this process in order to elucidate the underlying mechanisms that regulate eye morphogenesis. Our initial interest in analyzing Bves expression in the eye was to test our overall hypothesis that Bves regulates epithelial adhesion and movement during organogenesis. The current study demonstrates that Bves localizes to epithelial precursors of the eye early in embryogenesis. Early in development, Bves is localized to the apical regions of epithelial precursors in the cornea, lens and retina. Later in morphogenesis and in the adult, Bves is redistributed in a cell type-specific manner. Finally, morpholino knockdown experiments with a cultured human corneal cell line suggest that Bves may play a role in epithelial movement during corneal sheet formation and regeneration *in vitro*.

Bves is expressed in all three epithelial sources that contribute to the cornea, lens and retina. During the initial morphogenetic period of eye development when the epithelial primordia are simple and non-stratified, Bves remains at the apical region of each epithelium. In the case of the retina, Bves is localized to the apical regions of the neural and pigmented retina prior to and at the time of optic cup apposition in the chick (see Figure 34C) and mouse (data not shown). Later when the definitive layers of the retina are formed, Bves expression persists but is redistributed within specific layers of the differentiated retina. In addition, it colocalizes with components of the adherens and tight junctions. This is not surprising as proteins of tight, adherens and gap junctions have been shown to colocalize during epithelial formation and movement (Vasioukhin and Fuchs, 2001). During the stratification and differentiation of retinal and corneal epithelia, Bves localization varies in the specific epithelial strata (Figures 34 and 36). In the case of the lens, Bves expression is retained at the apical portion of the anterior epithelium, possibly participating in maintaining this structure as an epithelium but permitting lateral cellular movement that is necessary for replenishing primary fibers (Figure 35).

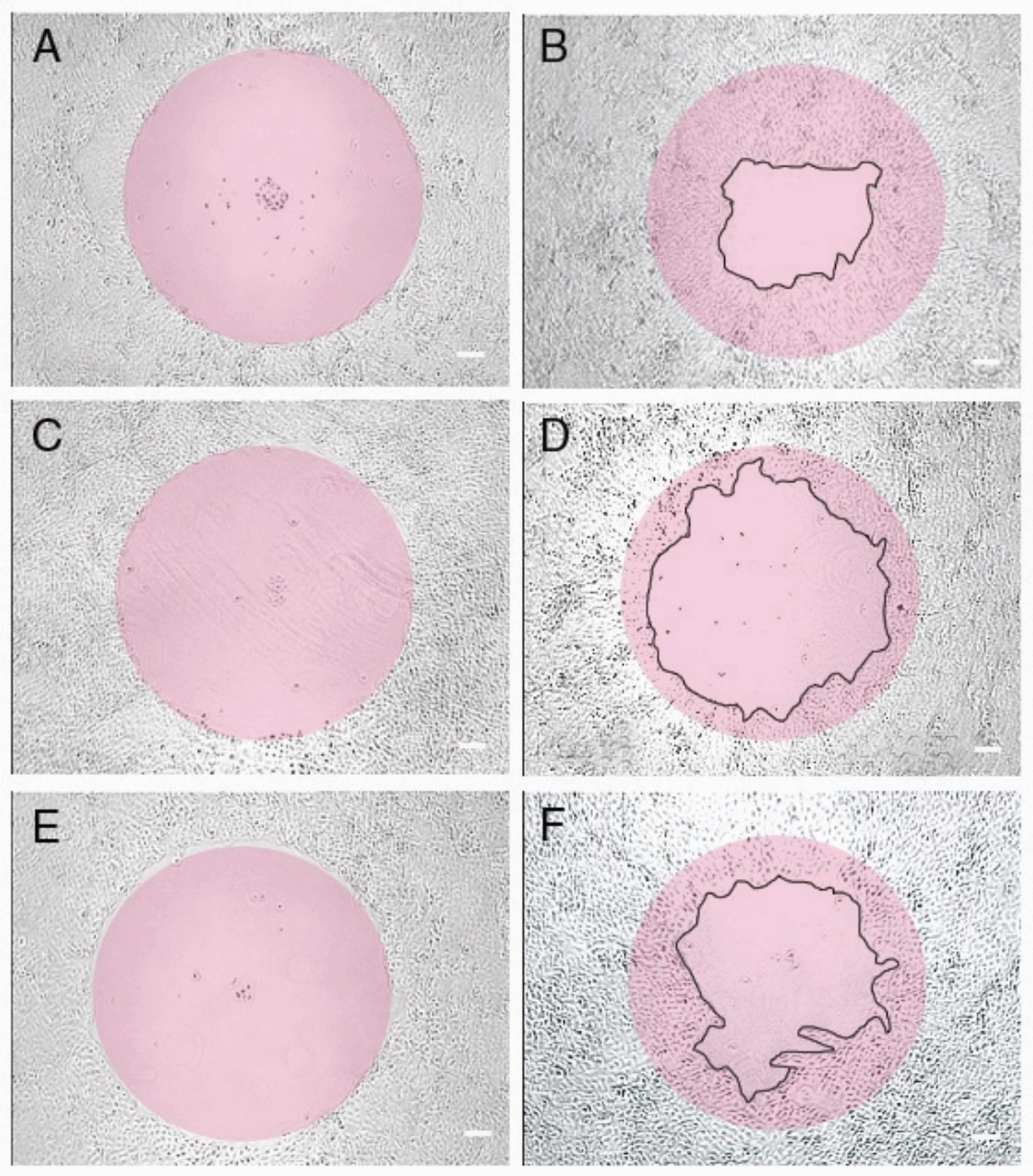

Figure 40. Quantification of corneal epithelial regeneration. The original wound area in cells treated with *Bves* morpholino (A), control morpholino (B), and no treatment (C) are shown in pink. The movement or growth of cells into this area 24 hours post-wound is shown respectively in these same cultures by the black outline (B,D,F). All scale bars = 50 um.

Table 1. Corneal growth after wounding. Percentage of the average area of cell growth of these groups at selected time points is presented. Growth of the no treatment group is subtracted from the experimental groups and given as zero. Variation is standard error of the mean.

	Bves morpholino	control morpholino	no treatment
24 hours	64 ±2 * †	54 ±4	55 ±3
48 hours	71 ±2 * †	63 ±3	55 ±4
72 hours	73 ±2 * †	65 ±3	60 ±5

* † $p < 0.05$ Bves morpholino vs. control morpholino and no treatment. No significant difference
between control morpholino and no treatment, N=7, SEM is reported.

Interestingly, the expression of Bves appears to be lost after the lens epithelial cells differentiate into lens fibers. This is seen in the sharp demarcation of Bves expression at the lens bow region. The redistribution of Bves in the adult retina, lens and cornea may also suggest a related but second function for this protein in the differentiated eye.

Bves expression in the developing and adult cornea is particularly interesting. First, Bves is expressed in the surface ectoderm prior to corneal induction (Figure 35H). As the corneal placode forms, Bves is expressed in these cells but is not restricted to them and is seen in the adjacent, non-corneal ectoderm. Still, as seen in figure 36, Bves protein quickly becomes restricted to the cornea during its stratification as expression is lost at the corneal-scleral junction**.** Corneal-specific expression of other gene products, such as keratin 12, has been observed during the stratification stage of development (Kiritoshi et al., 1991; Rodrigues et al., 1987).

Our current *in vitro* studies on the formation and regeneration of corneal epithelium suggest a role for Bves in wound healing. In these cultures, Bves is observed at points of cell-cell contact during corneal sheet formation. This is consistent with our previous work showing

that Bves staining at the cell surface precedes that of cadherins in cultured epicardial cells and suggests that the role of Bves in the initiation of cell-cell interaction may be conserved (Wada et al., 2003). Likewise in our wound healing experiments, Bves (Figure 38) and other markers of cell adhesion complexes (data not shown) are not seen at the surface of cells that migrate into the gap between the wound surfaces. At present, we are not certain whether these migratory cells have completely lost contact with epithelial cells juxtaposed to the wound surface or whether the cells maintain partial adhesion with their neighbors. In either case, the loss of these diverse adhesion markers in specific cells suggests a coordinated mechanism for activated migration during wound healing *in vitro*.

When cultures are treated with Bves morpholino in an effort to disrupt protein function, specific changes in epithelial sheets are observed that are not seen in control groups. First, epithelial sheets in morpholino-treated cultures are more irregular in appearance. These samples have many regions where epithelial continuity is breached. In these regions, Bves localization as visualized by antibody staining is diminished or lost. We postulate that morpholino treatment decreases the level of Bves protein in these cells. In turn, cells become less adherent or interactive with each other resulting in disorganization of the epithelial sheet. Next, morpholino-treated cultures show a distinct difference from control cultures in response to wounding. In these experiments, treated cells move into the wound area more rapidly than non-treated preparations. Our interpretation of these results is that impairment of Bves function leads to instability of cell-cell adhesion or interaction at the wound surface resulting in an early release of cells into the wound. Still, these cells do not form a continuous epithelium during the process of regeneration leading to the irregular nature of the newly formed sheet that we previously observed in non-injured, morpholino-treated samples.

The present data must be viewed in light of recent studies on the *pop1*-null mouse (Andree et al., 2002). In those studies, no overt developmental phenotype was seen in the eye or any other organ system although a delay in muscle regeneration, possibly due to a delay in myoblast fusion was observed. There are several facets to consider when comparing the two studies. First, there is a significant difference between examining *in vivo* development of a whole embryo and analysis of epithelial integrity and repair *in vitro.* The Andree et al. (2000) study correctly focused on potential phenotypes in striated muscle as the transcript is seen at its highest levels in these tissues and did not examine potential problems in optic development or repair. However, this study did indeed observe a phenotype in muscle regeneration that may reflect a problem in cell-cell interaction. It should be noted that the experiments we present here are extremely simple and highly focused. Obviously, additional experiments concerning Bves function and function of the *popeye* gene family as a whole must be conducted to delineate the actions of these proteins in development and repair.

In summary, this study demonstrates the expression of the Bves in embryonic and adult ocular tissues. While the role of Bves in organogenesis of the eye and maintenance of the tissue morphology in adult life has yet to be determined, our studies inhibiting Bves function in corneal epithelial sheet formation and regeneration suggest a role for this protein in these processes.

CHAPTER V

CONCLUSION

Epithelial movements/cell adhesion during developmental events

The participation of Bves in gastrulation movements and eye morphogenesis, although two seemingly different processes occurring at different times during embryogenesis and involving distinct series of differentiative events, provides a common link that supports our hypothesis that Bves plays an adhesive role in epithelial movement and morphogenesis during development. I decided to test this hypothesis by examining Bves function in *X. laevis* gastrulation due to the previous data suggesting Bves is a cell adhesion molecule (Wada et al., 2001), *bves* expression during early embryogenesis, and the simplicity and capability to manipulate the *X. laevis* early embryo. As a second example of Bves function in epithelia, I chose to examine eye formation after I determined strong Bves protein expression in the lens and retina, a resultant eye phenotype in the *X. laevis* embryo after morpholino injection, and the availability of an easily manipulative *in vitro* model of corneal epithelial function. As different as these two developmental processes are, Bves appears to provide a common adhesive function that extends not only to these two events, but many other developmental events that merit further research.

