肺の枝分かれ構造の
数理モデル化と
その実験的検証

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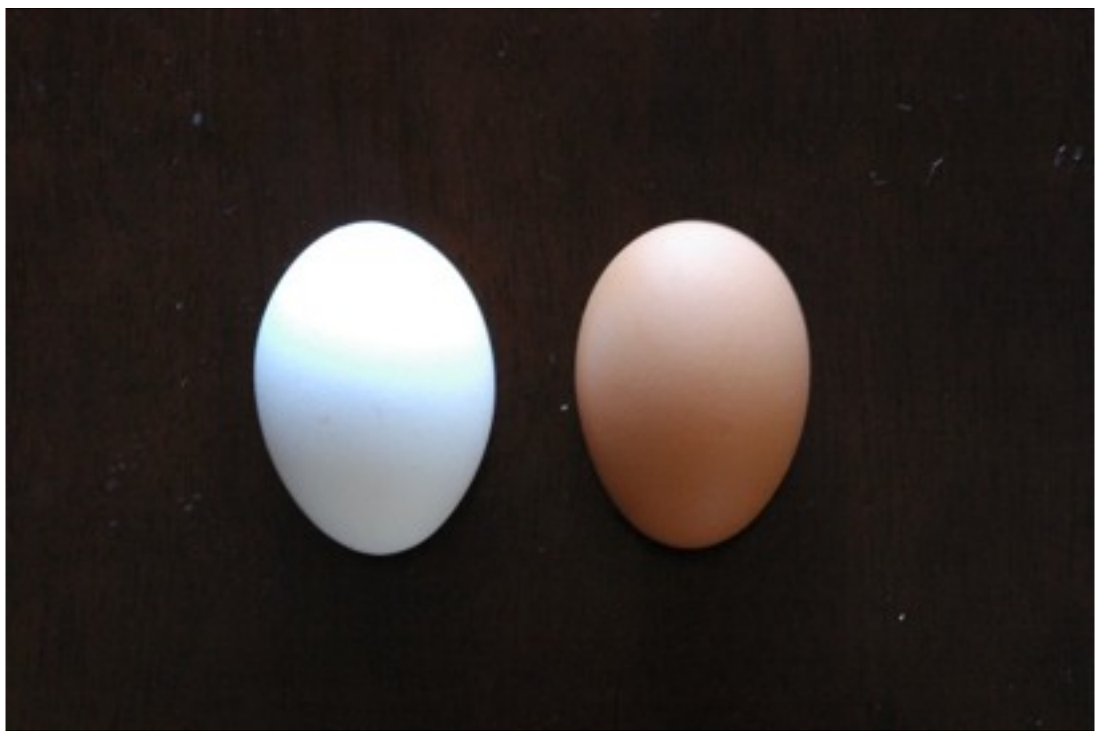
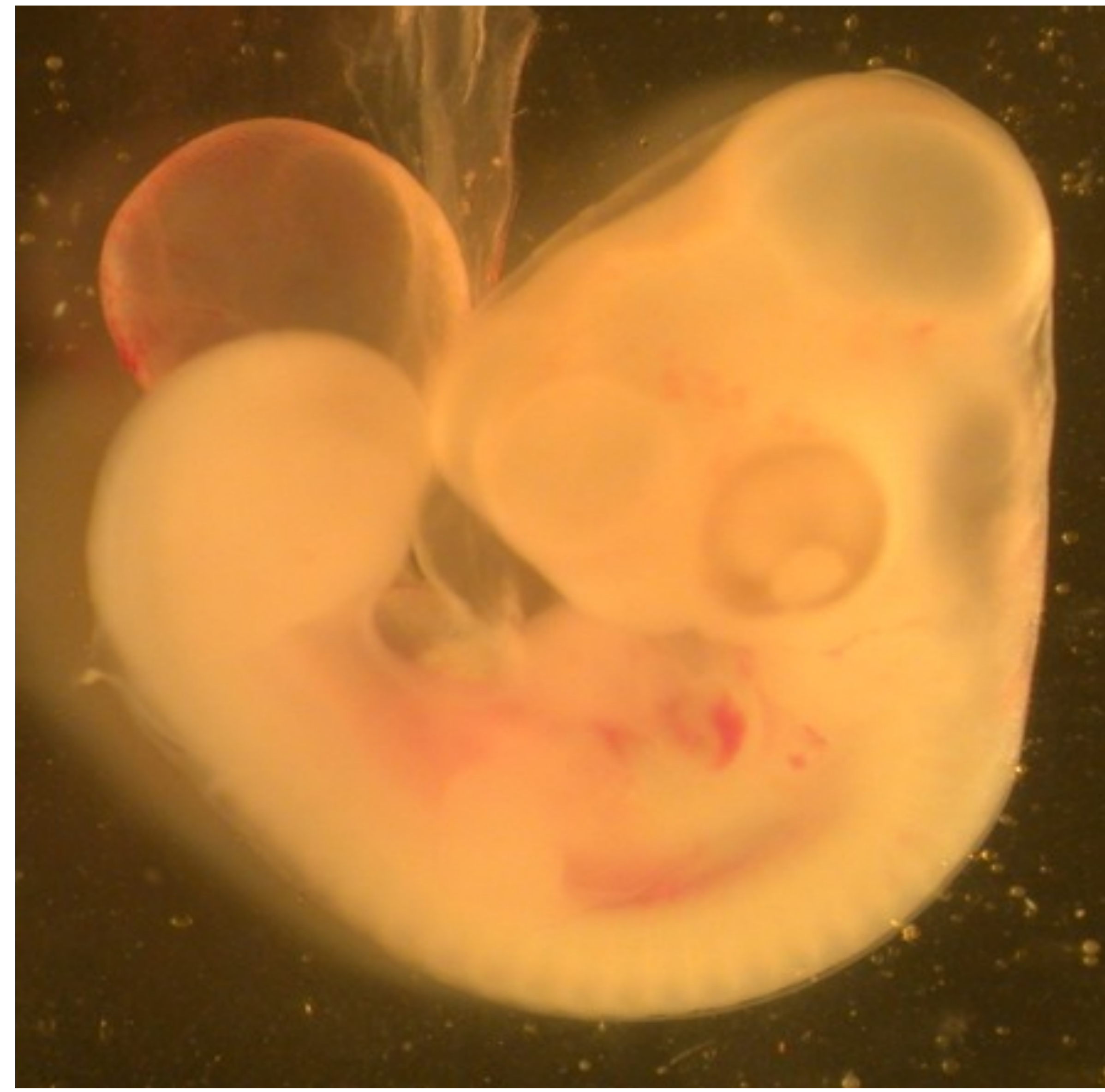
生物のかたちは不思議

発生生物学





2. 滞在費・研究活動費

滞在費・研究活動費は，次の「一日当たりの滞在費・研究活動費」に基づいて計算したうえ，原則として6か月ごとに支給します。

第1回目の送金先は，「派遣手続調書」（様式1）で指定された銀行口座，第2回目以降は「銀行口座届」（様式4）で指定された銀行口座です。

なお，派遣期間開始が年度当初（4月期）の場合，事務手続きの都合上第1回目の送金が派遣期間開始に間に合わないこともあります。あらかじめご承知おきください。

（一日当たりの滞在費・研究活動費）

区　　分	滞在費・研究活動費
甲　地　方	14,400円
乙　地　方	11,600円
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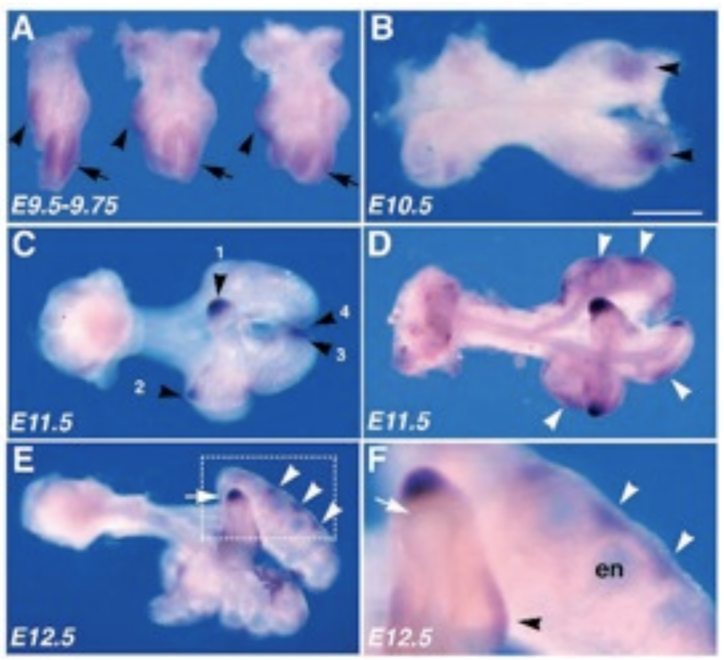
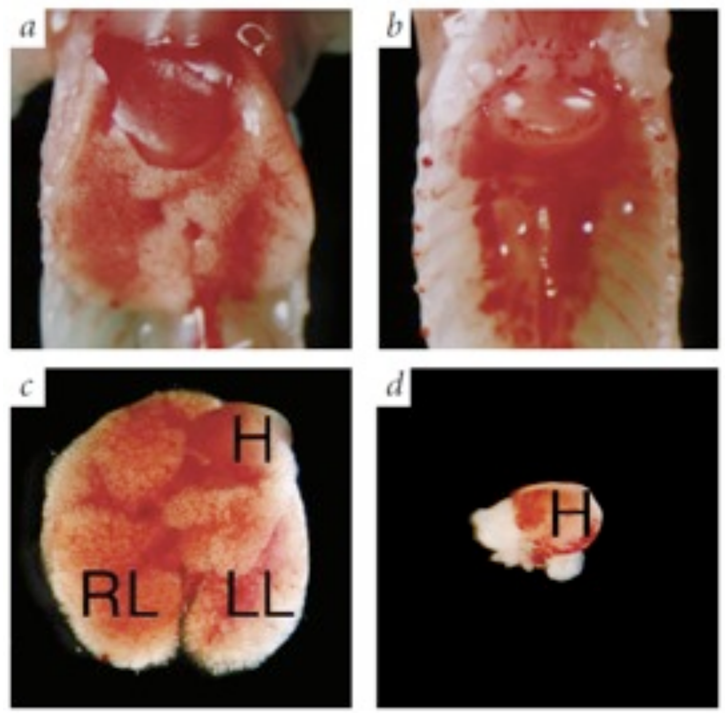
発生生物学における情報爆発



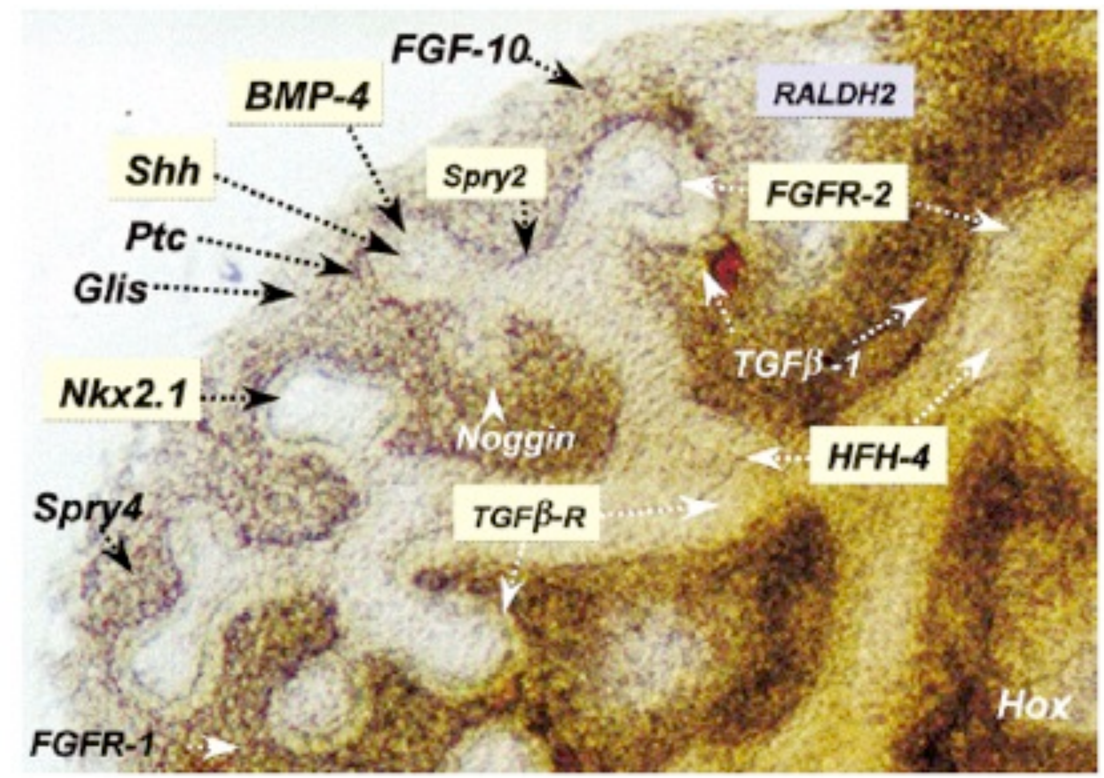
FGF10

Development 124, 4867-4878 (1997)



FGF10 KO

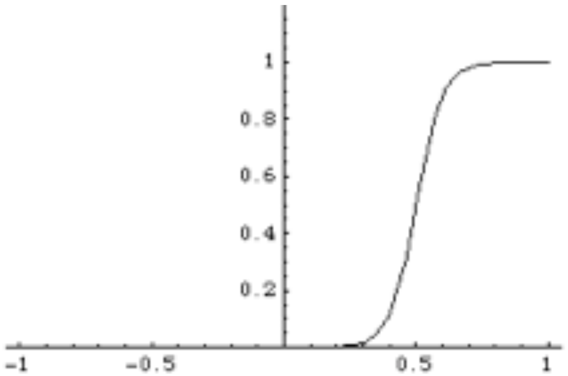
Nat Gen. 21, 138- (1999)

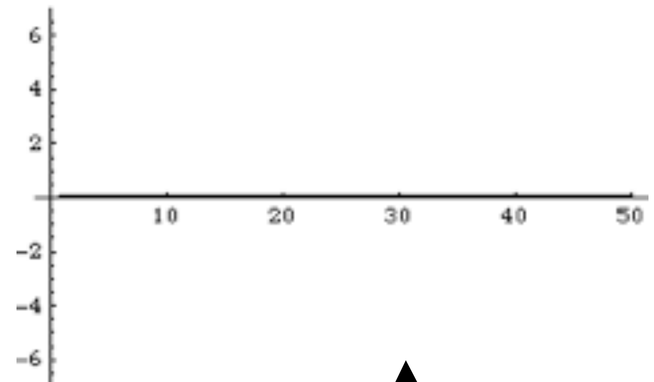


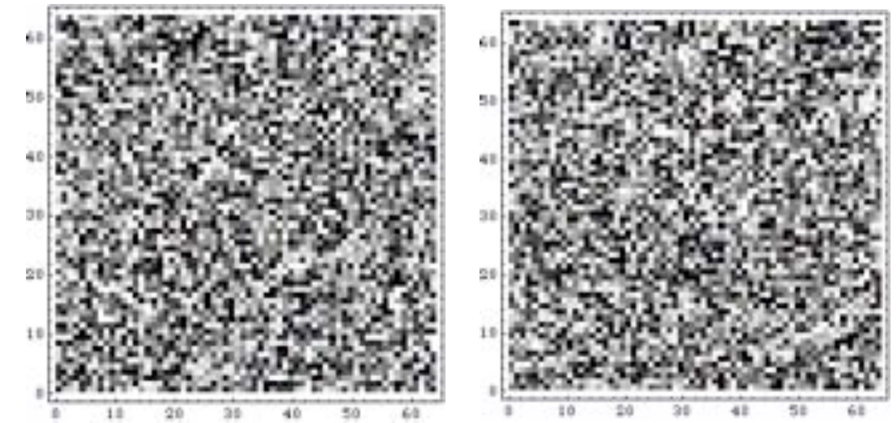
Review

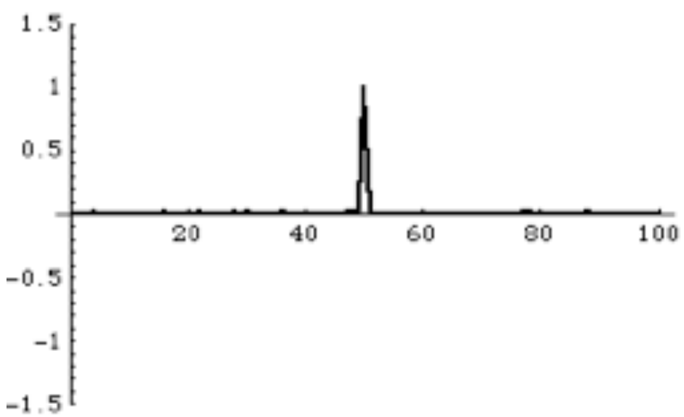
Dev Dyn 219:121-130 (2000)

