, C. J., K. L. Rinehart and A. N. Spencer (1992). Isolation of the neuropeptide less than Glu-Trp-Leu-Lys-Gly-Arg-Phe-NH2 (Pol-RFamide II) from the hydromedusa Polyorchis penicillatus. *Biochem Biophys Res Commun* **183**(2): 375-82.

Grimmelikhuijzen, C. J. and J. A. Westfall (1995). The nervous systems of cnidarians. *Exs* **72**: 7-24.

Grimmelikhuijzen, C. J., M. Williamson and G. N. Hansen (2002). Neuropeptides in cnidarians. *Can J Zool* **80**: 1690 - 1702.

Groger, H., P. Callaerts, W. J. Gehring and V. Schmid (2000). Characterization and expression analysis of an ancestor-type Pax gene in the hydrozoan jellyfish Podocoryne carnea. *Mech Dev* **94**(1-2): 157-69.

Groger, H. and V. Schmid (2001). Larval Development in Cnidaria: A Connection to Bilateria? *Genesis* **29**: 100 - 114.

Gruschus, J., D. Tsao, L. Wang, M. Nirenberg and J. Ferretti (1997). Interactions of the vnd/Nk-2 Homeodomain with DNA by Nuclear Magnetic Resonance Spectroscopy: Basis of Binding Specficity. *Biochemistry* **36**: 5372 - 5380.

Gruschus, J. M., D. H. Tsao, L. H. Wang, M. Nirenberg and J. A. Ferretti (1999). The three-dimensional structure of the vnd/NK-2 homeodomain-DNA complex by NMR spectroscopy. *J Mol Biol* **289**(3): 529-45.

Gu, X. and W. Huang (2002). Testing the parsimony test of genome duplications: a counterexample. *Genome Res* **12**(1): 1-2.

Guazzi, S., M. Price, M. De Felice, G. Damante, M.-G. Mattei and R. Di Lauro (1990). Thyroid nuclear factor 1 (TTF1) contains a homeodomain and displays a novel DNA binding specificity. *Embo J* **9**: 3631 - 3639.

Guigo, R., I. Muchnik and T. F. Smith (1996). Reconstruction of ancient molecular phylogeny. *Mol Phylogenet Evol* **46**: 189 - 213.

Hadzi, J. (1909). Uber das Nervensystem von *Hydra*. *Arb. Yool. Ist. Wien* **17**: 225 - 268.

Halder, G., P. Callaerts, S. Flister, U. Walldorf, U. Kloter and W. J. Gehring (1998). Eyeless initiates the expression of both sine oculis and eyes absent during Drosophila compound eye development. *Development* **125**(12): 2181-91.

Halder, G., P. Callaerts and W. J. Gehring (1995). Induction of ectopic eyes by targeted expression of the eyeless gene in Drosophila. *Science* **267**(5205): 1788-92.

Hamdan, H., H. Liu, C. Li, C. Jones, M. Lee, R. deLemos and P. Minoo (1998). Structure of the human Nkx2.1 gene. *Biochim Biophys Acta* **1396**(3): 336-48.

Hansen, G., M. Williamson and C. Grimmelikhuijzen (2000). Two-color double-labelling *in situ* hybridisation of whole-mount *Hydra* using RNA probes for five different *Hydra* neuropeptide preprohormones: evidence for colocalization. *Cell Tissue Res* **301**: 245 - 253.

Harrison, P., R. C. Babcock, G. Bull, J. Oliver, C. Wallace and B. Willis (1984). Mass spawning in tropical reef corals. *Science* **223**: 1186 - 1189.

Harrison, P. L. and C. C. Wallace (1990). Ecosystems of the World. Z. Dubinsky. Amsterdam, Elsevier: 133 - 207.

Harvey, R. (1996). NK-2 Homeobox Genes and Heart Development. *Developmental Biology* **178**: 203 - 216.

Hasson, P., B. Muller, K. Basler and Z. Paroush (2001). Brinker requires two corepressors for maximal and versatile repression in Dpp signalling. *Embo J* **20**(20): 5725-36.

Hatano M, Roberts C, Minden M, C. W and K. S (1991). Deregulation of a homeobox gene, HOX11, by the t(10;14) in T cell leukaemia. *Science* **253**(5015): 79 - 81.

Hauck, B., W. J. Gehring and U. Walldorf (1999). Functional analysis of an eye specific enhancer of the eyeless gene in Drosophila. *Proc Natl Acad Sci U S A* **96**(2): 564-9.

Hauptmann, G. (2001). One-, two-, and three-color whole-mount in situ hybridization to Drosophila embryos. *Methods* **23**(4): 359-72.

Hayashi, T., K. T and S. K (1998). Specification of Primary Pigment Cell and Outer Photoreceptor Fates by *BarH1* Homeobox Gene in the Developing *Drosophila* Eye. *Developmental Biology* **200**: 131 - 145.

Hayward, D., J. Catmull, J. Reece-Hoyes, H. Berghammer, H. Dodd, S. Hann, D. Miller and E. Ball (2001). Gene Structure and Larval Expression of *cnox-2Am* from the coral *Acropora millepora*. *Dev Genes Evol* **211**: 10 - 19.

Hayward, D. C., G. Samuel, P. C. Pontynen, J. Catmull, R. Saint, D. J. Miller and E. E. Ball (2002). Localized expression of a dpp/BMP2/4 ortholog in a coral embryo. *Proc Natl Acad Sci U S A* **99**(12): 8106-11.

Heidari, M., K. L. Rice, U. R. Kees and W. K. Greene (2002). Expression and purification of the human homeodomain oncoprotein HOX11. *Protein Expr Purif* **25**(2): 313-8.

Heinemeyer, T., E. Wingender, I. Reuter, H. Hermjakob, A. E. Kel, O. V. Kel, E. V. Ignatieva, E. A. Ananko, O. A. Podkolodnaya, F. A. Kolpakov, N. L. Podkolodny and N. A. Kolchanov (1998). Databases on transcriptional regulation: TRANSFAC, TRRD and COMPEL. *Nucleic Acids Res* **26**(1): 362-7.

Heitzler, P., M. Bourouis, L. Ruel, C. Carteret and P. Simpson (1996). Genes of the Enhancer of split and *achaete-scute* complexes are required for a regulatory loop between Notch and Delta during lateral signalling in *Drosophila. Development* **122**: 161 - 171.

Heitzler, P. and P. Simpson (1991). The Choice of Cell Fate in the Epidermis of *Drosophila. Cell* **64**: 1083 - 1092.

Herring, B. P., A. M. Kriegel and A. M. Hoggatt (2001). Identification of Barx2b, a serum response factor-associated homeodomain protein. *J Biol Chem* **276**(17): 14482-9.

Higashijima, S., Kojima T, Michiue T, Ishimar S, Emori Y and Saigo K (1992). Dual *Bar* homeo box genes of *Drosophila* required in two photoreceptor cells, R1 and R6, and primary pigment cells for normal eye development. *Genes and Development* **6**: 50 - 60.

Higashijima, S., Michiue T, E. Y and S. K (1992). Subtype determination of Drosophila embryonic external sensory organs by redundant homeo box genes BarH1 and BarH2. *Genes and Development* **6**: 1005 - 1018.

Hill, A., A. Wagner and M. Hill (2002). Hox and paraHox genes from the anthozoan Parazoanthus parasiticus. *Mol Phylogenet Evol* **28**: 529 - 535.

Hislop, N. R. (2003). Characterisation of anterior patterning genes in the staghorn coral, *Acropora millepora*. School of Pharmacy and Molecular Sciences. Townsville, James Cook University**:** 199.

Hjalt, T. A. and J. C. Murray (1999). The human BARX2 gene: genomic structure, chromosomal localization, and single nucleotide polymorphisms. *Genomics* **62**(3): 456-9.

Hobmayer, B., F. Rentzsch, K. Kuhn, C. M. Happel, C. C. von Laue, P. Snyder, U. Rothbacher and T. W. Holstein (2000). WNT signalling molecules act in axis formation in the diploblastic metazoan Hydra. *Nature* **407**(6801): 186-9.

Holland, P. (1991). Cloning and Evolutionary Analysis of *msh*-like homeobox genes from mouse, zebrafish and ascidian. *Gene* **98**: 253 - 257.

Holland, P. (1998). Major Transitions in Animal Evolution: A Developmental Genetic Perspective. *Amer. Zool.* **38**: 829 - 842.

Holland, P. (2001). Beyond the Hox: how widespread is homeobox gene clustering? *J. Anat* **199**: 13 - 23.

Holland, P. W. and J. Garcia-Fernandez (1996). Hox genes and chordate evolution. *Dev Biol* **173**(2): 382-95.

Holland, P. W., J. Garcia-Fernandez, N. A. Williams and A. Sidow (1994). Gene duplications and the origins of vertebrate development. *Dev Suppl*: 125-33.

Holley, S. A., P. D. Jackson, Y. Sasai, B. Lu, E. M. De Robertis, F. M. Hoffmann and E. L. Ferguson (1995). A Conserved System for Dorsal-Ventral Patterning in Insects and Vertebrates involving sog and chordin. *Nature* **376**: 249 - 253.

Horiike, T., K. Hamada, S. Kanaya and T. Shinozawa (2001). Origin of eukaryotic cell nuclei by symbiosis of Archaea in Bacteria is revealed by homology-hit analysis. *Nat Cell Biol* **3**(2): 210-4.

Horton, A. C., N. R. Mahadevan, I. Ruvinsky and J. J. Gibson-Brown (2003). Phylogenetic analyses alone are insufficient to determine whether genome duplication(s) occurred during early vertebrate evolution. *J Exp Zoolog Part B Mol Dev Evol* **299**(1): 41-53.

Hoshiyama, D., H. Suga, N. Iwabe, M. Koyanagi, N. Nikoh, K. Kuma, F. Matsuda, T. Honjo and T. Miyata (1998). Sponge Pax cDNA related to Pax-2/5/8 and ancient gene duplications in the Pax family. *J Mol Evol* **47**(6): 640-8.

Hovde, S., C. Abate-Shen and J. Geiger (2001). Crystal Structure of the Msx-1 Homeodomain/DNA Complex. *Biochemistry* **40**: 12013 - 12021.

Hsieh-Li, H. M., D. P. Witte, J. C. Szuckshi, M. Weinstein, H. Li and S. S. Potter (1995). Gsh-2: a murine homeobox gne expressed in the developing brain. *Mech Dev* **50**: 177 - 186.

Hu, G., H. Lee, S. Price, M. Shen and C. Abate-Shen (2001). *Msx* Homeobox genes inhibit differentiation through upregulation of *cyclin D1*. *Development* **128**: 2373 - 2384.

Hughes, A. L. (1999). Phylogenies of developmentally important proteins do not support the hypothesis of two rounds of genome duplication early in vertebrate history. *J Mol Evol* **48**: 565 - 576.

Hughes, A. L., J. da Silva and R. Friedman (2001). Ancient genome duplications did not structure the human Hox-bearing chromosomes. *Genome Res* **11**(5): 771-80.

Hughes, C. L. and T. C. Kaufman (2002). Hox Genes and the Evolution of the Arthropod Body Plan. *Evol Dev* **4**(6): 459 - 499.

Hummel, T., K. Krukkert, J. Roos, G. Davis and C. Klambt (2000). Drosophila Futsch/22C10 is a MAP1B-like protein required for dendritic and axonal development. *Neuron* **26**(2): 357-70.

Hyman, L. H. (1940). Metazoa of the Tissue Grade of Construction - The Radiate Phyla - Phylum Cnidaria. The Invertebrates: Protozoa through Ctenophora. New York, McGraw-Hill Book Company Inc: 365 - 641.

Irvine, K. D. and C. Rauskolb (2001). Boundaries in development: formation and function. *Annu Rev Cell Dev Biol* **17**: 189-214.

Isshiki, T., M. Takeichi and A. Nose (1997). The role of the *msh* homeobox gene during *Drosohpila* neurogenesis: implication for the dorsoventral specification of the neuroectoderm. *Development* **124**: 3099 - 3109.

Iwabe, N., K. Kuma and T. Miyata (1996). Evolution of gene families and relationship with organismal evolution: rapid divergence of tissue-specific genes in the early evolution of chordates. *Mol Biol Evol* **13**(3): 483-93.

Iwao, K., T. Fujisawa and M. Hatta (2002). A cnidarian neuropeptide of the GLWamide family induces metamorphosis of reef-building corals in the genus Acropora. *Coral Reefs* **21**: 127 - 129.

Jagla, K., M. Bellard and M. Frasch (2001a). A Cluster of *Drosophila* Homeobox Genes Involved in Mesoderm Differentiation Programs. *BioEssays* **23**: 125 - 133.

Jagla, K., M. Bellard and M. Frasch (2001b). A cluster of Drosophila homeobox genes involved in mesoderm differentiation programs. *BioEssays* **23**(2): 125-33.

Jagla, K., I. Stanceva, G. Dretzen, F. Bellard and M. Bellard (1994). A distinct class of homeodomain proteins is encoded by two sequentially expressed Drosophila genes from the 93D/E cluster. *Nucleic Acids Res* **22**(7): 1202-7.

Jagla, T., F. Bellard, Y. Lutz, G. Dretzen, M. Bellard and K. Jagla (1998). ladybird determines cell fate decisions during diversification of Drosophila somatic muscles. *Development* **125**(18): 3699-708.

Jagla, T., Y. Bidet, J. P. Da Ponte, B. Dastugue and K. Jagla (2002). Cross-repressive interactions of identity genes are essential for proper specification of cardiac and muscular fates in Drosophila. *Development* **129**(4): 1037-47.

Jakob, W., S. Sagasser, S. Dellaporta, P. Holland, K. Kuhn and B. Schierwater (2004). The Trox-2 Hox/ParaHox gene of Trichoplax (Placozoa) marks an epithelial boundary. *Dev Genes Evol* **214**(4): 170-5.

Jang, C. C., J. L. Chao, N. Jones, L. C. Yao, D. A. Bessarab, Y. M. Kuo, S. Jun, C. Desplan, S. K. Beckendorf and Y. H. Sun (2003). Two Pax genes, eye gone and eyeless, act cooperatively in promoting Drosophila eye development. *Development* **130**(13): 2939-51.

Jiao, R., M. Daube, H. Duan, Y. Zou, E. Frei and M. Noll (2001). Headless flies generated by developmental pathway interference. *Development* **128**(17): 3307-19.

Jimenez, F., L. Martin-Morris, L. Velasco, H. Chu, J. Sierra, D. Rosen and K. White (1995). *vnd*, a gene required for early neurogenesis of *Drosophila*, encodes a homeodomain protein. *The EMBO Journal* **14**(14): 3487 - 3495.

Jin, S., S. Martinek, W. Joo, J. Wortman, N. Mirkovic, A. Sali, M. Yandell, N. Pavletich, M. Young and A. Levine (2000). Identification and Characterization of a p53 Homologue in *Drosophila melanogaster. Proc. Natl. Acad. Sci. USA* **97**(13): 7301 - 7306.

Jones, F., Kioussi C, Copertino D, Kallunki P, H. B and E. G (1997). *Barx2*, a new homeobox gene of the *Bar* class, is expressed in neural and craniofacial structures during development. *Proc. Natl. Acad. Sci. USA* **94**: 2632 - 2637.

Jordan, I. K., Y. I. Wolf and E. V. Koonin (2004). Duplicated genes evolve slower than singletons despite the initial rate increase. *BMC Evol Biol* **4**(1): 22.

Jun, S. and C. Desplan (1996). Cooperative Interactions Between Paired Domain and Homeodomain. *Development* **122**: 2639 - 2650.

Jun, S., R. V. Wallen, A. Goriely, B. Kalionis and C. Desplan (1998). Lune/eye gone, a Pax-like protein, uses a partial paired domain and a homeodomain for DNA recognition. *Proc Natl Acad Sci U S A* **95**(23): 13720-5.

Kalidas, S. and D. Smith (2002). Novel Genomic cDNA Hybrids Produce Effective RNA Interference in Adult *Drosophila. Neuron* **33**: 177 - 184.

Kamachi, Y., M. Uchikawa, A. Tanouchi, R. Sekido and H. Kondoh (2001). Pax6 and SOX2 form a co-DNA-binding partner complex that regulates initiation of lens development. *Genes Dev* **15**(10): 1272-86.

Kammandel, B., K. Chowdhury, A. Stoykova, S. Aparicio, S. Brenner and P. Gruss (1999). Distinct cis-essential modules direct the time-space pattern of the Pax6 gene activity. *Dev Biol* **205**(1): 79-97.

Kappen, C. (2000). Analysis of a complete homeobox gene repertoire: implications for the evolution of diversity. *Proc Natl Acad Sci U S A* **97**(9): 4481-6.

Kappen, C., K. Schughart and F. H. Ruddle (1993). Early evolutionary origin of major homeodomain sequence classes. *Genomics* **18**(1): 54-70.

Kasahara, H., A. Usheva, T. Ueyama, H. Aoki, N. Horikoshi and S. Izumo (2001). Characterization of homo- and heterodimerization of cardiac Csx/Nkx2.5 homeoprotein. *J Biol Chem* **276**(7): 4570-80.

Kennedy, M., R. Gonzalez-Sarmiento, U. Kees, F. Lampert, N. Dear, T. Boehm and T. Rabbitts (1991). Hox11, a homeobox-containing T-cell oncogene on human chromosome 10q24. *Proc. Natl. Acad. Sci. USA* **88**: 8900 - 8904.

Kennerdell, J. R. and R. W. Carthew (1998). Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell* **95**(7): 1017-26.

Kidwell, M. G. (2002). Transposable elements and the evolution of genome size in eukaryotes. *Genetica* **115**(1): 49-63.

Kimura, M. (1983). The Neutral Theory of Molecular Evolution, Cambridge University Press.

Kiss, I. I., J. Szabad and J. Major (1978). Genetic and developmental analysis of puparium formation in Drosophila. *Mol Gen Genet* **164**: 77 - 83.

Knirr, S., N. Azpiazu and M. Frasch (1999). The role of the NK-homeobox gene slouch (S59) in somatic muscle patterning. *Development* **126**(20): 4525-35.

