iPS細胞からつくる腎臓オルガノイド研究

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ヒト器官形成研究チーム

28/9/2021 Glycoforum

オルガノイドとは?

Organoid = Organ + oid (臓器もどき)

一般的な定義:組織幹細胞が自己組織化して形成される3次元組織

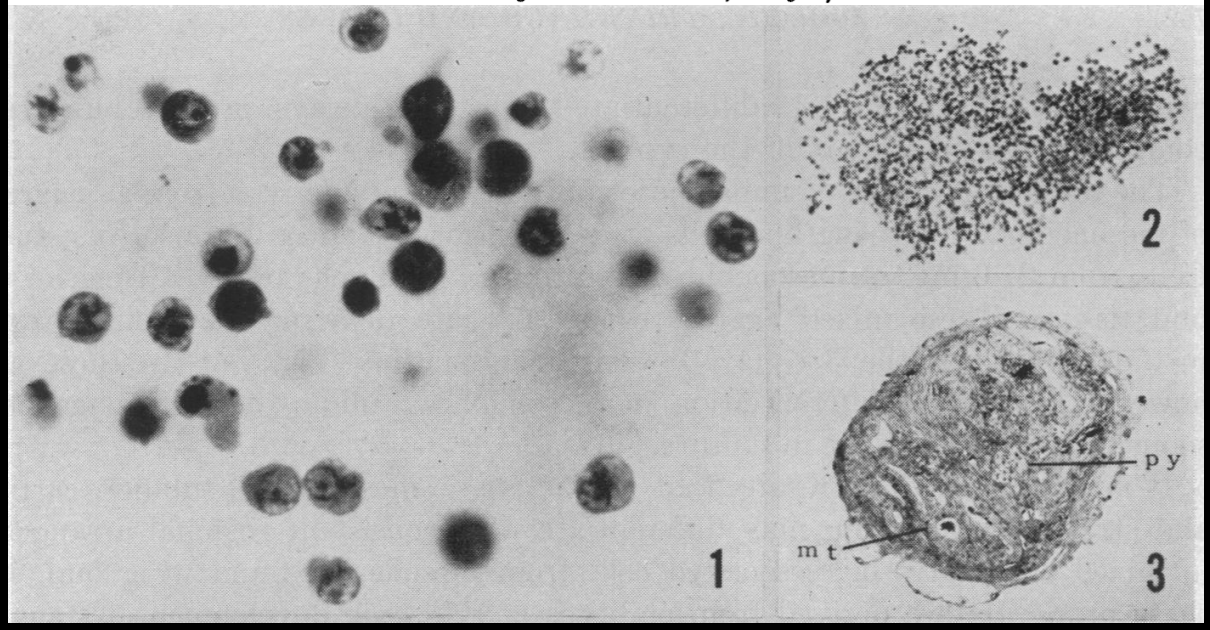
元々は、胎仔組織細胞の解離再集合体

*DIFFERENTIATION IN CULTURE OF MIXED AGGREGATES OF DISSOCIATED TISSUE CELLS**

By J. P. Trinkaus and Peggy W. Groves

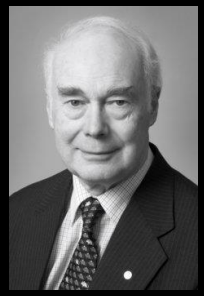
Osborn Zoological Laboratory, Yale University

Communicated by J. S. Nicholas, July 1, 1955



(Trinkaus et al., *PNAS* 1955)

Embryoid body
（胚様体）



Sir Martin J. Evans

Establishment in culture of pluripotential cells from mouse embryos

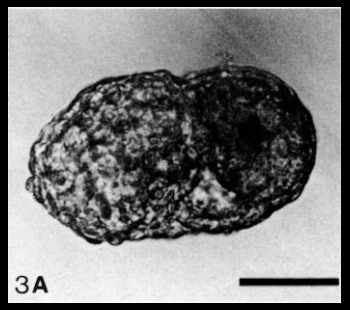
M. J. Evans* & M. H. Kaufman†

Departments of Genetics* and Anatomy†, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK

Pluripotential cells are present in a mouse embryo until at least an early post-implantation stage, as shown by their ability to take part in the formation of chimaeric animals[1] and to form teratocarcinomas[2]. Until now it has not been possible to establish progressively growing cultures of these cells *in vitro*, and cell lines have only been obtained after teratocarcinoma formation *in vivo*. We report here the establishment in tissue culture of pluripotent cell lines which have been isolated directly from *in vitro* cultures of mouse blastocysts. These cells are able to differentiate either *in vitro* or after innoculation into a mouse as a tumour *in vivo*. They have a normal karyotype.

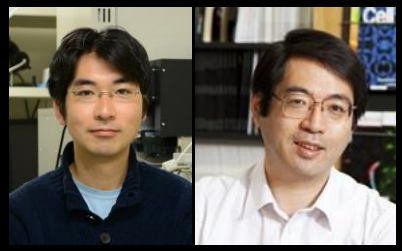
（Evans et al., *Nature* 1981）

胚様体



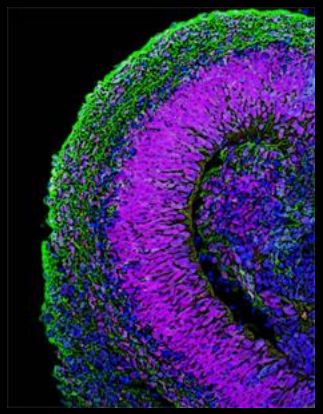
（Doetschman et al., *Development* 1985）

幹細胞由来のオルガノイド



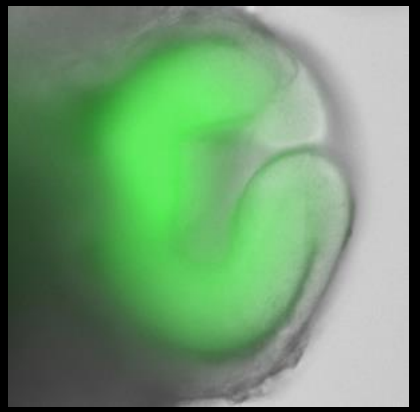
M. Eiraku　　Y. Sasai

自己組織化脳



（Eiraku et al., *Cell Stem Cell* 2008）

自己組織化眼杯



（Eiraku et al., *Nature* 2011）

オルガノイドは生命現象として興味深いだけでなく、応用利用にも期待されている。

オルガノイド技術の可能性

CellPress

Cell Stem Cell

Short Article

Zika Virus Depletes Neural Progenitors in Human Cerebral Organoids through Activation of the Innate Immune Receptor TLR3

Jason Dang,[1,2,6] Shashi Kant Tiwari,[1,6] Gianluigi Lichinchi,[1,3] Yue Qin,[1] Veena S. Patil,[1] Alexey M. Eroshkin,[4] and Tariq M. Rana[1,5,*]

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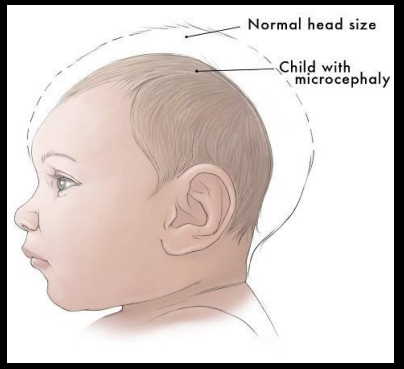
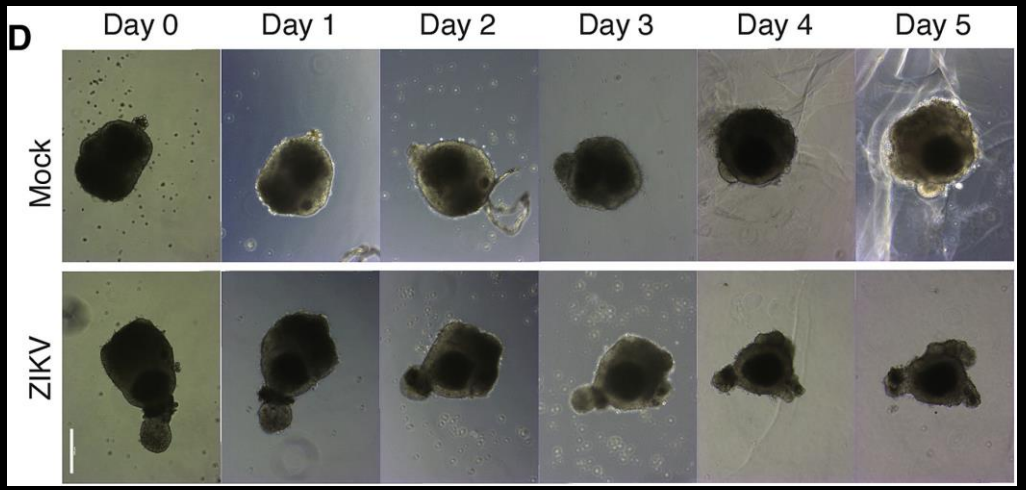
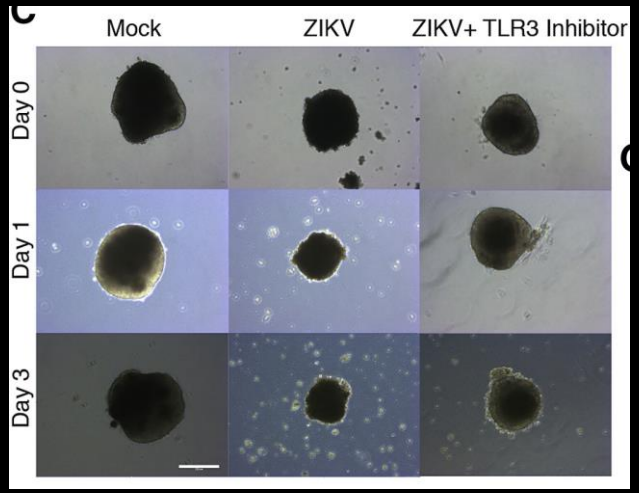
[2]Department of Bioengineering, University of California San Diego, La Jolla, CA 92093, USA
[3]Graduate School of Biomedical Sciences
[4]Bioinformatics core
Sanford Burnham Prebys Medical Discovery Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA
[5]Institute for Genomic Medicine, University of California San Diego, La Jolla, CA 92093, USA
[6]Co-first author
*Correspondence: trana@ucsd.edu
http://dx.doi.org/10.1016/j.stem.2016.04.014



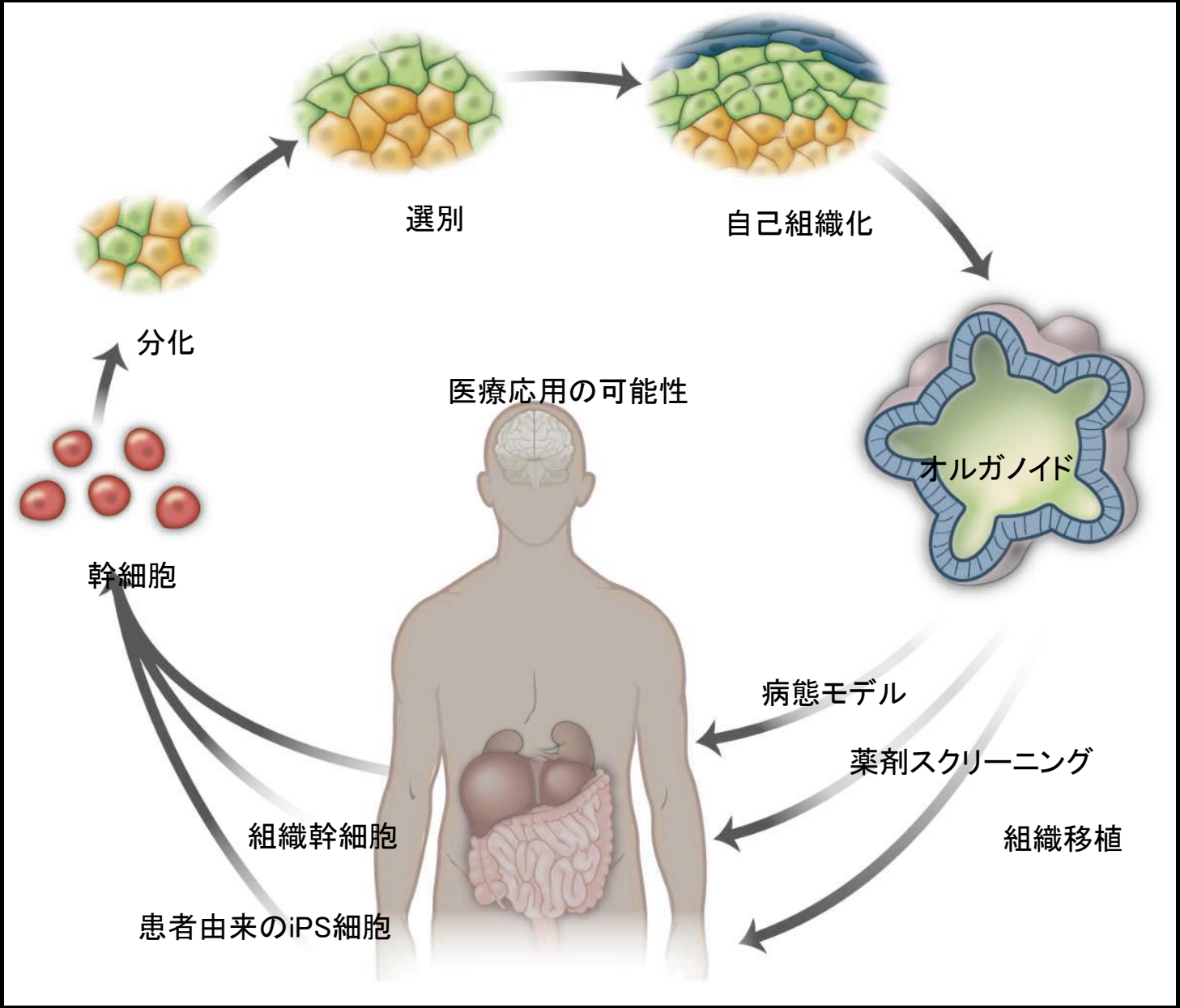
妊娠中に蚊の媒介するジカウィルスに感染すると、出生児に小頭症が発症するリスクが高まる。





