The *C. elegans* pharynx: a model for organogenesis[*]

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Abstract

The *C. elegans* foregut (pharynx) has emerged as a powerful system to study organ formation during embryogenesis. Here I review recent advances regarding cell-fate specification and epithelial morphogenesis during pharynx development. Maternally-supplied gene products function prior to gastrulation to establish pluripotent blastomeres. As gastrulation gets under way, pharyngeal precursors become committed to pharyngeal fate in a process that requires PHA-4/FoxA and the Tbox transcription factors TBX-2, TBX-35, TBX-37 and TBX-38. Subsequent waves of gene expression depend on the affinity of PHA-4 for its target promoters, coupled with combinatorial strategies such as feed-forward and positive-feedback loops. During later embryogenesis, pharyngeal precursors undergo reorganization and a mesenchymal-to-epithelial transition to form the linear gut tube. Surprisingly, epithelium formation does not depend on cadherins, catenins or integrins. Rather, the kinesin ZEN-4/MKLP1 and CYK-4/RhoGAP are critical to establish the apical domain during epithelial polarization. Finally, I discuss similarities and differences between the nematode pharynx and the vertebrate heart.

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1. The pharynx as a model for organ development

Four characteristics of the *C. elegans* pharynx make it a powerful system to study organogenesis. First, *C. elegans* is transparent and the complete cell-lineage is known (Sulston et al., 1983), making it possible to follow organogenesis from the earliest stages of primordium formation to the terminal steps of differentiation and morphogenesis. Second, the mature pharynx is simple and well-characterized. It is composed of 95 nuclei that can be grouped into seven cell types. The position and morphology of these cells have been characterized at the ultrastructural level (Albertson and Thomson, 1976). In addition, there are antibodies and GFP reporters that mark individual cell types or developmental stages within the pharynx (http://www.wormbase.org/db/searches/expr_search). These tools are invaluable for detailed studies of wildtype and mutant embryos at the level of individual cells. Third, pharynx development is robust. Embryos with abnormal development in other tissues can still produce a well-differentiated pharynx. For example, an embryo that cannot undergo normal morphogenesis arrests as a ball of cells with a differentiated pharynx (Ahnn and Fire, 1994; Chanal and Labouesse, 1997; Storfer-Glazer and Wood, 1994; Terns et al., 1997). This characteristic enables researchers to focus on molecules likely to play a direct role in pharynx formation without the problems associated with indirect effects. Fourth, formation of the pharynx faces similar developmental challenges to those of organs in more complex animals and uses conserved molecular pathways to meet those challenges. For example, the pharynx is composed of cells with different embryonic origins (Sulston et al., 1983), similar to the polyclonal origin of most vertebrate organs. The *pha-4* locus (discussed below) is critical to specify pharyngeal identity, regardless of ancestry (Mango et al., 1994). The mammalian orthologues of *pha-4* are the FoxA proteins, and, like *pha-4*, FoxA2 is essential for gut development in all organisms studied to date (Carlsson and Mahlapuu, 2002).

2. Anatomy of the pharynx

We have a detailed knowledge of the anatomy of the pharynx, based on ultrastructural studies by Donna Albertson (Albertson and Thomson, 1976). The pharynx is a bilobed, linear tube encased in a basement membrane. It can be subdivided into six sections, which are, from anterior to posterior, the buccal cavity, procorpus, metacorpus (anterior bulb), isthmus, terminal bulb and pharyngeal-intestinal valve (see Figure 1). I define the pharynx as those cells of the foregut that express PHA-4 and that are lost in *pha-4* mutants, which includes two additional types of cells not encompassed by the pharyngeal basement membrane (arcade cells, pharyngeal intestinal valve cells; Horner et al., 1998; Mango et al., 1994). These criteria identify 95 nuclei that can be subdivided into seven cell types: arcade cells, muscles, epithelia, neurons, glands, marginal cells and valves (Albertson and Thomson, 1976). Along the longitudinal axis, there are eight sections of muscles and three sections of marginal cells; these make up the bulk of the pharynx (see Figure 2). Radially, the muscles and marginal cells are organized with three-fold symmetry around the pharyngeal lumen. These cells have characteristics of epithelia, with adherens junctions and an apical surface that faces the lumen (Albertson and Thomson, 1976). Posteriorly, a toroid of six valve cells connects pm8, the last pharyngeal muscle, to the intestine. Anteriorly, the pharynx attaches to the buccal cavity and exterior epidermis via nine arcade cells and nine epithelial cells, organized into three rings. Five gland cells and twenty neurons are embedded within the muscle/marginal cell epithelium. The neurons extend processes either dorsally or along the left and right subventral surfaces and synapse onto muscles or nerves. The gland cells contain processes that open into the pharyngeal lumen. The glands appear to secrete vesicles through these processes just before hatching, at each larval molt and during feeding. The nature of the secretions is unknown but may aid in degrading chitin and cuticle, and in digesting food. The pharyngeal lumen is lined with cuticle, which connects to the cuticle of the epidermis. Specialized fingers that project into the lumen of the terminal bulb may function as teeth or a sieve during feeding. For excellent images and an in-depth description of pharyngeal and epithelial morphology, see Wormatlas and the online WormBook.

Figure 1. Nuclei of the pharynx. The *C. elegans* digestive tract is an epithelial tube consisting of the buccal cavity (lower panel, yellow), foregut or pharynx (green), midgut or intestine (orange) and hindgut (blue). Nuclei within the pharynx (upper panel) are shown as red muscles, purple neurons, orange epithelia, pink marginal cells and brown glands. Not shown: arcade cells and pharyngeal intestinal valves.

Figure 2. Pharynx anatomy. The pharynx is composed of eight layers of muscles (pm1-8, green) separated by structural marginal cells (mc1-3, pink). These are arranged with three-fold rotational symmetry, as shown in the cross section. Kindly reproduced with permission from Z.F. Altun & D.H. Hall's Alimentary System in WormAtlas. Right-click or control click for high resolution image.

3. Early control of pharyngeal development-the ABa lineage

The pharynx is generated polyclonally during embryogenesis: at the 4-cell stage, two blastomeres, ABa and EMS, contribute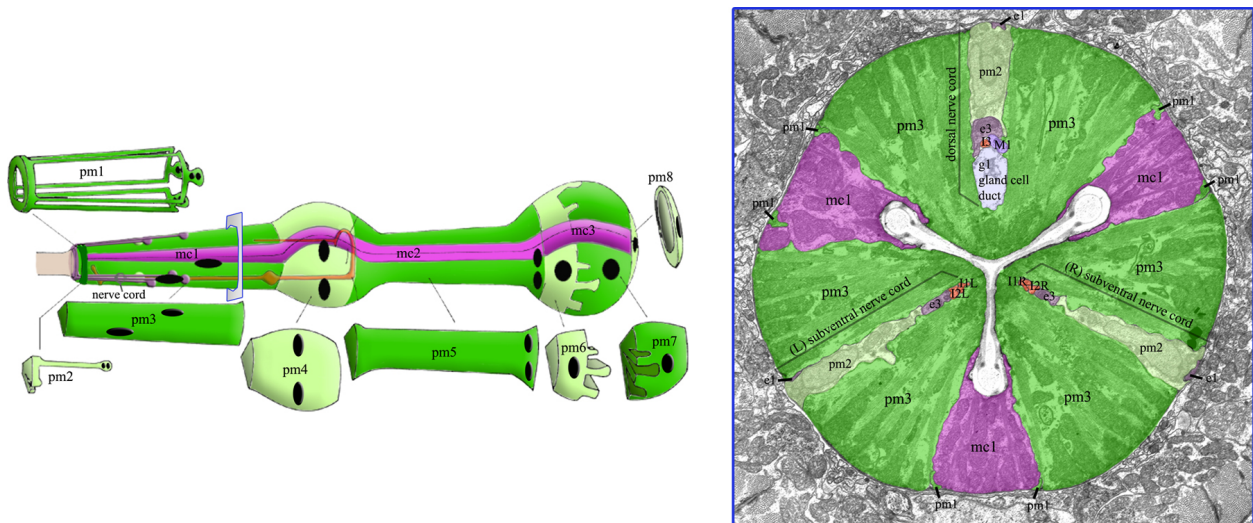 descendants to the pharynx, whereas their sisters do not (see Figure 3). Prior to gastrulation at the 28-cell stage, most early blastomeres are pluripotent and give rise to multiple cell types. For example, ABa and EMS each produces both pharyngeal cells and non-pharyngeal cells such as epidermis or body wall muscle (Sulston et al., 1983).
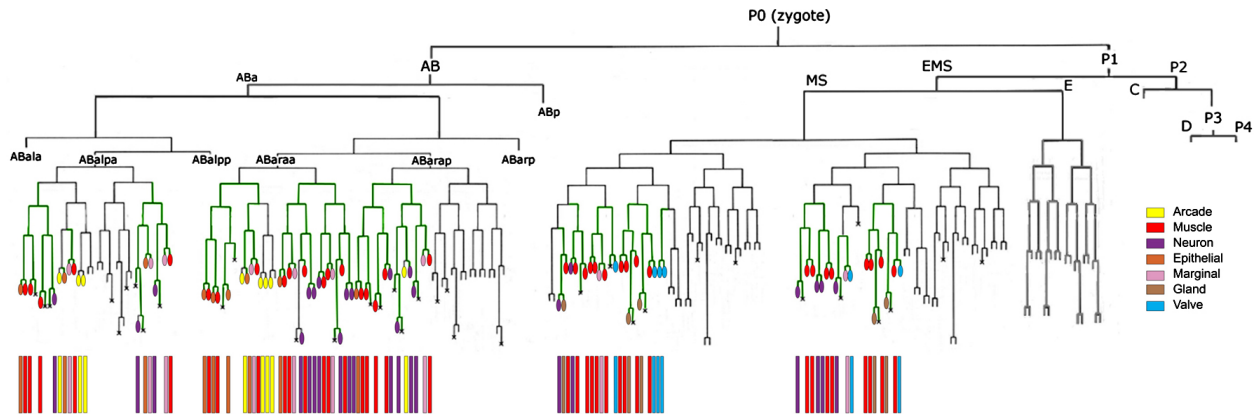