Kobayishi, M., S. Takezawa, K. Hara, R. Yu, Umesono Y, K. Agata, M. Taniwaki, K. Yasuda and K. Umesono (1999). Identification of a Photoreceptor Cell-specific Nuclear Receptor. *Proc. Natl. Acad. Sci. USA* **96**: 4814 - 4819.

Koehler, K., T. Franz and T. N. Dear (2000). Hox11 is required to maintain normal Wt1 mRNA levels in the developing spleen. *Dev Dyn* **218**(1): 201-6.

Koizumi, K., C. Lintas, M. Nirenberg, J. S. Maeng, J. H. Ju, J. W. Mack, J. M. Gruschus, W. F. Odenwald and J. A. Ferretti (2003). Mutations that affect the ability of the vnd/NK-2 homeoprotein to regulate gene expression: transgenic alterations and tertiary structure. *Proc Natl Acad Sci U S A* **100**(6): 3119-24.

Kojima, T., Ishimaru S, Higashijima S, Takayama E, Akimaru H, Sone M, E. Y and S. K (1991). Identification of a different-type homeobox gene, *BarH1*, possibly causing Bar (B) and *Om*(1D) mutations in *Drosophila. Proc. Natl. Acad. Sci. USA* **88**: 4343 - 4347.

Kojima, T., Sato, M and Saigo, K (2000). Formation and Specification of Distal Leg Segments in *Drosophila* by dual *Bar* homeobox genes, *BarH1* and *BarH2*. *Development* **127**: 769-778.

Kolkman, J. A. and W. P. Stemmer (2001). Directed evolution of proteins by exon shuffling. *Nat Biotechnol* **19**(5): 423-8.

Kondrashov, F. A., I. B. Rogozin, Y. I. Wolf and E. V. Koonin (2002). Selection in the evolution of gene duplications. *Genome Biol* **3**(2): RESEARCH0008.

Kopan, R. (2002). Notch: A Membrane-bound Transcription factor. *J Cell Sci* **2002**(115): 1095 - 1097.

Kortschak, R. D., G. Samuel, R. Saint and D. J. Miller (2003). EST analysis of the cnidarian Acropora millepora reveals extensive gene loss and rapid sequence divergence in the model invertebrates. *Curr Biol* **13**(24): 2190-5.

Koshiba, K., A. Kuroiwa, H. Yamamoto, K. Tamara and H. Ide (1998). Expression of Msx Genes in Regenerating and Developing Limbs of Axolotol. *The Journal of Experimental Zoology* **282**: 703 - 714.

Kourakis, M. and M. Martindale (2000). Combined-Method Phylogenetic Analysis of Hox and ParaHox Genes of the Metazoa. *Journal of Experimental Zoology (Mol Dev Evol)* **288**: 175 - 191.

Koyanagi, M., K. Ono, H. Suga, N. Iwabe and T. Miyata (1998a). Phospholipase C cDNAs from sponge and hydra: antiquity of genes involved in the inositol phospholipid signaling pathway. *FEBS Lett* **439**(1-2): 66-70.

Koyanagi, M., H. Suga, D. Hoshiyama, K. Ono, N. Iwabe, K. Kuma and T. Miyata (1998b). Ancient gene duplication and domain shuffling in the animal cyclic nucleotide phosphodiesterase family. *FEBS Lett* **436**(3): 323-8.

Kruse, M., A. Mikoc, H. Cethovic, V. Gamulin, B. Rinkevich, I. M. Muller and W. E. Muller (1994). Molecular Evidence for the Presence of a Developmental Gene in the Lowest Animals: Identificiation of a Homeobox-like gene in the Marine Sponge *Geodia cydonium. Mech. Ageing Dev.* **77**: 43 - 54.

Kuhn, K., B. Streit and S. B (1999). Isolation of Hox Genes from the Scyphozoan *Cassiopeia xamachana*: Implications for the Early Evolution of Hox Genes. *Journal of Experimental Zoology (Mol Dev Evol)* **285**: 63 - 75.

Kuhn, K., B. Streit and B. Schierwater (1996). Homeobox genes in the cnidarian Eleutheria dichotoma: evolutionary implications for the origin of Antennapedia-class (HOM/Hox) genes. *Mol Phylogenet Evol* **6**(1): 30-8.

Kumar, J. P. and K. Moses (2001). Expression of evolutionarily conserved eye specification genes during Drosophila embryogenesis. *Dev Genes Evol* **211**(8-9): 406-14.

Kuzuoka, M., T. Takahashi, C. Guron and R. Raghow (1994). Murine homeobox-containing gene, Msx-1: analysis of genomic organization, promoter structure, and potential autoregulatory cis-acting elements. *Genomics* **21**(1): 85-91.

Lake, J. A. (1988). Origin of the eukaryotic nucleus determined by rate-invariant analysis of rRNA sequences. *Nature* **331**(6152): 184-6.

Lander, E. S., L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. LeVine, P. McEwan, K. McKernan, J. Meldrim, J. P. Mesirov, C. Miranda, W. Morris, J. Naylor, C. Raymond, M. Rosetti, R. Santos, A. Sheridan, C. Sougnez, N. Stange-Thomann, N. Stojanovic, A. Subramanian, D. Wyman, J. Rogers, J. Sulston, R. Ainscough, S. Beck, D. Bentley, J. Burton, C. Clee, N. Carter, A. Coulson, R. Deadman, P. Deloukas, A. Dunham, I. Dunham, R. Durbin, L. French, D. Grafham, S. Gregory, T. Hubbard, S. Humphray, A. Hunt, M. Jones, C. Lloyd, A. McMurray, L. Matthews, S. Mercer, S. Milne, J. C. Mullikin, A. Mungall, R. Plumb, M. Ross, R. Shownkeen, S. Sims, R. H. Waterston, R. K. Wilson, L. W. Hillier, J. D. McPherson, M. A. Marra, E. R. Mardis, L. A. Fulton, A. T. Chinwalla, K. H. Pepin, W. R. Gish, S. L. Chissoe, M. C. Wendl, K. D. Delehaunty, T. L. Miner, A. Delehaunty, J. B. Kramer, L. L. Cook, R. S. Fulton, D. L. Johnson, P. J. Minx, S. W. Clifton, T. Hawkins, E. Branscomb, P. Predki, P. Richardson, S. Wenning, T. Slezak, N. Doggett, J. F. Cheng, A. Olsen, S. Lucas, C. Elkin, E. Uberbacher, M. Frazier, R. A. Gibbs, D. M. Muzny, S. E. Scherer, J. B. Bouck, E. J. Sodergren, K. C. Worley, C. M. Rives, J. H. Gorrell, M. L. Metzker, S. L. Naylor, R. S. Kucherlapati, D. L. Nelson, G. M. Weinstock, Y. Sakaki, A. Fujiyama, M. Hattori, T. Yada, A. Toyoda, T. Itoh, C. Kawagoe, H. Watanabe, Y. Totoki, T. Taylor, J. Weissenbach, R. Heilig, W. Saurin, F. Artiguenave, P. Brottier, T. Bruls, E. Pelletier, C. Robert, P. Wincker, D. R. Smith, L. Doucette-Stamm, M. Rubenfield, K. Weinstock, H. M. Lee, J. Dubois, A. Rosenthal, M. Platzer, G. Nyakatura, S. Taudien, A. Rump, H. Yang, J. Yu, J. Wang, G. Huang, J. Gu, L. Hood, L. Rowen, A. Madan, S. Qin, R. W. Davis, N. A. Federspiel, A. P. Abola, M. J. Proctor, R. M. Myers, J. Schmutz, M. Dickson, J. Grimwood, D. R. Cox, M. V. Olson, R. Kaul, N. Shimizu, K. Kawasaki, S. Minoshima, G. A. Evans, M. Athanasiou, R. Schultz, B. A. Roe, F. Chen, H. Pan, J. Ramser, H. Lehrach, R. Reinhardt, W. R. McCombie, M. de la Bastide, N. Dedhia, H. Blocker, K. Hornischer, G. Nordsiek, R. Agarwala, L. Aravind, J. A. Bailey, A.

Bateman, S. Batzoglou, E. Birney, P. Bork, D. G. Brown, C. B. Burge, L. Cerutti, H. C. Chen, D. Church, M. Clamp, R. R. Copley, T. Doerks, S. R. Eddy, E. E. Eichler, T. S. Furey, J. Galagan, J. G. Gilbert, C. Harmon, Y. Hayashizaki, D. Haussler, H. Hermjakob, K. Hokamp, W. Jang, L. S. Johnson, T. A. Jones, S. Kasif, A. Kaspryzk, S. Kennedy, W. J. Kent, P. Kitts, E. V. Koonin, I. Korf, D. Kulp, D. Lancet, T. M. Lowe, A. McLysaght, T. Mikkelsen, J. V. Moran, N. Mulder, V. J. Pollara, C. P. Ponting, G. Schuler, J. Schultz, G. Slater, A. F. Smit, E. Stupka, J. Szustakowski, D. Thierry-Mieg, J. Thierry-Mieg, L. Wagner, J. Wallis, R. Wheeler, A. Williams, Y. I. Wolf, K. H. Wolfe, S. P. Yang, R. F. Yeh, F. Collins, M. S. Guyer, J. Peterson, A. Felsenfeld, K. A. Wetterstrand, A. Patrinos, M. J. Morgan, J. Szustakowki, P. de Jong, J. J. Catanese, K. Osoegawa, H. Shizuya, S. Choi and Y. J. Chen (2001). Initial sequencing and analysis of the human genome. *Nature* **409**(6822): 860-921.

Landgraf, M., T. Bossing, G. M. Technau and M. Bate (1997). The origin, location, and projections of the embryonic abdominal motorneurons of Drosophila. *J Neurosci* **17**(24): 9642-55.

Langenau, D. M., T. Palomero, J. P. Kanki, A. A. Ferrando, Y. Zhou, L. I. Zon and A. T. Look (2002). Molecular cloning and developmental expression of Tlx (Hox11) genes in zebrafish (Danio rerio). *Mech Dev* **117**(1-2): 243-8.

Latchman, D. (1999). POU Family Transcription Factors in the Nervous System. *Journal of Cellular Physiology* **179**: 126 - 133.

Laughon, A. (1991). DNA binding specificity of homeodomains. *Biochemistry* **30**(48): 11357-67.

Lee, Y. S. and R. W. Carthew (2003). Making a better RNAi vector for Drosophila: use of intron spacers. *Methods* **30**(4): 322-9.

Leuzinger, S., F. Hirth, D. Gerlich, D. Acampora, A. Simeone, W. Gehring, R. Finkelstein, K. Furukubo-Tokunaga and H. Recihert (1998). Equivalence of the fly orthodenticlegene and the human OTX genes in embryonic brain development of Drosophila. *Development* **125**: 1703 - 1710.

Lewin, B. (1997). Genes VI. Oxford, Oxford University Press.

Lewis, S. and C. Konradi (1996). Analysis of DNA-Protein Interactions in the Nervous System Using the Electrophoretic Mobility Shift Assay. *Methods: A Companion to Methods in Enzymology* **10**: 301 - 311.

Li, S., S. M. Price, H. Cahill, D. K. Ryugo, M. M. Shen and M. Xiang (2002). Hearing loss caused by progressive degeneration of cochlear hair cells in mice deficient for the Barhl1 homeobox gene. *Development* **129**(14): 3523-32.

Li, W. H., Z. Gu, H. Wang and A. Nekrutenko (2001). Evolutionary analyses of the human genome. *Nature* **409**(6822): 847-9.

Linecum, J., A. Fannon, K. Song, Y. Wang and D. Sassoon (1998). Msh Homeobox Genes Regulate Cadherin-Mediated Cell Adhesion and Cell-Cell Sorting. *Journal of Cellular Biochemistry* **70**: 22 - 28.

Lister, J. A., J. Close and D. W. Raible (2001). Duplicate mitf genes in zebrafish: complementary expression and conservation of melanogenic potential. *Dev Biol* **237**(2): 333-44.

Liu, J., T. Wilson, J. Milbrandt and M. Johnston (1993). Identifying DNA-Binding SItes and Analyzing DNA-Binding Domains Using a Yeast Selection System. *Methods: A Companion to Methods in Enzymology* **5**: 125 - 137.

Locascio, A., M. Manzanares, M. J. Blanco and M. A. Nieto (2002). Modularity and reshuffling of Snail and Slug expression during vertebrate evolution. *Proc Natl Acad Sci U S A* **99**(26): 16841-6.

Lockett, T. J. (1990). A bacteriophage lambda DNA purification procedure suitable for the analysis of DNA from either large or multiple small lysates. *Anal Biochem* **185**(2): 230-4.

Logan, C., R. Wingate, I. McKay and A. Lumsden (1998). *Tlx-1* and *Tlx-3* Homeobox Gene Expression in Cranial Sensory Ganglia and Hidbrain of the Chick Embryo: Markers of Patterned Connectivity. *The Journal of Neuroscience* **18**(14): 5389 - 5402.

Lohmann, J. U., I. Endl and T. C. Bosch (1999). Silencing of developmental genes in Hydra. *Dev Biol* **214**(1): 211-4.

Long, M. (2001). Evolution of novel genes. *Curr Opin Genet Dev* **11**(6): 673-80.

Long, M., S. J. de Souza, C. Rosenberg and W. Gilbert (1996). Exon shuffling and the origin of the mitochondrial targeting function in plant cytochrome c1 precursor. *Proc Natl Acad Sci U S A* **93**(15): 7727-31.

Loosli, F., M. Kmita-Cunisse and W. J. Gehring (1996). Isolation of a Pax-6 homolog from the ribbonworm Lineus sanguineus. *Proc Natl Acad Sci U S A* **93**(7): 2658-63.

Loureiro, J. and M. Peifer (1998). Roles of Armadillo, a Drosophila catenin, during central nervous system development. *Curr Biol* **8**(11): 622-32.

Lowe, C. J. and G. A. Wray (1997). Radical alterations in the roles of homeobox genes during echinoderm evolution. *Nature* **389**(6652): 718-21.

Lowe, C. J., M. Wu, A. Salic, L. Evans, E. Lander, N. Stange-Thomann, C. E. Gruber, J. Gerhart and M. Kirschner (2003). Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. *Cell* **113**(7): 853-65.

Lu, M., Gong G, S. W and H. A (1991). The *tcl-3* proto-oncogene altered by chromosomal translocation in T-cell leukaemia codes for a homeobox protein. *The EMBO Journal* **10**(10): 2905 - 2910.

Lu, S., L. Dogarad, M. Murtha and F. Ruddle (1992). Expression Pattern of a murine homeobox gene, *Dbx*, displays extreme spatial restriction in empryonic forebrain and spinal cord. *Proc. Natl. Acad. Sci. USA* **89**: 8053 - 8057.

Luke, G. N., L. F. Castro, K. McLay, C. Bird, A. Coulson and P. W. Holland (2003). Dispersal of NK homeobox gene clusters in amphioxus and humans. *Proc Natl Acad Sci U S A* **100**(9): 5292-5.

Lundin, L. (1999). Gene Duplications in early metazoan evolution. *Cell and Developmental Biology* **10**: 523 - 530.

Lundin, L. G. (1993). Evolution of the vertebrate genome as reflected in paralogous chromosomal regions in man and the house mouse. *Genomics* **16**(1): 1-19.

Lynch, M. (1999). The Age and Relationships of the Major Animal Phyla. *Evolution* **53**: 319 - 325.

Lynch, M. (2002). Genomics. Gene duplication and evolution. *Science* **297**(5583): 945-7.

Lynch, M. and J. S. Conery (2000). The evolutionary fate and consequences of duplicate genes. *Science* **290**(5494): 1151-5.

Lynch, M. and J. S. Conery (2003a). The evolutionary demography of duplicate genes. *J Struct Funct Genomics* **3**(1-4): 35-44.

Lynch, M. and J. S. Conery (2003b). The origins of genome complexity. *Science* **302**(5649): 1401-4.

Lynch, M. and A. Force (2000). The probability of duplicate gene preservation by subfunctionalization. *Genetics* **154**(1): 459-73.

Lynch, M., M. O'Hely, B. Walsh and A. Force (2001). The probability of preservation of a newly arisen gene duplicate. *Genetics* **159**(4): 1789-804.

MacKenzie, A., L. Purdie, D. Davidson, M. Collinson and R. E. Hill (1997). Two enhancer domains control early aspects of the complex expression pattern of *Msx1. Mech Dev* **62**: 29 - 40.

Mackie, G. O. (1990). The Elementary Nervous System Revisited. *Amer. Zool.* **30**: 907 - 920.

Maizel, A., O. Bensaude, A. Prochiantz and A. Joliot (1999). A Short Region of its Homeodomain is necessary for Engrailed nuclear export and secretion. *Development* **126**: 3183 - 3190.

Mannervik, M., Y. Nibu, H. Zhang and M. Levine (1999). Transcriptional Coregulators in Development. *Science* **284**: 606 - 6009.

Martin, A. (2001). Is tetralogy true? Lack of support for the "one-to-four rule". *Mol Biol Evol* **18**(1): 89-93.

Martin, V. J. (2000). Reorganisation of the nervous system during metamorphosis of a hydrozoan planula. *Invertebrate Biology* **119**: 243- 253.

Martin, V. J., C. L. Littlefield, W. E. Archer and H. R. Bode (1997). Embryogenesis in hydra. *Biol. Bull.* **192**: 345 - 363.

Martindale, M. Q., K. Pang and J. R. Finnerty (2004). Investigating the origins of triploblasty: 'mesodermal' gene expression in a diploblastic animal, the sea anemone Nematostella vectensis (phylum, Cnidaria; class, Anthozoa). *Development* **131**(10): 2463-74.

Martinez, D., D. Bridge, L. M. Masuda-Nakagawa and P. Cartwright (1998). Cnidarian homeoboxes and the zootype. *Nature* **393**: 748 - 749.

Martinez, P. and C. T. Amemiya (2002). Genomics of the HOX gene cluster. *Comp Biochem Physiol B Biochem Mol Biol* **133**(4): 571-80.

Mastick, G., R. McKay, T. Oligino, K. Donovan and J. Lopez (1995). Identification of Target Genes Reguated by Homeotic Proteins in *Drosophila melanogaster* Through Genetic Selection of *Ultrabithorax* Protein-Binding Sites in Yeast. *Genetics* **139**: 349 - 363.

