Instituto Juan March
de Estudios e Investigaciones

73 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Co-sponsored by
EMBO EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

Workshop on

Development and Evolution

Organized by

G. Morata and W. J. Gehring

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INTRODUCTION

Ginés Morata and Walter J. Gehring

The study of evolution has been a principal goal of biologists ever since Darwin published "The Origin of Species". After more than a century, a large amount of data have been accumulated, mostly from the fossil record and comparative anatomy and embryology. It has provided a historical picture of life in the planet; how different animal and plant phyla have appeared, flourished and in many cases extinguished. For many years the prevailing theme in these studies was the richness and diversity of animals. The differences between classes of animals -- mosaic versus regulative eggs, radial versus spiral cleavages, protozoa versus multicellular animals, annelids, arthropod, mollusks, versus echinoderms, ascidians and vertebrates -- were emphasized as a source of speculations on how they had evolved.

The irruption of molecular biology brought a revolution into the field of evolution, firstly providing novel ways to classify the different animal groups (especially from sequence comparison of rRNAs see, for example, Adoutte and Philipe, 1993), and secondly from the discovery of the universality of the major developmental mechanisms. In fact, the search for common mechanisms has been a driving force of Molecular Biology ever since the discovery of the universality of DNA or of the genetic code.

Considering only the Animal Kingdom, there presently are over two millions species that show a overwhelming diversity of sizes, shapes and life cycles. It is hard to imagine that there may be some general unifying principles behind that seemingly intractable complexity. And yet we are finding that those principles exist. The formation of new species during evolution is in essence the generation of new forms and shapes of body parts, so it is intimately connected with modifications in the functioning of developmental processes that generate morphological variations. That is, the explanation of the divergence that occurred during evolution will require the understanding of developmental processes.

During the last decade we have witnessed very significant progress in the field of Developmental Biology, in the larger part due to the introduction of the new molecular hardware: There have been profound insights into problems such as the generation of the diversity along the anteroposterior or proximal distal body axes or the signalling mechanisms underlying pattern formation. It has to be said that the leading edge has been the fruitfly *Drosophila*, while other model organisms like the mouse or the nematode *C. elegans* followed track. Typically, the genes involved with a particular process have been identified in the fly and subsequently the homologue genes have been found in other organisms, usually the mouse. The next step concerns

the mechanism, which once has been worked out in the fly, it has been shown to operate also in the other species.

A paradigmatic case is the *Hox* complex. The *Hox* genes had been studied (under another name) in *Drosophila* for more that 60 years and genetic studies (see Lewis, 1978) have determined that: 1) they generate much of the body's morphological diversity, 2) they probably function by regulating the activity of other, subsidiary, genes, 3) they are clustered in the chromosome, 4) they function following a collinear rule, and 5) they probably derive from a common ancestor.

The advent of molecular biology, and in particular the discovery by the groups of Walter Gehring and Matt Scott of the homeobox, which encodes a DNA binding polypeptide, provided a molecular marker for *Hox* genes and suggested a molecular mechanism of *Hox* function based on transcriptional regulation. But above all, the homeobox provided a key to enter into the genome of other organisms, worms, mice, humans, in which the *Hox* genes would have been very difficult or impossible to detect by conventional genetic analysis. It did not take long to demonstrate that all the properties described in *Drosophila* for the *Hox* complex applied to the mouse and generally to other organisms. The implication is that the generation of the morphological diversity along the A/P body axis follows a rule common to all animals and was invented only once in evolution. The conservation of structure and function of the *Hox* complex in species belonging to such distant animal phyla like Arthropods and Chordates implied the *Hox* complex had appeared before these groups diversified in the lower Cambrium, around 540 million years ago. This is the time when the famous Cambric explosion occurred, in which all extant animal groups suddenly appeared. It is hard to resists the temptation to speculate that the sudden emergence of so much morphological diversity is connected with the developmental invention of the Hox complex. The conservation affects not only to *Hox* complex itself, but also to cofactors like the *extradenticle* gene, that performs a similar function for the *Hox* complexes of *Drosophila* and the mouse.

It is worth mentioning that there are some body regions that do not have *Hox* gene activity but are instead specified by other genes. The development of anterior head requires *empty spiracles (ems), orthodenticle (otd)* and *buttonhead(btd),* two of which (*ems* and *otd*) are homeobox genes known to be conserved in vertebrates where they are expressed in the brain. At the other end of the body, the homeobox gene *caudal* specifies terminal structures and, correspondingly, the vertebrate homologues are also expressed in the posterior body regions where they presumably play a similar instructive role.

A high degree of conservation is also observed in other genetic processes not connected with the anterior posterior polarity of the body. The best known case is *Pax6* (*eyeless*), a paired class gene found to be responsible for eye development in arthropods, mollusks and vertebrates. Work mainly from Walter Gehring's laboratory indicates eyes appeared only once in evolution and involved a genetic operation of which the gene *eyeless* (or its repeat *twin of eyeless*) is the triggering event. The same operation is still being used after 540 million years in the whole of the Animal Kingdom.

Finally, new insights have been made in the mechanism of pattern formation, until recently one of the least known of developmental phenomena. The gene *hedgehog* plays a central role in the cell patterning of *Drosophila* by itself and by inducing the activity of the other signalling genes decapentaplegic and wingless. The vertebrate homologue *Sonic hedgehog* is implicated in a positional signalling mechanism with many similarities to that found in *Drosophila*.

As mentioned above molecular biologists have accustomed us to think in terms of common principles, but not even the molecular biologists have prepared us for the universality of developmental mechanisms we are finding. This atmosphere of surprise still permeates every paper on this subject.

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OPENING REMARKS

Peter A. Lawrence

Evolution and Development, some Reminiscences

The relationship between development and evolution (mostly what development can tell us about evolution rather than the other way around) is not a new subject of interest. I remember when I was an undergraduate in Cambridge (1959), being taught about Amphioxus and what it might reveal about the segmentation of the vertebrate head. In those days the mainstay of zoology was comparative anatomy and comparative embryology. And the new subject of molecular biology was not part of my undergraduate course. So I went to lectures by Carl Pantin on the phyla and phylogeny of the animal kingdom. Unofficially I could go out of the zoology department to a hidden cell and hear Sydney Brenner's lectures on bacterial genetics, including the work of Jacob and Monod on the *lac* operon.

The prevailing theme of Pantin's lectures was the richness and diversity of animals and speculations on how they had evolved. The differences between classes of animals were the driving force behind the lectures: mosaic versus regulative eggs; radial versus spiral cleavage; annelids, arthropods and molluscs versus echinoderms, ascidians and vertebrates. These matters gave the lectures intellectual bite, and we students were asked to write essays on the many groups of animals that did not fit easily into the schemes, such as rotifers, pogonophora, sipunculids, coelenterates, etc. I mention these things to make two points:

First, the almost fascist domination of molecular biology as the subject of our time has since extinguished the general knowledge of diversity of animals. This applies to most taught biology since the mid 60s, meaning nearly everyone who does research nowadays.

Second, partly because of this influence, we have become accustomed to the idea that we should look for similarity and common mechanisms rather than investigate the differences between animals.

But not even the molecular biologists have prepared us for the universality of developmental mechanisms. I expect that many talks at this meeting will give examples as to how amazingly similar mechanisms of development are. This atmosphere of surprise still permeates every paper on this subject. It is worth thinking a moment as to what are the sources of this surprise. One source I think is simply the bewildering diversity of animal form. It is a leftover of the old zoology, that represented by my lectures at Cambridge, by the classification, the emphasis on the many separate groups of animals and their differences. The other source is much more primitive, I believe it comes from the same prejudice that fired the rejection of Darwin's theories, how could we humans be related to monkeys? It was just unacceptable. It is even more unacceptable to think that we sophisticated beings could have so much in common with slugs, worms and things that creep in the night.

Now I would like to make some outsider's comments on the modern subject of development and evolution. Many papers in the field are really modern dress versions of comparative anatomy and embryology, but others have yielded profound insights. One is the finding that the rhombomeres of vertebrates are similarly designed to the parasegments of insects.

Partly because the field is so trendy, I think it is being overindulged and rather soft conclusions about evolution are given more weight than they deserve. These problems are partly the outcome of the methods we have, that are powerful and in some respects too easy. Thanks to transgenic flies and mice, and the yeast GAL4 system, it is feasible to express in one animal genes from another or to express endogenous genes ectopically. I believe these experiments should be interpreted with more caution than they often are. Many of the experiments consist of driving gene expression at a high level, and often concern transcription factors. Usually little or no attempt is made to find out how much the level of the alien or ectopic protein being driven compares with the level of the homologous protein *in vivo*. This is important as transcription factors seem to work as complexes bound combinatorially, in diverse sets, to the DNA. This could mean that large amounts of an alien protein can do odd things.

For example it has been shown that DNA contains a mass of binding sites varying widely in their affinities for ligands. A protein introduced from mice might bind poorly to sites in *Drosophila*. Yet if it was present in manyfold excess, it could produce outcomes that looked specific, but might not be. I mean that the specificity could be written more by the other proteins that are there than by the protein that is added. So one and the same outcome might be produced by a number of different alien genes. Ginés Morata will present some examples of this. As he and Gabrielle Rieckhof will also discuss there is the possibility that genes that affect the import of transcription factors to the nucleus, such as extradenticle, can change the outcome of ectopic expression experiments quite dramatically.

There is another possible problem that might affect particularly homeobox genes. Homeobox genes are included amongst the selector genes in the terminology of García-Bellido, they have to remain switched on in parts of the embryo to determine what we have called the genetic address, meaning the combination of selector genes that tells the cells which part of the body they are to construct. If the genetic address is changed then the body part is also changed. These genes must remain active and inactive in the appropriate places. Such genes are often subject to autoregulation, a mechanism to keep them switched on during development. So if a similar gene is introduced ectopically into a fly, one has to ask whether the morphological effects, extra eyes or wings for example, are produced directly by the introduced protein or because it turns on the endogenous fly gene.

But apart from these more detailed criticisms I would like to encourage a new direction. I think the field should go full circle. As I mentioned, in the 40s and 50s the main interest of zoology was comparative anatomy and comparative embryology. In the present day the concern of both evolutionary and developmental biologists has been to show that these differences are not as profound as my ex-professor Carl Pantin thought. That most, maybe all metazoa have a common origin and that the genome and many fundamental mechanisms of organisation have been conserved. Now I think we should get over that surprise. We should start to enjoy and investigate the differences between animals again. After all it is the differences between animals that are the main product of evolution, and consequently of most interest to evolutionists. The commonality of mechanism should be more the foodstuff of developmental biologists.

Peter A. Lawrence

Session 1: Evolution at the molecular level

Chairperson: Thomas C. Kaufman

An updated phylogeny of the Metazoa: implications on the evolution of development.

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In spite of the fact that each of the 30-35 metazoan phyla displays diagnostic features allowing unambiguous assignment of species to a given phylum, construction of phylogenetic schemes linking these phyla into a robust evolutionary tree on the basis of anatomical characters has proven difficult. This is due to the absence of a nested series of homologous characters among these phyla.

The advent of molecular phylogeny raised the hope that these difficulties might be overcome. The outcome, however, is more complex that expected. First, a number of ribosomal RNA based molecular phylogenies yielded clearly aberrant results such as the polyphyly of molluscs. Second, several major nodes of the phylogeny resisted resolution, up to a recent period, *i.e.* took the form of an unresolved "bush". The presentation will first recapitulate the sources of difficulties in phylogenetic reconstruction, subdividing them into two very distinct types, those caused by artefacts (uneven rates of substitutions generating "long branches attraction", mutational saturation, sampling bias,...) and those caused by the real historical situation, namely the occurrence of rapid evolutionary radiations. It will be shown, in particular, that failure to resolve some nodes in a tree may in fact be taken as a positive result indicative of a evolutionary radiation, once artefacts are excluded. Such an approach has proven quite interesting in discussing the Cambrian radiation (ref. 1).

Recent analyses, specially that of Aguinaldo *et al.* (ref. 2), have taken great care to avoid artefacts and have obtained strikingly and potentially far reaching conclusions. They show the Bilateria to be subdivided into the two traditional clades of protostomes and deuterostomes but, within the protostomes, several major new findings emerge: the protostomes are divided into two very large groups, the *Ecdysozoa*, comprising arthropods but also nematodes and the *Lophotrochozoa*, comprising all the animals displaying either a trocophore larvae (annelids, molluscs, but also nemertines, and platyhelminthes) or a lophophore (brachiopods,...). Thus, several phyla that were previously thought to hold a much more basal position in the metazoan tree under the assumption of "primitiveness" of their coelomic organization are raised much higher in the tree, well within coelomates. Such is the case of Platyhelminthes which, as "acoelomates" were thought to represent the earliest emerging bilaterian phylum and, most importantly, of Nematodes which were considered as "primitive" and grouped with several other more minor phyla in the ill-defined "pseudo-coelomates". In both cases, we may therefore be dealing with secondarily simplified organisms. This is obviously of great importance in interpreting both the genomic and developmental features of these organisms.