Figure 3. The pharynx is generated polyclonally. The *C. elegans* cell lineage, with emphasis on the sublineages that generate pharyngeal cells (green lines). Pharyngeal cell types are denoted by color: yellow arcade cells, red muscles, purple neurons, orange epithelia, pink marginal cells, brown glands and blue valves. Right-click or control click for high resolution image.

The production of pharyngeal cells by ABa and EMS depends on at least two distinct molecular pathways. The ABa pathway is dependent on intercellular signaling between blastomeres and on the Notch receptor orthologue *glp-1* (see Figure 4 and Table 1; Priess et al., 1987; Priess and Thomson, 1987). *glp-1* RNA is donated to the embryo by the mother (i.e., maternally) and is selectively translated in ABa and its sister ABp (Evans et al., 1994). Multiple events during embryogenesis depend on Notch signaling, and I focus only on those involved in pharyngeal development. An unidentified GLP-1 ligand generated by EMS descendents activates the LAG-1 transcription factor at the 12–15-cell stage (Christensen et al., 1996; Hutter and Schnabel, 1994; Lambie and Kimble, 1991; Mango et al., 1994; Moskowitz et al., 1994). LAG-1 in turn activates the organ selector gene *pha-4* (Smith and Mango, 2006), described below, and the REF-1 family of bHLH transcription factors (Neves and Priess, 2005). The REF-1 family is comprised of six genes distantly related to the *E(spl)* family of repressors: *ref-1, hlh-25, hlh-26, hlh-27, hlh-28, hlh-29*. Each gene contains two bHLH domains and a basic region. However, these factors lack other sequences typically found in *E(spl)* proteins, such as the Orange domain and the terminal W/YRPW sequence (Neves and Priess, 2005). In other species, this motif mediates binding to co-repressors. Thus, it is uncertain how REF-1 family members recruit co-repressors such as UNC-37/groucho, although genetic evidence supports a repressor function (Neves and Priess, 2005).

Table 1. Summary of genes involved in pharynx development. Genes implicated in pharynx development are listed from earliest stages of specification to later events. Aph: anterior pharynx absent Daf: dauer defective, M/Z: maternal or zygotic contribution of RNA or protein, Pha: pharynx absent, pm: pharyngeal muscle, PPa: posterior pharynx absent, Pun: pharynx unattached, Rcp: receptor, TF: transcription factor, Zn: zinc, ?: unknown.

Gene	Homology	M/Z	Expression (initiation)	Pha phenotype	Targets	Binding sequence	Upstream genes	References
glp-1	Notch Rcp	M	ABa@4	Aph	*ref-1* *pha-4*	N/A	?	Kalb et al., 1998; Mango et al., 1994; Priess et al., 1987
lag-1	Su(H) TF	M	Broadly	Aph	*ref-1* *pha-4*	RTGGGAA	*glp-1*	Christensen et al., 1996; Smith and Mango, 2006
skn-1	Zipper TF	M	EMS@4	Pha	*ref-1* *end-1*	G/ATCAT + A/T	?	Bowerman et al., 1992; Maduro et al., 2005b; Mango et al., 1994; Neves and Priess, 2005
med-1/2	GATA-like Zn TF	M, Z	EMS@4 and maternal	Ppa	*end-1/3* *hlh-25*	RRRAGT-ATAC	*skn-1*	Broitman-Maduro et al., 2005; Maduro et al., 2001
ref-1	bHLH TF	Z	ABa@26 ABp@4 EMS@24	none	*tbx-37* *tbx-2*	CANNTG	*lag-1*	Neves and Priess, 2005; Smith and Mango, 2006
tbx-37/38	T-box	Z	ABa@24	Aph	*pha-4?*	?	*ref-1* in some cell types	Good et al., 2004
pha-4	FoxA	Z	ABa@44 MS@28	Pha	*many*	TRTTKRY	*lag-1* *tbx-37/38* *med-1/2* *glp-1*	Gaudet and Mango, 2002; Good et al., 2004; Maduro et al., 2005b; Mango et al., 1994; Smith and Mango, 2006
htz-1	H2A.Z histone variant	Z	Broadly from the 28-cell stage	Delayed activation	*myo-2, R07B1.9*	N/A	?	Updike and Mango, 2006
tbx-2	T-box	Z	ABa@8E	ABa muscles absent	*pha-4* *ceh-22?*	?	*ref-1* in some cells *tbx-37/38?*	Chowdhury et al., 2006; Smith and Mango, 2006
ceh-22	Nkx2-5 Homeobox TF	Z	pm3-5, pm7	Indistinct BM Around pharynx	*myo-2*	CACTTAT	*pha-4* *ceh-22* *ceh-2* *pha-2*	Kalb et al., 1998; Mango et al., 1994; Okkema and Fire, 1994; Okkema et al., 1997
peb-1	FLYWCH Zn TF	Z	Broadly	Glands distended	*myo-2*	YDTGCCRW	?	Beaster-Jones and Okkema, 2004; Thatcher et al., 2001
daf-12	NHR Zn TF	Z	Broadly	Daf	*myo-2* *ceh-22*	AGTGCA	*daf-9*	Ao et al., 2004

Gene	Homology	M/Z	Expression (initiation)	Pha phenotype	Targets	Binding sequence	Upstream genes	References
pha-1	Novel	Z	Broadly	Arrested diffn, loss of TF expression	*ceh-22* *pha-4*	NA or ?	?	Fay et al., 2004; Granato et al., 1994; Schnabel and Schnabel, 1990
pha-2	Homeobox	Z	Pm5, I4, epi	pm4, pm5, morph	*ceh-22* *ceh-2*			Avery, 1993; Morck et al., 2004
ast-1	ETS	Z	Head	Pun	?	?	?	Schmid et al., 2006
myo-2	Myosin Heavy chain	Z	pm1-pm8			NA	*pha-4* *ceh-22* *peb-1* *daf-12* *daf-3*	Ao et al., 2004; Gaudet and Mango, 2002; Mango et al., 1994; Okkema and Fire, 1994; Thatcher et al., 2001
unc-39	six4/5	Z	Mesoderm Arc	Pun metacorpus	?	?	?	Yanowitz et al., 2004
eya-1	Eyes absent	Z	Broadly ≥ bean	Thin, asymmetric	?	NA	?	Furuya et al., 2005
ubc-9	E2 sumo ligase	?	?	ABa muscles absent	?	NA	?	Chowdhury et al., 2006
gei-17	E3 sumo ligase	?	?	Thick isthmus	?	NA	?	Chowdhury et al., 2006
ceh-24	Nkx TF	Z	pm8	None	?	?	?	Harfe et al., 1998
ceh-2	Homeobox ems, EMX	Z	I3, NSM, M3, pm2, e2	Poor feeding, M3 defective		?	?	Aspock et al., 2003
ceh-43	Distal-less homeobox	Z	Non-pha neurons, epidermis	Pun, anterior leakage				Aspock and Burglin, 2001; Burglin and Aspock, 1999