Masuda-Nakagawa, L. M., H. Groer, B. L. Aerne and V. Schmid (2000). The HOX-like gene Cnox2-Pc is expressed at the anterior region in all life cycle stages of the jellyfish Podocoryne carnea. *Dev Genes Evol* **210**(3): 151-6.

Matera, I., T. Bachetti, R. Cinti, M. Lerone, L. Gagliardi, F. Morandi, M. Motta, F. Mosca, G. Ottonello, R. Piumelli, J. G. Schober, R. Ravazzolo and I. Ceccherini (2002). Mutational analysis of the RNX gene in congenital central hypoventilation syndrome. *Am J Med Genet* **113**(2): 178-82.

Mc Donald, J., S. Holbrook, T. Isshiki, J. Weiss, C. Doe and D. Mellerick (1998). Dorsoventral patterning in the *Drosohphila* central nervous system: the *vnd* homeobox gene specifies ventral column identity. *Genes and Development* **12**: 3603 - 3612.

McClintock, J. M., M. A. Kheirbek and V. E. Prince (2002). Knockdown of duplicated zebrafish hoxb1 genes reveals distinct roles in hindbrain patterning and a novel mechanism of duplicate gene retention. *Development* **129**(10): 2339-54.

McMillan, J., D. Yellowlees, A. J. Heyward, P. Harrison and D. Miller (1988). Preparation of high molecular weight DNA from hermatypic corals and its use for DNA hybridization and cloning. *Marine Biology* **98**: 271 - 276.

Medina, M., A. Collins, J. Siberman and M. Sogin (2001). Evaluating Hypotheses of Basal Animal Phylogeny using Complete Sequences of Large and Small Subunit rRNA. *Proc. Natl. Acad. Sci. USA* **98**(17): 9707 - 9712.

Meech, R., H. Makarenkova, D. B. Edelman and F. S. Jones (2003). The homeodomain protein Barx2 promotes myogenic differentiation and is regulated by myogenic regulatory factors. *J Biol Chem* **278**(10): 8269-78.

Meinhardt, H. (2002). The radial-symmetric hydra and the evolution of the bilateral body plan: an old body became a young brain. *BioEssays* **24**(2): 185-91.

Mellerick, D. and M. Nirenberg (1995). Dorsal-Ventral Patterning Genes Restrict NK-2 Homeobox Gene Expression to the ventral half of the Central Nervous System of *Drosophila* Embryos. *Dev Biology* **171**: 306 - 316.

Merabet, S., Z. Kambris, M. Capovilla, H. Berenger, J. Pradel and Y. Graba (2003). The hexapeptide and linker regions of the AbdA Hox protein regulate its activating and repressive functions. *Dev Cell* **4**(5): 761-8.

Meyer, A. and M. Schartl (1999). Gene and genome duplications in vertebrates: the one-to-four (-to-eight in fish) rule and the evolution of novel gene functions. *Curr Opin Cell Biol* **11**(6): 699-704.

Miles, A. and D. J. Miller (1992). Genomes of diploblatic organisms contain homeoboxes: sequence of *eveC*, an *evenskipped* homolog from the cnidarian *Acropora formosa. Proc R Soc Lond B Biol Sci* **248**: 159 - 161.

Miller, D. (1999). What the Cnidaria tell us about Pax evolution. *Zoological Studies* **38**(4): 367 - 372.

Miller, D. J. and E. E. Ball (2000). The coral Acropora: what it can contribute to our knowledge of metazoan evolution and the evolution of developmental processes. *BioEssays* **22**(3): 291-6.

Miller, D. J., Hayward, D.C., Reece-Hoyes, J.S., Scholten, I., Catmull, J., Gehring, W.J., Callaerts, P., Larsen, J.E., and Ball, E.E. (2000). Pax gene diversity in the basal cnidarian Acropora millepora (Cnidaria; Anthozoa): Implications for the evolution of the Pax gene family. *Proc Natl Acad Sci U S A* **97**: 4475 - 4480.

Miller, D. J. and A. Miles (1993). Homeobox genes and the zootype. *Nature* **365**: 215 - 216.

Minguillon, C. and Garcia-Fernandez (2003). Genesis and Evolution of the *Evx* and *Mox* genes and the extended Hox and ParaHox gene clusters. *Genome Biology* **4**(2): 12.1 - 12.8.

Misquitta, L. and B. M. Paterson (1999). Targeted disruption of gene function in Drosophila by RNA interference (RNA-i): a role for nautilus in embryonic somatic muscle formation. *Proc Natl Acad Sci U S A* **96**(4): 1451-6.

Miyata, T. and H. Suga (2001). Divergence pattern of animal gene families and relationship with the Cambrian explosion. *BioEssays* **23**(11): 1018-27.

Mochizuki, K., H. Sano, S. Kobayashi, C. Nishimiya-Fujisawa and T. Fujisawa (2000). Expression and evolutionary conservation of nanos-related genes in Hydra. *Dev Genes Evol* **210**(12): 591-602.

Mokady, O., M. Dick, D. Lackschewitz, B. Schierwater and L. Buss (1998). Over one-billion years of head conservation? Expression of an ems class gene in the Hydractinia symbiolongicarpus (Cnidaria: Hydrozoa). *Proc Natl Acad Sci U S A* **95**: 3673 - 3678.

Moore, R. C. and M. D. Purugganan (2003). The early stages of duplicate gene evolution. *Proc Natl Acad Sci U S A* **100**(26): 15682-7.

Moran, J. V., R. J. DeBerardinis and H. H. Kazazian, Jr. (1999). Exon shuffling by L1 retrotransposition. *Science* **283**(5407): 1530-4.

Morgan, R. (2004). Pax6 is a direct, positively regulated target of the circadian gene Clock. *Dev Dyn* **230**(4): 643-50.

Morris, S. (2000). The Cambrian "Explosion": Slow-fuse or megatonnage? *Proc. Natl. Acad. Sci. USA* **97**(9): 4426 - 4429.

Morse, A. N. C., K. Iwao, M. Baba, K. Shimoike, T. Hayashibara and M. Omori (1996). An Ancient Chemosensory mechanism brings new life to coral reefs. *Biol. Bull.* **191**: 149 - 154.

Muhr, J., E. Andersson, M. Persson, T. Jessell and J. Ericson (2001). Groucho-Mediated Transcriptinal Repression Establishes Progenitor Cell Pattern and Neuronal Fate in the Ventral Neural Tube. *Cell* **104**: 861 - 873.

Muller, P., N. Yanze, V. Schmid and J. Spring (1999). The Homeobox Gene Otx of the jellyfish Podocoryne carnea: Role of a Head Gene in Striated Muscle and Evolution. *Dev Biol* **216**: 582 - 594.

Murtha, M. T., J. F. Leckman and F. H. Ruddle (1991). Detection of homeobox genes in development and evolution. *Proc Natl Acad Sci U S A* **88**: 10711 - 10715.

Nadeau, J. H. and D. Sankoff (1997). Comparable rates of gene loss and functional divergence after genome duplications early in vertebrate evolution. *Genetics* **147**(3): 1259-66.

Naito, M., H. Ishiguro, T. Fujisawa and Y. Kurosawa (1993). Presence of eight distinct homeobox-containing genes in cnidarians. *FEBS Lett* **333**(3): 271-4.

Nakamura, M., W. Nishida, S. Mori, K. Hiwada, K. Hayashi and K. Sobue (2001). Transcriptional activation of beta-tropomyosin mediated by serum response factor and a novel Barx homologue, Barx1b, in smooth muscle cells. *J Biol Chem* **276**(21): 18313-20.

Nambu, P. and J. Nambu (1996). The *Drosophila* fish-hook gene encodes a HMG domain protein essential for segmentation and CNS development. *Development* **122**: 3467 - 3475.

Nelson, C. E., B. M. Hersh and S. B. Carroll (2004). The regulatory content of intergenic DNA shapes genome architecture. *Genome Biol* **5**(4): R25.

Newberry, E. P., T. Latifi, J. T. Battaile and D. A. Towler (1997). Structure-function analysis of Msx2-mediated transcriptional suppression. *Biochemistry* **36**(34): 10451-62.

Nielson, C. (1995). Animal Evolution: Interrelationships of the Living Phyla. Oxford; New York, Oxford University Press.

Niimi, T., M. Seimiya, U. Kloter, S. Flister and W. Gehring (1999). Direct Regulatory Interaction of the eyeless protein with an eye-specific enhancer in the *sine oculis* gene during eye induction in *Drosophila*. *Development* **126**: 2253 - 2260.

Nordstrom, K. (2003). Evolution of eyes: Pax, gene duplications and morphology. Department of Cell and Organism Biology. Sweden, Lund University: 120.

Nordstrom, K., I. Scholten, J. Nordstrom, D. Larhammar and D. Miller (2003a). Mutational analysis of the Acropora millepora PaxD paired domain highlights the importance of the linker region for DNA binding. *Gene* **320**: 81-7.

Nordstrom, K., R. Wallen, J. Seymour and D. Nilsson (2003b). A simple visual system without neurons in jellyfish larvae. *Proc R Soc Lond B Biol Sci* **270**(1531): 2349-54.

Nose, A., T. Isshiki and M. Takeichi (1998). Regional Specification of Muscle Progenitors in *Drosophila*: The Role of the *msh* Homeobox Gene. *Development* **125**(215 - 223).

Odden, J. P., S. Holbrook and C. Q. Doe (2002). Drosophila HB9 is expressed in a subset of motoneurons and interneurons, where it regulates gene expression and axon pathfinding. *J Neurosci* **22**(21): 9143-9.

Odorico, D. M. and D. J. Miller (1997). Internal and external relationships of the Cnidaria: implications of primary and predicted secondary structure of the 5'-end of the 23S-like rDNA. *Proc R Soc Lond B Biol Sci* **264**(1378): 77-82.

Ohno, S. (1969). The role of gene duplication in vertebrate evolution. The Biological Basis of Medicine. London, Academic Press: 109 - 132.

Ohno, S. (1970). Evolution by Gene Duplication. Berlin, Heidelberg, New York, Springer-Verlag.

Ohno, S. (1996). The notion of the Cambrian pananimalia genome. *Proc Natl Acad Sci U S A* **93**(16): 8475-8.

Ohno, S., U. Wolf and N. Atkin (1968). Evolution from fish to mammals by gene duplication. *Hereditas* **59**: 169 - 187.

Ono, K., H. Suga, N. Iwabe, K. Kuma and T. Miyata (1999). Multiple protein tyrosine phosphatases in sponges and explosive gene duplication in the early evolution of animals before the parazoan-eumetazoan split. *J Mol Evol* **48**(6): 654-62.

Onuma, Y., S. Takahashi, M. Asashima, S. Kurata and W. J. Gehring (2002). Conservation of Pax 6 function and upstream activation by Notch signaling in eye development of frogs and flies. *Proc Natl Acad Sci U S A* **99**(4): 2020-5.

Overton, P. M., L. A. Meadows, J. Urban and S. Russell (2002). Evidence for differential and redundant function of the Sox genes Dichaete and SoxN during CNS development in Drosophila. *Development* **129**(18): 4219-28.

Owens, B. M., Y. X. Zhu, T. C. Suen, P. X. Wang, J. F. Greenblatt, P. E. Goss and R. G. Hawley (2003). Specific homeodomain-DNA interactions are required for HOX11-mediated transformation. *Blood* **101**(12): 4966-74.

Pabst, O., H. Herbrand and A. Hans-Henning (1998). Nkx 2.9 is a Novel Homeobox Transcription Factor Which Demarcates Ventral Domains in the Developing Mouse. *Mech Dev* **73**: 85 - 93.

Pailhoux, E., G. Veyssiere, S. Fabre, C. Tournaire and C. Jean (1992). The genomic organisaton and DNA sequence of the mouse vas deferens androgen-regulated protein gene. *J. Steroid Biochem. Mol. Biol.* **42**: 561 - 568.

Papalopulu, N. and C. Kintner (1996). A Xenopus gene, Xbr-1, defines a novel class of homeobox genes and is expressed in the dorsal ciliary margin of the eye. *Dev Biol* **174**(1): 104-14.

Papayannopoulos, V., Tomlinson A, Panin V, R. C and K. Irvine (1998). Dorsal-Ventral Signaling in the *Drosophila* Eye. *Science* **281**: 2031 - 2034.

Patterson, K., O. Cleaver, W. Gerber, F. White and P. Krieg (2000). Distinct Expression Patterns for Two Xenopus Bar Homeobox Genes. *Dev Genes Evol* **210**: 140 - 144.

Patterson, K. D. and P. A. Krieg (1999). Hox11-family genes XHox11 and XHox11L2 in xenopus: XHox11L2 expression is restricted to a subset of the primary sensory neurons. *Dev Dyn* **214**(1): 34-43.

Patthy, L. (1999). Genome evolution and the evolution of exon-shuffling--a review. *Gene* **238**(1): 103-14.

Patthy, L. (2003). Modular assembly of genes and the evolution of new functions. *Genetica* **118**(2-3): 217-31.

Pebusque, M. J., F. Coulier, D. Birnbaurm and P. Pontarotti (1998). Ancient large-scale genome duplications: phylogenetic linkage analyses shed light on chordate genome evolution. *Mol Biol Evol* **15**: 1145 - 1159.

Pennacchio, L. A. and E. M. Rubin (2001). Genomic strategies to identify mammalian regulatory sequences. *Nat Rev Genet* **2**(2): 100-9.

Pernet, V., M. Anctil and C. J. Grimmelikhuijzen (2004). Antho-RFamide-containing neurons in the primitive nervous system of the anthozoan Renilla koellikeri. *J Comp Neurol* **472**(2): 208-20.

Pevzner, P. and G. Tesler (2003). Genome rearrangements in mammalian evolution: lessons from human and mouse genomes. *Genome Res* **13**(1): 37-45.

Phelps, C. and A. Brand (1998). Ectopic Gene Expression in *Drosophila* using GAL4 System. *Methods: A Companion to Methods in Enzymology* **14**: 367-379.

Philippe, H., A. Germot and D. Moreira (2000). The new phylogeny of eukaryotes. *Curr Opin Genet Dev* **10**(6): 596-601.

Phillips, G. (1997). Structure and Dynamics of Green Fluorescent Protein. *Current Opinion in Structural Biology* **7**: 821 - 827.

Phillips, K. and B. Luisi (2000). The Virtuoso of Versatility: POU Proteins that Flex to Fit. *Journal of Molecular Biology* **302**: 1023 - 1039.

Piccin, A., A. Salameh, C. Benna, F. Sandrelli, G. Mazzotta, M. Zordan, Rosato E, K. P and R. Costa (2001). Efficient and Heritable Functional Knock-out of an Adult Phenotype in *Drosophila* using a GAL4-driven Hairpin RNA incorporating a heterologous Spacer. *Nucleic Acids Research* **29**(12).

Pigliucci, M. (2002). Buffer Zone. *Nature* **417**: 598-599.

Pires-daSilva, A. and R. J. Sommer (2003). The evolution of signalling pathways in animal development. *Nat Rev Genet* **4**(1): 39-49.

Plaza, S., D. M. De Jong, W. J. Gehring and D. J. Miller (2003). DNA-binding characteristics of cnidarian Pax-C and Pax-B proteins in vivo and in vitro: no simple relationship with the Pax-6 and Pax-2/5/8 classes. *J Exp Zoolog B Mol Dev Evol* **299**(1): 26-35.

Poggi, L., M. Carl, R. Vignali, G. Barsacchi and J. Wittbrodt (2002). Expression of a medaka (Oryzias latipes) Bar homologue in the Differentiating Central Nervous System and Retina. *Mech Dev* **114**: 193 - 196.

Pollard, S. L. and P. W. Holland (2000). Evidence for 14 homeobox gene clusters in human genome ancestry. *Curr Biol* **10**(17): 1059-62.

Powers, T. P., J. Hogan, Z. Ke, K. Dymbrowski, X. Wang, F. H. Collins and T. C. Kaufman (2000). Characterization of the Hox cluster from the mosquito Anopheles gambiae (Diptera: Culicidae). *Evol Dev* **2**(6): 311-25.

Price, M., D. Lazzaro, T. Pohl, M. G. Mattei, U. Ruther, J. C. Olivo, D. Duboule and R. Di Lauro (1992). Regional Expression of the Homeobox Gene Nkx 2.2 in the developing mammalian forebrain. *Neuron* **8**: 241 - 255.

Prince, V. and B. Pickett (2002). Splitting Pairs: The Diverging Fates of Duplicated Genes. *Nature Reviews Genetics* **3**: 827 - 837.

Punzo, C., M. Seimiya, S. Flister, W. J. Gehring and S. Plaza (2002). Differential interactions of eyeless and twin of eyeless with the sine oculis enhancer. *Development* **129**(3): 625-34.

Qian, Y., M. Billeter, G. Otting, M. Muller and W. Gehring (1989). The Structure of the *Antennapedia* homeodomain is determined by NMR spectorscopy in solution: Comparison with prokaryotic repressors. *Cell* **59**: 573 - 580.

Qiu, M., K. Shinanura, L. Sussel, S. Chen and J. L. R. Rubenstein (1998). Control of anteroposterior and dorsoventral domains of Nkx-6.1 gene expression relative to other Nkx genes during vertebrate CNS development. *Mech Dev* **72**: 77 - 88.

Queitsch, C., T. Sangster and S. Lindquist (2002). Hsp90 as a capacitor of phenotypic variation. *Nature*: 1 - 7.

Quiring, R., U. Walldorf, U. Kloter and W. J. Gehring (1994). Homology of the eyeless gene of Drosophila to the Small eye gene in mice and Aniridia in humans. *Science* **265**(5173): 785-9.

Raff, R. A. (2000). Evo-devo: the evolution of a new discipline. *Nat Rev Genet* **1**(1): 74-9.

Raju, K., S. Tang, I. D. Dube, S. Kamel-Reid, D. M. Bryce and M. L. Breitman (1993). Characterization and developmental expression of Tlx-1, the murine homolog of HOX11. *Mech Dev* **44**(1): 51-64.

Reichert, H. (2002). Conserved Genetic Mechanisms for Embryonic Brain Patterning. *Int J Dev Biol* **46**(1): 81 - 87.

Reichert, H. and A. Simeone (2001). Developmental genetic evidence for a monophyletic origin of the bilaterian brain. *Philos Trans R Soc Lond B Biol Sci* **356**(1414): 1533-44.