Because of the uncertainties of phylogenetic reconstruction based on nucleotide sequences, it is quite satisfactory that some of the unexpected results just described are confirmed by a very different approach based on a qualitative analysis of hox gene duplications and signature amino acids. Using such an approach, Balavoine (ref. 3) has recently obtained quite suggestive evidence for placement of the Platyhelminthes within the Lophotrochozoa. In short, topological congruence between completely inbdependent sets of characters presently appears to provide the most decisive argument in phylogenetic reconstruction.

Should these recent results prove to be true, they have deep implications for the

reconstruction of the last common ancestor of bilateria. It already appears, that this ancestor was much more elaborate in terms of its morphology developmental processes and corresponding gene arrays than was commonly assumed under a more "gradist" view.

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3. Balavoine, G. *C. R. Acad. Sci.* **320**, 83-94 (1997).

Evolution of Paired Domains

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Pax proteins are a family of transcription factors with a highly-conserved paired domain; many members also contain a paired-type homeodomain and/or an octapeptide. Nine mammalian *Pax* genes are known and classified into four subgroups: *Pax-1/9*, *Pax-2/5/8*, *Pax-3/7* and *Pax-4/6*. Most of these genes are involved in nervous system development. In particular, *Pax-6* is a key regulator that controls eye development in vertebrates and *Drosophila*. Although the *Pax-4/6* subgroup seems to be more closely related to *Pax-2/5/8* than to *Pax-3/7* or *Pax-1/9*, its evolutionary origin is unknown. We therefore searched for a *Pax-6* homolog and related genes in *Cnidaria*, which is the lowest phylum of animals that possess a nervous system and eyes. A sea nettle (a jellyfish) genomic library was constructed and two pax genes (*Pax-A* and *-B*) were isolated and partially sequenced. Surprisingly, unlike most known *Pax* genes, the paired box in these two genes contains no intron. In addition, the complete cDNA sequences of brown hydra *Pax-A* and *-B* were obtained. Hydra Pax-B contains both the homeodomain and the octapeptide, whereas hydra Pax-A contains neither. DNA binding assays showed that sea nettle Pax-A and - B and hydra Pax-A paired domains bound to a Pax-5/6 site and a Pax-5 site, though hydra Pax-B paired domain bound neither. An alignment of all available paired domain sequences revealed two highly conserved regions, which

cover the DNA binding contact positions. Phylogenetic analysis showed that Pax-A, and especially Pax-B, were more closely related to Pax-2/5/8 and Pax-4/6 than to Pax-1/9 or Pax-3/7 and that the *Pax* genes can be classified into two supergroups: *Pax-A/Pax-B/Pax-2/5/8/4/6* and *Pax-1/9/3/7*. From this analysis and the gene structure we propose that modern *Pax-4/6* and *Pax-2/5/8* genes evolved from an ancestral gene similar to Cnidarian *Pax-B*, having both the homeodomain and the octapeptide.

In addition, we inferred the paried domain sequences at several important ancestral nodes of the PAX protein tree and used a panel of paired domain binding sequences to study their sequence binding specificities. We have identified several key amino acid residues for the evolutionary changes of binding specificity of the paired domain.

Sectorial gene repression in development

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Not only are most structural genes, or, rather, most functional subunits that compose them, extremely old, but so are (1,2) many gene interaction patterns, as has been beautifully demonstrated for some such patterns over these last few years, with Walter Gehring in the lead (3). Among the very ancient inventions in the realm of the mechanisms of gene interaction is the simultaneous repression of members of a group of homologous genes. Such sectorial repression or "superrepression" plays an important role in development. Two main types of sectorial repression may be distinguished. One is the developmental type, in which case different parts of the gene complex become activable over portions of the complex that extend to different points from its 5' toward its 3' limits; the susceptibility of the individual genes to be transcriptionally activated in various regions of organismal time and space depends on the order of the genes along the chromosome (e.g., *Hox* genes). There is, secondly, the terminal differentiation type of sectorial repression, in which duplicate genes are either collectively superrepressed or none of them is. (A dependency of activability of repressed individual genes on gene order can, in this case, be introduced at a second, subordinate level of control, as in the human β-globin complex.) The shift from the presence of to the release from superrepression is operationally defined as a shift from a relative insensitivity of DNA to certain endonucleases such as DNAse I to a so-called intermediate sensitivity to these endonucleases.

The higher order structures established in superrepression can be thought of as formed through the cooperative action (4) of various protein factors that form complexes among themselves and with DNA. Four interdependent parameters may indeed play special roles in the formation and the stability of these complexes: cooperativity, competition, mass action (5, 6), and hysteresis effects. At least in the case of developmental superrepression, it is likely that a competition occurs among superrepressible desoxyribonucleoprotein domains for "locking molecules"(5), of which the *Drosophila* polycomb protein is one example (7). The role played by the parameters cited can, in principle, (a) explain why the superrepressed part of the developmental gene complex, in a given sector of the organism, does not in all cells possess the same extension, a phenomenon that is also reflected in position effect variegation; and (b) suggest that sectorial repression of genes may offer opportunities of developmental switches between stable states (+ and -) of activability of genes. Hysteresis implies that, once the superrepressed complex has been formed through cooperativity, it will be buffered against fluctuations in the concentrations of locking molecules and will not easily come apart when a given species of locking molecule hits a trough. Again, counterparts to these phenomena are expected to occur in position effect variegation,

which now seems to share fundamental traits with mechanisms of normal development, contrary to what has previously been thought.

As a further contribution to development, and through both the competition and hysteresis effects, sectorial repression would appear to offer a mechanism for stable genic switches. In addition, the distribution over genomes of superrepressed gene sectors may provide a sound molecular basis for the distinction among cell types.

Also, the analysis of the cellular distribution of superrepressed sectors released from superrepression should permit one to study the evolution of cell types and to draw phylogenetic trees of cell types.

All body parts use mostly the same genes. If this generalization is true, it implies that differences among body parts are mostly attributable to differences in gene regulation. There must therefore be mechanisms whereby gene regulation becomes different in different parts of the organism. Evolutionary evidence suggests that new organs, such as wings in arthropods, tend to appear in most segments (or parasegments) of the organism. This observation is, then, to be interpreted as follows: in arthropods (with equivalent situations probable for other organisms), wings or other morphological formations are mostly the result of certain changes in a regulatory pattern that must be thought, basically, to apply to the organism as a whole. In order for arthropod wings not to spread over most segments, a regulatory mechanism must be set up whereby wing formation is inhibited only in those segments to which wings are not to spread. Such a regulatory mechanism has apparently been provided by the evolution of developmental superrepression. If different segments use essentially the same genes, with some exceptions, any morphological acquisitions that are not to appear repetitively will require local regulatory *compensations* for the regulatory changes that have occurred. This is probably why *hox* genes and related genes have so many things to do: where appropriate, they have to be able to neutralize any morphological innovation -- probably their most general effect. The answers to the challenge are developmental genes that are activable only in certain parts of the body because it is only in these parts that the genes fall outside of a superrepressed chromosomal sector. The obligatory repression of most morphological traits in most, but not all parts of the organism could be referred to as evolutionary morpholysis, complementary to morphogenesis. Morphogenesis tends to involve the whole organism, morpholysis, parts of it. The stability over eons of the compensatory regulatory processes of morpholysis is probably in part attributable to the properties of sectorial repression.

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Session 2: Body plans and body parts (1)

Chairperson: Ginés Morata

Evolution of segmentation genes

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The interplay between maternal and zygotic segmentation genes that form the segment pattern of the embryo is very well understood in *Drosophila*. There is a hierarchy of transcription factors acting upon each other via diffusion controlled protein gradients. This mode of pattern formation seems to be particularly adapted to the syncytial mode of embryogenesis in *Drosophila*. However, previous results have shown that at least the pairrule genes *hairy* and *even skipped* are also utilized in *Tribolium* embryos which show a cellular mode of embryogenesis at later stages.

We have further investigated in how far other genes of the segmentation gene hierarchy show conserved or divergent expression patterns in the short germ embryo of *Tribolium*. Results will be shown for the *Tribolium* homologues of *hunchback*, *caudal*, *Krüppel*, *empty spiracles*, *orthodenticle*, *sloppy paired*, *tailless* and *forkhead*. Most of the expression patterns of these genes appear to be highly conserved between *Drosophila* and *Tribolium*, indicating that the whole segmentation gene hierarchy may be conserved.

To test the degree of conservation of regulatory elements between the two species, we have made reporter gene constructs with upstream sequences from *Tribolium* gene sequences and transformed them into *Drosophila*. The analysis of the resulting expression pattern suggests that the regulatory interactions are highly conserved, allowing to study the regulation of *Tribolium* genes in *Drosophila*. Using this system, it is even possible to make particular inferences on the functional changes in regulatory sequences that have led to the long germ mode of embryogenesis found in *Drosophila*. In particular, it seems evident that a functional homologue of *bicoid* must exist in *Tribolium*, but that the regulation of the segmentation function of *hunchback* has evolved only in long germ embryos. The activator of *hunchback* in short germ embryos appears to be *caudal*.

We have also started to investigate the role of regulatory changes versus changes in amino acid sequences as potential sources of evolutionary novelties. We find that a large fraction of genes exists in the *Drosophila* genome that evolves with very high substitution rates. Regulatory changes, on the other hand, seem to occur relatively infrequently in these genes and even less frequently in the known segmentation genes. These results are not in line with the notion that evolutionary changes are primarily driven by the evolution of regulatory interactions.

Baculovirus Mediated Gene Misexpression:
A Potential Method for Analyzing the Evolution of Pattern Formation

Daniel Oppenheimer, William Browne, and Nipam H. Patel
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Genetic analysis of development in several model organisms has led to the identification of many genes that, when mutated, are capable of creating marked changes in developmental pattern and morphology. Subsequent comparative analysis of gene expression in numerous organisms has raised the possibility that changes in expression of some of these genes may account for evolutionary changes in pattern formation(1). For example, extensive work on the homeotic genes of Drosophila clearly indicate their importance in establishing the body plan of this insect. Recent work in crustaceans suggests that a number of striking changes in the regulation of some of these homeotic genes may account for evolutionary changes in morphology that are reminiscent of homeotic transformations (2). While great progress has been made from these types of comparative analyses, it has been difficult to test the hypotheses derived from these studies, in part because of the difficulty in manipulating gene expression outside of a relatively small number of model systems.

We have been attempting to develop a gene misexpression system that will be applicable to a wide variety of organisms. One system under development, the use of baculovirus to mediate gene misexpression, appears to be a promising approach. The particular baculovirus we are using, AcMNPV, can complete its life cycle in only a very limited range of moth hosts. We have shown, however, that it is capable of infecting embryonic cells from a wide range of organisms. In our initial studies, we used a lacZ reporter to monitor introduced gene expression and various aspects of virus infection. We have found that viral infection and subsequent lacZ expression can occur quite early in development and, to a some degree, we can control the extent and location of infection. To test the actual usefulness of this system in perturbing development and analyzing gene function, we engineered a baculovirus carrying the Drosophila wingless gene. In wingless mutants of Drosophila, ectodermal expression of engrailed disappears after germband extension since wingless is normally required to maintain engrailed expression. Injection of the wingless containing virus into Drosophila embryos homozygous for a transcript null allele of wingless is capable of rescuing engrailed expression when infected cells are close to or within the engrailed competent domain of the ectoderm. Injection of the wingless carrying virus into wild-type Drosophila embryos is capable of generating naked cuticle in regions that should contain ventral denticle bands. Finally, injection of this wingless containing virus into the beetle, Tribolium castaneum, resulted in the posterior expansion of engrailed stripes during early development. This result in Tribolium suggests that this beetle utilizes wingless to maintain engrailed expression as does Drosophila, and also, as in Drosophila, the Tribolium ectoderm appears to contain an engrailed competent domain presumably set-up by the action of pair-rule prepatterning.

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The Diversity of Development within Insects

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Drosophila development is now understood in great detail. Early patterning involves the diffusion of transcription factors in the syncytial cytoplasm of the blastoderm stage embryo. By the time of cellularisation, the entire body plan is already defined by the expression of segmentation and homeotic genes.