One of the REF-1 downstream targets, direct or indirect, is a pair of T-box genes called *tbx-37* and *tbx-38*. TBX-37 and TBX-38 constitute a pair of closely related, redundant factors that are activated at the 24-cell stage in the eight ABa descendents and required for a subset of these cells to generate pharynx (Good et al., 2004). Their restricted expression within these cells depends on repression by REF-1 family members, since inactivation of REF-1 family genes leads to widespread TBX-37/38 in blastomeres that normally would never normally express these genes (Neves and Priess, 2005). Within ABa-derived cells destined to form the pharynx, initiation of TBX-37/38 expression precedes REF-1 activity, which explains why GLP-1 activity does not block pharyngeal development in this subset of cells. The T-box family of transcription factors are defined by the T-box DNA binding domain, and can function as either activators or repressors (Wilson and Conlon, 2002). There are at least 20 T-box transcription factors in the *C. elegans* genome and surprisingly, several of them function as redundant pairs e.g., *tbx-37* and *tbx-38*, *tbx-8* and *tbx-9* (Andachi, 2004; Good et al., 2004; Pocock et al., 2004). The combination of Notch signaling and TBX-37/38 function activate the organ selector gene *pha-4*/FoxA to initiate pharyngeal development (Good et al., 2004; Kalb et al., 1998; Mango et al., 1994). Thus, selectivity of *pha-4* activation within the AB lineage depends on the co-incidence of two distinct cues-GLP-1/Notch and TBX-37/38. For additional discussion on Notch Signaling, see Notch signaling in the *C. elegans* embryo. For more information on transcription factors see chapters by Okkema and Krause, and Blackwell and Walker in the Molecular biology section of WormBook.
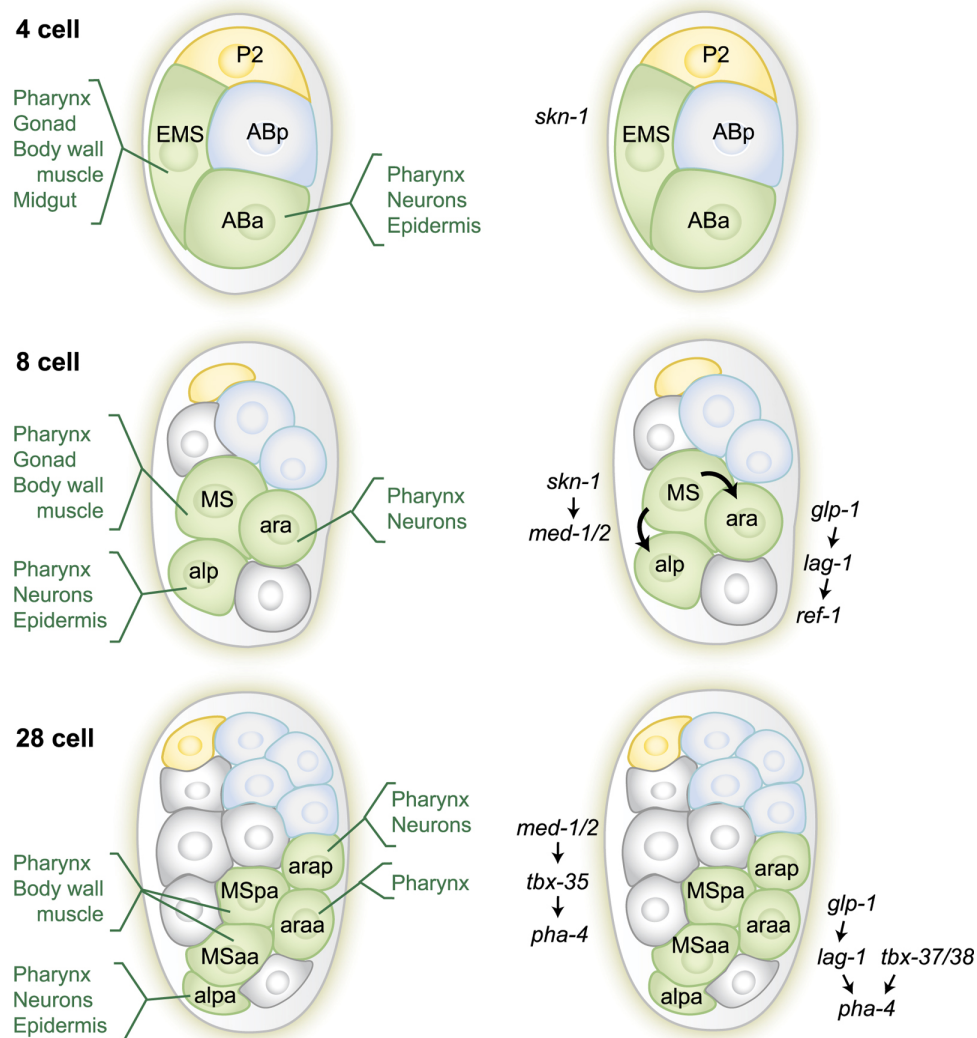
Figure 4. Early pharyngeal development. Features of pharyngeal development from the 4-cell stage to the 28-cell stage. This period is under control of maternal factors, but transitions to zygotic control with the activation of *tbx-35, tbx-37, tbx-38* and *pha-4*. Left panels show cells and the cell types they produce. Right panels illustrate genetic regulatory relationships. The pharynx is generated from green blastomeres (ABa and EMS at the 4-cell stage). Descendents of green cells that do not produce pharyngeal cells are grey.

4. Early control of pharyngeal development-the EMS lineage

The EMS pathway of pharyngeal development does not require *glp-1* signals (see Figure 4; Good et al., 2004; Priess et al., 1987; Priess and Thomson, 1987). Rather, EMS depends on the activity of two other maternal genes to generate pharyngeal cells, namely *skn-1* and *pop-1* (Bowerman et al., 1992; Lin et al., 1995). *skn-1* encodes an bZIP-related transcription factor that functions at the 4-8 cell stage to specify the EMS blastomere (Bowerman et al., 1993; Bowerman et al., 1992). In the absence of *skn-1*, no pharynx is produced because EMS descendents are transformed into their cousin, the C blastomere, and C does not generate Notch ligands to signal to ABa. *pop-1* encodes a Tcf/Lef-1 homolog that is enriched in the nucleus of a daughter of EMS, called MS, by the action of the *wingless* signalling pathway and *lit-1* kinase (Lin et al., 1995; Lo et al., 2004). When *pop-1* activity is reduced, MS develops like E and MS-derived pharyngeal cells are lost (Lin et al., 1995). E can produce pharyngeal-inducing cues and so the anterior pharynx is still made. The role of POP-1 in MS is to repress genes that promote E fate (Broitman-Maduro et al., 2005; Calvo et al., 2001; Maduro et al., 2002; Shetty et al., 2005).

The MS and E blastomeres also express the GATA-like factors *med-1* and *med-2* at the 7-cell stage. The *med* gene products are contributed maternally (Maduro et al., 2006) and are also activated by SKN-1 (Maduro et al.,

2006; Maduro et al., 2001). Loss of both *med* genes leads to conversion of MS fate into that of C, leading to loss of the posterior pharynx (Goszczynski and McGhee, 2005; Maduro et al., 2006; Maduro et al., 2001). There is currently debate regarding the contribution of the MED factors, with one report suggesting few embryos have an MS to C fate transformation (Goszczynski and McGhee, 2005) and other studies suggesting a greater proportion of embryos are affected (Maduro et al., 2006; Maduro et al., 2001). The MED factors bind the consensus sequence RAGTATAC, which differs from the canonical GATA site WGATAR. Strikingly, a search of the genome for MED consensus sites accurately predicted many MED factor target genes. Among these targets were multiple transcription factors: *tbx-35*, *sox-1, hlh-25, hlh-27, hlh-28*, and *hlh-29* (Broitman-Maduro et al., 2005). One of these, *tbx-35,* is expressed within MS descendants, and is required to produce the bulk of body wall muscles and pharyngeal cells by MS descendants (Broitman-Maduro et al., 2006). Intriguingly, *tbx-35* is closely related to *tbx-37* and *tbx-38*, revealing a similar requirement for Tbox factors for both ABa and MS pharyngeal development. A second target of SKN-1 within MS are *ref-1* family members, which may repress TBX-37/38 in these cells (Neves and Priess, 2005).

5. The transition from maternal to zygotic control

LAG-1, TBX-35, TBX-37/38 and the MED factors initiate the zygotic phase of pharyngeal development. The phenotypes associated with the zygotic regulators differ from those observed with the maternal genes. In *glp-1, skn-1* and *pop-1* embryos, all of the cell types that normally derive from a given blastomere are affected, not just pharyngeal cells. In these mutant embryos, ABa or EMS descendants follow the cell lineage and differentiation patterns characteristic of other early blastomeres (Bowerman et al., 1992; Hutter and Schnabel, 1994; Lin et al., 1995; Priess et al., 1987). These phenotypes suggest that early blastomeres acquire unique identities that are specified by maternal genes like *glp-1, skn-1* and *pop-1*. By contrast, later in development, cells that are destined to make the pharynx and that derive from different cell lineages, coordinate their development to form an integrated, functioning structure. The genes that function during these later processes have phenotypes that affect a specific tissue or organ rather than a cell lineage, for example, *pha-4*, *ceh-22*, *tbx-2* (Chowdhury et al., 2006; Mango et al., 1994; Okkema and Fire, 1994; Okkema et al., 1997; Smith and Mango, 2006). LAG-1, TBX-35, TBX-37/38 and the MED factors contribute to the transition from maternal to zygotic control-their mutant phenotypes affect cell lineages, but their target genes, described below, are geared towards forming tissues and organs (Broitman-Maduro et al., 2006; Good et al., 2004; Maduro et al., 2005a; Smith and Mango, 2006).

