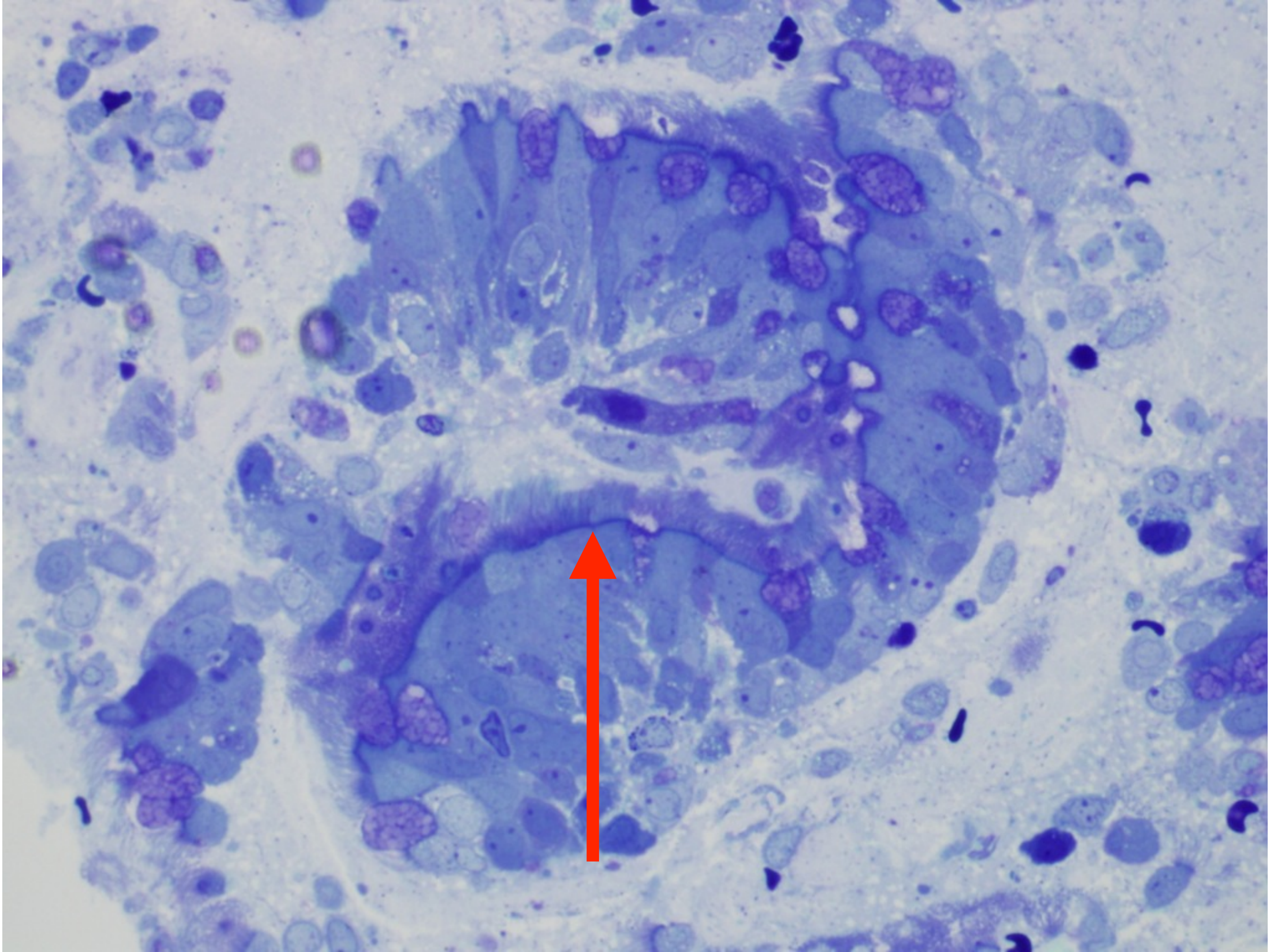
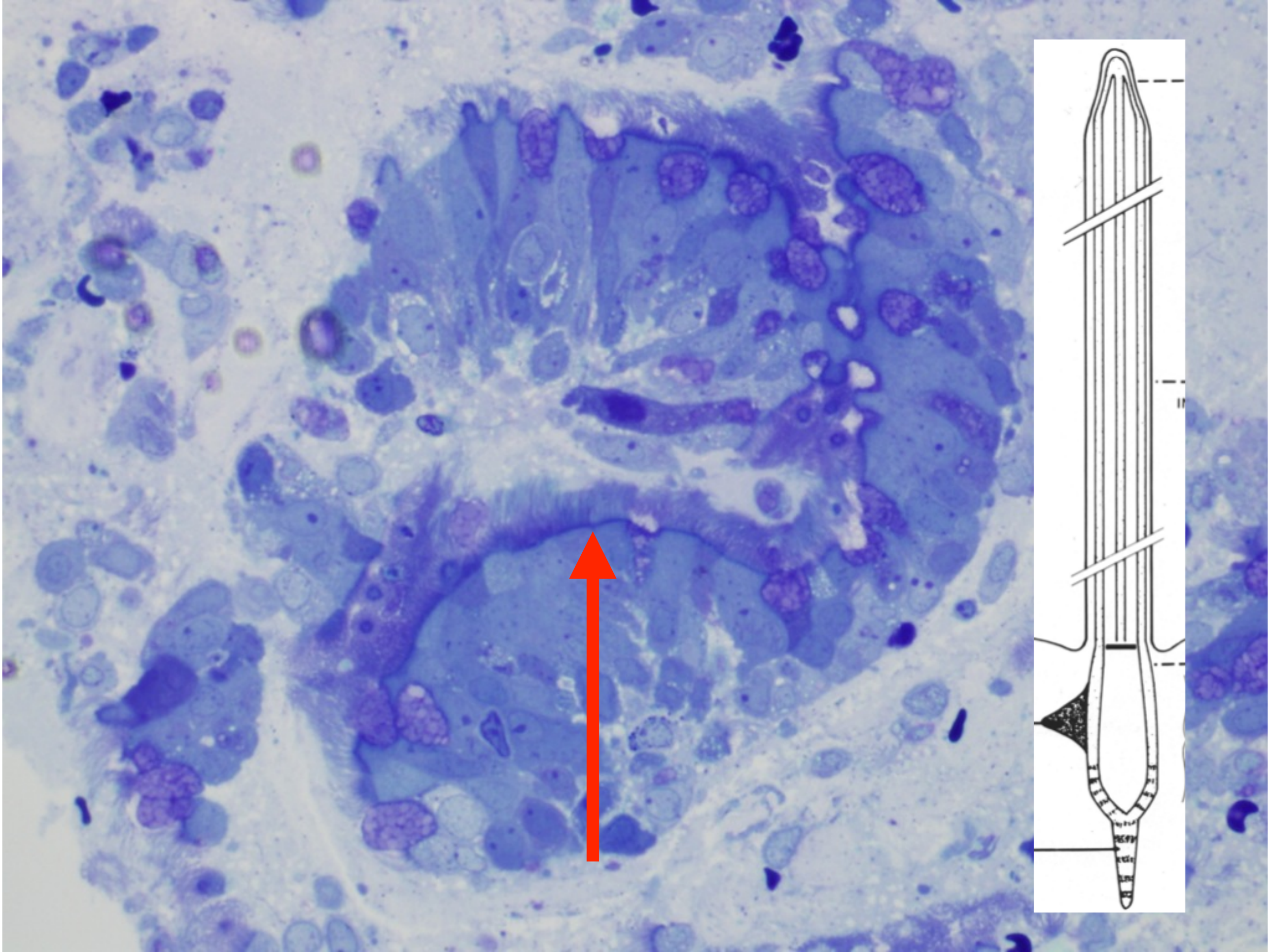
Disorders of Cilia

Ted Pysher, M.D.





Disorders of Cilia

Biology of cilia

Primary ciliary dyskinesia - disorders of motile cilia

Laterality defects - disorders of nodal cilia

The ciliopathies - disorders of non-motile (primary) cilia

Distribution of Cilia

Present in all eukaryotes except fungi and higher plants

Confined to sensory neurons in most invertebrates

Single non-motile cilium present on most cells in vertebrates

Single motile cilium present on cells in the primitive node

Multiple motile cilia present on respiratory, Fallopian, and ependymal lining cells; and one in spermatozoan flagella

Evolution of Cilia and Flagella

Bacteria

Flagella $\overset{?}{\rightleftharpoons}$ Type III transport/injection system

Archaea

Homologous to Type IV pili (not bacterial flagella)