反応拡散系によるパターン形成

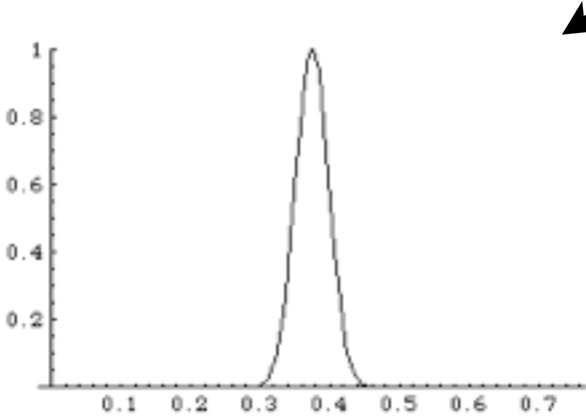








$$\mathbf{u}' = f(\mathbf{u}) + \mathbf{D}\Delta\mathbf{u}$$



実験：多変数（20-100）

理論：1-3変数

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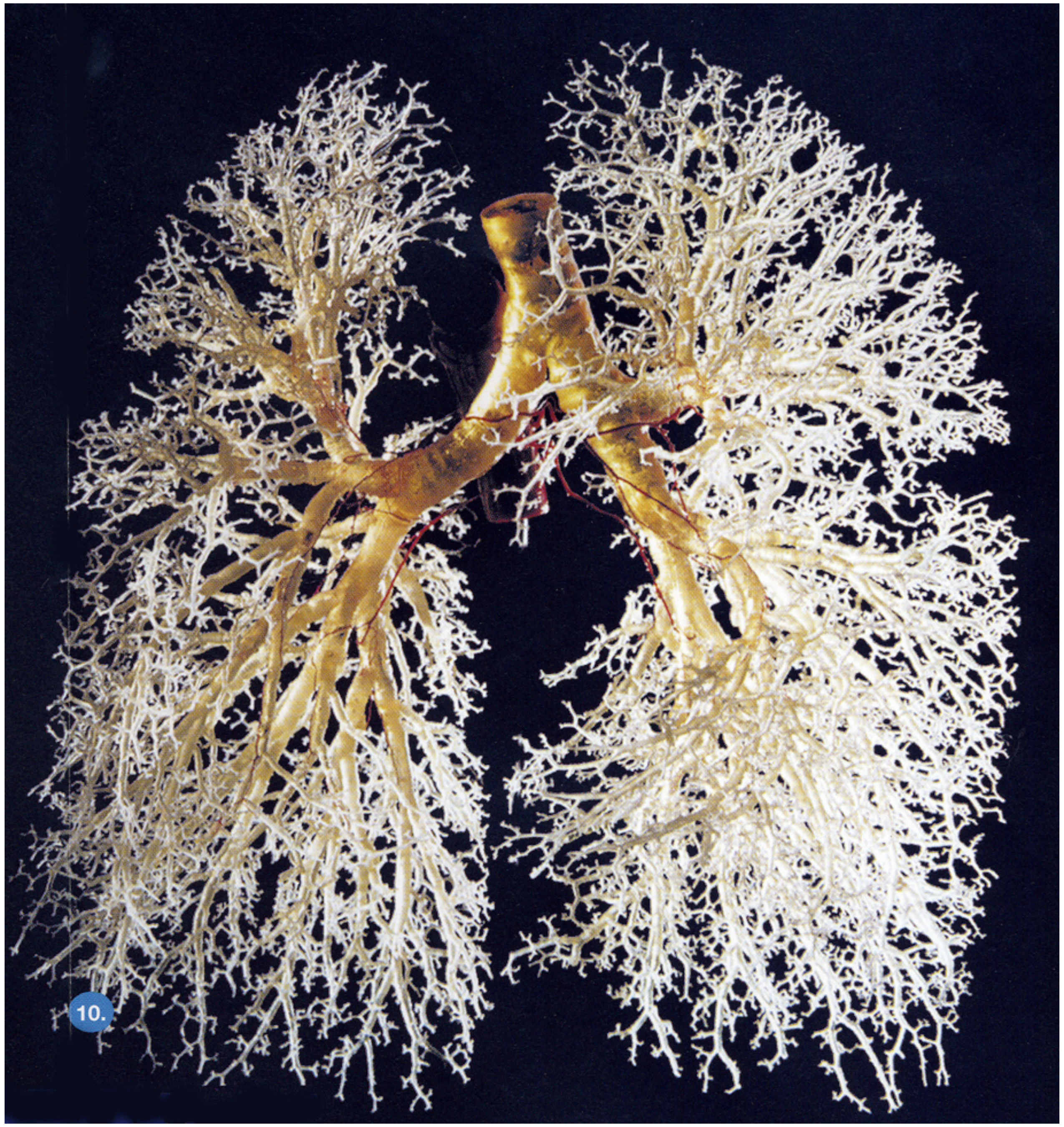
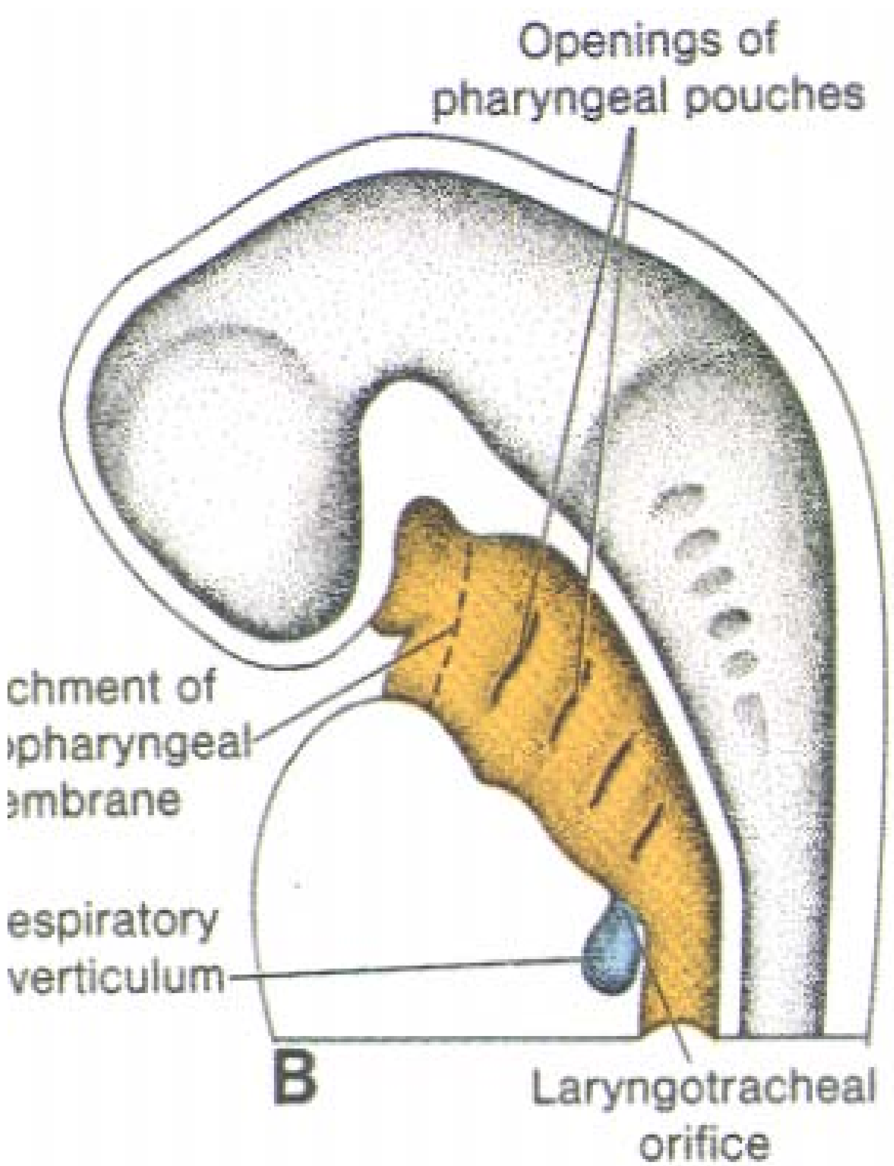
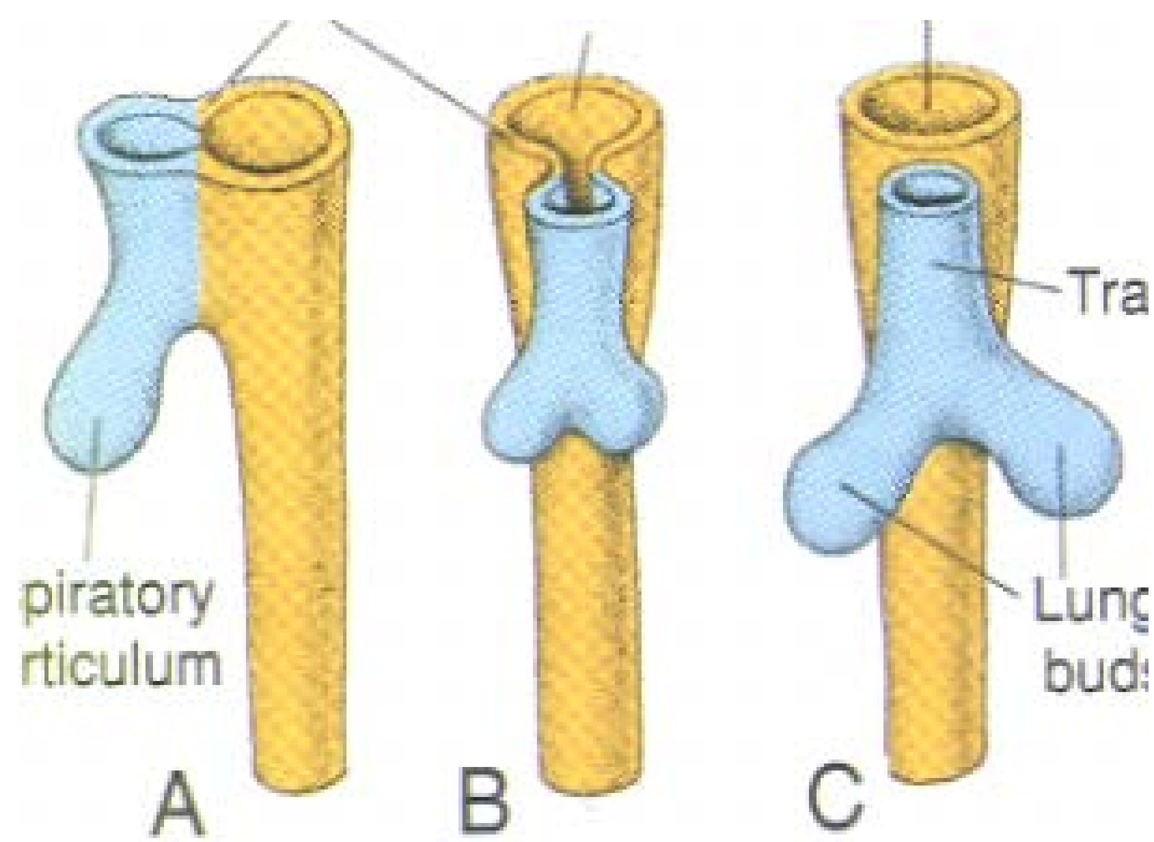
どのように対応をつけるか？

2. 肺の枝分かれ形成



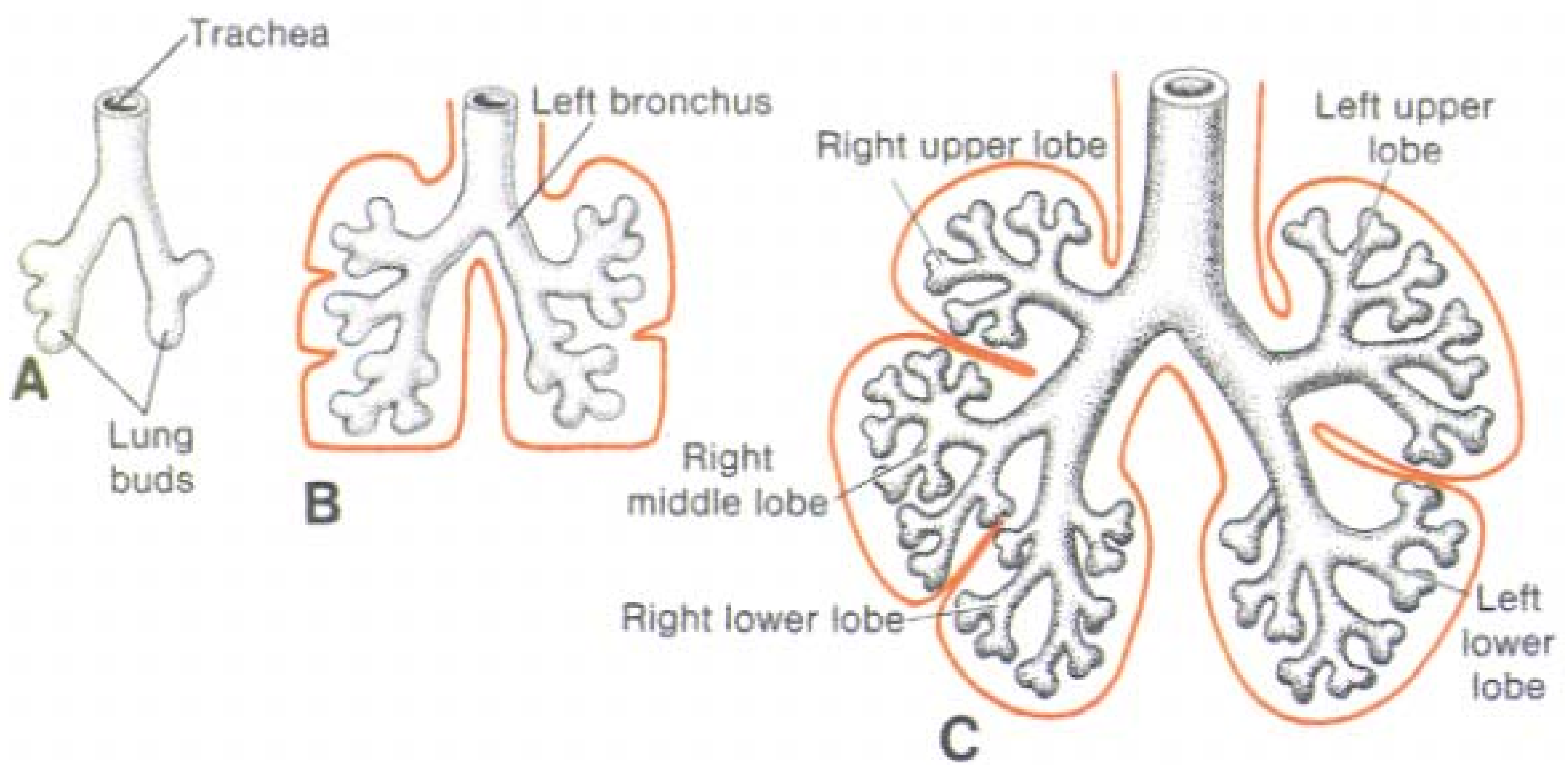
肺の枝分かれの形成





Langman’s medical embryology

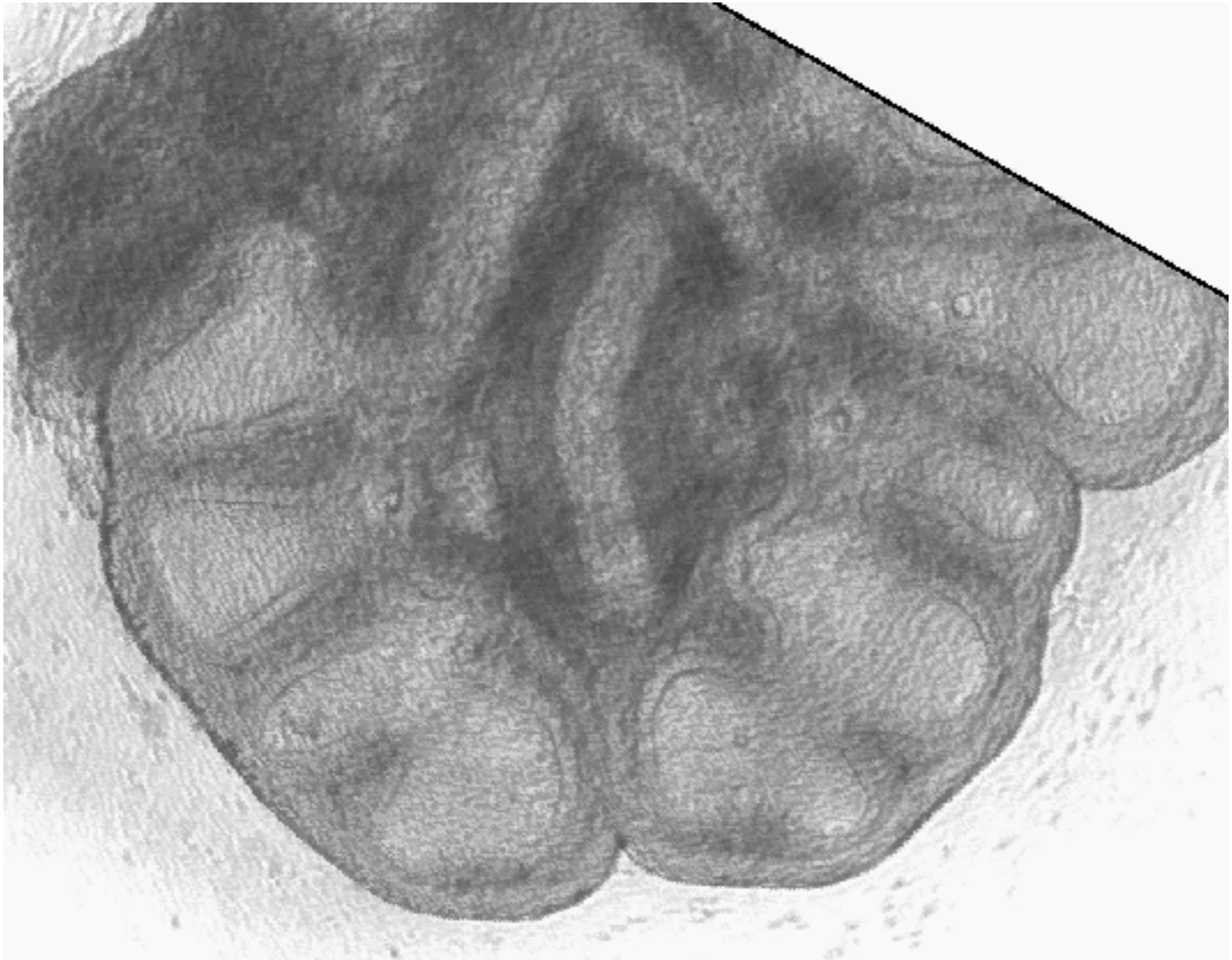
肺の枝分かれの形成



上皮と間葉の相互作用

Langman's medical embryology

器官培養系



関係する遺伝子

Table 1. Examples of mutations in mouse giving a reported lung and/or tracheal phenotype

Gene symbol	Gene name	Expression pattern	Phenotype	Reference
Signaling molecule				
Egfr	Epidermal growth factor receptor	Epithelium and mesenchyme	Impaired branching and deficient alveolization	Miettinen et al. (1997)
Fgf18	Fibroblast growth factor 18	Mesenchyme	Deficient alveolization	Usui et al. (2004)
Fgf9	Fibroblast growth factor 9	Epithelium and pleura	Impaired branching, reduced mesenchyme	Colvin et al. (2001)
Grem1	Gremlin 1	Epithelium and mesenchyme	Deficient alveolization	Michos et al. (2004)
Hip1	Huntingtin-interacting protein 1	Mesenchyme	Impaired branching	Chuang et al. (2003)
Shh	Sonic hedgehog	Epithelium	Impaired branching, tracheoesophageal fistula	Litingtung et al. (1998)
Tgfb3	Transforming growth factor, β3	Epithelium and pleura	Impaired branching	Kaartinen et al. (1995)
Wnt7b	Wingless-related MMTV integration site 7B	Epithelium	Vascular defect, reduced mesenchyme	Shu et al. (2002)
Catnnb1	β-Catenin	Epithelium	Impaired branching, proximal/distal specification	Mucenski et al. (2003)
Ltbp4	Latent transforming growth factor β binding protein 4	Not reported	Pulmonary emphysema	Sterner-Kock et al. (2002)
Wnt5a	Wingless-related MMTV integration site 5A	Mesenchyme and epithelium	Increased branching, tracheal defect	Li et al. (2002)
Fgf10	Fibroblast growth factor 10	Mesenchyme	Lung agenesis	Sekine et al. (1999)
Fgfr2b	Fibroblast growth factor receptor 2b	Epithelium	Lung agenesis	De Moerlooze et al. (2000)
Fgf8	Fibroblast growth factor 8	Not reported	Right pulmonary isomerism	Fischer et al. (2002)
Acvr2b	Activin receptor IIB	Not reported	Right pulmonary isomerism	Oh and Li (1997)
Nodal	Nodal	Not reported	Right pulmonary isomerism	Lowe et al. (2001)
Lefty1	Left right determination factor 1	Not reported	Left pulmonary isomerism	Meno et al. (1998)
Traf4	Tnf receptor associated factor 4	Not reported	Tracheal defect	Shiels et al. (2000)
Fgfr3/Fgfr4	Fibroblast growth factor receptor 3/4	Epithelium and mesenchyme	Deficient alveolization	Weinstein et al. (1998)
Nog	Noggin	Mesenchyme	Lobation defect	Weaver et al. (2003)
Transcription factor				
Cebpa	CCAAT/enhancer binding protein (C/EBP), α	Epithelium	Hyperproliferation of type II cells	Sugahara et al. (2001)
Foxa1/Foxa2	Forkhead box A1/A2	Epithelium	Impaired branching, reduced smooth muscle	Wan et al. (2005)
Foxf1a	Forkhead box F1a	Mesenchyme	Impaired branching, lobation defect	Lim et al. (2002)
Hoxa5	Homeobox A5	Mesenchyme	Impaired branching, tracheal defect	Aubin et al. (1997)
Klf2	Kruppel-like factor 2 (lung)	Not reported	Impaired sacculation	Wani et al. (1999)
Mycn	Neuroblastoma myc-related oncogene 1	Epithelium	Impaired branching	Moens et al. (1992)
Trp63	Transformation-related protein 63	Epithelium	Tracheobronchial defect	Daniely et al. (2004)
Titf1	Thyroid transcription factor 1	Epithelium	Loss of distal lung fate, impaired branching, tracheoesophageal fistula	Kimura et al. (1996)
Nfib	Nuclear factor I/B	Epithelium and mesenchyme	Sacculation defect	Steele-Perkins et al. (2005)
Sox11	SRY-box-containing gene 11	Epithelium	Hypoplastic lung	Sock et al. (2004)
Tcf21	Transcription factor 21 (Pod1)	Mesenchyme	Impaired branching	Quaggin et al. (1999)
Rarb/Rara	Retinoic acid receptor α/β	Epithelium and mesenchyme	Left lung agenesis and right lung hypoplasia	Mendelsohn et al. (1994)
Pitx2	Paired-like homeodomain transcription factor 2	Mesenchyme	Right pulmonary isomerism	Lin et al. (1999)
Foxj1	Forkhead box J1	Epithelium	Left-right asymmetry, loss of ciliated cells	Brody et al. (2000)
Gata6	GATA-binding protein 6	Epithelium	Impaired sacculation	Yang et al. (2002)
Gli2/Gli3	GLI-Kruppel family member GLI2/GLI3	Mesenchyme	Lung agenesis	Motoyama et al. (1998)
Ascl1	Achaete-scute complex homolog-like 1	Neuroendocrine cells	Loss of neuroendocrine cells	Ito et al. (2000)
Others				
Eln	Elastin	Mesenchyme	Deficient alveolization	Wendel et al. (2000)
Lmnb1	Lamin B1	Epithelium and mesenchyme	Deficient alveolization	Vergnes et al. (2004)
Lama5	Laminin α5	Epithelium and pleura	Defective lobation	Nguyen et al. (2002)
Pcaf	p300/CBP-associated factor	Epithelium and mesenchyme	Defective proximal and distal epithelial cell differentiation	Shikama et al. (2003)
Adam17	A disintegrin and metallopeptidase domain 17	Epithelium	Impaired epithelial differentiation, impaired branching	Zhao et al. (2001) Peschon et al. (1998)
Crh	Corticotropin releasing hormone	Epithelium	Defective epithelial and mesenchymal maturation	Muglia et al. (1999)
Pthlh	Parathyroid hormone-like peptide	Epithelium	Deficient alveolization	Rubin et al. (2004)
Itga3	Integrin α3	Epithelium	Impaired branching	Kreidberg et al. (1996)
Cutl1	Cut-like 1	Epithelium	Impaired epithelial differentiation	Ellis et al. (2001)