Whereas ABa and EMS are pluripotent, their descendants become lineally restricted (see Figure 3). Around the 200 cell stage, ABa and EMS descendants are born that will produce either all pharyngeal cells or no pharyngeal cells (Sulston et al., 1983). The lineage restriction seen at this time applies to pharyngeal fate generally, not to individual cell types found within the pharynx. For example, one particular pharyngeal precursor born at the 200-cell stage (ABaraaapa) divides twice to generate four pharyngeal cells: one muscle, one epithelial cell, one arcade cell and one marginal cell (Sulston et al., 1983). This separation of pharyngeal and non-pharyngeal cell lineages was the first clue that cells acquire a general pharyngeal organ identity.

6. The organ selector gene *pha-4*

The central regulator of pharynx development is *pha-4,* a FoxA transcription factor (Horner et al., 1998; Kalb et al., 1998; Mango et al., 1994). No pharyngeal primordium is formed in embryos lacking *pha-4,* and at least a proportion of pharyngeal cells develop into ectoderm, which can be visualized by the ectodermal markers LIN-26 and ELT-3 (Horner et al., 1998; Labouesse and Mango, 1999; Kiefer et al., 2006). Conversely, expression of *pha-4* throughout the embryo can induce pharyngeal fate in a subset of embryonic blastomeres (Horner et al., 1998; Labouesse and Mango, 1999). No zygotic gene besides *pha-4* has been found that can mutate to a pharynx-less phenotype (S. Mango, unpublished, J. Priess, pers. comm., J. Rothman, pers. comm.), suggesting that the maternal ABa and MS pathways converge on *pha-4*. Thus, *pha-4* behaves as an organ selector gene (Mann and Carroll, 2002) that specifies pharynx identity for both ABa and MS-derived blastomeres.

Transcription of *pha-4* is activated at the 28-cell stage in MS descendants, the 44-cell stage in ABa descendents (Baugh et al., 2003; Good et al., 2004; Horner et al., 1998; Smith and Mango, 2006) and continues to be expressed in all pharyngeal cells throughout life (Alder et al., 2003; Azzaria et al., 1996; Horner et al., 1998; Kalb et al., 1998). *pha-4* is also expressed in midgut and hindgut cells, at varying levels, and is required to generate anterior hindgut cells vir and rep (Mango et al., 1994). In the pharynx, the timing of activation suggests that *pha-4* may be regulated directly by LAG-1 and TBX-37/38 within ABa descendents at the 24-cell stage (Good et al., 2004), and TBX-35 in the MS lineage (Broitman-Maduro et al., 2006). However, it is likely that additional, undiscovered regulators exist.

7. Commitment to pharyngeal fate

When do embryonic cells commit to pharyngeal fate? Three observations suggest that embryonic cells are pluripotent up to the 4E stage (equivalent to 50-100 cells; embryos are staged by the number of endodermal or E cells), but subsequently lose their plasticity. First, at the 2E stage and earlier, individual blastomeres contribute to multiple cell types, as revealed by the *C. elegans* cell lineage (Sulston et al., 1983). One cell division later (4E stage, 50-100 cells), many cells give rise to descendants that contribute to only a single tissue or organ. Second, blastomeres at the 2E and 4E stages can adopt alternate fates in response to forced ubiquitous expression of heterologous cell fate regulators (Fukushige and Krause, 2005; Horner et al., 1998; Labouesse and Mango, 1999; Zhu et al., 1998; Kiefer et al., 2006). However, early blastomeres can no longer adopt alternate fates when challenged with an ectopic cell-fate regulator at ≥8E stage (Labouesse and Mango, 1999; Kiefer et al., 2006). Third, most genes required for cell identity are expressed by the 4-8E stage. These include *skn-1, pha-4, med-1/2, tbx-35, tbx-37/38* and *tbx-2* (Bowerman et al., 1992; Chowdhury et al., 2006; Good et al., 2004; Horner et al., 1998; Priess et al., 1987; Smith and Mango, 2006). Genes expressed later often have subtler phenotypes involving morphology or differentiation, (e.g., *ceh-22, pha-1, ceh-24* (Fay et al., 2004; Harfe et al., 1998; Okkema and Fire, 1994; Okkema et al., 1997; Schnabel and Schnabel, 1990). A temperature-sensitive configuration of *pha-4* (Kaltenbach et al., 2005) revealed that reduction of *pha-4* before the 4E-stage led to loss of pharyngeal identity whereas inactivation of *pha-4* at later stages affected morphogenesis but not pharyngeal fate (Kiefer et al., 2006). These observations suggest that cells transition from pluripotent to committed at approximately the 4-8E stage.

Three strategies ensure that embryonic blastomeres develop into pharyngeal cells and do not stray towards another identity (see Figure 5). The first is positive feedback loops between pairs of pharyngeal regulators. For example, a positive regulatory loop between PHA-4 and TBX-2 contributes to production of pharyngeal muscles. TBX-2 expression initiates at the 8E stage and is required to maintain PHA-4 expression within ABa-derived pharyngeal muscle precursors (Chowdhury et al., 2006; Smith and Mango, 2006). Loss of either *pha-4* or *tbx-2* leads to reduced expression of the other transcription factor and absence of ABa-derived pharyngeal muscles. In other animals, TBX-2 orthologues function as repressors (Naiche et al., 2005) and, in *C. elegans*, TBX-2 interacts with components of the SUMO-conjugating pathway by yeast two hybrid (Chowdhury et al., 2006). In other organisms, SUMO is a repressive mark for transcription (Gill, 2005). Thus, it is unclear whether the positive regulatory loop between PHA-4 and TBX-2 reflects direct activation or indirect effects.
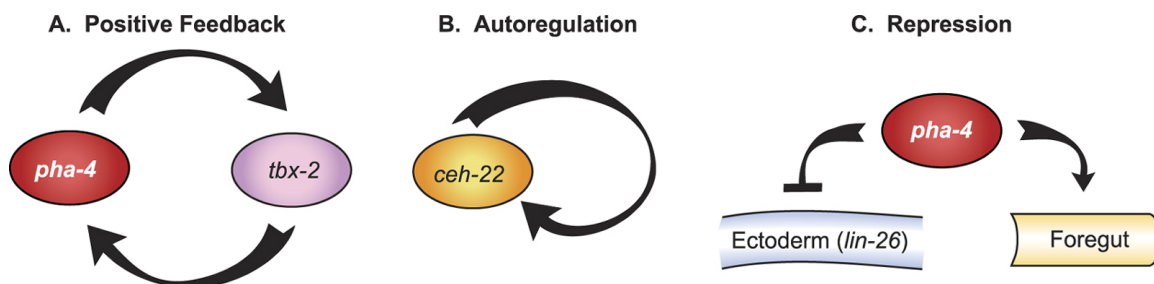

Figure 5. Strategies for cell fate commitment. Commitment to pharyngeal fate depends on A. positive feedback loops, B. positive autoregulation and C. repression of alternative fates.

A second strategy for cell fate commitment is auto-regulation (see Figure 5). For example, *ceh-22* contains two enhancer elements, distal and proximal, which control initiation and maintenance of *ceh-22* expression, respectively (Kuchenthal et al., 2001; Vilimas et al., 2004). The proximal enhancer carries a CEH-22 binding site that is necessary and sufficient for activity. Thus, maintenance of *ceh-22* transcription depends on positive autoregulation through the proximal enhancer, which likely contributes to robust, stable *ceh-22* expression and pharyngeal muscle fate.

The third strategy for cell fate commitment is transcriptional repression, which is important to inhibit alternative cell fates (see Figure 5). *pha-4* is necessary and sufficient to inhibit ectodermal fate, and inhibits expression of ectodermal genes such as *lin-26* or *elt-3* (Horner et al., 1998; Kiefer et al., 2006). The *lin-26* locus contains the CISg regulatory element, which is peppered with consensus, conserved PHA-4 sites (Landmann et al., 2004), and which binds PHA-4::YFP within pharyngeal cells, suggesting repression by PHA-4 could be direct (Kiefer et al., 2006). Repression of ectodermal fate also requires the NuRD complex and the TRIM factor TAM-1, which associates with PHA-4 in yeast two-hybrid assays (Kiefer et al., 2006; Li et al., 2004). Intriguingly, other

proteins bearing a TRIM motif or RING finger domain mediate transcriptional repression via binding to the NuRD subunit Mi-2, suggesting a possible link between these factors (Shimono et al., 2003).

8. Downstream of PHA-4: patterning the pharynx primordium

How are individual cell types generated within the pharynx? This question has been difficult to address because the phenotypes associated with loss of individual pharyngeal cells can be subtle. Moreover, there appears to be significant redundancy for the underlying molecular mechanisms. Redundancy can be at the level of gene duplication (e.g., the six REF-1 family members; Neves and Priess, 2005) or at the level of pathways for non-homologous genes (e.g., *ceh-22/Nkx2.5* and *pha-1*/DUF1114 (Okkema et al., 1997)). For these reasons, reverse genetics and genomic approaches will likely be very useful for deciphering the regulatory network that governs the latter stages of pharyngeal development (Table 2).