Eukaryotes

Symbiotic

spirochete + eukaryote or archaebacterium

invasion by centriole-like virus

Endogenous

assembly of cytoplasmic tubulins and dyneins

Functions of Cilia

Cell motility

Directing fluid flow across a surface

Sensory perception

Chemical

Mechanical

Thermal

Osmolal

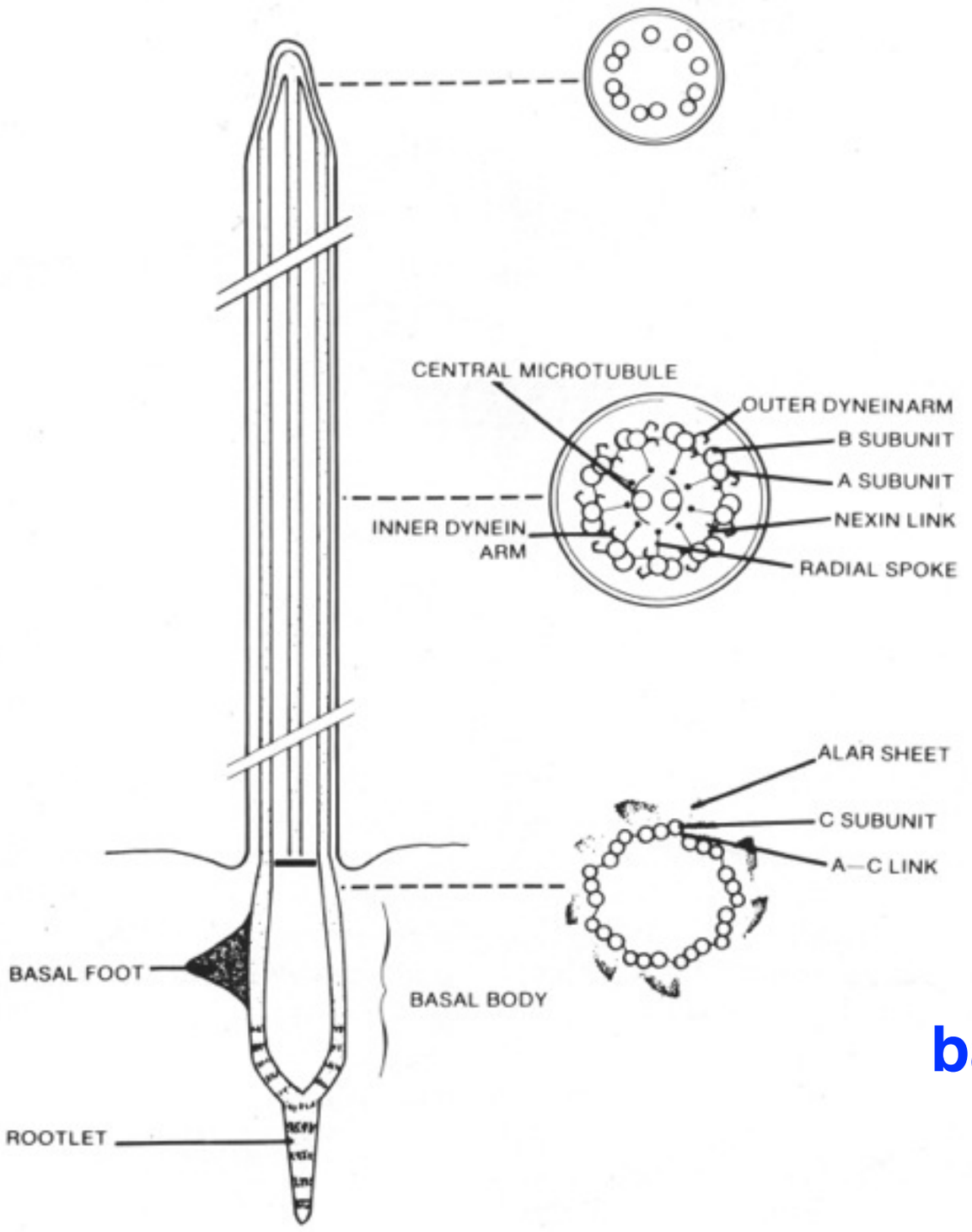
Light

Gravity

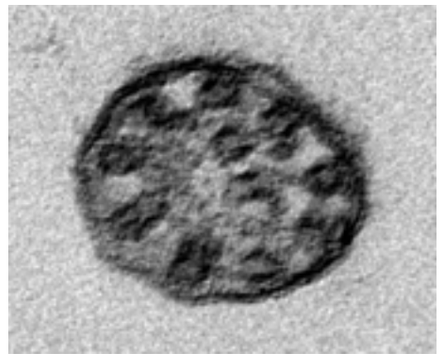
Signal transduction

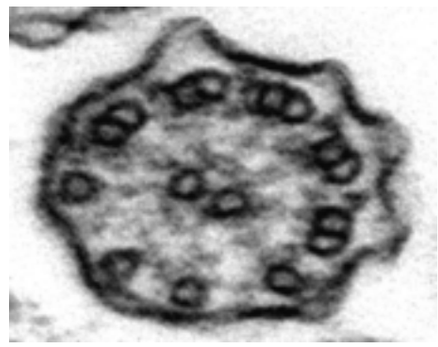
Cell cycle regulation

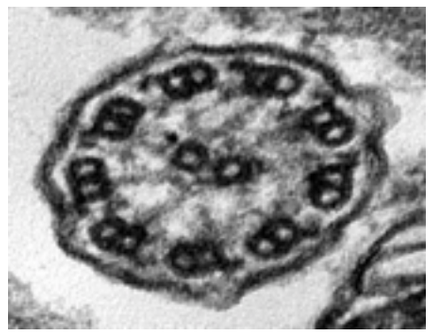
Normal anatomy of a motile cilium

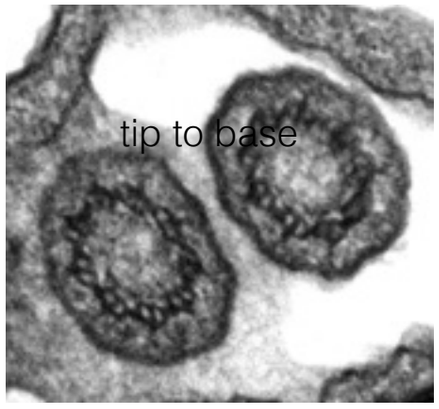


tip







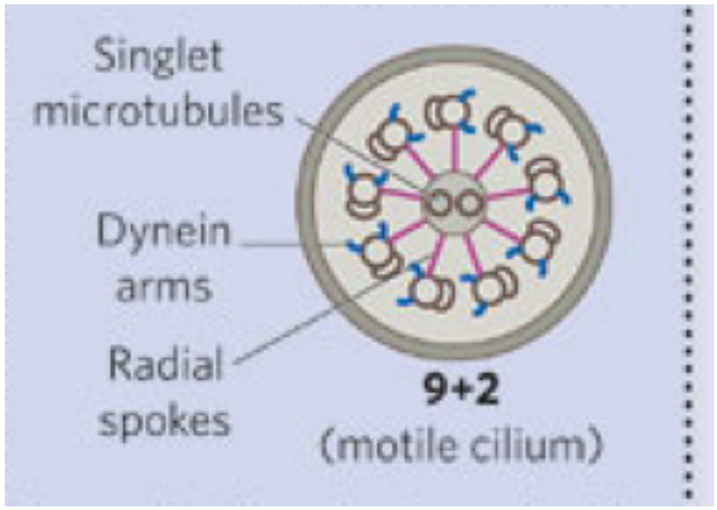


transition zone

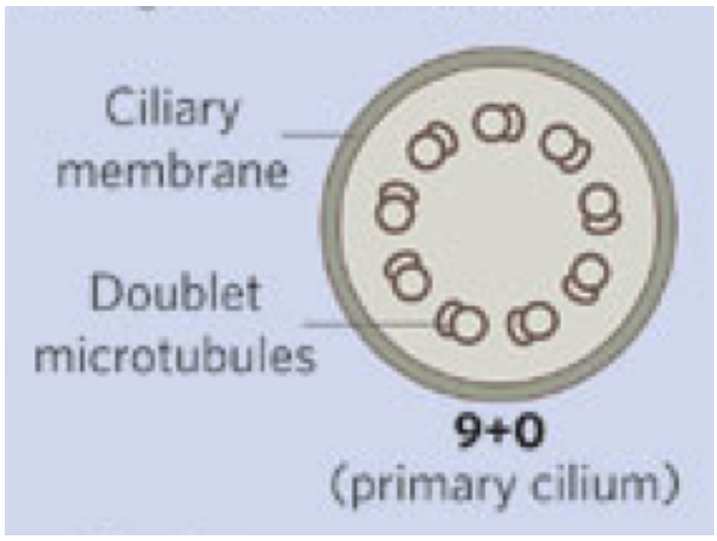
basal body

Two Types of Cilia

Motile



Primary
(non-motile)

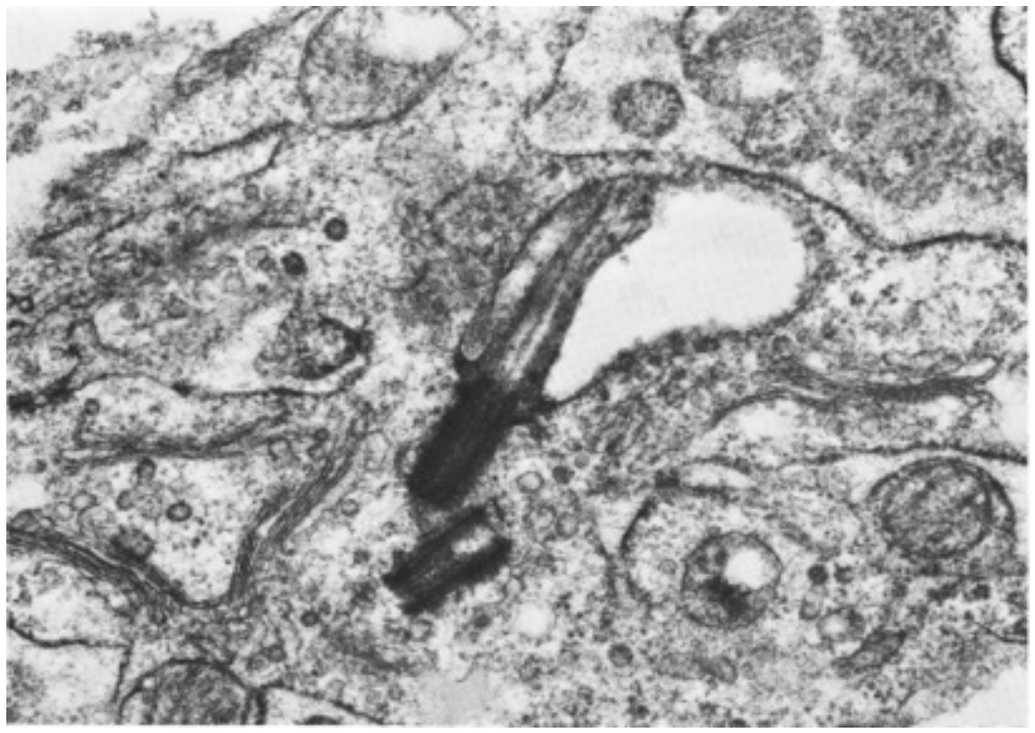


Ainsworth C. Cilia: Tails of the unexpected. Nature 2007;448:638-41

The centriole cycle

The primary cilium is disassembled as the cell re-enters the cell cycle

The mother and daughter centrioles detach from the cell membrane
Each centriole begins to duplicate at the G1 to S transition
The new daughter centrioles grow during G2



Mahjoub MR. Organogenesis 2013;9(2):61-69

Nigg EA, Stearns T. Nat Cell Biol 2011; 13:1154

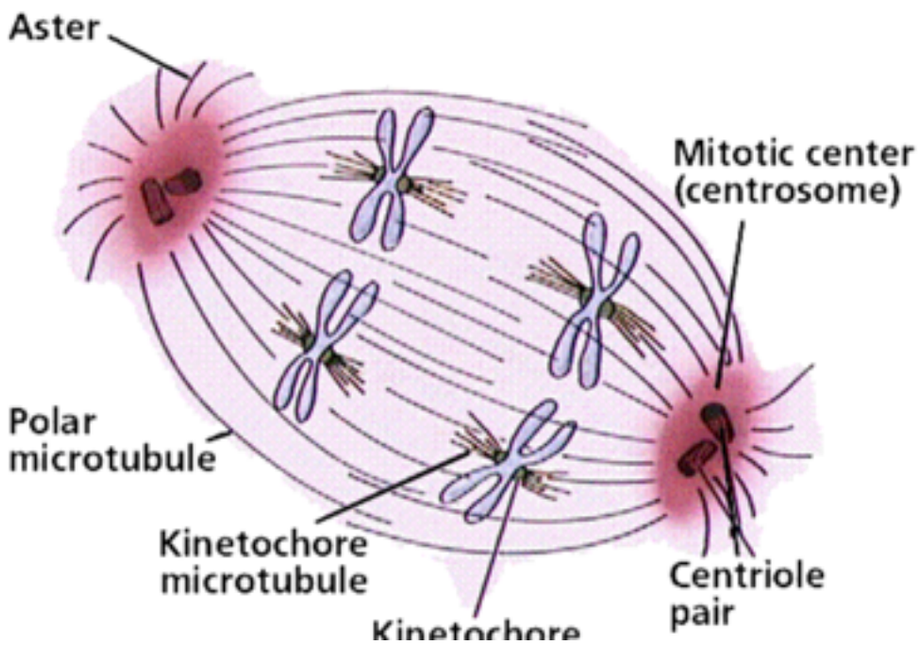
The centriole cycle

The primary cilium is disassembled as the cell re-enters the cell cycle

The mother and daughter centrioles detach from the cell membrane
Each centriole begins to duplicate at the G1 to S transition
The new daughter centrioles grow during G2

The two new pairs move to opposite sides of the cell and establish the spindle poles

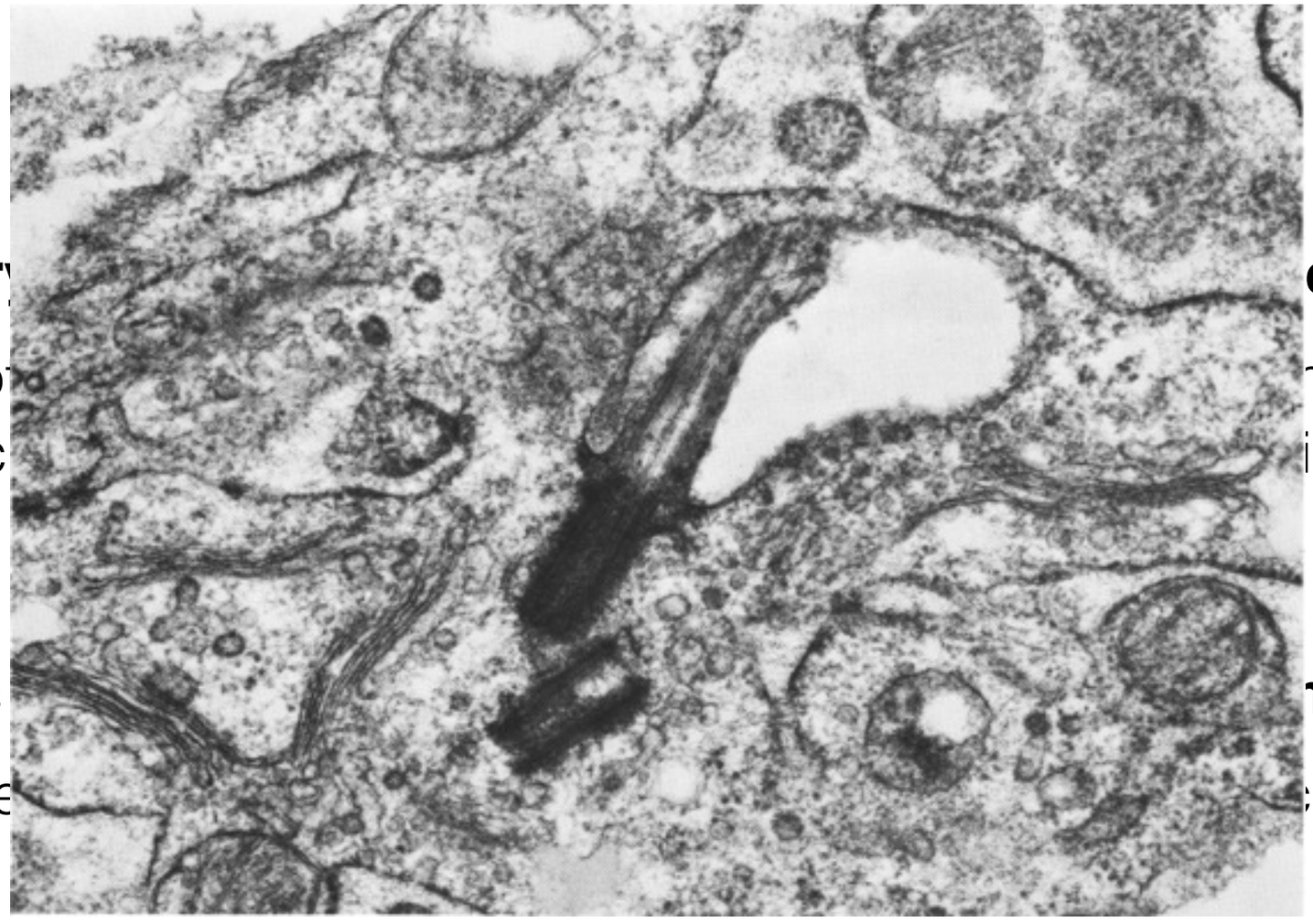
Each pair is segregated by the spindle so each daughter cell receives one pair



Mahjoub MR. Organogenesis 2013;9(2):61-69

Nigg EA, Stearns T. Nat Cell Biol 2011; 13:1154

The centriole cycle



The primar **e cell cycle**

The mo nembrane
Eac ition

The two new pairs **n the spindle poles**

Each pair is se ceives one pair

The older (“mother”) of the two centrioles docks in the plasma membrane

The axoneme is elongated and the ciliary membrane acquires specialized receptors and channels via intraflagellar transport during G1