Development　133, 1611-（2006）

生体のシステムは

複雑すぎる

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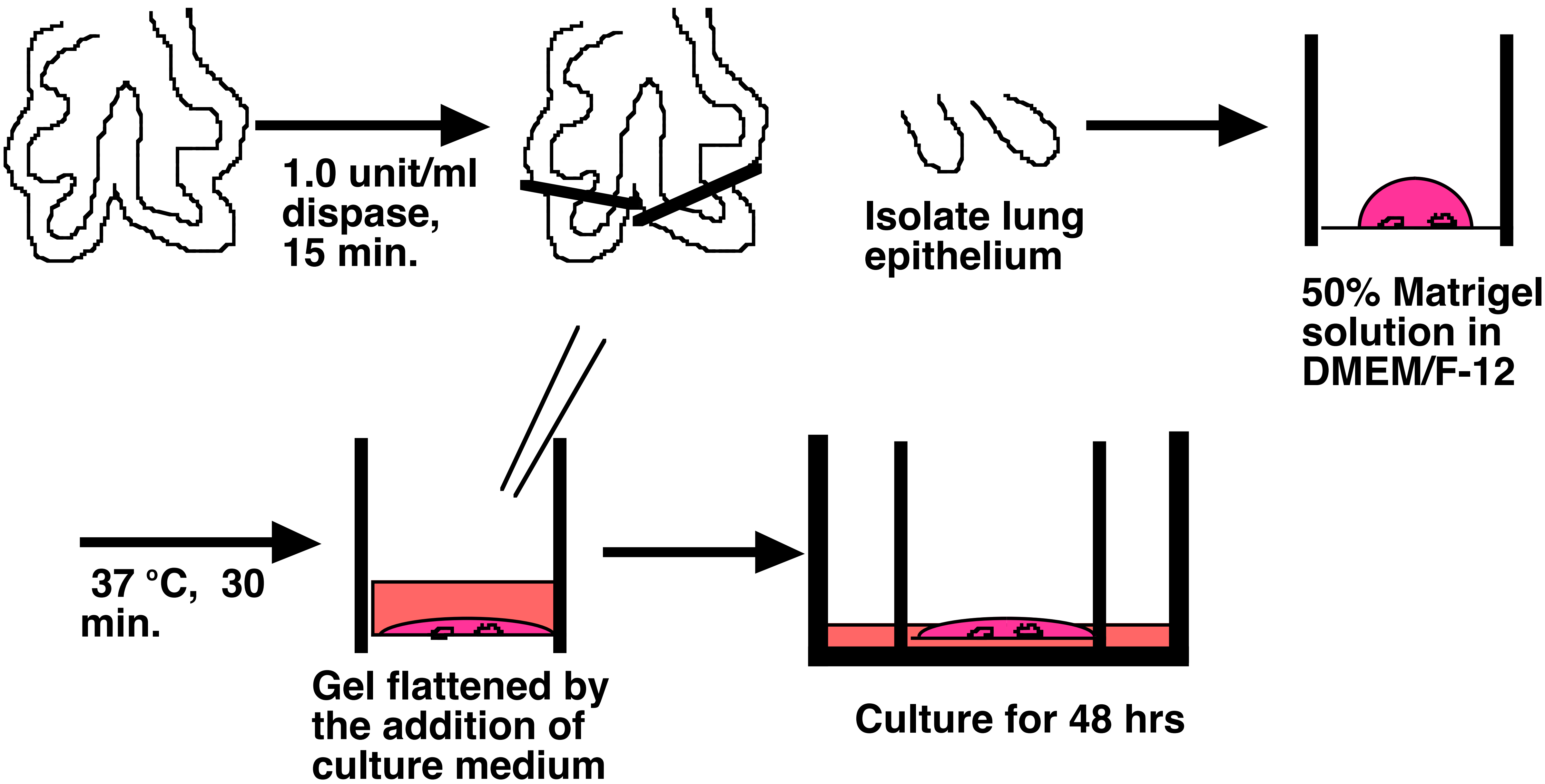
出来るだけ簡単な

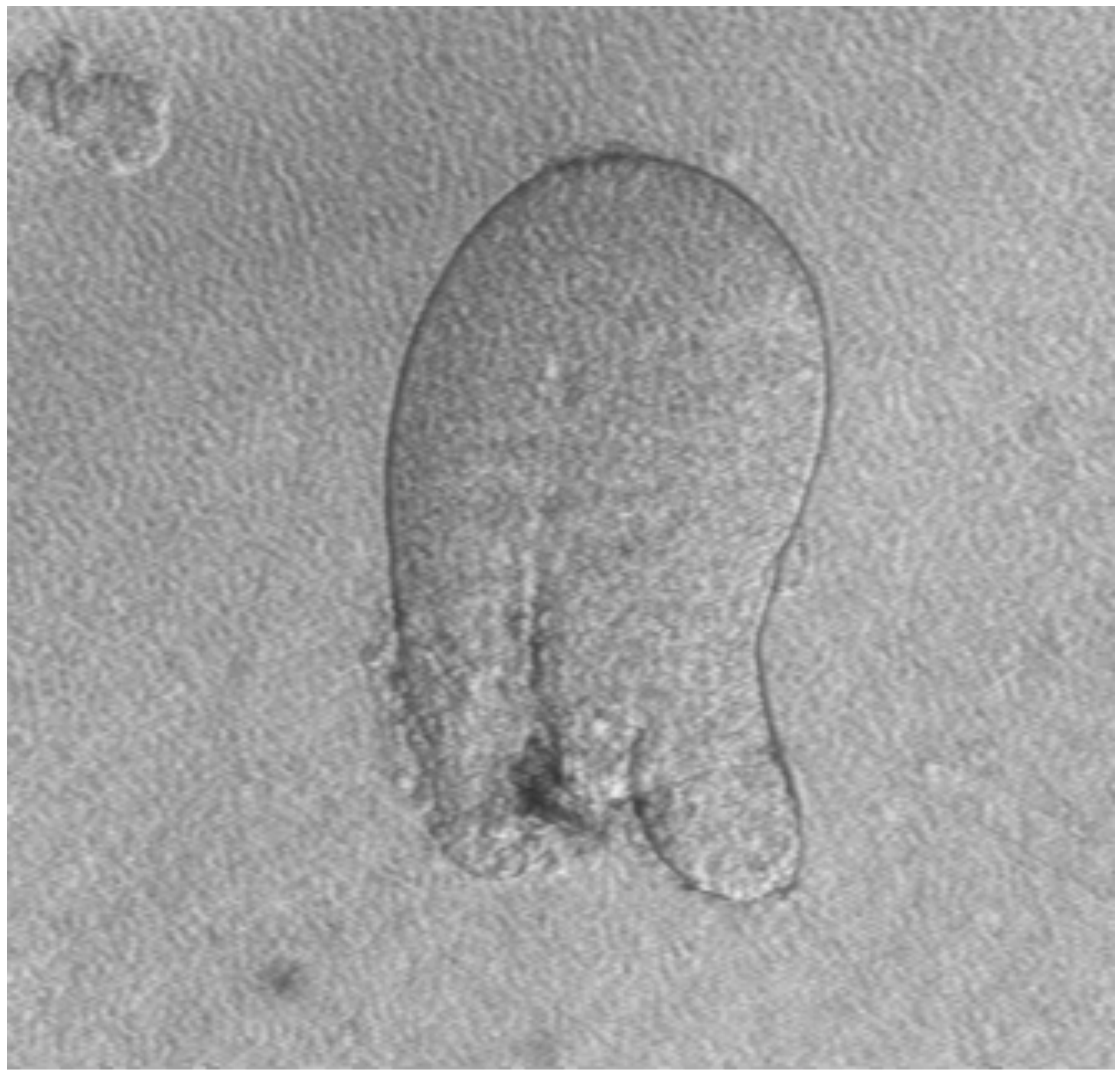
実験系を作る

上皮のみを単離した培養系

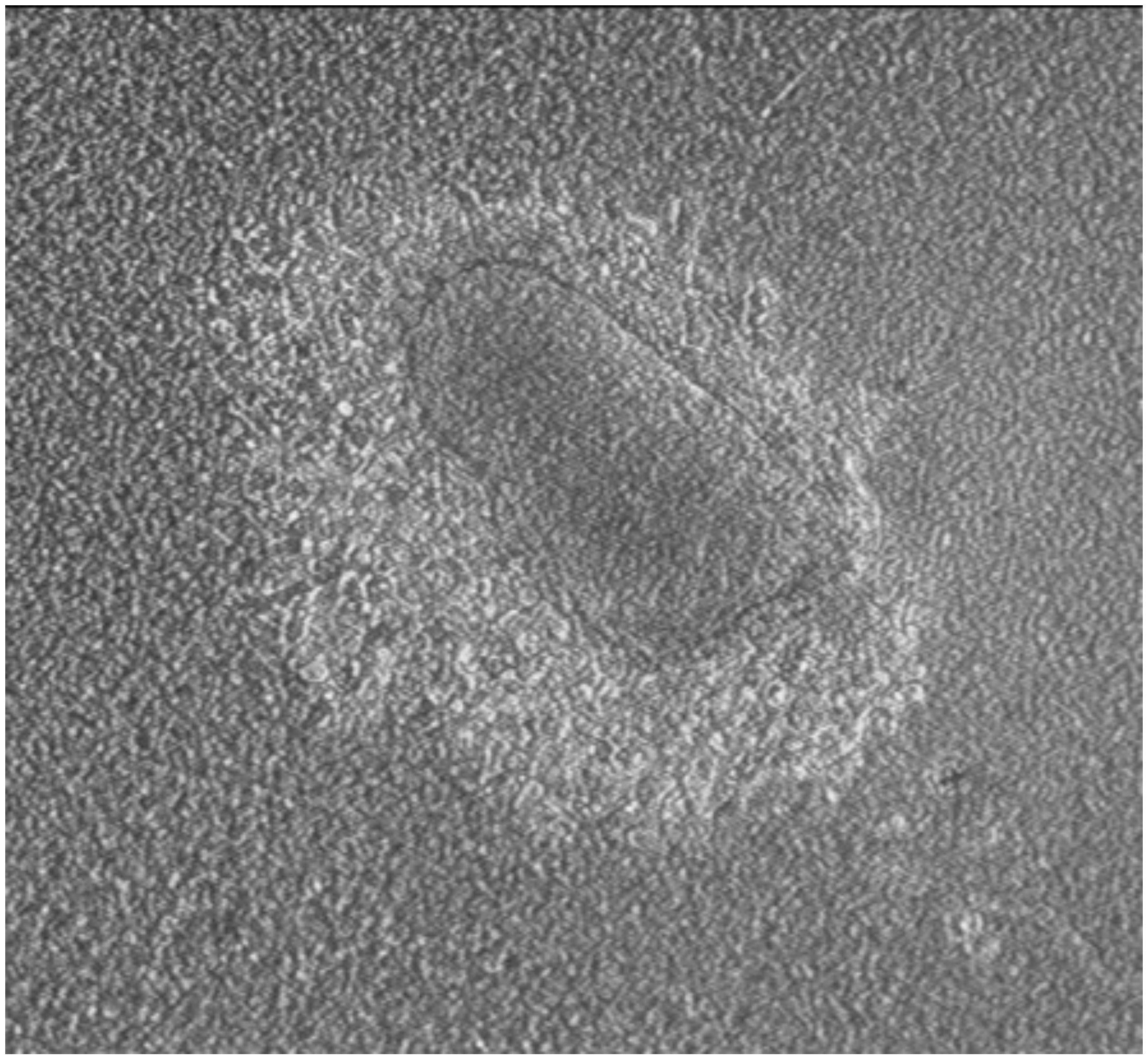


Ito & Nogawa, Development 121(4) 1015-22 (1995)

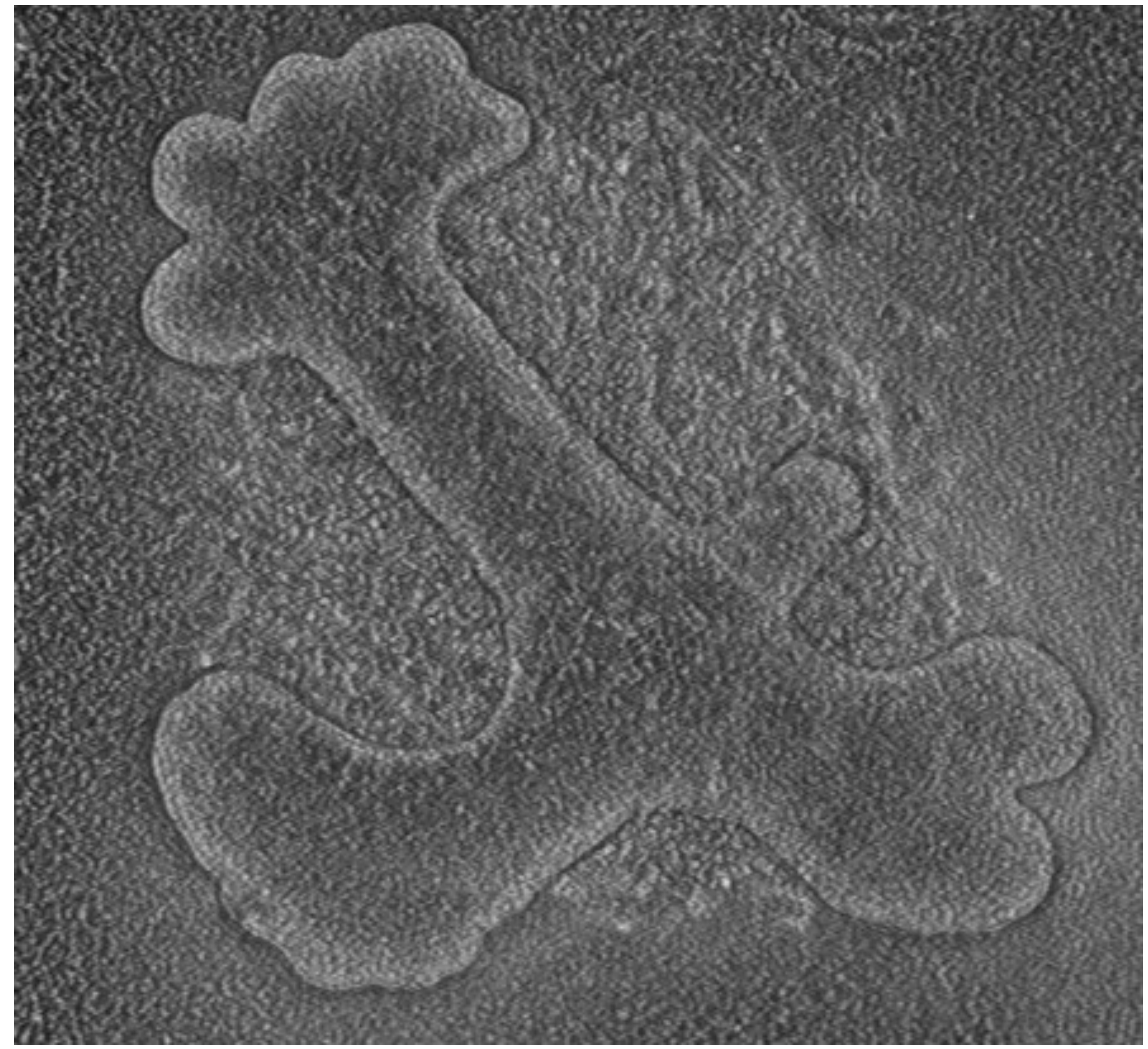
上皮だけでも
枝分れの形成は起きる



培地にFGF が必要



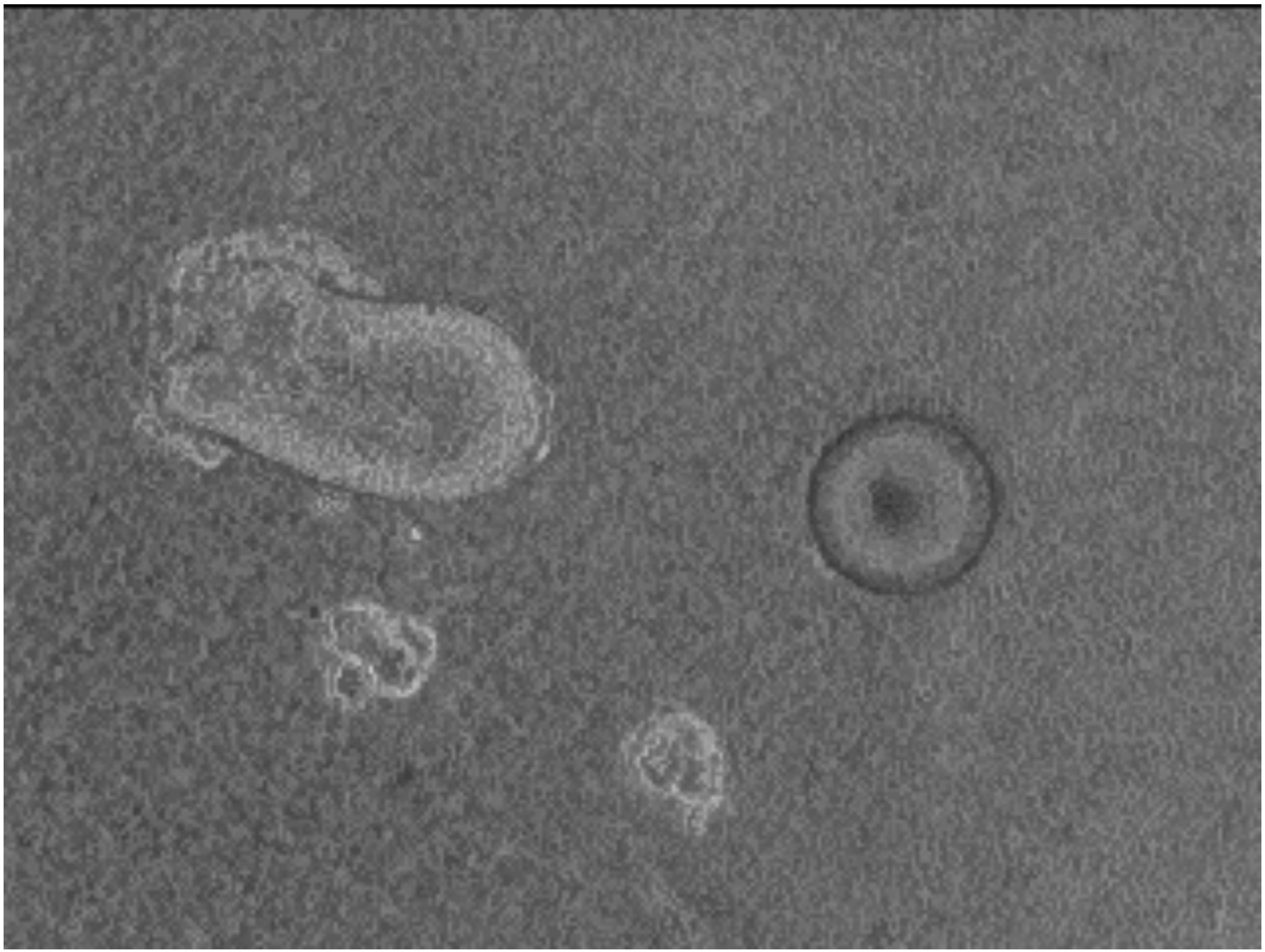
10 ng/ml FGF1



100 ng/ml FGF1

Ito & Nogawa, Development 121(4) 1015-22 (1995)

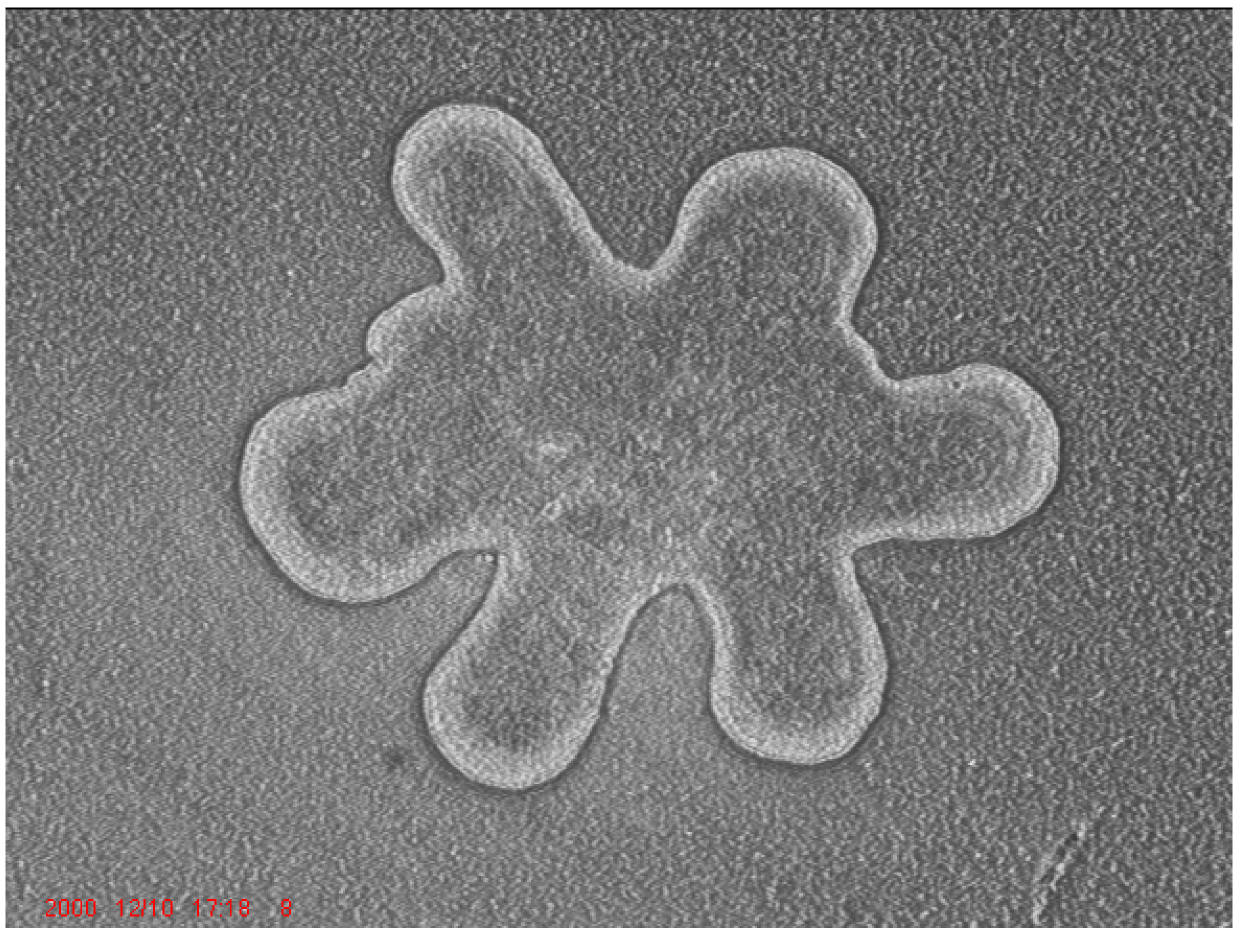
肺上皮はFGFの濃度の高い方に向かって伸びる



Bellusci et al., Development 124(23) 4867-78 (1997)