There are many cell adhesion mechanisms that regulate the way that cells are organized in tissues. Stable cell interactions are required to maintain mature tissue integrity, while morphogenesis of developing tissue requires dynamic changes in cell adhesion in both epithelial and mesenchymal cells. Among these molecules, cadherins and integrins are involved with

mechanisms of cell movement. Cadherins are homophilic transmembrane adhesion molecules that are important in cell recognition and cell sorting during development (Takeichi, 1991). These molecules are required for cells to remain tightly associated with each other and their assembly is essential for the stability of many other cell adhesion and junctional proteins. Adhesion is also involved in the cell arrangements needed to produce various tissue architectures. Condensation or compaction of cells occur in many developing tissues and organ rudiments by reinforcing cell recognition and sorting processes that cause the segregation of cells into distinct regions of tissue. Examples include the requirement of E-cadherin in the epithelial formation of the mouse blastocyst (Fleming and Johnson, 1988) and epiboly movements during *X. laevis* gastrulation (Levine et al., 1994), EP/C-cadherin for convergent extension of *X. laevis* animal cells (Lee and Gumbiner, 1995), and N-cadherin participation in the condensation of mesenchymal cells during chondrogenesis (Oberlender and Tuan, 1994).

Adhesion processes are also associated with cell motility and cell migration. The same adhesion processes that regulate compaction and condensation are the ones that mediate looser noncompacted adhesion necessary in migrating cells, either in epithelium or mesenchymal cells. In turn, morphogenetic changes in tissues can take place by local cell rearrangements. Epithelial and mesenchymal cell migration often results in changes in the shapes of tissues and the embryo as a whole. Cells can either migrate individually, as in chick gastrulation, or as an adherent piece of tissue, as occurs in amphibian gastrulation. Local cell rearrangements involve intercellular motility where cells move with respect to their neighbors. An important example of this is during optic vesicle formation. It is imperative that a specific group of neuroectodermal cells migrate or move laterally to form the optic stalk. In this case, these cells move as an epithelial sheet, maintaining cell association as the structure changes shape. It is unknown what signals are

involved with the induction of epithelial remodeling, but the maintenance of epithelial integrity through cell junction complexes is vital for continued formation of the optic nerve and retina. As shown in Chapter 4, Bves expression in the forming retina and lens suggests a function for Bves in maintaining epithelial integrity as these cell populations migrate and change shape. Studies are underway to examine Bves in the morphogenesis of the eye using the *X. laevis* as a model system (discussed below).

Cell adhesion is critical for proper embryonic development and adult tissue regeneration. Cell adhesion molecules play an essential role in tissue organization and allow for proper tissue and organ physiological functions. Regulation of tissue morphogenesis requires complex interactions between adhesion molecules, the cytoskeleton, and multiple signaling pathways. The synergism between physical adhesion and developmental signaling supplies the means to tightly integrate physical aspects of tissue morphogenesis with cell growth and differentiation, allowing for proper patterning of the embryo. While no Bves signaling events are known, inhibition of Xbves function *in vivo* results in the inhibition of epithelial movement and gastrulation, illustrating the importance of proper cell adhesion during development.

A delicate balance of cell adhesion is necessary to maintain structural integrity of the cleaving embryo, cell migration during gastrulation, cell sorting for organogenesis, and cell regeneration during adulthood. An ever-changing pattern of cadherin expression is observed throughout development that separates distinct groups of cells by aggregation. From the very first cleavage event, maternal EP/C- and XB/U-cadherin are localized on all newly formed membranes (Heasman et al., 1994b; Muller et al., 1994). These proteins provide interblastomere adhesion necessary to form the blastocoel cavity that will set up the structural organization to prevent cell intermixing and allow for proper cell migration during gastrulation (Nieuwkoop,

1973). Without theses two critical cadherins during cleavage stage, the *X. laevis* embryo would loose its structural integrity and fall apart (Heasman et al., 1994b; Muller et al., 1994). Inhibition of the *Xbves* maternal message by injection of antisense primers into the *X. laevis* oocyte would be indicative of Xbves adhesive activity before gastrulation. Based on mRNA and protein expression, I predict that Xbves is important in maintaining cell-cell adhesion in the cleaving embryo.

Gastrulation requires changes in cell morphology, cell adhesion, and cell migration that reorganize the embryo into three specific germ layers. In the *X. laevis laevis,* the organism I have focused my research on, morphogenetic movements include epiboly of the animal cap, convergent extension of the involuting and non-involuting marginal zone and migration of involuted mesodermal cells on the blastocoel roof (Keller et al., 1992; Keller and Tibbetts, 1989; Wilson and Keller, 1991; Winklbauer, 1990; Winklbauer and Nagel, 1991; Winklbauer et al., 1992). Cell adhesion must be modulated in an intricate way when cells slide past each other during gastrulation or when boundaries are formed during germ layer or notochord formation. Another member of the cadherin family, E-cadherin, is important during these gastrulation movements. Unlike XB/U- and EP/C-cadherin, E-cadherin is not expressed until after mid-blastula transition (MBT) (Choi and Gumbiner, 1989; Levi et al., 1991). Studies have shown that E-cadherin is necessary for maintaining ectodermal integrity during epiboly and dominant negative E-cadherin expressing embryos are unable to complete gastrulation (Levine et al., 1994). This is a prime example of how expression of different cadherin family members switch on and off according to necessity. This helps us make predictions about possible phenotypes when examining the novel cell adhesion molecule, Bves.

Organogenesis is yet another example of the involvement of cadherins and epithelial movement in development. The first event in organogenesis of the central nervous system is initiated by the interaction between the dorsal mesoderm and its overlying ectoderm. The chordamesoderm directs the ectoderm to form a neural tube that will differentiate into the brain and spinal cord (Balak et al., 1987; Dettlaff, 1983; Dickinson et al., 1995). N-cadherin is expressed exclusively in cells of the neural plate that will fold into the neural tube, while outlying epidermal cells express E-cadherin (Detrick et al., 1990). The N-cadherin expressing cells sort from the E-cadherin expressing cells, causing aggregation amongst cells that will later differentiate into proper cell types. This is a classic example of how cell adhesion molecules regulate cell sorting required for organogenesis. Participation of various cadherin members during cleavage, gastrulation, and organogenesis has proven to be essential for proper development. The dynamic expression of cadherin family members throughout various developmental processes provides an example of how Bves may play a role in multiple processes during development.

Characterization of Bves

bves is a novel gene product first identified from a subtractive hybridization of stage 18 chick hearts (Reese et al., 1999). This stage represents a dynamic time during heart formation in which heart tube looping, myocyte proliferation, trabeculation, and epicardial differentiation occurs. Reese et al. (1999) first described this gene product in chicken as uniquely expressed in the proepicardial organ and a subset of its progeny that give rise to vascular smooth muscle in the coronary arteries of the heart. Development of chick Bves specific antibodies allowed continued study by Wada et al. (2001) that showed dynamic subcellular localization in cells that

formed an epithelial sheet and those that delaminated to form freely migrating mesenchyme. Immunocytochemical analysis of rat epicardial cells with the Bves and E-cadherin antibodies show Bves localization at the cell surface in cells that formed cell-cell junction (Wada et al., 2001). Additionally, L-cells transfected with *bves* displayed adhesive properties, compared to nontransfected cells (Wada et al., 2001). Andree *et al.* (2000) subsequently reported identification of three family members called POP1-3, POP1a being identical to *bves*, and the others showing significant homology. The diverse expression pattern of these additional members implies additional roles of this gene family through various stages of embryogenesis. These data, along with our understanding of cell adhesion in epithelial sheet formation and dynamic cell movement, and *bves* RNA expression in the early *X. laevis* embryo are suggestive of *bves* playing a role in epithelial cell adhesion during gastrulation movements, including epiboly and involution. Still, no *in vivo* studies show a phenotype and probable Bves function in development. Thus, my experiments used a broad approach to examine gene function. In these studies, I looked for cellular and tissue phenotypes that may be predicted by previous examples of the traditional adhesion molecules.

***Xbves* participation in *X. laevis* gastrulation**

X. laevis gastrulation involves the coordination of several distinct events driven by region-specific movements that are associated with a complex balance of spatially and temporally regulated cell adhesion (Montero and Heisenberg, 2003; Winklbauer, 1990; Winklbauer and Nagel, 1991). The beginning of gastrulation is marked by changes in shape of bottle cells at the site of the blastopore (Hardin and Keller, 1988). These endodermal bottle cells invaginate in the marginal zone, permitting the marginal cells to involute through the blastopore

lip and continue to pass into the interior as a sheet. The involuting cells at the leading edge of the mesodermal mantle migrate along the inner surface of the blastopore roof towards the animal pole, forming the archenteron (Hardin and Keller, 1988; Keller and Danilchik, 1988). These sets of movement are driven by active repacking of the cells, where cells cooperatively form and align lamellipodia creating interdigitation of cells, resulting in narrowing and elongation of tissue (Keller et al., 1992; Keller and Tibbetts, 1989). This forms the force necessary for the cells to converge toward the dorsal midline and extend along the antero-posterior axis, also known as convergent extension. During this time, the external epithelium in the region of the animal pole spreads to take the place of the cell sheets that have turned inward (Keller and Tibbetts, 1989; Shih and Keller, 1992; Wilson et al., 1989). Migrating mesodermal cells on the blastocoel roof express integrins that bind to the extracellular matrix protein, fibronectin, covered along the roof (Beddington and Smith, 1993; Winklbauer, 1990). Cells undergoing convergent extension in the marginal zone express EP/C-cadherin and XB/U-cadherin (Kuhl et al., 1996; Lee and Gumbiner, 1995; Zhong et al., 1999), while the epiboly of the ectoderm depends upon E-cadherin (Levine et al., 1994). From these studies, we conclude that cell adhesion molecules play a role in cell movements, both in cell adhesion and cell detachment, during early *X. laevis* embryogenesis that are vital for the proper formation of the three germ layers and continued normal development. Given the expression pattern of *Xbves* in early embryogenesis and previous data suggesting a role in cell-cell adhesion, my work tested the hypothesis that Bves played a role in the epithelial movements governing gastrulation.

Adhesion molecules have been shown to be very important in *X. laevis* gastrulation (Brieher and Gumbiner, 1994; Choi and Gumbiner, 1989; Levi et al., 1991; Montero and Heisenberg, 2003). This is illustrated in developing embryos that have been depleted of or

express dominant negative forms of cadherin proteins (Heasman et al., 1994b; Heasman et al., 2000; Kuhl et al., 1996; Levine et al., 1994). C/EP-cadherin is a major cadherin expressed in the blastula and early gastrula and is responsible for cellular adhesion of the early embryo (Heasman et al., 1994b; Lee and Gumbiner, 1995). Heasman et al. (1994b) showed that injection of antisense oligonucleotides into oocytes depletes maternal C/EP-cadherin mRNA and protein levels and results in disrupted blastomere adhesion in whole embryos and in reaggregation assays. C/EP-cadherin at the cell surface is decreased when animal cap explants are induced by activin to undergo tissue elongation in convergent extension (Brieher and Gumbiner, 1994; Smith et al., 1987; Zhong et al., 1999). Dominant negative expression and overexpression of wild type C/EP-cadherin in embryos result in disrupted gastrulation movements suggesting a proper equilibrium of cell adhesion is required for normal morphogenesis (Lee and Gumbiner, 1995).