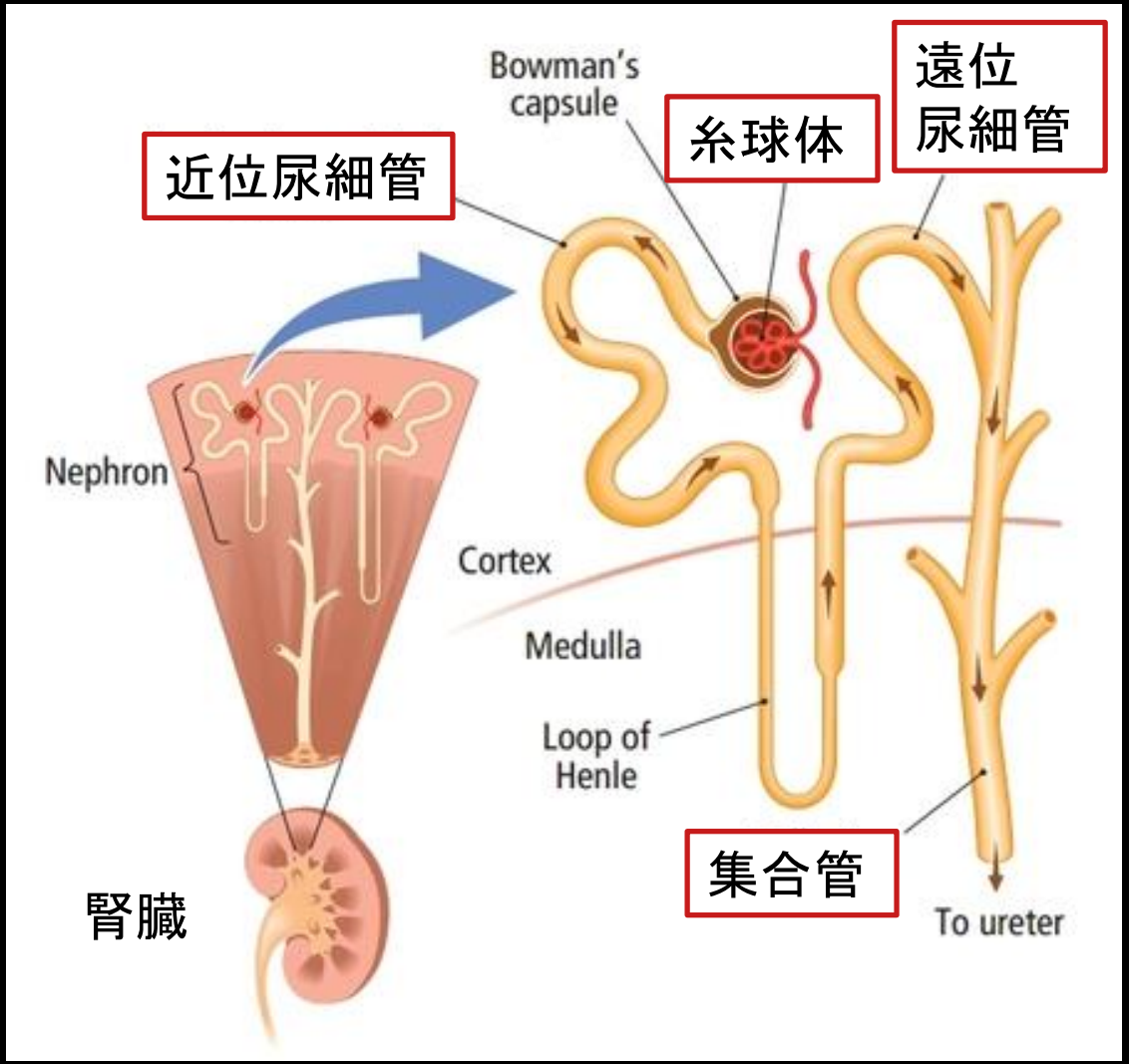
（J. Dong et al., *Cell Stem Cell* 2016）

オルガノイド技術の可能性

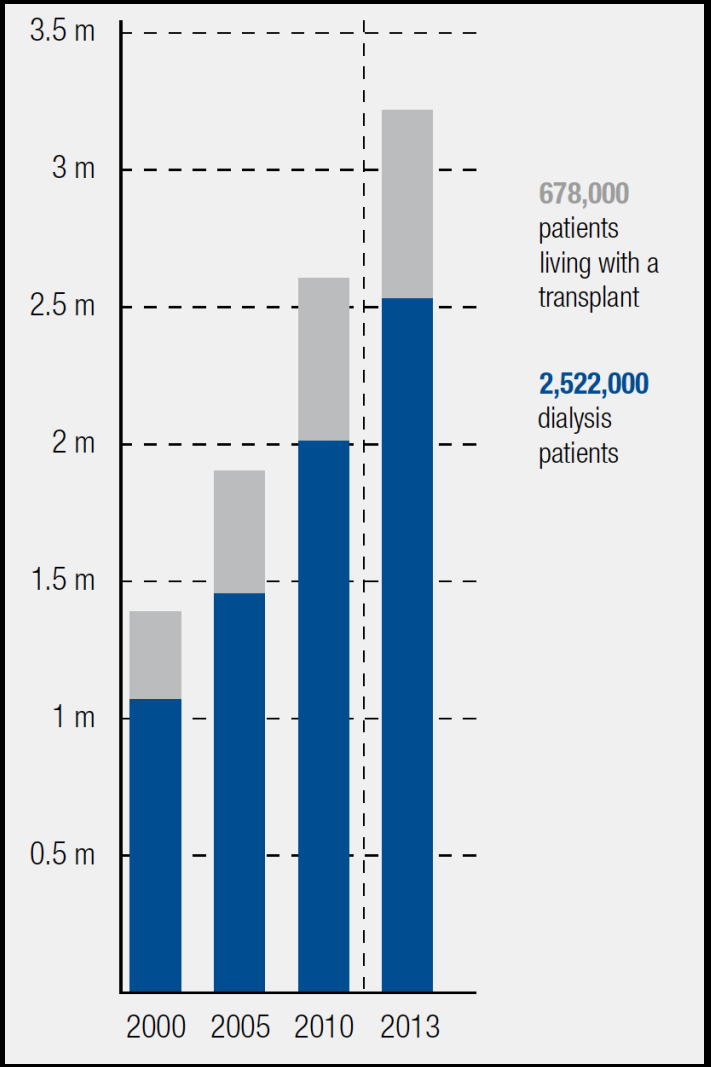


（Lancaster and Knoblich, Science 2014より改変）

末期腎不全患者数は毎年6～7%ずつ増加

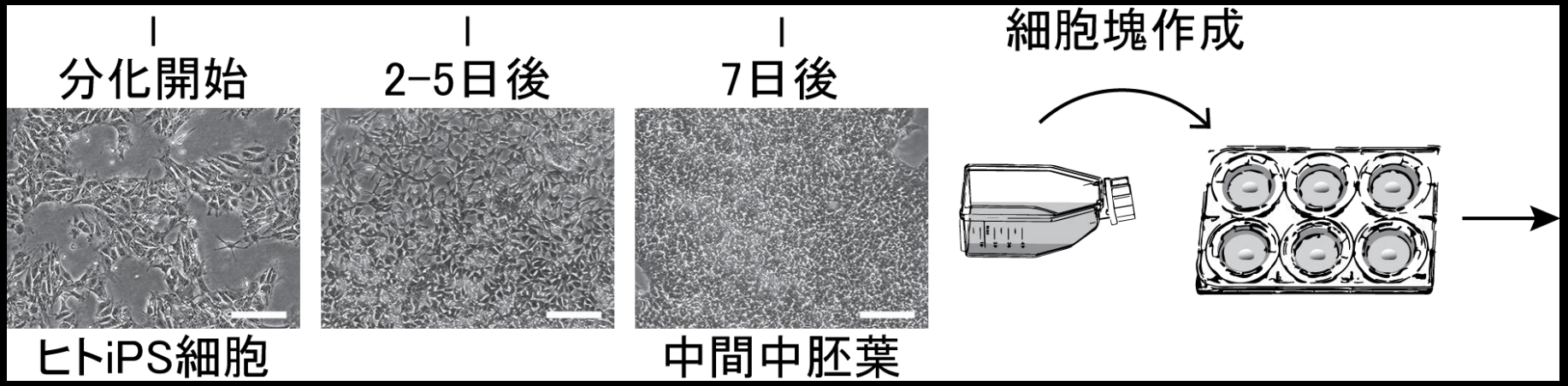


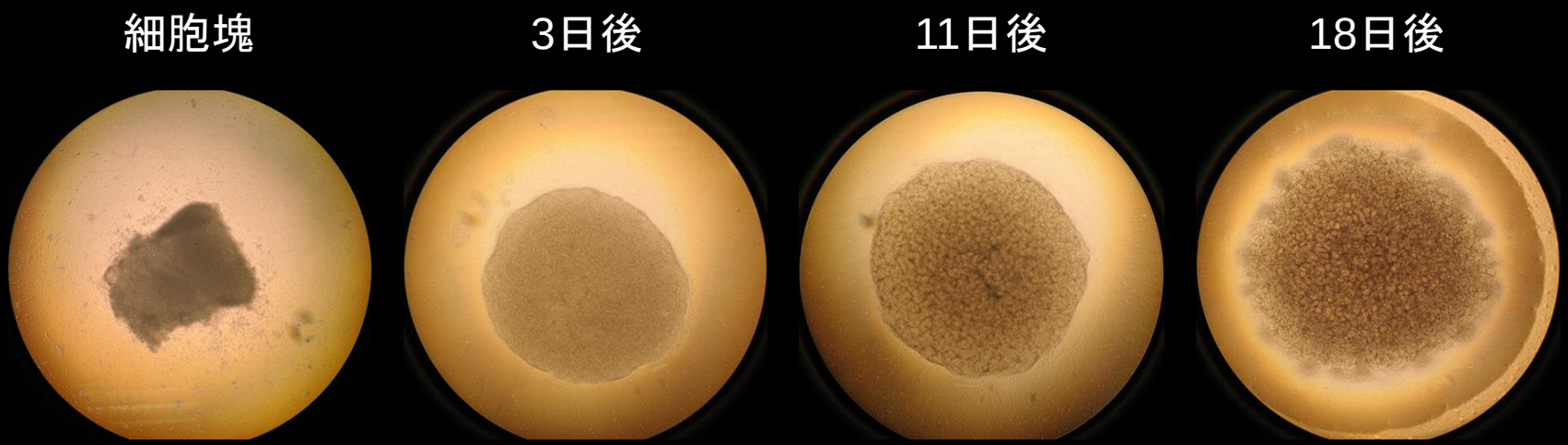
leavingcertbiology.net (Chapter 37: The Human Urinary System) より改変



(Fresenius Medical Care Deutschland. 2014)

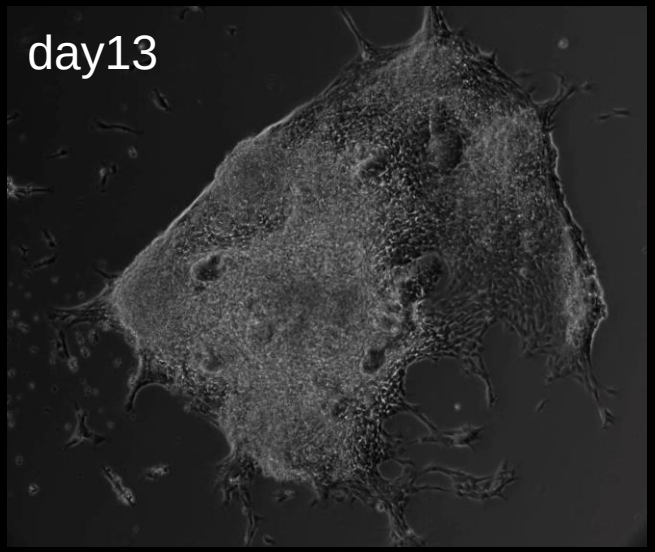
3次元培養による腎臓オルガノイドの作成

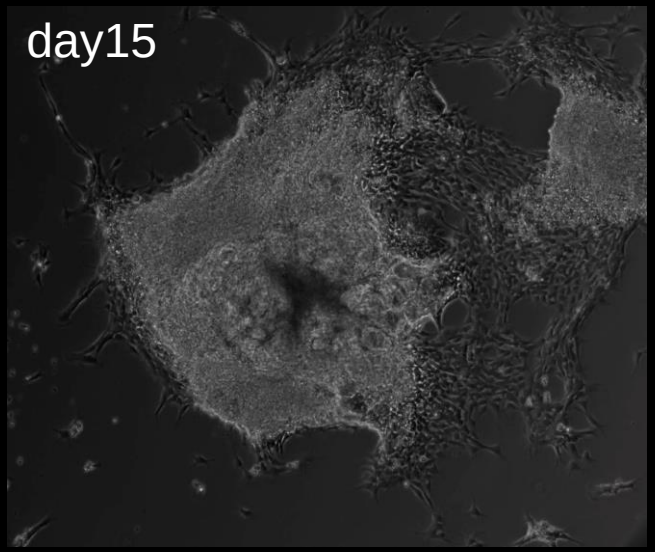


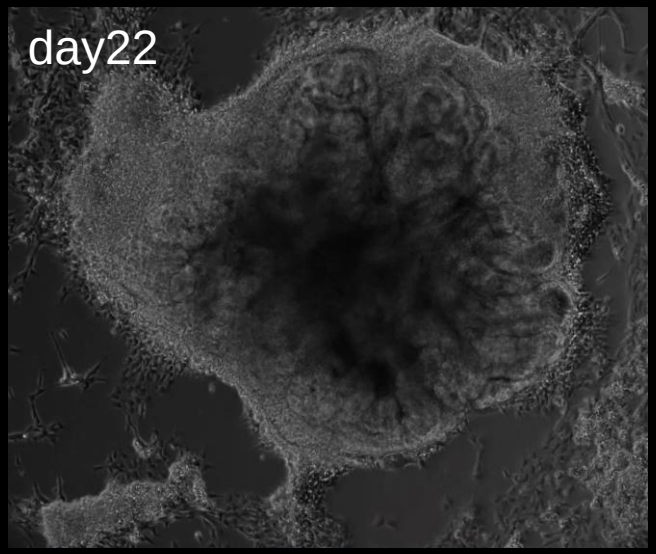


(Takasato et al., *Nature* 2015)