細胞外基質の分解

＞嚢胞形成



Control

2 μg/ml collagenase I

FGFが大事。

細胞外基質も関係する。

枝分れのできる原理は？

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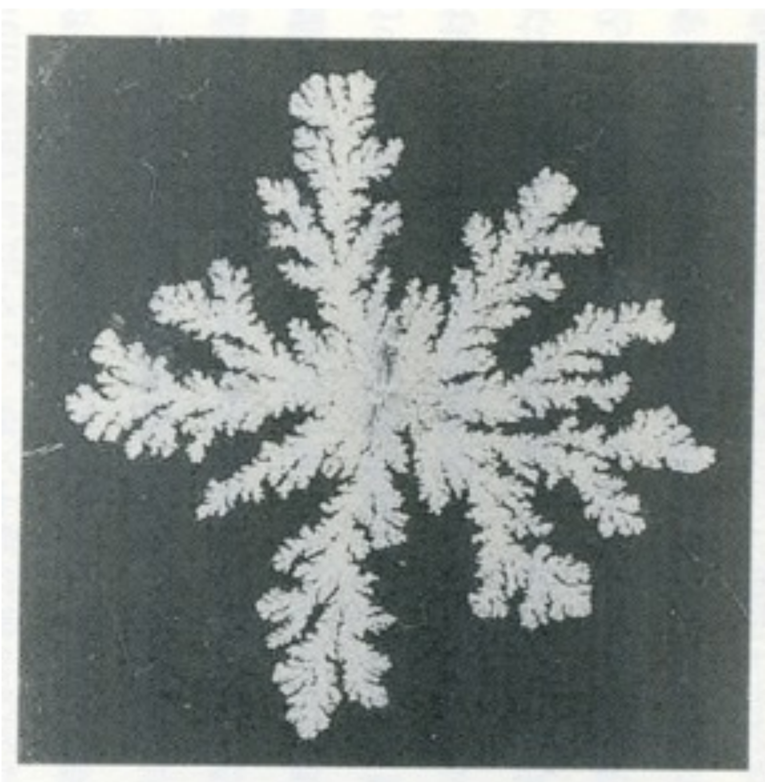
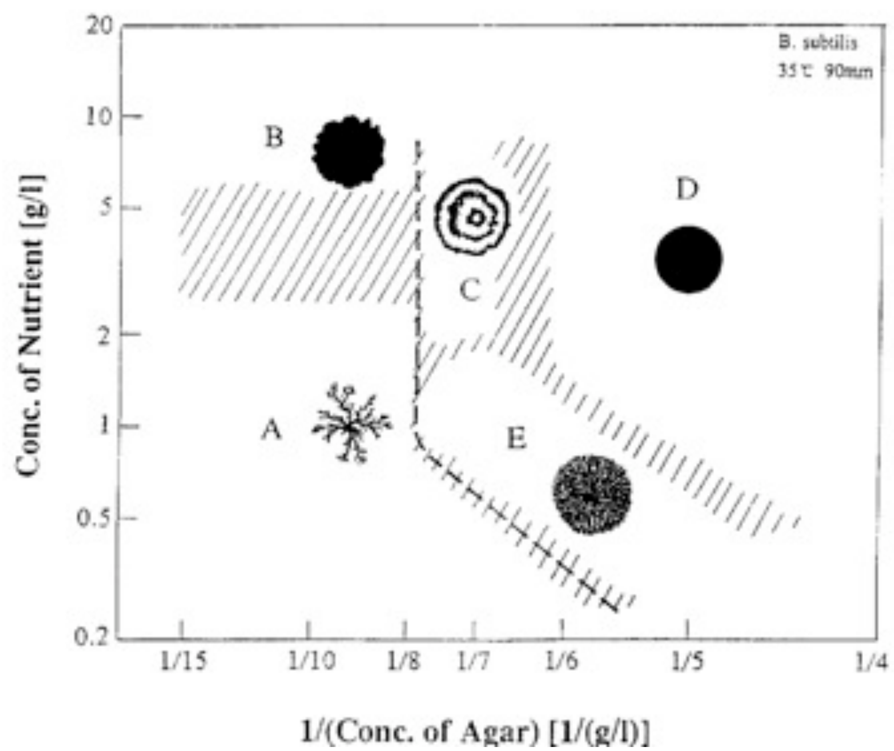
別の系に学ぶ

バクテリアコロニーの

パターン形成





松下貢先生

（中大）

低栄養下でバクテリアのコロニーが

様々なパターンをつくる

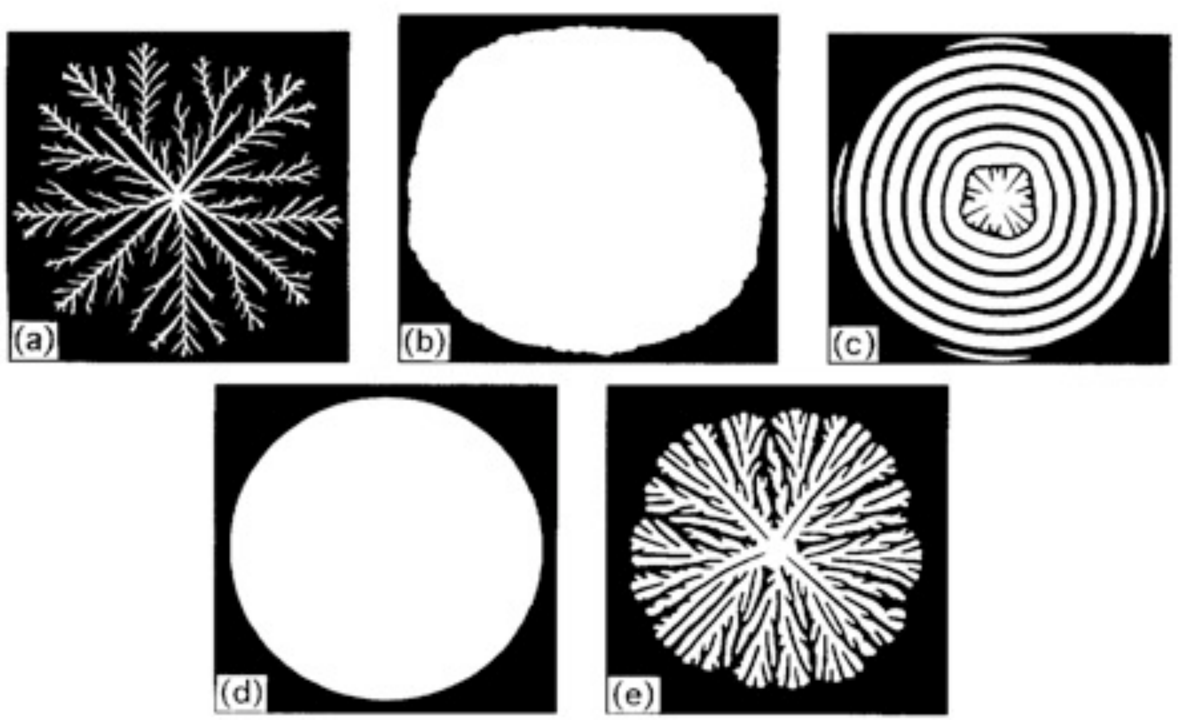
三村モデル

$$\frac{\partial b}{\partial t} = \nabla(d(b,n)\nabla b) + \varepsilon g(n)b - a(b,n)b\,,$$

$$\frac{\partial s}{\partial t} = a(b,n)b\,,$$

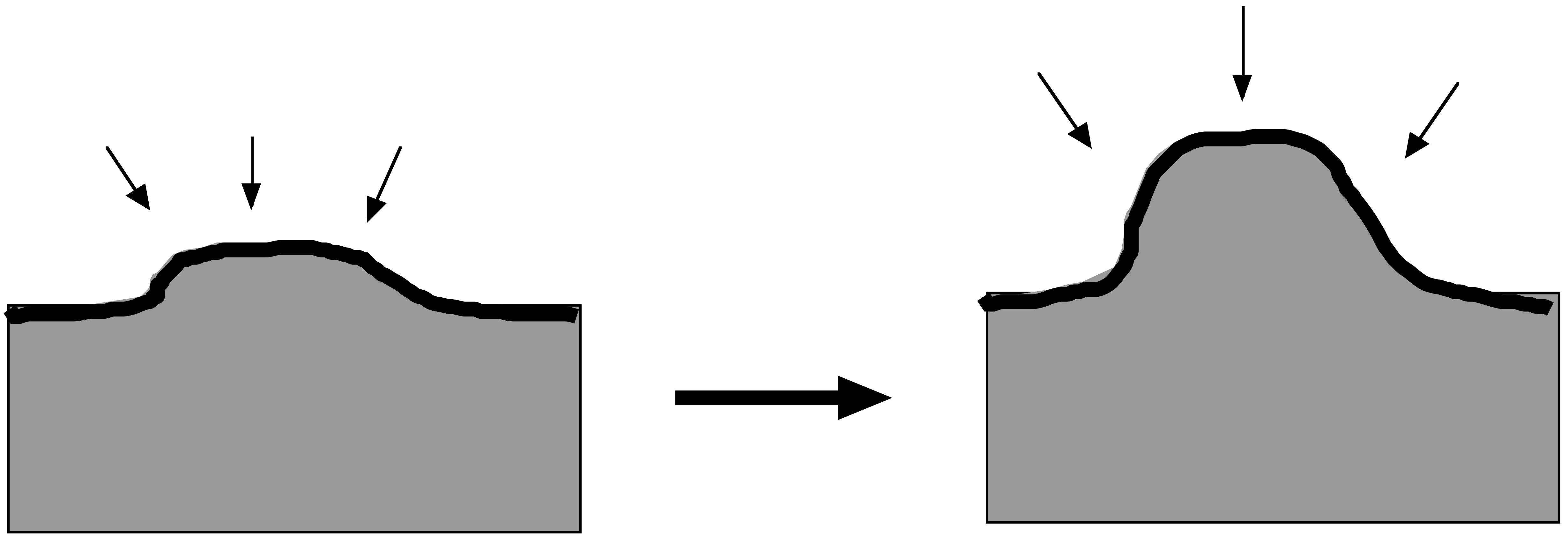
$$\frac{\partial n}{\partial t} = \nabla^2 n - g(n)b\,.$$

三村昌泰先生（明大）



Mimura et al., Physica A 249, 517- (1998)

“突起の部分が早くのびる”



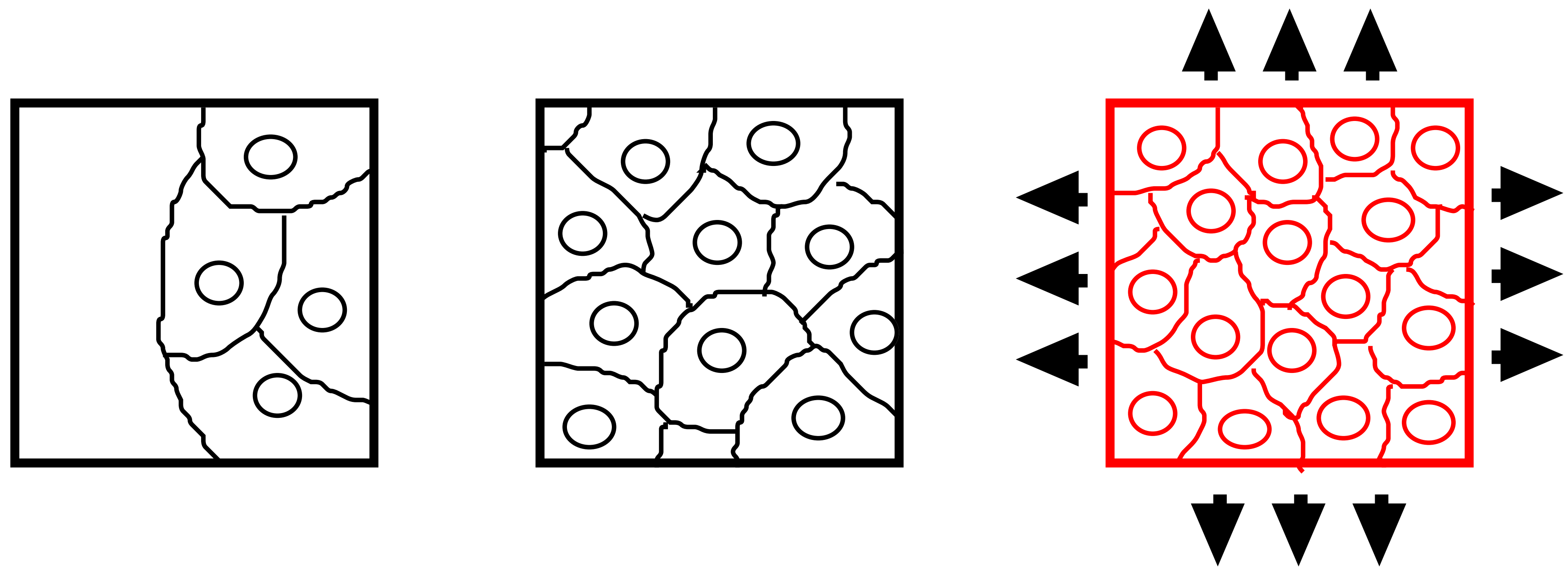
対応が薄々見える

- FGF = 栄養
- 肺の上皮細胞=バクテリアの細胞密度

FGF欠乏モデル

- 細胞密度　c(x, y, t)
- FGF 濃度 n(x, y, t)
- 細胞はFGFを消費して成長する
- FGF はゲルの中を拡散する
- 細胞は一定の細胞密度を保つように動く

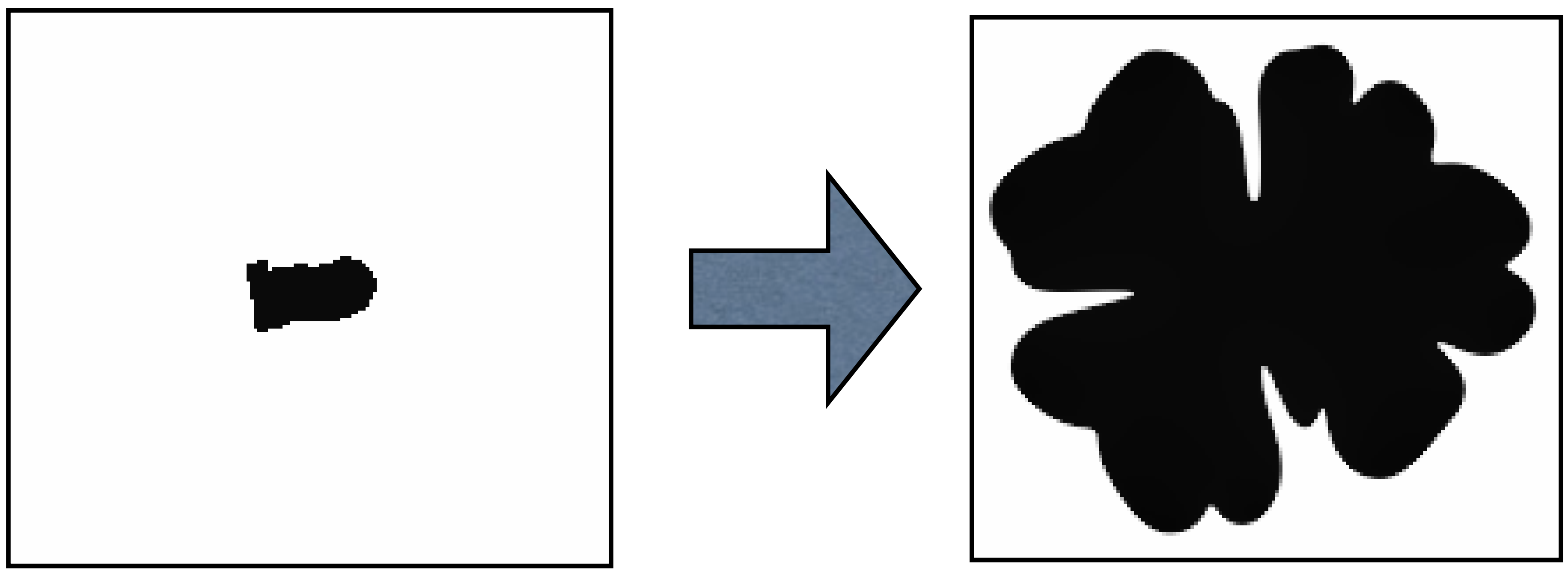
細胞運動の定義



c (Cell Density)	$<c_0$	c_0	$>c_0$
J (cell movement)	0	0	$\Delta(c-c_0)$

=Volume-of-fluid method (J. A. Sethian, Level Set Methods and Fast Marcing Methods)

数値計算



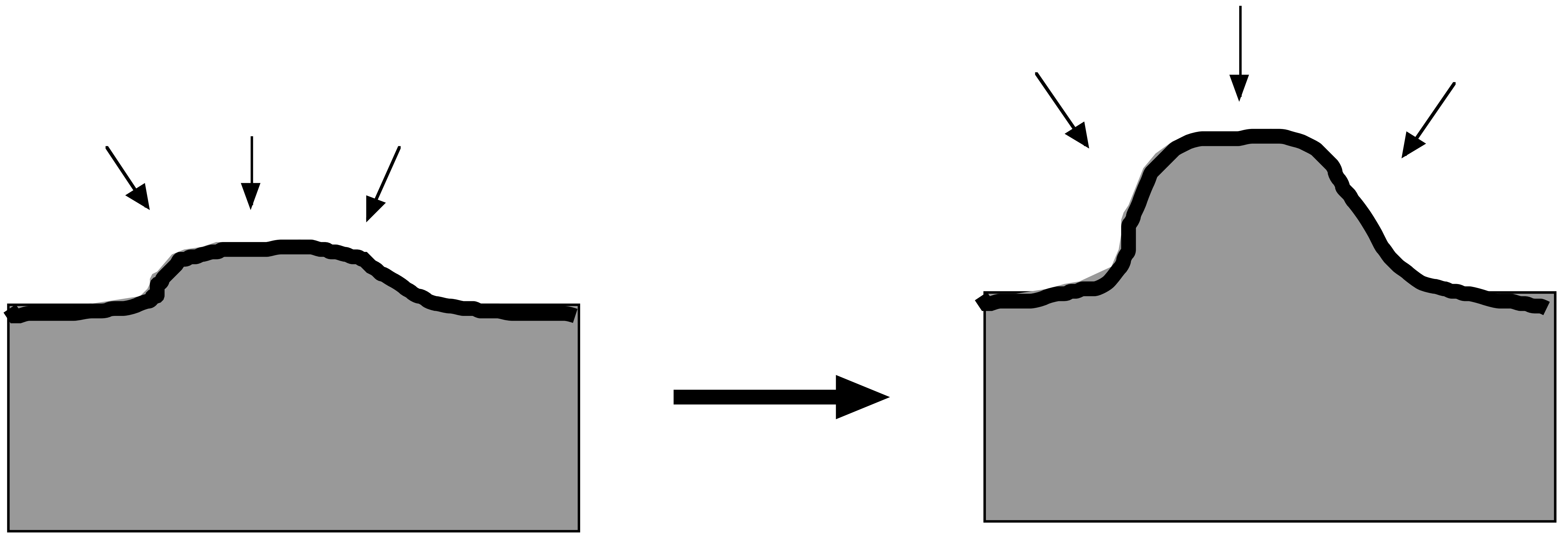
$$f(c,n) = \frac{5cn}{1+3n}, p = 1.0$$

$$d_1 = 4.0, d_2 = 1.7$$

$$n_0 = 0.5, c_0 = 1.0$$

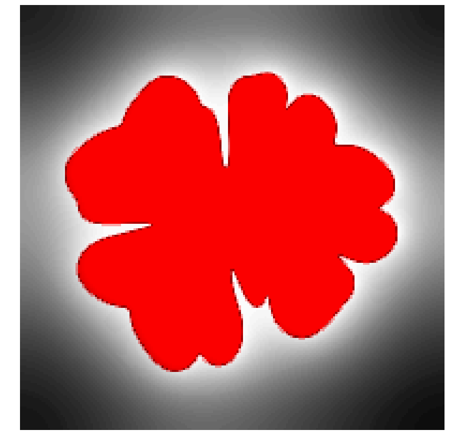
Miura & Shiota, MOD 116, 29-38(2002)

“突起の部分が早くのびる”