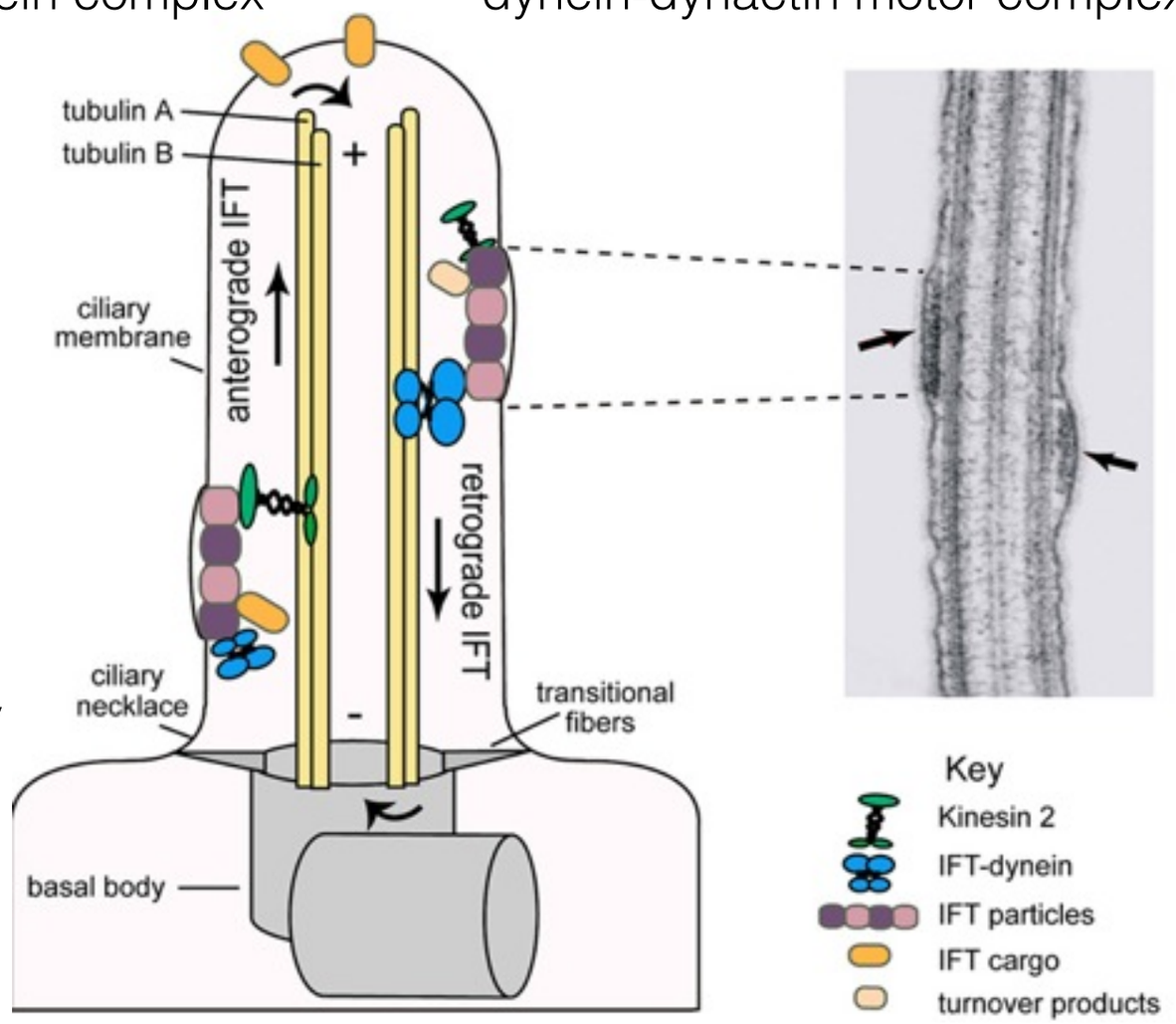
Mahjoub MR. Organogenesis 2013;9(2):61-69

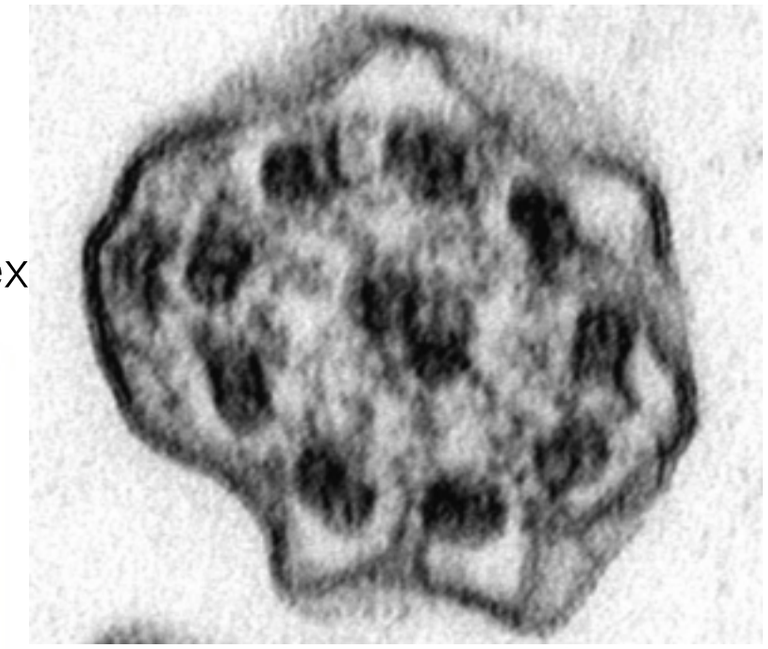
Nigg EA, Stearns T. Nat Cell Biol 2011; 13:1154

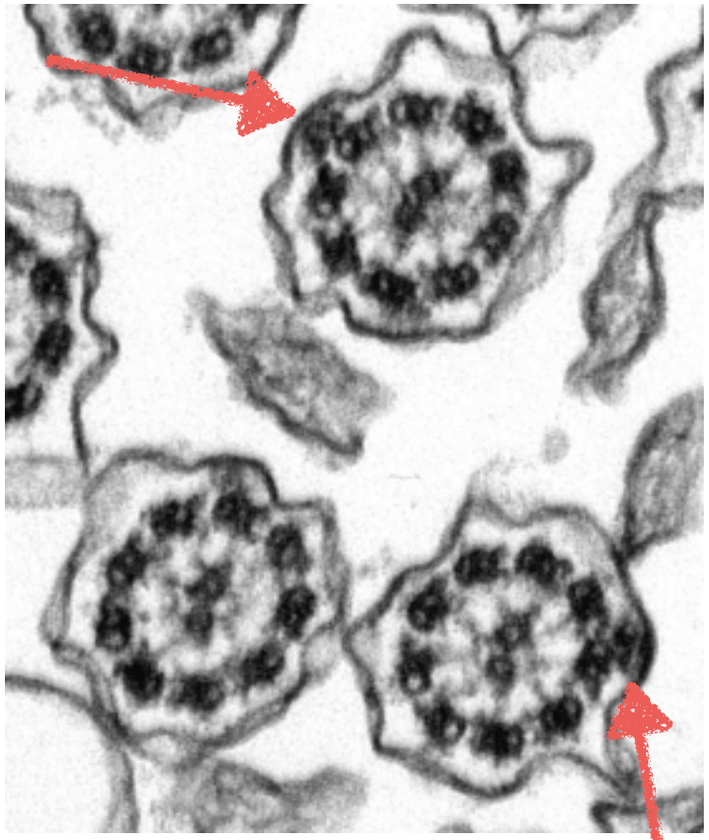
Intraflagellar Transport

Anterograde (toward tip)
kinesin motor protein complex

Retrograde (from tip)
dynein-dynactin motor complex







Loading at basal body and transition zone

The ciliary pore complex limits the transfer of cytoplasmic proteins

http://www.bioscience.org/2008/v13/af/2871/fig1.jpg

video @ www.ovguide.com

Signaling at the Cilium

Pathways shown to utilize the cilium for signaling

sonic hedgehog (Shh)
canonical Wnt
non-canonical Wnt
PDGFA
EGFR
Notch
mTOR

Baker K, Beales PL. Am J Med Genet 2009; 151C: 281

Disorders of Cilia

Biology of cilia

Primary ciliary dyskinesia - disorders of motile cilia

Laterality defects - disorders of nodal cilia

The ciliopathies - disorders of non-motile (primary) cilia

Primary Ciliary Dyskinesia

1904	**Siewert**	**Bronchiectasis and situs inversus**
1933	**Kartagener**	**Sinusitis, bronchiectasis, situs inversus**
1943	Hodge	First EM description of cilia
1947	**Torgersen**	**Lung-nose syndrome**
1954	Fawcett & Porter	First EM cross sections
1959	Afzelius	Described dynein arms
1975	Afzelius, Pedersen	Absent dynein arms
1979	Sturgess and Turner	Radial spoke deficiency
1980	Sturgess and Turner	Absence of central microtubules

Primary Ciliary Dyskinesia

1904	Siewert	Bronchiectasis and situs inversus
1933	Kartagener	Sinusitis, bronchiectasis, situs inversus
1943	**Hodge**	**First EM description of cilia**
1947	Torgersen	Lung-nose syndrome
1954	**Fawcett & Porter**	**First EM cross sections**
1959	**Afzelius**	**Described dynein arms**
1975	Afzelius, Pedersen	Absent dynein arms
1979	Sturgess and Turner	Radial spoke deficiency
1980	Sturgess and Turner	Absence of central microtubules

Primary Ciliary Dyskinesia

1904	Siewert	Bronchiectasis and situs inversus
1933	Kartagener	Sinusitis, bronchiectasis, situs inversus
1943	Hodge	First EM description of cilia
1947	Torgersen	Lung-nose syndrome
1954	Fawcett & Porter	First EM cross sections
1959	Afzelius	Described dynein arms
1975	**Afzelius, Pedersen**	**Absent dynein arms**
1979	**Sturgess and Turner**	**Radial spoke deficiency**
1980	**Sturgess and Turner**	**Absence of central microtubules**

Diagnosing PCD

Clinical History

Prevalence - 1:16,000

75% of full term neonates with PCD have neonatal respiratory distress requiring supplemental oxygen

Infants and children have chronic recurrent ear, nose, sinus, and lower airway infections

Bronchiectasis is almost uniformly present by adulthood

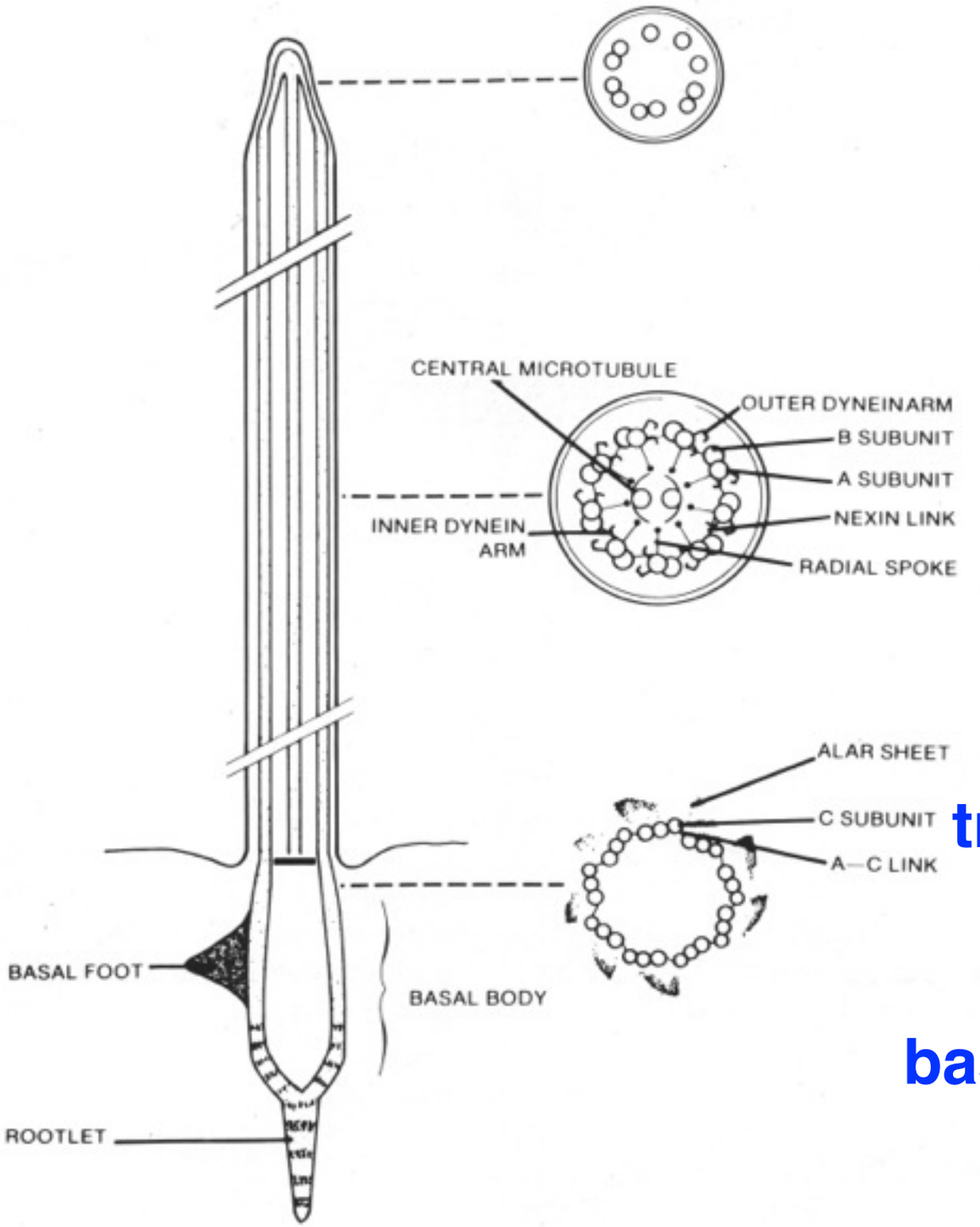
Situs inversus totalis (without physiologic consequences) is present in 50% of patients
25% of patients with situs inversus totalis have PCD