XB/U-cadherin is a second maternal cadherin expressed ubiquitously on all newly formed cell membranes up to neurula stage (Kuhl et al., 1996; Muller et al., 1994). As previously shown with C/EP-cadherin, XB/U-cadherin is also required for interblastomere adhesion (Heasman et al., 1994b; Lee and Gumbiner, 1995; Muller et al., 1994). Kuhl et al. (1996) examined the role of XB/U-cadherin in gastrulation by injection of a dominant-negative form under the control of a CMV promoter. In embryos expressing high doses of dominant negative XB/U-cadherin, the mesoderm failed to roll over the blastopore lip, resulting in impaired mesoderm involution and migration. The authors hypothesized that XB/U-cadherin is involved in convergent extension movements because in embryos with mutant protein the mesoderm is pushed aside the blastopore and does not migrate over the blastopore lip (Kuhl et al., 1996). Normally, convergent extension of the dorsal involuting marginal zone pushes cells

over the lip (Gerhart and Keller, 1986). When low doses of mutant XB/U-cadherin were applied, embryos proceeded through gastrulation normally, but exhibited a posteriorized phenotype later in development, including malformations of the brain and eye anlagen (Kuhl et al., 1996). Specifically, the eye anlagen had failed to separate from brain tissue in the earliest migration events of the eye vesicle. Additionally, wild type XB/U-cadherin transfected L-cells showed calcium dependent aggregation compared to transfection of mutant forms (Finnemann et al., 1997; Muller et al., 1994). These data confirm that XB/U-cadherin is required for multiple developmental events during cleavage, gastrulation, and organogenesis.

Finally, E-cadherin is a zygotic transcript first expressed at MBT, just before gastrulation (Choi and Gumbiner, 1989; Levi et al., 1991). During gastrulation, E-cadherin expression is restricted to the outer epithelial cells and is more highly expressed in animal cells compared to vegetal cells (Angres et al., 1991). When these two-cell embryos were injected with a dominant negative form of E-cadherin, embryos appeared relatively normal until gastrulation, supporting expression data that E-cadherin is not required until gastrulation processes. At mid-gastrulation holes appeared in the ectoderm of the animal hemisphere, and injected embryos were not able to complete gastrulation due to disruption in epiboly movements (Levine et al., 1994). This phenotype was rescued with co-injection of E-cadherin RNA, but not C-cadherin (Levine et al., 1994). Additionally, when dominant negative N-cadherin was injected, embryos did not display the ectodermal ripping phenotype, but instead resulted in a neural tube defect, as expected (Levine et al., 1994). Again, this selective interference is consistent with the homophilic adhesion specificity of cadherins and the dynamic expression of specific family members of adhesion molecules. The cadherin phenotypes derived from these experiments and the functional data collected provided a model for a solid experimental design in the *Xbves* studies.

The phenotypes observed with disruption of these various forms of adhesion molecules serve as a model for the analysis of Bves function and would predict specific phenotypes for Bves disruption if Bves function is involved with epithelial movement and/or adhesion.

X. laevis embryos have allowed us to examine the role of *bves* in the dynamic cell migration processes during gastrulation. The presence of a maternal as well as zygotic *Xbves* transcript indicates that this gene may play a role during many stages of development. As well, the dynamic spatio-temporal pattern of mRNA expression suggests that *Xbves* may participate in maintaining the epithelial adhesion of a group of cells, while allowing them to migrate past or separate from groups of non-*Xbves* expressing cells. Whole mount *in situs* and immunofluorescence shows *Xbves* mRNA and protein are expressed in the surface ectoderm throughout gastrulation. During this time, the external epithelium in the region of the animal pole spreads to take the place of the cell sheets that have turned inward forming the archenteron and extends to cover the entire external surface of the embryo (Keller and Tibbetts, 1989; Shih and Keller, 1992; Wilson et al., 1989). This mechanism of epiboly is driven by an increase in cell number coupled with change of cell shape and integration of several deep layers into one sheet of cells (Keller and Tibbetts, 1989; Keller, 1980).

Morpholino knock down experiments have shown us that embryos with depleted Xbves protein fail to complete gastrulation. Animal cells that normally express Xbves at the cell surface fail to cover the vegetal cells of the yolk plug in morpholino-injected embryos. Embryos fail to continue development and die shortly afterward. This halt in epiboly movements is also seen in dominant negative E-cadherin expressing embryos (discussed above) (Levine et al., 1994). Furthermore, embryos coinjected with *Xbves* morpholino and *Xbves* rescue RNA can complete gastrulation and continue to develop with minimal defects. Detection of Xbves protein

by immunoreactivity with Xbves antiserum in morpholino-injected embryos reveals a significant reduction in protein levels in morpholino versus uninjected embryos. Thus, specificity of the injected morpholino for *Xbves* RNA is confirmed both by rescue experiments and immunofluorescence data. Therefore, it can be deduced that Xbves is the gene product responsible for this halt in gastrulation.

Detection of *goosecoid* and *Xbra* transcripts in *Xbves* morpholino-injected embryos by whole mount *in situ* hybridization and bisectioning these embryos show that induction of these genes are not affected, but involution and migration of cells into the embryo have been inhibited. Finally, *Xbves* transfected L-cells forms a significant larger percentage of aggregates versus vector-transfected cells, suggesting Xbves confers adhesiveness. These results suggest that the finely balanced equilibrium of cell adhesion and the resultant phenotype seen in embryos lacking Bves is also disrupted.

Bves expression in the eye and function in corneal epithelial sheet regeneration

The discovery that Bves plays an essential adhesive role in epithelial movement and morphogenesis during gastrulation is not the end of the story. The role of Bves during gastrulation may be just one of many developmental events in which Bves participates. Eye formation is a second developmental event in which I postulate that Bves plays a potential role in epithelial movement. The dynamic expression of Bves in epithelia during optic vesicle formation, optic cup fusion, lens development, and cornea maturation is elucidatory to a function in this developmental event. Bves protein is detected in the epithelial layer of the neuroectoderm as it evaginates laterally to form the optic vesicle. Bves expression remains strong as the optic vesicle invaginates and the two apical epithelial surfaces face each other and fuse to form the

retina. While the inner retina layer differentiates into nine neural retina layers, Bves remains highly expressed in the photoreceptor layer, derived from the neuroectodermal epithelia. The outer epithelial layer of the retina differentiates into the pigmented retinal epithelium, and Bves also remains strongly expressed in this single cell layer throughout development. During lens and cornea placode formation, Bves is exclusively expressed in these cells and omitted from surrounding epithelia. Expression remains high in the primary lens epithelium during lens fiber formation and in the corneal epithelium throughout development and into adulthood. This dynamic expression pattern of Bves in epithelial cell populations that are undergoing active reshaping and morphogenesis suggest a function in epithelia during development.

Functional *in vitro* experiments performed in cell culture support the findings from the *in vivo X. laevis* experiments. Morpholino treatment of wounded corneal cells to knock down the expression of Bves protein disrupts the equilibrium of cell adhesion and results in increased rate of migration and irregular sheet reformation. Rather than the generation of a confluent epithelial sheet after wounding with strong cell surface Bves expression, morpholino-treated cell cultures display many gaps and holes between neighboring cells and limited Bves expression. These results illustrate a second example supporting our hypothesis that Bves serves as a cell adhesion molecule during epithelial morphogenesis and migration.

Though gastrulation and eye development occur at different times during development and result in two very different structures, they serve as examples of a fundamental role Bves serves throughout embryogenesis. *Xbves* is expressed in a distinct group of epithelial and migrating cells in the *X. laevis* embryo. *In vivo* studies in the *X. laevis* embryo show that Xbves is required for proper migration of epithelial animal cells. The same migration defect is seen in an *in vitro* model of corneal epithelial cells. When a confluent sheet of corneal cells is scratch

wounded, cells delaminate and start migrating to cover up the wound and reform an epithelial sheet. However, when cells are treated with Bves morpholino, the cells are unable to properly migrate and reassemble the epithelial sheet. Additionally, Bves expression in the eye includes epithelia that require reshaping and morphogenesis to form proper ocular structures suggesting a function in eye morphogenesis. Taken together, these studies have shown that Bves is important in proper epithelial cell migration and morphogenesis.

CHAPTER VI

FUTURE AIMS

The data presented in this dissertation has shown that Bves is a cell adhesion molecule that is involved either directly or indirectly with epithelial migration during morphogenetic processes, specifically gastrulation and eye formation and regeneration. Others have shown the importance in proper regulation and function of cell adhesion molecules in epithelial cell integrity and migration. Therefore, a discussion of cell-cell adhesion and cell junctions is warranted.

Cell-cell adhesion during early *X. laevis* development

Cell-cell adhesion is a mechanism that is critical to all stages of development for a variety of processes, including blastocoel formation, germ layer formation, neural tube formation, and axis specification. A complex networking of membrane, cytoskeletal and signaling molecules is required for proper cellular function. Epithelia are polarized cells that provide a barrier between tissue compartments, as well as separating the organism from its environment. In this section I will discuss the three major types of cell junctions: occluding junctions, anchoring junctions, and gap junctions, as illustrated in Figure 41, and give an example of how each serves a function in early embryogenesis. At the cellular level, junctional complexes and their resident proteins are responsible for separating apical and basolateral compartments. I will end the discussion with my hypothesis on the possible function of Bves in these junctions and propose experiments to test this hypothesis.

Cell junctions

The two roles of occluding junctions, also referred to as tight junctions, are to function as barriers to the diffusion of membrane proteins between apical and basolateral domains of the plasma membrane and to seal neighboring cells together so that water soluble molecules cannot "leak" between cells. These properties that result in the forming of a "fence" between the apical and basolateral membranes are crucial for maintaining a polarized epithelial cell sheet and

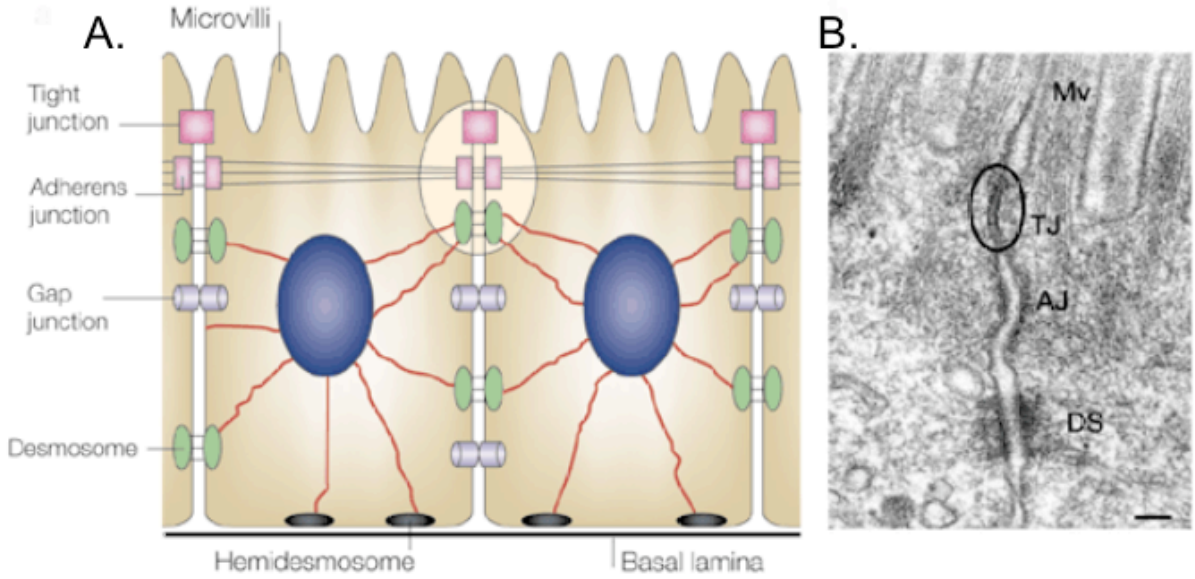

Figure 41. Junctional complexes in epithelial cells. (A) Schematic diagram of cell-cell adhesion complexes between two epithelial cells. (B) Electron micrograph of tight junction (TJ), adherens junction (AJ), and desmosome (DS). (MV: microvilli)

properly facilitating directional transport of molecules into and out of the cell. The four-transmembrane proteins that form tight junctions in epithelia are occludin and members of the claudin family (Furuse et al., 1998; Furuse et al., 1993). Major cytoplasmic components of the tight junction complex are the zonula occludens, ZO-1, ZO-2, and ZO-3, thought to cluster the transmembrane proteins through interactions in their C-terminal PDZ domain (Fanning et al., 1999). Claudins interact directly with tight junction-specific, membrane-associated guanylate kinase homologues, ZO-1-3, and indirectly with AF-6 and the myosin-binding molecule cingulin

(Heiskala et al., 2001). These protein-protein interactions promote scaffolding of the tight junction transmembrane proteins and provide a link to the actin cytoskeleton for transducing regulatory signals to and from tight junctions. Overexpression studies on a recently discovered *X. laevis* claudin family member, *Xcla*, caused cells to adhere more tightly and prevented normal cell dispersion, whereas injection of *Xcla* containing a mutated PDZ domain resulted in a broad dispersion of large groups or clumps of cells in the gastrulating *X. laevis* embryo (Brizuela et al., 2001). Additional evidence supporting the importance of claudins lies in the ability of *Xcla* to affect cell adhesion and migration, but also to influence left-right asymmetries during early *X. laevis* development (Brizuela et al., 2001). These data show that a tight junction claudin family member in *X. laevis* is required to maintain the proper balance of cell adhesion during gastrulation. Taken together, these studies suggest that alteration of tight junction components lead to major problems in epithelial function, specifically in gastrulation.