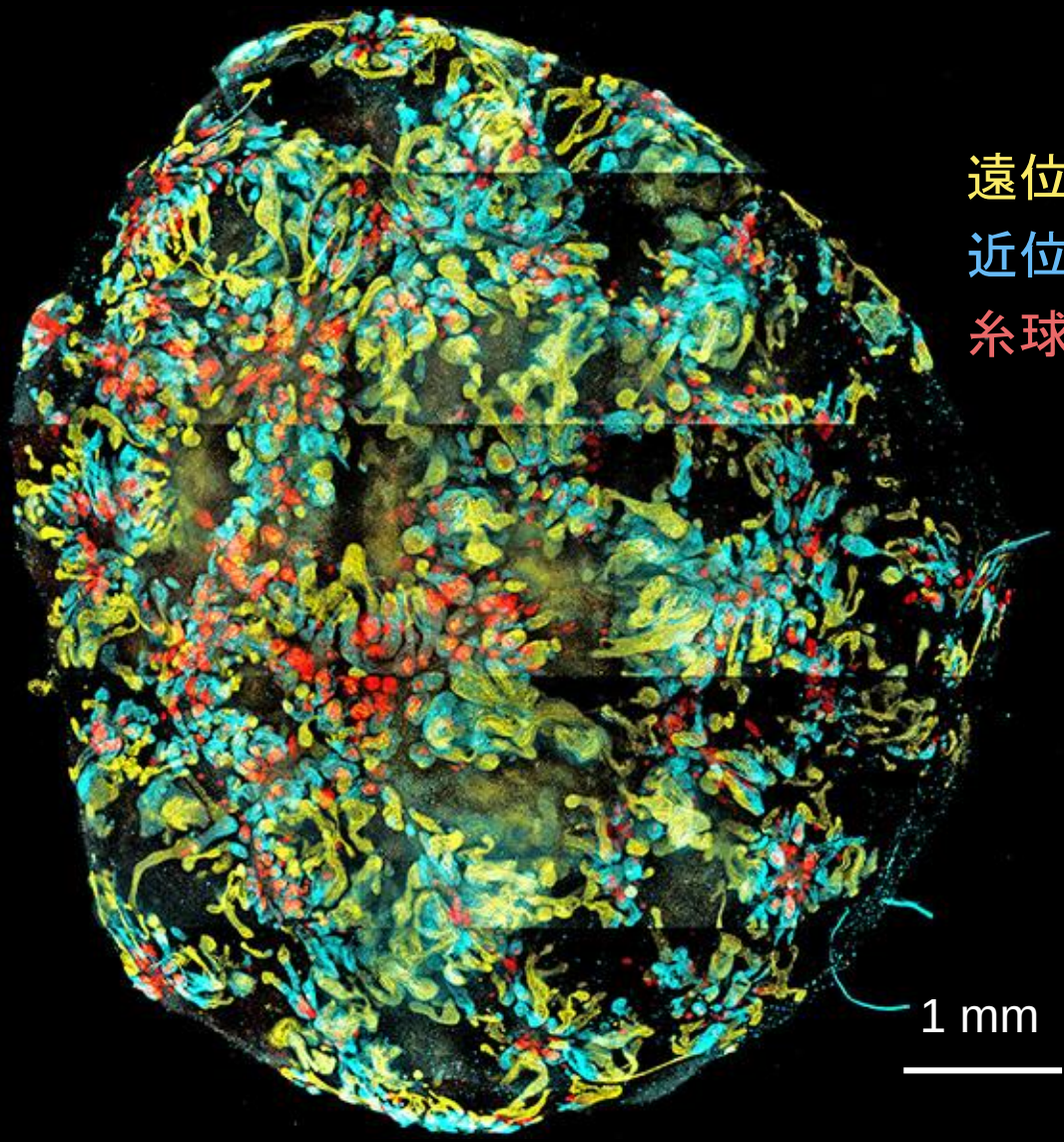
腎臓オルガノイド形成時の経時観察







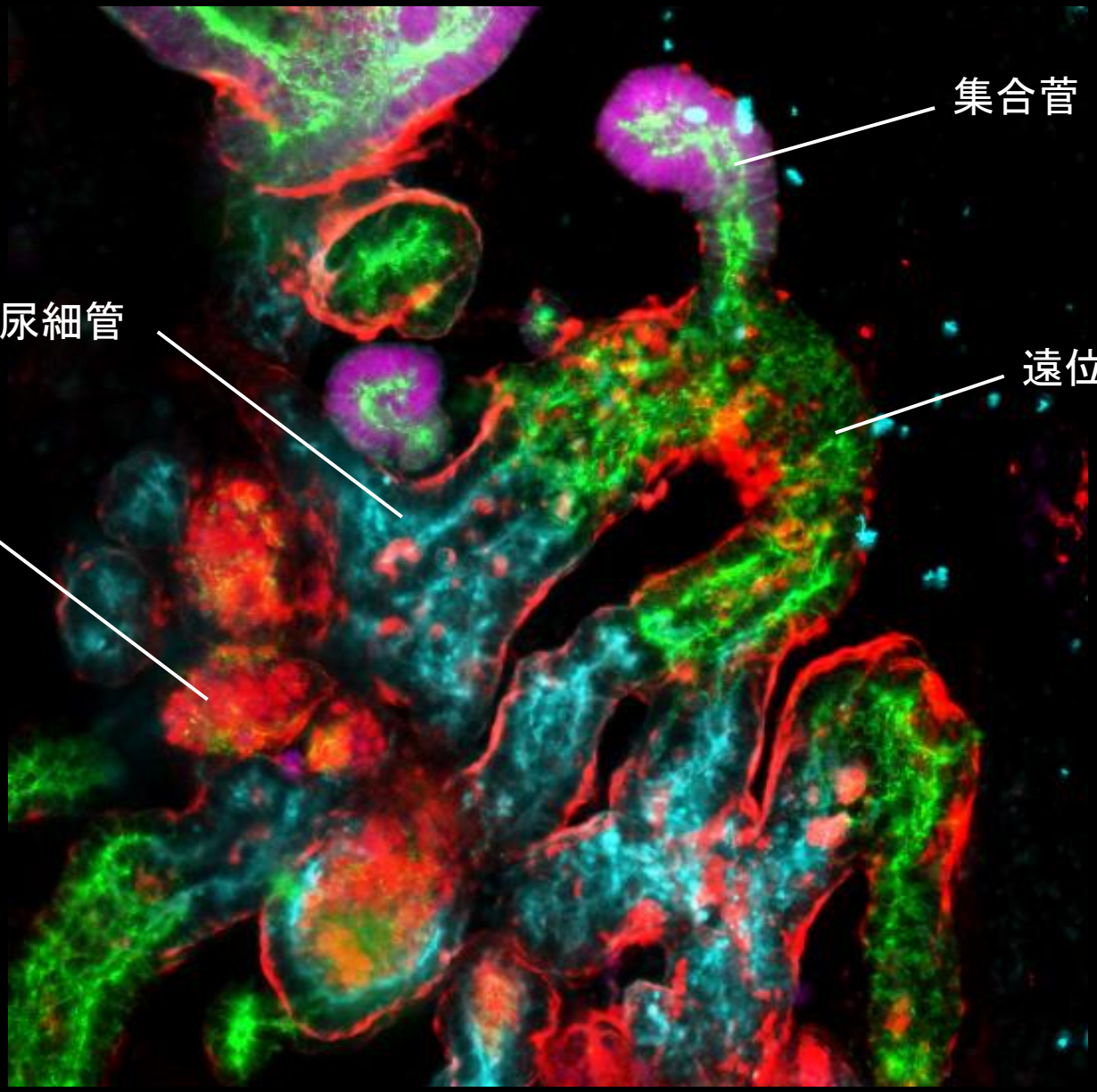
腎臓オルガノイド内には多数のネフロンが発生している



(Takasato et al., *Nature* 2015)

自己組織化されたネフロンは各部位を持つ

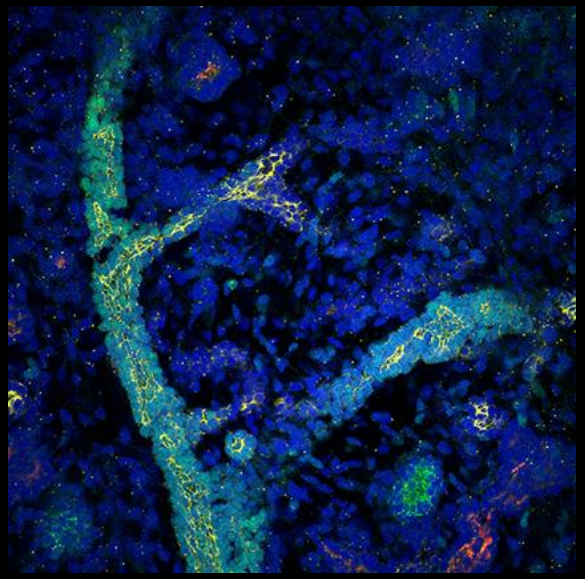
GATA3
ECAD
LTL
WT1



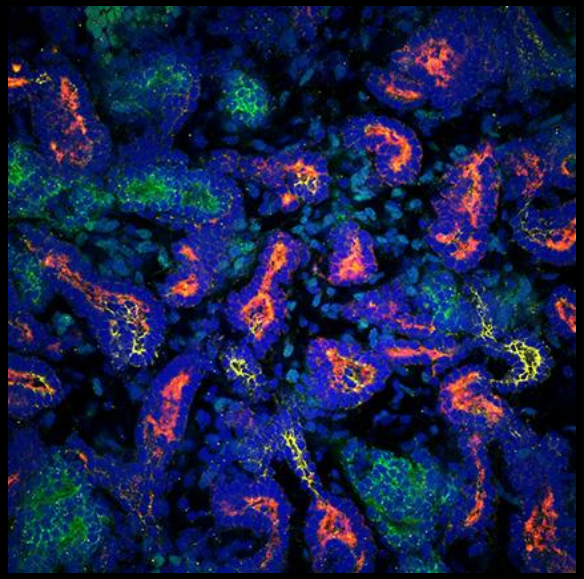
(Takasato et al., *Nature* 2015)

底部に集合管、中部に尿細管、上部に糸球体

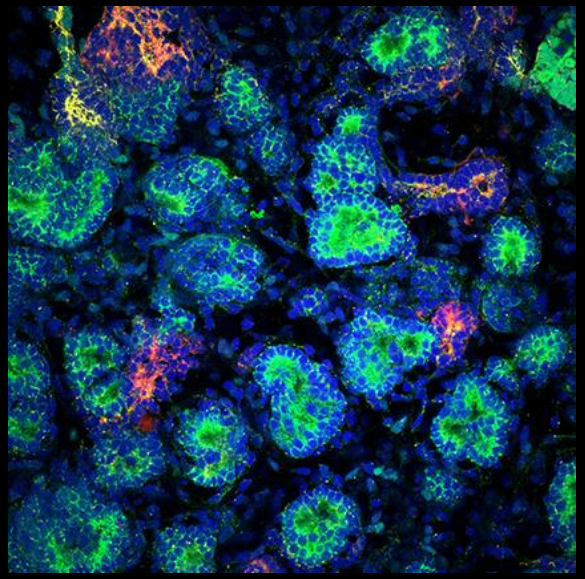
底部

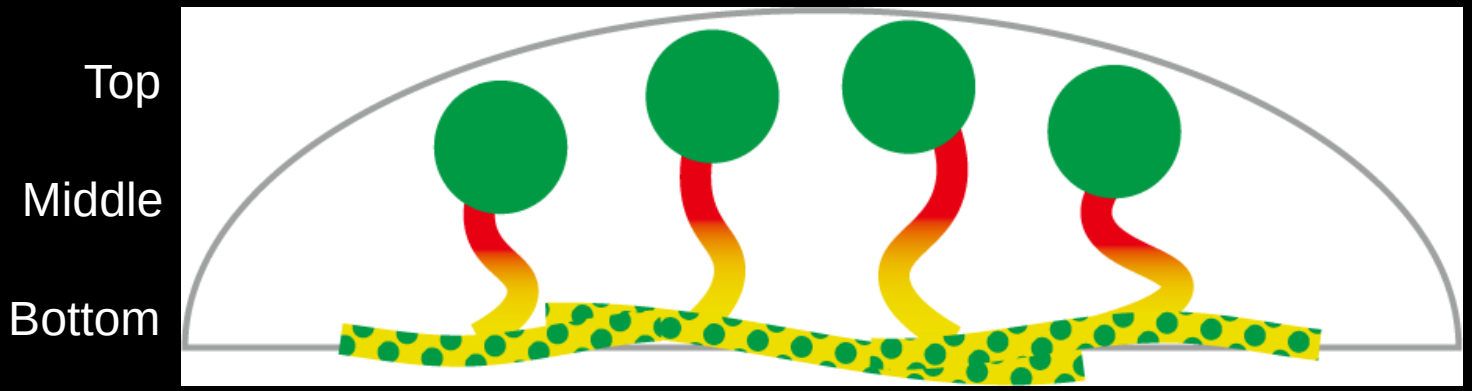


中部



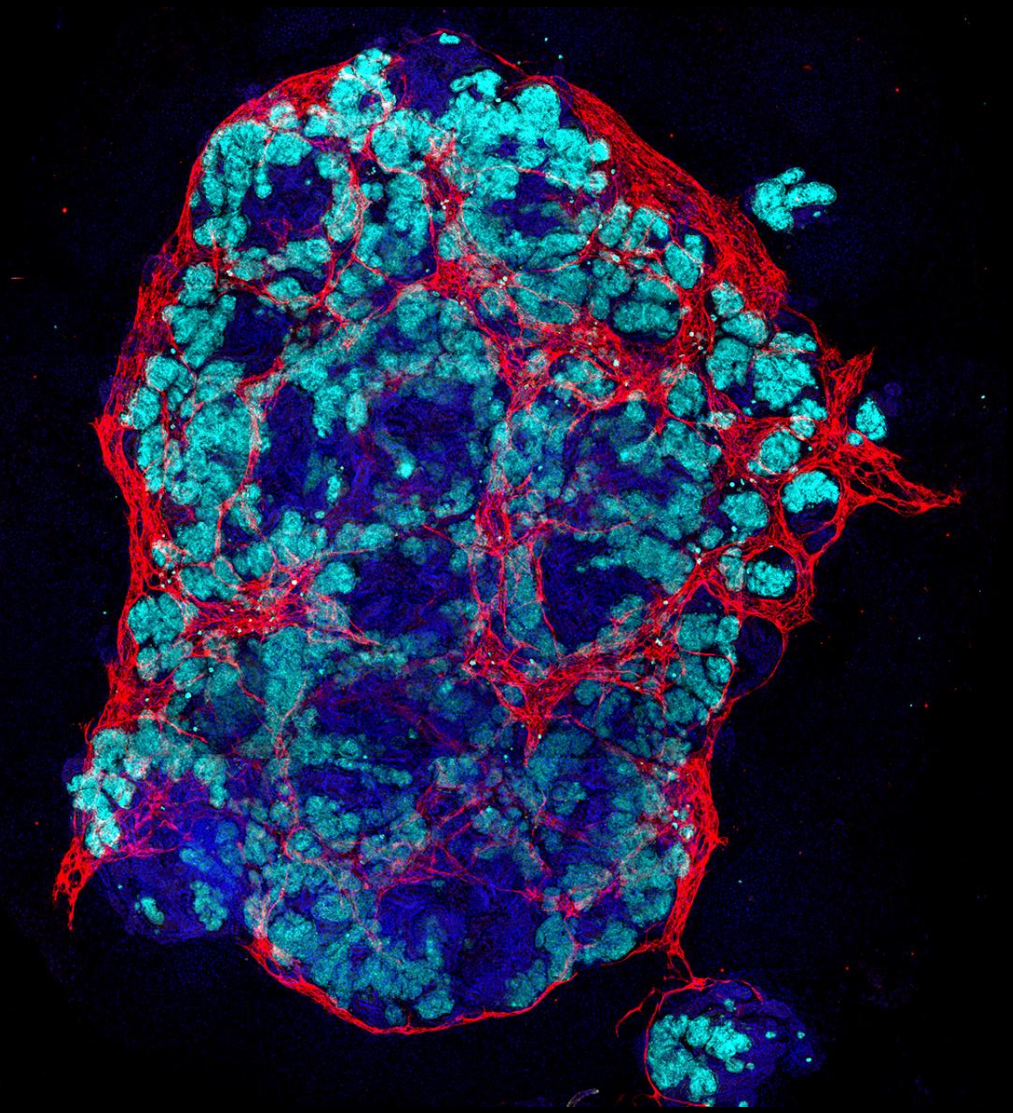
上部





(Takasato et al., *Nature* 2015)