Syncytial development is not universal within the insects. Many lower insects make much or all of their segment pattern after cellularisation – a point made particularly clear by our recent studies in the Locust, which show that the blastoderm becomes cellular even before the aggregation of cells to form the embryonic primordium [2]. Yet more remarkable is the diversity of development among the parasitic *Hymenoptera* [see Grbic Abstract in this volume]. Species within the same family may exhibit extreme differences in early embryogenesis: The *Braconidae* contain ectoparasitic species laying yolky eggs which show syncytial development similar to that of *Drosophila*, and endoparasitic species laying small, yolk-free eggs which undergo total cleavage and "short germ" patterning .

It remains unclear how the patterning mechanisms that act in the *Drosophila* embryo relate to those that act in a cellular environment. However, the rapid transitions observed among the parasitic *Hymenoptera* suggest that the ability to generate pattern after cellularisation has been retained in some form even by those orders that typically make pattern in the syncytial blastoderm.

It is now clear that at least two of the atypical *Drosophila Hox* genes - *zen* and *ftz* - derive from ancient members of the *Hox* cluster that have evolved particularly rapidly within the insect lineage [3,4]. We suggest that the developmental role of these genes changed in the ancestors of the insects, with a loss of many of the functional constraints that act on the 'canonical' *Hox* genes.

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THE DIVERGENT HOMEODOMAIN OF BICOID INTERACT DIFFERENTIALLY WITH DNA AND RNA

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Homeodomains (HDs) are known as DNA binding domain with a key role in transcriptional control[1]. The homeodomain-containing protein BICOID (BCD) is the anterior determinant in *Drosophila*. *bcd* evolved very rapidly and it has been found only in dipterans. In addition to its transcriptional regulation properties, BCD was recently showed to control gene expression at the translational level[2, 3]. The target of such a function is the maternal *caudal (cad)* mRNA. *cad* mRNA is evenly distributed in the embryo and a protein gradient of CAD is formed by gradual suppression of translation by BCD[4]. In contrast to BCD, CAD is broadly conserved among Phyla forming protein gradients.

Biochemical evidence showed that the BCD-HD interacts with a specific RNA sequences whithin the 3'UTR of *cad* mRNA. These results provide a novel property for HDs, in addition to the DNA binding and, a dual role for BCD controlling both transcription and translation in early embryogenesis. We analyzed the DNA and RNA binding properties of BCD. Basic aminoacid residues in the helix-3 are important for DNA recognition as well for RNA recognition. Some of them are only involved in RNA but not DNA binding. That makes the BCD-HD different than other HDs.

Are those properties unique for the BCD-HD? Can we predict other HDs binding RNA? The R-rich motif of the BCD-HD involved in translational control is unique among the know HDs. However, the structural analysis of specific RNA-binding motifs (ribosomal protein L11[5] and viral transactivators[6]) showed striking similarities with HDs. In addition, some new functions different than transcription have been described for HD-containing proteins. We will discuss the evolution of the BCD-HD at the light of the new data and the conservation of the CAD gradient in response to factors different than BCD.

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Lateral signalling in development: on equivalence groups and assymetric developmental potential.

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Notch-mediated lateral signalling takes place between equivalent cells that then adopt alternative fates. An example is the epidermal-neural choice in the *Drosophila* neuro-ectoderm. All cells initially produce both the ligand and the receptor, but, with time, one cell(s) comes to dominate(s) and signals to the other(s). Resolution depends upon a feedback loop within each cell, linking production of the ligand with activation of the receptor. The choice of signalling cell is in some cases, such as the thoracic microchaetes, random. A random choice of cell fate could arise from stochastic fluctuations in the turnover of different components of the signalling pathway, causing small differences that can be amplified by the feedback loop In other cases, such as that of the thoracic macrochaetes, the outcome is biased and the same cell is generally chosen to become the dominant signalling cell. In the case of the dorso-central macrochaetes, two signals have been shown to bias the choice of cell fate by increasing the levels of one component of the feedback loop. Finally, during embryonic neurogenesis in *Drosophila*, a highly derived process, choice of the neuroblasts is almost completely predetermined. Notch-mediated signalling is still required but 80% of the precursors segregate normally in the absence of the feedback loop. It is likely that the Notch pathway is extremely old. A random choice of fate generated through lateral signalling is probably a ancient process in terms of evolution.

Session 3: Body plans and body parts (2)

Chairperson: Michael Akam

Homeotic Genes and the Evolution of Arthropod Body Plans and Body Parts

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One of the first explicit hypotheses concerning the relationship between the evolution of specific genes and the morphological evolution of animals was put forth by Ed Lewis in 1978. His suggestion that homeotic (or *Hox*) gene diversity expanded in the course of arthropod and insect evolution has inspired comparative studies of *Hox* gene evolution and regulation in many taxa. In order to trace the evolution of the arthropod *Hox* genes further back through arthropod evolution, we cloned the *Hox* genes from a myriapod and an onychophoran. Since the onychophora are a sister group to the arthropods, the complement of *Hox* genes shared between onychophora and arthropods reflects the condition prior to the origin and radiation of the arthropods. Despite their limited segmental diversity, all insect *Hox* genes are found in both taxa, including the trunk *Hox* genes *Ultrabithorax* and *abdominal-A* (*abd-A*) as well as orthologs of the *fushi tarazu* gene. These results and comparative analysis of *Hox* gene expression demonstrate that a complete arthropod *Hox* gene family existed in the ancestor of the onychophoran/arthropod clade and that arthropod diversity arose subsequently through changes in the regulation of *Hox* genes and their targets.

One specific model of adaptive modification of body plans is the evolution of the haltere in two-winged Dipteran insects from a four-winged ancestor under the control of the *Ubx* gene. Although the four-winged phenotype of *Ubx* mutants have been known for decades, it is not known how *Ubx* modifies a developing wing field to a haltere field. By exploiting recent advances in understanding wing patterning, we examined the expression and regulation of several candidate genes involved in patterning features that differ between the wing and haltere. We have identified five *Ubx*-regulated genes that suggest how haltere size, shape, venation, and bristle pattern differ from the wing. Interestingly, some of these genes are not differentially regulated between the forewing and hindwing of butterflies, suggestions that they fell under *Ubx* control in the course of Dipteran evolution.

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Genetic subdivisions of the arthropod leg

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Based on fossil evidence and in comparative anatomy, Snodgrass (1935) suggested that the arthropod leg is subdivided into two major parts: the proximal part or 'coxopodite' that would be an expansion of the body wall, and the distal region or 'telopodite', that would be the genuine appendage. Recently we have presented evidence that in *Drosophila* and in the crustacean *Artemia* these two regions correspond to the functional domains of the homeobox genes *extradenticle (exd)* and *Distal-less (Dll)* (Gonzalez-Crespo and Morata, 1996). Moreover, we observed that development of the *Dll* domain requires normal activity of the *hh* pathway whereas the *exd* domain does not.

We will present recent results obtained in our laboratory (and in collaboration with Richard Mann's group in Columbia) about the functional interactions between *exd*, *Dll* and the *hh* pathway.

We find that in early embryonic development the Dll and Exd proteins are coincidental in the nuclei of the cells in the leg primordia, but by late embryogenesis Exd becomes cytoplasmic, and therefore ineffective, in those cells. *Dll* acts as a negative regulator of Exd nuclear transport, because in *Dll*$^-$ mutants Exd remains nuclear in the leg primordia. The regulation of Exd subcellular localisation by *Dll* persists throughout imaginal disc development because the elimination of *Dll* function during late third instar results in nuclear accumulation of Exd in the leg imaginal cells.

We have also studied the interactions between *exd* and the *hh* pathway. The *hh* signal induces the *dpp* and *wg* transduction pathways respectively in the dorsal and ventral regions of the leg (Basler and Struhl, 1994). We have examined the effect of loss or gain of function of *exd* on the activity of *dpp* response genes *optomotor-blind (omb)* and *dachsund (dac)* (Nellen et al 1996; Lecuit and Cohen, 1997). The expression of these two genes is normally restricted to regions within the *Dll* domain. We find that *exd*$^-$ cells in the *exd* domain are able show *omb* and *dac* expression, although not all the cells respond equally. Moreover, ectopic expression of *exd* in the *Dll* domain represses *omb* and *dac* activity.

The previous observations indicate that *exd* prevents the establishment of the *hh* pathway and prompted us to look at the effect of forcing the pathway in the *exd* domain. We induced clones in the *exd* domain in which the *dpp* pathway is constitutively activated by expressing a ligand independent form of the Dpp receptor (Nellen et al. 1996). These clones produce leg outgrowths and in some cases differentiate pattern elements characteristic of the *Dll* domain. They contain *Dll* activity and the Exd product accumulates in the cytoplasm. We also examined whether the ventral component of the hh transduction pathway, *wg*, also represses *exd* activity. The induction of *wg*$^+$ clones in the *exd* domain also results in ectopic *Dll* expression and consequently in elimination of Exd from the cells nuclei. These results indicate that the *hh* pathway represses *exd* activity, probably throughout activation of *Dll*. In normal development, once it is established, the maintenance of *Dll* activity by the *hh* pathway ensures that the Exd protein remains in the cytoplasm.

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EVOLUTIONARY PARALLELS BETWEEN DROSOPHILA AND VERTEBRATE LIMBS

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It has become clear that during embryogenesis the same genes are used time and again in different cells and tissues. Thus, one of the problems of understanding development is the identification of common genes, or groups of genes (syntagma), that are used to fulfill specific developmental functions. An effective approach to this has been to examine, in evolutionarily distant organisms, functionally similar structures whose formation might present similar developmental problems. One of the most fruitful models is the study of the development of limbs in Drosophila and vertebrates. Whilst it is evident that at the morphological level, both during embryogenesis and in the adult stage, the limbs of Drosophila and vertebrates are quite distinct, the similarities at the molecular level, during early embryogenesis, suggests that they are evolutionarily related. This begs the question whether the limbs of these two organisms are true homologous structures. One possibility is that the last common ancestor of vertebrates and Drosophila possessed some form of primitive appendage which has subsequently evolved to form the present day structures. An alternative possibility is that the limbs in Drosophila and vertebrate arose independently as novel outgrowths from the main body. If the first possibility is correct, which goes against current thinking, then the molecular similarities between the limbs are to be expected and the limbs should be considered homologous structures. The molecular and morphological differences would have arisen as the result of natural selection in the different phyla. On the other hand, if they cannot be classed as homologous, then we need to explain why so many of the same genes are employed in both structures.

We and others have demonstrated remarkable similarities in the identities and functions of the genes which pattern the limbs of both Drosophila and vertebrates. In particular, the genes that establish the anteroposterior (Hedgehog, Patched and BMP-DPP) and proximodistal axes (Fringe, Serrate, Notch, Distaless). We have now isolated vertebrate homologues (Apterous, Lmx and Wnts) of some of the genes that control dorsoventral patterning in Drosophila wing development. While we are far from knowing all the genes involved in the control of limb outgrowth and patterning, we will attempt to discuss how the interactions between the known genes combine to regulate limb development in different organisms. The existence of conserved functional multi gene units or syntagmata between the Drosophila and vertebrate limbs has led us to speculate upon the existence of a very ancient genetic program that is sufficient to induce directional growth and that this program was then put to use many times during evolution, giving rise to the wide variety of appendages observed today during the embryogenesis of different organisms. As to the question of whether Drosophila and vertebrate limbs should be considered homologous structures by using these functionally conserved multi gene units as molecular markers, we would conclude that at some fundamental level the Drosophila and vertebrate limbs are, after all, ancient homologues.

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The master control genes for morphogenesis and evolution of the eye

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The homeobox identifies a number of **master control genes** specifying the body plan along the antero-posterior axis. We have isolated the *Pax-6* homologue of *Drosophila* which contains both a paired box and a homeobox, and corresponds to the *eyeless* gene. The first *eyeless (ey)* mutation was isolated in 1915 by Hoge, but only in 1994 we found out that *ey* is homologous to *Small eye* in the mouse and *Aniridia* in humans. This came as a surprise since previously it had been assumed that the compound eyes of insects had evolved independently of the single lens eyes of vertebrates, and that the eyes of vertebrates and cephalopods were the product of independent convergent evolution. We now have identified *Pax-6* homologs in flatworms, ribbon worms, insects, cephalopods, sea urchins, ascideans and vertebrates. These genes show a very high degree of sequence conservation and even the intron splice sites are highly conserved, indicating that they are true homologs.