The junctional complex in epithelial cells also contains gap junctions, although these are not strictly known as mediators of adhesion. Instead, gap junctions are created by channel forming protein molecules that allow passive diffusion of ions and small water-soluble molecules from cell to cell (Warner, 1987). This type of transport provides intercellular communication through coordinating activities of individual cells by sharing metabolites and ions, such as electrically coupled cardiomyocytes or regulating axis pattern during early development (Caveney, 1985; Guthrie and Gilula, 1989; Levin and Mercola, 1998). Members of the connexin family of gap junction proteins have been cloned and characterized, and share the potential to form a bundle of four helical hydrophobic transmembrane domains (Gimlich et al., 1990; Milks et al., 1988). A chimeric connexin that functions as a dominant-negative inhibitor of intercellular communication injected into 8-cell stage *X. laevis* embryo leads to delamination of

surface ectoderm in the gastrulating embryo (Paul et al., 1995), whereas injection of a blocking antibody against connexin into *X. laevis* embryos, results in blockage of communication at the 32-64 cell stage (Warner et al., 1984). Additionally, the asymmetrical expression pattern of connexin 43, and heterotaxia (uncoordinated inversions of some sided organs) resulting from ventral induction or dorsal inhibition of connexin 43 (Levine et al., 1994), suggests that connexin 43 is involved in coordinating dorsal-ventral and left-right polarity in the early embryo (Guthrie et al., 1988; Olson and Moon, 1992). These results illustrate that gap junction proteins are involved in cell communication and adhesion in part (Paul et al., 1995), as well as axis formation between blastomeres in the early *X. laevis* embryos. Therefore, by altering proteins that regulate cell adhesion and/or interaction, epithelial function and ultimately embryogenesis are affected. As well, altering a protein involved primarily in communication can also alter adhesion. Note the interplay: thus, while Bves has not been definitively proven to be an adhesion molecule, altering its function could change cell adhesion.

The third type of junctions, anchoring junctions, connect the cytoskeletal elements of a cell to either those of another cell or to the extracellular matrix, and include adherens junctions, desmosomes, and hemidesmosomes. Cell-matrix adherens junctions connect the cells to the extracellular matrix by connecting their actin filaments to various proteins in the matrix, such as fibronectin, vitronectin, collagens, and laminin (Albelda and Buck, 1990; Ruoslahti, 1991). In the gastrulating *X. laevis* embryo, fibronectin is localized to the roof of the blastocoel and interaction with cells expressing integrins is thought to play a role in the migration of invaginated mesodermal cells along the roof (Beddington and Smith, 1993). Studies have shown that inhibition of integrin/fibronectin interaction in *X. laevis* results in delayed blastopore closure, axial defects and ectodermal thickening (Johnson et al., 1993; Ramos and DeSimone,

1996; Ramos et al., 1996). Additionally, injection of blocking antibodies against the cell-binding domain of fibronectin *in vivo* have shown that fibronectin is required for the cellular rearrangements that drive epiboly in the marginal zone at gastrulation (Marsden and DeSimone, 2001). Lack of fibronectin is correlated with blastocoel roof thickening and a loss of deep cell polarity (Marsden and DeSimone, 2001). While we think Bves-Bves self-interaction is critical to function, a possible role of Bves could be in cell-matrix interactions, since their disruption can cause changes in epithelial function. Therefore, future studies must consider possible effects on cell-cell and/or cell-matrix activity. These alterations could be primary or secondary effects.

Cell-cell adherens junctions are mediated by the calcium-dependent cadherin family members that connect the actin filaments of adjacent cells, forming an adhesion belt in epithelial sheets (Hirano et al., 1987; Ozawa and Kemler, 1992; Takeichi, 1990). Cadherin-based adhesion has been well characterized and is the primary adhesion system in early *X. laevis* development (Huber et al., 1996), as illustrated by the expression of cadherin family members during cleavage stage. XB/U- and EP/C-cadherin are two cell adhesion molecules that are expressed on all newly formed cell membranes and are responsible for keeping the blastomeres together up to gastrulation stage (Heasman et al., 1994b). If antisense primers against EP/C-cadherin are introduced into the oocyte, the resulting blastomere displays loss of cell-cell adhesion and obliteration of the blastocoel (Heasman et al., 1994b). During gastrulation, zygotic E-cadherin is required for cells to participate in epiboly movements of the animal cap during gastrulation (Levine et al., 1994), while maternal XB/U- and EP/C-cadherin loose their original expression pattern and are ubiquitously expressed throughout. NF-protocadherin (NFPC) is expressed within the deep cells of the embryonic ectoderm and in the neural folds (Bradley et al., 1998). Ectopic expression in *X. laevis* shows that NFPC mediates cell adhesion within the ectoderm

(Bradley et al., 1998). Also, expression of a dominant-negative NFPC disrupts the integrity of the ectoderm, causing deep layer cells to dissociate, but leaving the outer layer intact (Bradley et al., 1998). After gastrulation, a fourth cadherin member, N-cadherin is required for neural tube segregation and closure (Detrick et al., 1990). The cadherin family is an example of critical and dynamic gene expression of adhesion molecules throughout the early stages of *X. laevis* development. These studies show that adhesion is essential for epithelial migration, integrity, and remodeling. Therefore, epithelial integrity is important not only in separating apical and basolateral compartments as discussed above, but also in maintaining epithelial sheets during morphogenesis.

It is important to remember that cell-cell adhesion is not only mediated by the interaction of extracellular binding domains of these cell surface molecules, but is also accompanied by attachment to the cortical cytoskeleton inside the cell. The cytoskeleton assists and stabilizes the lateral clustering of the adhesion molecules to facilitate multipoint binding and to provide tensile strength. The cytoskeleton plays a major role in transmitting and distributing mechanical stresses within the cell as well as in their conversion into a chemical response. This switch into chemical signaling events is a fundamental cellular process that occurs at cell-extracellular matrix contacts, known as focal adhesions (Jamora and Fuchs, 2002). Focal adhesions evolve from small dot-like adhesion sites termed focal complexes: nascent integrin-mediated adhesions formed during lamellipodial protrusions (Geiger and Bershadsky, 2001; Rottner et al., 1999). Focal complexes and focal adhesions are associated with different sub-domains of actin cytoskeleton. Cell motility is initiated by the extension of protrusions, usually in the form of broad lamellipodia. Protruding lamellipodia contain a dense, rapidly polymerizing branching network of actin filaments. Without proper formation and maintenance of adhesion molecules and focal adhesion complexes, cell motility and migration is affected.

A third element that regulates cell-cell adhesion is cell signaling. The Wnt pathway is a well-studied signaling cascade that is implicated in developmental patterning processes (Huelsken et al., 2000; Klingensmith and Nusse, 1994; Larabell et al., 1997; Maloof et al., 1999; Schneider et al., 1996). The cadherin-associated cytoplasmic protein armadillo/ß-catenin controls Drosophila segment polarity (McCrea et al., 1991; Peifer and Wieschaus, 1990; Wieschaus et al., 1984) and induces the dorsal-ventral and anterior-posterior body axes in *X. laevis* (Funayama et al., 1995; Guger and Gumbiner, 1995; Heasman et al., 2000; Montross et al., 2000). Tissue polarity, or planar cell polarity, is a property shown by some epithelia, when the cells become polarized within the plane of the epithelium, along an axis perpendicular to the apical-basal axis of the cell. Experiments have shown that cell-cell adhesion is regulated through the Wnt/Fz signaling pathway during gastrulation (Djiane et al., 2000; Medina et al., 2000; Medina and Steinbeisser, 2000; Tada and Smith, 2000). Convergent extension movements are inhibited in *X. laevis* explants overexpressing the Fz7 receptor (Djiane et al., 2000). Disruption in the Wnt pathway may be due to a loss of planar cell polarity in marginal zone cells (Djiane et al., 2000; Wallingford et al., 2000). Interactions of members of the wingless/Wnt family with frizzled receptors are responsible for establishment of planar cell polarity (Djiane et al., 2000; Tada and Smith, 2000). Additionally, the integrin-dependent binding of deep cells to fibronectin is sufficient to drive membrane localization of Dishevelled-GFP (Marsden and DeSimone, 2001). Therefore, cell adhesion and cell polarity pathways merge and interact to regulate cell rearrangements during gastrulation.

Taken together, these studies demonstrate that disruption of elements of the tight, adherens, and gap junctions lead to inhibition of epithelial function. Most importantly for the current discussion, epithelial movements in *X. laevis* gastrulation are often disrupted with perturbation of Xbves function. Therefore, it is likely that Bves is involved somehow in the

large scope of cell adhesion. It is the duty of my colleagues to determine exactly what role Bves plays in epithelial assembly and maintenance. In the next section I will outline a number of experiments designed to examine the mechanism of Bves function.

Bves experiments during gastrulation

The discovery of the *bves* gene family and its potential role in epithelial movement and cell adhesion may prove fundamental in furthering our understanding of the dynamic cell adhesion processes that are so crucial in early embryogenesis. While the results I obtained using *in vitro* models of epithelial sheet formation and repair, and *in vivo* models of epithelial movement during gastrulation and eye developments may appear diverse, a single underlying theme is reiterated: mainly that epithelial movement and function is altered with disruption of Bves function. There are a multitude of experiments that need to be performed in order to further elucidate the precise mechanism by which Bves functions. The experiments I propose would test the most fundamental elements changed by alteration of Bves activity. The reason for this approach is that little is known about Bves. Only two papers, one from my work, have tested Bves function (Andree et al., 2002). No interacting partners have been identified and knock-out mice reveal no developmental phenotype. Thus, experiments are needed to test Bves function. Key among future experiments is the analysis of how individual cell shape and movement are affected. First, the gastrulation phenotype produced by Xbves depleted embryos needs to be further analyzed. By labeling a subset of morpholino-injected cells with a dye, cell populations lacking Xbves protein can be tracked through development. Migration and adhesiveness of these cells can be compared with noninjected, Bves expressing cells in the same embryo as an internal control. The expression and localization of other epithelial cell adhesion

markers such as cadherins, fibronectin, and NFPC will be analyzed to determine whether loss of Bves at the cell surface affects expression of these markers in the deep migrating cells during gastrulation. Fluorescenated membrane protein marker can be introduced into specific cells and changes in cell shape can be analyzed in morpholino-injected embryos, since intercalation, convergent extension, and/or epiboly may be affected. Additionally, electron microscopy will allow for examination of cell morphology and cell junction assembly in morpholino-injected embryos. As noted above, disruption of junctional proteins can cause widespread inactivation of junctional complexes. Quantification of junction complexes would clarify whether Bves affects junctional assembly. With reference to epithelial movements, we would look for involution of bottle cells into the blastopore lip and the extent, if any, of archenteron formation. Further, cell morphology and organization of surface cells during epiboly can be compared between morpholino-injected and control injected embryos. Determining that cell shape, movement, and junctional complexes are altered will be vital in determining Bves function in gastrulation.