Richardson, M. (1999). Vertebrate Evolution: the Developmental Origins of Adult Variation. *BioEssays* **21**: 604 - 613.

Rincon-Limas, D. E., C. Lu, I. Canal, M. Calleja, C. Rodriguez-Esteban, J. Izpisua-Belmonte and J. Botas (1999). Conservation of the expression and function of apterous orthologs in *Drosophila* and mammals. *Proc Natl Acad Sci U S A* **96**(5): 2165 - 2170.

Roberts, C. W., A. M. Sonder, A. Lumsden and S. J. Korsmeyer (1995). Development expression of Hox11 and specification of splenic cell fate. *Am J Pathol* **146**(5): 1089-101.

Roberts, D. B. (1986). Drosophila: a practical approach. Oxford, IRL Press.

Ruddle, F. H., J. L. Bartels, K. L. Bentley, C. Kappen, M. T. Murtha and J. W. Pendleton (1994). Evolution of Hox genes. *Annu Rev Genet* **28**: 423-42.

Ruiz-Gomez, M., S. Romani, C. Hartmann, H. Jackle and M. Bate (1997). Specific muscle identities are regulated by Kruppel during Drosophila embryogenesis. *Development* **124**(17): 3407-14.

Russo, M. T., A. Donizetti, A. Locascio, S. D'Aniello, A. Amoroso, F. Aniello, L. Fucci and M. Branno (2004). Regulatory elements controlling Ci-msxb tissue-specific expression during Ciona intestinalis embryonic development. *Dev Biol* **267**(2): 517-28.

Ruvinsky, I., L. M. Silver and J. J. Gibson-Brown (2000). Phylogenetic Analysis of T-box genes demonstrates the importance of amphioxus for understanding evolution of the vertebrate genome. *Genetics* **156**: 1249 - 1257.

Saha, M. S., R. R. Miles and R. M. Grainger (1997). Dorsal-ventral patterning during neural induction in Xenopus: assessment of spinal cord regionalization with xHB9, a marker for the motor neuron region. *Dev Biol* **187**(2): 209-23.

Saito, T., K. Sawamoto, H. Okano, D. Anderson and K. Mikoshiba (1998). Mammalian BarH Homologue is a Potential Regulator of Neural bHLH Genes. *Developmental Biology* **199**: 216 - 225.

Salvini-Plawen, L. V. and E. Mayr (1977). On the evolution of photoreceptors and eyes. *Evol Biol* **10**: 207 - 263.

Sambrook, J., E. F. Fritsch and T. Maniatis (1989). Molecular cloning: a laboratory manual. Cold Spring Harbour, Cold Spring Harbour University Press.

Samuel, G., D. Miller and R. Saint (2001). Conservation of a DPP/BMP signaling pathway in the nonbilateral cnidarian Acropora millepora. *Evol Dev* **3**(4): 241-50.

Sandstrom, D. J., C. A. Bayer, J. W. Fristrom and L. L. Restifo (1997). Broad-complex transcription factors regulate thoracic muscle attachment in Drosophila. *Dev Biol* **181**(2): 168-85.

Sato, M., T. Kojima, T. Michiue and K. Saigo (1999). *Bar* homeobox genes are latitudinal prepattern genes in the developing *Drosophila* notum whose expression is regulated by the concerted functions of *decapentaplegic* and *wingless*. *Development* **126**: 1457 - 1466.

Satokata, I. and R. Maas (1994). Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet* **6**: 348 - 356.

Saunders, K., K. Koizumi, W. Odenwald and M. Nirenberg (1998). Neuroblast pattern formation: Regulatory DNA that confers the vnd/NK-2 homeobox gene pattern on a reporter gene in transgenic lines of *Drosophila*. *Proc. Natl. Acad. Sci. USA* **95**: 8316 - 8321.

Scheider, K. (1890). Histologie von *Hydra fusca* mit besonderer Berucksichtigung des Nervensystems der Hydropolypen. *Arch. mikrosk. Anat* **35**: 321 - 379.

Schierwater, B. and R. Desalle (2001). Current problems with the zootype and the early evolution of Hox genes. *J Exp Zool* **291**(2): 169-74.

Schierwater, B. and K. Kuhn (1998). Homology of Hox genes and the zootype concept in early metazoan evolution. *Mol Phylogenet Evol* **9**(3): 375-81.

Schierwater, B., M. Murtha, M. Dick, F. H. Ruddle and L. W. Buss (1991). Homeoboxes in cnidarians. *J. Exp. Zool.* **260**: 413 - 416.

Scholz, C. B. and U. Technau (2003). The ancestral role of Brachyury: expression of NemBra1 in the basal cnidarian Nematostella vectensis (Anthozoa). *Dev Genes Evol* **212**(12): 563-70.

Schroeder, M. D., M. Pearce, J. Fak, H. Fan, U. Unnerstall, E. Emberly, N. Rajewsky, E. D. Siggia and U. Gaul (2004). Transcriptional control in the segmentation gene network of Drosophila. *PLoS Biol* **2**(9): E271.

Schubert, M., L. Z. Holland, N. D. Holland and D. K. Jacobs (2000). A phylogenetic tree of the *Wnt* genes based on all available full-length sequences, including five from the cephalochordate amphioxus. *Mol Biol Evol* **17**: 1896 - 1903.

Schummer, M., Scheurlen I, S. C and G. B (1992). HOM/HOX homeobox genes are present in hydra (*Chlorohydra viridissima*) and are differentially expressed during regeneration. *The EMBO Journal* **11**(5): 1815 - 1823.

Schwartz, R. and E. Olson (1999). Building the Heart Piece by Piece: Modularity of *cis*-elements regulating *Nkx2-5* transcription. *Development* **126**: 4187 - 4192.

Scott, M. P., J. W. Tamkun and G. W. Hartzell, 3rd (1989). The structure and function of the homeodomain. *Biochim Biophys Acta* **989**(1): 25-48.

Seimiya, M. and W. J. Gehring (2000). The Drosophila homeobox gene optix is capable of inducing ectopic eyes by an eyeless-independent mechanism. *Development* **127**(9): 1879-86.

Seimiya, M., H. Ishiguro, K. Miura, Y. Watanabe and Y. Kurosawa (1994). Homeobox-containing genes in the most primitive metazoa, the sponges. *Eur J Biochem* **221**(1): 219-25.

Semenza, G. L., G. L. Wang and R. Kundu (1995). DNA binding and transcriptional properties of wild-type and mutant forms of the homeodomain protein Msx2. *Biochem Biophys Res Commun* **209**(1): 257-62.

Seo, H. C., R. B. Edvardsen, A. D. Maeland, M. Bjordal, M. F. Jensen, A. Hansen, M. Flaat, J. Weissenbach, H. Lehrach, P. Wincker, R. Reinhardt and D. Chourrout (2004). Hox cluster disintegration with persistent anteroposterior order of expression in Oikopleura dioica. *Nature* **431**(7004): 67-71.

Shen, W. and G. Mardon (1997). Ectopic eye development in Drosophila induced by directed dachshund expression. *Development* **124**(1): 45-52.

Shenk, M. A., H. R. Bode and R. E. Steele (1993a). Expression of Cnox-2, a HOM/HOX homeobox gene in hydra, is correlated with axial pattern formation. *Development* **117**(2): 657-67.

Shenk, M. A., L. Gee, R. E. Steele and H. R. Bode (1993b). Expression of Cnox-2, a HOM/HOX gene, is suppressed during head formation in hydra. *Dev Biol* **160**(1): 108-18.

Shimeld, S. M. and P. W. Holland (2000). Vertebrate innovations. *Proc Natl Acad Sci U S A* **97**(9): 4449-52.

Shirasawa, S., A. Arata, H. Onimaru, K. A. Roth, G. A. Brown, S. Horning, S. Arata, K. Okumura, T. Sasazuki and S. J. Korsmeyer (2000). Rnx deficiency results in congenital central hypoventilation. *Nat Genet* **24**(3): 287-90.

Shivdasani, R. (2002). Molecular Regulation of Vertebrate Early Endoderm Development. *Dev Biology* **249**: 191 - 203.

Shopland, L. S., K. Hirayoshi, M. Fernandes and J. T. Lis (1995). HSF access to heat shock elements in vivo depends critically on promoter architecture defined by GAGA factor, TFIID, and RNA polymerase II binding sites. *Genes and Development* **9**: 2756 - 2769.

Sidow, A. (1996). Genome duplications in the evolution of early vertebrates. *Curr Opin Genet Dev* **6**: 715 - 722.

Sinha, S., M. D. Schroeder, U. Unnerstall, U. Gaul and E. D. Siggia (2004). Cross-species comparison significantly improves genome-wide prediction of cis-regulatory modules in Drosophila. *BMC Bioinformatics* **5**(1): 129.

Skeath, J. (1998). The *Drosophila* EGF receptor controls the formation and specification of neuroblasts along the dorsal-ventral axis of the *Drosophila* embryo. *Development* **125**: 3301 - 3312.

Skeath, J., G. Panganiban and S. Carroll (1994). The *ventral nervous system defective* gene controls proneural gene expression at two distinct steps during neuroblast formation in *Drosophila. Development* **120**: 1517 - 1527.

Skeath, J. B. (1999). At the Nexus between pattern formation and cell-type specification: the generation of individual neuroblast fates in the *Drosophila* embryonic central nervous system. *BioEssays* **21**: 922-931.

Skeath, J. B. and S. Thor (2003). Genetic control of Drosophila nerve cord development. *Curr Opin Neurobiol* **13**(1): 8-15.

Skrabanek, L. and K. H. Wolfe (1998). Eukaryotic genome duplication - where's the evidence? *Curr Opin Genet Dev* **8**: 694 - 700.

Slack, J. M., Holland P.W. and G. C.F. (1993). The Zootype and the Phylotypic Stage. *Nature* **361**: 490 - 492.

Smith, D. M. and C. J. Tabin (1999). Chick Barx2b, a marker for myogenic cells also expressed in branchial arches and neural structures. *Mech Dev* **80**(2): 203-6.

Smith, K. M., L. Gee, I. L. Blitz and H. R. Bode (1999a). CnOtx, a Member of the Otx Gene Family, has a role in Cell Movement in Hydra. *Dev Biol* **212**: 392 - 404.

Smith, N. G., R. Knight and L. D. Hurst (1999b). Vertebrate genome evolution: a slow shuffle or a big bang? *BioEssays* **21**(8): 697-703.

Smith, S. B., H. C. Ee, J. R. Conners and M. S. German (1999c). Paired-homeodomain transcription factor PAX4 acts as a transcriptional repressor in early pancreatic development. *Mol Cell Biol* **19**(12): 8272-80.

Smith, S. T. and J. B. Jaynes (1996). A conserved region of engrailed, shared among all en-, gsc-, Nk1-, Nk2- and msh-class homeoproteins, mediates active transcriptional repression in vivo. *Development* **122**(10): 3141-50.

Smolenicka, Z., F. Pani, K. J. Hwang, J. M. Gruschus and J. A. Ferretti (2003). Sequence of a conserved region of a new sea urchin homeobox gene from the NK family. *Cell Biol Int* **27**(2): 81-7.

Soullier, S., P. Jay, F. Poulat, J. Vanacher, P. Berta and V. Laudet (1999). Diversification Pattern of the HMG and SOX family members during evolution. *Journal of Molecular Evolution* **48**: 517 - 527.

Spring, J. (2003). Major transitions in evolution by genome fusions: from prokaryotes to eukaryotes, metazoans, bilaterians and vertebrates. *J Struct Funct Genomics* **3**(1-4): 19-25.

Spring, J., N. Yanze, C. Josch, A. M. Middel, B. Winninger and V. Schmid (2002). Conservation of Brachyury, Mef2, and Snail in the myogenic lineage of jellyfish: a connection to the mesoderm of bilateria. *Dev Biol* **244**(2): 372-84.

Stathopoulos, A. and M. Levine (2002). Dorsal Gradient Networks in the *Drosophila* embryo. *Dev Biol* **246**: 57 - 67.

Steele, R. E., S. E. Hampson, N. A. Stover, D. F. Kibler and H. R. Bode (2004). Probable horizontal transfer of a gene between a protist and a cnidarian. *Curr Biol* **14**(8): R298-9.

Stephenson, T. A. (1926). British Sea Anenomes. London, The Ray Society.

Stern, C. D. (2002). Induction and initial patterning of the nervous system - the chick embryo enters the scene. *Curr Opin Genet Dev* **12**(4): 447-51.

Stewart, M., C. Murphy and J. W. Fristrom (1972). The recovery and preliminary characterization of X chromosome mutants affecting imaginal discs of Drosophila melanogaster. *Dev Biol* **27**(1): 71-83.

Strecker, T., K. Kongsuwan, J. Lengyel and J. Merriam (1986). The zygotic mutant *tailless* affects the anterior and posterior ectodermal regions of the *Drosophila* embryo. *Dev Biol* **113**: 64 - 76.

Suga, H., M. Koyanagi, D. Hoshiyama, K. Ono, N. Iwabe, K. Kuma and T. Miyata (1999). Extensive gene duplication in the early evolution of animals before the parazoan-eumetazoan split demonstrated by G proteins and protein tyrosine kinases from sponge and hydra. *J Mol Evol* **48**(6): 646-53.

Suga, H., K. Kuma, N. Iwabe, N. Nikoh, K. Ono, M. Koyanagi, D. Hoshiyama and T. Miyata (1997). Intermittent divergence of the protein tyrosine kinase family during animal evolution. *FEBS Lett* **412**(3): 540-6.

Sumoy, L., L. Craim, M. Escarceller, M. Nadal, M. Gratacos, M. Pujana, X. Estivill and B. Peral (2000). HMG20A and HMG20B map to human chromosomes 15q24 and 19p13.3 and constitute a distinct class of HMG-box genes with ubiquitous expression. *Cytogent Cell Genet* **88**: 62-67.

Sun, H., D. P. Dickinson, J. Costello and W. H. Li (2001). Isolation of Cladonema Pax-B genes and studies of the DNA-binding properties of cnidarian Pax paired domains. *Mol Biol Evol* **18**(10): 1905-18.

Sun, H., S. Merugu, X. Gu, Y. Y. Kang, D. P. Dickinson, P. Callaerts and W. H. Li (2002). Identification of essential amino acid changes in paired domain evolution using a novel combination of evolutionary analysis and in vitro and in vivo studies. *Mol Biol Evol* **19**(9): 1490-500.

Sun, H., A. Rodin, Y. Zhou, D. Dickinson, D. Harper, D. Hewett-Emmett and L. Wen-Hsiung (1997). Evolution of Paired Domains: Isolation and Sequencing of Jellyfish and Hydra *Pax* Genes Related to *Pax-5* and *Pax-6*. *Proc. Natl. Acad. Sci. USA* **94**: 5156 - 5161.

Sussel, L., O. Marin, S. Kimura and J. L. Rubenstein (1999). Loss of Nkx2.1 Homeobox Gene Function Results in a Ventral to Dorsal Molecular Respecification within the Basal Telencephalon: Evidence for a Transformation of the Pallidum into the Striatum. *Development* **126**: 3359 - 3370.

Svane, I. and P. Dolmer (1995). Perception of light at settlement: a comparative study of two invertebrate larvae, a scyphozoan planula and a simple ascidian tadpole. *J Exp Marin Biol Ecol* **187**: 51 - 61.

Tanabe, Y., C. William and T. M. Jessell (1998). Specification of motor neuron identity by the MNR2 homeodomain protein. *Cell* **95**(1): 67-80.

Tang, H. K., S. Singh and G. F. Saunders (1998). Dissection of the transactivation function of the transcription factor encoded by the eye developmental gene PAX6. *J Biol Chem* **273**(13): 7210-21.

Tang, S. and M. L. Breitman (1995). The optimal binding sequence of the Hox11 protein contains a predicted recognition core motif. *Nucleic Acids Res* **23**(11): 1928-35.

Tardent, P. and E. Frei (1969). Reaction patterns of dark- and light-adapted Hydra to light stimuli. *Experientia* **25**(3): 265-7.

Technau, U. (2001). Brachyury, the blastopore and the evolution of the mesoderm. *BioEssays* **23**: 788 - 794.

Technau, U. and H. R. Bode (1999). HyBra1, a Brachyury homologue, acts during head formation in Hydra. *Development* **126**(5): 999-1010.

Thaler, J., K. Harrison, K. Sharma, K. Lettieri, J. Kehrl and S. L. Pfaff (1999). Active suppression of interneuron programs within developing motor neurons revealed by analysis of homeodomain factor HB9. *Neuron* **23**(4): 675-87.

Tissier-Seta, J. P., M. L. Mucchielli, M. Mark, M. G. Mattei, C. Goridis and J. F. Brunet (1995). Barx1, a new mouse homeodomain transcription factor expressed in cranio-facial ectomesenchyme and the stomach. *Mech Dev* **51**(1): 3-15.

Tomarev, S. I., P. Callaerts, L. Kos, R. Zinovieva, G. Halder, W. Gehring and J. Piatigorsky (1997). Squid Pax-6 and eye development. *Proc Natl Acad Sci U S A* **94**(6): 2421-6.

Treisman, J. E. (1999). A conserved blueprint for the eye? *BioEssays* **21**(10): 843-50.

Tsao, D. H., J. M. Gruschus, L. H. Wang, M. Nirenberg and J. A. Ferretti (1994). Elongation of helix III of the NK-2 homeodomain upon binding to DNA: a secondary structure study by NMR. *Biochemistry* **33**(50): 15053-60.

Tsao, D. H., J. M. Gruschus, L. H. Wang, M. Nirenberg and J. A. Ferretti (1995). The three-dimensional solution structure of the NK-2 homeodomain from Drosophila. *J Mol Biol* **251**(2): 297-307.

Tsuda, M., K. Kamimura, H. Nakato, M. Archer, W. Staatz, B. Fox, M. Humphrey, S. Olson, T. Futch, V. Kaluza, E. Siegfried, L. Stam and S. B. Selleck (1999). The cell-surface proteoglycan Dally regulates Wingless signalling in Drosophila. *Nature* **400**(6741): 276-80.

Tucker, A. S., K. L. Matthews and P. T. Sharpe (1998). Transformation of tooth type induced by inhibition of BMP signaling. *Science* **282**(5391): 1136-8.