In order to find out whether *ey* is a master control gene, we have constructed a gain-of-funtion mutant and used the Gal-4 system to ectopically express *ey* in the wing, leg and antennal imaginal discs Ectopic eyes were induced by switching on *ey* alone. This identifies *ey* as a **master control gene** for eye morphogenesis. The ectopic eyes are morphologically normal and contain functional photoreceptors. Subsequently, we have also tested the mouse *Small eye* gene and the *Pax-6* homolog from the squid in *Drosophila* and found that they both can induce ectopic *Drosophila* eyes. Therefore, we propose that *ey* is the "universal" master control gene for eye morphogenesis and evolution, and that the various types of eyes evolved from a single eye prototype.

Recently, we have discovered that in higher insects the *eyeless* gene has been duplicated, and that the *twin-of-eyeless (toy)* gene acts upstream of *ey* in the hierarchy. *toy* induces *ey* which in turn activates the *sine oculis*, the next target gene downstream in the cascade. We are progressively determining the hierarchy of the controlling genes in the eye developmental pathway leading to the various eye types.

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EXCLUSIVE AND COORDINATE EXPRESSION OF *RHODOPSIN* GENES

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The fly retina is composed of three different types of ommatidia, depending on the nature of the *rhodopsin* genes expressed in the R7 and R8 inner photoreceptors. These receptors integrate light information to detect polarized and/or color light. In the dorsal margin, both R7 and R8 express *rh3*. In the rest of the retina, two types of ommatidia with a stochastic distribution express either *rh3* in R7 and *rh5* in R8, or the *rh4-rh6* pair. However, there is an almost absolute coordination between the types of rh genes expressed in R7 and R8 (*rh3/5* or *rh4/6*). We are studying the mechanisms underlying the exclusive expression of *rh3* and *rh4* in R7 (or *rh5* and *rh6* in R8) as well as the signaling events between the R7 and R8 cells which allow the coordinate expression of the correct pair of *rh* genes in each ommatidium. The availability of highly specific promoters and of genetic tools offers a unique opportunity to understand this fascinating problem.

Our recent studies on the *rh* promoters have defined short regulatory elements required for the highly specific expression of the *rh3-5* genes. A conserved homeodomain binding site present in all *rh* genes, including those of vertebrates, mediates Pax-6 function and provides photoreceptor identity. The upstream elements of the different *rh* genes, which share no homology (even for *rh3* and *rh4* which are both expressed in R7 cells) establish the photoreceptor subtype expression. An analysis of mutated and chimeric promoters between different *rh* genes led us to suggest that exclusion of *rh3* and *rh4* involves a negative regulation of *rh4* and a positive activation of *rh3*. This has allowed us to generate an R7 specific promoter that is expressed in both *rh3*- and *rh4*-type R7 cells. We have identified a repeated 6bp site mediating R7-specific expression of *rh4*, as well as a broader region required for *rh4* repression in *rh3*-expressing R7 cells. In the *rh3* promoter, we have identified sites (multiple K50 homeodomain sites) required for its specific expression and the gene acting on these sites: *orthodenticle*, although expressed in all photoreceptors, is only activated and required for *rh3* expression. We are now screening for the proteins binding to the identified sequences and to the conserved sites whose function has not yet been elucidated.

An other approach to identify genes required in the pupal/adult eye is to generate differential cDNA libraries from isolated ommatidia. Using specific *in vivo* labeling of subtypes of ommatidia (*rh3/5* or *rh4/6*), we have generated libraries from ~50 isolated ommatidia containing only *rh3/5*, *rh4/6* or *rh6* cells.

We are also using a genetic approach to identify genes involved in the exclusive and coordinate expression of the inner photoreceptor *rh* genes. For this purpose, we have generated enhancer trap lines using a GFP reporter gene and looking at specific expression in subsets of R7 or R8 cells. We have also designed a genetic screen for genes which can modulate the expression pattern of GFP reporter genes specifically expressed in subsets of R7 or R8. Since most of these genes should have earlier function in other cell-cell communication processes, we are using an F1 screen with somatic clones generated with the FRT system and a flp gene specifically expressed in the eye (generously provided by Barry Dickson).

One possible model for the interaction described is that the expression of one *rhodopsin* gene is able to repress expression of another through a signal transduction pathway independent of light. Using *rh* mis-expression and analysis of the two types of R7 and R8 cells, we want to understand how and at which stages the coordination of *rhodopsin* expression in R7 and R8 cells is achieved.

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Session 4: Homeobox genes

Chairperson: Denis Duboule

The Homeotic Genes in Insect and Arthropod Evolution, Thomas C. Kaufman - HHMI Indiana University, Bloomington, Indiana, USA

Our laboratory has focused over the past several years on investigating the homeotic Antennapedia complex [ANT-C] in Drosophila melanogaster. This group of tightly linked genes was defined in this lab and shown to be responsible for the specification of segmental identity in the anterior thorax and head of the embryo and adult. In addition to investigating the function of the ANT-C genes in D. melanogaster (Diptera) we are comparing the expression patterns of proboscipedia (pb), labial(lab), Deformed (Dfd) and Sex combs reduced (Scr) among insects representative of the orders Siphonaptera (Ctenocephalides felis), Hemiptera (Oncopeltus fasciatus), Orthoptera (Acheta domestica), and Thysanura (Thermobia domestica). The homologs of ANT C genes from non-Drosophila insects have been cloned by performing PCR with degenerate primer pairs on embryonic cDNA pools (RT-PCR). These RT-PCR products have been cloned, sequenced and used as probes for in-situ hybridization to embryos. The homologs are highly conserved and are ?85% similar at the predicted amino acid level. As expected the expression of homeotic genes in some pattern elements were found to be highly conserved while other elements proved to be variable. In some cases the highly conserved patterns are correlated with functions that would seem critical to the development and formation of the body plan of all insects while the more variable elements correspond to functions involved in sculpting the basic insect body plan. In most cases it is the pattern of homeotic gene expression in the adult Drosophila primordia, rather than the larval, which most closely resembles the pattern of homeotic gene expression in the other insect orders. More recently we have expanded our analyses to include crustaceans (Armadillium vulgarae), millipedes (Oxidus gracilis) and spiders (Achaearanea tepidiorum) from which we have been able to identify a majority of the Hom-C genes. Data obtained on the spatial patterns of homeotic gene expression from these non-insect arthropods should make it possible to deduce the ancestral pattern of expression and infer the changes in this pattern which occurred during the evolution of the arthropoda.

PLANARIAN HOMEOBOXES: NEW INSIGHTS TO MORPHOGENETIC MECHANISMS IN REGENERATION

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Planarians are the stereotypical representatives of the simplest triploblastic organism in the tree of life, possessing three tissue layers, bilateral symmetry, cephalization (two cephalic ganglia and two ventral nerve cords) and complex organ systems such as: simple eyes, secretory organs, and sensory systems in the auricles. They are acoelomate and the digestive system is formed by a central pharnyx and a blind gut lacking an anus. These phenotypic traits place flatworms basally in a metazoan phylogeny. But, phylogenetic studies by molecular analyses of 18S ribosomal RNA (1,2), and more recently by the comparative analysis of planarian Hox genes, locate the Platyhelminthes as a sister group of Lophotrochozoa in the Protostomia.

The Hox genes encode a family of transcription factors that contain the homeodomain. They are organized in clusters and determine the antero-posterior polarity of a vast number of metazoa (3). Thus far, the roles and genomic organization of these remain unclear in the lower triploblasts (Platyhelminthes). Planarians (Tricladida) are well known for their high regenerative capacity, they can regenerate bidirectionally: anteriorly (head regeneration), posteriorly (tail regeneration), or bilaterally: left to right and right to left. This gives us the possibility to study the function of Hox cluster genes in spatial situations of pattern restitution that are impossible to produce in development or amphibian regeneration. To identify potential molecular markers involved in planarian regeneration and determine their number and genomic organization we performed an exhaustive search for related Hox genes in two Platyhelminthes: Triclads and Policlads.

Here we examine the sequences of ten Hox genes isolated from both Platyhelminthes, and the expression patterns of six Hox genes, (*Dthox-A* to *-G*) isolated from *Girardia tigrina*. (Tricladida). Sequence comparisons reveal high similarities to anterior and medial groups of coelomate Hox genes, with higher similarity to Protostomia. Spatial expression studies from intact and regenerating triclads show two types of patterns of expression: 1) a ubiquitous expression of *Dthox -A, -E, -G* and *-F* in intact animals, and very early, synchronous and colocalized expression in the regenerative tissue (blastema and postblastema) suggesting a non homeotic function for these genes (4); 2) A nested and colinear, according orthology, expression along the antero-posterior axis of *Dthox -D* and *-C* in intact animals, and differential activation or deactivation according to an antero-posterior pattern during regeneration. These results indicate that triclads have two types of Hox genes (perhaps two Hox clusters), that either conserve or loose the differential spatial expression which is linked to homeotic function in such model systems as *Drosophila*. The extension of this study to Policlad development will clarify the role of these genes in axial polarity determination and maintenance (work in progress).

In our search for non Hox homeobox genes we have isolated representatives of several homeobox families. Three of the homeobox families that show an interesting pattern of expression and have been extensively studied in our lab are the gap cephalic, POU and Pax-6 genes. We have isolated one Otx gene (*Dtotx)* (in collaboration with A. Stornaiuolo and E. Boncinelli from H. San Raffaele, Milan). *Dtotx* is located in intact adults in the anterior and medial planarian axis. Its expression during regeneration has

been quantified by RNAse protection of anterior and posterior regenerative tissue (blastema) and by whole mount *in situ* hybridization. By both methods we found a early and higher expression in the anterior (head) blastemas than in the posterior (tail) ones. Since Platyhelminthes are considered the most basal phylum with a clear cephalization, the *Dtotx*-expression supports the ancestral role of Otx genes in cephalization. We also identified one *Pax-6* gene. It encodes a single transcript with extensive, although lower, sequence identity and several conserved splice sites with the known *Pax-6* genes of vertebrates and invertebrates. *Dtl'ax-6* is expressed in regenerating and fully grown eyes, and evidence has also been obtained for low level expression in other body regions. Since Pax-6 is required for the development of the eye in both vertebrates and invertebrates, it has been suggested that the photoreceptor cells of these animals have a monophyletic origin (5). One of the downstream genes of *Pax-6* is *sine oculis*. A homologous gene in planarians has been recently isolated, and show a higher transcript levels in the cephalic and pharynx regions (*Pax-6* and *sine oculis* genes have been studied in collaboration with P. Callaerts and W. Gehring from Biozentrum, Univ. of Basel). We have also isolated three members of the POU homeobox family (*GTPOU-1, 3*and *4*). Polyclonal and monoclonal antibodies raised against GTPOU-1 fusion protein detect the presence of GTPOU-1 protein in the central (cephalic ganglia) and peripheral (sub epidermal and sub muscular plexus) nerve cells located exclusively in the most anterior third of the planarian. Most of the representatives of the class III POU genes, to which *GTPOU-1* belongs are responsible for nerve cell determination. Studies of GTPOU-1 pattern distribution during regeneration showed a great nerve cell plasticity, indicating that regeneration is a general process that affects all the structures, including regions far from the wound. This supports the existence of morphallactic processes in planarian regeneration.

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Conserved and divergent aspects of homeobox gene expression in *C. elegans* & evolution of ancient TALE homeobox genes.

Talk of Thomas R. Bürglin, Biozentrum, University of Basel, Switzerland

In our lab we have been analyzing a diverse set of homeobox genes. *ceh-2* is an orthologue of the *Drosophila* gene *ems*. It is expressed in the anterior of the pharynx. Thus, while it is expressed in the anterior of the animals as expected, we observe no expression in the brain. To further investigate head development, we make GFP reporter constructs for the two *otd*-like genes *ceh-36* and *ceh-37*. Expression is observed in sensory neurons of the brain, which correlates as expected with the expression patterns in flies and vertebrates. We also are examining the expression pattern of four *sine-oculis* like genes in *C. elegans*. Currently we cannot resolve the evolutionary history of the so genes. Two of the genes seem to be a *C. elegans* or nematode specific duplication, as the genes are next to each other on the chromosome. Expression of one of the genes is seen in the pharynx.

The gene *ceh-14* is a LIM homeobox gene, a member of the LIM3 family. We see expression in sensory neurons in the head and in the tail, as well as expression in epidermal type tissues, i.e. the spermathica, and the hypodermis. The expression in the hypodermis has the form of a gradient, apparently controlled by an extracellular signal. The vertebrate orthologues of *ceh-14* are expressed in motorneurons of the spinal cord. The much simpler ventral cord of *C. elegans* does not seem to require *ceh-14*.