To determine whether *Xbves* participates in convergent extension movements, animal caps assays can be conducted in the presence of activin (Brieher and Gumbiner, 1994; Broders and Thiery, 1995; Hadeball et al., 1998; Zhong et al., 1999). Animal caps with depleted Xbves protein can be treated with activin, which induces gastrulation movements. If Xbves is vital to convergence extension movements, then activin treated *Xbves* morpholino-injected caps will not elongate, unlike wild type animal caps. Inhibition of animal cap elongation would suggest that Xbves is important in regulating proper epithelial function that is necessary in convergent extension cell rearrangements and movements. Through closer examination and detection of morpholino-injected cells during gastrulation movements we will be able to clarify the precise role *Xbves* plays during these processes.

To date, there are no known Bves binding proteins. A yeast two-hybrid screen is currently underway to determine whether Bves interacts with other molecules that serve as anchoring proteins to the cytoskeleton or as signaling molecules. It has been well documented that cell-cell adhesion is regulated through the Wnt/Fz signaling pathway during gastrulation (Djiane et al., 2000; Medina et al., 2000; Medina and Steinbeisser, 2000; Tada and Smith, 2000). Induction of this pathway will be analyzed by expression analysis of members of this pathway in Bves depleted embryos to determine whether Bves may be linked to the Wnt/Fz pathway.

While several alternative experiments can be proposed, the most likely outcome is that movements of embryonic epithelium are initiated at the onset of gastrulation, as predicted by my previous studies. This may be a result of the initial translation of *Xbves* RNA and/or presence of maternal Xbves protein prior to the inception of morpholino blockade. If a subset of cells were morpholino-injected and labeled, we can predict that these cells would be altered in their movement relative to other epithelial cells during gastrulation processes. These cells may lose contact with other cells undergoing epiboly and/or involution. This is reminiscent of my recently published data showing morpholino treatment of corneal epithelial cells leads to disruption of intact sheets and alteration of cell morphology. Analysis of cell shape using membrane integrated GFP protein (supplied by Dr. Chris Wright) may reveal these cell shape changes. It may be possible to monitor alterations in junctional complexes in these cells with coupling immunofluorescence of cadherins. These expected results would determine exactly why gastrulation movements are halted when Xbves function is inhibited.

Bves eye phenotype

Another important element to investigate is the phenotype I discovered in *X. laevis*

embryos injected with a lower amount of *Xbves* morpholino (20ng) into one cell of a two-cell embryo. While these embryos successfully developed through gastrulation, there are developmental malformations seen at the tadpole stage. Similar phenotypes are observed in embryos morpholino-injected to block XB/U-cadherin function (Kuhl et al., 1996). Since the *Xbves* morpholino was introduced into only half of the embryo, one side of the embryo will serve as an internal control while the other side will be the experimental case. Most noticeably, there is a missing eye on the injected side of the embryo, while the uninjected eye appears normal (Figure 42). The lens fails to separate from the surface ectoderm in the earliest lens morphogenetic events of lens formation (Figure 42I, arrowhead). This study supports our findings and lays the groundwork for future Bves studies in *X. laevis* embryos. Our preliminary studies suggest the outgrowth of the optic vesicle is initiated but proper growth or extension of the optic cup for interaction with surface ectoderm is disrupted. Thus, a viable lens is not formed and subsequent eye development is impeded. Additionally, there appears to be decreased pigment demarcations on the affected side of the embryo suggesting inhibition of neural crest and/or melanocyte migration and differentiation. Initial observation of transverse tissue sections of these injected embryos show asymmetrical tissue organization, including a unilateral pattern of somite differentiation. This finding is similar to the phenotype seen in mutant XB/U-cadherin expressing *X. laevis* embryos presented by Kuhl et al. (1996). As mentioned above, when low doses of mutant XB/U-cadherin were expressed, embryos proceeded through gastrulation normally, but exhibited malformations of the brain and eye anlagen (Kuhl et al., 1996). It is especially interesting to note the appearance of surface ectoderm on the injected side. There is a thickened epidermis and an irregular outpocketing of cells (Figure 42I, arrow). Thus we can propose that epidermal cells have altered cell-cell interaction and /or adhesion. It will be

interesting to analyze junctional proteins and complexes in these specimens. Again analysis with antibodies to junctional proteins and electron microscopy to observe junctions can be employed. These results will serve as supporting evidence to conclusions drawn from the gastrulation studies.

The eyeless phenotype seen in Bves morpholino-injected embryos is a classic example of inhibition of epithelial activity. An evolutionary example of eyeless species is the cave fish, which diverged through the geographical separation of the *Astyananx mexicanus*, resulting in an eyed surface-dwelling and eyeless cave-dwelling form (Borowsky and Wilkens, 2002; Roy, 1971; Strecker et al., 2003). Eye formation in the cave fish is initiated during embryogenesis. but the lens vesicle later degenerates, and the cornea, iris and other optic tissues are absent or rudimentary (Jeffery, 2001; Yamamoto and Jeffery, 2000). The authors postulate that an unknown inductive signal from the lens is involved in cave fish eye degeneration and transplantation of the surface-dwelling fish into the cave-dwelling fish is sufficient to rescue eye development (Yamamoto and Jeffery, 2000). This natural model of eye degeneration would be a fundamental model to compare with Xbves eyeless embryos. Future examination of these low dose Xbves morpholino-injected embryos will include using a dye tracer to track morpholino injection and distribution. Determination of exactly what processes are disrupted during eye development will be assessed by detection of markers for the eye, including *Pax 6*, *rhodopsin*, and *crystalline* (Hirsch and Harris, 1997; Negishi et al., 1990; Perron et al., 1998; Yamamoto and Jeffery, 2000). While further analysis is needed to track injected cells, specify the tissue types and examine the precise malformations, morpholino-injected *X. laevis* embryos serve as an ideal

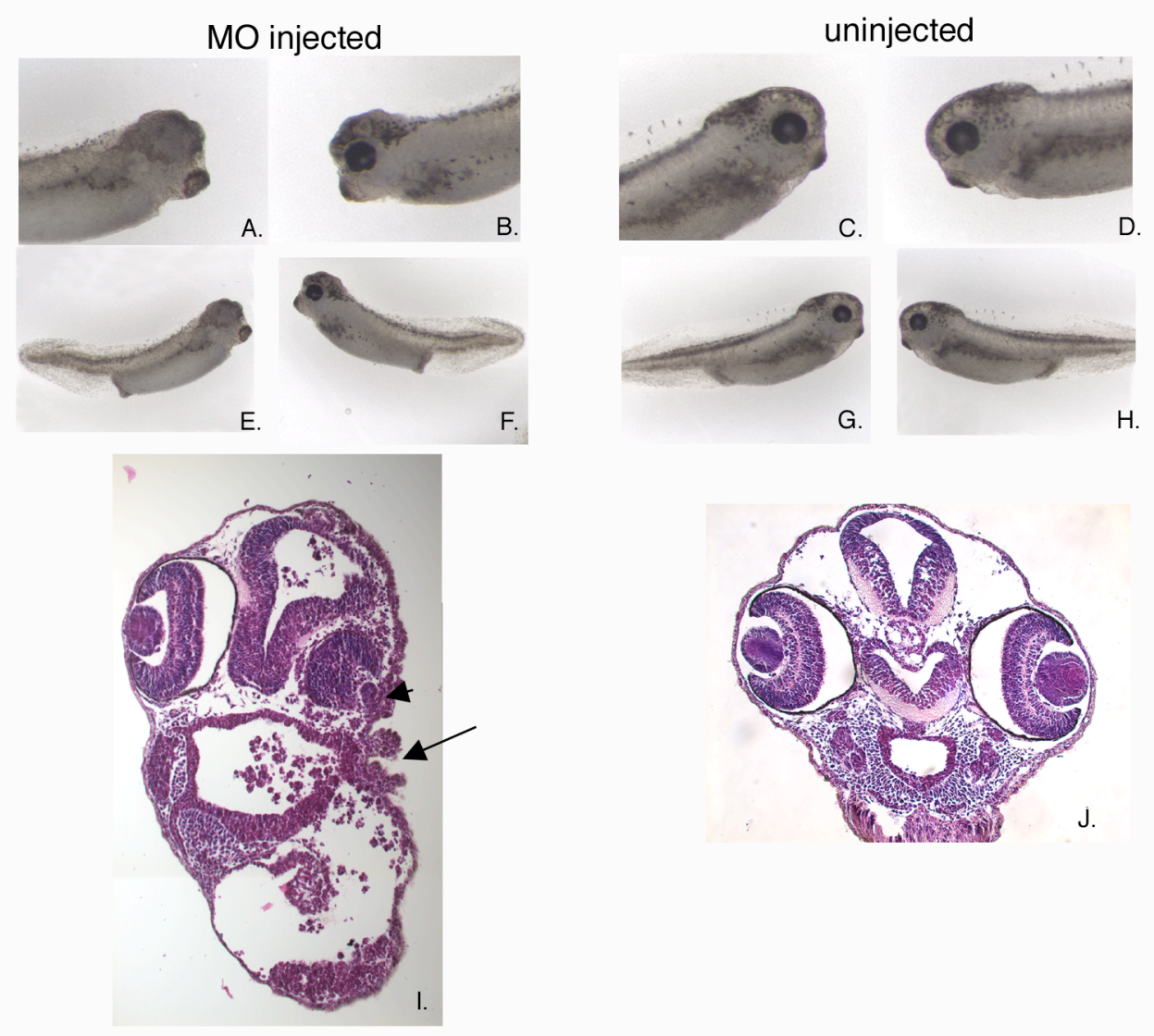

Figure 42. Injection of low doses of *Xbves* morpholino results in an eyeless phenotype. (A-H) Embryos injected with a low dose of *Xbves* morpholino (A-B, E-F) into one of two cells develop without an eye on the injected side, compared with uninjected (C-D, G-H) embryos. Each group of figures (A,B,C,D, and E,D,G,H) illustrates both sides of a single embryo in high and low magnification. (I-J) Transverse tissue sections through the low dose (I) and uninjected (J) embryos.

system to investigate Xbves adhesive function in organogenesis. The ability to rescue the described morpholino phenotypes provides excellent means to show specificity and validation of collected data.

In conclusion, data derived from this dissertation provide strong *in vitro* and *in vivo* evidence that *bves* plays an adhesive role in epithelial adhesion and morphogenetic movements during gastrulation in *X. laevis* and eye morphogenesis. Combined with previous data that show *bves* participates in epithelial to mesenchymal transition in epicardium and coronary artery development, as well as data that show *bves* expression across many tissues, the data presented here support the idea that one gene product can participate in a variety of developmental processes. This study is an important contribution to understanding the overall larger concept of how *bves* functions in embryogenesis and the adult. My data are the first to show that *bves* participates both during development and during adult tissue regeneration. What is not known is how Bves fits into current models of cell adhesion and cell-cell interaction at cell junctions. More so, discovery of what, if any, proteins interact with Bves would be very telling to its function. The biologic function and participating members of many signaling pathways have been well documented. If *bves* is found to be part of a known pathway, then the doors may open up to a vast array of information impacting *bves* function.