Table 2. Algorithms. Tools for genomic approaches to transcription control: Algorithms that can be used to identify potential regulatory sequences for known or novel transcription factors, to construct regulatory pathways or analyze microarrays.

Name	URL	Use	References
BioProspector	http://ai.stanford.edu/~xsliu/BioProspector/	Find enriched sequence motifs via Gibbs sampling and Markov models	Liu et al., 2001
BioTapestry	http://labs.systemsbiology.net/bolouri/software/BioTapestry/	Visualization of gene regulatory networks	Longabaugh et al., 2005
CisOrtho	http://dev.wormbase.org/CisOrtho/	Identify conserved targets of worm transcription factors whose DNA binding specificity is known	Bigelow et al., 2004
ClusterBuster	http://cagt.bu.edu/page/ClusterBuster_about	Identify clusters of pre-specified motifs in nucleotide sequences	Frith et al., 2003
Co-Bind	http://ural.wustl.edu/software.html	Identify target sites for cooperatively binding transcription factors	GuhaThakurta and Stormo, 2001
Compare Prospector	http://ai.stanford.edu/~iliu/CompareProspector/index.html	Discover enriched sequence motifs conserved across species	Liu et al., 2004
ConSite	http:/www.phylofoot.org/consite	Discover enriched sequence motifs conserved across species	Sandelin et al., 2004
Cytoscape	http://www.cytoscape.org/	Network data integration, visualization, and analysis	Shannon et al., 2003
GSEA	http://www.broad.mit.edu/gsea/	Determine whether a set of genes shows significant differences between two biological states	Mootha et al., 2003
Improbizer	http://www.cse.ucsc.edu/~kent/improbizer/improbizer.html	Identify statistically enriched sequences in DNA or RNA	Ao et al., 2004
JASPAR	http://jaspar.genereg.net	Open-access repository for transcription factor binding site profiles	Vlieghe et al., 2006
MEME/MAST	http://meme.sdsc.edu/meme/intro.html	Motif Discovery and Search	Bailey and Elkan, 1994; Bailey and Gribskov, 1998

Name	URL	Use	References
ModuleFinder	http://the_brain.bwh.harvard.edu/PSB2005MFSuppl/index.html	Evaluate the likelihood that a genomic region is a cis regulatory module for an input set of transcription factors according to: homotypic site clustering; heterotypic site clustering; and evolutionary conservation	Philippakis et al., 2005
MultiFinder	http://the_brain.bwh.harvard.edu/multifinder.html	Motif search using AlignACE, MDscan, BioProspector and MEME	Huber and Bulyk, 2006
PAZAR	http://sourceforge.net/projects/pazar	A framework to allow multiple boutique databases to function independently within a larger system; a public repository for regulatory data	Wasserman et al., unpublished
RSAT	http://rsat.ulb.ac.be/rsat/	Series of modular computer programs to detect regulatory signals	van Helden, 2003
TRANSFAC	http://www.gene-regulation.com/pub/databases.html	Database of eukaryotic transcription factors and their binding sites	Knuppel et al., 1994
Worm Enhancer	http://wormenhancer.org/Main	Find clusters of binding sites in the genome.	Markstein, Markstein, Levine, unpublished

9. Temporal control during organogenesis

Patterning the pharyngeal primordium depends on successive programs of gene expression. How is this achieved? One input is PHA-4 itself (see Figure 6). Promoter analyses suggest that PHA-4 directly regulates many genes expressed in the pharynx, including genes active both early and late, (Gaudet and Mango, 2002; Gaudet et al., 2004; Kalb et al., 1998; Kuchenthal et al., 2001; Smith and Mango, 2006; Vilimas et al., 2004). The affinity of PHA-4 for its binding sites contributes to the timing of target gene activation (Gaudet and Mango, 2002; Gaudet et al., 2004). Mutations that alter the affinity of PHA-4 binding sites higher or lower shift the onset of target gene expression earlier or later, respectively. These temporal shifts occur in the context of the promoter, and are not an absolute predictor of transcriptional activation of target genes. Moreover, they depend on recruitment of the histone variant HTZ-1 for a subset of pharyngeal promoters (Updike and Mango, 2006). Thus, PHA-4 may function as a competence factor (Zaret, 2002) that modulates chromatin to prime a promoter for activation.

A second means for temporal control is feed-forward regulation (see Figure 6). For example, PHA-4 activates *ceh-22/Nkx2.5* and both PHA-4 and CEH-22 activate *myo-2*, which encodes a myosin heavy chain expressed in terminally-differentiating pharyngeal muscles (Gaudet and Mango, 2002; Kalb et al., 1998; Mango et al., 1994; Okkema and Fire, 1994; Okkema et al., 1997). The involvement of CEH-22 in *myo-2* transcription helps explain why the *myo-2* promoter fires late in embryogenesis, even though it possesses a high affinity PHA-4 binding site. However, CEH-22 is expressed by mid-embryogenesis (bean stage), whereas *myo-2* transcription initiates later (two-fold stage), suggesting additional inputs are involved.

Combinatorial regulation is the third means to control timing (see Figure 6). A single PHA-4 binding site, similar to what exists in most promoters, is not sufficient to activate expression (Gaudet et al., 2004). Multiple PHA-4 binding sites can activate transcription throughout the pharynx, but this configuration is rarely seen in natural promoters (Gaudet et al., 2004). Bioinformatic searches for sequences over-represented in pharyngeal gene promoters led to the discovery of two motifs associated with early-expressed pharyngeal genes and two associated with late-expressed pharyngeal genes (Gaudet et al., 2004). These early and late cis elements were tested for biological function in two ways. One was necessity: were the sequences required for expression of natural

pharyngeal promoters? Mutation of the candidate sites revealed whether natural promoters required the sequences for normal expression. The second was sufficiency - could three copies of a candidate element introduced upstream of the *pes-10* basal promoter activate GFP transcription? For these kinds of assays to succeed, GFP reporters were introduced into worms without vector sequences, which can contain cryptic pharyngeal enhancers (Hope, 1991; Young and Hope, 1993). In addition, low concentrations of reporter DNA (≤2ng/ul) were used, as were more likely to recapitulate endogenous expression (Gaudet and Mango, 2002; Gaudet et al., 2004). In Table 2 I list algorithms and databases that may be helpful for identifying transcription factor binding sites within groups of genes.
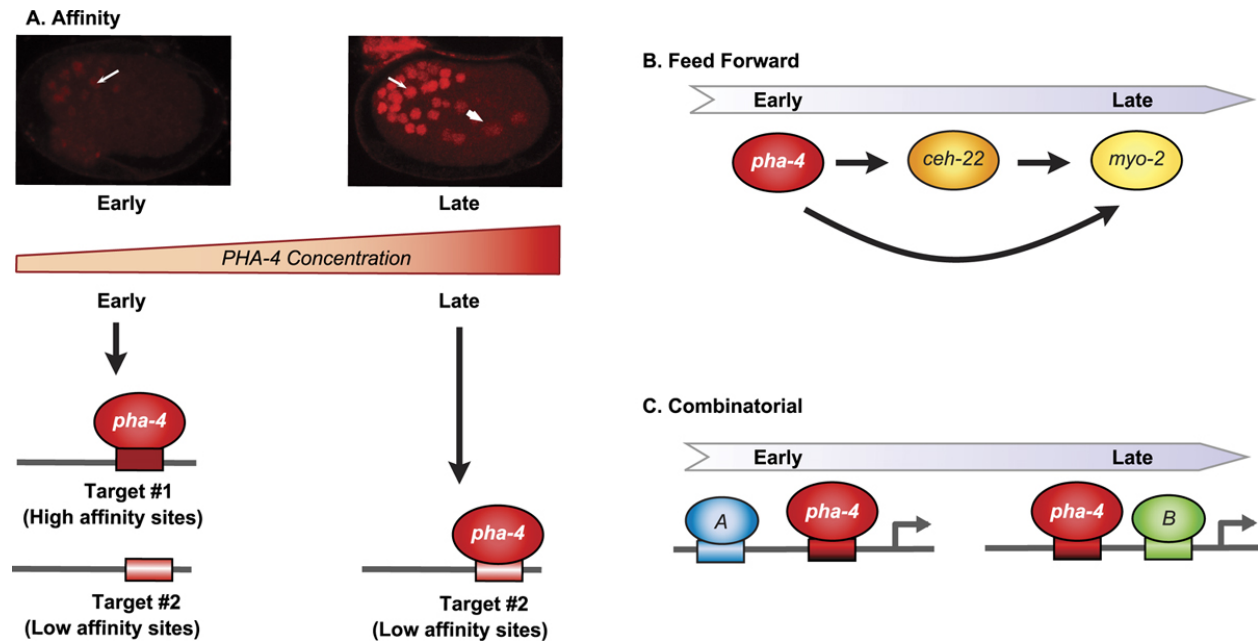

Figure 6. Strategies for temporal control. A. As embryogenesis proceeds, PHA-4 protein accumulates. Affinity of PHA-4 protein for its DNA binding site contributes to early (high affinity) vs. late (lower affinity) onset of expression. B. Feed-forward regulation contributes to late onset expression of target genes, including those with high affinity PHA-4-binding (e.g., *myo-2*). C. Pharyngeal genes are regulated by additional factors (A and B) in addition to PHA-4. These can include both activators and repressors.