Urbach, R. and G. M. Technau (2003). Segment polarity and DV patterning gene expression reveals segmental organization of the Drosophila brain. *Development* **130**(16): 3607-20.

Valerius, M. T., H. Li, J. L. Stock, M. Weinstein, S. Kaur, G. Singh and S. S. Potter (1995). Gsh-1: a novel murine homeobox gene expressed in the central nervous system. *Dev Dyn* **203**: 337 - 351.

van Heyningen, V. and K. A. Williamson (2002). PAX6 in sensory development. *Hum Mol Genet* **11**(10): 1161-7.

Venkatesh, T., N. Holland, L. Holland, M. Su and R. Bodmer (1999). Sequence and developmental expression of ampioxus *AmphiNk2-1*: Insights into the evolutionary origin of the vertebrate thyroid gland and forebrain. *Dev Genes Evol* **209**: 254 - 259.

Venter, J. C., et al (276 co-authors) (2001). The Sequence of the Human genome. *Science* **291**: 1304 - 1351.

von Ohlen, T. and C. Q. Doe (2000). Convergence of dorsal, dpp, and egfr signaling pathways subdivides the drosophila neuroectoderm into three dorsal-ventral columns. *Dev Biol* **224**(2): 362-72.

Wagner, G. P., C. Amemiya and F. Ruddle (2003). Hox cluster duplications and the opportunity for evolutionary novelties. *Proc Natl Acad Sci U S A* **100**(25): 14603-6.

Walter, J. and M. Paulsen (2003). The potential role of gene duplications in the evolution of imprinting mechanisms. *Hum Mol Genet* **12 Spec No 2**: R215-20.

Wang, C. C., T. Brodnicki, N. G. Copeland, N. A. Jenkins and R. P. Harvey (2000). Conserved linkage of NK-2 homeobox gene pairs *Nkx2-2/2-4* and *Nkx2-1/2-9* in mammals. *Mammalian Genome* **11**: 466 - 468.

Wang, L., R. Chmelik and M. Nirenberg (2002). Sequence-specific DNA binding by the vnd/NK-2 homeodomain of *Drosophila*. *Proc. Natl. Acad. Sci. USA* **99**(20): 12721-12726.

Wang, W., X. Chen, H. Xu and T. Lufkin (1996). Msx3: A novel murine homologue of the *Drosophila* msh homeobox gene restricted to the dorsal embryonic central nervous system. *Mech Dev* **58**: 203 - 215.

Wang, Y. and X. Gu (2000). Evolutionary patterns of gene families generated in the early stage of vertebrates. *J Mol Evol* **51**(1): 88-96.

Watada, H., R. Mirmira, J. Kalamaras and M. German (2000). Intramolecular Control of Transcriptional Activity by the Nk2-specific domain in Nk-2 Homeodomain Proteins. *Proc. Natl. Acad. Sci. USA* **97**(17): 9443 - 9448.

Wawersik, S. and R. L. Maas (2000). Vertebrate eye development as modeled in Drosophila. *Hum Mol Genet* **9**(6): 917-25.

Wegner, M. (1999). From Head to Toes: the multiple facets of Sox proteins. *Nucleic Acids Research* **27**(6): 1409 - 1420.

Weiler, S., J. Gruschus, D. Tsao, L. Yu, L. Wang, M. Nirenberg and J. Ferretti (1998). Site-directed Mutagenesis in the vnd/NK-2 Homeodomain. *The Journal of Biological Chemistry* **273**: 10994 - 11000.

Weiss, J., Von Ohlen T, Mellerick D, Dressler G, D. C and M. Scott (1998). Dorsoventral patterning in the *Drosophila* central nervous system: the *intermediate neuroblasts defective* homeobox gene specifies intermediate column identity. *Genes and Development* **12**: 3591 - 3602.

Wellik, D. M. and M. R. Capecchi (2003). Hox10 and Hox11 genes are required to globally pattern the mammalian skeleton. *Science* **301**(5631): 363-7.

Wellik, D. M., P. J. Hawkes and M. R. Capecchi (2002). Hox11 paralogous genes are essential for metanephric kidney induction. *Genes Dev* **16**(11): 1423-32.

Wendel, J. F. (2000). Genome evolution in polyploids. *Plant Mol Biol* **42**(1): 225-49.

Westwood, J. T., J. Clos and C. Wu (1991). Stress-induced oligomerisation and chromosomal relocalisation of heat-shock factor. *Nature* **353**: 82 - 827.

Wiens, M., A. Mangoni, M. D'Esposito, E. Fattorusso, N. Korchagina, H. C. Schroder, V. A. Grebenjuk, A. Krasko, R. Batel, I. M. Muller and W. E. Muller (2003). The molecular basis for the evolution of the metazoan bodyplan: extracellular matrix-mediated morphogenesis in marine demosponges. *J Mol Evol* **57** **Suppl 1**: S60-75.

William, C. M., Y. Tanabe and T. M. Jessell (2003). Regulation of motor neuron subtype identity by repressor activity of Mnx class homeodomain proteins. *Development* **130**(8): 1523-36.

Williams, S. C., C. R. Altmann, R. L. Chow, A. Hemmati-Brivanlou and R. A. Lang (1998). A highly conserved lens transcriptional control element from the Pax-6 gene. *Mech Dev* **73**(2): 225-9.

Willmer, P. (1990). Invertebrate Relationships: Patterns in Animal Evolution. Cambridge; New York, Cambridge University Press.

Wilson, D., G. Sheng, T. Lucuit, N. Dostatni and C. Desplan (1993). Cooperative Dimerization of Paired Class Homeo Domains on DNA. *Genes and Development* **7**: 2120 - 2134.

Wilson, M. and P. Koopman (2002). Matching SOX: partner proteins and co-factors of the SOX family of transcriptional regulators. *Curr Opin Genet Dev* **12**(4): 441-6.

Wolf, Y. I., F. A. Kondrashov and E. V. Koonin (2000). No footprints of primordial introns in a eukaryotic genome. *Trends Genet* **16**(8): 333-4.

Wolfe, K. H. and D. C. Shields (1997). Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* **387**(6634): 708-13.

Wu, K. H., M. L. Tobias, J. W. Thornton and D. B. Kelley (2003). Estrogen receptors in Xenopus: duplicate genes, splice variants, and tissue-specific expression. *Gen Comp Endocrinol* **133**(1): 38-49.

Xiang, B., S. Weiler, M. Nirenberg and J. Ferretti (1998). Structural Basis of an embryonically lethal single Ala - Thr mutation in the vnd/NK2 homeodomain. *Proc. Natl. Acad. Sci. USA* **95**: 7412 - 7416.

Xu, P. X., X. Zhang, S. Heaney, A. Yoon, A. M. Michelson and R. L. Maas (1999). Regulation of Pax6 expression is conserved between mice and flies. *Development* **126**(2): 383-95.

Yagi, Y., T. Suzuki and S. Hayashi (1998). Interaction Between *Drosophila* EGF receptor and vnd determines three dorsoventral domains of the neuroectoderm. *Development* **125**: 3625 - 3633.

Yang, L. and N. E. Baker (2003). Cell cycle withdrawal, progression, and cell survival regulation by EGFR and its effectors in the differentiating Drosophila eye. *Dev Cell* **4**(3): 359-69.

Yanze, N., J. Spring, C. Schmidli and V. Schmid (2001). Conservation of Hox/ParaHox-Related Genes in the Early Development of a Cnidarian. *Dev Biol* **236**: 89 - 98.

Yokoyama, S. and R. Yokoyama (1989). Molecular evolution of human visual pigment genes. *Mol Biol Evol* **6**(2): 186-97.

Yu, R., Chiang, M, Tanabe, T, Kobayashi, M, Yasuda, K, Evans, R and Umesono, K (2000). The Orphan Nuclear Receptor Tlx Regulates Pax2 and is Essential for Vision. *Proc. Natl. Acad. Sci. USA* **97**(6): 2621 - 2625.

Yu, R., M. McKeown, R. Evans and K. Umesono (1994). Relationship between *Drosophila* gap gene *tailless* and a vertebrate nuclear receptor TLX. *Nature* **370**: 375 - 379.

Yu, W. P., S. Brenner and B. Venkatesh (2003). Duplication, degeneration and subfunctionalization of the nested synapsin-Timp genes in Fugu. *Trends Genet* **19**(4): 180-3.

Zaffran, S., G. Das and M. Frasch (2000). The NK-2 Homeobox Gene *scarecrow (scro)* is expressed in pharynx, ventral nerve cord and brain of *Drosophila* embryos. *Mechanisms of Development* **94**: 237-241.

Zhang, H., K. M. Catron and C. Abate-Shen (1996a). A role for the Msx-1 homeodomain in transcriptional regulation: residues in the N-terminal arm mediate TATA binding protein interaction and transcriptional repression. *Proc Natl Acad Sci U S A* **93**(5): 1764-9.

Zhang, H., G. Hu, H. Wang, P. Sciavolino, N. Iler, M. M. Shen and C. Abate-Shen (1997). Heterodimerization of Msx and Dlx homeoproteins results in functional antagonism. *Mol Cell Biol* **17**(5): 2920-32.

Zhang, J. (2003). Evolution by gene duplication: an update. *TRENDS in Ecology and Evolution* **18**(6): 292 - 298.

Zhang, N., W. Shen, A. Ho and M. Lu (1996b). Three distinct domains in the HOX-11 homeobox oncoprtoein are required for optimal transactivation. *Oncogene* **13**: 1781 - 1787.

Zhao, G. and J. Skeath (2002). The Sox-Domain Containing Gene *Dichaete/fish-hook* acts in concert with *vnd* and *ind* to regulate cell fate in the *Drosophila* neuroectoderm. *Development* **129**: 1165-1174.

Zipursky, S. L., T. R. Venkatesh, D. B. Teplow and S. Benzer (1984). Neuronal development in the Drosophila retina: monoclonal antibodies as molecular probes. *Cell* **36**: 15 - 26.

Appendix A – Abbreviations

aa	amino acid
Ab	antibody
amp	ampicillin
AP	alkaline phosphatase
arm	armadillo
A/P	anterior/posterior
BCIP	5-bromo-4-chloro-3-indolyl phosphate
bp	base pair
°C	degrees Celsius
cDNA	complementary deoxyribonucleic acid
Ci	Curie(s)
cm	centimetre
CNS	central nervous system
cpm	counts per minute
DAB	3,3-diaminobenzidine
ddH$_2$O	double distilled water
DEPC	diethyl pyrocarbonate
DIG	digoxygenin
DNA	deoxyribonucleic ac id
DNase	dexyribonuclease
dATP	deoxyribo-adenine triphosphate
dCTP	deoxyribo-cytosine triphosphate
dGTP	deoxyribo-guanine triphosphate
dNTP	deoxyribo-nucleotdie triphosphates (dATP, dCTP, dTTP, dGTP)
dTTP	deoxyribo-thymine triphosphate
dUTP	deoxyribo-uracil triphophate
D/V	dorsal/ventral
E. coli	*Escherichia coli*
EDTA	ehylenediamine tetraacetic acid
Fl	fluorescein
g	gram
HD	homeodomain
HEPES	N-2-hydroxyethylpiperazine-N]-2-ethane sulphonic acid
h	hour(s)
HRP	horseradish peroxidase
I	inosine
kan	kanamycin
kb	kilobase pair(s)
LB	Luria-Bertani Media
M	molar
min	minutes(s)
μg	microgram (10^{-6}g)
μL	microlitre (10^{-6}L)
μM	micromolar (10^{-6}M)
mg	milligram(s)

mL	millilitre(s)
mol	mole(s)
MOPS	3-(N-morpholino)prepanesulphonic acid
mm	millimetre (10^{-3}m)
mM	millimolar (10^{-3}M)
mRNA	messenger ribonucleic acid
NBT	nitro blue tetrazolium
ng	nanogram(s) (10^{-9}g)
O/A	oral/aboral
OP	octapeptide
ORF	open reading frame
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PD	paired domain
PEG	polyethylene glycol
pfu	plaque forming unit(s)
pH	unit of acidity/alkalinity
RNA	ribonucleic acid
RNase	ribonuclease
RNAi	ribobucleic acid interference
SEM	scanning electron microscope
TE	Tris.HCl-EDTA buffer
Tris	tris(hydroxymethyl)aminomethane
tRNA	transfer ribonucleic acid
U	unit(s)
UAS	upstream activator sequence
UTR	untranslated region
UV	ultraviolet
VNC	ventral nerve cord
v/v	units volume per units volume
w/v	units weight per units volume

Appendix B – Creation of an *ind*-GAL4 driver line

Although *ind* was cloned as a direct target of Vnd and the motif likely to mediate this interaction is known (Weiss *et al.*, 1998), no enhancers have been identified and its promoter has not been characterised. This lack of information on *ind* regulation represents a major problem in terms of construction of an ind-GAL4 line.

The strategy adopted was to generate constructs containing different portions of the region immediately 5' of the likely transcription start site (promoter); in addition, the intention was to generate additional constructs in which promoter fragments were fused (using the technique of splicing by overlap extension - see section 2.4.15) to an 80bp candidate regulatory region located downstream of the *ind* coding sequence. This region contains several Vnd binding sites, and is hereafter referred to as the Weiss fragment (Weiss *et al.*, 1998). Each of the constructs were designed to include *KpnI* and *NotI* restriction sites on their 5' and 3' ends respectively to enable directional cloning into the equivalent sites of the pG4PN vector (which was kindly supplied by Dr Coral Warr, Monash University). Constructs were generated by Mr Niko Frank under my direct supervision. The constructs for this work are summarised schematically as Fig 7.1.

Each of the fragments was initially amplified from genomic DNA extracted from wild type *Drosophila*. A common reverse primer was used in all constructs (6_prom_rev 5'-aaggaaaaagcggccgccttgggtttctggggta) while additional primers used in the production of each construct varied and occasionally overlapped depending upon the particular requirements (Construct 1: 1_Weiss_for 5'-ggggtaccagttccttgctcaagtgtgc; 2_Weiss_rev 5'-cgagtgtgttcggcctacctgctgacccag; 5_prom_for 5'-ctgggtcagcaggtaggccgaacacactcgctc; Construct 2: 1_Weiss_for; 4_prom_for 5'- ctgggtcagcaggtaaatcgcagacaaaaaacggaa; 3_Weiss_rev 5'-tttttgtctgcgatttacctgctgacccag; Construct 3: 7_prom_for 5'-ggggtaccaatcgcagacaaaaaacggaa). Each construct was subcloned into pGEM-T and sequenced before being isolated by restriction digest and cloned into the pG4PN vector. Plasmids were purified using a QIAGEN midprep purification kit before being injected into *white*$^-$ *Drosophila* embryos. Each of the constructs was injected into the dechorionisated eggs of 100 – 120 *Drosophila* and allowed to develop. Of these, for

