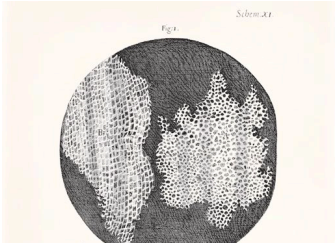
Introduction to Anthropogeny Lecture 7 ANTH203

Cellular Approaches

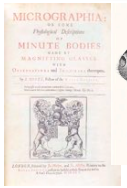


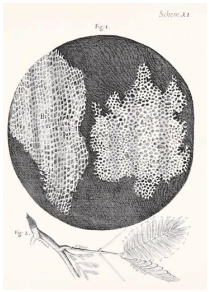
Pascal Gagneux

Thursday, November 9, 2023

Robert Hooke's drying of cells in a sliver of cork (oak bark)

Robert Hooke: naming the "cell"

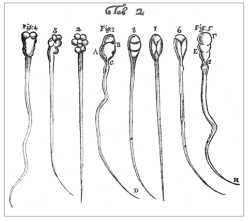


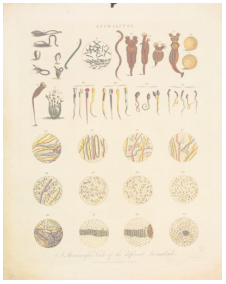


CELLS : 1665
BARE WALL TOOMS WHERE MONKS LIVE: CELLS

Robert Hooke's drying of cells in a sliver of cork (oak bark)

Antonie van Leeuwenhoek

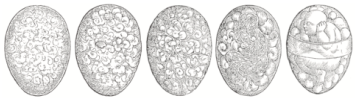




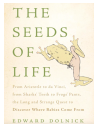
Animalcules, 1670s

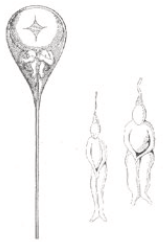
Antonie van Leeuwenhoek, detected "animalcules", moving microscopic "animals" in his own semen fluid.

Ovists and Spermists



Jacob Rüff, 1554





Nicolaas Hartsoeker, 1695

preformationists, ovists, and spearmints. Lazzaro Spalanzani and frogs in pants

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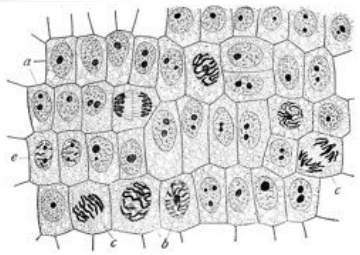
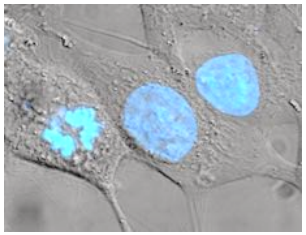
Onion skin for scale!



Onion (*Allium cepa*) root cells in different phases of the cell cycle (drawn by E. B. Wilson, 1900)

Onion skin is one cell thick!

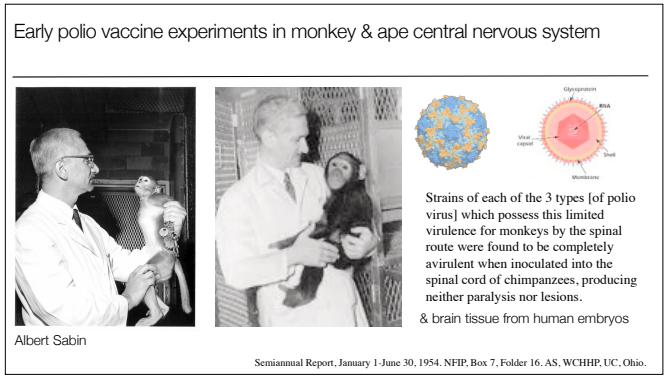
Earliest cell culture



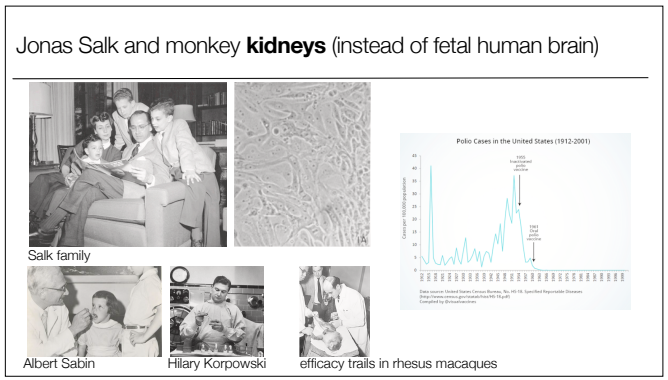
Blue staining shows nucleic acids (DNA) in nuclei of cells. Keeping cells alive outside the body. How to keep dying tissue alive.