To summate, my data are the first to demonstrate a conserved function *in vivo* using two variant systems. Clearly more work is needed to place Bves in a current model of cell adhesion and to explore further function in embryogenesis. However, my data show that Bves plays an adhesive role in epithelial movement and morphogenesis during development.

REFERENCES

Aberle, H., Butz, S., Stappert, J., Weissig, H., Kemler, R. and Hoschuetzky, H. (1994). Assembly of the cadherin-catenin complex in vitro with recombinant proteins. *J Cell Sci* **107 (Pt 12)**, 3655-63.

Aberle, H., Schwartz, H. and Kemler, R. (1996). Cadherin-catenin complex: protein interactions and their implications for cadherin function. *J Cell Biochem* **61**, 514-23.

Albelda, S. M. and Buck, C. A. (1990). Integrins and other cell adhesion molecules. *Faseb J* **4**, 2868-80.

Andree, B., Fleige, A., Arnold, H. H. and Brand, T. (2002). Mouse Pop1 is required for muscle regeneration in adult skeletal muscle. *Mol Cell Biol* **22**, 1504-12.

Andree, B., Hillemann, T., Kessler-Icekson, G., Schmitt-John, T., Jockusch, H., Arnold, H. H. and Brand, T. (2000). Isolation and characterization of the novel popeye gene family expressed in skeletal muscle and heart. *Dev Biol* **223**, 371-82.

Angres, B., Muller, A. H., Kellermann, J. and Hausen, P. (1991). Differential expression of two cadherins in Xenopus laevis. *Development* **111**, 829-44.

Araki-Sasaki, K., Ohashi, Y., Sasabe, T., Hayashi, K., Watanabe, H., Tano, Y. and Handa, H. (1995). An SV40-immortalized human corneal epithelial cell line and its characterization. *Invest Ophthalmol Vis Sci* **36**, 614-21.

Ashe, M., Pabon-Pena, L., Dees, E., Price, K. L. and Bader, D. (2004). LEK1 is a potential inhibitor of pocket protein-mediated cellular processes. *J Biol Chem* **279**, 664-76.

Balak, K., Jacobson, M., Sunshine, J. and Rutishauser, U. (1987). Neural cell adhesion molecule expression in Xenopus embryos. *Dev Biol* **119**, 540-50.

Balser, J. R. (1999). Structure and function of the cardiac sodium channels. *Cardiovasc Res* **42**, 327-38.

Beddington, R. S. and Smith, J. C. (1993). Control of vertebrate gastrulation: inducing signals and responding genes. *Curr Opin Genet Dev* **3**, 655-61.

Beebe, D. C. and Coats, J. M. (2000). The lens organizes the anterior segment: specification of neural crest cell differentiation in the avian eye. *Dev Biol* **220**, 424-31.

Borowsky, R. and Wilkens, H. (2002). Mapping a cave fish genome: polygenic systems and regressive evolution. *J Hered* **93**, 19-21.

Bower, D. J., Errington, L. H., Pollock, B. J., Morris, S. and Clayton, R. M. (1983). The pattern of expression of chick delta-crystallin genes in lens differentiation and in trans-differentiating cultured tissues. *Embo J* **2**, 333-8.

Bradley, R. S., Espeseth, A. and Kintner, C. (1998). NF-protocadherin, a novel member of the cadherin superfamily, is required for Xenopus ectodermal differentiation. *Curr Biol* **8**, 325-34.

Brady, J. P., Garland, D. L., Green, D. E., Tamm, E. R., Giblin, F. J. and Wawrousek, E. F. (2001). AlphaB-crystallin in lens development and muscle integrity: a gene knockout approach. *Invest Ophthalmol Vis Sci* **42**, 2924-34.

Branford, W. W. and Yost, H. J. (2002). Lefty-dependent inhibition of Nodal- and Wnt-responsive organizer gene expression is essential for normal gastrulation. *Curr Biol* **12**, 2136-41.

Brieher, W. M. and Gumbiner, B. M. (1994). Regulation of C-cadherin function during activin induced morphogenesis of Xenopus animal caps. *J Cell Biol* **126**, 519-27.

Brizuela, B. J., Wessely, O. and De Robertis, E. M. (2001). Overexpression of the Xenopus tight-junction protein claudin causes randomization of the left-right body axis. *Dev Biol* **230**, 217-29.

Broders, F. and Thiery, J. P. (1995). Contribution of cadherins to directional cell migration and histogenesis in Xenopus embryos. *Cell Adhes Commun* **3**, 419-40.

Caveney, S. (1985). The role of gap junctions in development. *Annu Rev Physiol* **47**, 319-35.

Cepko, C. L., Ryder, E. F., Austin, C. P., Walsh, C. and Fekete, D. M. (1993). Lineage analysis using retrovirus vectors. *Methods Enzymol* **225**, 933-60.

Chiambaretta, F., Blanchon, L., Rabier, B., Kao, W. W., Liu, J. J., Dastugue, B., Rigal, D. and Sapin, V. (2002). Regulation of corneal keratin-12 gene expression by the human Kruppel-like transcription factor 6. *Invest Ophthalmol Vis Sci* **43**, 3422-9.

Cho, K. W., Blumberg, B., Steinbeisser, H. and De Robertis, E. M. (1991). Molecular nature of Spemann's organizer: the role of the Xenopus homeobox gene goosecoid. *Cell* **67**, 1111-20.

Choi, Y. S. and Gumbiner, B. (1989). Expression of cell adhesion molecule E-cadherin in Xenopus embryos begins at gastrulation and predominates in the ectoderm. *J Cell Biol* **108**, 2449-58.

Cordenonsi, M., Mazzon, E., De Rigo, L., Baraldo, S., Meggio, F. and Citi, S. (1997). Occludin dephosphorylation in early development of Xenopus laevis. *J Cell Sci* **110 (Pt 24)**, 3131-9.

Cunliffe, V. and Smith, J. C. (1992). Ectopic mesoderm formation in Xenopus embryos caused by widespread expression of a Brachyury homologue. *Nature* **358**, 427-30.

Curran, M. E. (1998). Potassium ion channels and human disease: phenotypes to drug targets? *Curr Opin Biotechnol* **9**, 565-72.

D'Atri, F. and Citi, S. (2002). Molecular complexity of vertebrate tight junctions (Review). *Mol Membr Biol* **19**, 103-12.

Dahm, R., Bramke, S., Dawczynski, J., Nagaraj, R. H. and Kasper, M. (2003). Developmental aspects of galectin-3 expression in the lens. *Histochem Cell Biol* **119**, 219-26.

Danilchick, M., Peng, H. B. and Kay, B. K. (1991). Xenopus laevis: Practical uses in cell and molecular biology. Pictorial collage of embryonic stages. *Methods Cell Biol* **36**, 679-81.

de Iongh, R. U., Lovicu, F. J., Overbeek, P. A., Schneider, M. D., Joya, J., Hardeman, E. D. and McAvoy, J. W. (2001). Requirement for TGFbeta receptor signaling during terminal lens fiber differentiation. *Development* **128**, 3995-4010.

Detrick, R. J., Dickey, D. and Kintner, C. R. (1990). The effects of N-cadherin misexpression on morphogenesis in Xenopus embryos. *Neuron* **4**, 493-506.

Dettlaff, T. A. (1983). A study of the properties, morphogenetic potencies and prospective fate of outer and inner layers of ectodermal and chordamesodermal regions during gastrulation, in various Anuran amphibians. *J Embryol Exp Morphol* **75**, 67-86.

DiAngelo, J. R., Vasavada, T. K., Cain, W. and Duncan, M. K. (2001). Production of monoclonal antibodies against chicken Pop1 (BVES). *Hybrid Hybridomics* **20**, 377-81.

Dibner, C., Elias, S. and Frank, D. (2001). XMeis3 protein activity is required for proper hindbrain patterning in Xenopus laevis embryos. *Development* **128**, 3415-26.

Dickinson, M. E., Selleck, M. A., McMahon, A. P. and Bronner-Fraser, M. (1995). Dorsalization of the neural tube by the non-neural ectoderm. *Development* **121**, 2099-106.

Djiane, A., Riou, J., Umbhauer, M., Boucaut, J. and Shi, D. (2000). Role of frizzled 7 in the regulation of convergent extension movements during gastrulation in Xenopus laevis. *Development* **127**, 3091-100.

Ellinor, P. T., Yang, J., Sather, W. A., Zhang, J. F. and Tsien, R. W. (1995). Ca2+ channel selectivity at a single locus for high-affinity Ca2+ interactions. *Neuron* **15**, 1121-32.

Fagotto, F. and Gumbiner, B. M. (1994). Beta-catenin localization during Xenopus embryogenesis: accumulation at tissue and somite boundaries. *Development* **120**, 3667-79.

Fanning, A. S., Mitic, L. L. and Anderson, J. M. (1999). Transmembrane proteins in the tight junction barrier. *J Am Soc Nephrol* **10**, 1337-45.

Finnemann, S., Mitrik, I., Hess, M., Otto, G. and Wedlich, D. (1997). Uncoupling of XB/U-cadherin-catenin complex formation from its function in cell-cell adhesion. *J Biol Chem* **272**, 11856-62.

Fleming, T. P. and Johnson, M. H. (1988). From egg to epithelium. *Annu Rev Cell Biol* **4**, 459-85.

Fleming, T. P., Papenbrock, T., Fesenko, I., Hausen, P. and Sheth, B. (2000). Assembly of tight junctions during early vertebrate development. *Semin Cell Dev Biol* **11**, 291-9.

Freeman, T. (2000). High throughput gene expression screening: its emerging role in drug discovery. *Med Res Rev* **20**, 197-202.

Funayama, N., Fagotto, F., McCrea, P. and Gumbiner, B. M. (1995). Embryonic axis induction by the armadillo repeat domain of beta-catenin: evidence for intracellular signaling. *J Cell Biol* **128**, 959-68.

Furuse, M., Fujita, K., Hiiragi, T., Fujimoto, K. and Tsukita, S. (1998). Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol* **141**, 1539-50.

Furuse, M., Hirase, T., Itoh, M., Nagafuchi, A., Yonemura, S. and Tsukita, S. (1993). Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol* **123**, 1777-88.

Geiger, B. and Bershadsky, A. (2001). Assembly and mechanosensory function of focal contacts. *Curr Opin Cell Biol* **13**, 584-92.

Gerhart, J. and Keller, R. (1986). Region-specific cell activities in amphibian gastrulation. *Annu Rev Cell Biol* **2**, 201-29.

Gimlich, R. L., Kumar, N. M. and Gilula, N. B. (1990). Differential regulation of the levels of three gap junction mRNAs in Xenopus embryos. *J Cell Biol* **110**, 597-605.

Green, J. B., New, H. V. and Smith, J. C. (1992). Responses of embryonic Xenopus cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. *Cell* **71**, 731-9.

Guger, K. A. and Gumbiner, B. M. (1995). beta-Catenin has Wnt-like activity and mimics the Nieuwkoop signaling center in Xenopus dorsal-ventral patterning. *Dev Biol* **172**, 115-25.

Guthrie, S., Turin, L. and Warner, A. (1988). Patterns of junctional communication during development of the early amphibian embryo. *Development* **103**, 769-83.

Guthrie, S. C. and Gilula, N. B. (1989). Gap junctional communication and development. *Trends Neurosci* **12**, 12-6.

Hadeball, B., Borchers, A. and Wedlich, D. (1998). Xenopus cadherin-11 (Xcadherin-11) expression requires the Wg/Wnt signal. *Mech Dev* **72**, 101-13.

Hamburger, V. and Hamilton, H. L. (1992). A series of normal stages in the development of the chick embryo. 1951. *Dev Dyn* **195**, 231-72.