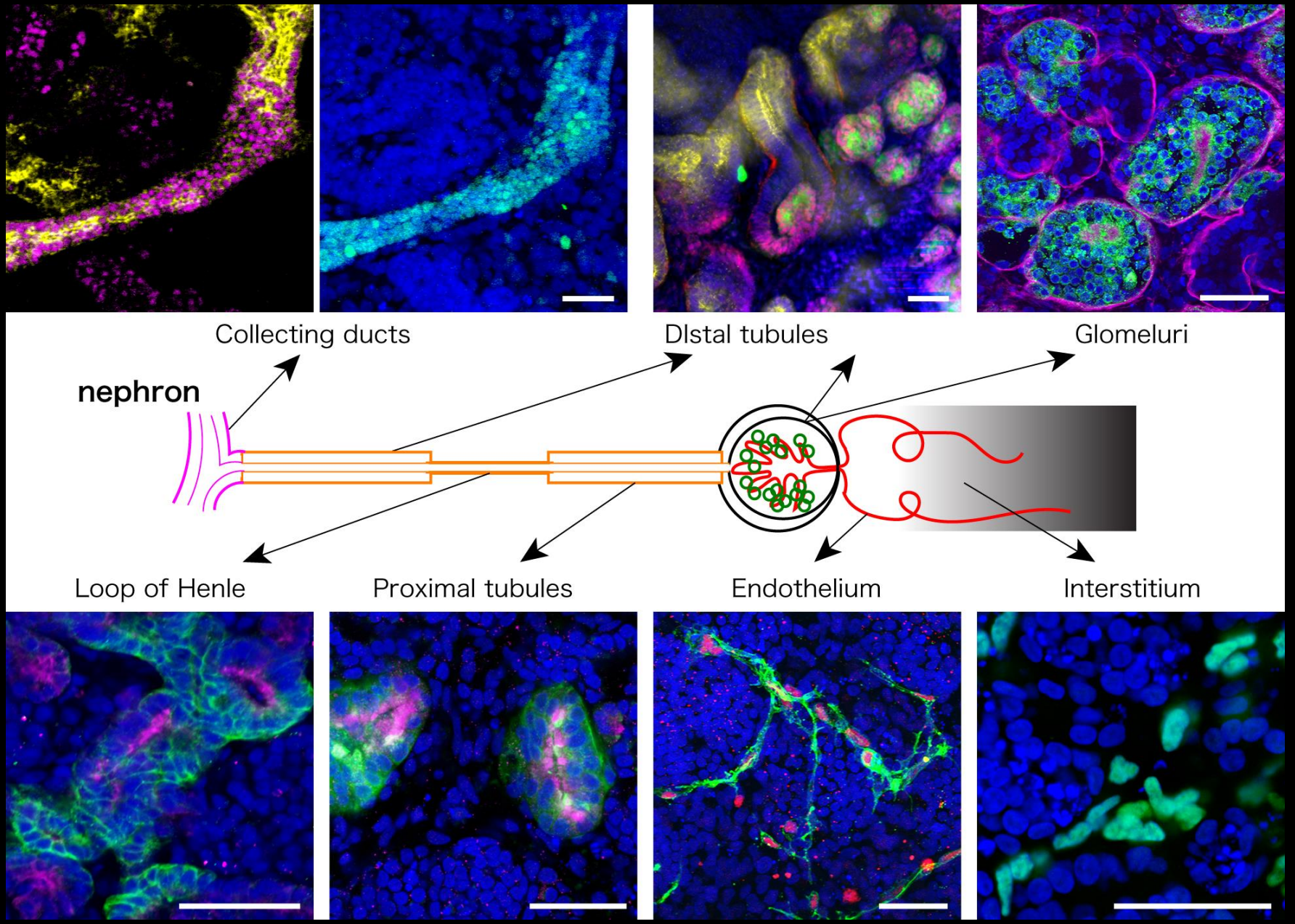
腎臓オルガノイドは血管網も有する



糸球体 血管網

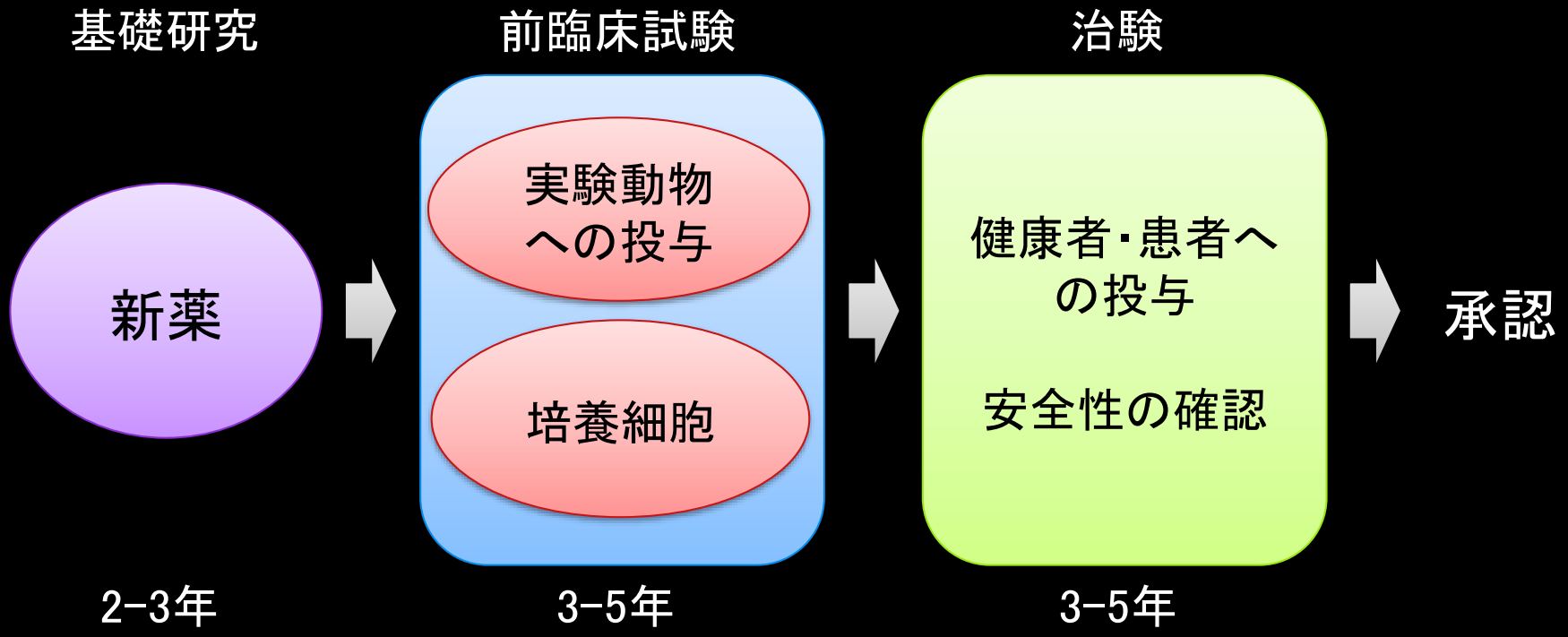
(Takasato et al., *Nature Protocols* 2016)

