6

General Conclusions

6.1 A LINK BETWEEN MORPHOLOGICAL AND MOLECULAR COMPLEXITY?

It is often assumed that there is a direct relationship between the morphological complexity of an organism and the number of developmental genes required for its specification, but the degree to which this is true is not clear. The Hox cluster duplications that have occurred within the chordate lineage are consistent with the notion of a correlation between morphological and molecular complexity. Whereas the simplest extant chordates (like other invertebrates) have a single Hox cluster (Ferrier and Holland, 2001; Garcia-Fernandez and Holland, 1994), at least two duplication events are thought to have led to the presence of four Hox clusters in the majority of vertebrates. Consistent with a link to body plan complexity, the most morphologically diverse group of metazoans, the bony fish, have undergone a further duplication event, leading (after secondary losses) to seven HOX clusters in the zebrafish (Amores et al., 1998) and medaka (Naruse et al., 2000), and five Hox clusters in the pufferfish (Amores et al., 1998).

During the course of this study, five new homeobox genes (corresponding to four distinct types – *Emx, Hbn, Arx* and *Dmbx1/2*) were cloned from *Acropora*, bringing the total number of homeobox gene classes cloned from this organism (excluding *Pax* genes) to 16. Given that *Acropora* has not been extensively studied, this probably represents a minor fraction of the total number present. Previous studies indicate that other gene families show similar levels of complexity in *Acropora* – for example, there are at least four Pax genes (Catmull et al., 1998; Miller et al., 2000; Reece-Hoyes, 2001), and 10 distinct nuclear receptors (Grasso et al., 2001). This suggests a level of molecular complexity that is unexpected in a structurally simple animal – by comparison, *Drosophila* has approximately 80 homeobox genes and 21 nuclear receptors. The notion of cryptic complexity is also

supported by a preliminary EST project in progress; of approximately 500 ESTs with strong ($e > 10^{-6}$) database matches, a surprising proportion have no clear *Drosophila* or *Caenorhabditis* counterparts (Kortschak et al., submitted). This implies that gene loss in the model invertebrates is likely to have been more extensive than was previously recognised, and that the common ancestor of cnidarians and bilateral animals was remarkably complex (at least at the molecular level).

This assumed relationship between molecular and morphological complexity is, in some ways, reminiscent of the debate that raged 10-15 years ago on the issue of genome size and morphological complexity. Mammals have large genomes (Humans, ~3.15 Gb; Mouse, ~2.75 Gb) relative to the model invertebrates *Drosophila* (116,000 kb) and *Caenorhabditis* (97,000 kb), which are again large by comparison with the yeast *Saccharomyces cerevisiae* (~12,496 kb). However, this apparent pattern collapses when a more representative range of vertebrates, invertebrates and fungi are considered – salamanders and lungfish, for example, have extraordinarily large genomes (being 28 and 35 times greater than mammalian genomes respectively; reviewed in Ohno, 1970) and it is clear that neither *Saccharomyces* nor *Drosophila* are representative of their larger taxonomic groups.

One rationalization of the apparent paradox of molecular complexity in *Acropora* is that perhaps genetic regulatory mechanisms are significantly less complex in the Cnidaria. In bilateral animals, regulatory genes frequently play multiple roles in development, a phenomenon that is achieved through differential use of complex promoters and enhancer elements. For example, the *Drosophila even-skipped (eve)* gene, plays at least three distinct roles at different times during development (including segmentation, neurogenesis and posterior patterning; (Frasch et al., 1987). Each of these roles requires sophisticated control mechanisms – for example, the seven individual *eve* expression stripes observed during early fly segmentation are regulated by separate enhancers (Harding et al., 1989). Although very little is known about regulatory mechanisms in cnidarians, it is unlikely that such complexity will be found in the case of *eveC*, the *Acropora even-skipped* ortholog (Miles and Miller, 1992). Perhaps animal evolution has been characterised primarily by the increased complexity of genetic regulation rather than by changes in coding sequences -

animals such as cnidarians may have a similar number of genes to bilateral animals but may effectively use them in less 'efficient' ways.

6.2 *ACROPORA* AS A REPRESENTATIVE CNIDARIAN FOR EVO-DEVO PURPOSES.

As a sister group to the Bilateria, the Cnidaria are likely to be important in understanding the evolution of developmental mechanisms and genetic complexity. Within the Phylum Cnidaria, members of the basal class, the Anthozoa, are perhaps more likely to represent characteristics of the ancestral metazoan than are representatives of the more derived classes such as *Hydra* (class Hydrozoa). The Cnidaria are an extremely diverse phylum, whose classes are thought to have diverged deep in Pre-Cambrian time (Conway Morris, 1998; Conway Morris, 2000a; Conway Morris, 2000b). Therefore the use of cnidarians for 'evo-devo' purposes is likely to be complicated both by the recruitment of genes to cnidarian-specific functions and by heterogeneity of function within the phylum.

Representatives from at least 16 different homeobox gene families have been identified in *Acropora*, five of which were isolated during this project. Some of these genes are expressed during development in patterns consistent with their known functions in higher animals, whereas others are more likely to have cnidarian-specific functions. *emx-Am* is one example where function appears to have been conserved from cnidarians to vertebrates. Its restricted pattern of expression along the single (oral-aboral) axis in a subset of presumed neurons reflects the role of the *ems/Emx* gene family in the specification of anterior structures and brain patterning in bilaterians. How the *emx-Am* data relate to the adult expression pattern of the orthologous *Hydractinia* gene, *Cn-ems*, is unclear. In the hydrozoan, expression is limited to the endodermal epithelial cells (or digestive cells) of the hypostome. The function of the *Acropora Gsx* homolog, *cnox2-Am,* also appears to be conserved with its bilaterian counterparts. The expression of *cnox2-Am* in a subset of presumed neurons that is restricted along the oral-aboral axis in the early developmental stages of the coral mirrors the corresponding patterns of the *gsh/Gsx* family in dorsal-ventral patterning mechanisms in higher animals (Hayward et al., 2001). Hence, key

components of both the anterior-posterior (A/P) and dorsal-ventral (D/V) bilaterian patterning systems are differentially expressed along the single oral-aboral axis in *Acropora* planulae.