One stage in the preparation of the rabies vaccine: a rabbit brain on a square of muslin. Pasteur Institute, India, circa 1910.
Wellcome Library, London

Pasteur and rabies vaccination. Illustration showing an anti-rabies vaccination being given at the Pasteur Institute in Paris, France. French chemist and microbiologist Louis Pasteur (1822-1895, standing at right) used rabbits to prepare a rabies virus which was milder and had a shorter incubation period than the wild virus. A person who has been bitten by a rabid animal is inoculated with the vaccine, which rapidly stimulates immunity to the wild strain. The first human patient was successfully treated in 1885. This engraving is based on a 1887 painting by Laurent Lucien Gsell (1860-1944). Titled 'La vaccine de la rage', the original is held at the Institute of Bacteriology at the Louis Pasteur University, Strasbourg, France.



In 1936, Albert Sabin and Peter Olitsky at the Rockefeller Institute successfully grew poliovirus in a culture of brain tissue from a human embryo. The virus grew quickly, which was promising, but Sabin and Olitsky were concerned about using this as starting material for a vaccine, fearing nervous system damage for vaccine recipients. They tried to grow poliovirus in cultures using tissue that had been taken from other sources, but were unsuccessful.



In the 1950s as the national effort to develop a polio vaccine required the importation of more than 200,000 rhesus monkeys annually for 6 years (Eudey and Mack 1984). Many of these imported NHPs were caught wild in their natural habitat (NAS 1970)

Dr. L. James Lewis, an employee of Dr. Jonas Salk, injects a rhesus monkey with the polio vaccine. At first, he anesthetized the monkey, shaved his leg and then disinfected the skin. He then injected the vaccine into the muscle tissue. The photo was taken in 1955, four days before the release of the evaluation report on the polio vaccine. Photo: Bettmann/ Corbis

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nervous system damage for vaccine recipients. They tried to grow poliovirus in cultures using tissue that had been taken from other sources, but were unsuccessful.

India Rhesus monkeys, used in the USA by the millions

Indian rhesus monkeys (M. mulatta) were imported at a rate of 200,000 per year for at least six years and by the tens of thousands for the next 20 years…until the ban by India in 1978.

Early cell culture: limited growth

Monkeys provided fresh kidneys

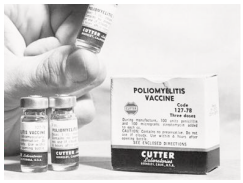
Mouth pipetting of kidney cell suspension

1950's primary cultures of monkey kidney cells

Modern tissue culture hood

Long way from primary kidney cell culture to stable cell lines.

Cutter Incident 1955

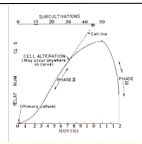


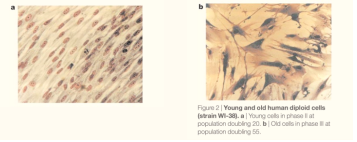
caused 40 000 cases of polio, leading to 200 children with varying degrees of paralysis and killing 10.

Fitzpatrick M. The Cutter Incident: How America's First Polio Vaccine Led to a Growing Vaccine Crisis. J R Soc Med. 2006;99(3):156.

Years later, in a suit brought against Cutter, the firm was found not negligent in making its vaccine because it had done its best making a new drug that was complicated to produce.
But it was found financially liable for the calamity it had caused during that spring of 1955.

Hayflick Limit: ~ 50 cell doublings….





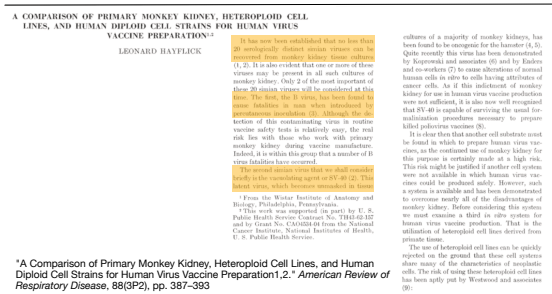
Over 750 million virus vaccine doses have been produced on **WI-38** or similar diploid cell strains. Hayflick established international standards for the production of human biologicals in passaged cells, which are still used today by the biotechnology industry

