reduction in the number of sex combs, or a proboscis to maxillary pulp transformation expected for loss of SCR activity. This indicates the PP2A-B’ activity is dispensable to development. A possible explanation for this observation is that the other PP2A activities assembled with distinct regulatory subunits compensate for the lack of PP2A-B’ activity.

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Program/Abstract # 406
Molecular fluctuations and interpreting spatial patterns, applied to Hunchback pattern formation
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Early Drosophila segmentation is specified by spatial gradients of transcriptional regulators, typically on hundreds to thousands of molecule copies per nucleus. Zygotic expression of segmentation genes can be affected by the fluctuations of these upstream gradients. Another, potentially greater, source of noise is the inherently stochastic nature of reactions involved in binding regulators, and transcript and protein production. From our experimentally-tested deterministic model of hunchback (hb) transcription under the control of Bicoid and Hb proteins, we made a stochastic version with which to investigate these issues. Real initial zygotic activation can be noisy; our simulations indicate that maternal Hb may reduce this, and could be one of its key biological functions. Multiple binding sites play a role in precise expression levels: simulations with high (wild-type) numbers of binding sites display less noise than those with lower (e.g. artificial promoter) numbers. Other flies display more or less bcd-binding sites than D. melanogaster (from 10 in Musca domestica to 4 in D. viridis). Our model robustly forms expression pattern across this evolutionary variation. We predict RNA pattern should be much noisier than protein pattern, and also show that Hb self-regulation can play a strong role in noise reduction. Overall, we find that the low copy numbers of both the DNA (numbers of promoter binding sites) and RNA can introduce strong noise into protein production, perhaps a more dominant effect than upstream ligand fluctuations.

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Program/Abstract # 407
Nerfin-1: A novel binding partner of Scalloped
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Scalloped (SD), a TEA/ATTS domain containing protein, is required for the proper development of Drosophila melanogaster. Despite being expressed in a variety of tissues, most of the work on SD has been restricted to understanding its role and function in patterning the adult wing. In the wing SD interacts with a cofactor, Vestigial (VG). Previous experiments have demonstrated that SD cannot activate transcription on its own and requires VG to form a functional transcriptional complex. The mammalian homolog of SD, TEF-1, is known to bind to several different co-factors. Work on these different cofactors has led to the identification of two SD protein:protein interaction domains: a vestigial interaction domain (VID) and a C-terminal trans-activating domain. Using a series of in vivo and in vitro experiments, we demonstrate that SD interacts with the Nervous finger-1 (Nerfin-1) protein. Nerfin-1 is a Zn transcription factor that is expressed in neural precursor cells and in the eye imaginal disc. Furthermore, we show that both Nerfin-1 and VG contain a similar domain that is able to recognize and bind to the VID.

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Program/Abstract # 409
Clearing up the fog in frog embryonic blood development
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The transcription factor Gata-1 and its cofactor Friend of Gata (Fog) are required for red blood cell (RBC) development in mice. Conversely, Fog acts exclusively as a negative regulator of blood in Drosophila. We propose to clarify its role in the evolutionary intermediate, Xenopus laevis. Our preliminary data suggest that in the context of RBC development, frogs are more like humans and mice than flies. Using morpholinos we have shown that knockdown of Fog in Xenopus embryos causes a reduction in primitive RBCs. Our findings are in contrast to the current model based on overexpression studies which predicts that Xenopus Fog inhibits RBC development by recruiting the co-repressor CtBP. To resolve these contradictory findings, we have generated a series of Fog mutants in which known repressor-binding domains have been disrupted. Over-expression of these mutants also results in loss of blood. Together with our morpholino studies, these data support the hypothesis that the reported loss of blood in Fog-overexpressing embryos is due to a dominant-negative squelching effect by which other limiting co-factors are sequestered away from the target promoter(s). To identify domains of Fog that are required for normal function or for squelching, we will overexpress mutant forms of Fog and ask whether they can rescue RBC development in Fog morphants, or suppress RBC development in wildtype embryos, respectively. This will allow us to identify functional domains that are required for normal erythropoiesis, or for squelching and to potentially identify novel binding partners that may be important for Fog’s role as an activator of blood development.

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Program/Abstract # 410
HMGA proteins in Xenopus laevis
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HMGA proteins are chromatin “architectural modifiers”, bearing three conserved “AT-hook” motifs with which they bind to DNA AT-rich regions to assist in gene transcription. We report the developmental expression of Xenopus laevis hmga2β (Xhmgax2β) and ofhxmgax (Xhmga), a gene encoding a highly divergent HMGA with eight AT-hooks. Xhmgax2β transcripts are first detected before the midblastula transition (MBT) by RT-PCR and then become more abundant. By in situ hybridisation (ISH), localized transcripts are first detected at neurula stages, in the presumptive central nervous system (CNS) and eye field. At tailbud and tadpole stages, Xhmga2β mRNA is detected in the CNS, in the otic vesicles, in neural crest cell derivatives, in the notochord and
in the medio-lateral mesoderm. Xhmgax expression is detected, by RT-PCR, from stage 2 to tadpole stage and then decreases at tadpole stage. Xhmgax mRNA is detected by ISH at the tadpole and tailbud stages. Localized mRNAs are present in various head regions, and in particular in the hindbrain, optic and otic vesicles, and in the branchial arches. We also investigated the biochemical properties of XHMGA2β. In GST-pull down assays, we found that, similar to murine HMGA1, it is able to interact with OTX/CRX homeodomain transcription factors. Furthermore, EMSA showed that the DNA-binding properties of XHMGA2β, but not of XHMGAx, are shared with the human homologue. In order to address the functional roles of both Xhmgax2β and Xhmgax, gain and loss of function experiments are underway. 5458