Hardin, J. and Keller, R. (1988). The behaviour and function of bottle cells during gastrulation of Xenopus laevis. *Development* **103**, 211-30.

Harland, R. M. (1991). In situ hybridization: an improved whole-mount method for Xenopus embryos. *Methods Cell Biol* **36**, 685-95.

Hausen, P. and Riebesell, M. (1991). The early development of Xenopus laevis : an atlas of the histology. Tübingen
Berlin ; New York: Verlag der Zeitschrift für Naturforschung ;
Springer-Verlag.

Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, P., Gumbiner, B., McCrea, P., Kintner, C., Noro, C. Y. and Wylie, C. (1994a). Overexpression of cadherins and underexpression of beta-catenin inhibit dorsal mesoderm induction in early Xenopus embryos. *Cell* **79**, 791-803.

Heasman, J., Ginsberg, D., Geiger, B., Goldstone, K., Pratt, T., Yoshida-Noro, C. and Wylie, C. (1994b). A functional test for maternally inherited cadherin in Xenopus shows its importance in cell adhesion at the blastula stage. *Development* **120**, 49-57.

Heasman, J., Kofron, M. and Wylie, C. (2000). Beta-catenin signaling activity dissected in the early Xenopus embryo: a novel antisense approach. *Dev Biol* **222**, 124-34.

Heasman, J., Wessely, O., Langland, R., Craig, E. J. and Kessler, D. S. (2001). Vegetal localization of maternal mRNAs is disrupted by VegT depletion. *Dev Biol* **240**, 377-86.

Heinemann, S. H., Terlau, H., Stuhmer, W., Imoto, K. and Numa, S. (1992). Calcium channel characteristics conferred on the sodium channel by single mutations. *Nature* **356**, 441-3.

Heiskala, M., Peterson, P. A. and Yang, Y. (2001). The roles of claudin superfamily proteins in paracellular transport. *Traffic* **2**, 93-8.

Henry, J. J., Carinato, M. E., Schaefer, J. J., Wolfe, A. D., Walter, B. E., Perry, K. J. and Elbl, T. N. (2002). Characterizing gene expression during lens formation in Xenopus laevis: evaluating the model for embryonic lens induction. *Dev Dyn* **224**, 168-85.

Hirano, S., Nose, A., Hatta, K., Kawakami, A. and Takeichi, M. (1987). Calcium-dependent cell-cell adhesion molecules (cadherins): subclass specificities and possible involvement of actin bundles. *J Cell Biol* **105**, 2501-10.

Hirsch, N. and Harris, W. A. (1997). Xenopus Pax-6 and retinal development. *J Neurobiol* **32**, 45-61.

Hitz, M. P., Pandur, P., Brand, T. and Kuhl, M. (2002). Cardiac specific expression of Xenopus Popeye-1. *Mech Dev* **115**, 123-6.

Houston, D. W. and Wylie, C. (2003). The Xenopus LIM-homeodomain protein Xlim5 regulates the differential adhesion properties of early ectoderm cells. *Development* **130**, 2695-704.

Hsieh, Y. W., Zhang, X. M., Lin, E., Oliver, G. and Yang, X. J. (2002). The homeobox gene Six3 is a potential regulator of anterior segment formation in the chick eye. *Dev Biol* **248**, 265-80.

Huber, O., Korn, R., McLaughlin, J., Ohsugi, M., Herrmann, B. G. and Kemler, R. (1996). Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. *Mech Dev* **59**, 3-10.

Hubrecht-Laboratorium (Embryologisch Instituut), Nieuwkoop, P. D. and Faber, J. (1967). Normal table of Xenopus laevis (Daudin). A systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis. Amsterdam,: North-Holland Pub. Co.

Huelsken, J., Vogel, R., Brinkmann, V., Erdmann, B., Birchmeier, C. and Birchmeier, W. (2000). Requirement for beta-catenin in anterior-posterior axis formation in mice. *J Cell Biol* **148**, 567-78.

Izutsu, Y., Tochinai, S. and Onoe, K. (2000). Loss of reactivity to pan-cadherin antibody in epidermal cells as a marker for metamorphic alteration of Xenopus skin. *Dev Growth Differ* **42**, 377-83.

Jamora, C. and Fuchs, E. (2002). Intercellular adhesion, signalling and the cytoskeleton. *Nat Cell Biol* **4**, E101-8.

Jean, D., Ewan, K. and Gruss, P. (1998). Molecular regulators involved in vertebrate eye development. *Mech Dev* **76**, 3-18.

Jeffery, W. R. (2001). Cavefish as a model system in evolutionary developmental biology. *Dev Biol* **231**, 1-12.

Johnson, K. E., Darribere, T. and Boucaut, J. C. (1993). Mesodermal cell adhesion to fibronectin-rich fibrillar extracellular matrix is required for normal Rana pipiens gastrulation. *J Exp Zool* **265**, 40-53.

Keller, R. (1991). Early embryonic development of Xenopus laevis. *Methods Cell Biol* **36**, 61-113.

Keller, R. and Danilchik, M. (1988). Regional expression, pattern and timing of convergence and extension during gastrulation of Xenopus laevis. *Development* **103**, 193-209.

Keller, R., Shih, J. and Domingo, C. (1992). The patterning and functioning of protrusive activity during convergence and extension of the Xenopus organiser. *Dev Suppl*, 81-91.

Keller, R. and Tibbetts, P. (1989). Mediolateral cell intercalation in the dorsal, axial mesoderm of Xenopus laevis. *Dev Biol* **131**, 539-49.

Keller, R. E. (1980). The cellular basis of epiboly: an SEM study of deep-cell rearrangement during gastrulation in Xenopus laevis. *J Embryol Exp Morphol* **60**, 201-34.

Kiritoshi, A., SundarRaj, N. and Thoft, R. A. (1991). Differentiation in cultured limbal epithelium as defined by keratin expression. *Invest Ophthalmol Vis Sci* **32**, 3073-7.

Klingensmith, J. and Nusse, R. (1994). Signaling by wingless in Drosophila. *Dev Biol* **166**, 396-414.

Knight, R. F., Bader, D. M. and Backstrom, J. R. (2003). Membrane topology of Bves/Pop1A, a cell adhesion molecule that displays dynamic changes in cellular distribution during development. *J Biol Chem* **278**, 32872-9.

Kojima, T., Kokai, Y., Chiba, H., Yamamoto, M., Mochizuki, Y. and Sawada, N. (2001). Cx32 but not Cx26 is associated with tight junctions in primary cultures of rat hepatocytes. *Exp Cell Res* **263**, 193-201.

Kuhl, M., Finnemann, S., Binder, O. and Wedlich, D. (1996). Dominant negative expression of a cytoplasmically deleted mutant of XB/U-cadherin disturbs mesoderm migration during gastrulation in Xenopus laevis. *Mech Dev* **54**, 71-82.

Kuroda, H., Inui, M., Sugimoto, K., Hayata, T. and Asashima, M. (2002). Axial protocadherin is a mediator of prenotochord cell sorting in Xenopus. *Dev Biol* **244**, 267-77.

Kwan, K. M. and Kirschner, M. W. (2003). Xbra functions as a switch between cell migration and convergent extension in the Xenopus gastrula. *Development* **130**, 1961-72.

Lambiase, A., Manni, L., Bonini, S., Rama, P., Micera, A. and Aloe, L. (2000). Nerve growth factor promotes corneal healing: structural, biochemical, and molecular analyses of rat and human corneas. *Invest Ophthalmol Vis Sci* **41**, 1063-9.

Larabell, C. A., Torres, M., Rowning, B. A., Yost, C., Miller, J. R., Wu, M., Kimelman, D. and Moon, R. T. (1997). Establishment of the dorso-ventral axis in Xenopus embryos is presaged by early asymmetries in beta-catenin that are modulated by the Wnt signaling pathway. *J Cell Biol* **136**, 1123-36.

Lee, C. H. and Gumbiner, B. M. (1995). Disruption of gastrulation movements in Xenopus by a dominant-negative mutant for C-cadherin. *Dev Biol* **171**, 363-73.

Lehmann-Horn, F. and Jurkat-Rott, K. (1999). Voltage-gated ion channels and hereditary disease. *Physiol Rev* **79**, 1317-72.

Leong, L., Menko, A. S. and Grunwald, G. B. (2000). Differential expression of N- and B-cadherin during lens development. *Invest Ophthalmol Vis Sci* **41**, 3503-10.

Levi, G., Gumbiner, B. and Thiery, J. P. (1991). The distribution of E-cadherin during Xenopus laevis development. *Development* **111**, 159-69.

Levin, M. and Mercola, M. (1998). Gap junctions are involved in the early generation of left-right asymmetry. *Dev Biol* **203**, 90-105.

Levine, E., Lee, C. H., Kintner, C. and Gumbiner, B. M. (1994). Selective disruption of E-cadherin function in early Xenopus embryos by a dominant negative mutant. *Development* **120**, 901-9.

Liu, Q., Londraville, R. L., Azodi, E., Babb, S. G., Chiappini-Williamson, C., Marrs, J. A. and Raymond, P. A. (2002). Up-regulation of cadherin-2 and cadherin-4 in regenerating visual structures of adult zebrafish. *Exp Neurol* **177**, 396-406.

Livesey, R. and Cepko, C. (2001). Neurobiology. Developing order. *Nature* **413**, 471, 473.

MacKinnon, R. (1995). Pore loops: an emerging theme in ion channel structure. *Neuron* **14**, 889-92.

Maloof, J. N., Whangbo, J., Harris, J. M., Jongeward, G. D. and Kenyon, C. (1999). A Wnt signaling pathway controls hox gene expression and neuroblast migration in C. elegans. *Development* **126**, 37-49.

Marsden, M. and DeSimone, D. W. (2001). Regulation of cell polarity, radial intercalation and epiboly in Xenopus: novel roles for integrin and fibronectin. *Development* **128**, 3635-47.

McCrea, P. D., Turck, C. W. and Gumbiner, B. (1991). A homolog of the armadillo protein in Drosophila (plakoglobin) associated with E-cadherin. *Science* **254**, 1359-61.

Medina, A., Reintsch, W. and Steinbeisser, H. (2000). Xenopus frizzled 7 can act in canonical and non-canonical Wnt signaling pathways: implications on early patterning and morphogenesis. *Mech Dev* **92**, 227-37.

Medina, A. and Steinbeisser, H. (2000). Interaction of Frizzled 7 and Dishevelled in Xenopus. *Dev Dyn* **218**, 671-80.

Meier, S. (1977). Initiation of corneal differentiation prior to cornea-lens association. *Cell Tissue Res* **184**, 255-67.

Milks, L. C., Kumar, N. M., Houghten, R., Unwin, N. and Gilula, N. B. (1988). Topology of the 32-kd liver gap junction protein determined by site-directed antibody localizations. *Embo J* **7**, 2967-75.

Montero, J. A. and Heisenberg, C. P. (2003). Adhesive crosstalk in gastrulation. *Dev Cell* **5**, 190-1.

Montross, W. T., Ji, H. and McCrea, P. D. (2000). A beta-catenin/engrailed chimera selectively suppresses Wnt signaling. *J Cell Sci* **113 (Pt 10)**, 1759-70.

Muller, H. A., Kuhl, M., Finnemann, S., Schneider, S., van der Poel, S. Z., Hausen, P. and Wedlich, D. (1994). Xenopus cadherins: the maternal pool comprises distinguishable members of the family. *Mech Dev* **47**, 213-23.

Nagafuchi, A. and Takeichi, M. (1989). Transmembrane control of cadherin-mediated cell adhesion: a 94 kDa protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. *Cell Regul* **1**, 37-44.