1962 – Hayflick entwickelt den ersten menschlichen diploiden Zellstamm WI-38 aus dem Lungengewebe eines drei Monate alten weiblichen Fötus. Diese Zellen werden bis heute in der Herstellung von Impfstoffen eingesetzt [10].
https://www.atsjournals.org/doi/pdf/10.1164/arrd.1963.88.3P2.387

Hayflick führte einen sechs Jahre andauernden Streit mit den nationalen Gesundheitsbehörden um die Rechte an der daraus entwickelten Zelllinie – und gewann. Seither dürfen amerikanische Forscher die Verwertungsrechte für ihre Entdeckungen behalten, auch wenn deren Forschung durch nationale Mittel finanziert wurde. Ein Kommentar von Hayflick hierzu wurde 2012 in Science veröffentlicht.

WI-38 is a diploid human cell line composed of fibroblasts derived from lung tissue of a 3-month-gestation female fetus. The fetus came from the elective abortion of a Swedish woman in 1962, and was used without her knowledge or permission.

Hayflick argued against the use of monkey cells….



A COMPARISON OF PRIMARY MONKEY KIDNEY, HETEROPLOID CELL LINES, AND HUMAN DIPLOID CELL STRAINS FOR HUMAN VIRUS VACCINE PREPARATION[1,2]

LEONARD HAYFLICK

It has now been established that no less than 20 serologically distinct simian viruses can be recovered from monkey kidney tissue cultures (1, 2). It is also evident that one or more of these viruses may be present in all such cultures of monkey kidney. Only 2 of the most important of these 20 simian viruses will be considered at this time. The first, the B virus, has been found to cause fatalities in man when introduced by percutaneous inoculation (3). Although the detection of this contaminating virus in routine vaccine safety tests is relatively easy, the real risk lies with those who work with primary monkey kidney during vaccine manufacture. Indeed, it is within this group that a number of B virus fatalities have occurred.

The second simian virus that we shall consider briefly is the vacuolating agent or SV-40 (2). This latent virus, which becomes unmasked in tissue cultures of a majority of monkey kidneys, has been found to be oncogenic for the hamster (4, 5). Quite recently this virus has been demonstrated by Koprowski and associates (6) and by Enders and co-workers (7) to cause alterations of normal human cells in vitro to cells having attributes of cancer cells. As if this indictment of monkey kidney for use in human virus vaccine production were not sufficient, it is also now well recognized that SV-40 is capable of surviving the usual formalinization procedures necessary to prepare killed poliovirus vaccines (8).

It is clear then that another cell substrate must be found in which to prepare human virus vaccines, as the continued use of monkey kidney for this purpose is certainly made at a high risk. This risk might be justified if another cell system were not available in which human virus vaccines could be produced safely. However, such a system is available and has been demonstrated to overcome nearly all of the disadvantages of monkey kidney. Before considering this system we must examine a third in vitro system for human virus vaccine production. That is the utilization of heteroploid cell lines derived from private tissue.

The use of heteroploid cell lines can be quickly rejected on the ground that these cell systems share many of the characteristics of neoplastic cells. The risk of using these heteroploid cell lines has been aptly put by Westwood and associates (9):

[1] From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania.

[2] This work was supported (in part) by U. S. Public Health Service Contract No. TH43-62-157 and by Grant No. CA04554-04 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

"A Comparison of Primary Monkey Kidney, Heteroploid Cell Lines, and Human Diploid Cell Strains for Human Virus Vaccine Preparation1,2." *American Review of Respiratory Disease*, 88(3P2), pp. 387–393

Leonard Hayflick's warning about SV40 another viruses.

Simian vacuolating virus 40. SV40

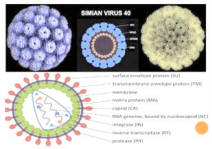
Some of the polio vaccine administered from 1955–1963 was contaminated with a virus, called simian virus 40 (SV40) a macaque polyomavirus that can induce cancer in rodents.

An estimated 10-30% of polio vaccines administered in the US were contaminated with simian virus 40 (SV40).

~30 million Americans were exposed to SV40 via contaminated vaccines.

The virus codes a protein known as the T-antigen, which regulates viral replication and inactivates tumor suppressor genes (p53)

Structure

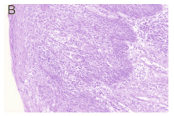


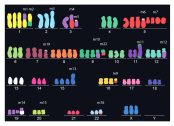
Rollison, D. E. M., and K. V. Shah. 2001. The epidemiology of SV40 infection due to contaminated polio vaccines: relation of the virus to human cancer, p. 561-584. *In* K. Khalili and G. L. Stoner (ed.), Human polyomaviruses: molecular and clinical perspectives. Wiley-Liss, Inc., New York, N.Y.