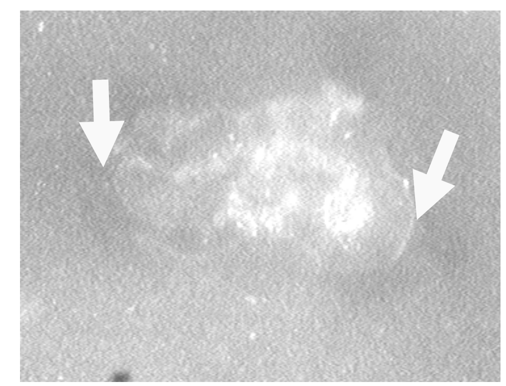


FGFが消費されているか？

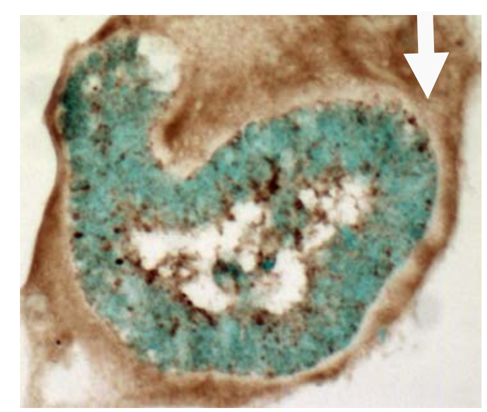
FGF distribution in numerical simulation



Cy3-labeled FGF

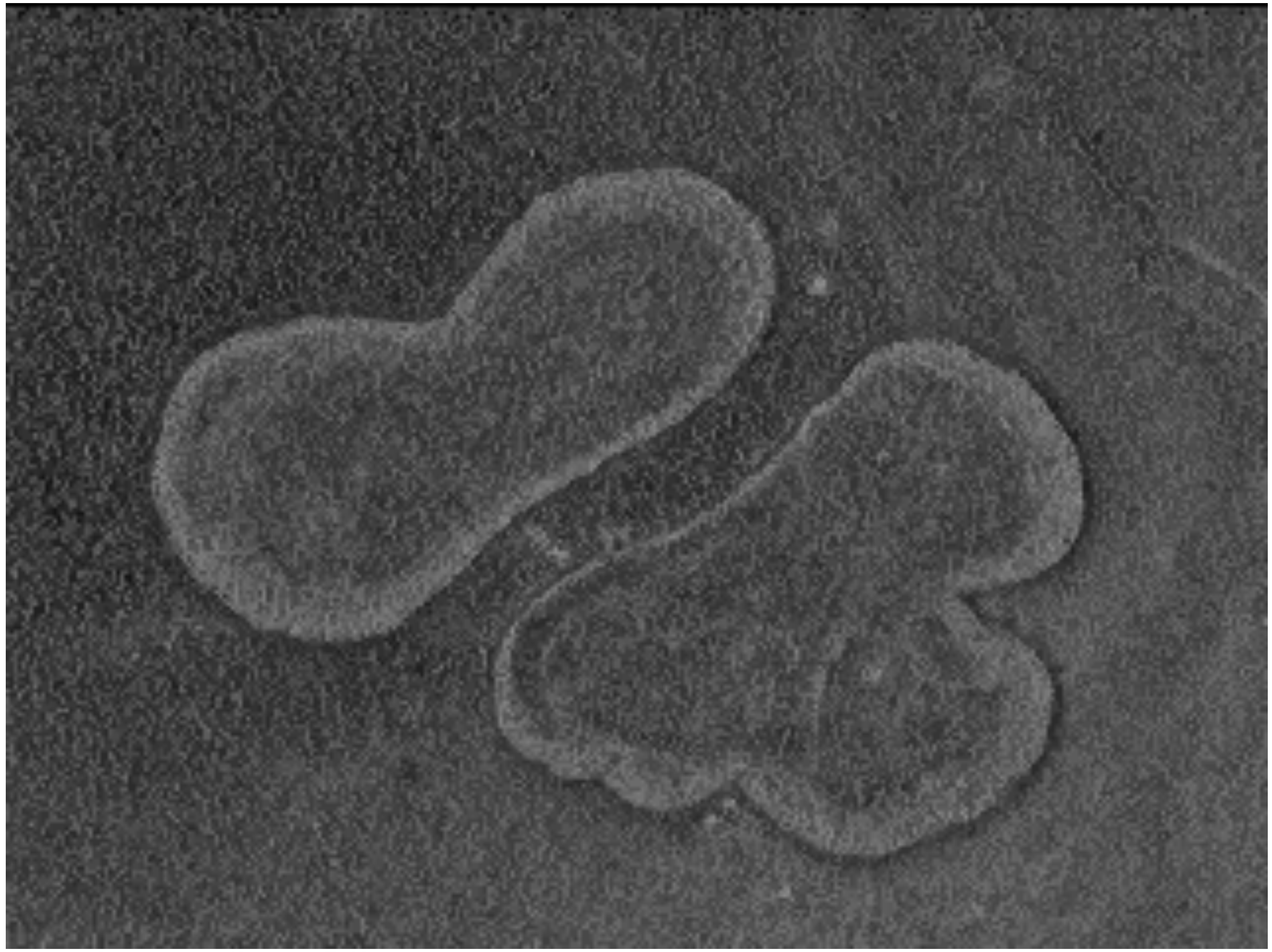


FGF immunohistochemistry



Miura & Shiota, MOD 116, 29-38(2002)

遮蔽効果



間のFGF濃度が低くなる＞枝が出ない

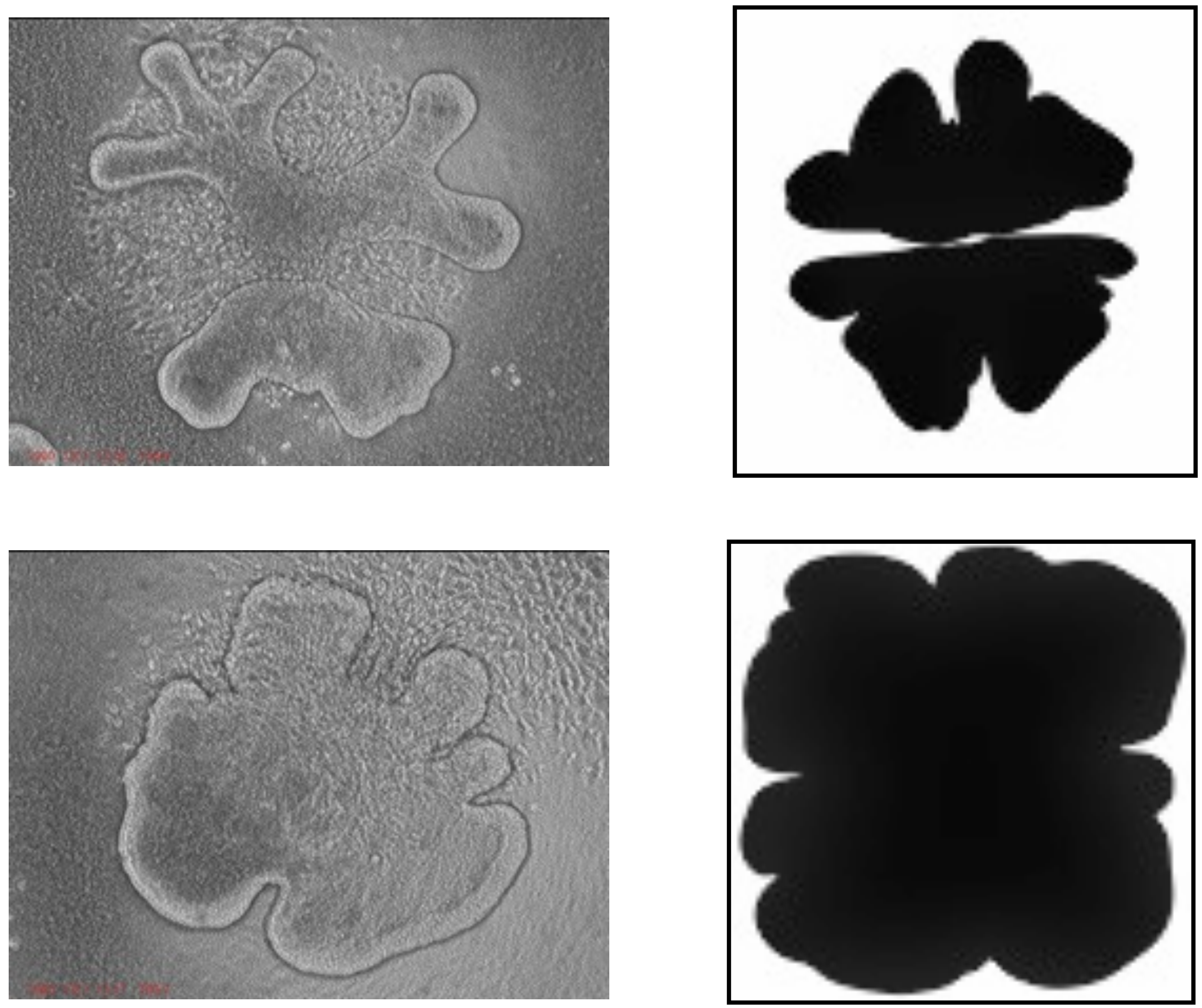
Miura & Shiota, MOD 116, 29-38(2002)

遮蔽効果の抑制

Experiment Simulation

Low FGF

High FGF



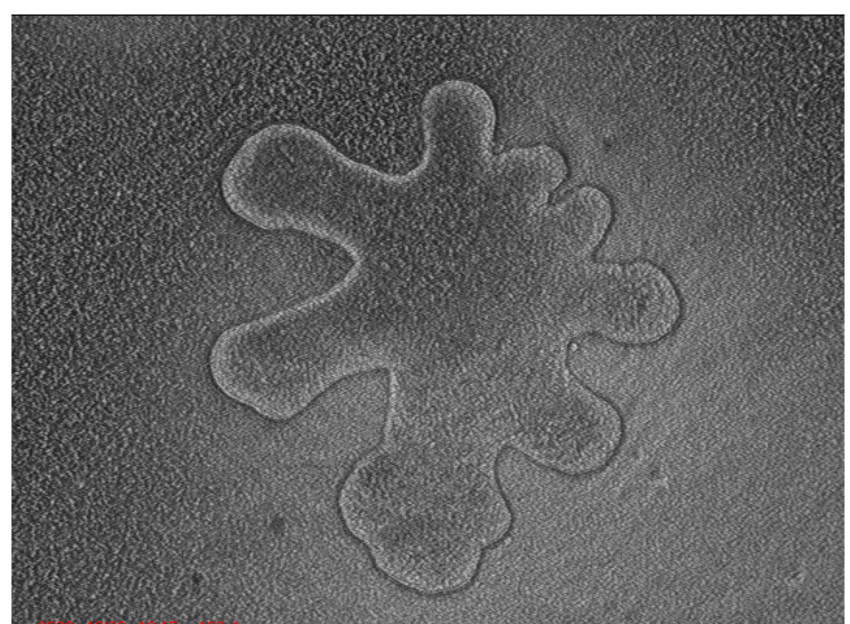
Miura & Shiota, MOD 116, 29-38(2002)

高濃度のFGFによる形態変化

Experiment Simulation

500 ng/ml FGF1

100 ng/ml FGF1



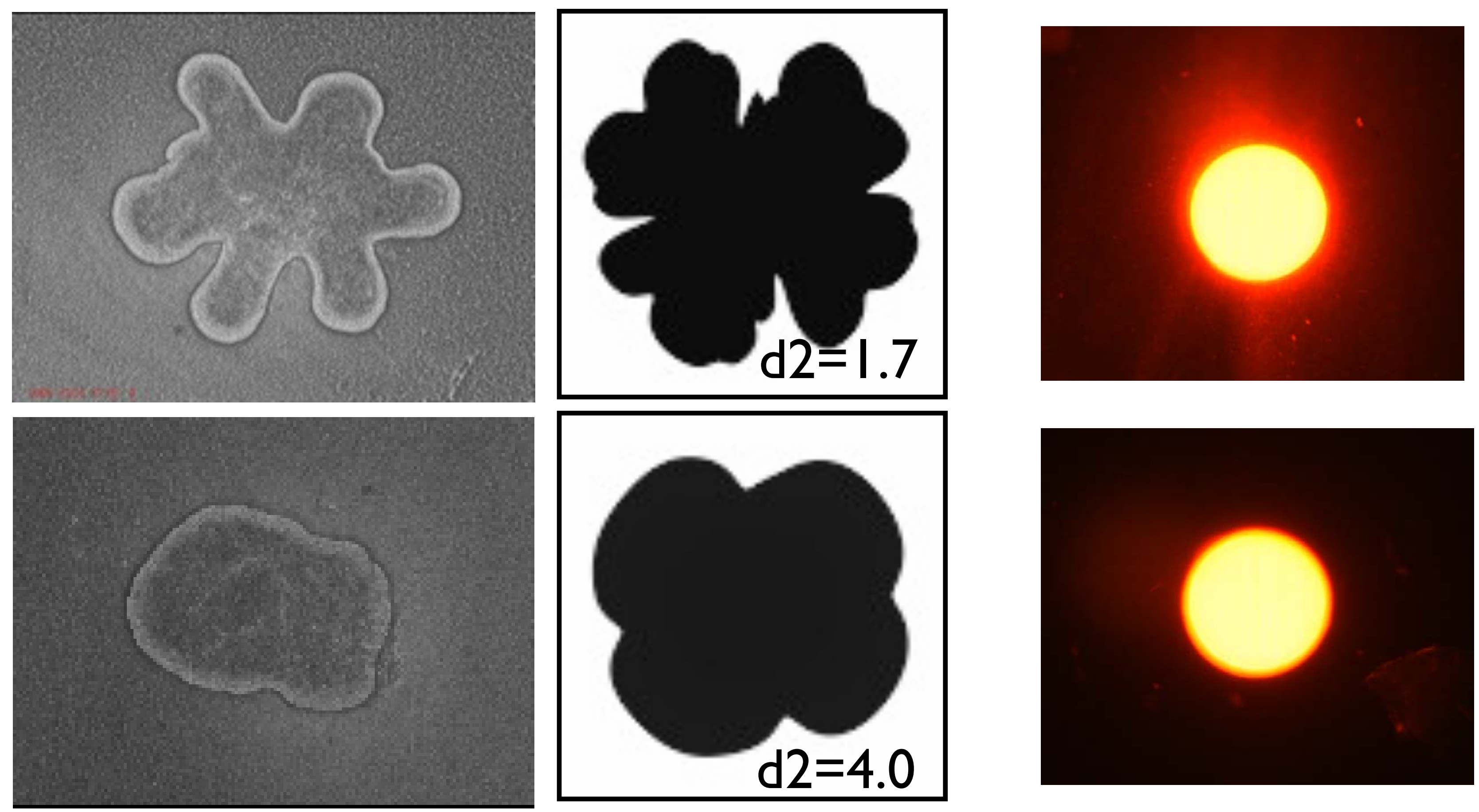
Miura & Shiota, MOD 116, 29-38(2002)

細胞外基質の分解と拡散係数

Experiment Simulation FGF-Cy3 beads

Control

Collagenase(+)



Miura & Shiota, MOD 116, 29-38(2002)

in vitro の肺上皮の
枝分れのメカニズムは
（だいたい）理解した.

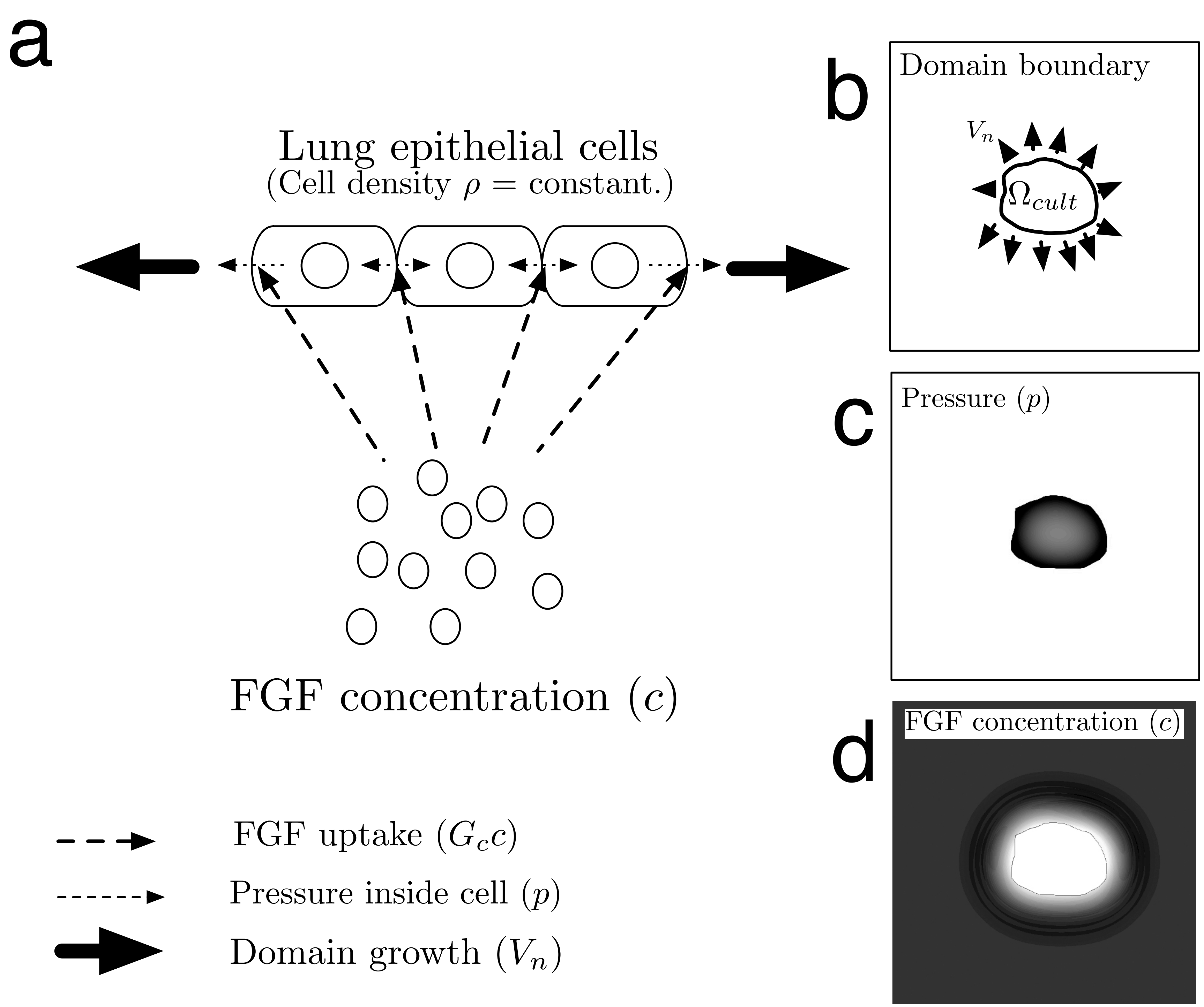
Miura & Shiota, MOD 116, 29-38(2002)

モデルが
数理的に扱いにくい
（細胞運動の部分）

数学的な解析の出来る連続モデルの構築

(Heidelberg 大学 D. Hartmann 氏との共同研究）

自由境界モデルの定義



Hartmann & Miura, JTB 242, 862- (2007)

支配方程式

圧力　細胞の成長　組織片内

$$
\begin{aligned}
0 &= \alpha \Delta p(\boldsymbol{x};t) + \frac{G_\rho c(\boldsymbol{x};t)}{\rho} & (\boldsymbol{x};t) \in \Omega_{\text{cult.}}(t) \times (0,T] \\
\frac{\partial c(\boldsymbol{x};t)}{\partial t} &= D\Delta c(\boldsymbol{x};t) - \chi_{\Omega_{\text{cult.}}(t)}(\boldsymbol{x}) G_c c(\boldsymbol{x};t) & (\boldsymbol{x};t) \in \Omega \times (0,T].
\end{aligned}
$$