10. Cell-type regulation

The mechanisms that establish individual pharyngeal cell types are best understood for pharyngeal muscles. The early events, during gastrulation, rely on PHA-4 and the Tbox genes described above. Less is known about later differentiation of the pharyngeal muscles. A hallmark of pharyngeal muscle differentiation is transcription of *myo-2/*myosin exclusively within pharyngeal muscles. The *myo-2* promoter has been analyzed extensively (Ao et al., 2004; Gaudet and Mango, 2002; Okkema and Fire, 1994; Okkema et al., 1993). Two cis-regulatory elements, B and C, are required for full activity of *myo-2*. The B subelement binds CEH-22, which is expressed in a subset of pharyngeal muscles. The C subelement binds PHA-4 and PEB-1, a FLYWCH zinc finger factor related to *Mod(mdg4)*, which is involved in insulator function in *Drosophila* (Beaster-Jones and Okkema, 2004; Kalb et al., 2002; Thatcher et al., 2001). The nuclear hormone receptor DAF-12 also activates *myo-2* and mediates the modulation of *myo-2* in response to nutrition (Ao et al., 2004). Surprisingly, inactivation of the binding sites of each of these factors does not block *myo-2* expression. Conversely, Mutation of *pha-4* binding sites leads to a delay in *myo-2* activation (Gaudet and Mango, 2002). *ceh-22* is required for isolated B element activity but endogenous *myo-2* is still active in *ceh-22* mutants (Okkema et al., 1997). *peb-1* mutants die because they cannot shed their cuticle during molting, which may reflect feeding defects and/or additional functions of *peb-1* (Fernandez et al., 2004). Nevertheless, *myo-2* expression appears normal. *daf-12* mutations lower *myo-2* reporter expression but do not obliterate it (Ao et al., 2004). Thus, the organism uses multiple inputs to assure robust expression of pharyngeal myosin and no one factor is essential.

Expression and specification of other pharyngeal cell types is less well understood. The Six family homeodomain protein *unc-39* is expressed in the pharyngeal arcade cells. Mutants have a misshapen pharynx and sometimes arrest with a Pun (Pharynx UNattached) pharynx, however it is unclear if this phenotype represents loss of arcade cell identity or defective morphogenesis (Yanowitz et al., 2004).

Additional genomic-scale searches of pharyngeal promoters have identified potential new regulators (Ao et al., 2004; GuhaThakurta et al., 2004). Ao and colleagues used microarray analysis and TopoMap clustering to identify genes expressed in different pharyngeal cell types (Ao et al., 2004). These cohorts of genes were then used to search for new cis-regulatory elements that dictate expression in muscle or epithelia. Conversely, using a yeast one-hybrid approach, Deplancke and colleagues discovered factors that bound digestive tract promoters (Deplancke et al., 2006). The authors surveyed 112 gut promoters or regulatory elements for transcription factor binding using a yeast one-hybrid assay and discovered 283 interactions involving 72 promoters and 117 interacting factors (Deplancke et al., 2006). Most factors interacted with a small number of genes and conversely most genes had multiple factors binding, an average of four. The factors discovered in this screen are presumably those that can bind as monomers or homomers in yeast, suggesting this is just the tip of the iceberg.

11. Transcriptional strategies for organogenesis

It is intriguing to compare the transcriptional strategies of cell fate specification and differentiation for the pharynx vs. the midgut, two very different organs. Pharynx development depends on PHA-4, which functions at multiple stages of development and in all pharyngeal cell types. To achieve diversity, pharyngeal promoters are regulated by a combinatorial mechanism. This strategy depends on transcription factors that are weak activators. For example, when expressed ectopically, PHA-4 can change the fate of only a subset of embryonic cells to pharynx (Horner et al., 1998). When introduced into yeast, PHA-4 functions poorly as a transcriptional activator in one-hybrid assays (Kalb et al., 2002). The configuration of pharyngeal promoters also dampens the effect of any one factor. Pharyngeal promoters typically contain multiple cis-regulatory sites (Deplancke et al., 2006; Gaudet et al., 2004), but individual sites are often suboptimal for binding a given transcription factor and are present in only 1-2 copies (Gaudet and Mango, 2002). Thus, the input from any one transcription factor is minor, and promoter firing depends on multiple weak inputs at a promoter.

The midgut, on the other hand, is a simple organ, composed of one cell type that derives from a single precursor, the E blastomere. This simplicity is mirrored at the transcriptional level. The midgut depends on tiers of GATA transcription factors that function for only 1-2 cell divisions and elicit a more homogeneous transcriptional response (Maduro et al., 2005a). As a consequence, these regulatory GATA factors are more potent activators and probably do not rely heavily on combinatorial mechanisms to activate their target genes. For example, widespread expression of one of these GATA factors, *end-1,* can convert the entire embryo into midgut (Zhu et al., 1998) and another GATA factor, *elt-2,* is a potent activator in yeast one-hybrid assays (Kalb et al., 2002). Surprisingly, these tiers of GATA factors are genetically redundant, and their individual contributions are just beginning to be understood (Maduro et al., 2006). Analysis of MED-1/2 target genes reveals a surprisingly simple code: two copies of the invariant sequence RRRAGTATAC in a 100bp stretch and within 2kb of the ATG start codon. These rules predicted 21 MED target genes of which at least 12/15 behaved as expected for a MED target (Broitman-Maduro et al., 2005). Thus, the simplicity of the intestine is mirrored in a simpler transcriptional strategy: more potent transcription factors, less binding sequence heterogeneity and a simpler promoter architecture. It will be interesting, as more target genes emerge, to determine if these distinctions continue to hold true.

12. Morphogenesis

By mid-embryogenesis, gastrulation is finished, cell division is almost complete, and the pharyngeal primordium is visible as a ball of cells bordering the nascent midgut in the interior of the embryo (Portereiko and Mango, 2001; Sulston et al., 1983). The pharyngeal cells are attached to each other and to the midgut by adherens junctions (Leung et al., 1999), but are not yet connected to the buccal cavity. Over the next sixty minutes, the pharyngeal cells shift their position and organization to form a linear tube that links the digestive tract to the exterior (Portereiko and Mango, 2001). The initial event of morphogenesis is reorganization of the pharyngeal primordium. Pharyngeal epithelial cells reorient their apicobasal polarity from rostrocaudal to dorsoventral relative to the embryonic axes (see Figure 7A to B). This rearrangement alters the morphology of the pharynx from a cyst, with the apical surfaces located internally, to a short tube that extends from the midgut to the anterior edge of the pharyngeal primordium. This movement aligns the pharyngeal epithelial cells with the arcade cells. Next, the arcade cells form adherens junctions that link the pharynx and epidermis, to form a continuous epithelium (see Figure 7B to C). This event mechanically couples the buccal cavity to the pharynx and anterior epidermis. During the third stage of pharyngeal extension, cells of the pharynx, buccal cavity and epidermis appear to undergo a local contraction that pulls them tightly together (see Figure 7C to D). The remainder of the pharynx is presumably dragged forward by virtue of its attachment to the anterior pharynx. Once connected the pharynx undergoes additional morphogenesis, to produce the bi-lobed structure of the mature pharynx.
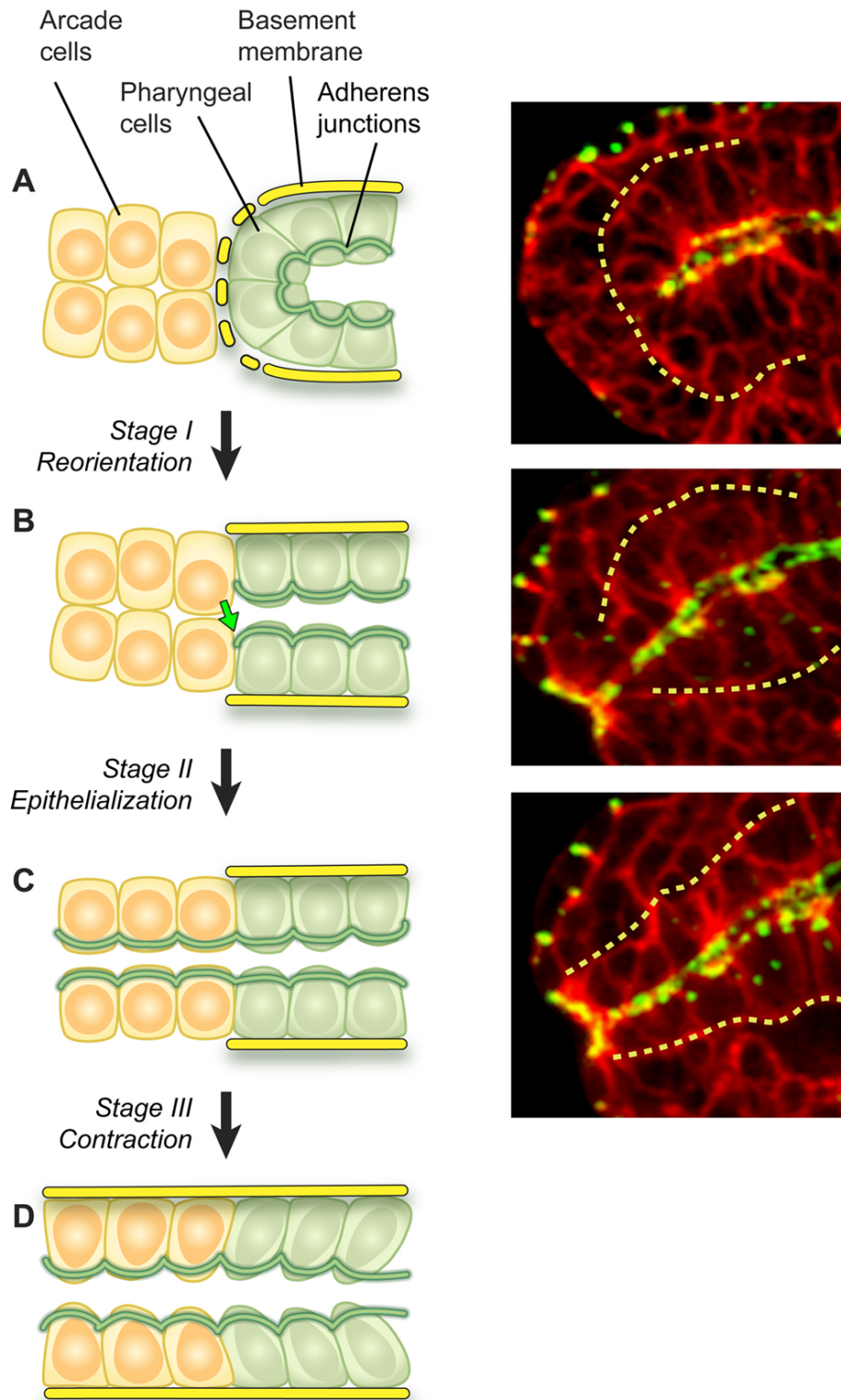
Figure 7. Pharyngeal morphogenesis. Left panels depict stages of Reorientation (A to B, Stage 1), Epithelialization (B to C, Stage 2) and Contraction (C to D, Stage 3). Yellow cells denote arcade cells, which are initially mesenchymal (A, B), but later become epithelialized (C, D). Green cells represent cells in the pharyngeal primordium. Right panels show midstage embryos stained for cell periphery (red, αUNC-70) and adherens junctions (green, MH27), merge is yellow. The basement membrane surrounding the pharynx is denoted by a dotted yellow line in both sets of panels.