SV40: a stowaway passenger in the monkey kidneys…..that inadvertently was injected into ~30 million Americans.

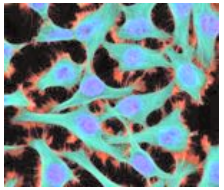
Immortal Cells: HeLa

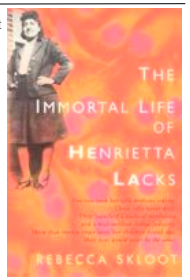
HPLV 18 transforms cervical cells, hyper aggressive cancer that killed patient Henrietta Lacks in Baltimore in 1951.





HeLa cells were critical for testing the polio vaccine as polio virus kills these cells





nuclei purple, microtubules blue, actin microfilaments red.

HeLa cells are rapidly dividing cancer cells, and the number of chromosomes varied during cancer formation and cell culture. The current estimate (excluding very tiny fragments) is a "hypertriploid chromosome number (3n+)" which means 76 to 80 total chromosomes (rather than the normal diploid number of 46) with 22–25 clonally abnormal chromosomes, known as "HeLa signature chromosomes."

Vaccines: Most Successful Intervention of Medicine

Inactivated: dead whole pathogen
Attenuated: live infectious pathogen manipulated to generate a non-pathogenic state.

Down sides:
not as good an antigen

potential reversal to pathogenic

Subunit vaccines: only part of the pathogen (surface glycoprotein) is used, non-infectious

not presenting diverse enough"face" of virus

Genetic vaccines: RNA or DNA encoding viral antigens In viral vector or lipid nanoparticle.

new, limited data on longterm risk

Viral vector vaccines: DNA from the virus is Inserted into the capsid of a Harmless virus as delivery vehicle.

little information on longterm risk, limited antigen

There are different ways of manufacturing vaccines.
Vaccines can have risks, but more than half a century of studies have shown that overall the benefits of mass-immunization far outweigh the risks to the individuals.

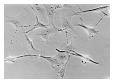
Growing virus to make vaccines:

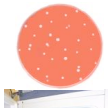
Primary tissue culture

Primate Kidneys

Hens' eggs

Cell lines

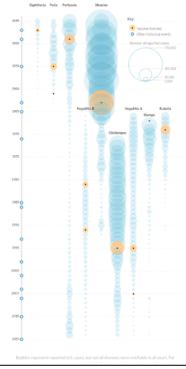




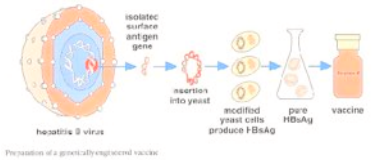
Bioreactor (cell-free)

N-1 at 40 L
Xcellerex XDR-200

The substrate used for making vaccine contributes to certain risks of the vaccine, e.e. Influenza vaccine made in chicken eggs can cause reactions in people who have egg allergies. Animal or human cell lines each carry risks of disease transmission, plant cells are also used, latest technology uses cell-free reactors to synthesize viral RNA (e.g. Pfizer)



Hepatitis B subunit vaccine

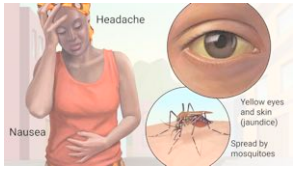


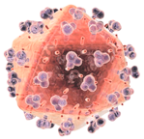
First successful anticancer vaccine

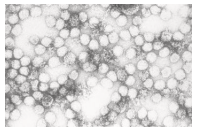
Your Hepatitis B vaccine was tested for safety in chimpanzees!

Studies by Alfred Prince and his team at the Vilab in Liberia
have paved the way for a Hepatitis B vaccine.The vaccine is now produced in yeast cells.

Yellow Fever, a flavivirus

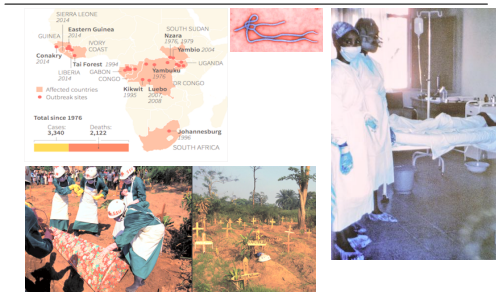






yellow fever is the only flavivirus that can be prevented with a very efficient vaccine. The yellow fever vaccine provides life long protection!