腎臓オルガノイドは全ての腎臓細胞を内包する



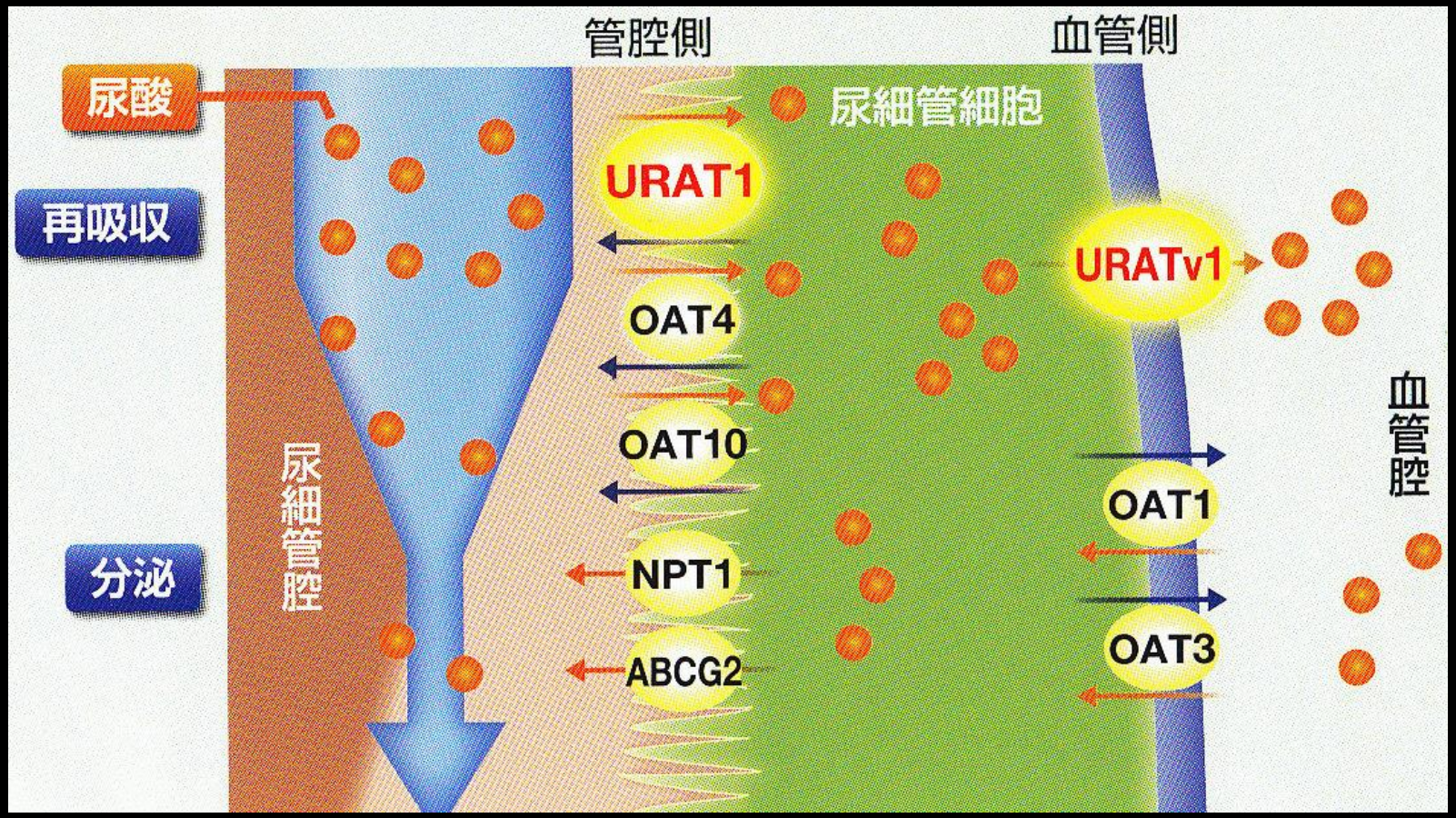
Scale = 100 μm

新薬開発時の腎毒性試験



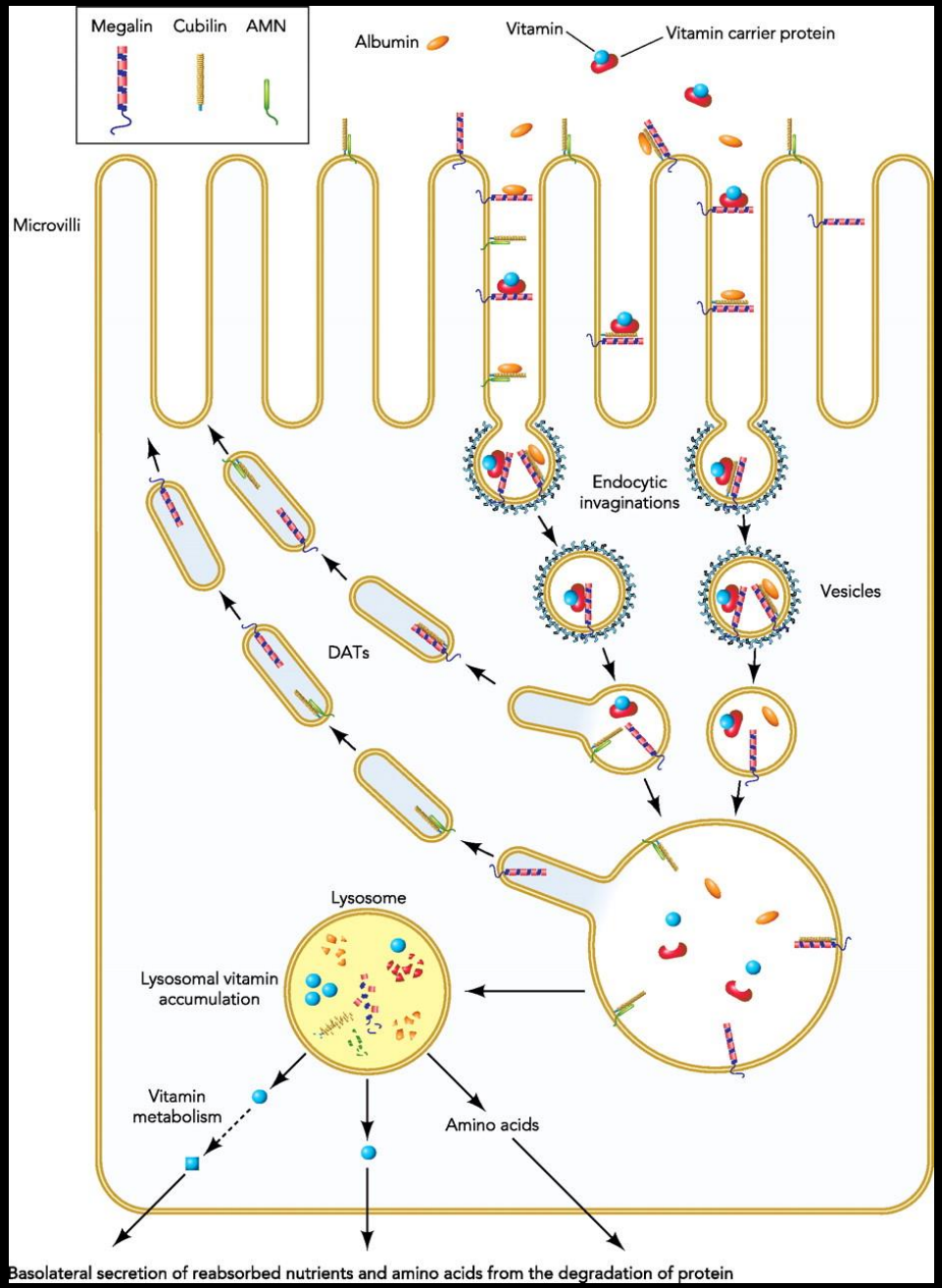
成功率：3万分の1
開発費用：数十から数百億円

尿細管の再吸収機能



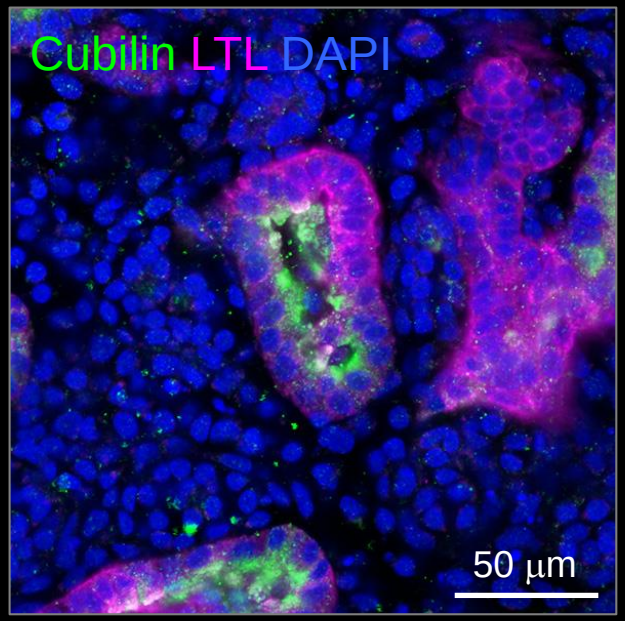
生理機能と薬物動態は一体である

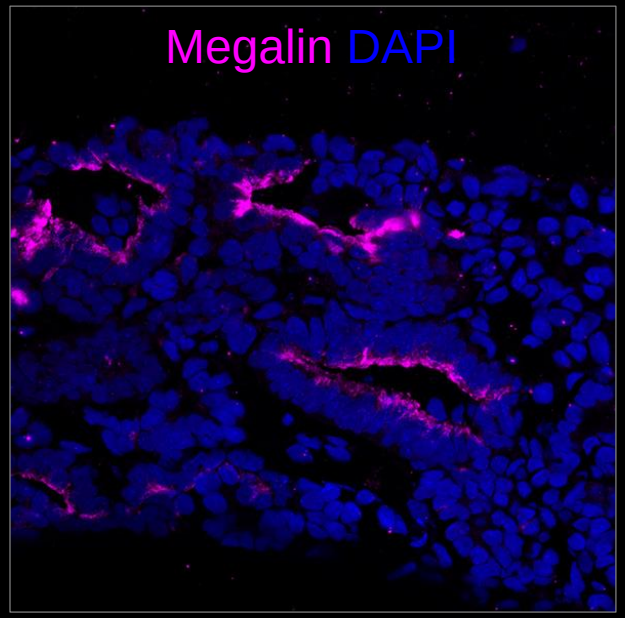
近位尿細管のエンドサイトーシス機能

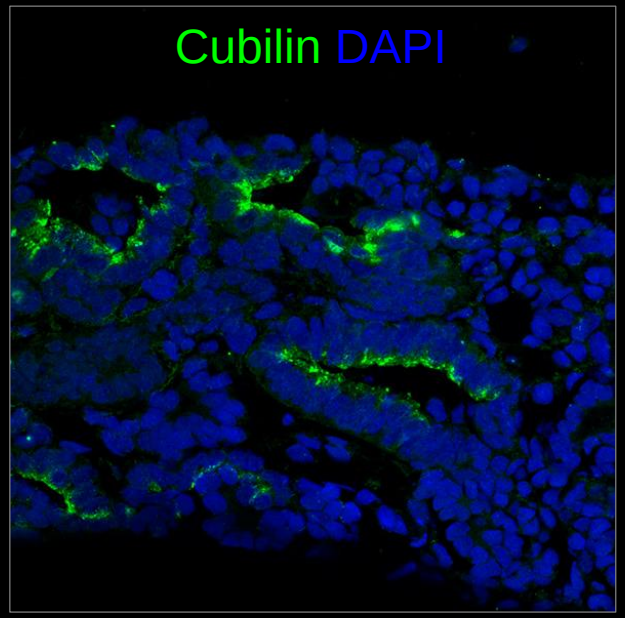


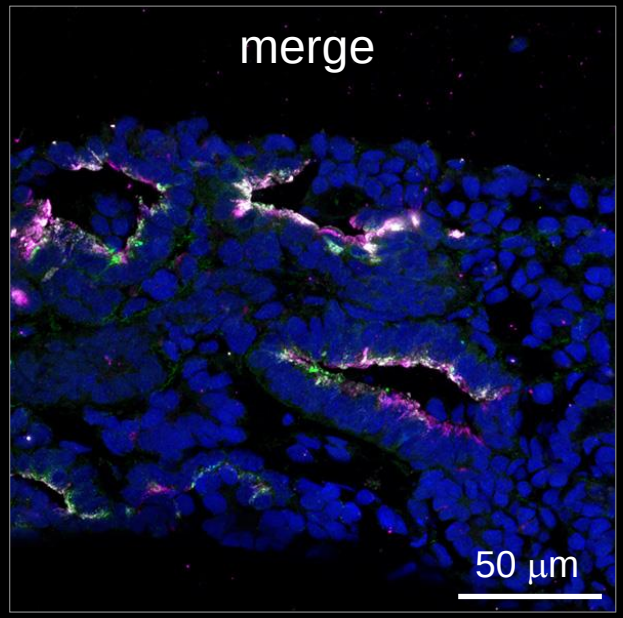
(Christensen et al, *Physiology* 2012)

腎臓オルガノイドの近位尿細管

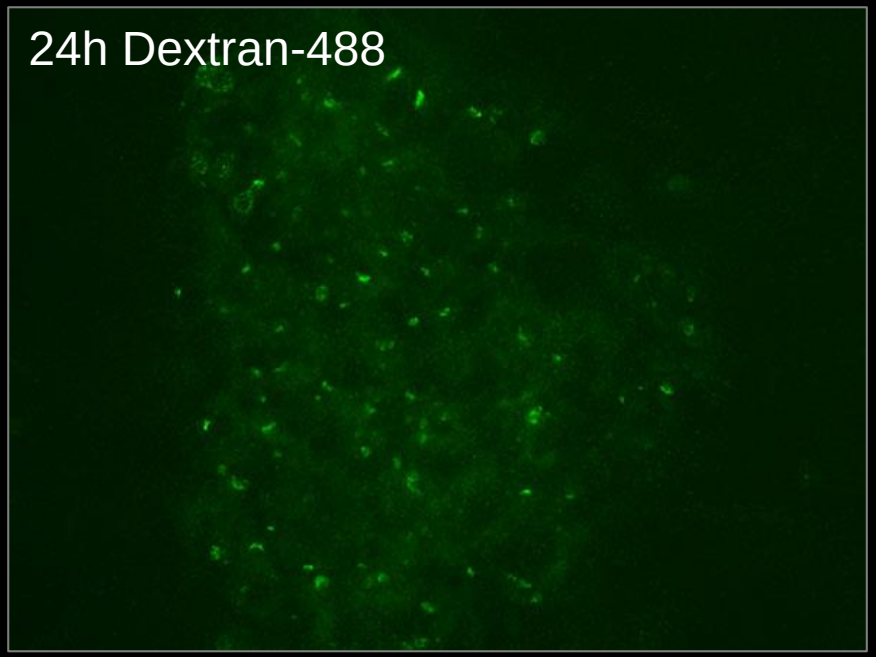




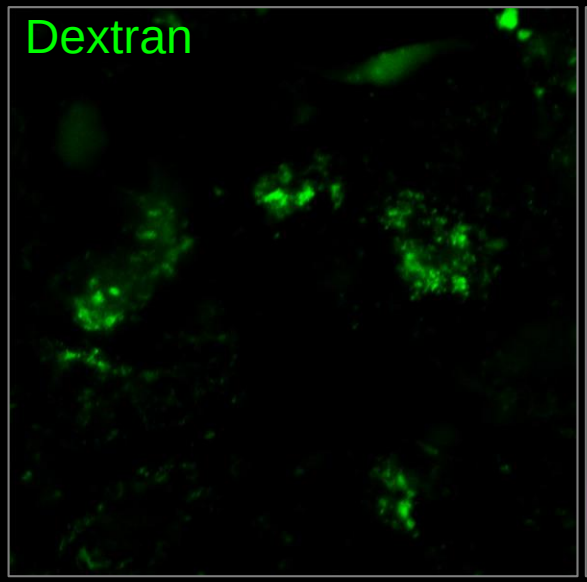


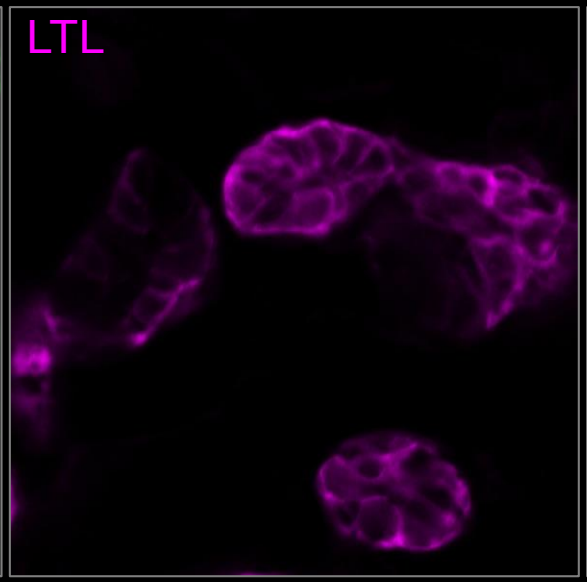


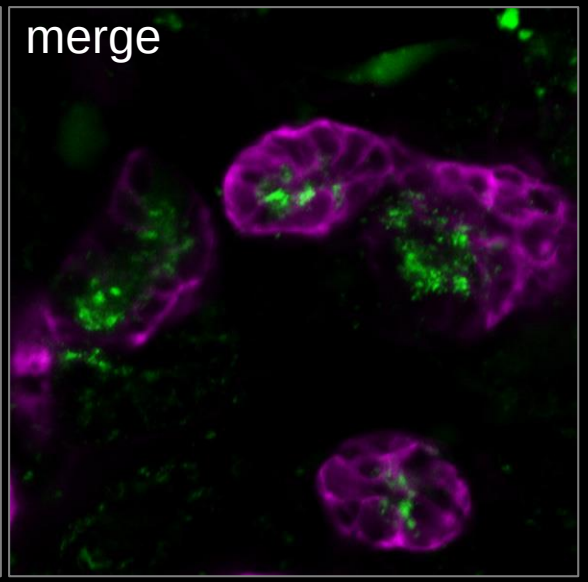
腎臓オルガノイドの近位尿細管はエンドサイトーシス機能を有す



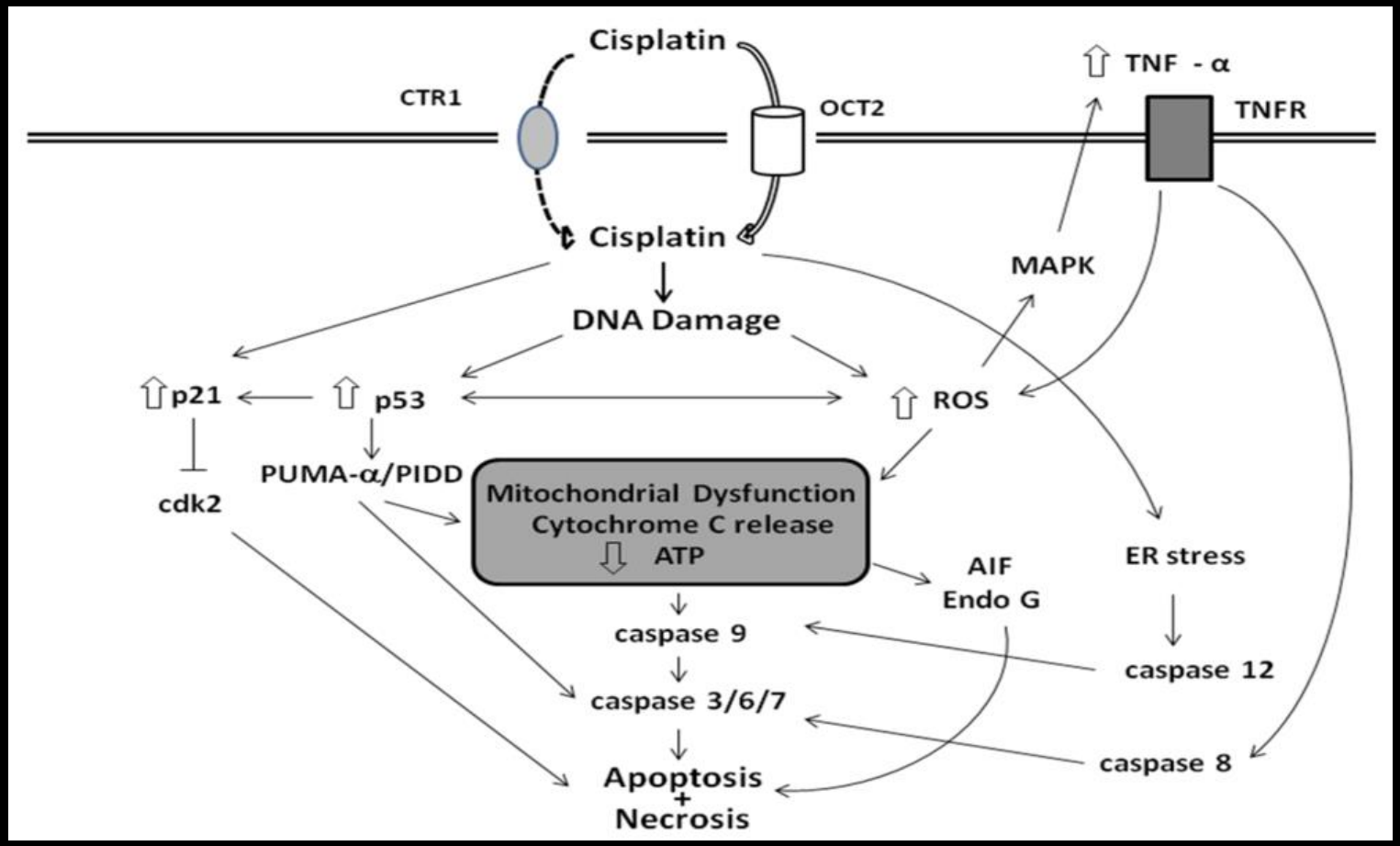
Dextran: a polysaccharide endocytosed by the PT