The *C. elegans* ONECUT genes are a novel class of cut homeobox genes, several of which are found in *C. elegans*. We see expression during early embryogenesis, in muscle and gut tissues, and an almost ubiquitous expression.

Analysis of the atypical homeobox gene *ceh-25* showed that there is alternative splicing within the homeodomain *. Two different types of homeodomains can be generated, which differ in the first third of the homeodomain. *ceh-25* belongs to the TALE superclass of homeobox genes. A comprehensive analysis of TALE superclass homeobox genes resulted in a new classification scheme: Currently, two classes are identified in plants, i.e., KNOX and BEL, two classes in fungi, i.e., CUP and M-ATYP, and four classes in animals, i.e. MEIS, Iroquois, PBC, and TGIF. Analysis of the MEIS class gene *ceh-25* revealed a new domain upstream of the homeodomain. Comparison of the MEIS domain with the plant KNOX domain revealed significant sequence similarity. Thus, in the case of MEIS and KNOX, a second domain has been conserved between plants and animals.

* T.R. Bürglin, (1997) Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. Nucl. Acids Res., in press

Genes and Factors That Are Involved in the Silk Gland Development and Silk Genes Transcription

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Through structural and functional analyses of the transcription factors for the silk genes we have planned to understand problems of embryonic silk gland formation as well as the silk genes transcription regulation (Suzuki, 1994).

Cell-free transcription systems for the silk genes were established (Tsuda and Suzuki, 1981) and elaborated to reconstitute differential transcription of the posterior silk gland-specific fibroin gene and the middle silk gland-specific sericin-1 gene (Suzuki et al., 1986; 1990). Major transcription factors for the fibroin gene are SGF-1 and -2 (Hui et al., 1990), while those for the sericin-1 gene are SGF-1 and -3 (Matsuno et al., 1989; 1990). SGF-1 which is present both in the posterior and the middle silk glands was purified, and its cDNA was cloned and identified as Fork head homologue (Mach et al., 1995). cDNA of SGF-3 which is present abundantly in the middle silk gland and scarcely in the posterior silk gland was obtained by a PCR cloning, and identified as Cf1a homologue (Fukuta et al., 1993). SGF-2 which is specific to the posterior silk gland and a key factor for the fibroin gene transcription was purified only recently by procedures including an affinity step, and identified as a huge complex of an apparent molecular mass 1.1 MDa accommodating about 12 subunits (K. Ohno et al., unpublished). Although exact stoichiometry of each subunit has not been elucidated yet, the complex includes 1 Lim homeodomain protein homologue, 3 Nuclear Lim Interactor homologues, 3 P25 variants, and several new proteins (K. Ohno et al., unpublished).

Both SGF-1 transcripts and protein were first detected in the most anterior and posterior regions of germ anlage, and later in the embryonic foregut and hindgut. By the end of embryo retraction stage both signals became detectable in the invaginating whole silk glands, and after the blastokinesis stage the products were restricted to the middle and posterior silk glands (Kokubo et al., 1996). The *SGF-1* expression in the silk gland is probably under the control of *Bombyx Scr* (Kokubo et al.,

1997a) because ectopic expression of *Bombyx Scr* in the *Nc/Nc* embryos (Itikawa, 1944; 1952) which lack *Bombyx Antp* (Nagata et al., 1996) caused ectopic formation of dwarf silk glands in the three thoracic segments accompanied with ectopic expression of *SGF-1* (Kokubo et al., 1997a). An interesting difference between the *Drosophila* Scr-Fkh and the *Bombyx* Scr-Fkh was recognized; *Bombyx* Scr disappears in the spots of the silk gland invagination where *Bombyx* Fkh will appear (Kokubo et al., 1997a).

SGF-3 in the embryos is first expressed in the prothoracic gland, and then in several tissues including the invaginating silk gland spots (Kokubo et al., 1997b). Later, the expression of SGF-3 in the silk gland was restricted to the anterior and the middle silk glands (Kokubo et al., 1997b). Since the *Drosophila* Cf1a expression was not detected in the *Drosophila* salivary gland, the above observation gives a clear distinction between *Drosophila* salivary gland and *Bombyx* silk gland development.

These observations as well as planned analyses of the expression patterns of each subunit of SGF-2 would clarify the complexity and the specificity in the process of silk gland development and the complex molecular basis of silk genes transcription regulation. These studies would naturally lead to reveal conservation and diversification between *Bombyx* silk gland and *Drosophila* salivary gland.

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***homothorax* ,which encodes a Extradenticle-related homeodomain protein, is required for the nuclear translocation of Extradenticle.** G.E. Rieckhof and R.S. Mann. Department of Biochemistry and Molecular Biophysics, Columbia University, 701 West 168th St., New York, NY 10032 Phone 212-305-2111, Fax 212-305-7924

We show that *homothorax* (*hth*) is required for the Hox genes to pattern the body of the fruit fly. *hth* is required for the nuclear localization of an essential Hox cofactor, Extradenticle (Exd) and encodes a homeodomain protein that shares extensive identity with the productive Meis1, a murine proto-oncogene. Meis1 is able to rescue *hth* mutant phenotypes, and can induce the cytoplasmic to nuclear translocation of Exd in cell culture and *Drosophila* embryos. Thus, Meis1 is a murine homologue of *hth*. Meis1 also specifically binds to Exd with high affinity *in vitro*. These data suggest a novel mechanism, that is conserved between mouse and flies, for regulating Hox activity that depends on the control of Exd's nuclear localization by a direct protein-protein interaction with the homeodomain protein encoded by *hth*.

Conservation of apterous Function and Regulation from Drosophila to vertebrates. Diego E. Rincón-Limas, John W. R. Schwabe, Cheng-Hsin Lu, Concepción Rodriguez-Esteban, Inmaculada Canal, Juan Carlos Izpisúa-Belmonte and Juan Botas. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030 and Gene Expression Laboratory, The Salk Institute, 10010 North Torrey Pines Road. La Jolla, CA 92037.

Mouse and human orthologues of Drosophila apterous were cloned to investigate the conservation of apterous regulation and function. Expression of the mouse orthologue (mlhx2) resembles apterous expression in flies suggesting that aspects of apterous regulation have been conserved from Drosophila to mammals. To test this hypothesis we generated transgenic mice with the enhancers that drive apterous expression in flies. We find that the apterous ventral nerve cord enhancer drives lacZ expression in the neural tube of transgenic mice in a pattern reminiscent of mlhx2 expression. In addition, a muscle-specific apterous enhancer also retains its tissue-specificity in transgenic mice. These results suggest an extraordinary conservation of the tissue-specific gene networks operating in the muscles and neural tubes of flies and mice. We also show that the human apterous orthologue (hlhx2) completely rescues the Drosophila apterous mutant phenotypes demonstrating the functional conservation of the Apterous protein.

Session 5: Vertebrate development

Chairperson: Walter J. Gehring

Genetic patterning of germ layers and body axes: insights from amphioxus

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Most multicellular animals have a triploblastic body organisation made of three layers of cells: ectoderm, mesoderm and endoderm. These so-called germ layers remain largely distinct in early development, but as embryogenesis proceeds their distinctiveness becomes less clear. For example, more than one germ layer may contribute to one structure, whilst experimental perturbation can cause cells to cross germ layer boundaries. Further, germ layers interact such that cellular changes in one germ layer have consequences for cells in adjacent germ layers. In vertebrates, the neural crest cells pose a problem for the germ layer concept, since they are ectodermal cells that in many ways behave as mesodermal cells, and may occupy the same physical space as mesoderm. How distinct then are germ layers? Do they utilise different sets of genes for embryonic patterning? Do they follow similar genetic patterning rules? Can genes be co-opted from one germ layer to another, or between tissue types within a germ layer?

Genes controlling patterning of ectodermal derivatives (including brain and spinal cord) are well characterised in vertebrates. Examples include Hox, Otx, Emx and Msx class homeobox genes encoding transcription factors. The Hox genes play key roles in axial patterning of ectodermal derivatives, and to some extent mesodermal derivatives, probably by assigning positional values to cells. Much less is known about genes that pattern endoderm (gut), but a few are known such as the IPF and Cdx homeoboxes.

We have investigated the evolution of these genes, focusing our attention on homeobox genes in the sister group of vertebrates: the cephalochordates or amphioxus.

First, we find that gene expression patterns of many amphioxus homeobox genes are consistent with roles in spatial patterning of embryonic ectoderm and endoderm. For example, amphioxus Hox[1] and Otx[2] genes are expressed in precise spatial regions of amphioxus ectoderm. The Otx gene marks the most rostral region, the cerebral vesicle, whilst the Hox genes are expressed in overlapping patterns with complex spatial modulation. These are just two of several examples where the regional restriction to gene expression is very similar in amphioxus and vertebrates.

Second, we find some surprisingly similarities in the way homeobox genes are regulated in endoderm and ectoderm, that suggest the nature of the ancestral patterning mechanism in the ancient animal embryos. On the basis of gene expression and gene sequences, we propose a novel hypothesis that suggests the Hox cluster and a twin of Hox cluster evolved by duplication, and this paved the way for germ layer origins[3].

Third, we find that amphioxus has fewer of each class of homeobox gene than do vertebrates[4]. For examples, amphioxus has a single Hox gene cluster[5], containing at least 12 tandemly arranged Hox genes. This contrast to the four gene clusters in mammals or Fugu, and the putative three clusters of lampreys[6]. Similarly, vertebrates have multiple Otx genes, whilst amphioxus has one. We can demonstrate that this reflects duplication in the vertebrate lineage and not loss in amphioxus, by careful molecular phylogenetic analyses, and by signature protein motifs that reveal the polarity of this change[7]. Many non-homeobox genes also show a greater number in vertebrates than in amphioxus. We propose that amphioxus retains a primitive and prototypical chordate genome and developmental strategy; in contrast, the vertebrate genome and vertebrate development is complicated by duplication.

Fourth, we are interested in the mechanism of duplication. We have data to argue that this gene duplication probably occurred by tetraploidy[8], and therefore affected all genes. Although all genes were duplicated, some have been lost. We are attempting to calculate rates of gene retention versus gene loss in different gene families, and we have uncovered cases of extreme gene loss (where vertebrates have secondarily reverted to the amphioxus number)[9]. These comparisons give insights into the evolutionary forces that have shaped the human genome, and help explain some unusual features of chromosome organisation such as the meaning of paralogy groups.

Fifth, comparison of Hox gene expression patterns in amphioxus and vertebrates suggest that homeobox genes have gained new roles after gene cluster duplication. These include co-option to other germ layers (eg. from ectoderm to mesoderm) abd co-option to new tissues within a germ layer (eg. from neural tube to neural crest cells). We are examining the mechanism of this functional divergence using a new strategy based on transgenic mice carrying amphioxus DNA[10]. These experiments test whether genes need to acquire new enhancers if they evolve new developmental roles.

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THE EVOLUTION OF COLINEARITY

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Vertebrate *Hox* gene are essential for the proper organization of the body plan during development. Inactivation of these genes usually leads to important alterations, or transformations, in the identification of the affected developing structures. *Hox* gene are activated following a progressive temporal sequence which is colinear with the position of these genes on their respective complexes, so that 'anterior' genes are activated earlier than 'posterior' ones (*temporal colinearity*). Slight modifications in the respective times of gene activation (heterochronies) may shift expression domains along the rostro-caudal axis and thus induce concurrent changes in morphologies. While the nature of the mechanism(s) behind temporal and spatial colinearities is unknown, it was proposed that such a mechanism relies on meta-*cis* interactions, i.e. may necessitate gene contiguity. Such a mechanism would be based on DNA-specific, rather than gene-specific, features such as chromatin configurations or DNA replication. The existence of such a meta-*cis* mechanism would explain the extraordinary conservation of this genetic system during evolution. In order to further investigate the nature of this mechanism, we have used a strategy of gene relocation as well as gene fusion based on recombination in ES cells. The results indicate that in vertebrates, colinearity is primarily depending upon gene accessibility, rather than gene activation. Potential mechanisms are proposed and the differences with the homeotic complexes of *Drosophila* will be emphasized.

The clustering of *Hox* genes has facilitated the global recruitment of this gene family to achieve novel functions. This is best examplified with the developing limbs where such genes are required for growth and patterning. In this case, colinear expression was designed in a different way, involving the presence of enhancer sequences located at various positions. The case of the *HoxD* complex in digit and genital development will be discussed.