Ebola, a filovirus vaccine rVSV-ZEBOV approved in 2019



A recently developed vaccine against ebola is a big hope for many. VSV-EBOV or rVSV-ZEBOV, sold under the brand name Ervebo, is a vaccine based on the vesicular stomatitis virus which was genetically modified to express a surface glycoprotein of Zaire Ebola virus

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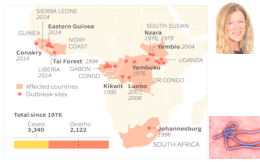
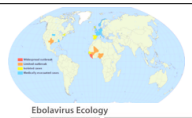
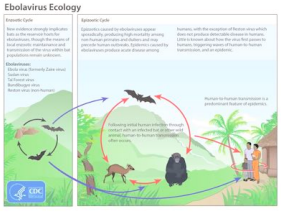
Ebola



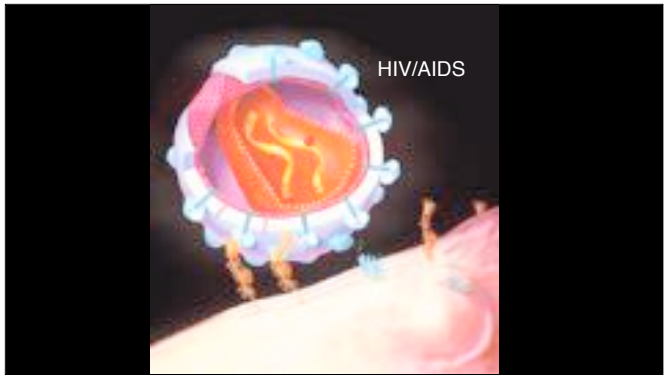




My friend and colleague was patient zero for the Ebola Ivory Coast outbreak in 1994.
She infected herself while helping a veterinarian conduct an autopsy of a dead wild chimpanzee.

HIV/AIDS: a chimpanzee zoonosis

It is now clear that HIV/AIDS emerged as a zoonosis bin Central Africa around the turn of the the 1900s.

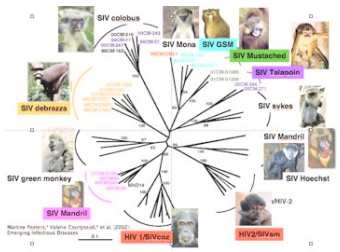


HIV infects T-lymphocytes in the blood stream, ultimately causing AIDS. Terese Winslow created this artwork to give scientists new insight into how HIV infects T-lymphocytes. The virion is shown in the first stage of infection, when the virion attaches to the surface of the T-cell. The molecules involved in this docking process are of particular interest to scientists, so she rendered them accurately according to the most up-to-date scientific information. These molecules include gp120 (the blue 'mushrooms' on the surface of the virus), CD4 (the long red molecules on the cell surface), and chemokine receptors (the groups of blue cylinders on the cell surface).

Again, no depiction of the many complex glycan molecules on both, the virus glycoprotein “mushrooms” or the host cell surface.

All other African primates have their own SIV

SIV in > 30 species of primates

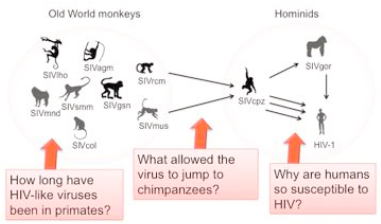


Humans only acquired HIV from African primates at the beginning of 1900s.

Peeters *et al.* **2002.** ***Emerg. Infect. Dis.***

Most African non-human primates each have their own versions of HIV, named SIV (simian immunodeficiency virus, a misnomer, as most other African primate species do not get sick).

What caused the virus to jump?



More than a million years in other African primates. Jump likely aided by bush meat hunting/butchering. The bases for human susceptibility are still being studied.