Heterotaxy (often associated with significant malformations) is present in ~6%

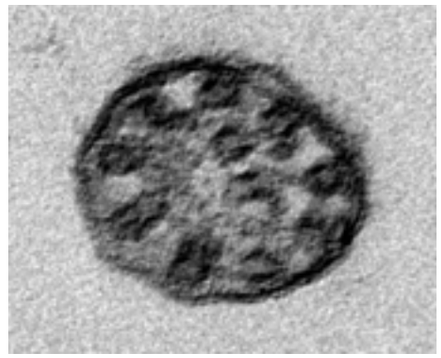
50% of males are infertile

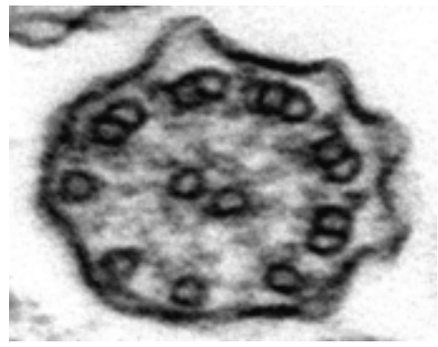
Zariwala MA, Knowles MR, Leigh MW. Primary Ciliary Dyskinesia. GeneReviews @ NCBI Bookshelf

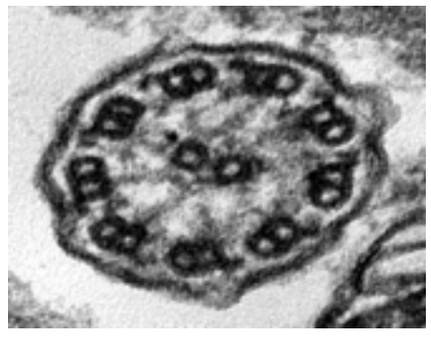
Diagnosing PCD

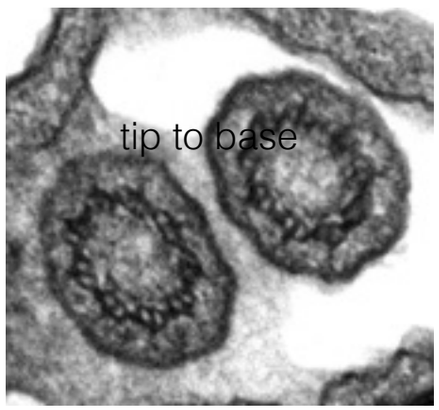


tip







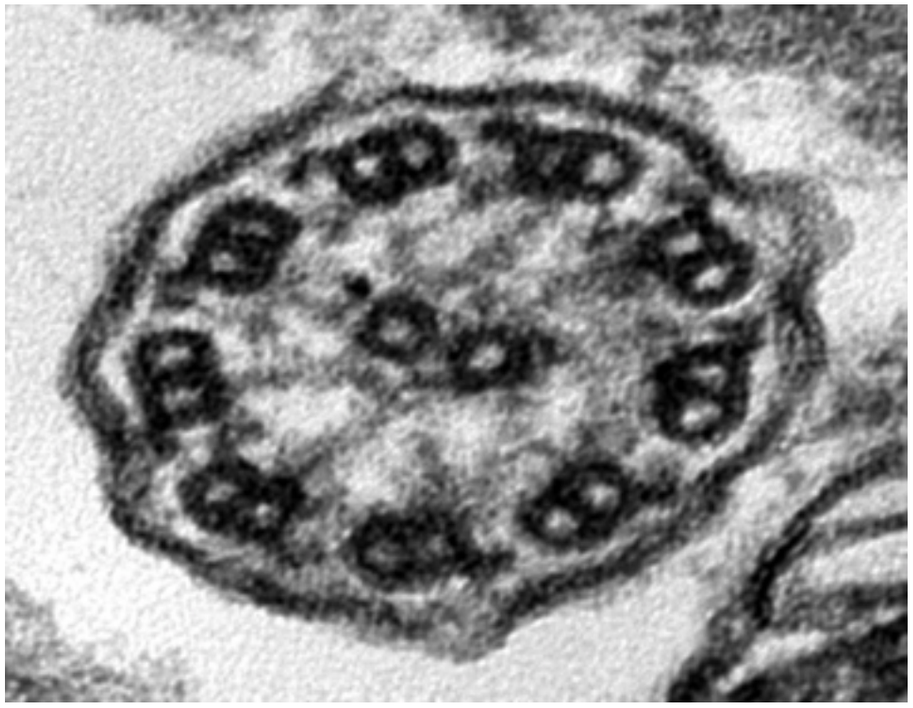


transition zone

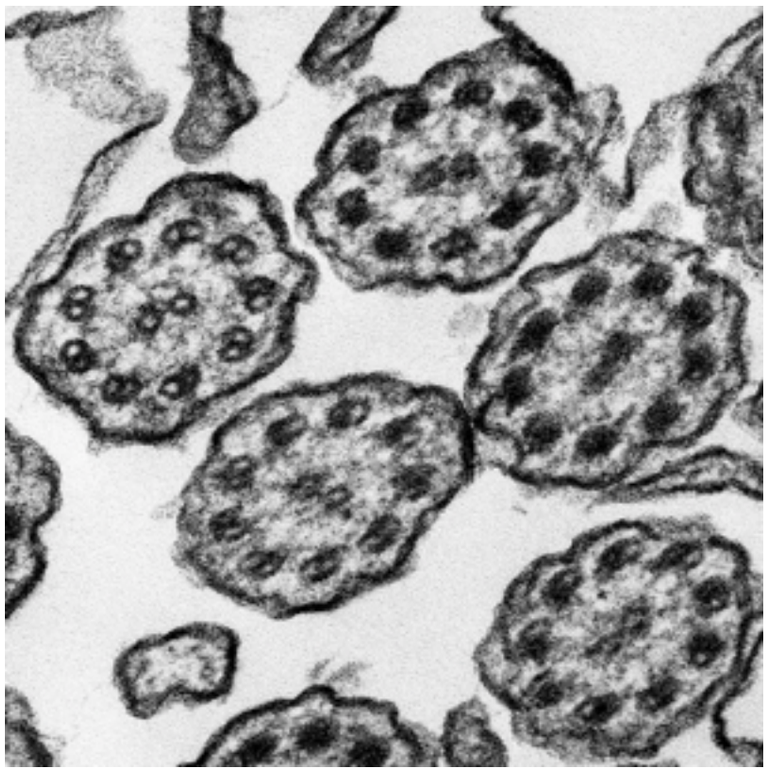
basal body

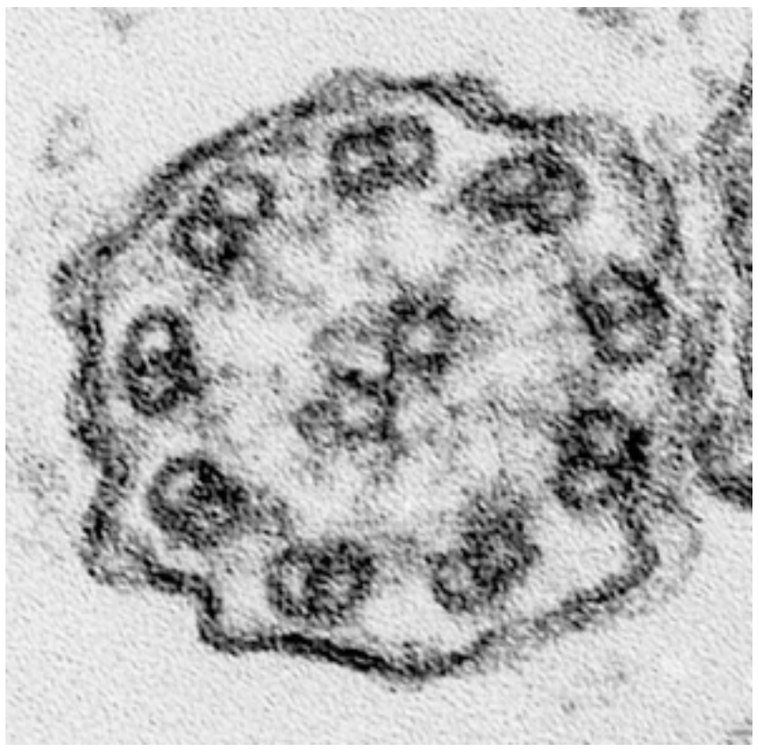
Normal anatomy of a motile cilium

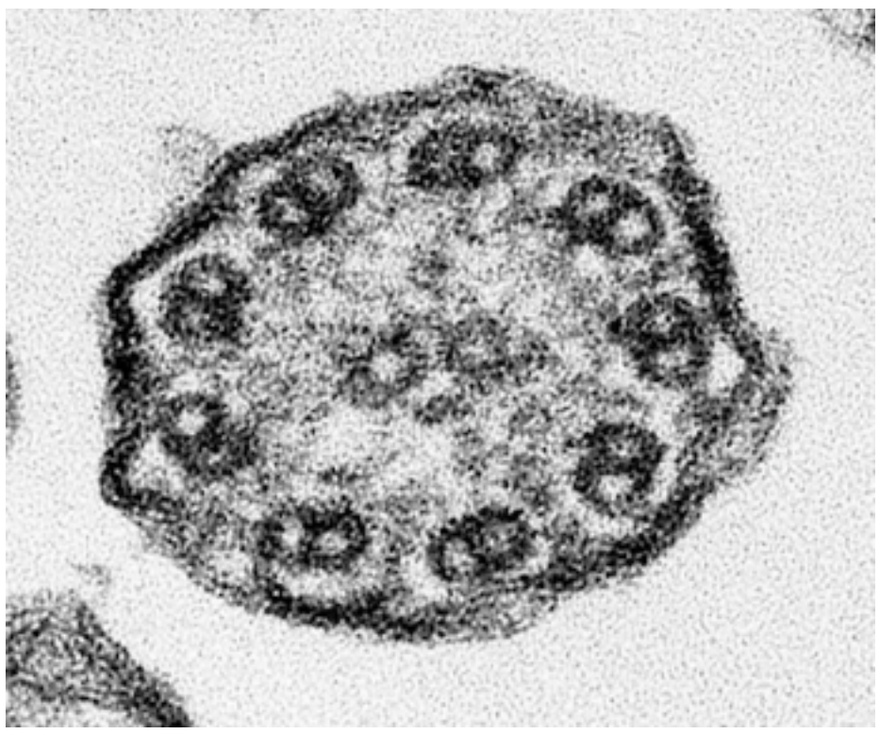
NORMAL



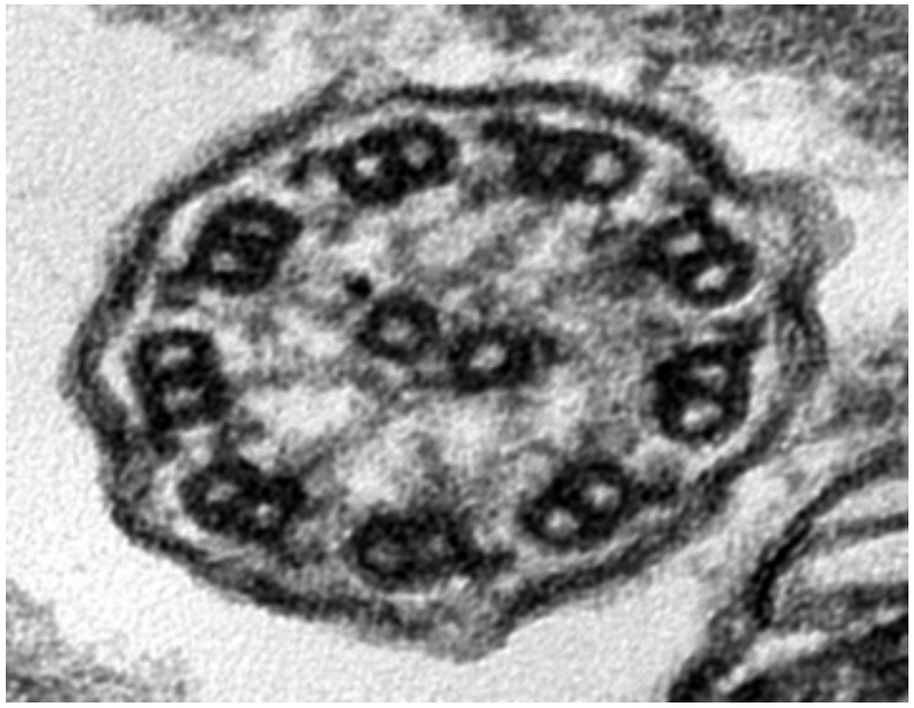
Dynein defect (>90% of cases with abnormal EM) - median and mean of <2 outer arms and/or <1 inner arm per cilium