Nasevicius, A. and Ekker, S. C. (2000). Effective targeted gene 'knockdown' in zebrafish. *Nat Genet* **26**, 216-20.

Negishi, K., Stell, W. K. and Takasaki, Y. (1990). Early histogenesis of the teleostean retina: studies using a novel immunochemical marker, proliferating cell nuclear antigen (PCNA/cyclin). *Brain Res Dev Brain Res* **55**, 121-5.

Niehrs, C., Keller, R., Cho, K. W. and De Robertis, E. M. (1993). The homeobox gene goosecoid controls cell migration in Xenopus embryos. *Cell* **72**, 491-503.

Nieuwkoop, P. D. (1973). The organization center of the amphibian embryo: its origin, spatial organization, and morphogenetic action. *Adv Morphog* **10**, 1-39.

Oberlender, S. A. and Tuan, R. S. (1994). Expression and functional involvement of N-cadherin in embryonic limb chondrogenesis. *Development* **120**, 177-87.

Olson, D. J. and Moon, R. T. (1992). Distinct effects of ectopic expression of Wnt-1, activin B, and bFGF on gap junctional permeability in 32-cell Xenopus embryos. *Dev Biol* **151**, 204-12.

Ozawa, M. and Kemler, R. (1992). Molecular organization of the uvomorulin-catenin complex. *J Cell Biol* **116**, 989-96.

Pancholi, S., Tullo, A., Khaliq, A., Foreman, D. and Boulton, M. (1998). The effects of growth factors and conditioned media on the proliferation of human corneal epithelial cells and keratocytes. *Graefes Arch Clin Exp Ophthalmol* **236**, 1-8.

Paul, D. L., Yu, K., Bruzzone, R., Gimlich, R. L. and Goodenough, D. A. (1995). Expression of a dominant negative inhibitor of intercellular communication in the early Xenopus embryo causes delamination and extrusion of cells. *Development* **121**, 371-81.

Peifer, M. and Wieschaus, E. (1990). The segment polarity gene armadillo encodes a functionally modular protein that is the Drosophila homolog of human plakoglobin. *Cell* **63**, 1167-76.

Perez-Garcia, M. T., Chiamvimonvat, N., Ranjan, R., Balser, J. R., Tomaselli, G. F. and Marban, E. (1997). Mechanisms of sodium/calcium selectivity in sodium channels probed by cysteine mutagenesis and sulfhydryl modification. *Biophys J* **72**, 989-96.

Perron, M., Kanekar, S., Vetter, M. L. and Harris, W. A. (1998). The genetic sequence of retinal development in the ciliary margin of the Xenopus eye. *Dev Biol* **199**, 185-200.

Pichaud, F. and Desplan, C. (2002). Pax genes and eye organogenesis. *Curr Opin Genet Dev* **12**, 430-4.

Ramos, J. W. and DeSimone, D. W. (1996). Xenopus embryonic cell adhesion to fibronectin: position-specific activation of RGD/synergy site-dependent migratory behavior at gastrulation. *J Cell Biol* **134**, 227-40.

Ramos, J. W., Whittaker, C. A. and DeSimone, D. W. (1996). Integrin-dependent adhesive activity is spatially controlled by inductive signals at gastrulation. *Development* **122**, 2873-83.

Reed, N. A., Castellini, M. A., Ma, H., Shearer, T. R. and Duncan, M. K. (2003). Protein expression patterns for ubiquitous and tissue specific calpains in the developing mouse lens. *Exp Eye Res* **76**, 433-43.

Reese, D. E. and Bader, D. M. (1999). Cloning and expression of hbves, a novel and highly conserved mRNA expressed in the developing and adult heart and skeletal muscle in the human. *Mamm Genome* **10**, 913-5.

Reese, D. E., Zavaljevski, M., Streiff, N. L. and Bader, D. (1999). bves: A novel gene expressed during coronary blood vessel development. *Dev Biol* **209**, 159-71.

Reneker, L. W., Silversides, D. W., Xu, L. and Overbeek, P. A. (2000). Formation of corneal endothelium is essential for anterior segment development - a transgenic mouse model of anterior segment dysgenesis. *Development* **127**, 533-42.

Rodrigues, M., Ben-Zvi, A., Krachmer, J., Schermer, A. and Sun, T. T. (1987). Suprabasal expression of a 64-kilodalton keratin (no. 3) in developing human corneal epithelium. *Differentiation* **34**, 60-7.

Rottner, K., Behrendt, B., Small, J. V. and Wehland, J. (1999). VASP dynamics during lamellipodia protrusion. *Nat Cell Biol* **1**, 321-2.

Roy, F. H. (1971). The blind fish of Mammoth Cave. *Ann Ophthalmol* **3**, 348-9.

Ruccione, K. (1999). Cancer and genetics: what we need to know now. *J Pediatr Oncol Nurs* **16**, 156-71.

Ruoslahti, E. (1991). Integrins. *J Clin Invest* **87**, 1-5.

Schneider, S., Steinbeisser, H., Warga, R. M. and Hausen, P. (1996). Beta-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech Dev* **57**, 191-8.

Schohl, A. and Fagotto, F. (2002). Beta-catenin, MAPK and Smad signaling during early Xenopus development. *Development* **129**, 37-52.

Shih, J. and Keller, R. (1992). Patterns of cell motility in the organizer and dorsal mesoderm of Xenopus laevis. *Development* **116**, 915-30.

Smith, J. C., Price, B. M., Green, J. B., Weigel, D. and Herrmann, B. G. (1991). Expression of a Xenopus homolog of Brachyury (T) is an immediate-early response to mesoderm induction. *Cell* **67**, 79-87.

Smith, J. C., Symes, K., Heasman, J., Snape, A. and Wylie, C. C. (1987). The Xenopus animal pole blastomere. *Bioessays* **7**, 229-34.

Snyders, D. J. (1999). Structure and function of cardiac potassium channels. *Cardiovasc Res* **42**, 377-90.

Spokony, R. F., Aoki, Y., Saint-Germain, N., Magner-Fink, E. and Saint-Jeannet, J. P. (2002). The transcription factor Sox9 is required for cranial neural crest development in Xenopus. *Development* **129**, 421-32.

Strecker, U., Bernatchez, L. and Wilkens, H. (2003). Genetic divergence between cave and surface populations of Astyanax in Mexico (Characidae, Teleostei). *Mol Ecol* **12**, 699-710.

Striessnig, J. (1999). Pharmacology, structure and function of cardiac L-type Ca(2+) channels. *Cell Physiol Biochem* **9**, 242-69.

Sugrue, S. P. and Zieske, J. D. (1997). ZO1 in corneal epithelium: association to the zonula occludens and adherens junctions. *Exp Eye Res* **64**, 11-20.

Summerton, J. and Weller, D. (1997). Morpholino antisense oligomers: design, preparation, and properties. *Antisense Nucleic Acid Drug Dev* **7**, 187-95.

Tada, M. and Smith, J. C. (2000). Xwnt11 is a target of Xenopus Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* **127**, 2227-38.

Takeichi, M. (1990). Cadherins: a molecular family important in selective cell-cell adhesion. *Annu Rev Biochem* **59**, 237-52.

Takeichi, M. (1991). Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* **251**, 1451-5.

Tan, C., Deardorff, M. A., Saint-Jeannet, J. P., Yang, J., Arzoumanian, A. and Klein, P. S. (2001). Kermit, a frizzled interacting protein, regulates frizzled 3 signaling in neural crest development. *Development* **128**, 3665-74.

Thoreson, M. A., Anastasiadis, P. Z., Daniel, J. M., Ireton, R. C., Wheelock, M. J., Johnson, K. R., Hummingbird, D. K. and Reynolds, A. B. (2000). Selective uncoupling of p120(ctn) from E-cadherin disrupts strong adhesion. *J Cell Biol* **148**, 189-202.

Tripathi, B. J., Tripathi, R. C., Livingston, A. M. and Borisuth, N. S. (1991). The role of growth factors in the embryogenesis and differentiation of the eye. *Am J Anat* **192**, 442-71.

Vasavada, T. K., DiAngelo, J. R. and Duncan, M. K. (2004). Developmental expression of pop1/bves. *J Histochem Cytochem* **52**, 371-8.

Vasioukhin, V. and Fuchs, E. (2001). Actin dynamics and cell-cell adhesion in epithelia. *Curr Opin Cell Biol* **13**, 76-84.

Vodicka, M. A. and Gerhart, J. C. (1995). Blastomere derivation and domains of gene expression in the Spemann Organizer of Xenopus laevis. *Development* **121**, 3505-18.

Wada, A. M., Reese, D. E. and Bader, D. M. (2001). Bves: prototype of a new class of cell adhesion molecules expressed during coronary artery development. *Development* **128**, 2085-93.

Wada, A. M., Smith, T. K., Osler, M. E., Reese, D. E. and Bader, D. M. (2003). Epicardial/Mesothelial cell line retains vasculogenic potential of embryonic epicardium. *Circ Res* **92**, 525-31.

Wallingford, J. B., Rowning, B. A., Vogeli, K. M., Rothbacher, U., Fraser, S. E. and Harland, R. M. (2000). Dishevelled controls cell polarity during Xenopus gastrulation. *Nature* **405**, 81-5.

Wang, I. J., Carlson, E. C., Liu, C. Y., Kao, C. W., Hu, F. R. and Kao, W. W. (2002). Cis-regulatory elements of the mouse Krt1.12 gene. *Mol Vis* **8**, 94-101.

Wang, X. and Feuerstein, G. Z. (1997). The use of mRNA differential display for discovery of novel therapeutic targets in cardiovascular disease. *Cardiovasc Res* **35**, 414-21.

Warner, A. E. (1987). The use of antibodies to gap junction protein to explore the role of gap junctional communication during development. *Ciba Found Symp* **125**, 154-67.

Warner, A. E., Guthrie, S. C. and Gilula, N. B. (1984). Antibodies to gap-junctional protein selectively disrupt junctional communication in the early amphibian embryo. *Nature* **311**, 127-31.

Wedlich, D. (2002). The polarising role of cell adhesion molecules in early development. *Curr Opin Cell Biol* **14**, 563-8.

Wieschaus, E., Nusslein-Volhard, C. and Kluding, H. (1984). Kruppel, a gene whose activity is required early in the zygotic genome for normal embryonic segmentation. *Dev Biol* **104**, 172-86.

Wilson, P. and Keller, R. (1991). Cell rearrangement during gastrulation of Xenopus: direct observation of cultured explants. *Development* **112**, 289-300.

Wilson, P. A., Oster, G. and Keller, R. (1989). Cell rearrangement and segmentation in Xenopus: direct observation of cultured explants. *Development* **105**, 155-66.

Winklbauer, R. (1990). Mesodermal cell migration during Xenopus gastrulation. *Dev Biol* **142**, 155-68.

Winklbauer, R. and Nagel, M. (1991). Directional mesoderm cell migration in the Xenopus gastrula. *Dev Biol* **148**, 573-89.

Winklbauer, R., Selchow, A., Nagel, M. and Angres, B. (1992). Cell interaction and its role in mesoderm cell migration during Xenopus gastrulation. *Dev Dyn* **195**, 290-302.

Xu, L., Overbeek, P. A. and Reneker, L. W. (2002). Systematic analysis of E-, N- and P-cadherin expression in mouse eye development. *Exp Eye Res* **74**, 753-60.

Yamamoto, Y. and Jeffery, W. R. (2000). Central role for the lens in cave fish eye degeneration. *Science* **289**, 631-3.

Zhong, Y., Brieher, W. M. and Gumbiner, B. M. (1999). Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. *J Cell Biol* **144**, 351-9.