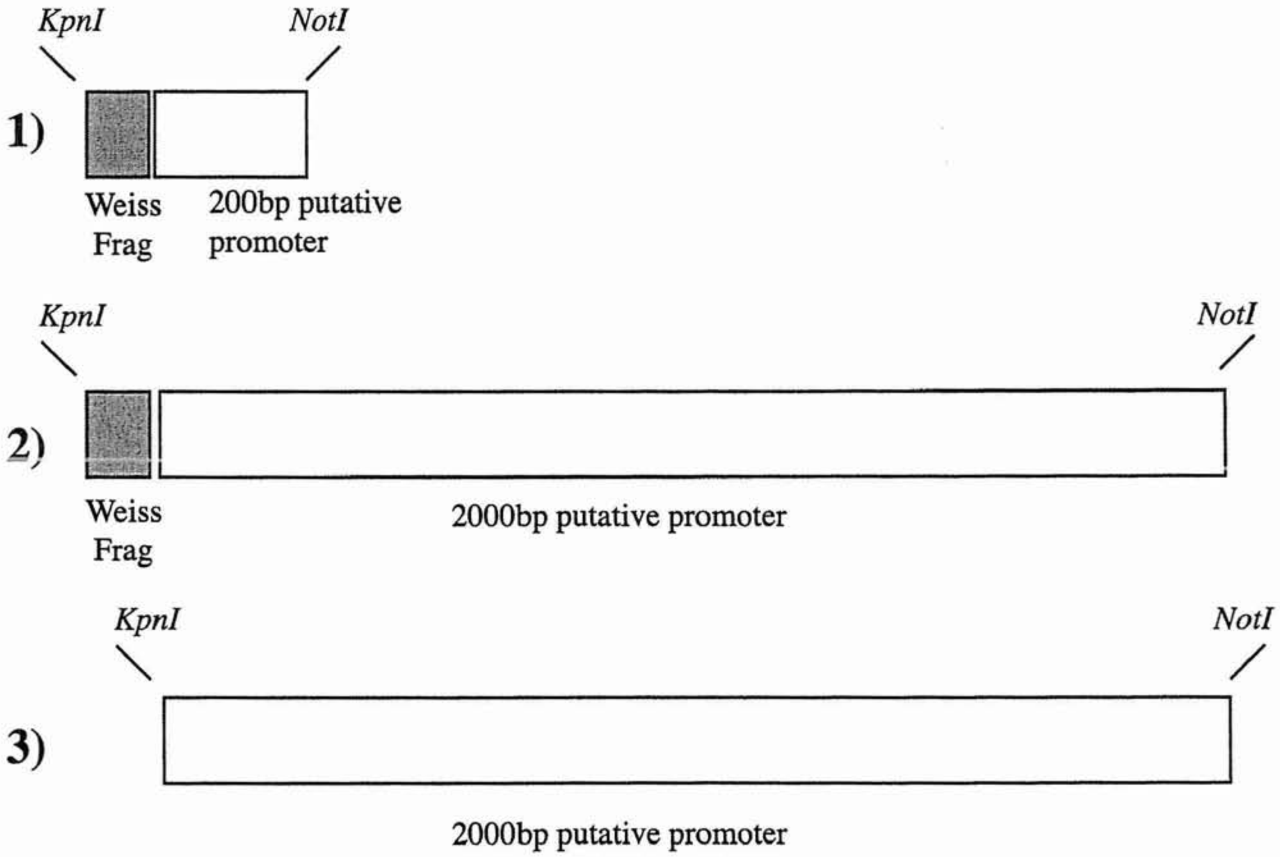


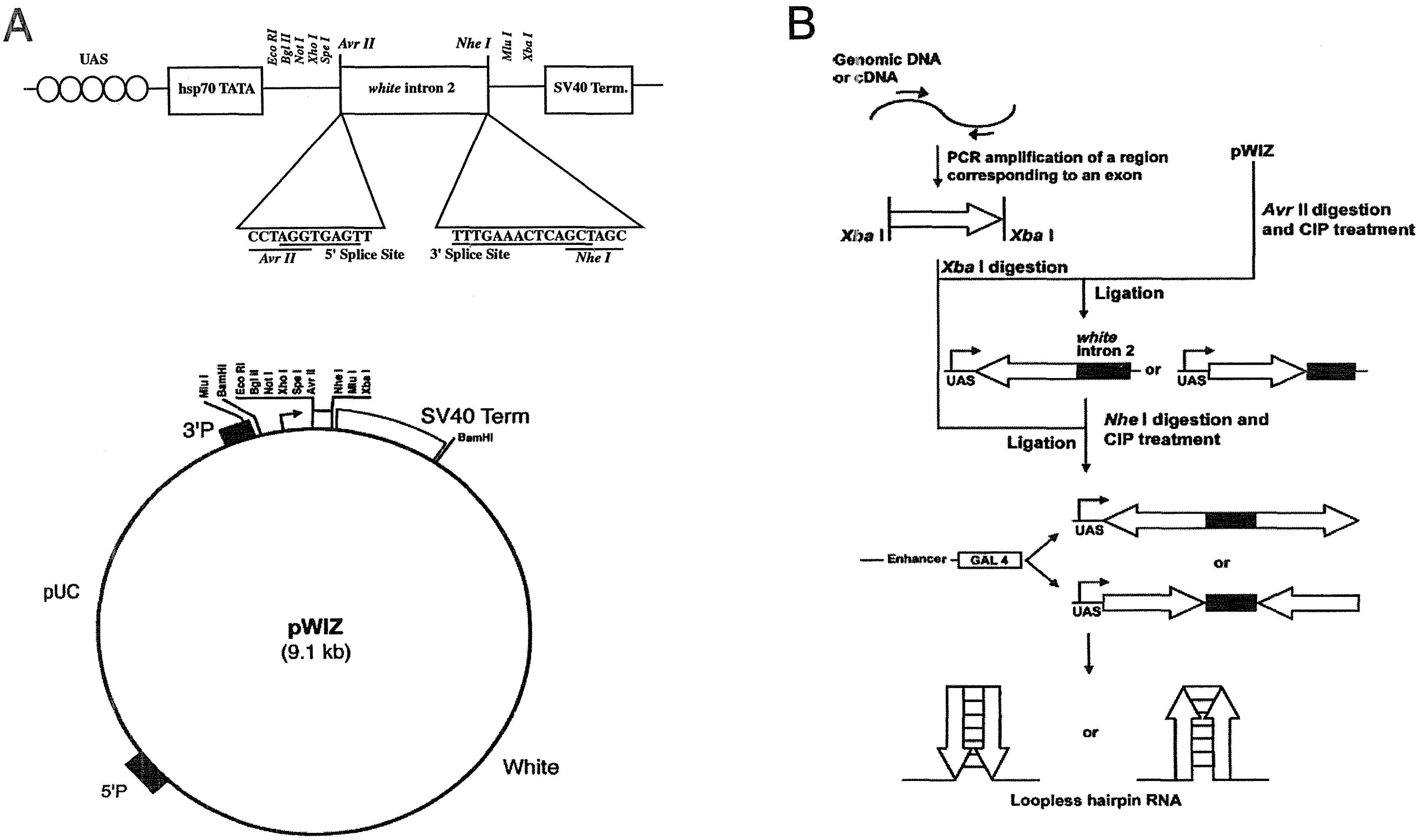
Figure 7.1: Schematic representaion of *ind*-GAL4 constructs. The grey shaded box inidcates the Weiss Fragment (see appendix B), while the open box indicates either 200bp or 2000bp *ind* promoter fragment as indicated. Constructs were generated via splicing by overlap extension and *KpnI* and *NotI* restriction sites engineered onto the 5' and 3' ends respectively to enable cloning into the pG4PN vector, upstream of the GAL4 activator. The numbers beside the constructs correspond to how they are referred to in the text. Note that constructs shown are not to scale.

construct (1) (see Fig 7.1) 60 flies hatched, and only 10 of these had successfully integrated the construct into their genome. For construct (2) and (3), approximately the same proportion of larvae hatched, however none of these had integrated the construct into their genomes. However, crossing the lines which had successfully integrated construct (1) with UAS-lacZ lines did not give any significant lacZ expression, so the experiment must be regarded as a failure. The generation and characterisation of additional *ind*-GAL4 constructs is continuing in collaboration with the laboratory of Prof G Technau at the University of Mainz.

Appendix C - RNAi as a tool to silence genes in Drosophila

Until fairly recently, targeted gene knockouts in *Drosophila* on the basis of sequence or position were difficult. Although *P*-element mutagenised fly lines are available from various stock centres, for many genes no mutant alleles or deficiency stocks are available. RNA interference (RNAi) is a powerful method for silencing genes at the post-transcriptional stage. Introduction of double-stranded RNA (dsRNA) triggers degradation of the mRNA bearing the same sequence and has been shown to be effective in a variety of organisms (Fire *et al.*, 1998; Kennerdell and Carthew, 1998; Baulcombe, 1999). Injection of a gene specific dsRNA into the early *Drosophila* embryo results in interference persisting throughout embryonic development (Kennerdell and Carthew, 1998; Misquitta and Paterson, 1999; Tsuda *et al.*, 1999), however effects on adult gene expression can be inconsistent and greatly reduced (Kennerdell and Carthew, 1998). However, the introduction of a genomic DNA/cDNA hybrid that is predicted to form hairpin dsRNA molecules following splicing was shown to effectively induce silencing of target genes in adult transgenic animals and could be stably expressed in transgenic *Drosophila* (Kalidas and Smith, 2002). Cloning of these fusions into a vector such as pUAST would allow targeted expression of the dsRNA hairpin via the GAL4-UAS system. For this reason, four cg13424 RNAi constructs were designed which were predicted to form differently sized dsRNA hairpins after splicing with differently sized loop regions between the hairpin. The constructs were designed to ensure that splice donor sites (GTNNGT) were not present in either orientation in the cDNA portion of the hybrid, encoded regions of homology between 190 and 450bp, and constructed so that the genomic DNA portion of the hybrid contained at least one intron. The genomic DNA and cDNA portions were spliced together using the PCR mediated technique, splicing by overlap extension, with outer primers designed to enable cloning into the *NotI* and *KpnI* sites of pUAST. Unfortunately, inverted repeat constructs such as these proved difficult if not impossible to clone, even with the presence of the intron in the genomic DNA portion of the construct and the use of SURE cells (Stratagene) which are deficient in *recBC sbcBC* and thus eliminate all known restriction systems which excise inverted repeats during replication of plasmid DNA. In addition, amplification of the genomic section of DNA required for the RNAi fusion often resulted in non-specific amplification products.

Recently, a simpler method of cloning inverted repeats for the production of an RNAi construct has become known, using the pWiz vector (WIZ for white intron zipper) (Lee and Carthew, 2003). Gene fragments are cloned upstream and downstream of the 74-nucleotide *white* intron, which is flanked by various restriction sites to facilitate cloning. The entire cassette is downstream of the UAS enhancer-promoter and upstream of the SV40 transcription termination site. The system is designed so that a single PCR fragment (as long as it doesn't contain consensus splice sites in either orientation as before) derived from only two PCR primers can be inserted on each side of the *white* intron. In addition, the vector includes *P*-element sites and a UAS sequence (as with pUAST) which allows for targeted expression of the dsRNAi hairpin loop in *Drosophila* (Lee and Carthew, 2003). The pWiz vector and cloning strategy is shown as Fig 7.2. Even with this vector, cloning proved difficult, and attempts were made to clone four cDNA fragments ranging from 230bp to 700bp of *cg13424*. Eventually, one of these (a 250bp inverted repeat) was cloned on either side of in pWiz in a 'tail-to-tail'manner (ie. with the 3' ends of the fragments on either side of the *white* intron), and verified by restriction mapping. Sequencing of the construct in pWiz could not be achieved, possibly due to the formation of a hairpin loop within the vector. Due to time constraints, this construct has not yet been transgenically expressed in *Drosophila*, however with time this will be achieved and expression of the RNAi hairpin driven by an appropriate GAL4 line. Production of additional *cg13424* RNAi/pWiz constructs will also be completed in order to determine which is most efficient in knocking out *cg13424* expression.


A
UAS
hsp70 TATA
Eco RI
Bgl II
Not I
Xho I
Spe I
Avr II
white intron 2
Nhe I
Mlu I
Xba I
SV40 Term.
CCTAGGTGAGTT
Avr II
5' Splice Site
3' Splice Site
TTTGAAACTCAGCTAGC
Nhe I
Mlu I
BamHI
Eco RI
Bgl II
Not I
Xho I
Spe I
Avr II
Nhe I
Mlu I
Xba I
3'P
SV40 Term
BamHI
pUC
pWIZ
(9.1 kb)
5'P
White
B
Genomic DNA
or cDNA
PCR amplification of a region corresponding to an exon
pWIZ
Xba I
Xba I
Avr II digestion and CIP treatment
Xba I digestion
Ligation
white intron 2
UAS
or
UAS
Nhe I digestion and CIP treatment
Ligation
UAS
Enhancer
GAL 4
or
UAS
or
Loopless hairpin RNA

Appendix D

Tandem organization of independently duplicated homeobox genes in the basal cnidarian Acropora millepora

Submitted to Sequence Corner, *Development, Genes and Evolution*

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Sequence corner

Tandem organization of independently duplicated homeobox genes in the basal cnidarian *Acropora millepora*

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ABSTRACT

A number of examples of independently duplicated regulatory genes have been identified in cnidarians, but the extent of this phenomenon and organization of these duplicated genes are unknown. Here we describe the identification of three pairs of independently duplicated homeobox genes in the anthozoan cnidarian, *Acropora millepora*. In each case, the pairs of paralogous genes are tightly linked, but the extent of sequence divergence implies that these do not reflect recent duplication events. Cnidarians have recently been shown to contain a number of genes previously assumed to have been vertebrate innovations; the presence of independently duplicated paralogs of a substantial number of regulatory genes adds a quantitative dimension to the likely complexity of cnidarian genomes.

INTRODUCTION

During vertebrate evolution, the Hox clusters and many other regulatory genes have been duplicated; hence regulatory genes that are present in single copies in *Drosophila* or amphioxus often have a number of vertebrate paralogs (Furlong and Holland, 2002). Although specific regulatory genes have been independently duplicated in other animal phyla (e.g. Minguillon et al., 2002), there has been an assumption that this phenomenon has been much more extensive in vertebrates and may have contributed to their evolutionary success.

Although limited data are presently available, it is clear that a significant number of regulatory genes have been independently duplicated in the Cnidaria. The *Hydra* nanos genes (Mochizuki et al., 2000) are the best-characterised example of this phenomenon, but other cases include Smad-1/5 (Samuel et al., 2001) and nuclear receptor (Grasso et al., 2001) genes in *Acropora*, snail and mox genes in *Nematostella* (Martindale et al., 2004), and paired-like genes in *Hydra* (Gauchat et al., 1998). Independent duplication of some genes is perhaps to be expected, as the Cnidaria may have been distinct from the bilaterian stem since deep in Pre-Cambrian time (Conway-Morris, 2000). However, the extent of gene duplication within the Cnidaria is unknown, as is the organization of these duplicated genes.

In the course of characterising the homeobox gene complement of *Acropora*, we have identified several cases of tightly linked paralogs, three of which are described here. Whilst one of these pairs may consist of pseudogenes, the other two pairs of genes are expressed and are therefore likely to be functional. The tandem duplicates are highly divergent, suggesting that their origins may not be recent. No comparable genomic data are yet available for any other cnidarian, but it is likely that independently duplicated genes are a common feature of cnidarian genomes, and may have contributed to the large genome sizes estimated for some members of the phylum. In addition to the duplication phenomenon described here, cnidarians share a surprising number of genes with vertebrates (Kortschak et al., 2003). The traditional assumption that a simple morphology correlates with a small transcriptome is therefore unlikely to hold; cnidarians are likely to have at least as many genes as *Drosophila* and *Caenorhabditis* and, in some cases, many more.

RESULTS AND DISCUSSION

Tandemly-organised NK2 genes related to *Drosophila* vnd

The *Acropora* genome contains a tandem pair of NK class homeobox genes related to the *Drosophila* vnd and the vertebrate Nkx2.1 type (see Fig. 1). The pair of genes was discovered during primer walking on a single genomic clone from the 5′ end of vnd1am. The two genes (vnd1-Am and vnd2-Am) are organised 'head-to-head' (Fig. 1a) with the start codons and homeoboxes only 7.4kb and 9kb apart respectively. Neither vnd1-Am nor vnd2-Am is likely to be a pseudogene as northern analysis indicates that both genes are expressed in planula larvae and adult *Acropora* colonies (data not shown). Complete or near-complete cDNAs for both vnd1-Am and vnd2-Am have been isolated using either 5′/3′ RACE (vnd1-Am) or by screening an adult *Acropora* cDNA library (vnd2-Am). In addition to the homeodomain (HD), both *Acropora* genes encode an Eh1 domain and vnd1-Am also contains an NK2SD; the former mediates interactions with Groucho class corepressors (Smith and Jaynes, 1996; Cowden and Levine, 2003) and is present in a range of different homeodomain proteins, whereas the latter is characteristic only of NK2 proteins and its function *in vivo* is unclear (Watada et al., 2000). The HDs encoded by vnd1-Am and vnd2-Am are 77% identical, and both are most closely related to an NK2 sequence from the sea anemone *Nematostella vectensis*. The closest non-cnidarian match for both vnd1-Am and vnd2-Am is *Branchiostoma floridae* Nkx2.2 (accession# AAD01958).

Unlike the other cases discussed here, there are bilaterian precedents for the pattern of organization of NK genes observed in *Acropora*, suggesting the possibility of synteny. In mouse and man, the four *vnd/Nkx2.1*-type genes are organised as two linked paralogous pairs; Nkx2.2/Nkx2.4 and Nkx2.1/Nkx2.9, reflecting an ancient duplication in which an ancestral NK2-type gene was duplicated to form a tandem gene pair, and the pair then duplicated to another chromosomal locus at a later time. Thus, genes are more similar to their duplicated partner on another chromosome than to their linked partner (Wang *et al*, 2000). On the basis of phylogenetic analysis (Fig. 1B) however, the *Acropora* NK2/*vnd* genes have been independently duplicated within the Cnidaria, thus their organisation does not reflect synteny between cnidarians and mammals. Both *Acropora* sequences fall into a clade that includes mouse and human Nkx2.2 and Nkx2.9 and *Drosophila* vnd, but within this the cnidarian sequences form a clearly distinct monophyletic group.

Acropora has two genes related to vertebrate Dmbx1.

Two paired-like genes of the K50-type (Galliot et al., 1999) are tightly linked and organised in tandem in the *Acropora* genome (see Fig. 2). The two genes are expressed, although in both cases only incomplete cDNA clones have so far been isolated. Database comparisons indicate that the HDs encoded by the two genes are closely related to the vertebrate Dmbx1-type, (Takahashi et al., 2002; Gogoi et al., 2002; Broccoli et al., 2002; Ohtoshi et al., 2002), and *Hydra* manacle (Bridge et al., 2000). Note that Dmbx1 has also been known as mbx (Kawahara et al., 2002), Atx (Martinez-Barbera et al., 2001), PaxB and otx3 (Zhang et al., 2002) in the literature. As in the case of a significant number of other *Acropora* genes (Kortschak et al., 2003), no clear orthologs of Dmbx1 appear to be present in either *Drosophila* or *Caenorhabditis*, reflecting secondary losses of this gene. The vertebrate Dmbx1 sequences are remarkably similar; the HDs in the zebrafish, chicken, mouse and human proteins are identical. The cnidarian and chordate Dmbx-related sequences appear to define a distinct family of K50 HD proteins that are closely related to, but distinct from, the Otx- and Ptx-types (see Fig. 2B).

The two *Acropora* Dmbx loci appear to represent a cnidarian specific duplication, as we could find no evidence for paralogous genes in any of the complete metazoan genomes (human, mouse, rat, zebrafish, pufferfish, *Ciona*, sea urchin). This duplication event presumably occurred in the deep past, as the corresponding proteins are highly divergent; the HDs have 65% identity, and the remainder of the sequences are virtually unalignable. Of the two coral proteins, Dmbx2-Am is more closely related to the chordate Dmbx1 type in the HD (77% vs 60% identity). On the basis of phylogenetic analysis, Dmbx1-Am appears to be the *Acropora* ortholog of manacle, although it is not yet clear whether both genes are present in *Hydra*.

Although the HDs of the Dmbx group are closely related (> 60% identity), the overall domain structures of the proteins differ significantly. In addition to the HD, the only other recognisable motif in the Dmbx2-Am protein is an octapeptide; it is not clear whether this is also present in Dmbx1-Am, as the sequence is incomplete. By contrast, the mouse and human Dmbx1 proteins contain OAR domains at their C-termini but no octapeptides, and manacle contains neither of these additional motifs.

A pair of Msx loci – genes or pseudogenes?

Two Msx-related homeobox genes are tightly linked in the genome of *Acropora millepora* – the linkage was discovered during the course of a primer walk from one of the genes (see Fig. 3). The genes are organised in a 'tail-to-tail' manner, the homeoboxes being a little under 3 kb apart. Single Msx genes have previously been cloned from two hydra species – *Chlorohydra viridissima* (Schummer et al., 1992) and *Hydra vulgaris* (Gauchat et al., 2000). The mammalian Msx loci are not linked.

