The Development and Function of Vertebrate Cilia

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Primary cilia
- Most cells in interphase and most differentiated cells
- Short and immotile
- 9+0 arrangement of microtubules
- Lack axonemal dynein arms
- Sensory function, required for signal transduction
- Dysfunction leads to pleiotropic effects manifest in syndromes like Bardet-Biedl syndrome

Vertebrate cells make two distinct kinds of cilia

<table>
<thead>
<tr>
<th>Motile cilia</th>
<th>Primary cilia</th>
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<tbody>
<tr>
<td>Long and motile</td>
<td>Short and immotile</td>
</tr>
<tr>
<td>9 pairs of microtubule doublets surrounding a central pair</td>
<td>9+0 arrangement of microtubules</td>
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<tr>
<td>Axonemal dynein arms</td>
<td>Lack axonemal dynein arms</td>
</tr>
<tr>
<td>Sensory function, required for fluid movement and cilia motility</td>
<td>Sensory function, required for fluid movement and cilia motility</td>
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<tr>
<td>Triples form cilia in the eye and olfactory systems</td>
<td>Cilia dysfunction in the eye and olfactory systems</td>
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<td>Axonemal dynein arms</td>
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Distribution of cilia in different groups of organisms

- Plants and fungi: No cilia
- Insects: Primary cilia and sperm flagella
- Vertebrates: Primary and motile cilia
- Nematodes: Primary cilia, no motile cilia
- Protozoans: Motile cilia and flagella

Ciliary development is intimately linked with the cell division cycle


How to build the motile cilium: the transcription factor Foxj1 and the motile ciliogenic program
In mammals, the winged helix (forkhead) transcription factor Foxj1 has been implicated in the biogenesis of motile cilia


Motility of nodal cilia induce left-right asymmetric development in vertebrate embryos


Summary

- Motile cilia are important for a number of developmental and physiological processes in vertebrates
- Foxj1 has a dedicated role in the induction of motile ciligenic program
- Foxj1 is both necessary and sufficient for motile cilia formation

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The zebrafish as a model system
- Can be easily bred and housed in high density laboratory conditions
- Produce large numbers of optically transparent embryos through external fertilization
- Amenable to forward and reverse genetic analysis as well as transgenesis
- Ease of high end imaging at cellular and sub-cellular resolution
- Nearly finished genome sequence
- Developmental and physiological processes have significant similarity with mammals and humans

Zebrafish foxj1a is expressed in tissues that differentiate motile cilia

Loss of Foxj1a compromises motile cilia formation in the zebrafish embryo

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Loss of Foxj1a affects left-right asymmetry of visceral organs

Foxj1a regulates expression of ciliogenic genes left-right dynein and centrin2

Foxj1a activity is sufficient for induction of left-right dynein and centrin2 expression

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Motile ciliogenic genes form a synexpression group and are expressed in ciliated tissues in a Foxj1a-dependent manner
http://zfin.org/cgi-bin/weblode?M:val=aa-2DB_home.apg
- Tektin
- Axonemal dynein intermediate chain
- Tubulin polymerization promoting protein
- Adenylate kinase
- EF-hand containing protein EFHC1
- WD-40 repeat containing protein
- TPR domain containing protein
A loss of expression of the ciliary genes in embryos deficient in Foxj1a, and their ectopic expression in embryos with ectopic Foxj1a activity

Promoters of ciliary genes are responsive to Foxj1a activity

Promoters of ciliary genes are bound by Foxj1a in vivo


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The Foxj1a induced ectopic cilia are motile

Adapted from Yu et al., Nat. Genet. 40: 1445-1453
Conclusions

- Foxj1 transcription factors are master regulators of the motile ciliogenic program – their activity is necessary as well as sufficient for the biogenesis of motile cilia
- Foxj1 directly regulates the expression of a suite of genes that are required to build the motile cilium

Primary cilia and hedgehog signaling: role of the iguana/DZIP1 protein

Hedgehog (Hh) signaling and patterning of the vertebrate embryo

Fig. 1

Fig. 2
Mechanism of Hh signal transduction and what can happen (in humans) when it goes wrong

Holoprosencephaly
Smith-Lemli-Opitz syndrome
Gorlin syndrome
Basal cell carcinoma
Medulloblastoma
Trichoepithelioma
Gastric cancer
Post-axial polydactyly

Hh signal transduction: connection with primary cilia


Ratio of activator to repressor forms of Gli transcription factors is altered in mice without primary cilia

Many essential components of the Hh pathway localize to basal bodies and ciliary axonemes.

Mutation of the zebrafish iguana (igu) gene disrupts Hh signal transduction.

iglu encodes a coiled-coil and zinc finger containing protein necessary for proper Gli activity.
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Mutation of the *igu* gene arrests primary cilia formation in the zebrafish embryo

Muscle cells  Retinal cells

Acetylated tubulin  β-catenin  DAPI

Adapted from Tay et al., (2010) Developmental Dynamics (in press)

Igu function is required for axonemal biogenesis

γ-tubulin  β-catenin  DAPI

Adapted from Tay et al., (2010) Developmental Dynamics (in press)

Compared to primary cilia, motile cilia formation has a less stringent requirement for Igu activity

Kupffer’s vesicle  Pronephric ducts

Acetylated tubulin  DAPI  Foxj1a

Adapted from Tay et al., (2010) Developmental Dynamics (in press)

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Conclusions
• The Igu protein is a newly identified component of the ciliogenic pathway.
• Igu localizes to the basal body and is required for the biogenesis of ciliary axonemes.
• The role of the primary cilium in Hh signal transduction is conserved in the zebrafish.
• Crucial role for the Igu proteins in the ciliogenic pathway, a role that is evolutionarily conserved in diverse organisms.

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