Table 3. Genes involved in pharyngeal morphogenesis. Genes that lead to a morphogenesis defect when mutaM/Z: maternal or zygotic contribution, phx: pharyngeal, Ref: references, ?: unknown.

Gene	Homology	M/Z	Cellular expression	Localization	Function	References
cyk-4	MgcRacGAP RhoGAP	M,Z	Broadly	?	Apicobasal polarity	Portereiko et al., 2004; Jenkins et al., 2006
zen-4	MKLP1 kinesin	M,Z	Broadly	?	Apicobasal polarity	Portereiko et al., 2004
ast-1	ETS	Z	Head	N/C	Pha attachment	Schmid et al., 2006
crp-1	*cdc-42* related	Z	Muscles and non-phx epithelia	Endosomes, trans-Golgi network	Apical trafficking	Jenna et al., 2005
let-413	scribble	Z	Epithelia	Basolateral	Confinement of CeAJ	Legouis et al., 2000; McMahon et al., 2001
die-1	Zinc finger TF	Z	Epithelia	Nuclear	Pha attachment	Heid et al., 2001
eff-1	Novel	Z	Muscles, non-phx fusing epithelia	Contact points	Epithelial fusion	Mohler et al., 2002; Shemer et al., 2004
elt-5/ egl-18	GATA TF	Z	Non-pha epidermis, neurons	Nuclear	Pha attachment	Koh et al., 2002; Koh and Rothman, 2001
fbl-1	Fibulin	Z	Non-phx	Basement membrane	Pha morphology	Muriel et al., 2005
ham-2	Zinc finger	Z	Embryo	Nuclear	Pha attachment	Baum et al., 1999
inx-3	Innexin	Z	Broadly, epithelia	Basal surface	Pha attachment pha morphology	Starich et al., 2003
pha-1	DUF1114	Z	Ubiquitous	Cytoplasmic	Pha attachment pha differentiation	Fay et al., 2004; Granato et al., 1994; Schnabel and Schnabel, 1990
ubc-18	Ubiquitin conj ez	?	?	?	Pha attachment	Fay et al., 2003
ari-1	Ariadne RING finger	?	Broadly	?	Pha attachment	Qiu and Fay, 2006
lin-35	Rb repressor	?	Broadly	Nuclear	Pha attachment	Fay et al., 2003
sma-1	βH-spectrin	Z	Epithelia	Apical	Pha elongation	McKeown et al., 1998
spc-1	α-spectrin	Z	Epithelia	Apical	Pha elongation	Norman and Moerman, 2002

Many factors affect pharynx morphogenesis, based on loss-of-function phenotypes, but it is unclear if these phenotypes reflect cell fate, differentiation or morphogenesis. Deletion of the ETS homologue *ast-1* leads to pharynx unattached (Pun) larvae that cannot feed (Schmid et al., 2006). By time-lapse videomicroscopy, pharyngeal development proceeds normally to the 1.5 fold stage when pharyngeal cells fail to attach to the buccal cavity. AST-1::GFP is expressed in the head, including a few unidentified pharyngeal cells. It is unclear whether the pharyngeal defects reflect a function for AST-1 in the pharynx or in surrounding head cells.
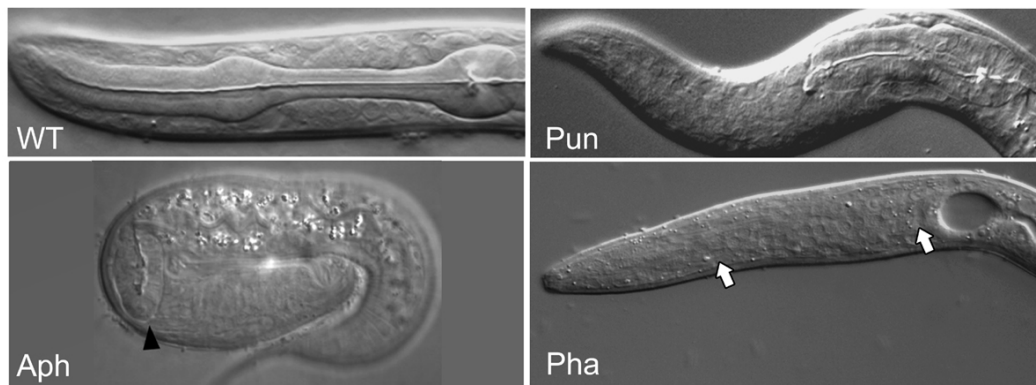
Figure 8. Pharyngeal phenotypes. Examples of pharyngeal phenotypes: wild type WT, pharynx unattached Pun (*zen-4*), anterior pharynx absent Aph (*aph-1*) and pharynx absent Pha (*pha-4*). Note the full size but unattached Pun pharynx compared to the smaller than normal Aph pharynx (arrowhead). In Pha animals, no pharynx is observed (arrows). Aph animal kindly reproduced from Goutte et al. (2002).

The *pha-2* homeobox is important for isthmus morphogenesis (Morck et al., 2004). Animals lacking *pha-2* have an abnormally thick pharyngeal isthmus, cells of the anterior bulb appear to mix with those of the isthmus, and CEH-22/Nkx2.5 is expressed inappropriately in pm5, which forms the isthmus (Morck et al., 2004). These data suggest that *pha-2* is required to distinguish pm5 fate or morphology, distinct from the other pharyngeal muscles.

The *ceh-43/distal-less* homolog is not obviously expressed in the pharynx but loss of *ceh-43* activity by RNAi leads to a detached pharynx, possibly because of problems with the epidermal epithelium (Aspock and Burglin, 2001). Similarly, loss of the GATA factor *elt-5* produces animals with Pun pharynges, likely due to epidermal defects (Koh et al., 2002).