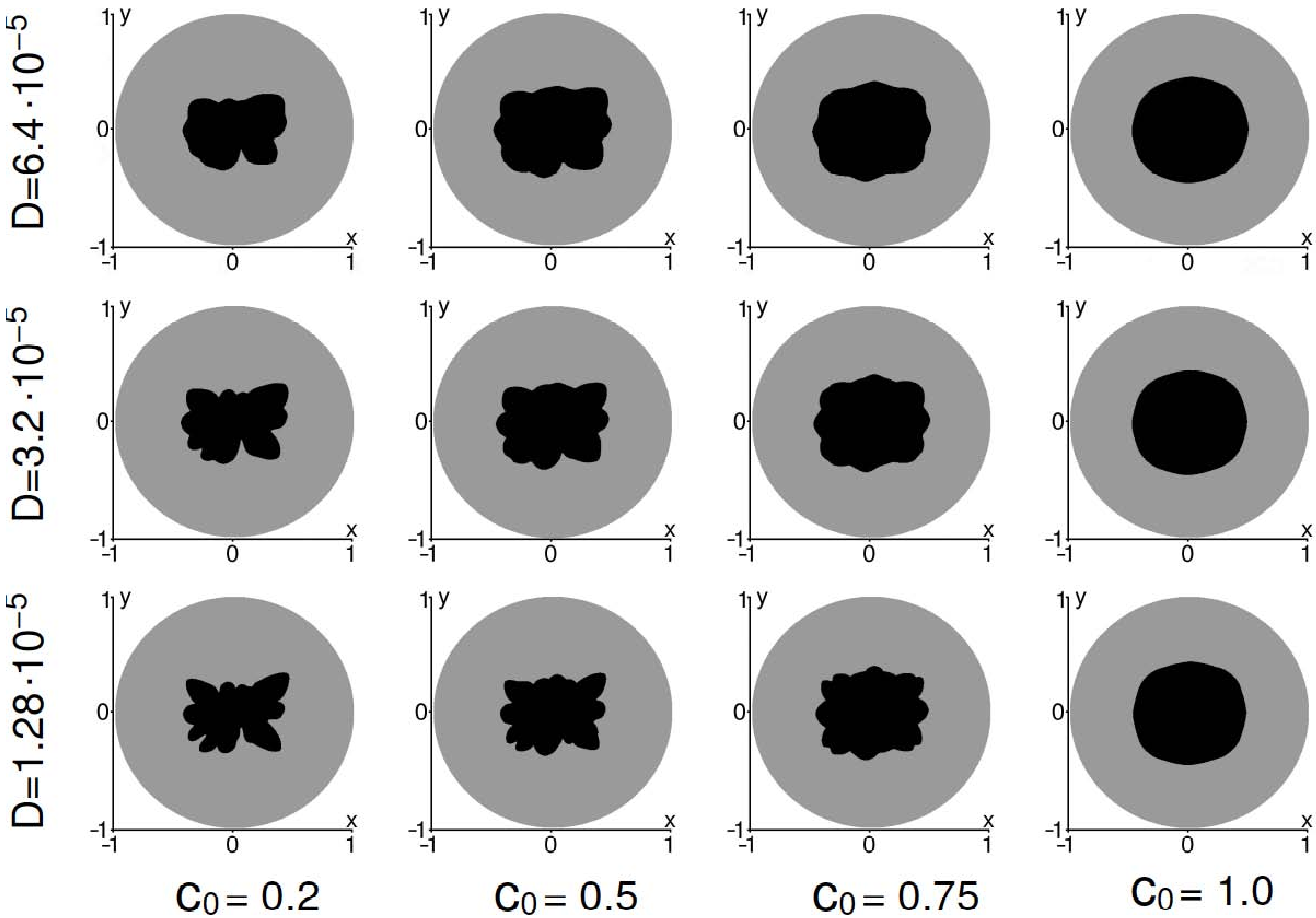
拡散　FGFの消費　全体

$$
V_n(\boldsymbol{x};t) = -\boldsymbol{\nu}(\boldsymbol{x};t) \cdot \alpha \nabla p(\boldsymbol{x};t) \qquad (\boldsymbol{x};t) \in \partial\Omega_{\text{cult.}}(t) \times (0,T]
$$

境界の速度　単位ベクトル　圧勾配　境界部分

Hartmann & Miura, JTB 242, 862- (2007)

Phase diagram

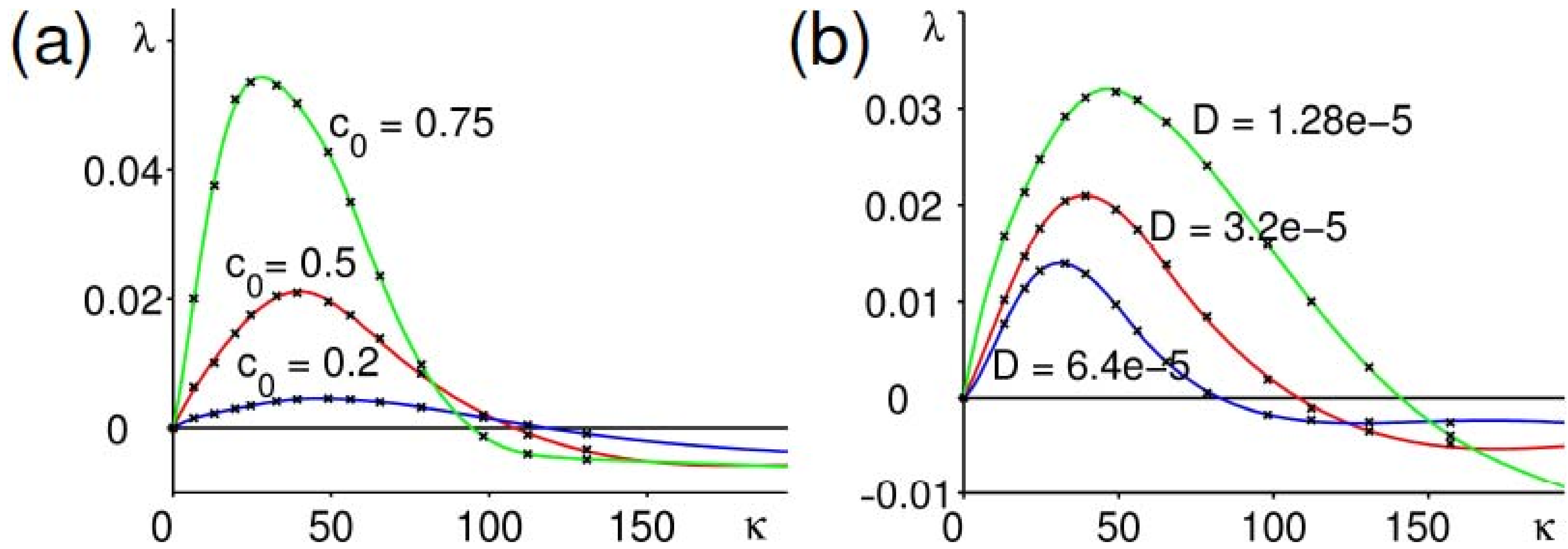


c0: FGFの初期濃度　　D: FGFの拡散係数

Hartmann & Miura, JTB 242, 862- (2007)

生成される枝の大きさの推定

（線形分散関係）

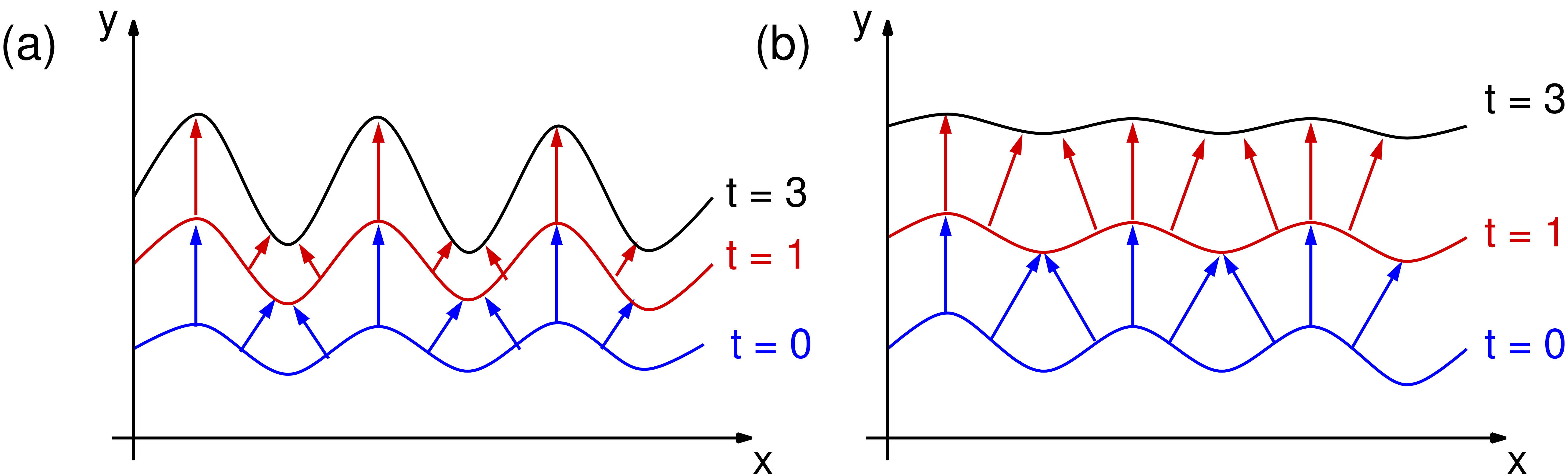


c0: FGFの初期濃度　D: FGFの拡散係数

Hartmann & Miura, JTB 242, 862- (2007)

直感的説明：

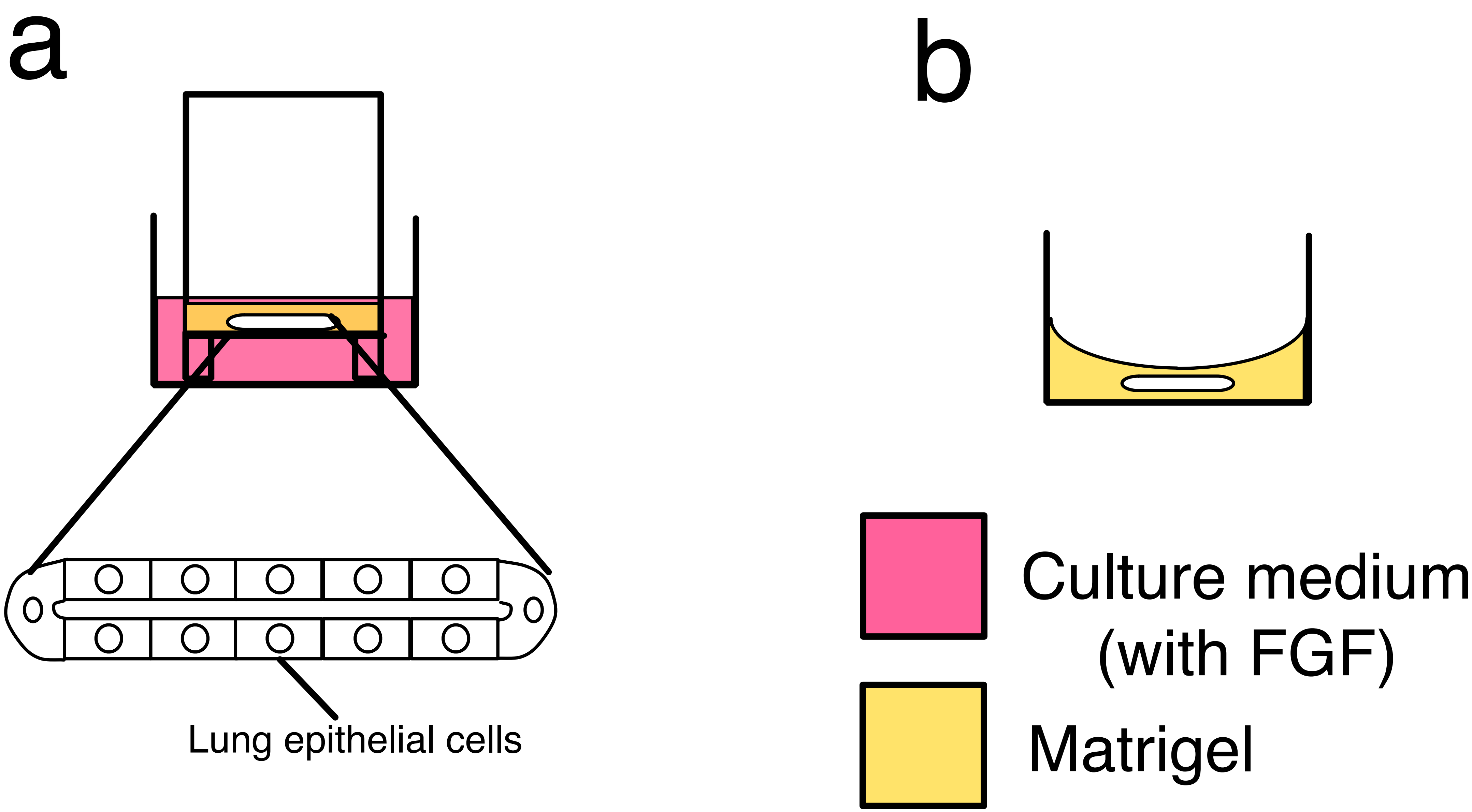
2つの極端な場合



FGF濃度が低い場合　　FGF濃度が高い場合

Hartmann & Miura, JTB 242, 862- (2007)

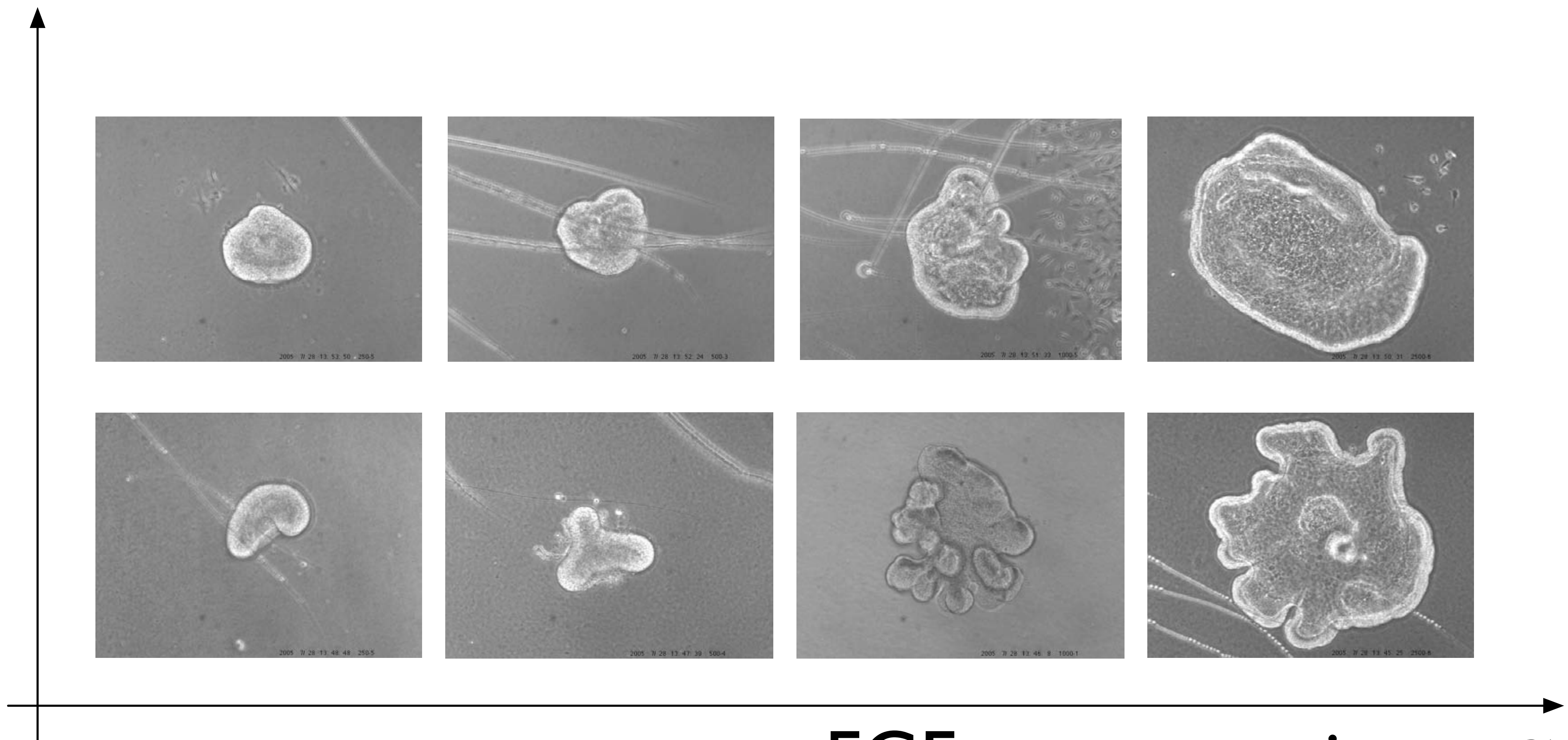
培養法を変える。



Hartmann & Miura, JTB 242, 862- (2007)

Phase diagramを描け。

拡散係数
$1/G_c$

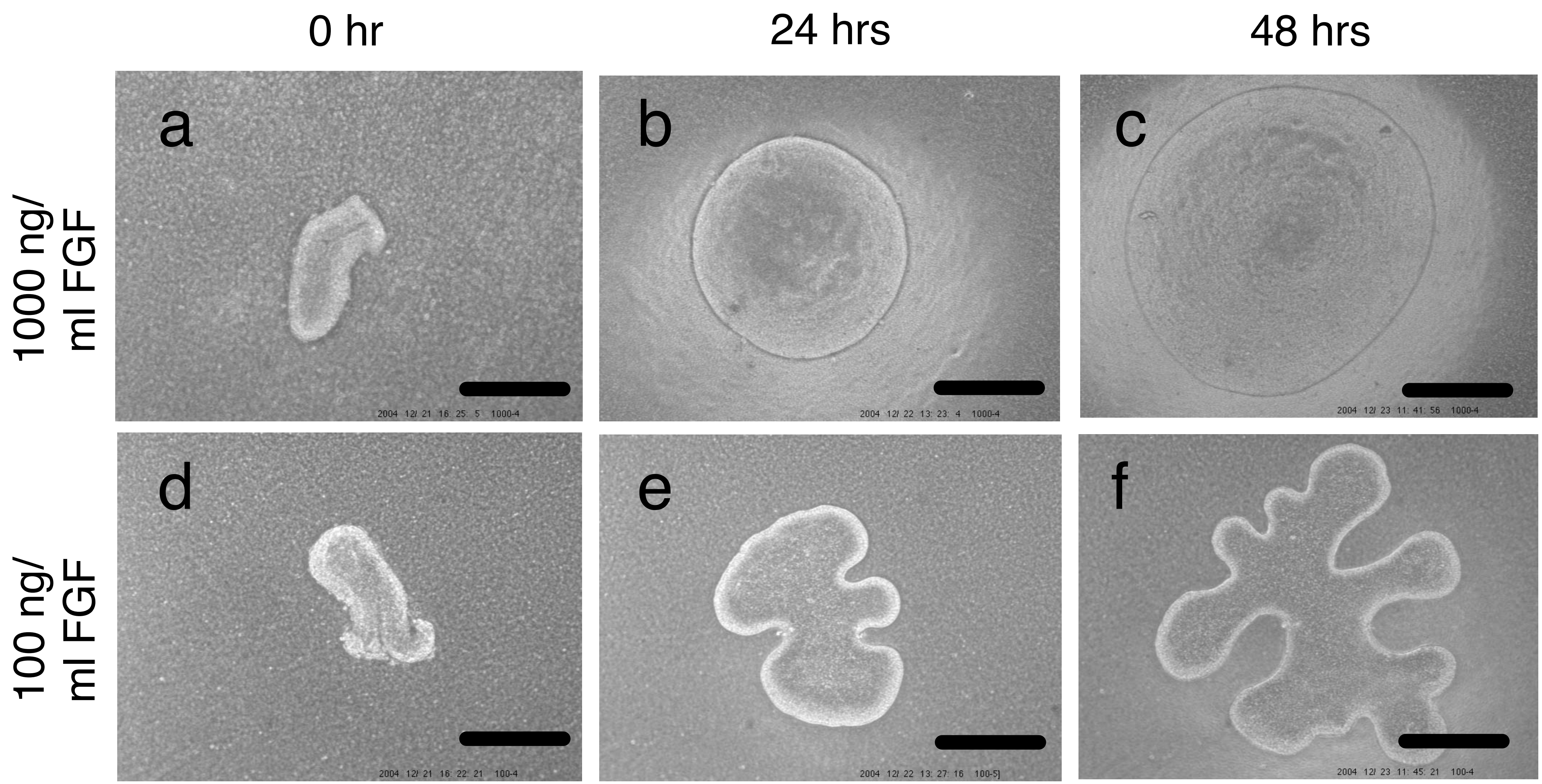


FGF concentration G_ρ

Hartmann & Miura, JTB 242, 862- (2007)

FGF濃度を
思い切り上げると、
枝分れでなく
嚢胞が出来るはずだ。

…できた！



Hartmann & Miura, JTB 242, 862- (2007)

論文は出るけど

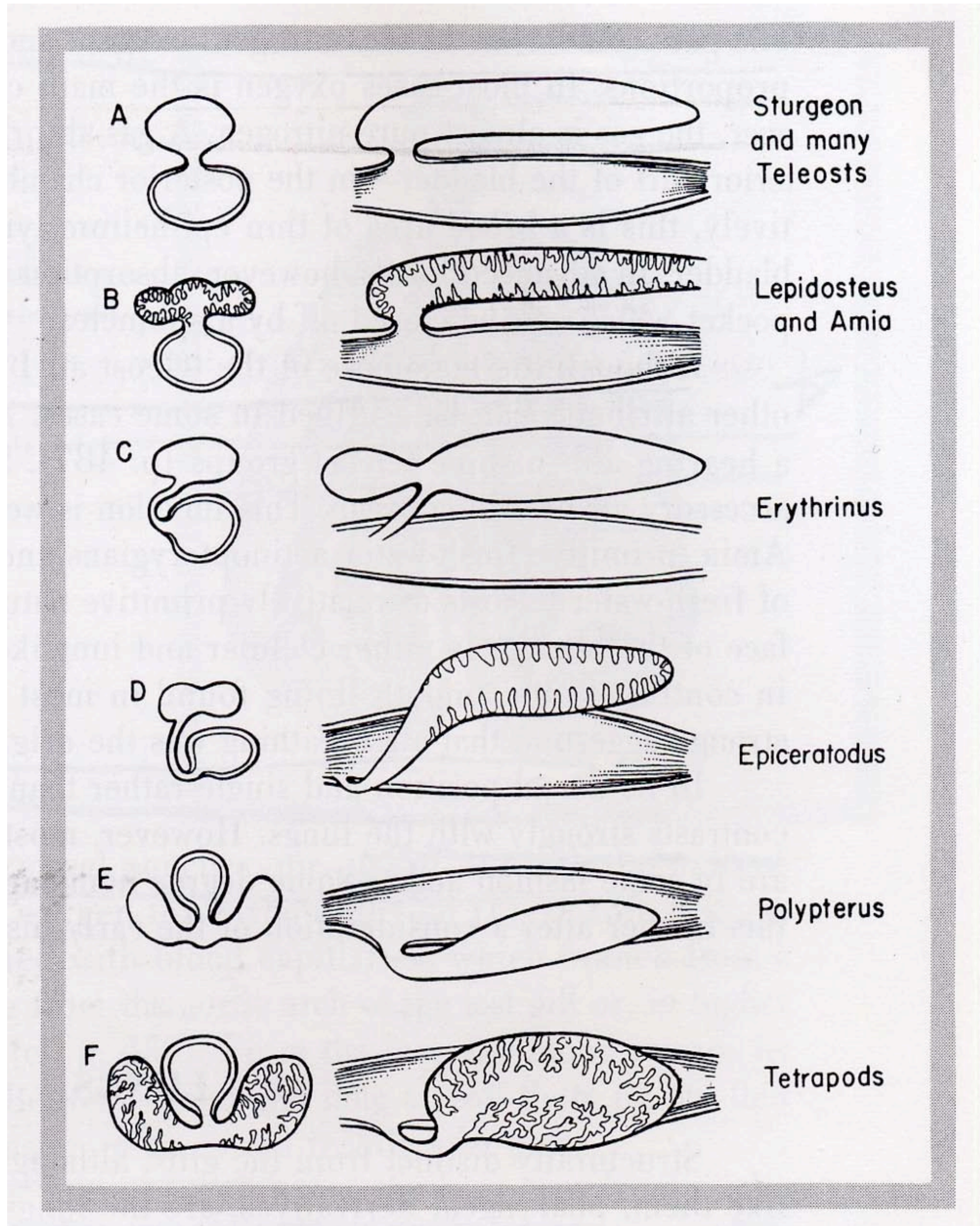
所詮 vitro...