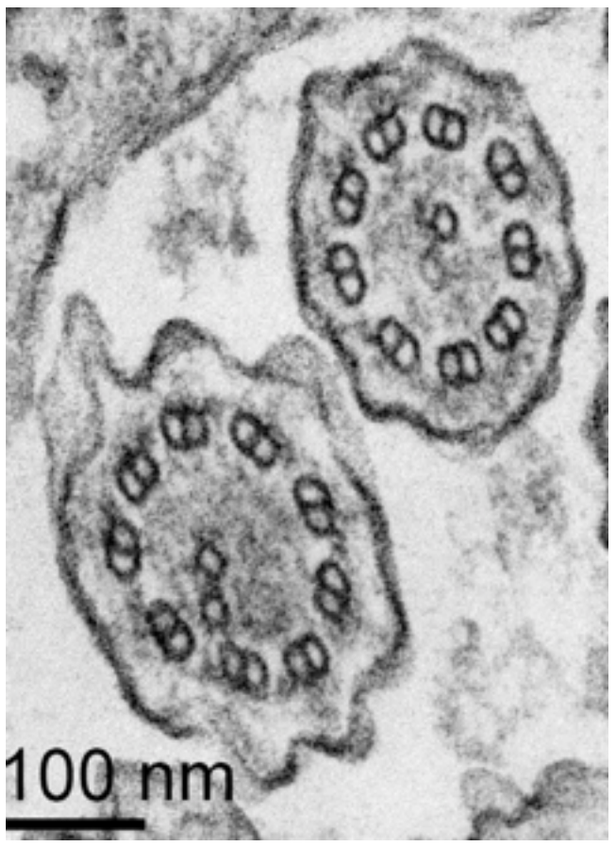


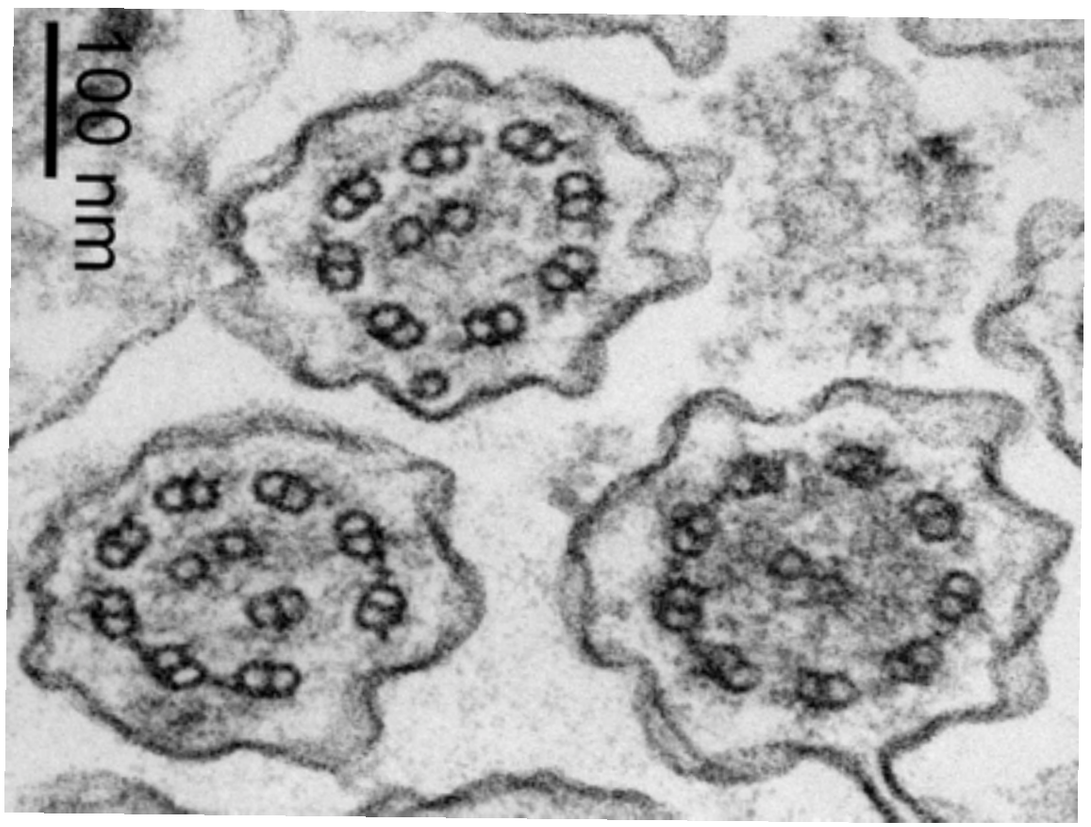


NORMAL

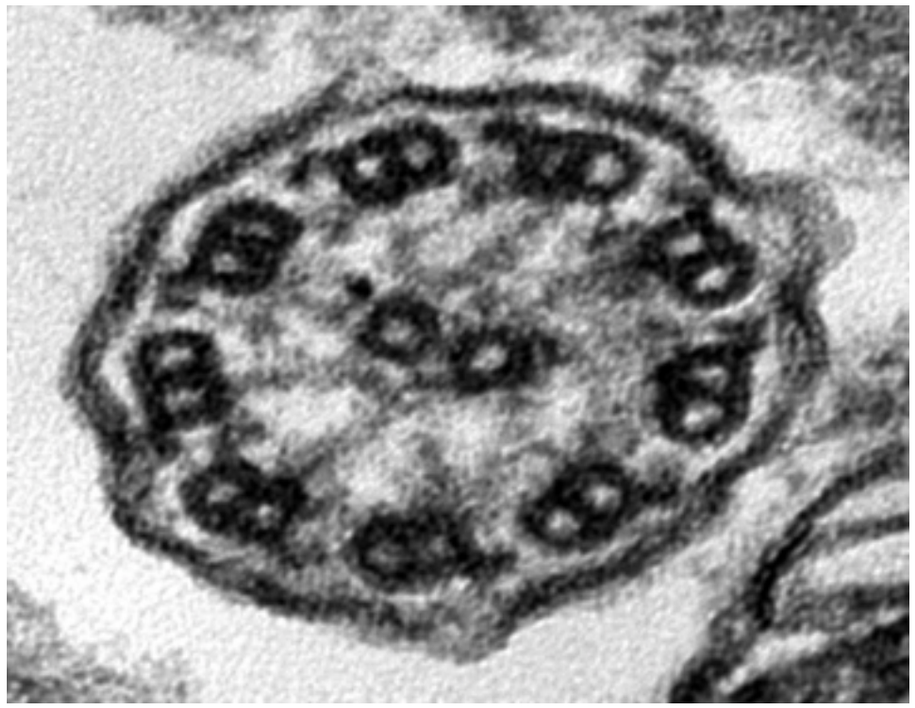


Spoke defect - >20% of cilia show meandering of the central pair of microtubules. Spokes are difficult to discern in clinical samples.

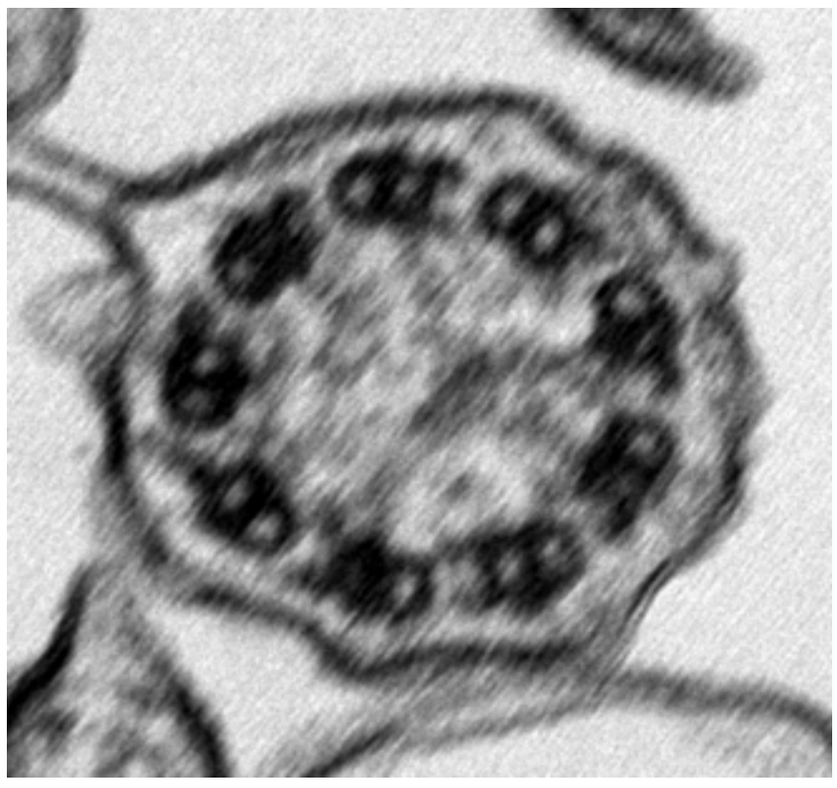


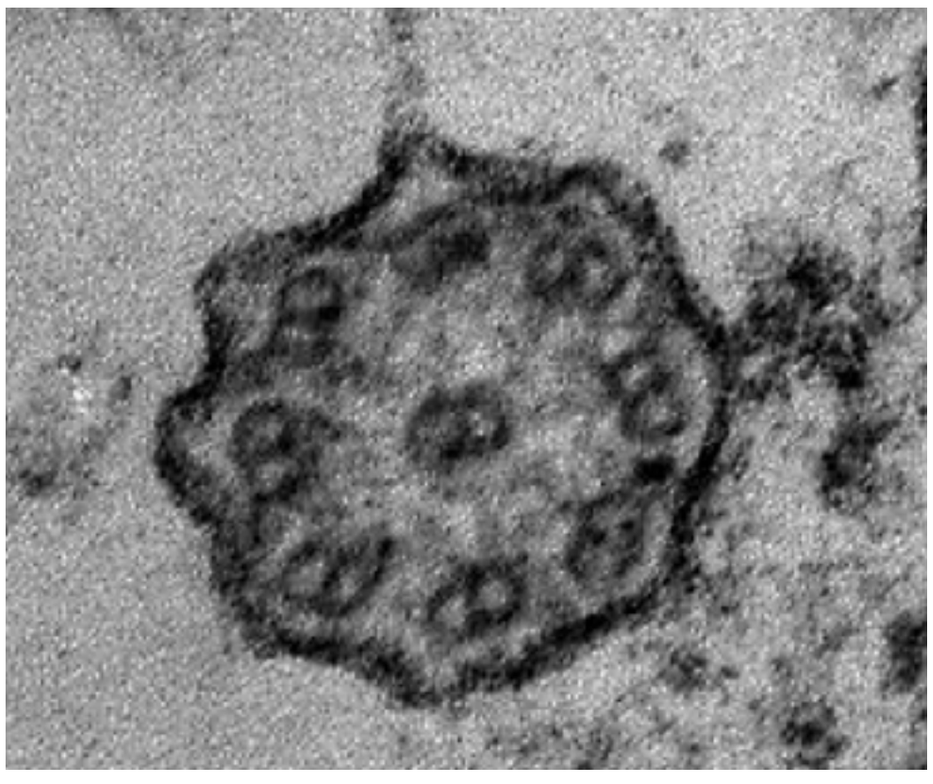


NORMAL

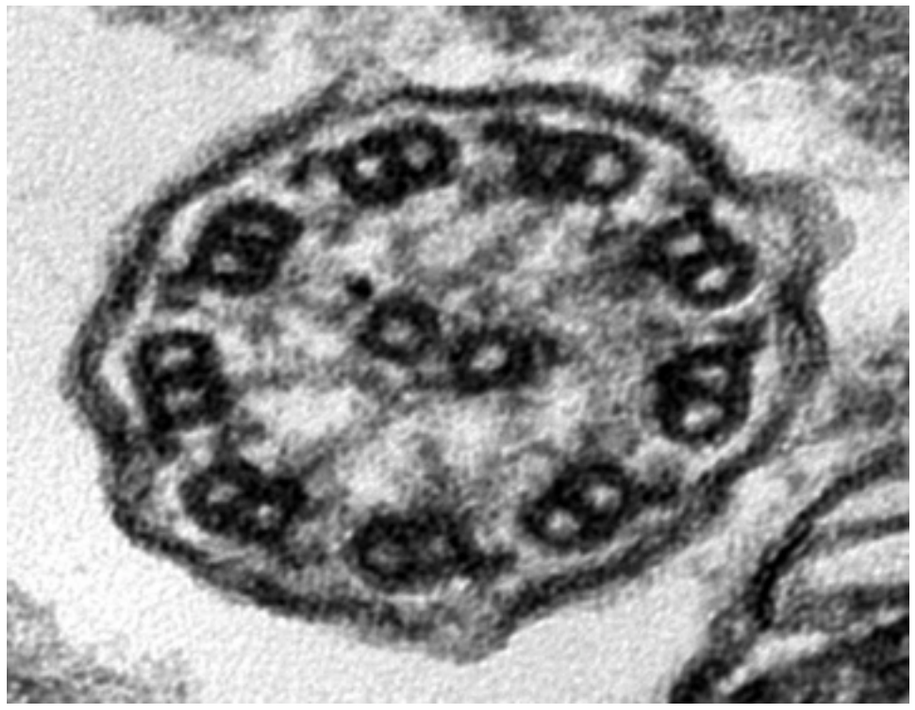


Central pair defect - >20% of cilia have no central pair of microtubules, and >20% show central transposition of a peripheral doublet (8+1 arrangement)