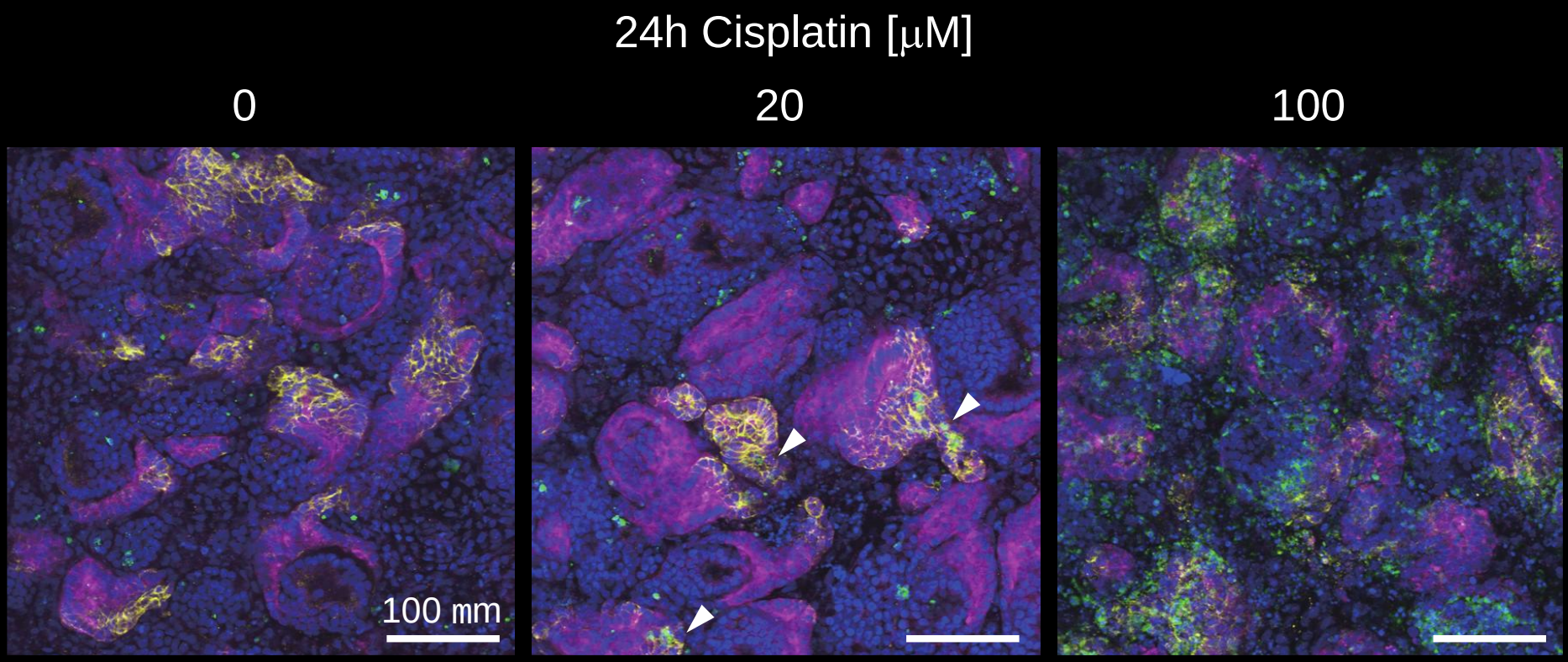


シスプラチンによる尿細管への腎毒性メカニズム

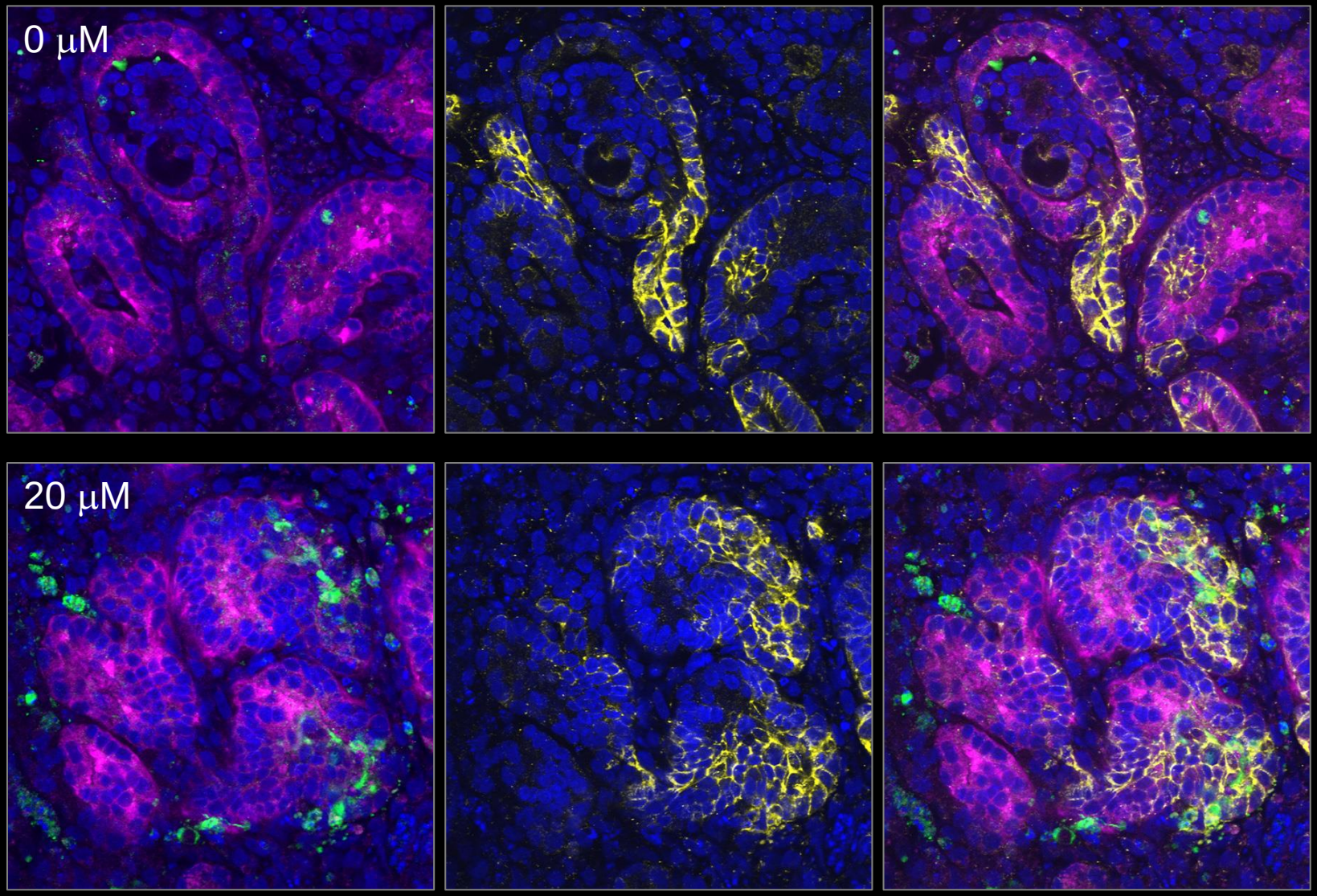


(RP Miller et al, *Toxins* 2010)

低用量のシスプラチンが組織特異的な細胞死を起こした



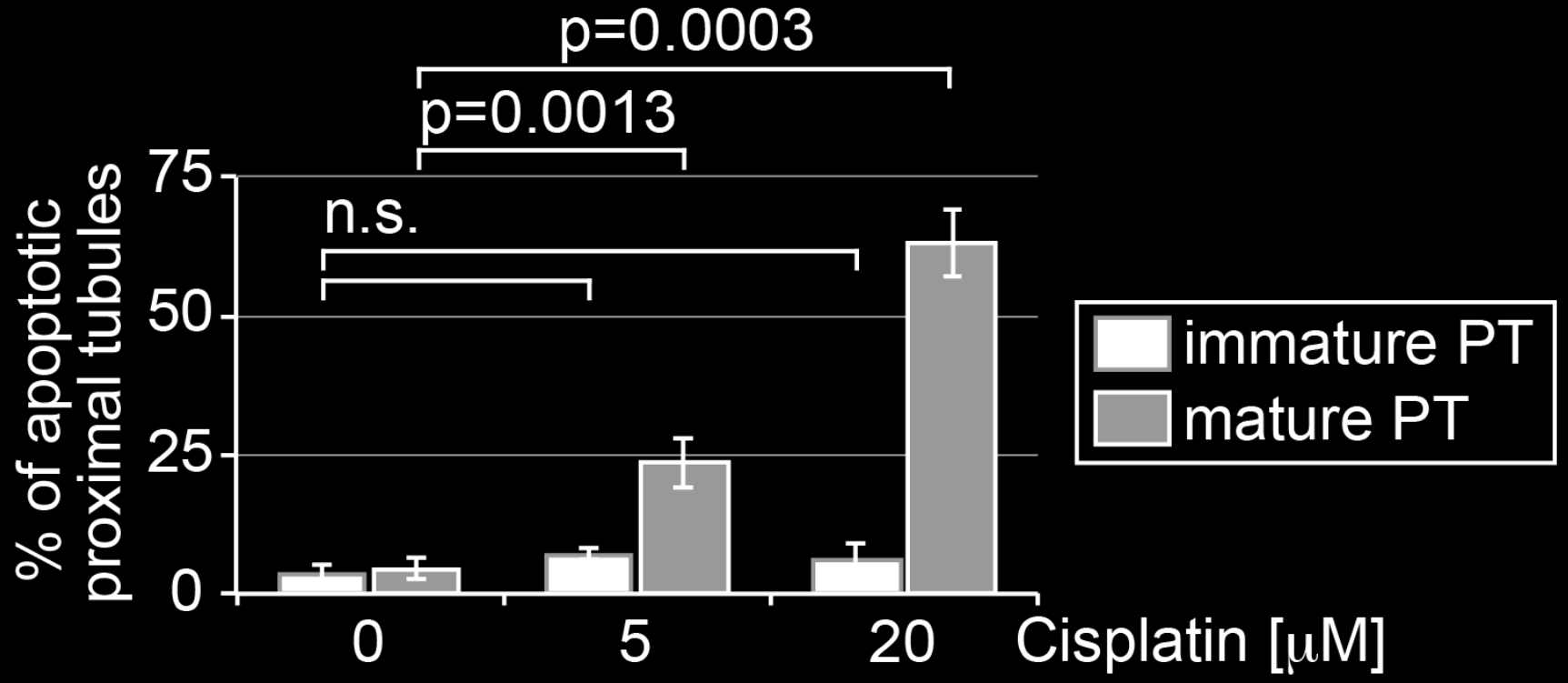
シスプラチンによる腎臓オルガノイドへの毒性試験



LTL ECAD cleaved-CASP3 DAPI

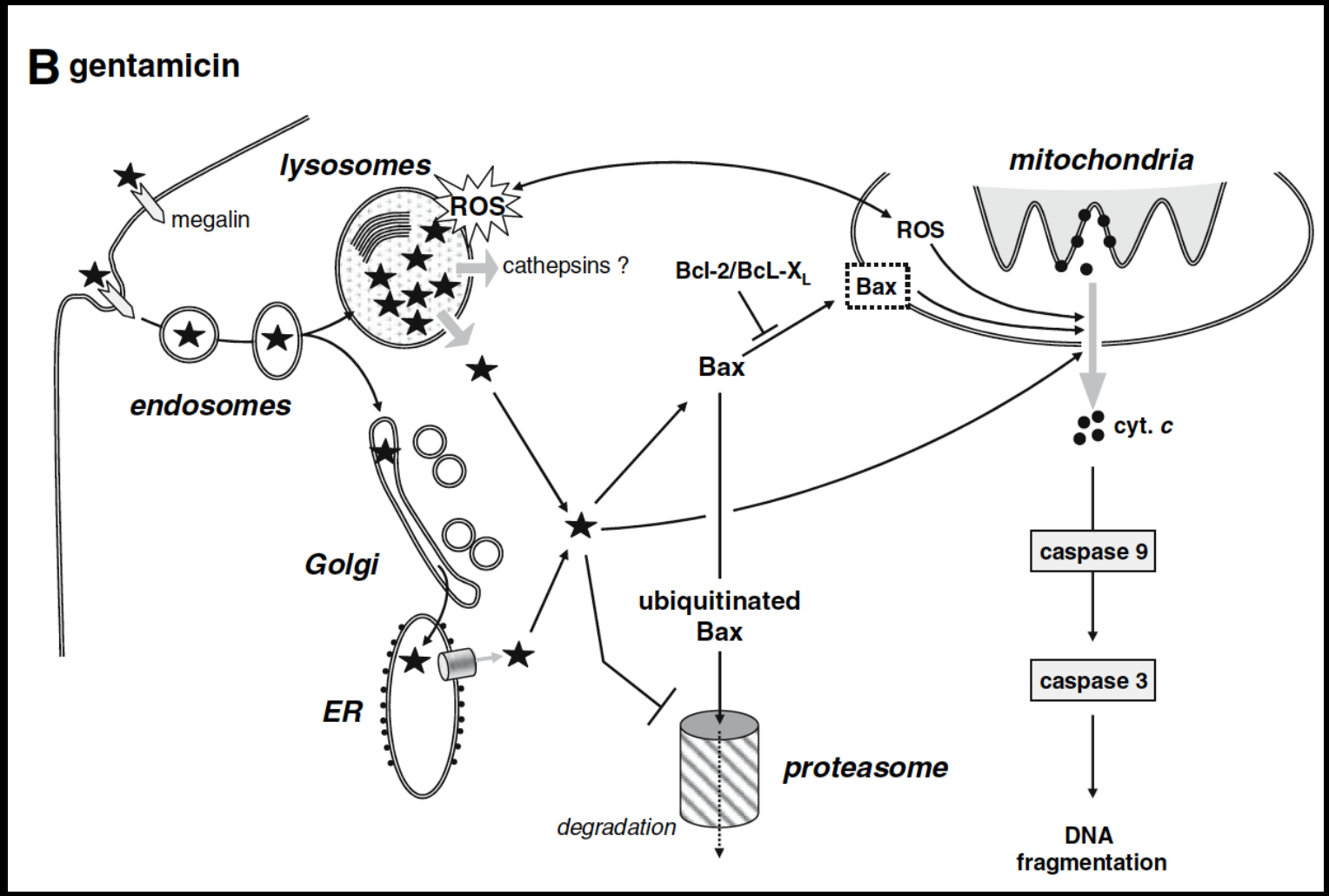
(Takasato et al., *Nature* 2015)