この結果で

in vivo の現象を

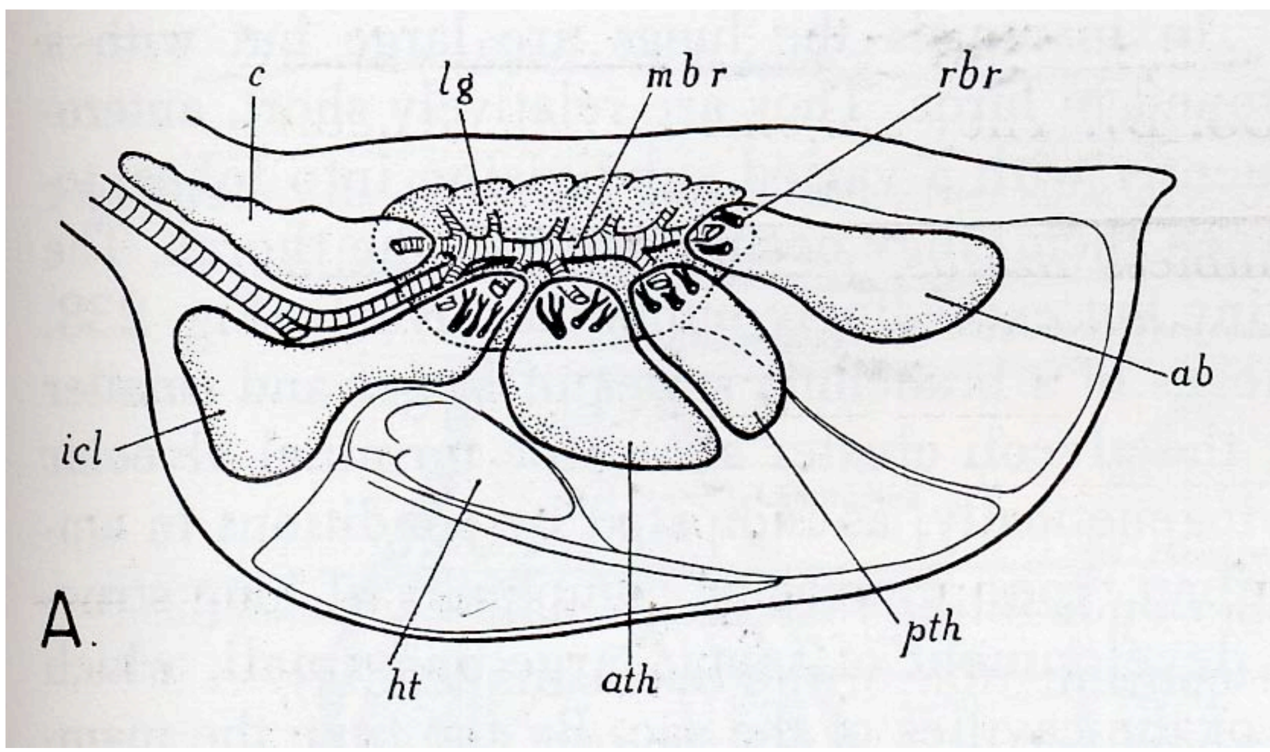
説明できないか？

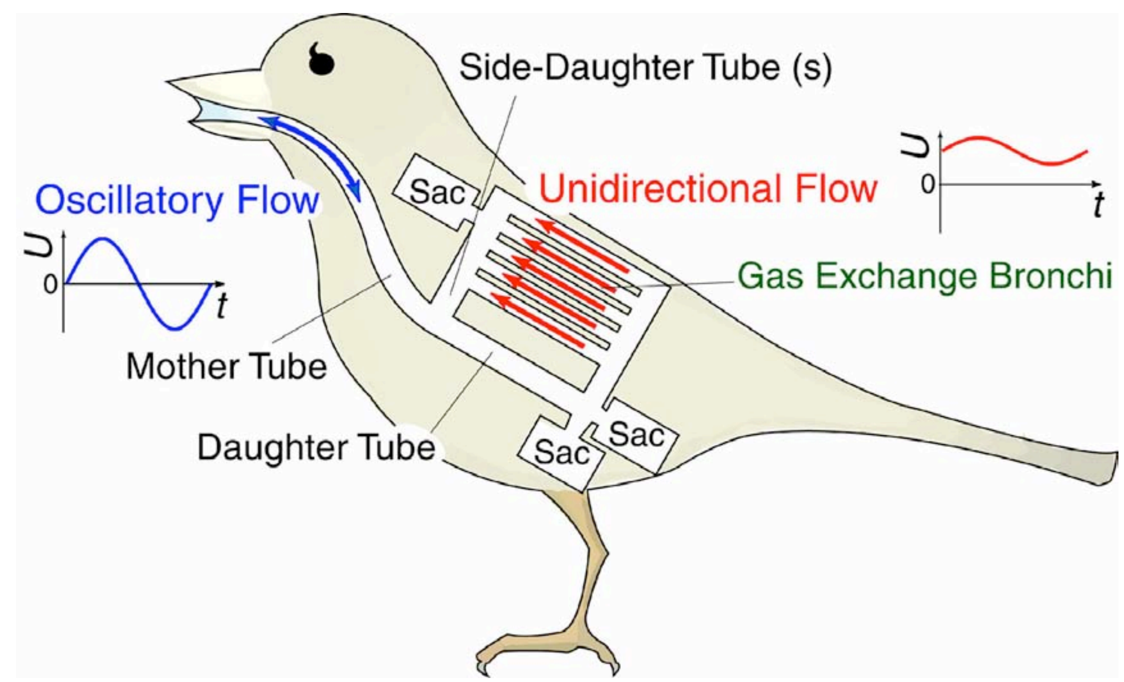
肺は浮き袋と相同



Romer “The Vertebrate Body” (1962)

鳥類肺の構造：
背側は分岐、腹側は嚢胞

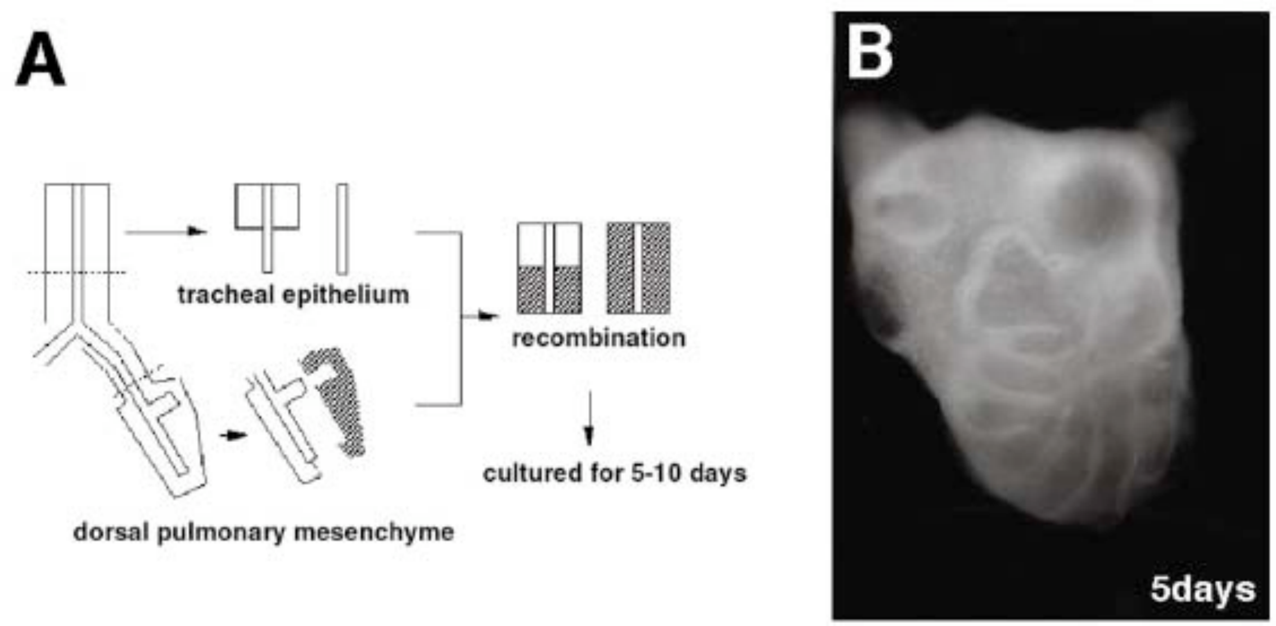


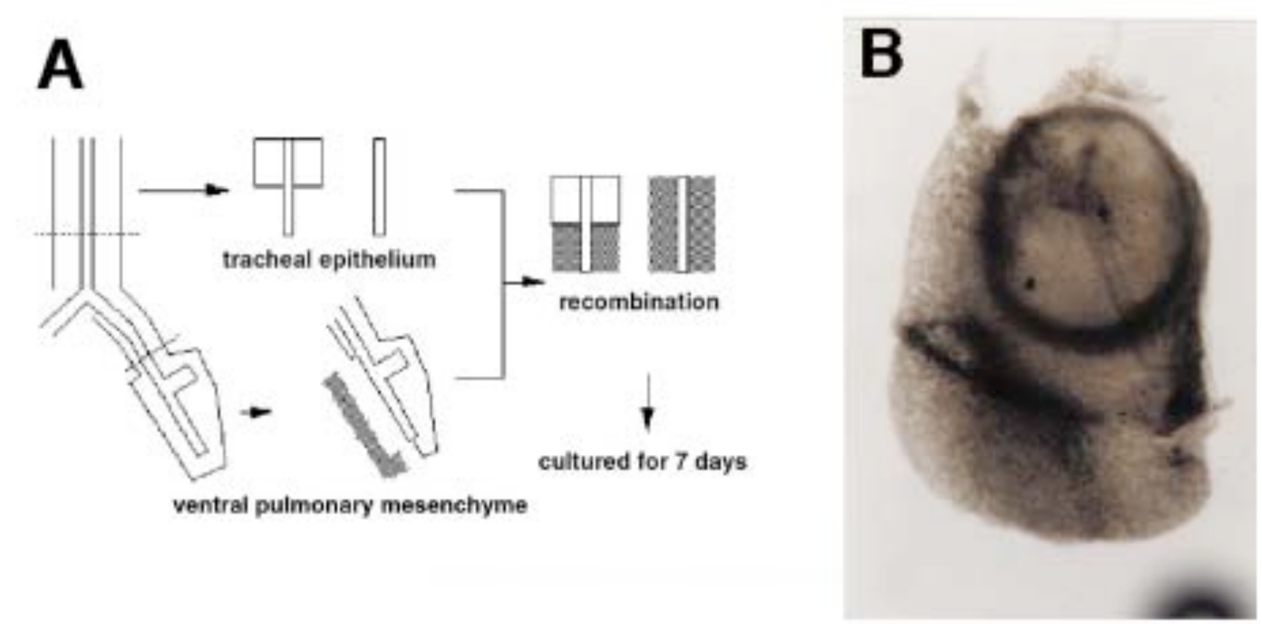


Romer “The Vertebrate Body” (1962)

構造の差は

間葉の性質から来る



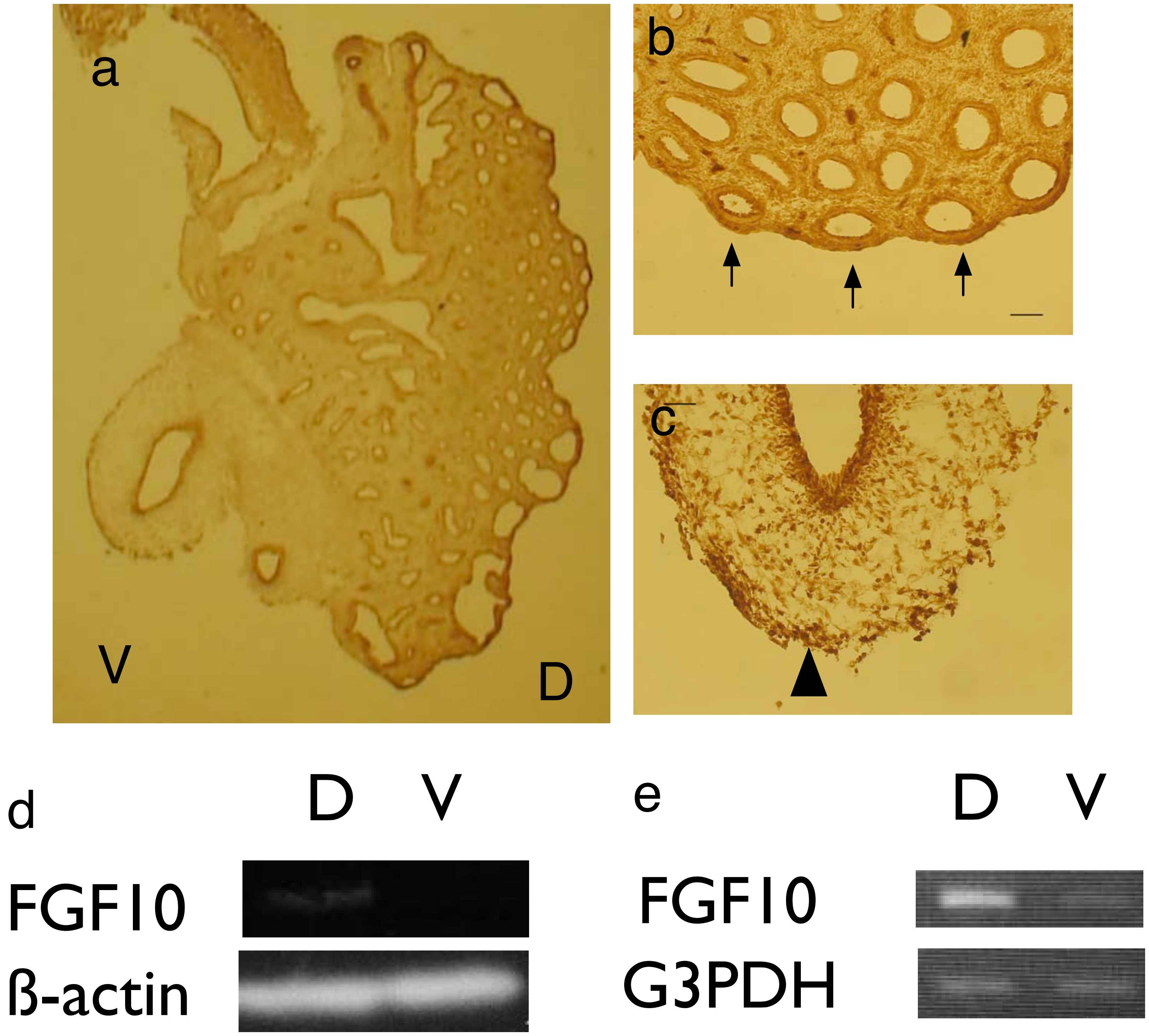


Sakiyama et al., Dev Biol, **2000**, *227*, 12-27

枝分れの代わりに囊胞を作るには？

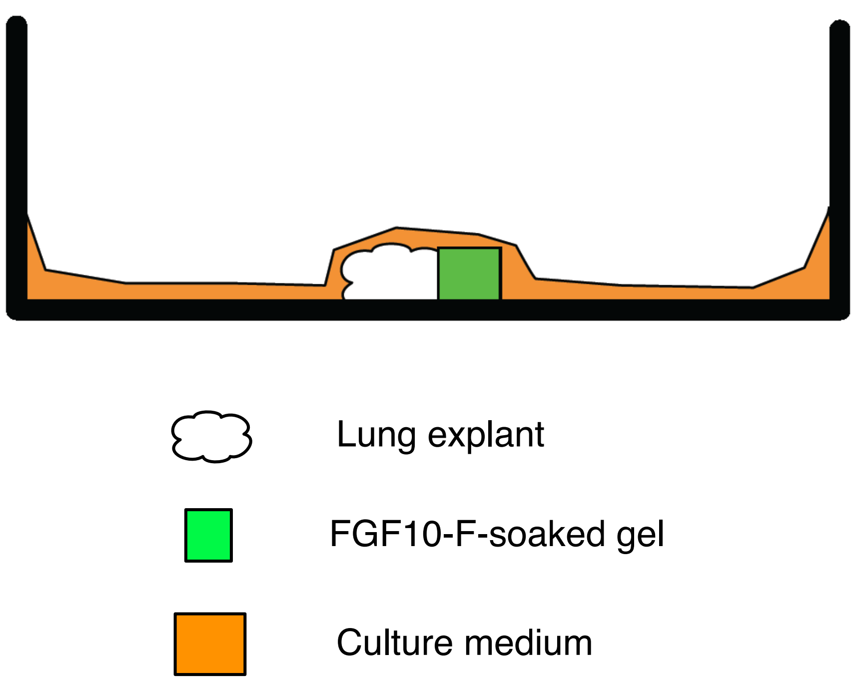
- FGFの濃度が非常に高い
- FGFの拡散係数が大きい

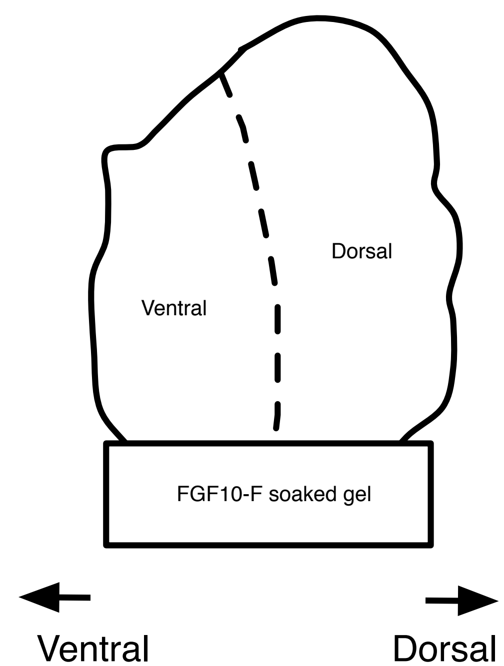
ニワトリ肺でのFGFの分布



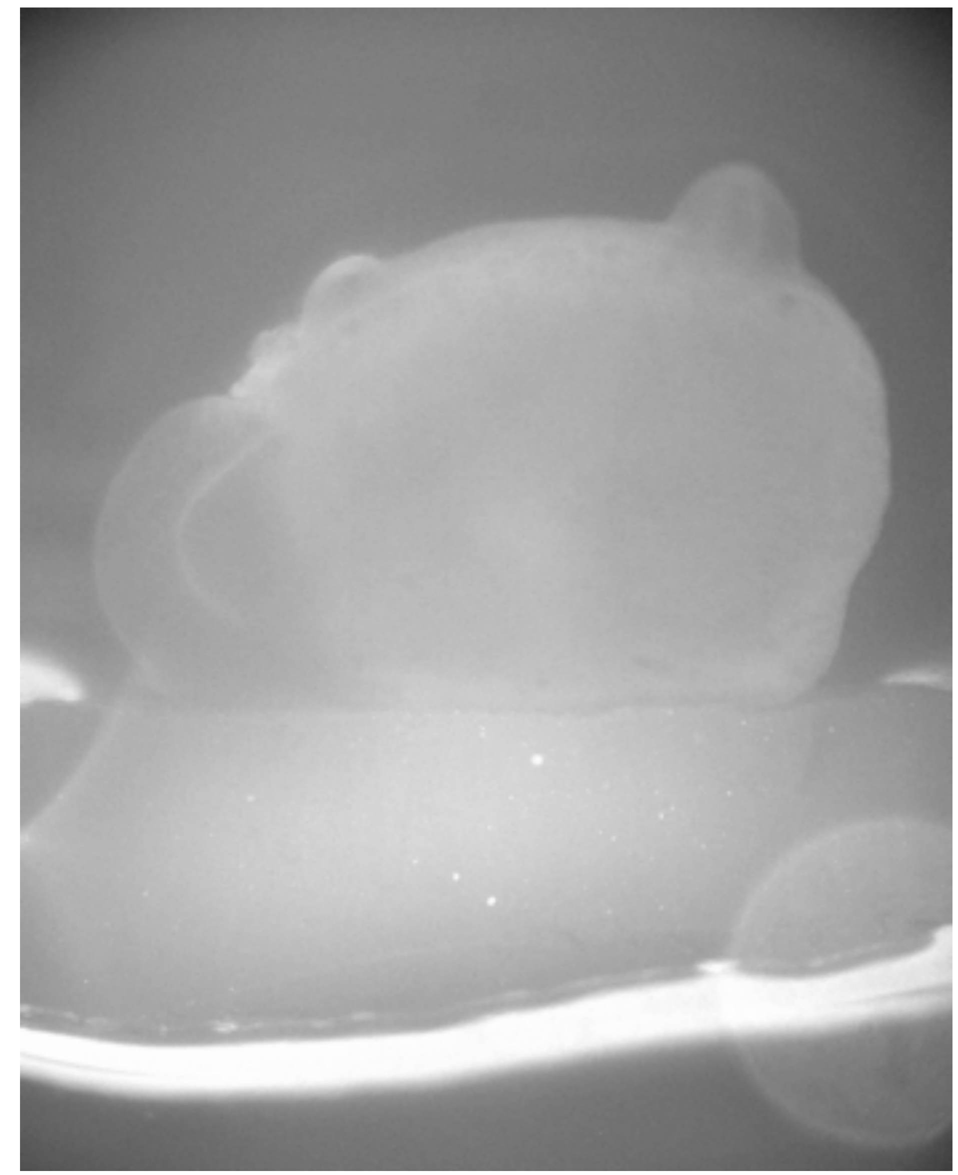
Miura et al., MOD 126, 160-(2009)