Phylogenetic analysis (Fig. 3B) clearly groups the HDs in both of these *Acropora* sequences with the Msx class, but the sequences are highly divergent and, although no stop codons are present in the homeoboxes, we suspect that these might be pseudogenes, on the following grounds. Firstly, no evidence could be obtained that the genes are expressed. Screening several million plaques from cDNA libraries corresponding to various developmental stages failed to identify cDNAs corresponding to Msx1-Am or Msx2-Am. Furthermore, quantitative RT-PCR using mRNA from all major developmental stages did not yield signals significantly above background. Secondly, the predicted amino acid sequences of the *Acropora* Msx HDs, particularly that of Msx2-Am, are highly derived and feature some dramatic amino acid substitutions at positions likely to be structurally significant. For example, the phenylalanine residue at HD position 16 (Phe16) in Msx2-Am appears to be unique; in all other HDs a leucine residue at this position is involved in HD core stabilisation. Residues known to be involved in DNA-binding specificity in the case of MSX1 (Hovde et al., 2001), but differing in the *Acropora* sequences, include Arg2 (Ser in Msx2-Am, Gly in Msx1-Am) and Arg58 (Lys in both *Acropora* sequences). In addition, the Glu30/Lys23 and Glu42/Arg31 salt bridges known to stabilise the MSX1 HD core (Hovde et al., 2001) are not possible in either of the *Acropora* HDs.

The cases described above imply that many regulatory genes have been independently duplicated in *Acropora*; of the 13 distinct classes of homeobox genes thus far identified in *Acropora* (excluding Pax genes), at least three exist as duplicated pairs that are tightly linked. Two highly similar Not loci are also tightly linked in *Acropora* (Hayward et al., unpublished) and the possibility of linked duplicates for many other genes cannot be ruled out. The paucity of genomic data for cnidarians means that the true extent of duplication events may be much greater than the cases documented here, and may have contributed significantly to the large genome sizes estimated for some cnidarians (David and Campbell, 1972; Zacharias et al., in press).

It is also unclear whether the specific duplications seen in *Acropora* occur throughout the Cnidaria. The degree of divergence between paralogs in *Acropora* (e.g. 77%, 65% and 53% identity in the Nkx, Dmbx and Msx HDs respectively) implies that the duplication events that gave rise to them are unlikely to have been recent. The divergence of the cnidarian classes is thought to have occurred deep in Pre-Cambrian time (Chen et al., 2002); hence some genes that are duplicated in *Acropora* may not be in (for example) *Hydra*. However, at least some cnidarian-specific duplication events precede the Anthozoa / Hydrozoa divergence; for example, duplicated nanos genes are present in both *Hydra* (Mochizuki et al., 2000) and *Acropora* (Go et al., unpublished). In other cases, such as that of the Msx genes, patterns of relatedness imply that the duplication events that gave rise to them may have post-dated the Anthozoa / Hydrozoa split. In the case of the Dmbx1-related genes, phylogenetic analyses reveal that one of the *Acropora* sequences (Dmbx1-Am) is likely to be orthologous with *Hydra* manacle (Bridge et al., 2000), whereas the other is more closely related to the vertebrate Dmbx sequences (Fig. 2B) This relationship, between related pairs of genes in a basal cnidarian and vertebrates, may indicate that the duplication of Dmbx genes predated the Anthozoa / Hydrozoa split. Hence, it is likely that a second Dmbx gene is, or was, present in the derived hydrozoan lineage.

Cnidarians share a surprising number of genes with vertebrates (Kortschak et al., 2003); hence cnidarian genomes are qualitatively complex. The independent duplication of a significant number of regulatory genes adds another dimension of complexity to cnidarian genomes. The traditional assumption that the morphological simplicity of cnidarians corresponds to a small transcriptome is therefore clearly incorrect; cnidarians are likely to have at least as many genes as the model invertebrates *Drosophila* and *Caenorhabditis* and, in some cases, many more.

References

Adachi J, Hagesawa M (1996) MOLPHY version 2.3: program for molecular phylogenetics based on maximum likelihood. Computer Sci Monogr 28:1-150

Bridge DM, Stover NA, Steele RE (2000) Expression of a novel receptor tyrosine kinase gene and a paired-like homeobox gene provides evidence of differences in patterning at the oral and aboral ends of hydra. Dev Biol 220: 253-262 DOI 10.1006/dbio.2000.9653

Broccoli V, Colombo E, Cossu G (2002) Dmbx1 is a paired-box containing gene specifically expressed in the caudal most brain structures. Mech Dev. 114: 219-223 DOI 10.1016/S0925-4773(02)00078-3

Chen JY, Oliveri P, Gao F, Dornbos SQ, Li CW, Bottjer DJ, Davidson EH (2002) Precambrian animal life: probable developmental and adult cnidarian forms from Southwest China. Dev Biol 248: 182-96 DOI 10.1006/dbio.2002.0714

Conway Morris S (2000) The Cambrian "explosion": slow-fuse or megatonnage? Proc Natl Acad Sci U S A. 97: 4426-4429

Cowden J, Levine M. (2003) Ventral dominance governs sequential patterns of gene expression across the dorsal-ventral axis of the neuroectoderm in the Drosophila embryo. Dev Biol 262: 335-349 DOI 10.1016/S0012-1606(03)00395-6

David CN, Campbell RD. (1972) Cell cycle kinetics and development of Hydra attenuata. I. Epithelial cells. J Cell Sci 11: 557-568

Furlong, R, Holland P (2002) Were Vertebrates Octoploid? Philos Trans R Soc Lond B Biol Sci 357: 531-544. DOI: 10.1098/rstb.2001.1035

Galliot B, de Vargas C, Miller D. (1999) Evolution of homeobox genes: Q50 Paired-like genes founded the Paired class. Dev Genes Evol 209: 186-197 DOI 10.1007/s004270050243

Gauchat D, Kreger S, Holstein T, Galliot B. (1998) Prdl-a, a gene marker for hydra apical differentiation related to triploblastic paired-like head-specific genes. Development 125: 1637-1645.

Gauchat D., Mazet F, Berney C, Schummer M, Kreger S, Pawlowski J, Galliot, B (2000) Evolution of Antp-class genes and differential expression of *Hydra* Hox/paraHox genes in anterior patterning. Proc. Natl. Acad. Sci. USA 97: 4493–4498

Gogoi RN, Schubert FR, Martinez-Barbera JP, Acampora D, Simeone A, Lumsden A (2002) The paired-type homeobox gene Dmbx1 marks the midbrain and pretectum. Mech Dev 114: 213-217 DOI 10.1016/S0925-4773(02)00067-9

Grasso LC, Hayward DC, Trueman JW, Hardie KM, Janssens PA, Ball EE. (2001) The evolution of nuclear receptors: evidence from the coral Acropora. Mol Phylogenet Evol. 21: 93-102. DOI 10.1006/mpev.2001.0994

Hovde S, Abate-Shen C, Geiger J (2001) Crystal Structure of the Msx-1 Homeodomain/DNA Complex. Biochemistry 40: 12013–12021 DOI 10.1021/bi0108148

Kawahara A, Chien CB, Dawid IB (2002) The homeobox gene mbx is involved in eye and tectum development. Dev Biol. 248: 107-117 DOI 10.1006/dbio.2002.0709

Kortschak RD, Samuel G, Saint R, Miller DJ. (2003) EST analysis of the cnidarian Acropora millepora reveals extensive gene loss and rapid sequence divergence in the model invertebrates. Curr Biol 13: 2190-2195 DOI 10.1016/j.cub.2003.11.030

Martindale MQ, Pang K, Finnerty JR (2004) Investigating the origins of triploblasty: 'mesodermal' gene expression in a diploblastic animal, the sea anemone Nematostella vectensis (phylum, Cnidaria; class, Anthozoa). Development 131: 2463-2474 DOI 10.1242/dev.01119

Martinez-Barbera JP, Signore M, Boyl PP, Puelles E, Acampora D, Gogoi R, Schubert F, Lumsden A, Simeone A (2001) Regionalisation of anterior neuroectoderm and its competence in responding to forebrain and midbrain inducing activities depend on mutual antagonism between OTX2 and GBX2. Development 128:4789-800

Minguillon C, Ferrier DE, Cebrian C, Garcia-Fernandez J (2002) Gene duplications in the prototypical cephalochordate amphioxus. Gene 287: 121-128 DOI 10.1016/S0378-1119(01)00828-9

Mochizuki K, Sano H, Kobayashi S, Nishimiya-Fujisawa C, Fujisawa T. (2000) Expression and evolutionary conservation of nanos-related genes in Hydra. Dev Genes Evol 210: 591-602
DOI: 10.1007/s004270000105

Ohtoshi A, Nishijima I, Justice MJ, Behringer RR (2002) Dmbx1, a novel evolutionarily conserved paired-like homeobox gene expressed in the brain of mouse embryos. Mech Dev 110: 241-244 DOI 10.1016/S0925-4773(01)00587-1

Samuel G, Miller D, Saint R. (2001) Conservation of a DPP/BMP signaling pathway in the nonbilateral cnidarian Acropora millepora. Evol Dev 3: 241-250

Schummer, M, Scheurlen I, Schaller C, Galliot B (1992) HOM/HOX homeobox genes are present in hydra (*Chlorohydra viridissima*) and are differentially expressed during regeneration. The EMBO Journal 11: 1815–1823

Smith, S, Jaynes, J. (1996) A conserved region of engrailed, shared among all en-, gsc-, Nk1-, Nk2- and msh-class homeoproteins, mediates active transcriptional repression in vivo. Development 122: 3141-3150

Takahashi T, Holland PW, Cohn MJ, Shimizu K, Kurokawa M, Hirai H. (2002) An orphan PRD class homeobox gene expressed in mouse brain and limb development. Dev Genes Evol. 212: 293-297. DOI 10.1007/s00427-002-0244-1

Wang, C, Brodnicki, T, Copeland, N., Jenkins, N, Harvey R (2000) Conserved linkage of NK-2 homeobox gene pairs *Nkx2-2/2-4* and *Nkx2-1/2-9* in mammals. Mammalian Genome 11: 466–468

Watada, H, Mirmira, R Kalamaras, J and German M (2000) Intramolecular control of transcriptional activity by the NK2-specific domain in Nk-2 homeodomain proteins. Proc. Natl. Acad. Sci. USA 97: 9443–9448

Zacharias H, Anokhin B, Khalturin K and Bosch TCG (2004) Genome sizes and chromosomes in the basal metazoan Hydra. In press to: Zoology.

Zhang Y, Miki T, Iwanaga T, Koseki Y, Okuno M, Sunaga Y, Ozaki N, Yano H, Koseki H, Seino S (2002) Identification, tissue expression, and functional characterization of Otx3, a novel member of the Otx family. J Biol Chem 277: 28065-29069 DOI 10.1074/jbc.C100767200

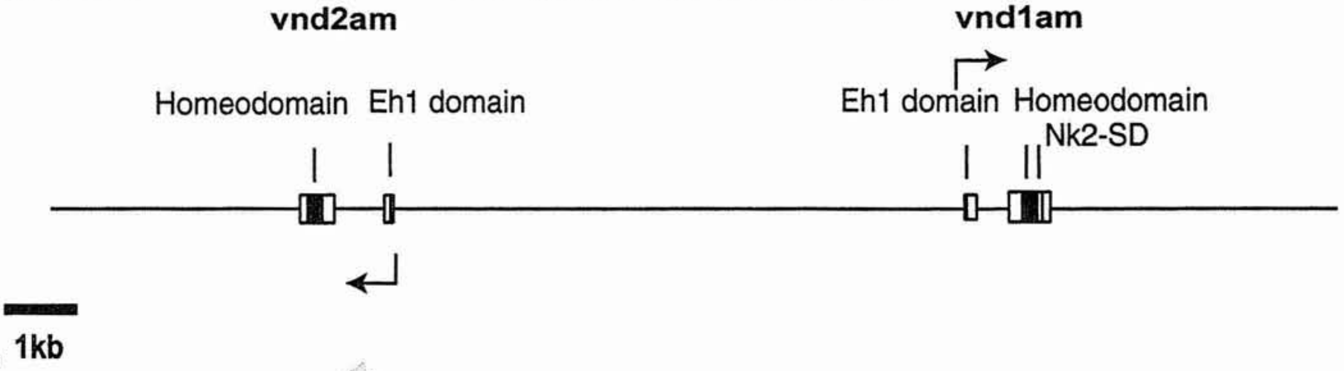
Figure Legends

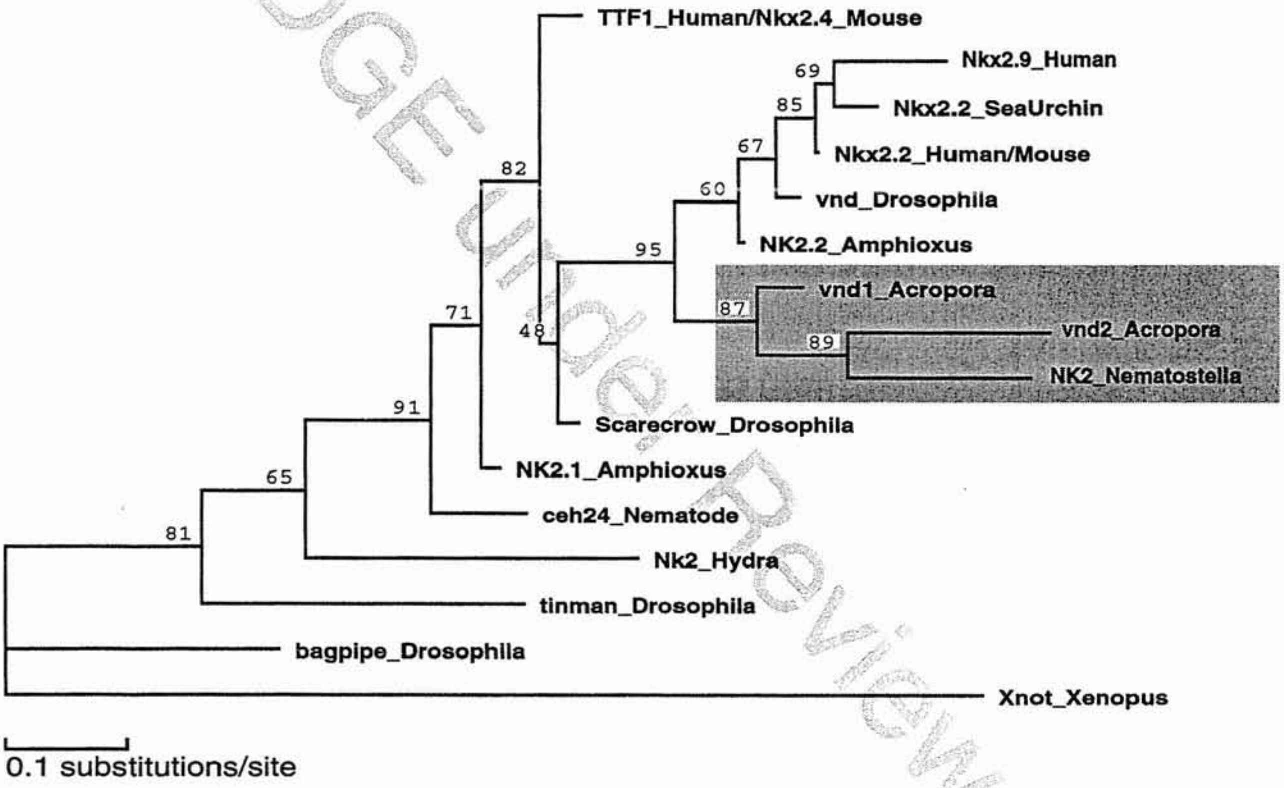
Fig. 1. Sequence comparisons and genomic organization of vnd-related genes in *Acropora*. (A) Structure of the genomic region containing the *vnd1-Am* and *vnd2-Am* loci. The arrows indicate the direction of transcription, and the boxes indicate exons. (B) The vnd1-Am and vnd2-Am homeodomains were subjected to Maximum-Likelihood phylogenetic analyses in MolPhy version 2.3 (Adachi and Hagesawa, 1996) using the Dayhoff model of protein evolution and local rearrangement of the NJ trees (1000 bootstraps). In these analyses, *Xenopus* Xnot (accession # CAA79629) served as outgroup. The shaded area indicates the clade

consisting of the cnidarian vnd-related sequences. (C) A Boxshade alignment of the homeodomain sequences used in the phylogenetic analyses. Database accession numbers of the sequences are: *Nematostella vectensis* NK2 (AAP88430), *Drosophila melanogaster* vnd (NM 057438), *Brachiostoma floridae* NK2.2 (AF032999), human TTF1 (NM 003317), human NKX2.2 (NM 002509), human NKX2.9 (AF000297), *Strongylocentrotus purpuratus* Nk2.2 (AAS58444), *Caenorhabditis elegans* ceh24 (NM 074018), *Hydra vulgaris* CnNK2 (AAB67611), *D. melanogaster* tinman (AAQ75417), *D. melanogaster* bagpipe (AAQ74415), *D. melanogaster* scarecrow (AAF26436) and *B. floridae* NK2.1 (AF077840).

Fig. 2. Sequence comparisons and genomic organization of *Dmbx1*-related genes in *Acropora*. (A) Structure of the genomic region containing the *Dmbx1-Am* and *Dmbx2-Am* loci. The arrows indicate the direction of transcription, and the boxes indicate exons. (B)Maximum-Likelihood phylogenetic analyses of Dmbx1-related homeodomain sequences, as described in Fig. 1B, using the *Drosophila* aristaless sequence (accession # NP 523834.1) as outgroup. The light shaded area indicates the clade consisting of the cnidarian Dmbx1-related sequences; the more darkly shaded area defines the Dmbx group. (C) A Boxshade alignment of the homeodomain sequences used in the phylogenetic analyses. Database accession numbers of the sequences are: *Hydra vulgaris* manacle (AF126249_1), mouse Dmbx1 (AAM78514.1), *Ciona intestinalis* CiDmbx (AAT66432.1), *Branchiostoma floridae* BfDmbx (AAT66431.1), *Drosophila melanogaster* Ptx1 (O18400), mouse Ptx2 (NP 035228.2), *D. melanogaster* orthodenticle (A35912), mouse Otx2 (AAH29667.1), human OTX1 (NP 055377.1), *H. vulgaris* Alx (AF295531), *H. vulgaris* prdla (CAA75668.1) and *H. vulgaris* prdlb (CAA75669.1).