シスプラチンによる腎臓オルガノイドへの毒性試験



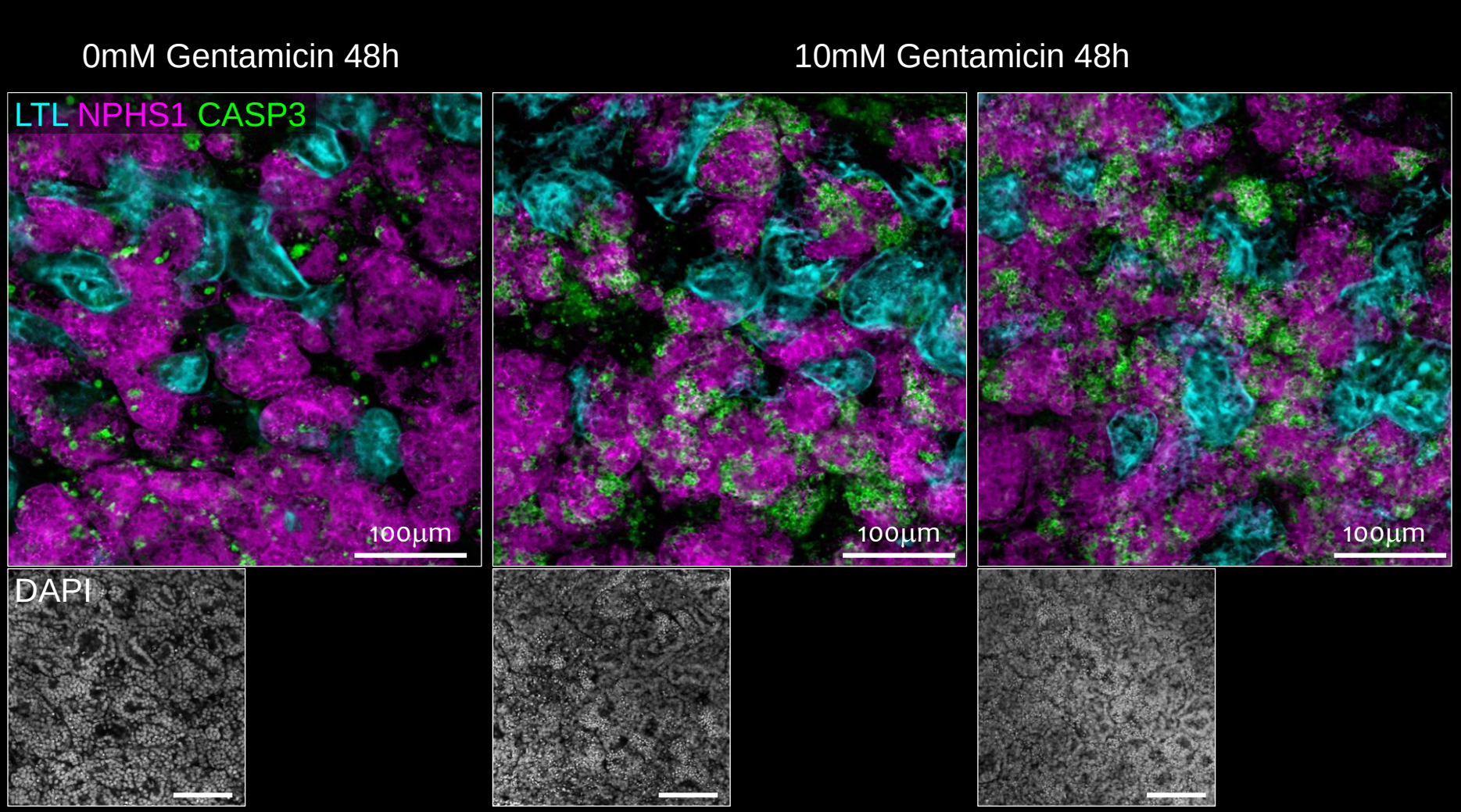
(Takasato et al., *Nature* 2015)

ゲンタマイシンはエンドサイトーシスによって尿細管に取り込まれる



(Servais H. Apoptosis 2008)

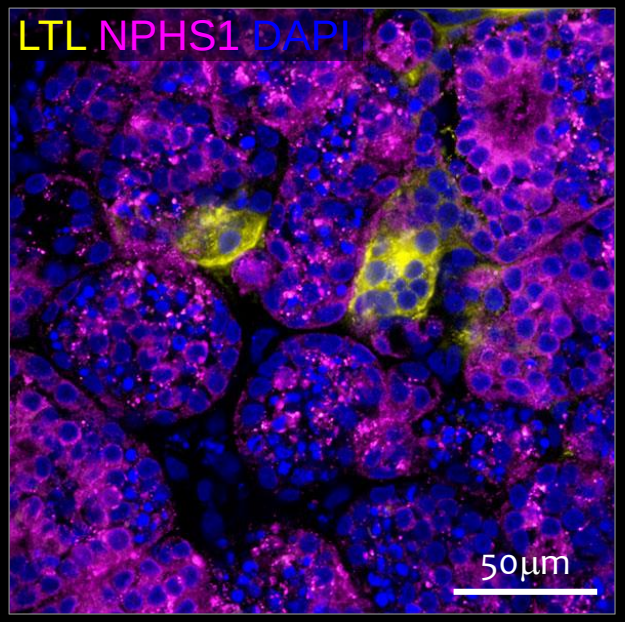
ゲンタマイシンは腎臓オルガノイドに細胞死を誘導する

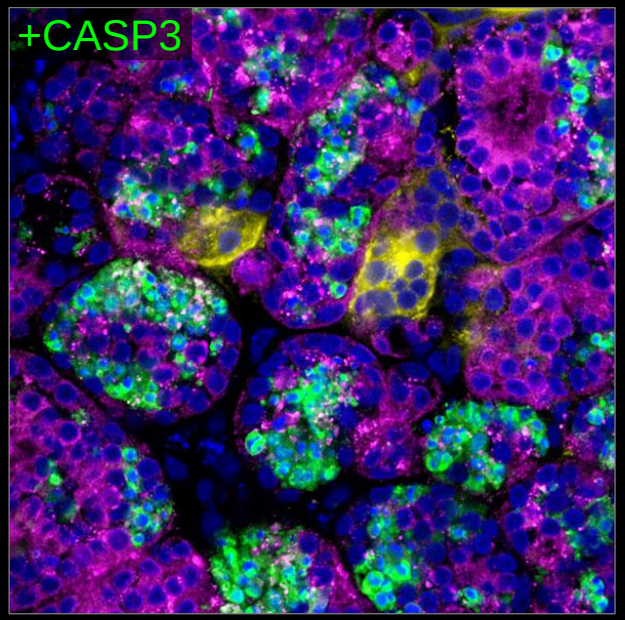


(*Unpublished*)

ゲンタマイシンは糸球体特異的にに細胞死を誘導する

#1





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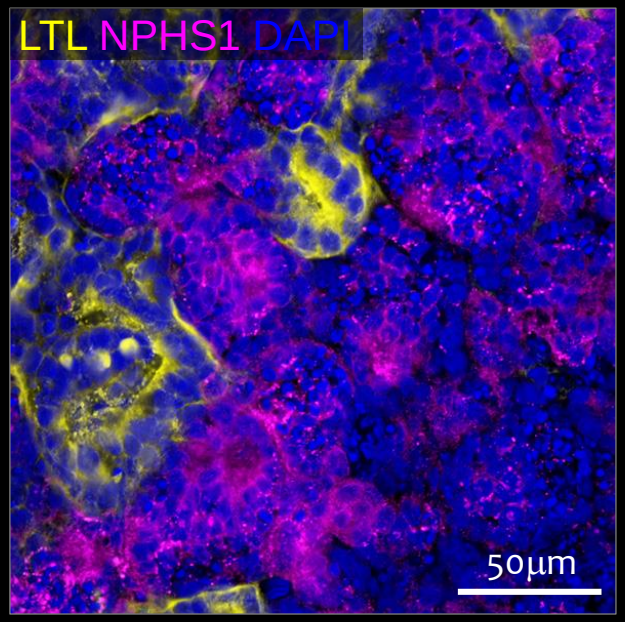
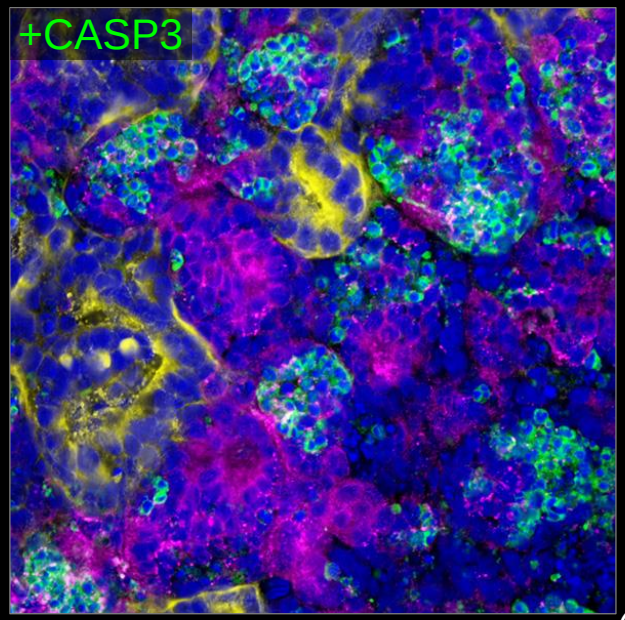
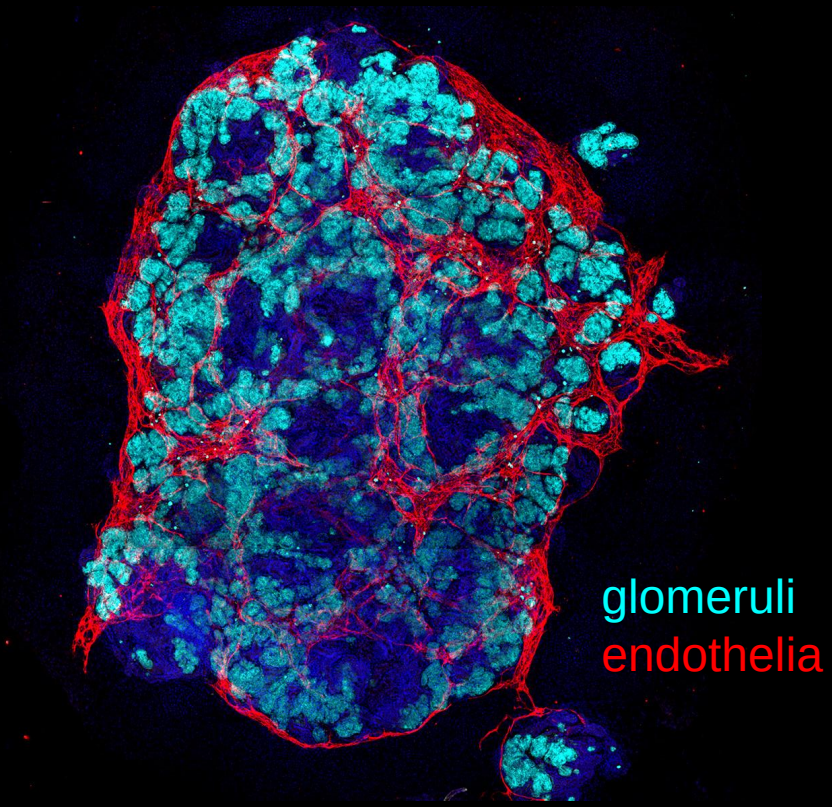




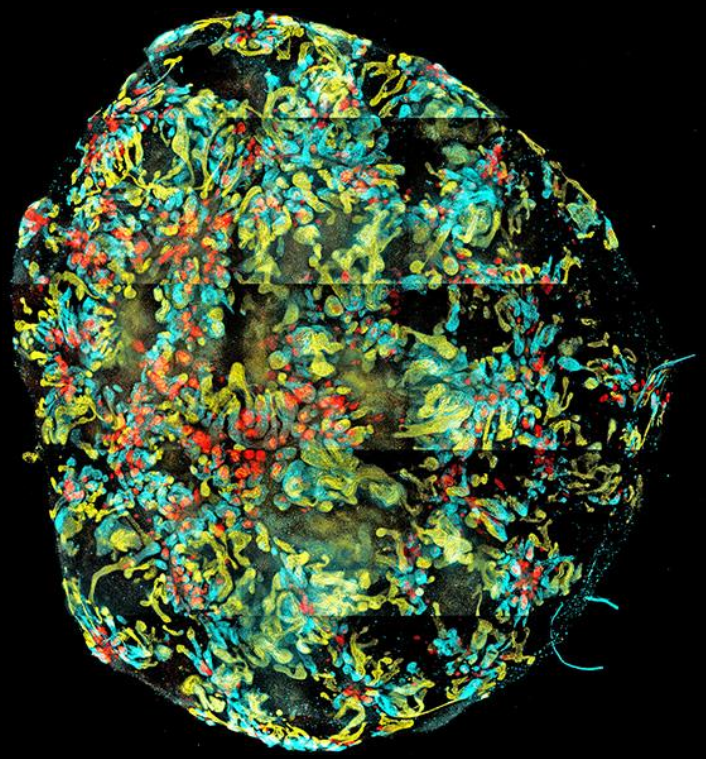
(*Unpublished*)