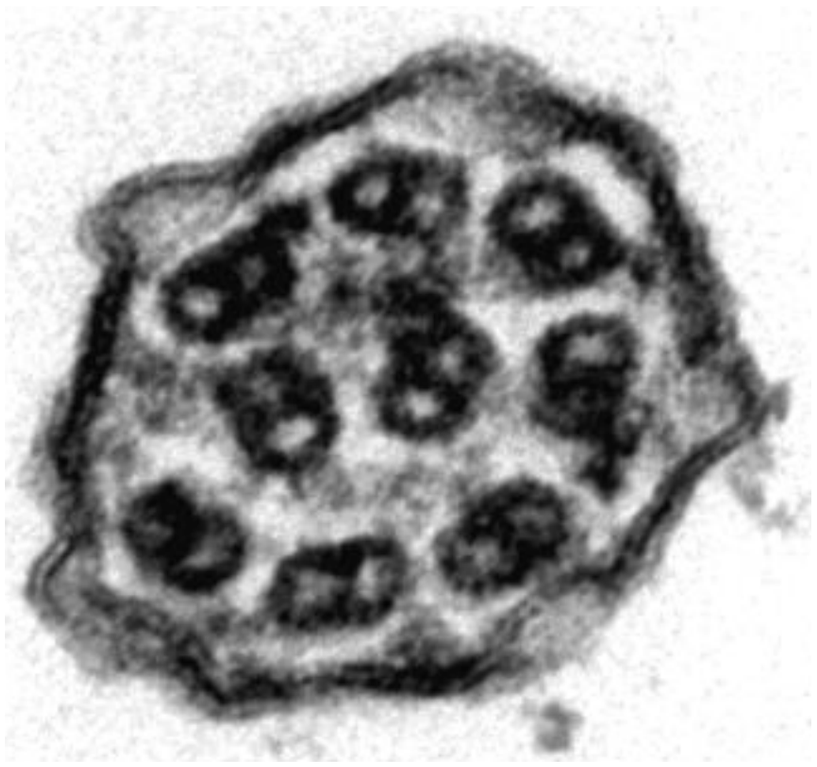


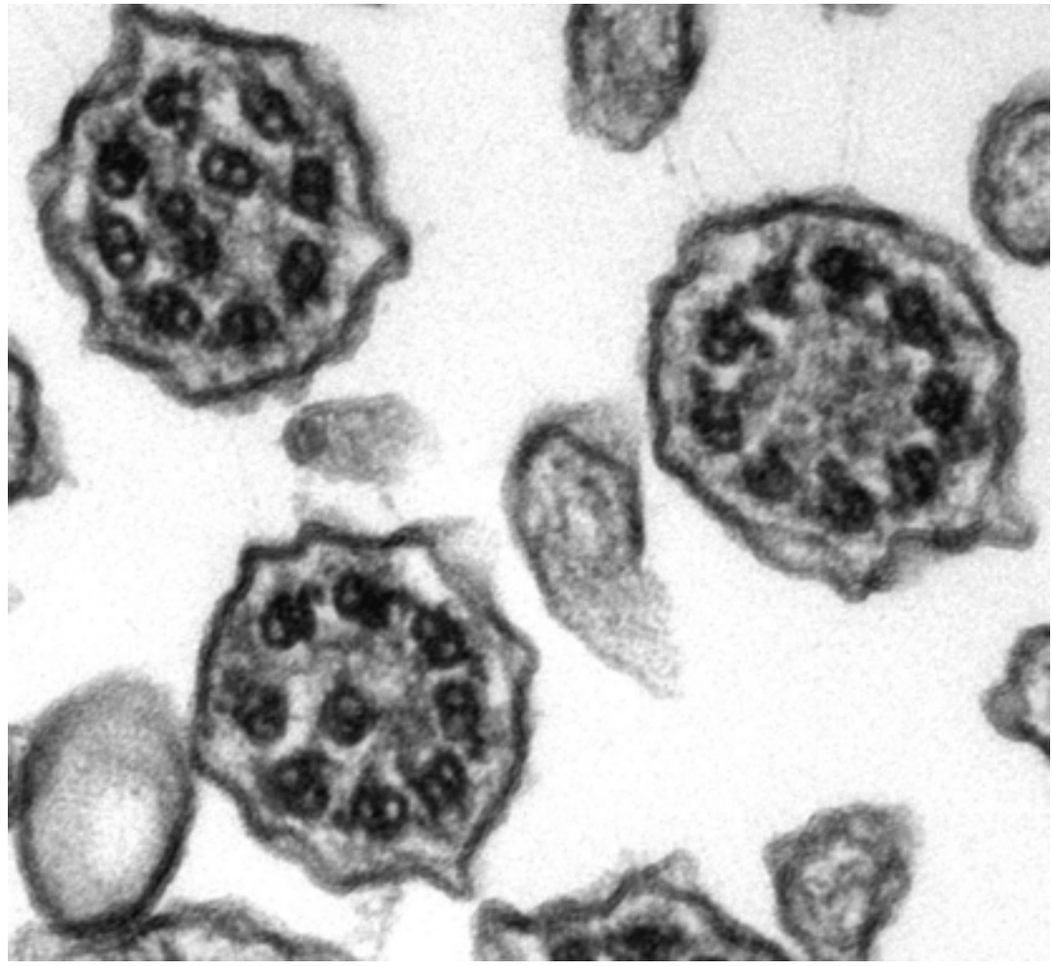


NORMAL



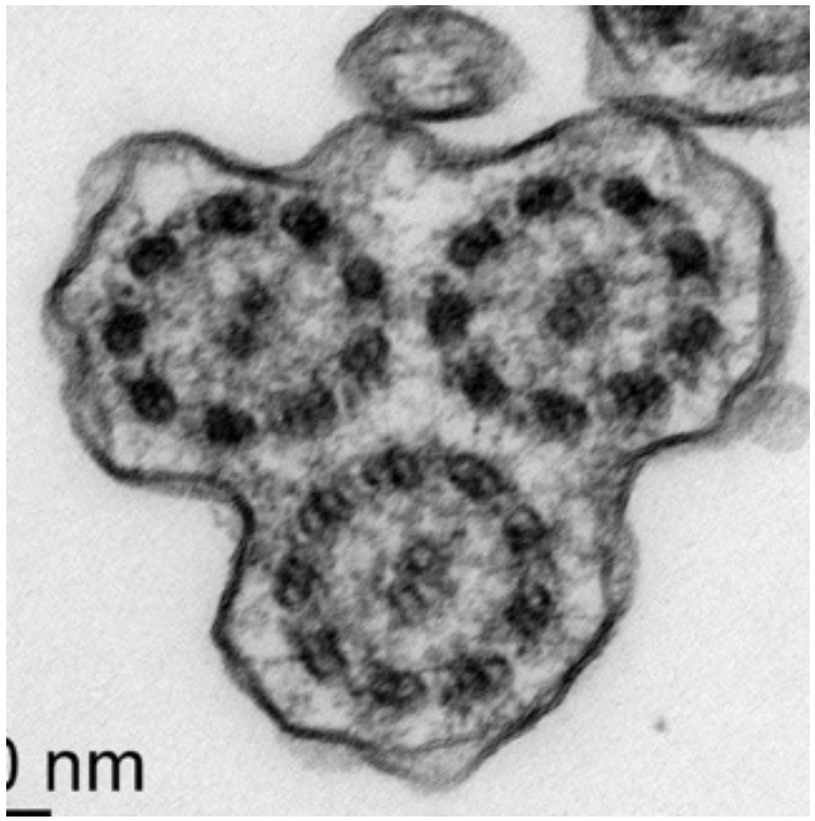
Nexin Link Defect - irregular contour to peripheral ring of doublets or jumbling of doublets. Links are difficult to discern in clinical samples.