Fig. 3. (A) Structure of the genomic region containing the *Msx1-Am* and *Msx2-Am* loci. The arrows indicate the direction of transcription, and the boxes indicate exons. (B) Maximum-Likelihood phylogenetic analyses of Msx1-Am and 2-Am homeodomains, as described in Fig. 1B, using the mouse Dlx1 sequence (accession # AAH79609) as outgroup. The shaded area indicates the clade consisting of the cnidarian Msx-related sequences. (C) A Boxshade alignment of the homeodomain sequences used in the phylogenetic analyses. Database accession numbers of the sequences are: *Hydra vulgaris* Msh (CAB88390), *Chlorohydra viridissima* Msh (CAA45912), *Drosophila melanogaster* Msh (NM 057976), mouse Msx2 (NP 038629), mouse Msx1 (AAH16426), *Danio rerio* mshE (NP 571348), *D. rerio* mshD (NP 571351), mouse Msx3 (AAH51983), *Branchistoma floridae* Msx (CAA10201), *D. rerio* mshB (U16311) and *Ciona intestinalis* MshA (M38581).





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Acropora vnd2	KRKRRVLFTKAQTFVLEKRFQQQRYLSAPEREELARIVHLTPAQVKIWFQNKRYKYRKQI	77/88
Nematostella NK2	KKKRRILFTKSQIFELERRFRQQKYLSANEREQLARIIDLTPTQVKIWFQNHRYKFKKQI	80/93
Drosophila vnd	KRKRRVLFTKAQTYELERRFRQQRYLSAPEREHLASLIRLTPTQVKIWFQNHRYKTKRAQ	83/90
Amphioxus Nk2.2	KRKRRVLFSKAQTYELERRFRQQRYLSAPEREHLARLIRLTPTQVKIWFQNHRYKCKRAQ	88/93
Human TTF1/Mouse Nkx2.4	RRKRRVLFSQAQVYELERRFKQQKYLSAPEREHLASMIHLTPTQVKIWFQNHRYKMKRQA	78/90
Human/Mouse Nkx2.4	KRKRRVLFSKAQTYELERRFRQQRYLSAPEREHLASLIRLTPTQVKIWFQNHRYKMKRAR	85/90
Human Nkx2.9	RKKRRVLFSKAQTLELERRFRQQRYLSAPEREQLASLLRLTPTQVKIWFQNHRYKLKRAR	80/90
Sea Urchin Nkx2.2	KRKRRVLFSKAQTYELERRFRQQRYLSAPEREHLASIIRLSPTQVKIWFQNHRYKLKRAR	88/90
Nematode ceh24	RRKRRVLFSQAQVYELERRFKQAKYLTAPEREQLANSIRLTPTQVKIWFQNHRYKCKRQE	78/88
Hydra CnNK2	RKKPRILFSQSQVMELGKKFKDQKYLSASERDQIANKLNLTPTQVKIWFQNKRYKCKKQT	62/85
Drosophila tinman	KRKPRVLFSQAQVLELECRFRLKKYLTGAEREIIAQKLNLSATQVKIWFQNRRYKSKRGD	63/75
Drosophila bagpipe	KKRSRAAFSHAQVFELERRFAQQRYLSGPERSEMAKSLRLTETQVKIWFQNRRYKTKRKQ	60/80
Drosophila scarecrow	RRKRRVLFTQAQVYELERRFKQQRYLSAPEREHLASLIHLTPTQVKIWFQNHRYKCKRQA	80/92
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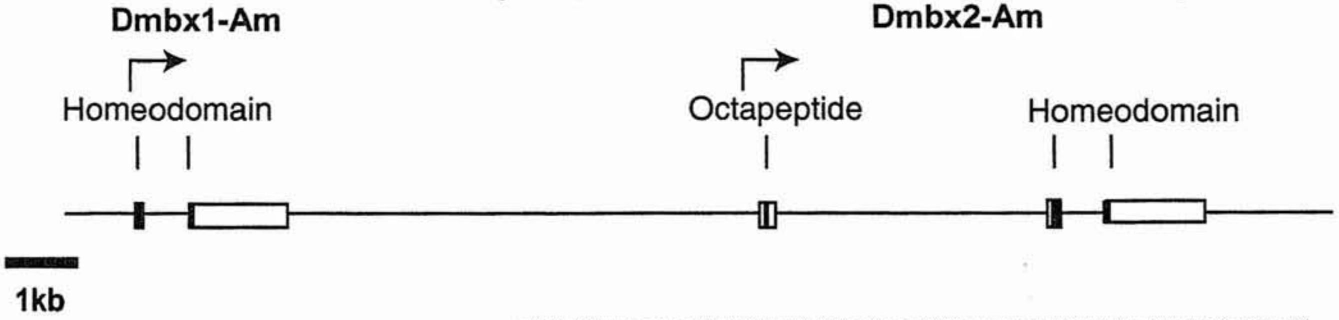

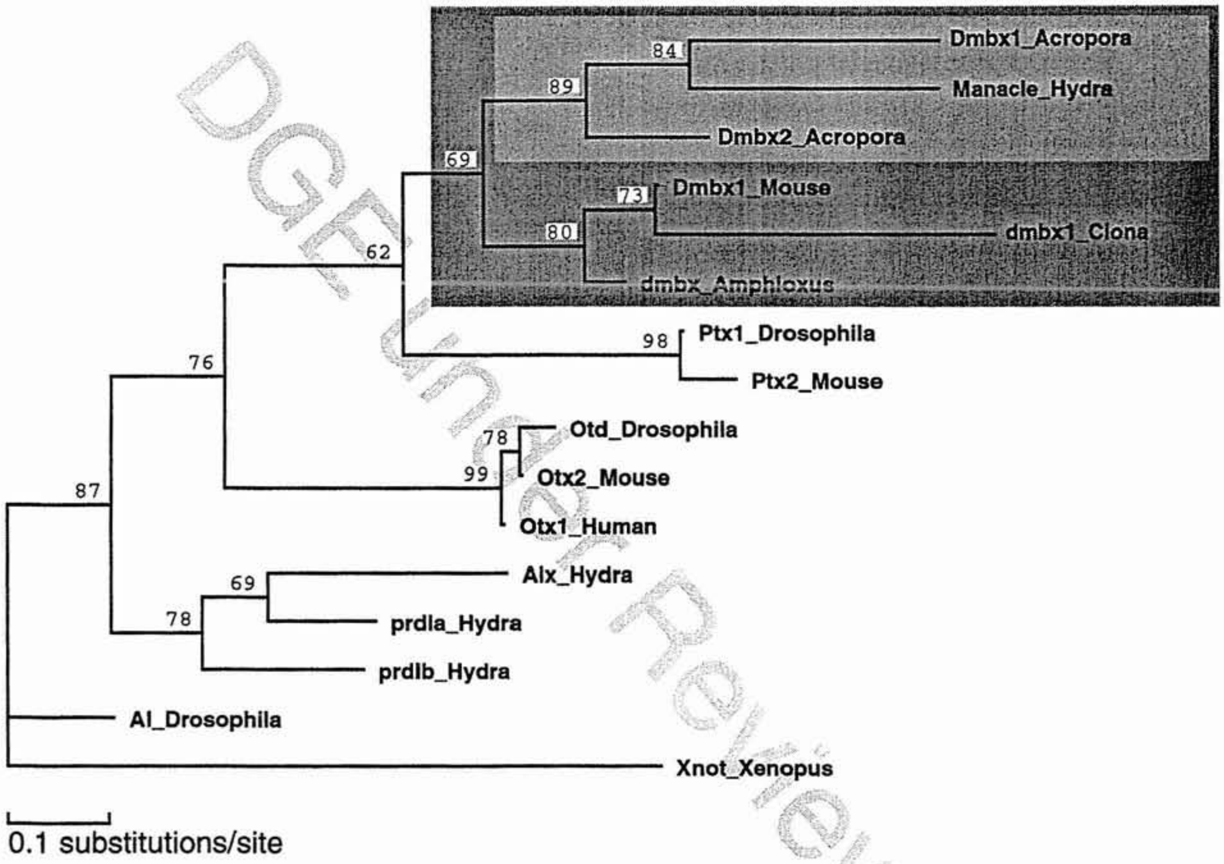

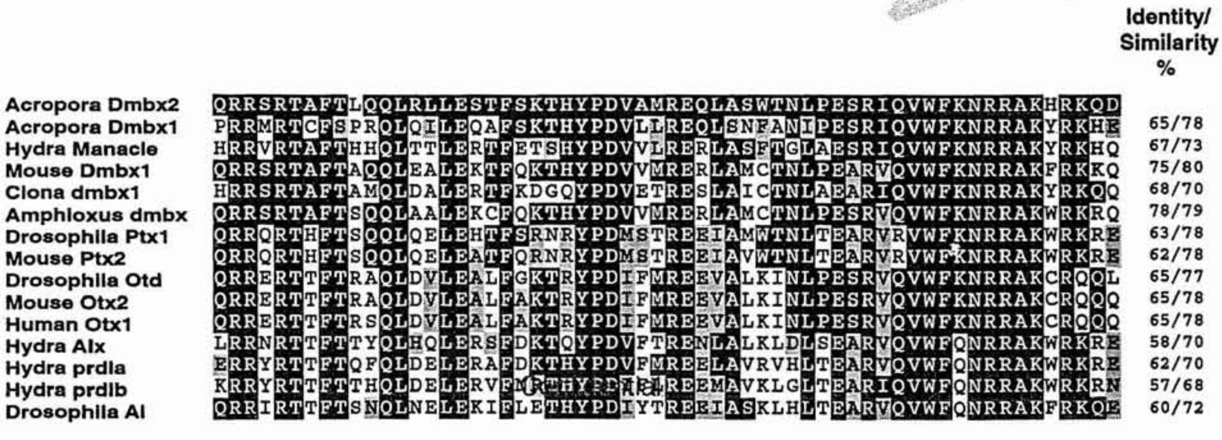

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Acropora Dmbx1	PRRMRTCFSPRQLQILEQAFSKTHYPDVLLREQLSNFANIPESRIQVWFKNRRAKYRKHE	65/78
Hydra Manacle	HRRVRTAFTHHQLTTLERTFETSHYPDVVLRERLASFTGLAESRIQVWFKNRRAKYRKHQ	67/73
Mouse Dmbx1	QRRSRTAFTAQQLEALEKTFQKTHYPDVVMRERLAMCTNLPEARVQVWFKNRRAKFRKKQ	75/80
Ciona dmbx1	HRRSRTAFTAMQLDALERTFKDGQYPDVETRESLAICTNLAEARIQVWFKNRRAKYRKQQ	68/70
Amphioxus dmbx	QRRSRTAFTSQQLAALEKCFQKTHYPDVVMRERLAMCTNLPESRVQVWFKNRRAKWRKRQ	78/79
Drosophila Ptx1	QRRQRTHFTSQQLQELEHTFSRNRYPDMSTREEIAMWTNLTEARVRVWFKNRRAKWRKRE	63/78
Mouse Ptx2	QRRQRTHFTSQQLQELEATFQRNRYPDMSTREEIAVWTNLTEARVRVWFKNRRAKWRKRE	62/78
Drosophila Otd	QRRERTTFTRAQLDVLEALFGKTRYPDIFMREEVALKINLPESRVQVWFKNRRAKCRQQL	65/77
Mouse Otx2	QRRERTTFTRAQLDVLEALFAKTRYPDIFMREEVALKINLPESRVQVWFKNRRAKCRQQQ	65/78
Human Otx1	QRRERTTFTRSQLDVLEALFAKTRYPDIFMREEVALKINLPESRVQVWFKNRRAKCRQQQ	65/78
Hydra Alx	LRRNRTTFTTYQLHQLERSFDKTQYPDVFTRENLALKLDLSEARVQVWFQNRRAKWRKRE	58/70
Hydra prdIa	ERRYRTTFTQFQLDELERAFDKTHYPDVFMREELAVRVHLTEARVQVWFQNRRAKWRKRE	62/70
Hydra prdIb	KRRYRTTFTTHQLDELERVFMRTHYPD[illegible]REEMAVKLGLTEARIQVWFQNRRAKWRKRN	57/68
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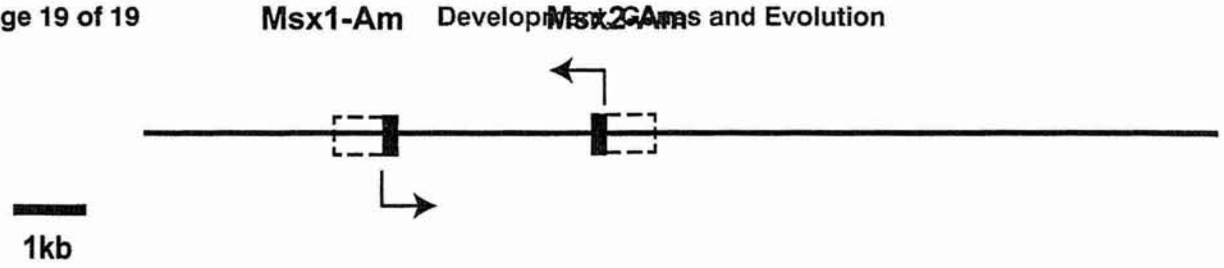

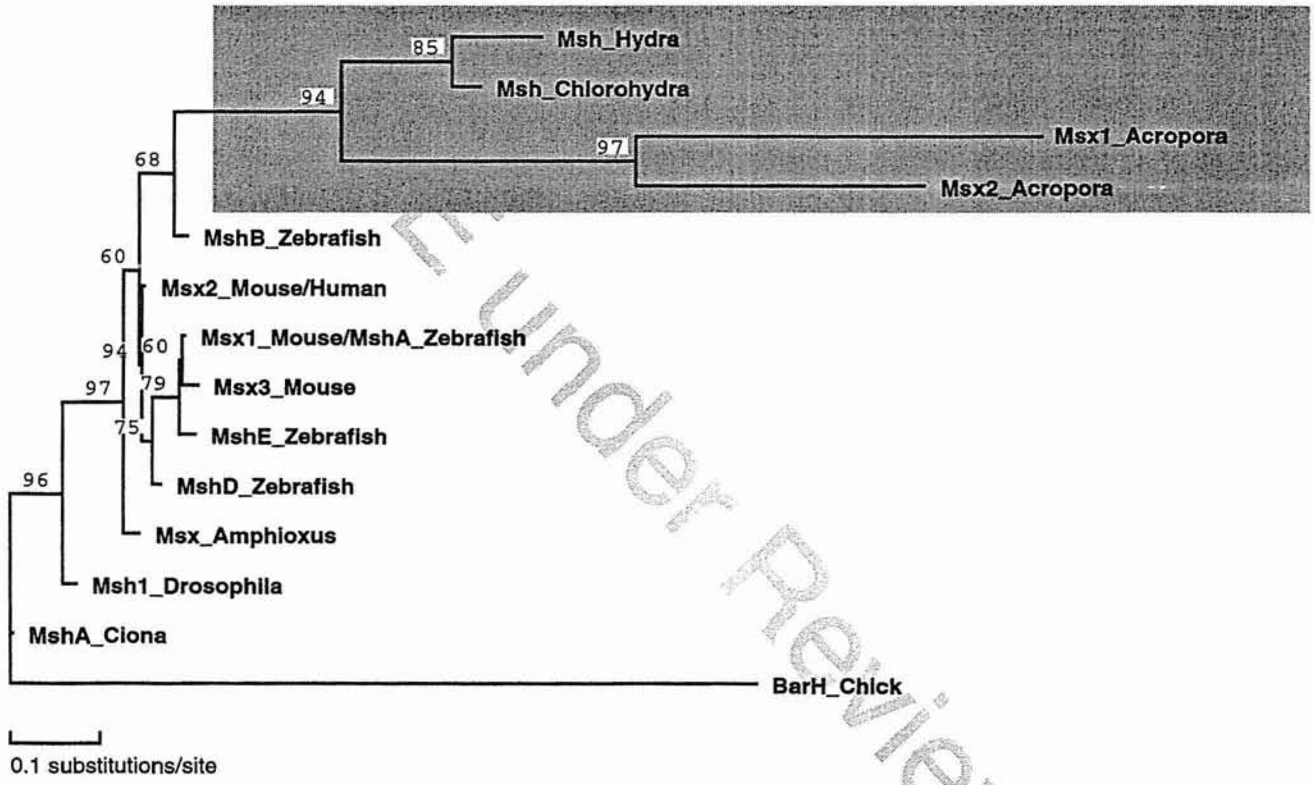

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Hydra Msh	NRKPRTPFSVNQLLALEQKFKRKQYLSISERSELSVMLRLTETQRKIWFQNRRAKQKRRT	50/62
Chlorohydra Msh	NRKPRTPFSVNQLLTLEQKFKRKQYLSISERAELSELLRLTETQIKIWFQNRRAKQKRSK	52/67
Drosophila Msh1	NRKPRTPFTTQQLLSLEKKFREKQYLSIAERAEFSSSLRLTETQVKIWFQNRRAKAKRLQ	50/65
Mouse/Human Msx2	NRKPRTPFTTSQLLALERKFRQKQYLSIAERAEFSSSLNLTETQVKIWFQNRRAKAKRLQ	52/67
Mouse Msx1/Zebrafish MshA	NRKPRTPFTTAQLLALERKFRQKQYLSIAERAEFSSSLSLTETQVKIWFQNRRAKAKRLQ	52/68
Zebrafish MshE	NRKPRTPFSTAQLLALERKFRQKQYLSIAERAEFSSSLSLTETQVKIWFQNRRAKAKRLQ	55/72
Zebrafish MshD	NRKPRTPFTTSQLLALERKFRQKQYLSIAERAEFSSSLTLTETQVKIWFQNRRAKAKRLQ	53/73
Mouse Msx3	NRKPRTPFTTAQLLALERKFHQKQYLSIAERAEFSSSLSLTETQVKIWFQNRRAKAKRLQ	52/68
Amphioxus Msx	NRKPRTPFTTQQLLALERKFRQKQYLSIAERAEFSASLNLTETQVKIWFQNRRAKAKRLQ	50/68
Zebrafish MshB	NRKPRTPFSTSQLLSLERKFRQKQYLSIAERAEFSNSLNLTETQVKIWFQNRRAKAKRLQ	53/65
Ciona MshA	NRKPRTPFTTQQLMSLEKKFREKQYLSIAERAEFSNSLSLTETQVKIWFQNRRAKSKRLQ	50/67