腎臓オルガノイドの課題

1. 糸球体の血管化



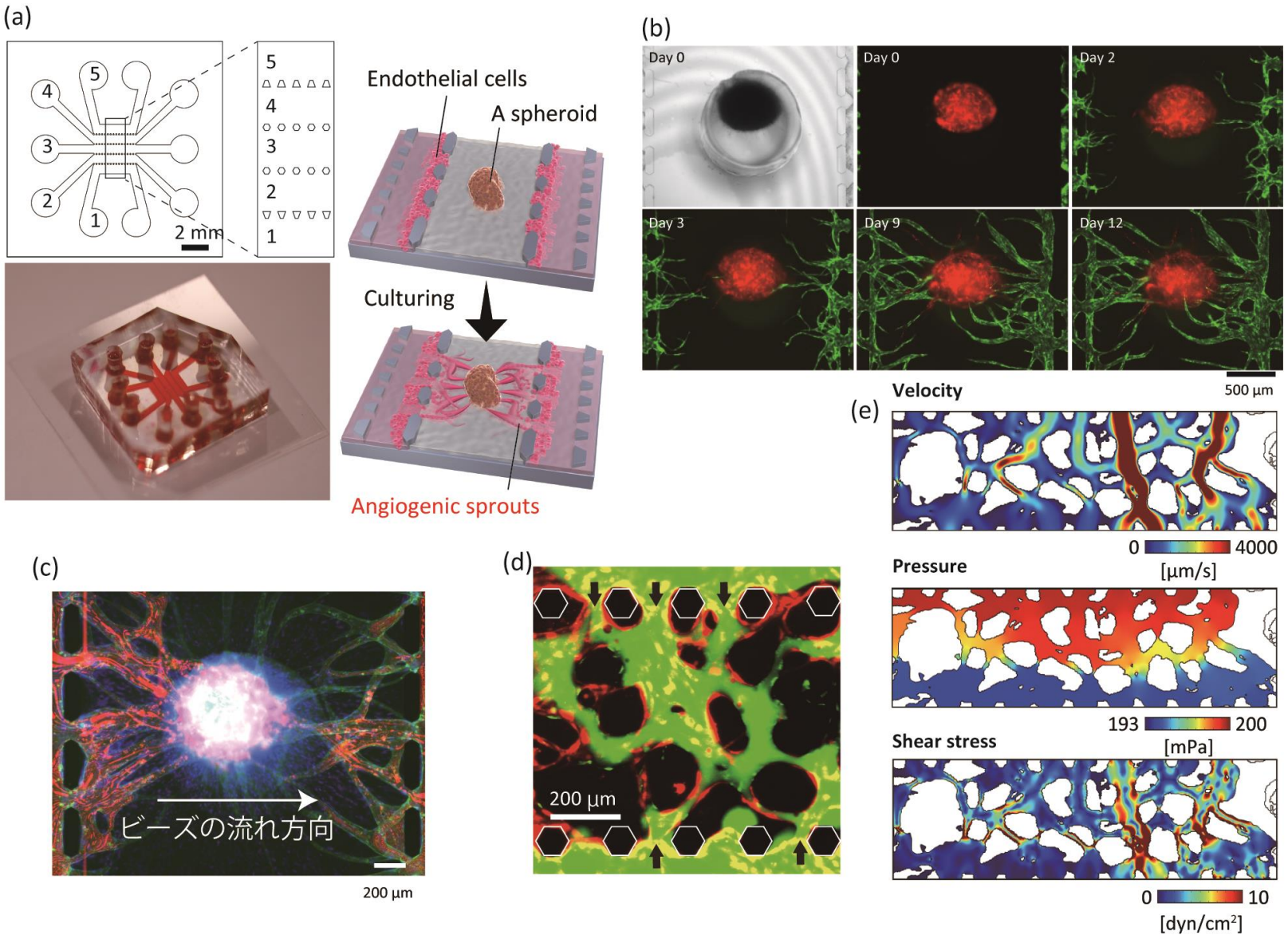
2. 膀胱との接続



3. 成熟度

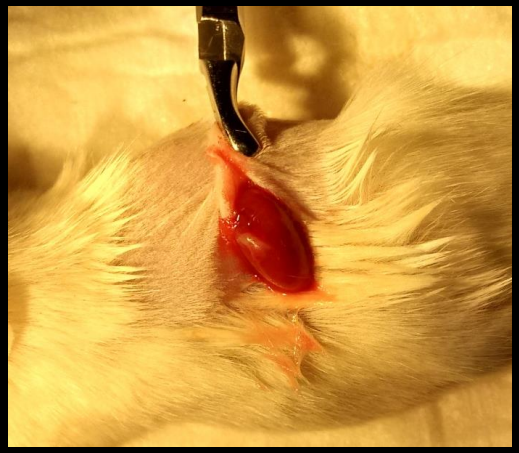
AMED事業での取り組み一部紹介

代表：横川 隆司、分担：荒岡（CiRA）、山下（CiRA）、高里（理研）、榎木（タカラバイオ）



オルガノイドは移植環境下で血管化される

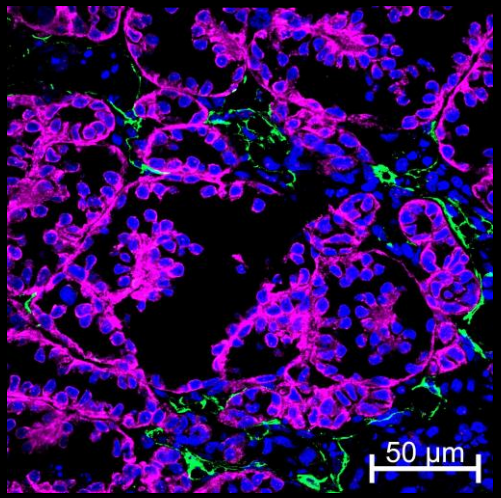
腎臓皮膜下に移植



7日後

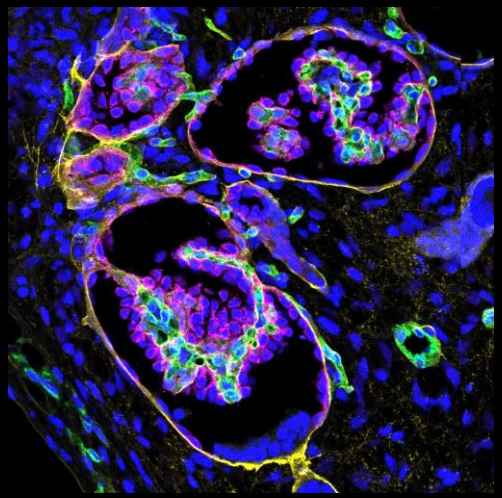
(*Stem Cell Reports, 2018*)

移植なし



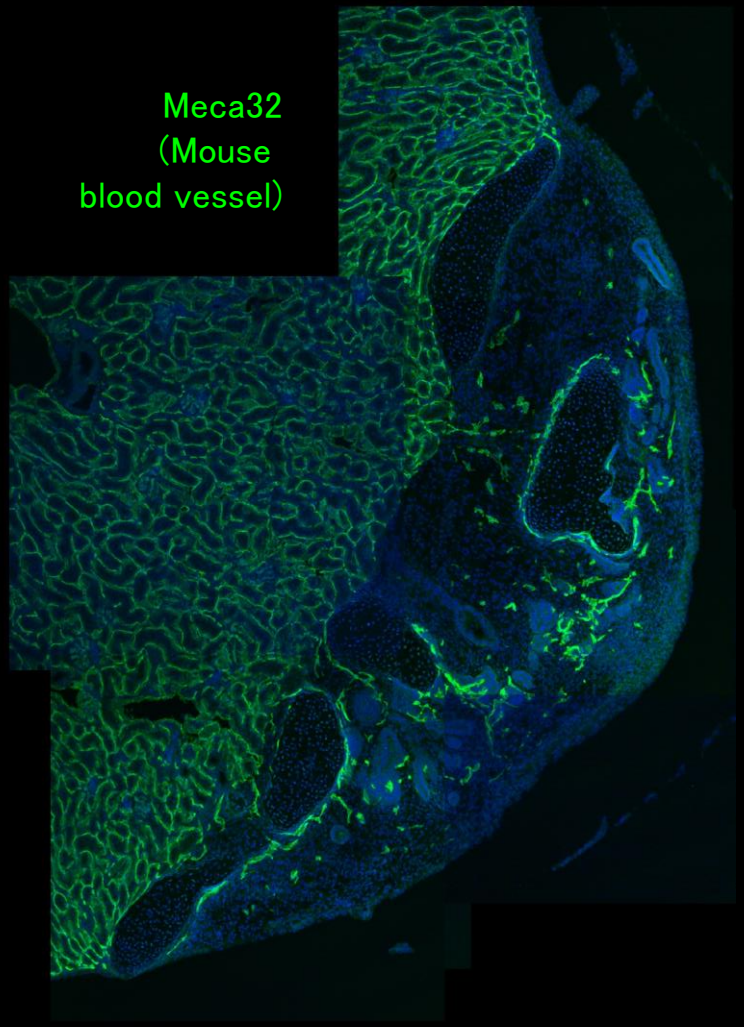
糸球体足細胞 血管

移植あり



(Yabuuchi)

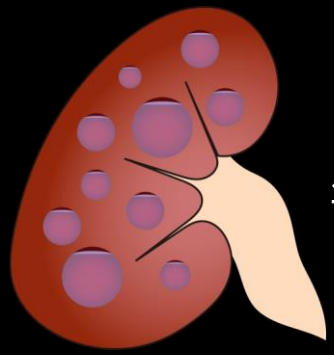
Meca32
(Mouse
blood vessel)



下部尿路系の一体的移植は水腎症を抑えられる

腎臓オルガノイド
単独

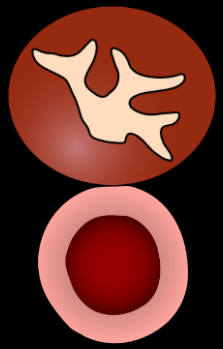
移植



生着失敗

水腎症様の症状を呈する

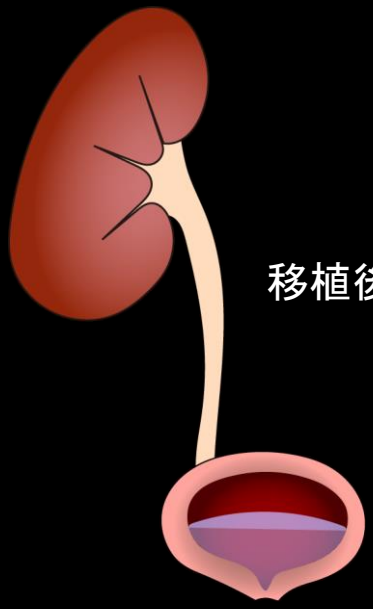
腎臓オルガノイド



膀胱オルガノイド

下部尿路系

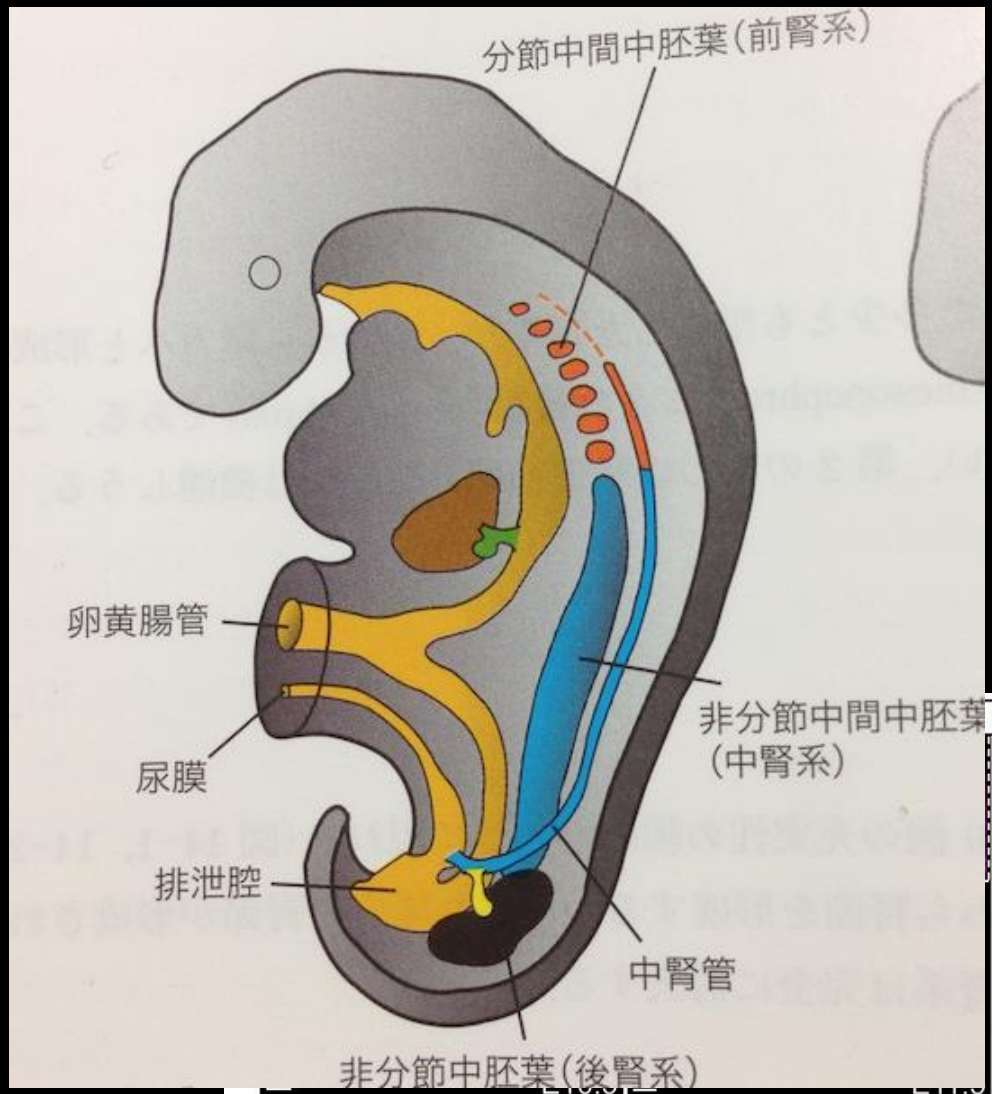
移植



移植後に成長可

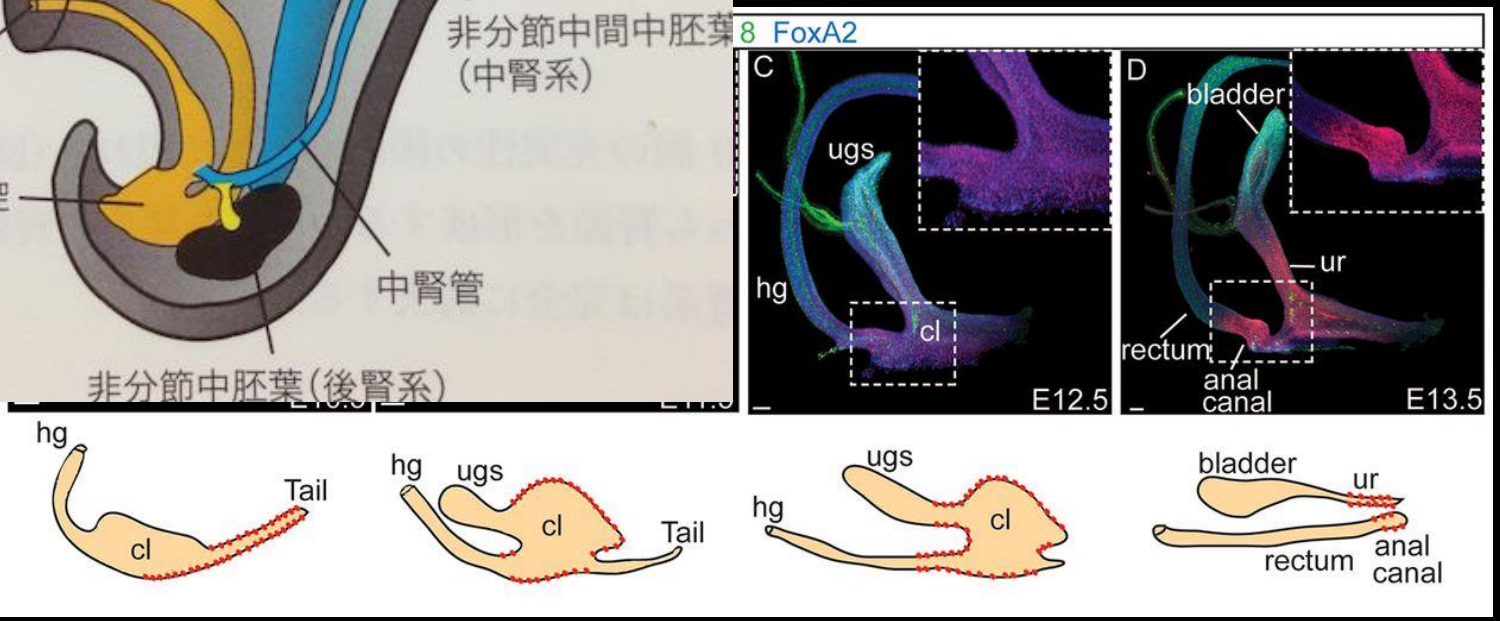
尿は膀胱に排出される

膀胱発生



iPS → 内胚葉 → 後腸

→ 総排泄腔→ 膀胱上皮



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