Program/Abstract # 411
Assessing the effects of Ca²⁺ activity on transcriptional regulators of neurotransmitter phenotype
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The acquisition of neurotransmitter phenotype is critical to the function of the nervous system; however, the developmental mechanisms by which neurons acquire neurotransmitter phenotypes are not well characterized. While the role of transcription factors has been emphasized in previous work, it has also been suggested that discrete frequencies of intracellular Ca²⁺ transients may be correlated with specific neurotransmitter phenotypes. We are interested in how Ca²⁺ activity interacts with programs of gene regulation. To determine if Ca²⁺ mediates gene expression at the transcriptional level, primary cell cultures in 0, 2, 10, 25, and 50 mM Ca²⁺ have been prepared from neural plate-stage Xenopus laevis embryos. We are using qRT-PCR to assess levels of expression of GABAergic and glutamatergic phenotypic markers as well those of transcription factors implicated in GABAergic versus glutamatergic phenotypes. We predict that levels of Ca²⁺ activity will be different in neurons cultured in different concentrations of Ca²⁺, which we are analysing with confocal microscopy and the Ca²⁺ indicator Fluo-4. If Ca²⁺ transients are influencing neurotransmitter phenotype determination at the transcriptional level, changes in activity may correspond with changes in the levels of expression of transcription factors regulating these phenotypes. Together, these data will provide insights into the interactions between Ca²⁺ activity and the developmental genetics underlying neurotransmitter phenotype.

Fibroblast Growth Factors (FGFs) are secreted molecules that activate the RAS/mitogen-activated protein kinase (MAPK) signaling pathway. In zebrafish development, FGF signaling is responsible for establishing dorsal polarity, maintaining the isthmic organizer, and ventricle formation. To understand how FGFs control these processes and dictate gene expression, we are studying the transcriptional regulation on an FGF target gene, dual specificity phosphatase 6 (dusp6). We have identified several putative DNA binding sites within the dusp6 promoter, including consensus sequences for Pea3 Ets transcription factors. Since several Ets factors are known transcriptional mediators of MAPK signaling, we hypothesized that Ets factors function to mediate FGF signaling processes. However, functional studies have been difficult due to redundant roles of Pea3 Ets factors in development. The importance of a specific Pea3 binding site within the dusp6 promoter was determined by EMSAs, and we demonstrate binding of an Etv5 Ets domain to this site. In addition, our gain-of-function studies show that over-expressing an activated form of Etv5 can induce dusp6 transcripts, thus indicating the importance of Ets factors to activate FGF target genes. In loss-of-function studies the concerted depletion of Ets proteins, Ern, Etv5, and Pea3, evoked phenotypes reminiscent of the fgf8 zebrafish mutant, including the disruption of the mid-hindbrain boundary and altered heart formation. These results reveal the requirement of Ets factors in maintaining FGF signaling in crucial developmental processes.

The acquisition of neurotransmitter phenotype is a critical step in the development of the central nervous system and requires extensive coordination of gene expression and intercellular signaling. While the terminal differentiation genes and many of the regulatory factors necessary for neurotransmitter phenotype specification have been identified, the timing of neurotransmitter phenotype acquisition and the precise mechanisms remain unclear. We have used fluorescent in situ hybridization and confocal microscopy to examine the expression of terminal differentiation genes of GABAergic (xGAD67, xGAT1, xVIAAT) and glycinergic (xGlyT2, xVIAAT) phenotypes in swimming tadpole-stage Xenopus laevis embryos. Our data show that despite the functional necessity for xVIAAT in GABAergic and glycinergic neurotransmission, 26% of cells expressing xGAD67 and 14% of cells expressing xGlyT2 in the spinal cord do not express xVIAAT. xGAD67 and xGAT1 also have significant areas of distinct, non-overlapping expression, even though both are necessary for GABAergic neurotransmission. Furthermore, approximately 15% of cells in the spinal cord expressing either xGAD67 or xGlyT2 coexpress the other. These data suggest that GABAergic and glycinergic neurotransmitter phenotype identities may not be firmly established or mutually exclusive during early development. We are analyzing the expression of transcription factors implicated in GABAergic and glycinergic specification to determine if different sets of factors specify cells which express multiple phenotype markers.