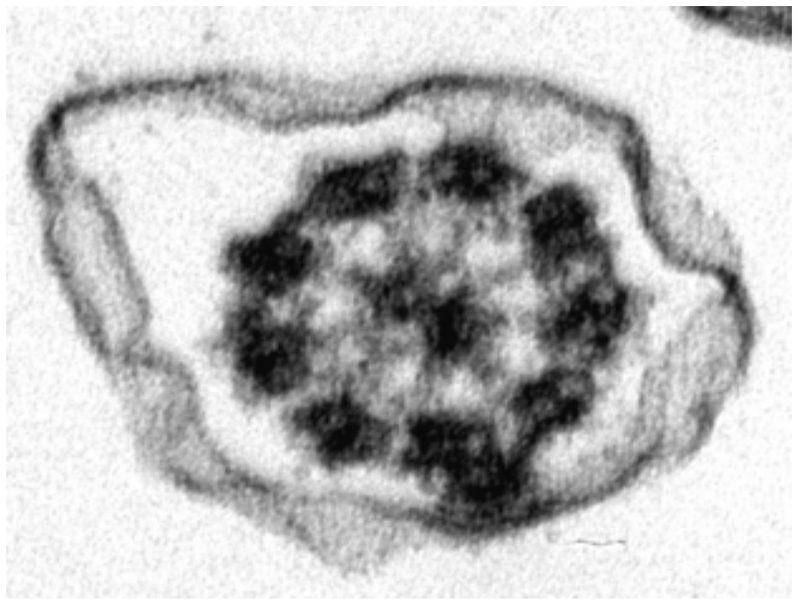


Acquired Lesions of Respiratory Cilia

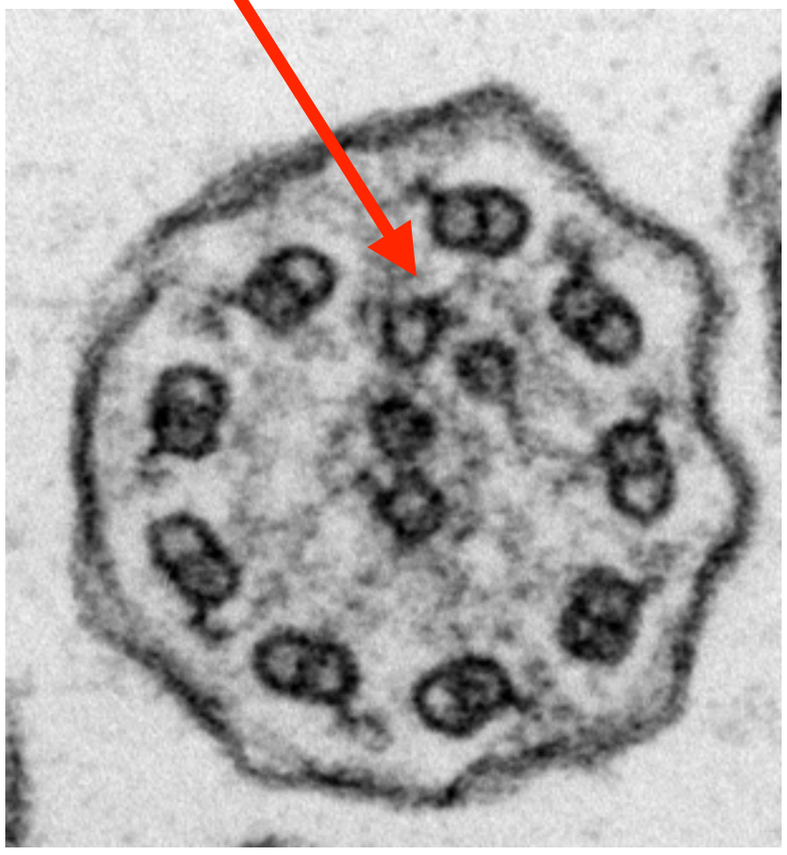
Compound cilium



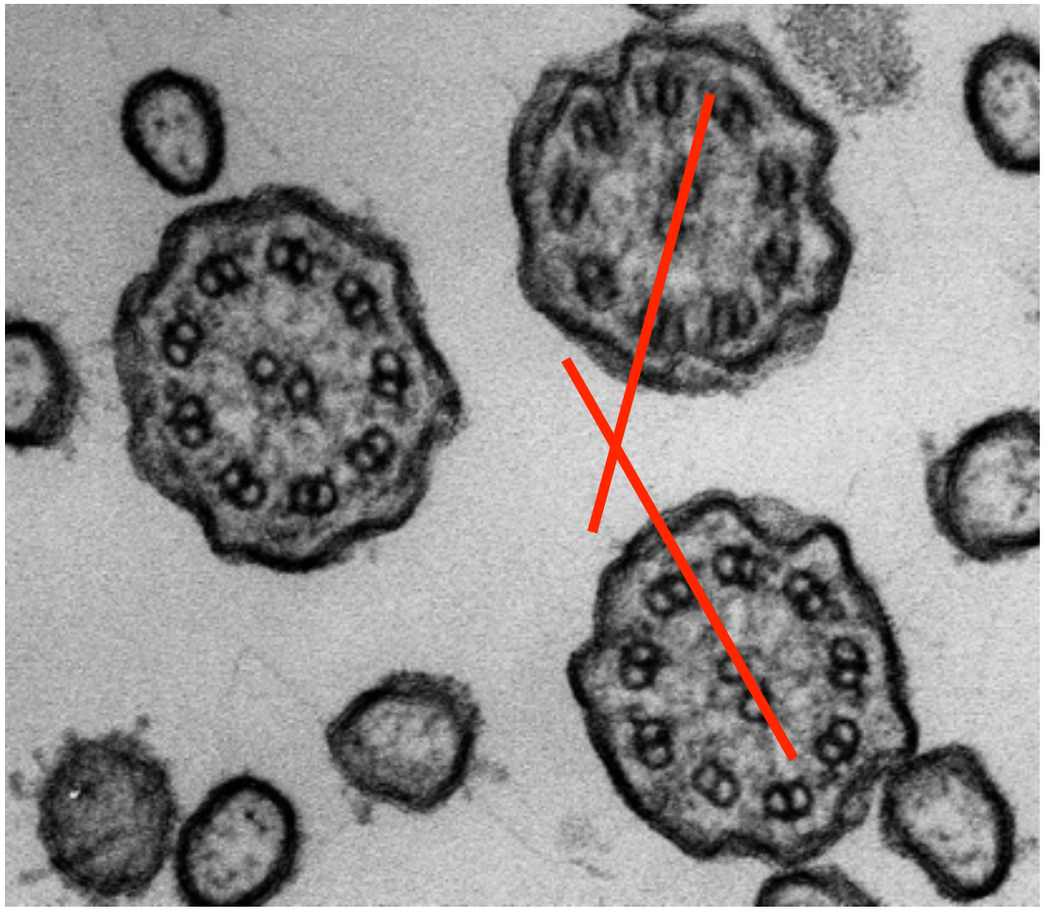
Excess ciliary membrane

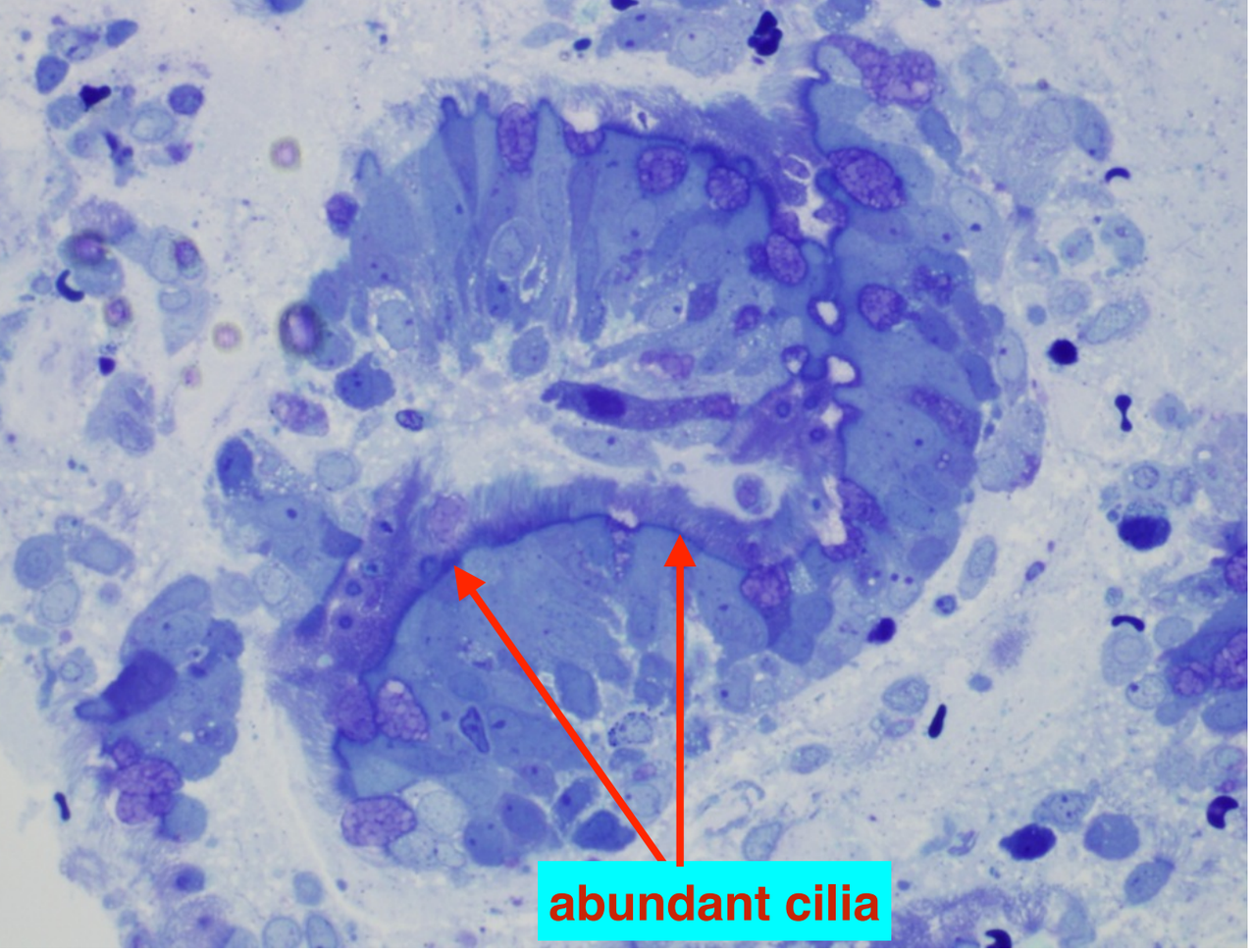


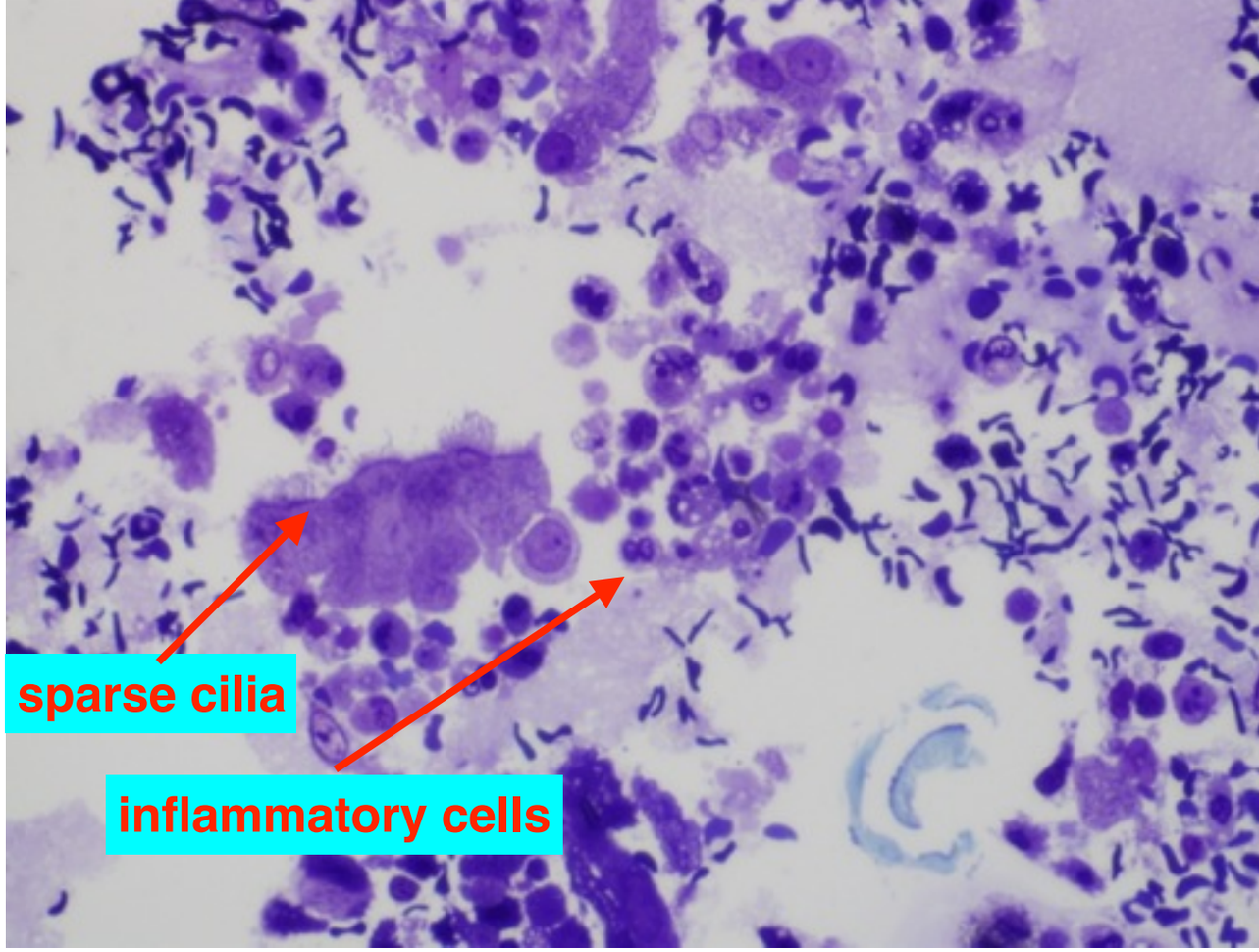
Accessory microtubules



Disorientation of cilia







If a study is clinically indicated

Treat and wait to allow the native epithelium to regenerate.
Obtain the sample from ***the most normal-appearing*** area of the airway.
Immediately immerse the sample (including brush) in EM fixative.
Consider an alternative way to make the diagnosis.

Diagnosing PCD

Genotype : TEM correlation

[% of all PCD]

Outer arm defect

DNAH5 [15-21]
DNAL1
DNAI1 [2-9]
DNAI2 [2]
TXNDC3
NME8 [4-5]
CCDC103
CCDC114

Cytoplasmic assembly

DNAAF2
DNAAF1 (LRRC50) [3]
DNAAF3
HEATR2
LRRC6
KTU

No EM lesion

DNAH11 [6]

Central pair defect

RSPH9
RSPH4A
HYDIN

Nexin link defect

CCDC39 [2-10]
CCDC40 [1-8]
CCDC164

Variable/unknown

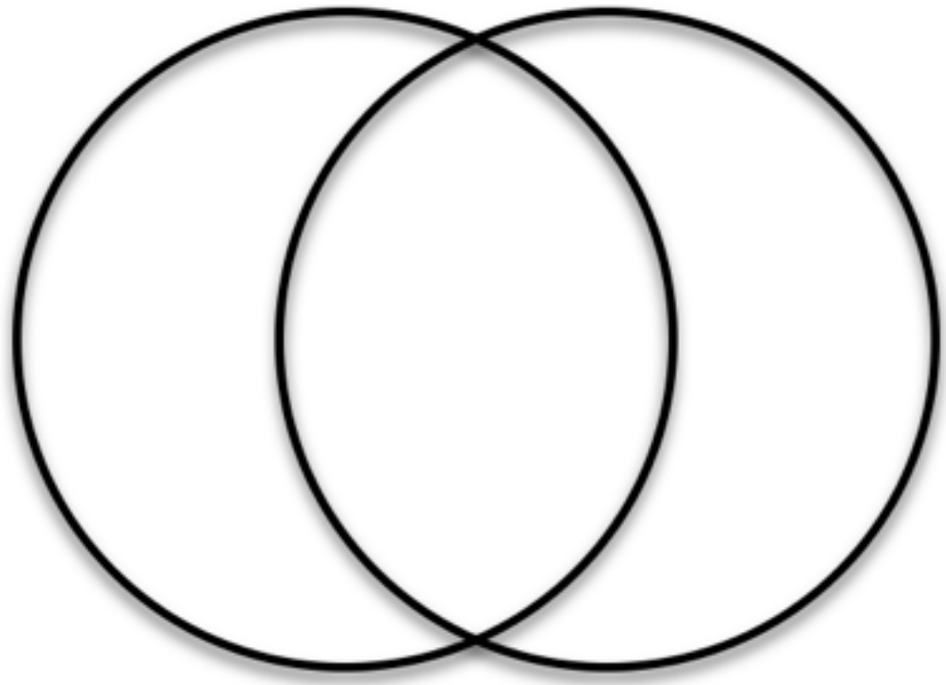
RPGR
OFD1

Omran H. Ultrapath XVI, 2012
Horani A, Brodie SL, Ferkol TW. Pediatr Res 2013, doi:10.1038/pr.2013.200
Zariwala MA, Knowles MR, Leigh MW. Primary Ciliary Dyskinesia. GeneReviews @ NCBI Bookshelf

Diagnosing PCD

EM Diagnosis

$$



Genetic Diagnosis

$$$$

Diagnosing PCD

Observation of Ciliary Beating

Direct observation

High speed video microscopy

Nitric Oxide Washout

PCD patients have one-tenth normal level of nasal NO

Mucociliary Clearance

Tracking of inhaled radiolabeled particles

Immunofluorescent Staining

Antibodies to outer Dynein Arm (ODA)-related genes-*DNAH5*, *DNAI2*

Antibodies to DNALI1 (inner arms) or GAS8/GAS11 (nexin-DRC)

Zariwala MA, Knowles MR, Leigh MW. Primary Ciliary Dyskinesia. GeneReviews @ NCBI Bookshelf