ニワトリ肺でのFGFの拡散





腹側　　　背側



Miura et al., MOD 126, 160-(2009)

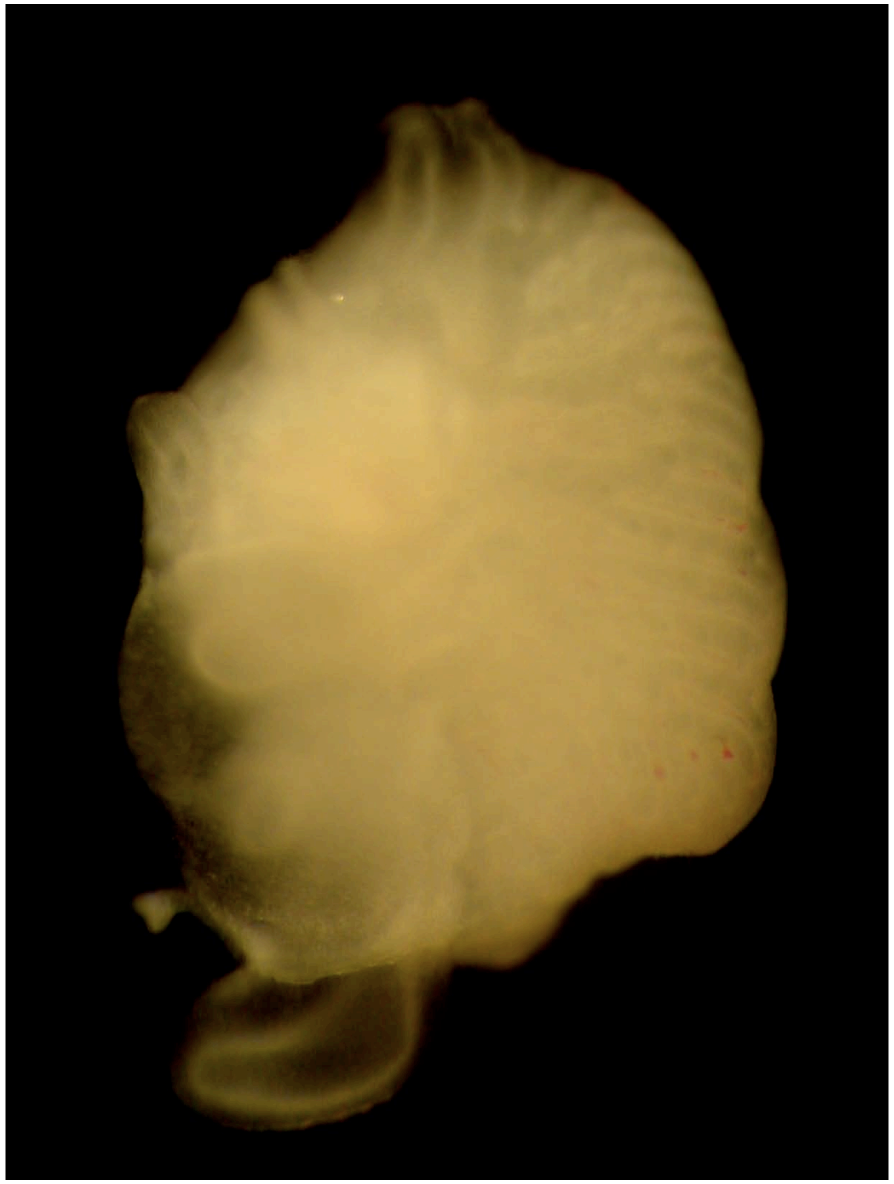
拡散が早いらしい

Miura et al., MOD 126, 160-(2009)

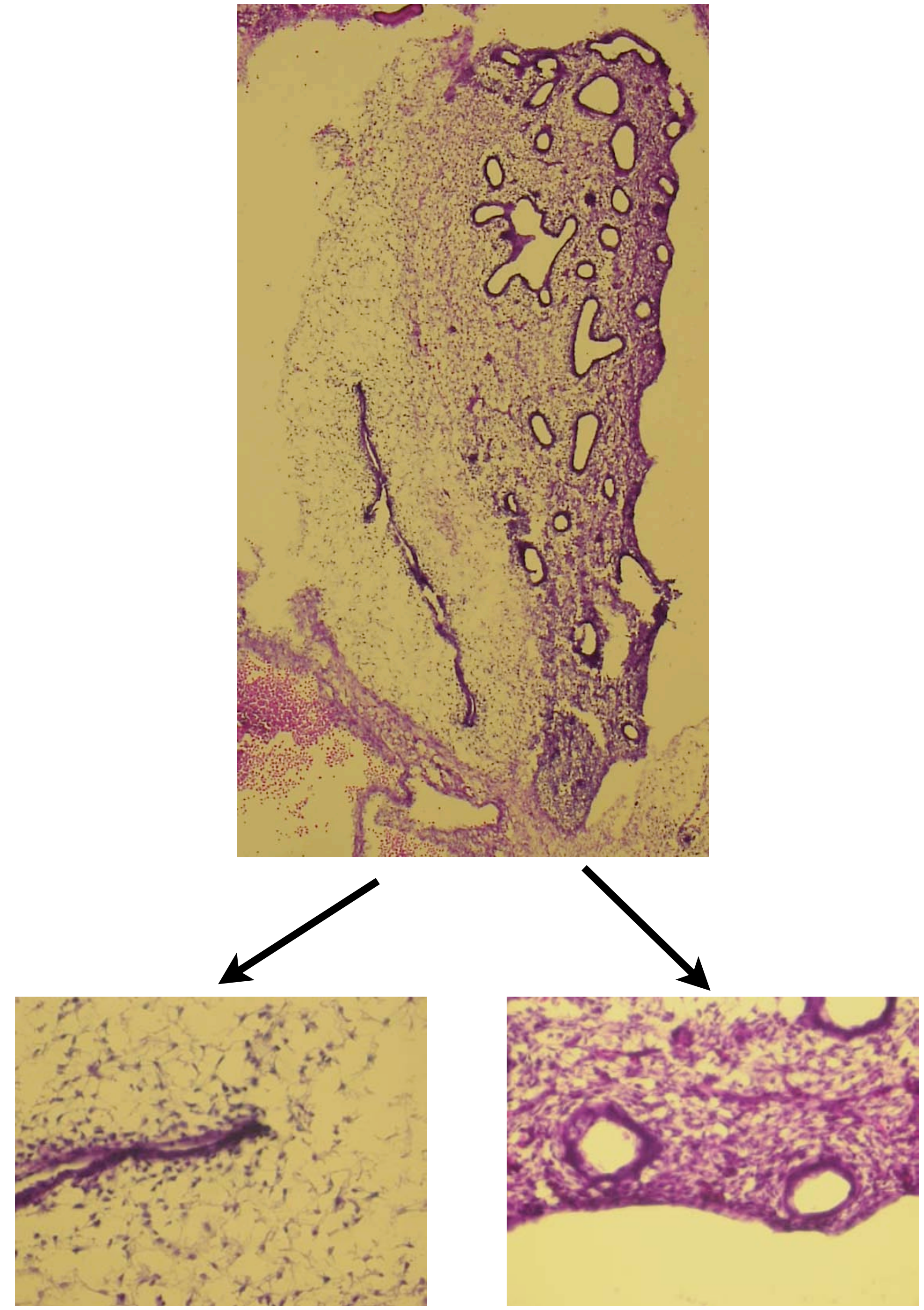
なぜ腹側で
拡散が早いのか？

Miura et al., MOD 126, 160-(2009)

組織構造



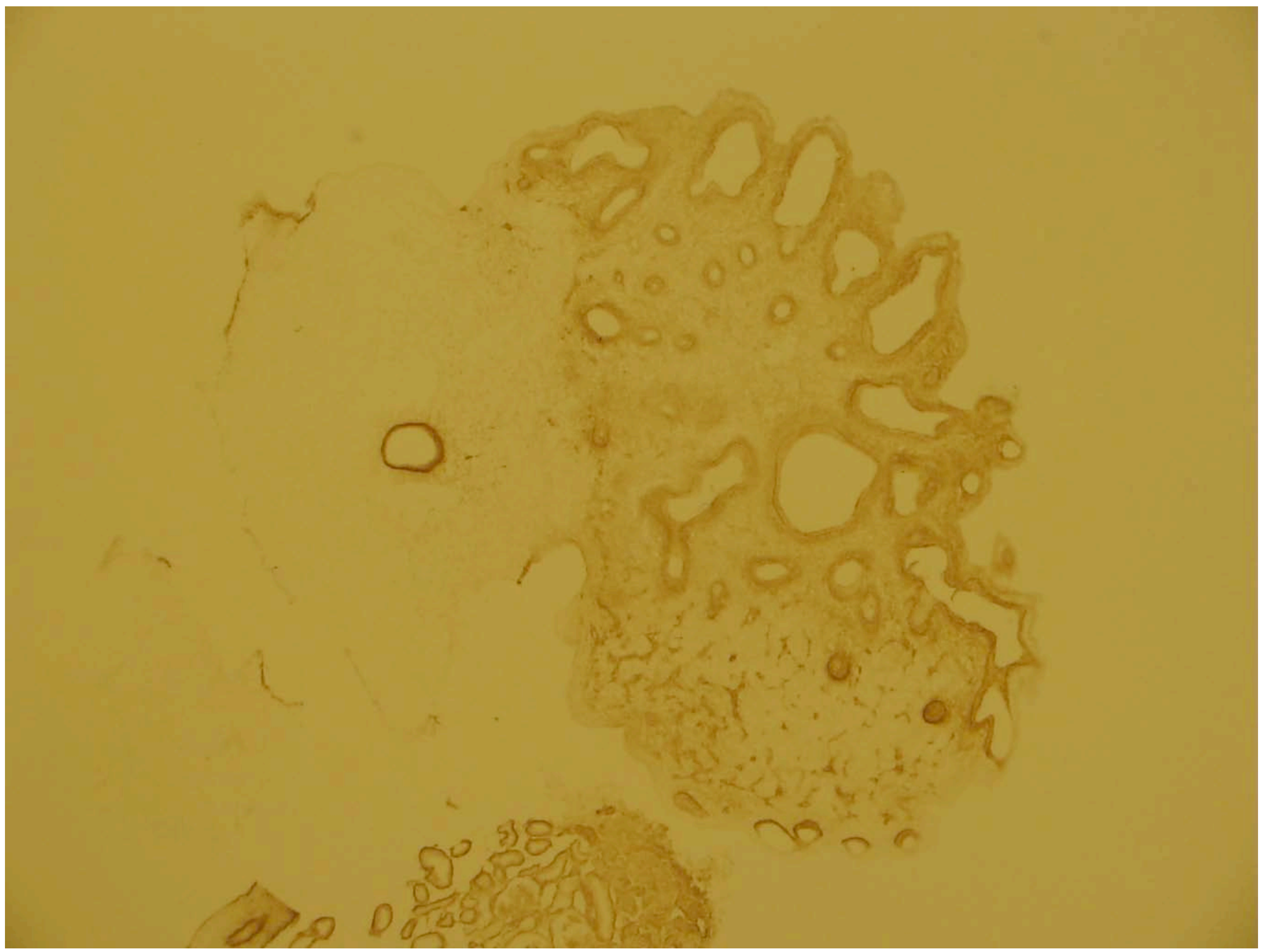
腹側　背側



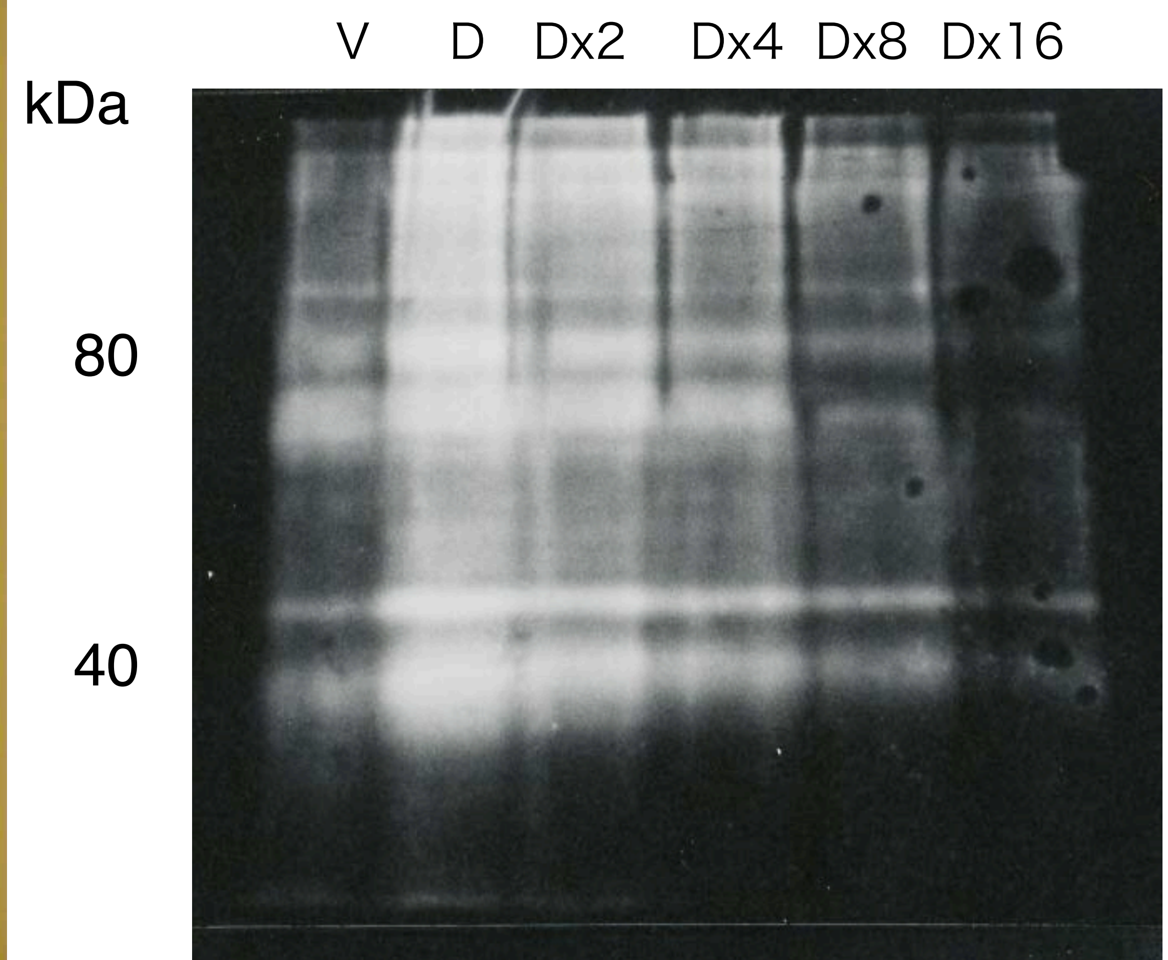
腹側　背側

Miura et al., MOD 126, 160-(2009)

細胞外基質：HSPG



腹側　　　　背側



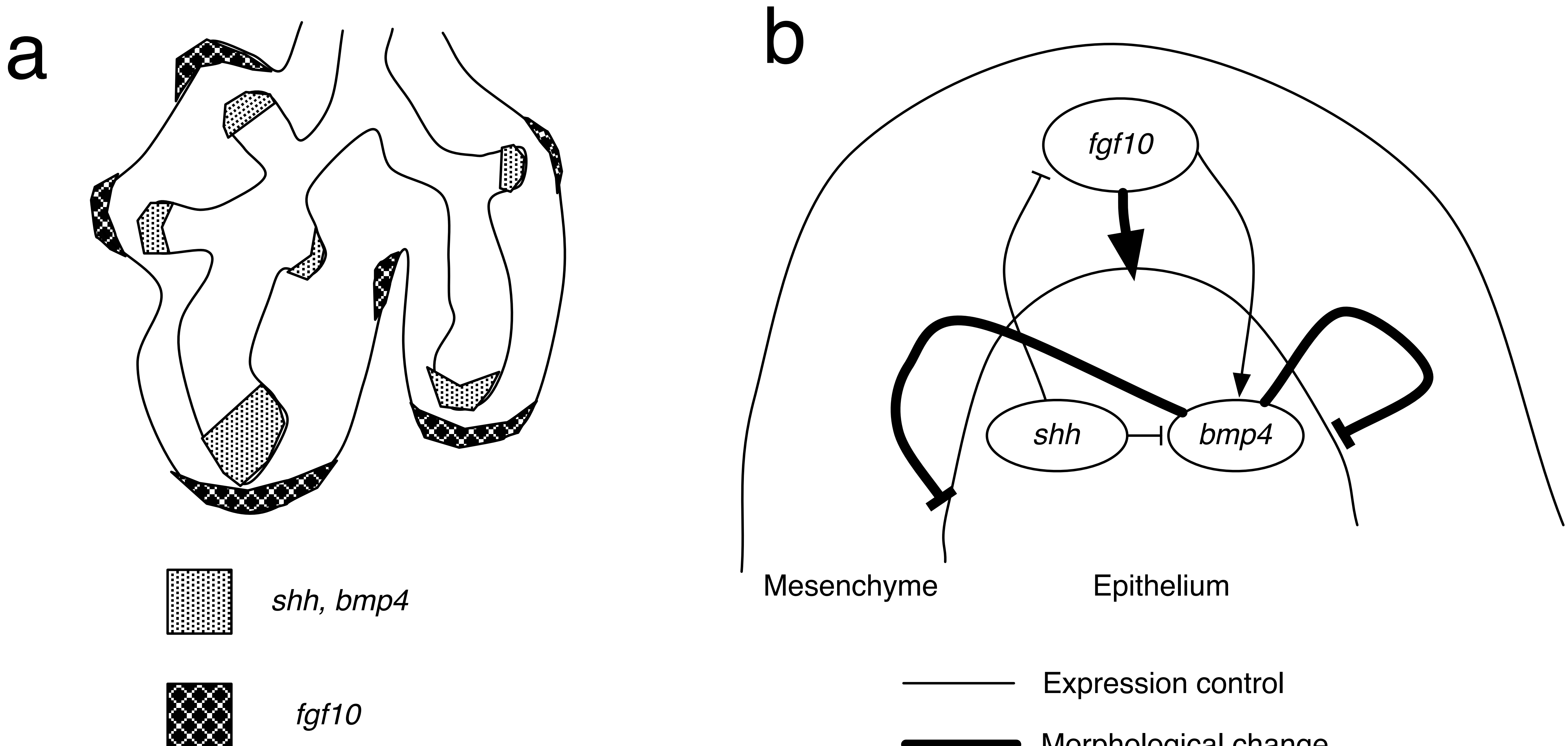
Miura et al., MOD 126, 160-(2009)

鳥類肺に生じる
嚢胞構造の生成機構を
in vitroのモデルの結果を
外挿して理解した。

Miura et al., MOD 126, 160-(2009)

まだすっきりしない。

In vivo の枝分れのメカニズムは？



Miura T., Curr Top Dev Biol 81, 291- (2008)