The *eya-1* locus is homologous to *eyes absent* and carries two HAD domains (Furuya et al., 2005). In other animals, *eyes absent* is a phosphatase co-factor for the *sine oculis* transcription factor and part of the eye regulatory circuit (Rebay et al., 2005). In worms, *eya-1* mutants arrest at the L1 or L2 stage with a thin pharynx and reduced pumping rates (Furuya et al., 2005). The pharyngeal bulb can be misshapen and the lumen stuffed with bacteria, suggesting a feeding defect. *eya-1* is partially redundant with *vab-3/pax-6* suggesting that the regulatory circuit that controls eye development in other animals may have adopted a new function for anterior development in *C. elegans*, which lacks eyes (Furuya et al., 2005).

die-1 encodes a zinc finger transcription factor expressed in many epithelia including the pharynx (Heid et al., 2001). *die-1* mutants can be detached from either the intestine or from the buccal cavity (Heid et al., 2001). DIE-1 is expressed in the pharynx, although the precise cells are unknown. DIE-1 binds multiple genes by yeast one-hybrid analysis, including genes many genes implicated in transcription (Deplancke et al., 2006).

sma-1 encodes βH(heavy)-spectrin, which is essential for the elongated form of the pharynx (McKeown et al., 1998). Mutants are viable but the procorpus and isthmus fail to elongate. Some of these defects may reflect the lack of body elongation rather than internal to the pharynx itself. Association of SMA-1 to apical surfaces of epithelia depends on α-spectrin (Norman and Moerman, 2002).

The transcriptional repressor *lin-35/Rb* and the ubiquitin conjugating enzyme *ubc-18* are required redundantly for the first stage of pharyngeal morphogenesis, Reorientation (Fay et al., 2003). Inactivation of both genes leads to arrested Pun animals (Fay et al., 2003). Similarly, inactivation of *pha-1* and either *ubc-18* or *ari-1/Ariadne* leads to an unattached pharynx phenotype (Fay et al., 2004; Qiu and Fay, 2006). One model to explain these complicated interactions is that over-expression of a factor "X" leads to a Pun phenotype. Transcriptional repression (e.g., *lin-35*) and protein degradation (*ubc-18* or *ari-1*) normally keep X in check. However, when both are inactivated, X excess inhibits pharyngeal morphogenesis. It is unclear what role the *pha-1* plays in this process. *pha-1* encodes a DUF1114 factor expressed in the cytoplasm (Fay et al., 2004).

13. Epithelium formation-the pharyngeal arcade cells

C. elegans epithelia resemble those of other organisms, with apical and basolateral domains (Knust and Bossinger, 2002). LET-413/scribble is localized to the basolateral domain, where it restricts the spread of components of adherens junction and the apical domain (Koppen et al., 2001; Legouis et al., 2000; McMahon et al., 2001), while the PAR-3/PAR-6 complex is confined to the apical domain (Leung et al., 1999). A single junction, the CeAJ, separates the apical and basolateral domains and has features of both adherens and tight junctions. For example, the CeAJ contains proteins that mediate adhesion such as HMR-1/cadherin, HMP-1/alpha-catenin, HMP-2/beta-catenin and VAB-9/claudin (Costa et al., 1998; Pettitt et al., 1996; Simske and Hardin, 2001). Located slightly more basally but still within the CeAJ are DLG-1/discs large and the coiled-coil protein AJM-1 (Bossinger et al., 2001; Koppen et al., 2001). The Fat subfamily cadherin *cdh-3* is expressed in the pharynx and would be predicted to localize to CeAJs (Pettitt et al., 1996).

What factors are required to build the pharynx epithelium? Surprisingly, many proteins implicated in the formation or maintenance of epithelia in other animals are apparently not essential in the *C. elegans* digestive tract. For example, homologues of Crumbs, cadherins, discs-large, ZO-1, α- or β-integrins do not give rise to obvious pharyngeal defects after being inactivated (Baum and Garriga, 1997; Costa et al., 1998; Drubin and Nelson, 1996; Pettitt et al., 1996; Williams and Waterston, 1994). These data suggest *C. elegans* may rely on additional molecules to establish epithelia. Intriguingly, studies with other organisms suggest alternative routes for epithelium formation exist in these animals also (Baas et al., 2004; Bilder et al., 2003; Harris and Peifer, 2004).

The kinesin-like protein *zen-4*/MKLP and its partner *cyk-4*/RhoGAP are required to polarize the arcade cells (Portereiko et al., 2004). Apical and adherens junction proteins fail to accumulate at the cell surface of arcade cells from *zen-4* mutants, even though these proteins are synthesized in the cell. Thus, *zen-4* and *cyk-4* appear important to target polarity proteins to the apical surface and CeAJ during polarization. Recent studies suggest that CYK-4 modulates cell polarity in other contexts by controlling RhoA activity and the contractile actomyosin cytoskeleton (Jenkins et al., 2006); perhaps this regulatory pathway will hold true for the pharyngeal epithelium as well.

LET-413, which contains a PDZ motif and leucine-rich repeats similar to *Drosophila scribble*, is localized to basolateral membranes of all epithelial cell types, including the pharynx (Chanal et al., 1997; Legouis et al., 2000). In epidermal cells lacking *let-413/scribble*, apical proteins such as PKC-3 are mislocalized, and CeAJ-associated proteins such as DLG-1 and AJM-1 remain along the lateral surface rather than becoming condensed into the junctional region as in wildtype (McMahon et al., 2001). Thus, LET-413 may function after ZEN-4 and CYK-4 to control adherens junction maturation and positioning.

14. Is the pharynx a heart?

There has been debate about a possible evolutionary relationship between the *C. elegans* pharynx and the vertebrate heart. Three observations suggest that the pharynx may be orthologous to the vertebrate heart. First, both the pharynx and heart are tubes that move material along their lumens using binucleate muscles (Albertson and Thomson, 1976; Kellerman et al., 1992). Both organs pump continuously, for the life of the organism. Second, both organs rely on similar electrical circuitry to control pumping. Contractions are synchronized by gap junctions that couple adjacent muscle cells, and contractions can continue in the absence of neuronal input (Avery and Horvitz, 1989; Bernstein and Morley, 2006; Starich et al., 1996; Starich et al., 2003). Contraction speed can be modulated by neurotransmitters that couple to GPCRs, including acetylcholine and serotonin (Hobson et al., 2003; Penela et al., 2006; Steger and Avery, 2004). The pharynx and heart rely on some similar types of channels such as the LQT potassium channels and L-type voltage-gated calcium channels (Avery and Horvitz, 1989; Raizen and Avery, 1994; Salkoff et al., 2005). Third, both organs rely on NKX transcription factors for their genesis: *ceh-22* for the pharynx and *Nkx2.5* for the zebrafish heart (Chen and Fishman, 1996; Okkema and Fire, 1994; Okkema et al., 1997). Moreover, Nkx2.5 can activate the CEH-22 target gene *myo-2* when expressed in *C. elegans* body wall muscles, and Nkx2.5 can rescue *ceh-22* mutants when expressed under control of the *ceh-22* promoter (Haun et al., 1998).

Despite these similarities, three observations suggest that parallels between the pharynx and heart may represent convergent evolution rather than true homology. First, while the heart is a mesodermal organ, the pharynx appears to be ectodermal. Topologically, the pharynx is connected to the epidermis and, like the epidermis, is lined with cuticle (Albertson and Thomson, 1976). Pharyngeal muscle is myoepithelial, displaying apical domains separated by adherens junctions from the basolateral surfaces, which contact a basal lamina (Albertson and Thomson, 1976; Portereiko and Mango, 2001). In *pha-4* mutant embryos, at least a portion of pharyngeal cells are

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transformed into ectodermal cell types, but not into mesodermal cell types (Horner et al., 1998; Kiefer et al., 2006). Second, the involvement of Nkx2.5 proteins for pharyngeal development may be misleading. In other organisms, Nkx2.5 factors are required for visceral muscle development as well as heart formation (e.g., *tinman* in *Drosophila* (Bodmer, 1993). This observation may explain why *Nkx2.5*, which is normally restricted to myocardiocytes in zebrafish, can nevertheless rescue visceral muscle development in *Drosophila Nkx/tinman* mutants (Park et al., 1998). Third, aspects of the electrical conductivity are different between the pharynx and heart. *C. elegans* lacks the voltage-gated sodium channel that typically initiates the action potential with a fast sodium spike in the heart (L. Avery, pers. comm.). The pharynx is excited by motor neurons via nicotinic receptors, similar to mammalian skeletal muscle (McKay et al., 2004; Raizen et al., 1995; Towers et al., 2005). The potassium channels that end the action potential are only distantly related between pharyngeal *exp-2* and cardiac hERG (Davis et al., 1999; Shtonda and Avery, 2005). These differences suggest that similarities between the two organs may represent convergent evolution between two muscular pumps faced with similar biological roles.

15. Conclusion

To form the pharynx, *C. elegans* faces developmental challenges that are similar to those of more complex animals and uses conserved molecular pathways to meet those challenges. With the ability to visualize individual cells during organogenesis and the development of powerful tools (genomics, forward and reverse genetics, molecular biology), scientists have begun to discover the genes required for cell fate specification and morphogenesis. Our challenge for the future is to uncover the function of these genes and to dissect the regulatory networks that drive these processes.

16. Acknowledgements

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