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Homeobox genes of the Emx and Otx family in development

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We characterized four vertebrate homeobox genes that play a role in the development and regionalization of the head. These four genes are *Emx1* and *Emx2*, related to a fruit fly gene termed *ems*, and *Otx1* and *Otx2*, related to a fruit fly gene termed *otd*. The four genes are expressed in extended regions of the developing rostral brain of mouse embryos, including the presumptive cerebral cortex and olfactory bulbs. At day 10 of development (E10) their expression domains are continuous regions contained within each other in the sequence *Emx1<Emx2<Otx1<Otx2*. The *Emx1* expression domain includes the dorsal telencephalon, *Emx2* is expressed in dorsal and ventral neurectoderm of the presumptive forebrain with an anterior boundary anterior to that of *Emx1* and a posterior boundary within the roof of presumptive diencephalon. The *Otx1* expression domain contains the *Emx2* domain and covers a continuous region including part of the telencephalon, the diencephalon and the mesencephalon. Finally, the *Otx2* expression domain contains the *Otx1* domain and practically covers the entire fore- and mid-brain. The first appearance of transcripts of the four genes is also sequential: *Otx2* is already expressed in the headfold at E7.5, followed by *Otx1* and *Emx2* in E8-8.5 and finally by *Emx1* in E9.5 mouse embryos. It seems reasonable to postulate a role of the four homeobox genes in establishing the identity of the various embryonic brain regions.

In particular, *Otx2* appears to play an early role in gastrulation and first specification of anterior head and rostral brain in mouse, frog, chick and zebrafish embryos and the two *Emx* genes play some role in the developing cerebral cortex. In this light, we analyzed *Emx* gene expression in avian and reptilian embryonic brains and *Otx2* activation in regenerating planarians.

Functional study of Amphioxus Hox regulatory elements in transgenic mice

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Cephalochordates are the closest living invertebrates group relative to vertebrates, and therefore extremely useful in studying the origin and evolution of the vertebrate body plan. Comparison of expression patterns of developmental genes from amphioxus with those of model vertebrate organisms has proven very informative to understand the basic building of a common antecesor. The next step is to try to compare the regulatory interactions between these genes and try to find out how small changes in regulation can carry to drastic reorganizations of morphology.

Hox genes complexes are responsible for anterior-posterior axial information in all animal groups studied so far. Protostomes (insects and nematodes) have only one complex while vertebrates have at least four, originated by duplication and divergence from a putative ancestor with a single complex. Amphioxus only complex resembles more closely that of vertebrates than protostomes, thereby representing a putative ancestor-like complex of present day vertebrate *Hox* genes.

In order to study the potential conservation of regulatory elements between amphioxus and vertebrates, we have carried out a systematic approach to test the ability of genomic regions from the 3' end of the complex (from *hox1* to *hox4*) to direct expression of a reporter gene (*lacZ*) in transgenic mice. Approximately 30 kb of DNA was subdivided in 7 different fragments and these were tested individually. In this way we have identified a number of elements that direct expression in the neural tube and in neural crest and its derivatives. Putative neural tube *Hox* regulatory elements from *hox1* and *hox3/hox4* seem to be present in amphioxus and function in a similar way to their mammalian counterparts from *hoxb1* and *hoxb3/hoxb4*. Nevertheless, rhombomere-specific enhancers were not identified, perhaps because of the lack of a clearly segmented region in the amphioxus posterior brainregion. Elements present in the vicinity of *hox2* gave rise to expression in neural crest populations streaming into the branchial arches and in cranial ganglia deriving from crest. This result came as a surprise as it is claimed that amphioxus does not have neural crest. The implications as well as the limitations of this kind of approach will be discussed.

XIRO, a *Xenopus* homolog of the *Drosophila* Iroquois-complex genes, controls the development of the neural plate

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The *Drosophila* homeoproteins Ara and Caup are members of a combinatory of factors (prepattern) that controls the highly localized expression of the proneural genes *achaete* and *scute*. We have identified two *Xenopus* homologs of *ara* and *caup*, *Xiro1* and *Xiro2*. Similarly to their *Drosophila* counterparts, they control the expression of a proneural gene (*XASH-3*) and, in addition, the size of the neural plate. Moreover, similarly to *ara* and *caup* the *Xiro* genes positively respond to overexpression of the *Drosophila cubitus interruptus* gene. These and other findings suggest the conservation of a genetic cascade that regulates proneural genes, and the existence in vertebrates of a prepattern of factors important to control the differentiation of the neural plate.

POSTERS

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EVIDENCE FOR A CLADE OF NEMATODES, ARTHROPODS, AND OTHER MOULTING ANIMALS

The arthropods consitute the most diverse animal group, but despite their rich fossil record and a century of phylogenetic study, their phylogenetic relationships remain unclear. Taxa previously proposed to be sister groups to the arthropods include Annelida, Onychophora, Tardigrada and others, but phylogenetic relationships have been conflicting. For example, onychophorans, like arthropods, periodically moult, have an arthropod arrangement of haemocoel, and have been related to arthropods in morphological and mitochondrial DNA sequence analyses. Like annelids, arthropods possess segmental nephirida and muscles that are a combination of smooth and obliquely striated fibers. Our phylogenetic analysis of 18S ribosomal DNA sequences indicates a close relationship between arthropods, nematodes, and all other moulting phyla. The results suggest that ecdysis (moulting) arose once and support the idea of a new clade, Ecdysozoa, containing moulting animals: arthropods, tardigrades, onychophorans, nematodes, nematomorphs, kinorhynchs and priapulids. No support is found for a clade of segmented animals, the Articulata, uniting annelids with arthropods. The hypothesis that nematodes are related to arthropods has important implications for developmental genetic studies using as model systems the nematode *Caenorhabditis elegans* and the arthropod *Drosophila melanogaster*, which are generally held to be phylogenetically distant from each other.

Role of *Xrx1* in *xenopus* eye development

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Eye development has been an active field of study ever since the beginning of the century. Although the main inductive events and morphogenetic movements have now been described in details, little is still known about the molecular mechanisms of these phenomena. We recently isolated *Xrx1*, a novel homeobox gene whose expression demarcates presumptive eye regions already at the end of gastrulation. Later on during development *Xrx1* transcripts are detected in pigmented epithelium, neural retina, diencephalon floor and pineal gland. Overexpression of *Xrx1* synthetic RNA leads to the generation of ectopic pigmented and neural retina as well as partial duplication of forebrain regions. The location of ectopic pigmented retina is always restricted between the eyes and the forebrain, a region that shows a transient competence to become retina in early development. As a complementary approach to study *Xrx1* function, we generated a fusion construct where the putative transactivation domain of *Xrx1* has been substituted by the *engrailed* repressor domain (*Xrx1*-EnR). When overexpressed in developing embryos, this chimeric protein is expected to bind and repress the transcription of *Xrx1* natural target genes. Microinjection of *Xrx1*-EnR RNA in dorsal blastomeres leads to suppression of eye development and, in most severe cases, to deletion of anterior head regions. Both "gain of function" and "loss of function" experiments suggest for *Xrx1* an important role in the development of the eye and anterior brain regions. The analysis of interactions between *Xrx1* and other homeobox genes expressed in the eye field (*Xsix3*, *Xpax2*, *Xpax6* etc.) is currently underway.

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Mechanisms controlling the nucleo-cytoplamsic localisation of EXD

The extradenticle protein (EXD) is a homeodomain transcription factor which has an important role regulating the DNA-binding specificity of homeotic selector proteins in *Drosophila*. Although EXD was initially thought to be a ubiquitously available cofactor, I have recently found that the subcellular localisation of EXD is under tight spatial, temporal and cell-type specific control (Aspland and White 1997). EXD is initially uniformly distributed but is excluded from nuclei until gastrulation. During the extended germ band stage protein remains predominantly cytoplasmic and does not accumulate in nuclei until germ band retraction. Nuclear accumulation occurs in a highly spatially regulated pattern. Later in development, in the wing and leg imaginal discs the subcellular localisation of EXD is also strongly patterned with distal regions exhibiting cytoplasmic EXD whereas in proximal regions EXD is nuclear. This correlates well with the functional requirement for EXD as proximal *exd*$^-$ clones show morphological changes whereas distal clones are normal. This post-transcriptional regulation of EXD subcellular localisation provides the basis for a novel level of control of homeotic gene function. As nuclear localisation is in general controlled by phosphorylation, the regulation of EXD localisation may link signal-transduction pathways with the regulation of homeotic gene target specificity. In this poster I will present the results of a study examining this possibility more fully. The results of my study on the regulation of EXD should be of general significance as there is a clear sequence and functional homology between *exd* and the vertebrate *Pbx* family.

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The murine *Hoxc-9* gene contains a structurally and functionally conserved enhancer responsive to *decapentaplegic* in transgenic flies.

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Reporter gene analysis of the *Hoxc-9* genomic region in transgenic mice allowed us to identify a positional enhancer in the *Hoxc-9* intron that drives expression in the posterior neural tube of midgestation mouse embryos in a *Hoxc-9* related manner. Sequence comparison to the chicken *Choxc-9* intron revealed the existence of two highly conserved sequence elements (CSEs) in a similar spatial arrangement. These structural similarities in the mammalian and avian lineage are mirrored by conserved function of the chicken *Choxc-9* intron in transgenic mice. Deletion analysis of the two introns suggests that full activity of both enhancers depends on cooperation between the two CSEs located close to the respective 5' and 3' splice sites. Following the paradigm of phylogenetically conserved developmental control mechanisms, *Drosophila* was used as a model organism to study the regulation of the *Hoxc-9* intragenic enhancer. Our data show that the mouse *Hoxc-9* enhancer acts in a conserved fashion in transgenic flies, conferring posteriorly restricted reporter gene expression to the developing central nervous system (CNS) in third instar larvae. Furthermore, this expression is apparently downregulated upon heat shock-induced overexpression of *decapentaplegic* (*dpp*), a fly homolog of members of the vertebrate transforming growth factor ß (TGFß) gene family. These results may encourage the use of transgenic flies in the genetic analysis of functionally conserved enhancers to gather clues about potential upstream regulators of vertebrate Hox genes.

Functional interactions between *exd* and the bithorax genes of *Drosophila*

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Exd is a *Drosophila* homeodomain protein closely related in the homeodomain to the Pbx family of mammalian proteins. One of the members of this family, *pbx1*, is rearranged in pre-B acute lymphoblastic leukemias (ALL), involving a t(1;19) (q23;p13) translocation. The translocation results in a replacement of the EA2 DNA binding domain with a homeodomain derived from *pbx1*.

Exd has been shown to be able to interact with some HOX proteins of *Drosophila* to modulate their specificity. It has been proposed, that Exd might therefore act as a cofactor of the Hox proteins in the specification of larval patterns during embryogenesis. Albeit of being a homeodomain protein, with a DNA binding domain, Exd is localized to the cytoplasm of the cell at the beginning of embryogenesis. Later on the protein translocates into the nuclei of the cells in the anterior part, but it remains cytoplasmic in the posterior segments of the embryo. The function of *exd* is at least in part regulated at the level of its subcellular localization, so that the nuclear *exd* seems to be the functional form of the protein. In this work we have investigated the regulation of the Exd intracellular distribution in *Drosophila* embryos. We demonstrate that the subcellular localization of Exd is regulated by a group of HOX genes, the bithorax complex genes *Ubx*, *abdA* and *AbdB* of *Drosophila*. All three HOX proteins are capable of preventing the translocation of Exd into the nucleus in the posterior segments of the embryo. We have also shown that the D-10 HOX protein, one of the mouse homologs to *AbdB*, is also able to interfere with the translocation of Exd into the nucleus when ectopically expressed during *Drosophila* development. Our results suggest that there might be a conserved mechanism by which HOX proteins regulate the presence of functional Exd protein in the nuclei of the cells.

We have also investigated the functional interactions between the *Drosophila* HOX protein Ubx and Exd. Clonal analysis show that nuclear Exd is necessary for the autoregulation of *Ubx* in *Drosophila* imaginal discs. Taken together our results suggest the presence of a regulatory loop by which the bithorax genes in *Drosophila*, and probably other HOX genes in vertebrates, regulate the amount of functional Exd/Pbx that goes into the nucleus and with which they interact to exert some of their functions.

Amphioxus *Hox* and *Evx* genes: Trunk Flexibility behind an archetypal cluster.

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We are interested in the evolution of developmental mechanisms during chordate evolution, and are studying the *Hox* and *Evx* genes of amphioxus (*Branchiostoma floridae*) in this context. Amphioxus has emerged as an excellent embryological and genetic model system in which to study vertebrate ancestry, and subsequent evolution (1). Amphioxus is a Cephalochordate, and hence is a member of the sister group of the vertebrates. It possesses chordate morphological characteristics, such as a notochord, somites, gill arches, a tail and dorsal nerve cord, but lacks vertebrate features such as a tripartite brain, extensive cephalisation, neural crest and myelinated nerves. At the genomic level amphioxus appears to reflect the pre-vertebrate state prior to the gene duplications and tetraploidisation events that occurred close to the origin of the vertebrates (2). The amphioxus Hox cluster provides the best example to illustrate this last point.