Disorders of Cilia

Biology of cilia

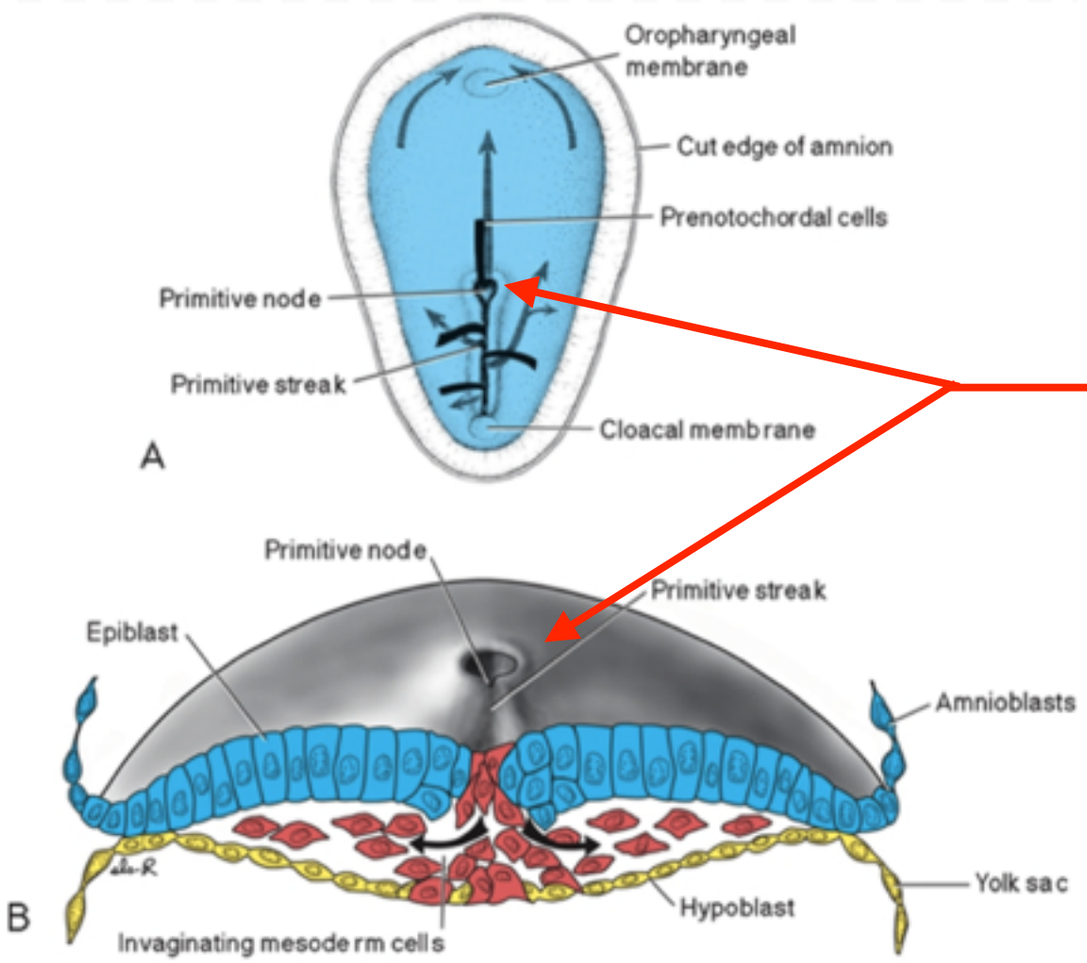
Primary ciliary dyskinesia - disorders of motile cilia

Laterality defects - disorders of nodal cilia

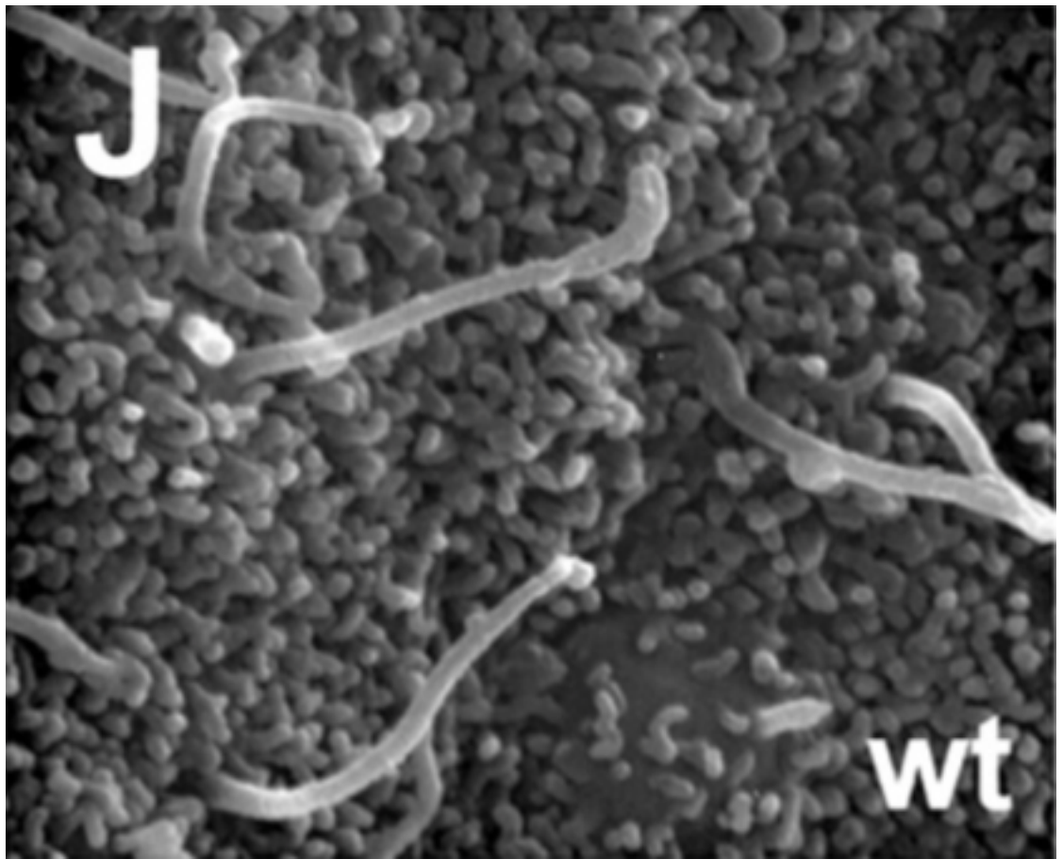
The ciliopathies - disorders of non-motile (primary) cilia

Nodal cilia - motile primary cilia

circular rather than whipping motion
unidirectional distribution of extracellular molecules
establish left-right asymmetry (left-right organizer)



www.study blue.com



mouse primitive node with 9+0 cilia

Hadjantonakis A-K, et al. PLoS ONE 2008;3(6):e2511

Disorders of Cilia

Biology of cilia

Primary ciliary dyskinesia - disorders of motile cilia

Laterality defects - disorders of nodal cilia

The ciliopathies - disorders of non-motile (primary) cilia

Recognition of the Ciliopathies

1867	Kowalevsky	single cilium present on a variety of vertebrate cells
1993	Kozminsky et al.	described intraflagellar transport (IFT)
2000	Pazour et al.	mouse PKD protein required for IFT
2003	Ansley et al.	BBS8 protein had *pilF* domain and localized to basal bodies
2004	Li et al.	ciliary proteome constructed, contained BBS5

Adams M. The primary cilium: an orphan organelle finds a home. Nature Education 3(9):54

Waters AM, Beales PL. Ciliopathies: an expanding disease spectrum. Pediatr Nephrol 2011;26(7):1039-56

Syndromes with Abnormal Ciliary Proteins

Alstrom S.
AR and AD polycystic kidney disease
Bardet-Biedl S.
Ellis-van Creveld S.
Jeune asphyxiating thoracic dystrophy
Joubert S.
Leber congenital amaurosis
McKusick-Kaufman S.
Meckel S.
nephronophthisis types 1-4
oral-facial-digital S., type I
primary ciliary dyskinesia
Senior-Loken S.
Sensenbrenner S.
short-rib polydactyly S.

Baker K, Beales PL. Am J Med Genet 2009; 151C: 281

Predicting Ciliopathies

Baker K, Beales PL. Am J Med Genet 2009; 151C: 281

Start with five established ciliopathies

BBS, OFD1, S-L, Meckel, Joubert

Find malformations that occur in at least three of these

retinitis pigmentosa	x		x	x	x	4
renal cystic disease	x	x	x	x	x	5
polydactyly	x	x		x	x	4
situs inversus/isomerism	x		x	x	x	4
mental retardation / dev delay	x	x		x	x	4
hypoplasia of corpus callosum	x	x		x	x	4
Dandy-Walker malformation	x		x	x	x	4
posterior encephalocele	mice			x	x	3
hepatic disease	x	x	x	x	x	5
	8	5	5	9	9	

Search OMIM for other syndromes with at least one pair of these malformations

Mapping the Ciliopathies

Excessive length

Meckel S.
Renal cysts
Nephronophthisis

Intraflagellar transport

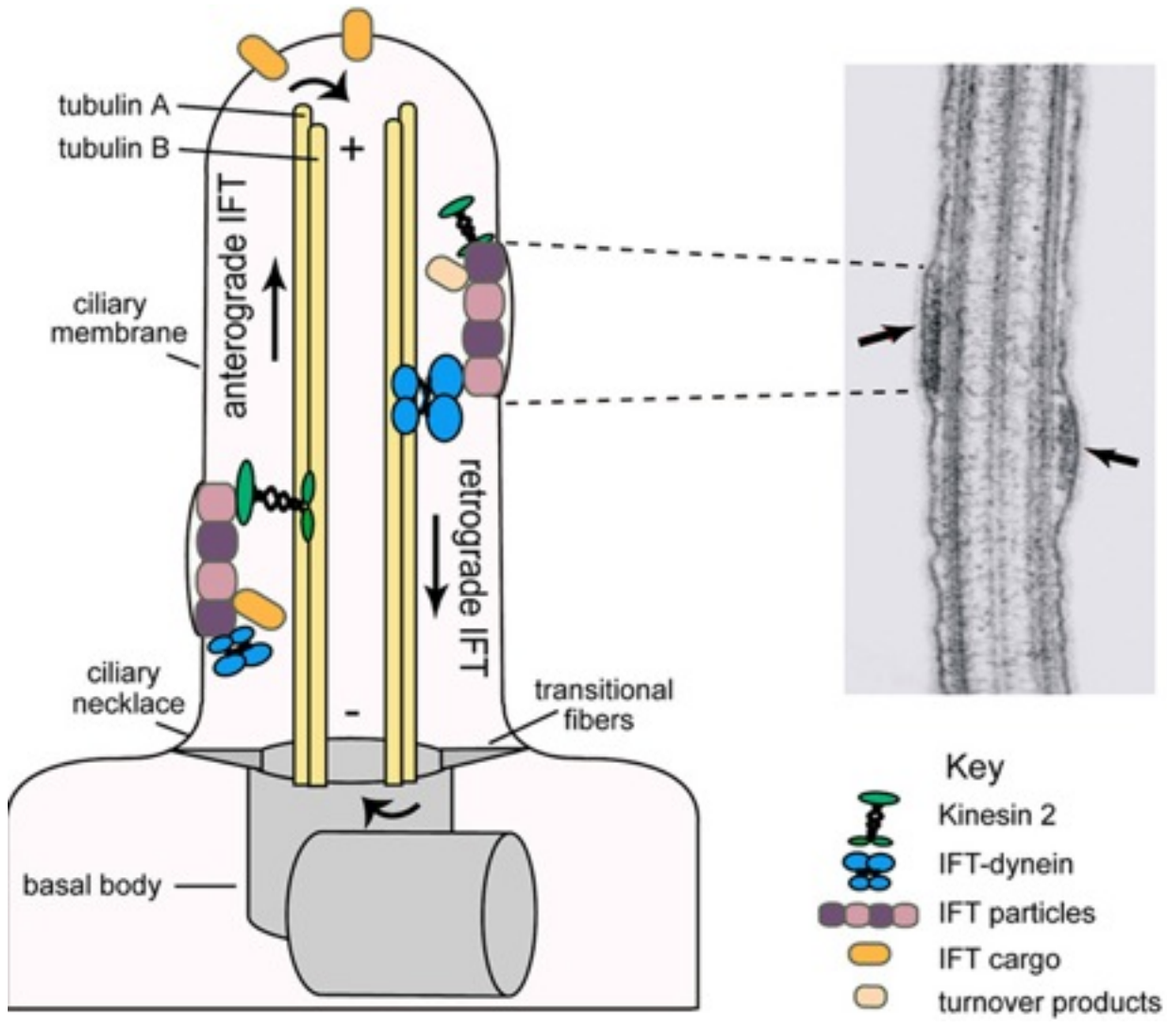
Renal cystic diseases
Hydrocephalus
Jeune S.
Sensenbrenner S. (Shh)
Situs inversus (kinesin motor)

Transition zone

Nephronophthisis
Meckel S.
OFD S.

Docking

Joubert S.
Meckel S.



Recruitment of membrane proteins

Bardet-Biedl S. - BBSome (complex of at least 8 BBS proteins)

Cell cycle modulators **Actin networks**

? other signaling disorders, channelopathies, cancer

Avasthi P, Marshall WF. Differentiation 2012;83:S30-42

Signaling at the Cilium

Pathways shown to utilize the cilium for signaling

sonic hedgehog (Shh)
canonical Wnt
non-canonical Wnt
PDGFA
EGFR
Notch
mTOR

Baker K, Beales PL. Am J Med Genet 2009; 151C: 281

Disorders of Cilia

Summary

Eukaryotes have three types of cilia - primary, nodal, and motile

Cilia develop from centrioles during interphase

Molecules move thru cilia via regulated trafficking at the base, and bidirectional intraflagellar transport

Primary ciliary dyskinesia is caused by a growing list of mutations that affect motile cilia

Situs inversus results from immotile nodal cilia

Ciliopathies are due to failed transport of receptor or signalling molecules in non-motile primary cilia