However, the expression patterns of these two genes present an anomaly that has yet to be resolved, since the *emx-Am* expression pattern would indicate that oral-aboral corresponds to anterior-posterior, while the *cnox2-Am* pattern would be consistent with oral-aboral corresponding to dorsal-ventral. Instead, these expression patterns imply that the two major bilaterian axes arose from a duplication event of a single axis in the last common ancestor.

In contrast to the cases of *emx-Am* and *cnox2-Am*, where aspects of function may be common to higher animals, it is difficult to relate the expression data for some *Acropora* genes to the roles of their counterparts in bilaterians. Whereas the *paired*-like homeobox gene *homeobrain* functions specifically in *Drosophila* brain patterning (Walldorf et al., 2000), *hbn-Am* appears to lack a neural-specific function in the coral, but is an early ectodermal marker. In this case, it is also unclear how the coral data relate to other cnidarians, as the *Hydra* ortholog of *hbn-Am*, *HyAlx,* is expressed in developing tentacles (Smith et al., 2000).

Whereas no vertebrate ortholog of *hbn-Am* has yet been identified, the *dmbx*-related genes reflect the (more common) scenario of apparent secondary gene loss in the fly. In this case, coral, *Hydra* and vertebrate orthologs are known, but no clear counterpart is present in *Caenorhabditis* or *Drosophila*. While the role of the *dmbx* genes in *Acropora* is unknown, function is unlikely to be conserved between *Hydra* and the mouse. In the mouse, *Dmbx1* is involved in early brain patterning, but the expression of *Hydra manacle* is restricted to the basal disk ectoderm.

In general, *Acropora* genes tend to show much higher levels of identity to their vertebrate rather than invertebrate counterparts, and this phenomenon is not restricted to specific gene classes (Kortschak et al., submitted). Similar patterns of relatedness have been found with

transcription factors such as Emx (Hislop et al., in prep) and Snail (Ball, unpublished, as well as cellular signaling molecules like Dpp and Smad (Samuel et al., 2001) and housekeeping components including methyl CpG-binding protein (Barnes, 2002). Likewise, in general much higher levels of identity are observed between *Acropora* and vertebrate sequences than between *Hydra* and vertebrate sequences (see, for example, Figure 3.4), reflecting the derived position of *Hydra*. These observations support the use of *Acropora*, rather than Hydra, as the model cnidarian for 'evo-devo' purposes.

6.3 DUPLICATED GENES IN THE CNIDARIA

Genome-wide duplications are thought to have been critical in enabling the early diversification of vertebrates (Holland et al., 1994; Holland, 1999). Conversely, tandem duplications are thought to be much less significant, and may actually have retarded diversification in some vertebrate lineages (Ohno, 1970). Some examples of apparently cnidarian-specific duplication of genes have been identified; the *Hydra nanos* genes (Mochizuki et al., 2000), *Podocoryne* tropomyosins (Groger et al., 1999) and the *Acropora Smad 1/5s* (Samuel et al., 2001).

Whilst the above examples suggest the possibility of widespread genome duplication within the Cnidaria, an unexpected number of tandemly-duplicated homeobox genes were discovered during the present study. This suggests tandem (rather than genome-wide) duplication events may have been common during cnidarian evolution, and may have contributed to the relatively large genomes of some cnidarians, such as *Hydra*. Phylum Cnidaria is morphologically diverse; the major classes are thought to have diverged in Pre-Cambrian time (Conway Morris, 1998; Conway Morris, 2000a; Conway Morris, 2000b). Therefore, it is possible that some genes that have been duplicated in *Acropora* may not be (for example) in *Hydra*. The *nanos* genes are an example where cnidarian-specific gene duplication is clearly ancient, as two orthologous genes are present in both *Hydra* (Mochizuki et al., 2000), and *Acropora* (Go et al., unpublished). *Hydra Cnnos2* appears to

have acquired a cnidarian-specific role in head morphogenesis, while *Cnnos1* is expressed specifically in germline cells, suggesting a common function with bilateral animals.

Evidence from the vertebrates suggests a link between genome-wide duplications and 'evolvability' (Kirschner and Gerhart, 1998), and begs the question, 'Is it possible that such a relationship also holds for the Cnidaria?' Although often thought of as an evolutionary failure, the Cnidaria are actually amongst the most successful of animal phyla. Is this merely a coincidence or a consequence of genome polyploidy? Given that the Cnidaria are thought to have split from the line leading to the bilateral Metazoa over 540 million years ago, it may not be surprising that extensive cnidarian-specific duplication has occurred. The question remains: are these cases indicative of an extensive independent genome duplication event in the Cnidaria, or are they the result of tandem duplications, as are common in *Caenorhabditis elegans* (Semple and Wolfe, 1999)? In the absence of a significant body of genomic data for any cnidarian, this question cannot be answered, but the *Acropora* data indicate that tandem duplication is certainly common. The jury is still out, but the case for the prosecution is strong.