Amphioxus has a single cluster, with no gaps, (it contains a member of each paralogy group, at least up to group 10) (3). A gene of a particular paralogy group is more closely related to its homologues in other clusters than to its neighbours within its own cluster. Vertebrates have 4 Hox clusters, none of which are complete, probably due to gene loss after the whole cluster duplications in the early vertebrates (2). The amphioxus cluster can thus be viewed as archetypal with respect to the clusters of vertebrates, at least up to paralogy group 10.

We have now continued the genomic walk further 5' from *AmphiHox10*, to allow a more thorough comparison to the vertebrates. The walk is still in progress, but so far we have isolated an *AmphiHox11*, and the 3' part of *AmphiHox12*. Although this walk is incomplete it has nevertheless become apparent that this posterior end of the cluster, *AmphiHox9-12*, is not as "archetypal" as the anterior end, *AmphiHox1-8*. *AmphiHox9-12* are not paralogues of the vertebrate *Hox9-12* genes, and appear to be the product of independent tandem duplications. Within the higher chordates (cephalochordates + vertebrates), one-to-one homologies can be seen between each anterior Hox group from 1-8. This strong conservation is not present in the posterior Hox cluster, where there has been higher levels of intergenic and interspecies sequence divergence (4), greater variability in relative intergenic distances, and independent origins of the vertebrate and cephalochordate groups 9-12. We call this phenomenon "Trunk Flexibility", to reflect the greater variability observed within the posterior chordate Hox genes, whose anterior boundaries of expression are within the chordate trunk.

We have also begun an investigation of *AmphiEvx* genes. Vertebrates have two *Evx* genes, one on the end of cluster A (*Evx1*) and one on the end of cluster D (*Evx2*). These locations may reflect either (1) the ancestral presence of *Evx* in the Hox clusters of chordates, or (2) the more recent movement of *Evx* onto the end of the clusters in vertebrates. The position of *Evx* relative to the cluster probably affects the function of the gene, since part of the expression of *Evx2* resembles what would be expected for a Hox gene more posterior (5') to *Hox13* (5).

We have isolated two amphioxus *Evx* genes (*EvxA* and *B*). These genes are called A and B as their sequence indicates that they are not one-to-one homologues of *Evx1* and 2, and hence are the result of an independent duplication event. We are in the process of mapping the genomic location of *A* and *B* by walking and chromosomal in situ hybridisation. The mapping of these genes and analysis of their expression patterns will help to establish some of the foundations from which the vertebrate body plan evolved.

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THE DROSOPHILA TERMINALIA IS ORGANIZED AS A DISTAL VENTRAL APPENDAGE. N. Gorfinkiel and I. Guerrero. Centro de Biología Molecular, Madrid, Spain.

In Drosophila the terminalia is composed by either the female or male genitalia and the sexual dimorphic analia that develop from a single genital imaginal disc. The terminalia can be considered as a derived appendage of the segmented polyramous limb of the common ancestor of all arthropods. In this sense, the genital disc would be of ventral origin like the leg and antennal discs. In accordance with this hypothesis, we have found that *Dll*, a homeobox gene that specifies ventral appendage development, is also expressed in female and male genital discs. In contrast with previous reports, clonal analysis of a *Dll* lack of function allele has shown that *Dll* is also required in the development of both male and female analia.

In the leg and antennal discs, *hh* expression in the P compartment induces Wg and Dpp in ventral and dorsal anterior cells respectively. *Dll* expression is activated by the combined action of Wg and Dpp which in turn are maintained by mutual repression. We have shown that ectopic *Dll* expression in the proximal region of the leg disc is able to induce ectopic *wg* and *dpp* and hence a secondary P/D axis.

In the genital disc this seems to be also the case; Wg and Dpp abut the *hh* expression domain and are overexpressed when Hh is ectopically expressed. Moreover, the Dll expression domain overlaps with the Wg and Dpp ones, both in the presumptive genitalia and analia, and ectopic Wg or Dpp results in the ctopic expression of Dll. We are currently studying the corresponding adult phenotypes of the lack of *Dll* function and also the consequences of ectopic expression of *Dll*. We are also analyzing whether the similarities between the leg and the genital disc extend to other pattern-forming genes.

These and other aspects of genitalia development will be discussed.

Evolution of development and changes in life history in insects

Miodrag Grbic

An important question in developmental biology is how phylogeny and life history interact to affect the course of evolution. Comparative studies indicate that developmental programs are broadly conserved within higher taxa and that fundamental regulatory pathways are often very similar between phyla. The Hox/HOM gene complex, for example, regulates anterior-posterior patterning in both arthropods and chordates, and may likely be used in pattern formation processes across all bilaterian metazoa. Such trends indicate that ancestry obviously plays a significant role in shaping how organisms develop. Comparisons between higher taxa also suggest that developmental mechanisms evolve slowly and that modifications in embryogenesis are usually associated with marked changes in adult morphology.

On the other hand ecologists and developmental biologists have identified several instances where distinct differences in embryonic development occur between closely related species without any concomitant changes in adult form. These studies indicate not only that alterations in embryogenesis can occur without major consequences for the adult body plan but that adaptations in early development may arise in response to changes in life history. An important goal is to now ask how phylogenetically widespread such punctuated changes in development might be, what kinds of life history shifts favor alterations in early development, and what mechanisms underly these changes?

If life history plays a role in shaping early development of insects, we would hypothesize that departures from the general insect developmental ground plan would most likely arise in groups whose eggs develop under conditions very different from those experierienced by insects generally. One such group is the parasitic wasps. Within this assemblage has also evolved polyembryonic species that form two or more individuals from a single egg .

The parasitic wasp *Copidosoma floridanum* represents the most extreme form of polyembryonic development known, forming up to 2000 embryos from a single egg. To understand the mechanisms of embryonic patterning in polyembryonic wasps and the evolutionary changes that led to this form of development we have analyzed embryonic development at the cellular level using confocal and scanning electron microscopy. *C. floridanum* exhibited three phases of embryogenesis: 1) early cleavage that leads to formation of a primary morula, 2) a proliferative phase that involves partitioning of embryonic cells into individual compartments and formation of thousands of morulae, and 3) morphogenesis whereby individual embryos develop into larvae. This developmental program represents a major departure from the insect developmental ground plan. The early developmental program of polyembryonic wasps shows several analogies with mammalian embryogenesis, including early separation of extraembryonic and embryonic cell lineages, formation of a morula and embryonic compaction. However, the late morphogenetic program of polyembryonic wasps, from germband formation to the completion of embryogenesis, proceeds in a fashion conserved in all insects. This suggests a lack of developmental constraints in early development, but strong constraints in the late developmental program (phylotypic stage). The development of *C. floridanum* and other polyembryonic insects suggests this form of development evolved in association with a shift in life history to endoparasitism.

Nonetheless, homologous elements of the *Drosophila* segmentation gene cascade function during polyembryony. Expression of the *Eve* antigen is detected in pre-gastrulation embryos from the posterior-end to 55% of the length of the newly formed primordium. This broad band of expression then rapidly resolves, anteriorally-posteriorally at gastrulation, into 15 stripes. No pair-rule expression pattern occurs in *C. floridanum* as seen in other advanced insects like *Drosophila* and beetles. Eve expression is followed by appearance of the En protein; beginning in the posterior 1-2 cells of the mandibular and labial segments followed by nearly simultaneous formation of the maxillary, thoracic and abdominal stripes. Expression of *C. floridanum* Ubx/Abd-A occurs in a conserved pattern during post-gastrulation from the posterior metathorax to the eighth abdominal segment.

If the shift to endoparasitism is important in the alterations observed in early development of polyembryonic wasps, we would expect that similar alterations would also be found in monoembryonic endoparasitoids. To examine the consequences of an endoparasitic life history for insect embryogenesis, we focused on two parasitic wasps from the same family. *Bracon hebetor* is ectoparasitic braconid that lays its eggs on the bodies of caterpillars, whereas *Aphidius ervi* is an endoparasitic braconid that lays its eggs in the hemocoel of aphids . As we would predict from life

history, *Bracon* lays yolky eggs with a rigid chorion. Embryogenesis also proceeds very similarly to *Drosophila* and the honeybee. Development begins by syncytial cleavage with the body plan becoming morphologically visible following cellularization of the blastoderm and gastrulation. Formation of the segmented germ band thereafter occurs nearly simultaneously in a classically long germband fashion. The similarity in these events with other long germband species are corroborated molecularly by the expression patterns observed for representative pair-rule, segment polarity and homeotic genes. As in *Drosophila*, an antigen for the pair-rule gene*Eve* is detected in *Bracon* as a broad domain prior to cellularization of the blastoderm followed by a pair-rule pattern in odd-numbered parasegments and later a segmentally reiterated pattern. Homologs of the segment polarity and homeotic genes *En* and *Ubx/Abd-A* is are likewise expressed in a temporal manner characteristic of other canonical long germband insects.

In contrast, the eggs laid by *Aphidius* are enclosed in a thin chorion and appear to be totally devoid of yolk. Cell injection experiments confirm that the *Aphidius* embryo undergoes holoblastic cleavage and that individual blastomeres become dye uncoupled by the 16-cell stage. Like *Copidosoma*, a morula stage embryo ruptures from the chorion enveloped by membrane of polar body origin. Following formation of a compacted blastula, however, the germband extends along the anterior-posterior axis in the manner of a short germband species like the grasshopper. During this process, no periodic or segmental expression of *Eve* antigen is detected even though Eve expression occurs later in several bilaterally paired neuroblasts in a pattern conserved in all insects . En stripes appear sequentially as the embryo initiates germ band extension that localizes to the posterior compartment of each segment, and Ubx/Abd-A expression in *Aphidius* is restricted to the abdomen in a conserved pattern to other insects.

The differences in early development of *Bracon* and *Aphidius* are as large as any described to date for insects in the comparative developmental literature. Yet, unlike grasshoppers and flies that reside in phylogenetically distant orders, these wasps occur in a monophyletic group of advanced insects. The differences in their early development, therefore, are not due to vastly different phylogenetic histories; rather we suggest the shift from an essentially free-living, terrestrial existance (*Bracon*) to development within another organism (*Aphidius*) has favored adaptations in *Aphidius* for survival in a new environment. In particular, the loss of yolk and a chorion in *Aphidius* would appear to be a key alteration in the shift from syncytial to holoblastic cleavage and corresponding alterations in expression of genes regulating anterior-posterior axis formation.

Conserved functions of mammalian Gli transcription factors in Hedgehog signaling pathway

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The Drosophila Gli zinc finger transcription factor Cubitus interruptus (Ci) plays dual roles in Hedgehog (Hh) signaling pathway. Genetic and molecular analysis has revealed that Ci functions as a downstream mediator of Hh signaling. Furthermore, *ci* function is required to repress *hh* transcription. In humans and mice, at least three *Gli* genes (*Gli1*, *Gli2* and *Gli3*) have been found. In humans, *GLI1* has been identified as an amplified gene in many forms of tumor and *GLI3* is mutated in two dominant genetic disorders, Greig cephalopolysyndactyly syndrome and Pallister-Hall syndrome. My laboratory is interested in studying the functions of the *Gli* genes during mammalian development. We report here the characterization of early embryonic phenotypes of *Gli2* and *Gli3* mutants. In *Gli2* homozygotes, floor plate differentiation is specifically abolished even in the presence of a normal notochord. Reduction of *Ptc* and *Gli1* expression indicates that *Gli2* homozygotes have diminished Sonic hedgehog (Shh) signaling. Interestingly, in *Gli2* homozygotes, motor neurons can be generated in the absence of floor plate differentiation. These observations suggest that *Gli2* is essential for floor plate differentiation and that, similar to Ci, Gli2 functions as a downstream mediator of Shh signaling. In *Gli3* homozygotes, anterior neural structures are severely affected. We demonstrate that this is due to ectopic *Shh* expression. Consistent with an upregulation of Shh signaling in the forebrain region, *Ptc* and *Gli1* expression is elevated. Interesingly, in addition to ectopic *Shh* expression, there is also a slight downregulation of *Ptc* and *Gli1* expression in the posterior part of the embryos. We find that the expression of *nodal*, a Shh-responsive gene, is specifically downregulated in the left lateral mesoderm. Furthermore, there is also ectopic expression of *nodal*. These observations suggest that Gli3 can function both as a downstream mediator of Shh signaling as well as a repressor of *Shh*. Our data indicate that the mammalian Gli transcription factors possess evolutionarily conserved functions in Hedgehog signaling.

Hindbrain neural crest establishes the vertebrate craniofacial pattern by defining muscle attachment sites and branchial arch polarity

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Cranial neural crest has long been suspected to be pivotal in patterning the vertebrate head and is thus thought to be the prime substrate of vertebrate head evolution (1). To investigate the influence of hindbrain segmentation on craniofacial patterning we have studied the long term fate of neural crest (NC) subpopulations of individual rhombomeres (r), using quail-chick chimeras (2). Mapping of all skeletal and muscle connective tissues developing from these small regions revealed several novel features of cranial neural crest with implications for our ideas on vertebrate head evolution.

To our surprise, we found that both the lower jaw and tongue skeleton are compound elements derived from neural crest populations of multiple branchial arches. These elements display an organisation which precisely reflects the rostrocaudal order of segmental crest deployment from the embryonic hindbrain. In these composite elements cryptic intraskeletal boundaries, which do not correspond with anatomical landmarks, form sharply defined interfaces between r1+r 2-, r4 and r6+7 crest. A highly constrained pattern of cranial skeletomuscular connectivity was found that precisely respects the positional origin of its constitutive crest: each rhombomeric population remains coherent throughout ontogeny, forming both the connective tissues of specific muscles and their respective attachment sites onto the neuro- and viscerocranium. Focal clusters of crest cells, confined to the attachment sites of branchial muscles, intrude into the otherwise mesodermal cranial base. In the viscerocranium, an equally strict, rhombomere-specific matching of muscle connective tissues and their attachment sites is found for all branchial and tongue (hypoglossal) muscles. Our study shows how individual rhombomeric neural crest populations can serve as the ultimate structural basis for craniofacial homology thoughout vertebrates. Their structural segmental coherence during ontogeny explains how cranial skeletomuscular pattern can be implemented and conserved despite radical evolutionary changes in the shapes of skeletal elements.

Our finding of maintained rostrocaudal levels of hindbrain neural crest populations is a prerequisite for the notion that branchial arches are secondary morphogenetic fields with discrete axes: the proximodistal axis as defined by the distance of a neural crest cell with respect to the embryonic brain and foregut endoderm and a rostrocaudal axis of branchial arch polarity. Selective ablations, heterotopic hindbrain neural crest grafts and subsequent expression analysis of signalling molecules elicited from discrete regions of the branchial arch ectoderm revealed that these polar patterns are set up and exquisitely regulated by individual underlying neural crest populations.

In a novel evolutionary scenario our experimental embryological studies illustrate the profound evolutionary implications of neural crest as a key vertebrate character: In a 'New Head' cranial neural crest modifies initially unpolar gill bars to branchial arches as polar structures by setting up polar sources of signalling molecules in the overlying head ectoderm. These signalling centers then influence in a feedback loop the underlying crest to form polarised skeletal elements which can evolve into sucking and biting apparatuses. The coherence of individually programmed crest populations establishes a precise network of skeletomuscular connectivity, always linking the visceral skeleton to the braincase via muscle connective tissues of the same rhombomeric origin. This allows evolutionary molecular changes on all three regionalising branchial arch axes during ontogeny without ever rendering their products, the visceral skeleton unfunctional. The elucidation of how exactly the molecular mechanisms of branchial arch regionalisation changed during vertebrate evolution are now lying ahead of us in a comparative approach of molecular embryology.

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The role of gravity and genetic redundancy in the Evolutionary Emergence of Multicellular Development and Life Spans.

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The increase in the number of Biological Experiments in Space carried out during the last 10 years has made quite obvious the following paradox: While experiments carried out with relatively simple biological systems, such as isolated and tissue culture cells, have given the overall consistent result that eucaryotic cells are able to sense and respond to the absence of gravity by modifying their reactions, specially those connected with cell proliferation and signal transduction pathways (for instance, Protein Kinase C activation), experiments in which more complex processes have been investigated, such as Biological Developmental Systems exposed to Microgravity have been surprisingly unaffected by the Space Environment. This is in spite of the fact that signal transduction pathways and cell proliferation are strongly involved in developmental decisions and mechanisms, and that currently, the range of developmental systems exposed to this abnormal environment is quite large (from nematodes, fruit flies, sea urchins, fishes and frogs, to name a few). In this presentation we will discuss the idea that at a certain phase during the evolutionary emergence of multicellular organisms the cues laid by generic forces such as gravity that were possibly involved in the organization of the primitive multicellular organisms may have become obscured/redundant by the appearance of new, environmental independent biological regulatory mechanisms capable of reproducing the same processes originally driven by the gravitational vector. The still poorly understood cellular and molecular mechanisms underlying these processes may be one of the reasons for the small effect of the absence of gravity on development, in the same way as the involvement of diffusion-controled autoorganizative processes has been very difficult to establish in present developmental systems, since new mechanisms based on transcriptionally related gene networks evolved possibly substituting the previous generic mechanisms. Due to the key importance of the coordination and performance of these different activities and operations for the overall process of development, biological systems evolved quite early during the appearance of Multicellularity the capability of imitating and assimilating the processes that had been before driven by generic and physical forces such as gravity, diffusion-driven reactions, etc. The increasingly recognised role of the redundancy of the biological processes involved in Development may be another aspect of the same phenomenon, guaranteeing the robustness and high probability of success of these critical processes. On the other hand, behavioural responses that may be important, for example, to set the life spans of organisms may be still readily susceptible to manipulation by external cues as experiments carried out in Space by our group with *Drosophila melanogaster* suggest.

Transcription factors regulating odontogenesis

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The molecular mechanisms governing the decision between molariform and incisiform patterns of rodent dentition are not yet known. Transcription factors are regulators of place-dependent morphogenesis and key coordinators of gene activity during developmental processes. Here, we analysed the expression of several transcription factors during mouse tooth development and studied its regulation in dental explants. *Otlx2/Rieg* is a homeobox gene involved in Rieger syndrome, a human disorder characterized by dental hypoplasia. *Otlx2/Rieg* expression distinguishes stomatodeal epithelium well before tooth initiation, and thereafter its expression becomes restricted to the epithelia of both molar and incisor primordia. Tissue recombination experiments showed that the maintenance of its expression requires signals from the mesenchyme. The recently identified homeodomain transcription factor *Barx1* is first expressed in mesenchyme of the first branchial arch, but during advanced developmental stages the gene is exclussively expressed in the mesenchyme of molar primordia and strictly complements the expression pattern of *Otlx2/Rieg* in dental epithelium. Finally, the *Sry*-related transcription factor *Sox9* is expressed in epithelial components and to a lesser degree in condensed mesenchyme of the developing teeth. These results suggest that *Otlx2/Rieg*, *Barx1* and *Sox9* participate in the hierarchical cascade of factors involved in the regulation of tooth morphogenesis.

CAUDAL GENE FUNCTION IN DROSOPHILA ADULT

Caudal is an homeobox containing gene first detected as maternal transcripts that form a gradient with maximum levels at the posterior pole of the egg (Mlodzik et al, 1985). This gradient is replaced later in development by zygotic transcripts wich also accumulate in the posterior of the embryo, parasegment 15 in the epidermis. Zygotic caudal mutants eliminate the tuft, the anal pads appear reduced and the anal sense organs look abnormal. The mutant phenotype closely correspond to the pattern of expression (McDonald&Struhl, 1986). The caudal gene is also expressed in the genital disc, specifically in a posterior portion of the dorsal epithelium that gives rise to the anal plates and the rectal ampulla of the hindgut in the adult, and both male and female flies carrying the cad1 allele lack anal plates.

We have used a new technique that allows direct visualization of gene patterns in adult Drosophila (Calleja, M.; Moreno, E.; Pelaz, S. and Morata, G. (1996) Visualization of Gene Expression in Living Adult Drosophila. Science, 274, 252-255.) to show -using a p-element insertion located at 38E by in situ to polytene chromosomes and wich behaves as an hipomorphic mutation of caudal- that caudal expression is restricted to female and male analia.

To study adult function of caudal we performed an ectopic expression experiment using GAL4-UAS method. From this experiment it is shown that ectopic expression of caudal protein can transform dorsal appendages like the wing -but not the haltere- towards analia and, as analia has a sexual dimorphism, transformation conserve this character. We have not been able to transform trunk regions towards analia.
The experiments suggest that: 1) caudal is an homeotic gene required for providing analia identity, 2) the analia is an appendage and 3) caudal is phenotipically suppressed by other homeotic genes like Ubx.
As there have been found several homologues of caudal in vertebrates and other insects with a similar pattern of expression and mutant phenotypes (for example Cdx2 haplo-insufficient mutant mice display a shortened or kinky tail) I propose that they are all members of a family of genes required for providing the homeotic information of terminal structures.

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Functional Dissection of the "YPWM" motif in Antennapedia.

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Protein sequence comparison of many homeotic gene products showed that in addition of the homedomain exist another highly conserved peptide region, the "YPWM" motif. This conserved motif is located upstream of the homeodomain in most of the homeotic genes and its connected to the homeodomain by its N-terminal flexible arm. Many homeotic genes share more than than just the tetrapeptide, Antennapedia (Antp) from different species share 12 amino acids in this region.

In order to test the functional role of this conserved region in Antp, we have analyzed a series of alanine substitution in transcriptional assays in cell culture and *in vivo* by expression in Drosophila embryos and larvae. We have found that the flanking regions of the conserved "YPWM" motif activate transcription and generate homeotic transformations very similar to the wild-type Antp embryos. Surprinsingly, alanine substitution of the "YPWM" motif had a dramatic effect in Drosophila, no homeotic transformations were observed in embryos. Compared to the wild-type Antp, the "YPWM" mutated proteins are over-expressed in Drosophila embryos at the same level, targeted to the nucleus, bind to target sites *in vitro* and the transcriptional activation potential had not been affected. Rather, it appears that were more effective activating the transcription.

Our results suggest that the conserved "YPWM" motif is crucial for the biological activity of the Antp protein and may be involved in protein-protein interactions *in vivo* that define target specificity.

bicoid as a phylogenetic addition to the insect body plan

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Both bicoid (bcd) and hunchback (hb) have been shown to act as morphogens in patterning the fly embryo along the anteroposterior axis. *hb* has been identified in a wide variety of arthropods and annelids, whereas *bcd* seems restricted to higher dipterans. Therefore, *bcd* might be a newly acquired gene which serves in head development and gradually replaces the function of the ancient morphogen hb.

Here we show that wild-type levels of *bcd* activity are sufficient to pattern the embryonic head in the absence of *hb* activity. However, hb becomes absolutely essential for bcd function, when the level of *bcd* activity is reduced to about half of wild-type level, an amount which is sufficient for normal anterior development in the presence of hb. bcd and hb are therefore able to synergize with each other in order to organize anterior development.

The problem in studying the specific roles of the morphogens bcd and hb lies in the bcd-dependent activation of *hb*. Thus, whenever *bcd* activity is altered, the *hb* activity is also indirectly changed. To overcome this problem, we have developed a system that allows us to study the two morphogens independently of each other.

Currently, we are investigating whether the bcd-dependent expression of *hb* is indeed required. It has previously been shown that zygotic *hb* expression can completely substitute for the lack of maternal hb. We have indications that maternal hb can similarly substitute for the zygotic, bcd-dependent *hb* expression. This suggests that the two morphogenetic systems, bcd and hb, do not need to be directly linked, which would reflect their independent evolutionary origins.

It has previously been shown, that bcd and hb have redundant functions in patterning the central region of the embryo. Here we show that bcd and hb have also redundant functions in suppressing the formation of posterior terminal structures at the anterior. However, only bcd seems able to induce the development of anterior terminal and head structures by itself. Therefore, bcd seems absolutely required and also sufficient for the establishment of the most anterior part of the fly embryo. This raises the question of how this region is defined in non-dipteran organisms.

To address this question, we are testing whether, in the presence of the hb gradient, the graded distribution of bcd is dispensible. Other studies have shown that anterior head structures develop even in the presence of low levels of bcd. We are examining whether bcd distributed equally along the anteroposterior axis is able to rescue the lack of normal bcd activity, as long as the hb gradient is unaltered. It is conceivable that bcd acts as a 'generic' activator which is required for the activation of anterior specific genes, but whose graded distribution is in principle unnecessary. This could lead to speculations about which other 'generic' activators might fulfill this role in organisms lacking the *bcd* gene.

List of Invited Speakers

Workshop on

DEVELOPMENT AND EVOLUTION

List of Invited Speakers

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