Perfect Storms

Colonial brutality and mass medical campaigns

Large urban centers and mass migrations

Intercontinental Medical Aid

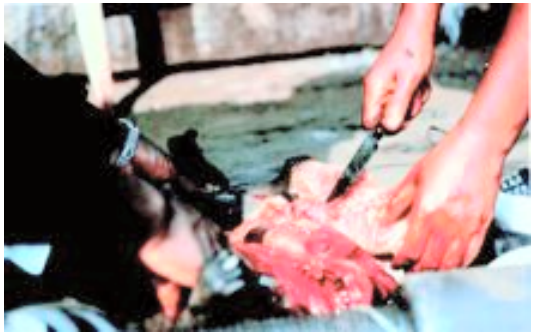
Blood Commerce

(Sex) Tourism and
IV Drug use

The convergence of colonial brutality, the first large urban centers (including sex workers), intercontinental medical aid, blood commerce (plasma pheresis businesses in Haiti), and sex tourism and IV drug use formed the perfect storm.

Bush meat trade

Apes are still hunted for their meat throughout tropical Africa, even in the cities, bush meat is valued much more highly than farmed meat.



perfect opportunity fro cross-species infections.

Polio vaccine studies in the Belgian Congo used hundreds of wild caught chimpanzees and bonobos for testing the efficacy and safety of vaccine.

These studies could not have cause the HIV1 epidemic which was well underway by the late 1950s.

Alexandre Jezierski on a monkey-hunting expedition for the Gabu-Nioka laboratory, 1954. *(Credit: G. Scott)*

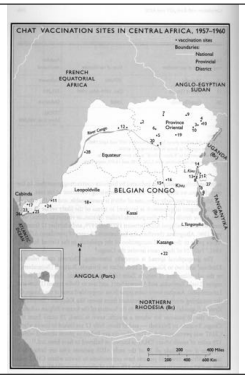
Chimp caught in a liana net by pygmies, at one of Rollais's base camps in the north of Province Oriental, 1958. *(Credit: G. Rollais)*

Two African assistants dismembering a dead chimp in the

Mass vaccination in Belgian Congo 1959

Mass vaccination in Belgian Congo 1959: suspected by some as possible origin of HIV/ AIDS

BUT
clearly not the case
rather HIV was already circulating at the time



The Alternative hypotheses about HIV origins:

1.Natural Transfer: infection by killing and butchering of apes for meat, more hunting in modern times, larger cities and more travel.

2. Natural Transfer & syringes (aided by rural clinics with rampant reuse of unsterilized hypodermic needles).

3.Oral Polio Vaccine (OPV), vaccine prepared on chimpanzee tissue cultures? infected with SIV and fed to ~1 million Africans in 1957-1960.

3 has been proven wrong, so likely a combination of 1 and 2.

Chimpanzee cells to Philadelphia

STUDIES OF LIVER FUNCTION TESTS IN CHIMPANZEES AFTER INOCULATION WITH HUMAN INFECTIOUS HEPATITIS VIRUS[1]

By

FRIEDRICH DEINHARDT,[2] GHISLAIN COURTOIS, PAULETTE DHERTE, PAUL OSTERRIETH, GASTON NINANE, GERTRUDE HENLE AND WERNER HENLE

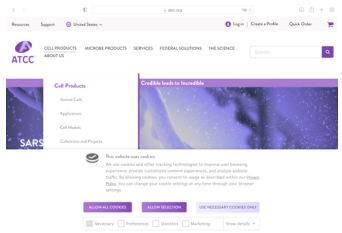
(Received for publication December 13, 1961)

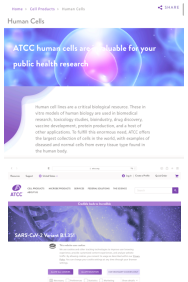
AM. J. HYG. 1962, VOL. 75: 311–321

Diarella and Camp Lindi sign. (Route d. Arbal.)

Tissue-culture studies. Additional efforts were made to isolate IH virus in chimpanzee kidney-tissue cultures. For these experiments minced pieces of chimpanzee kidneys were sent by air from Stanleyville to the Children's Hospital of Philadelphia. These were trypsinized within 24 hours after arrival. The total time between the removal of the kidneys and the preparation of tissue cultures varied from 3 to 6 days and good cultures were obtained from 5 out of 6 shipments. None of the 6 specimens revealed foamy agents or other latent viruses. Unfortunately, no evidence for the propagation of IH virus was obtained in any of the cultures inoculated with WH or no. 331 materials, and maintained for periods up to 3 weeks, or in second and third passages derived therefrom. Cultures were observed for cytopathology, development of interference to other cytopathogenic viruses, and staining with fluorescent antibodies (isothiocyanate-labeled human gamma globulin).

ATCC: American Type Culture Collection





The organization holds a collection of more than 3,000 human and animal cell lines and an additional 1,200 hybridomas.

ATCC or the American Type Culture Collection is a nonprofit organization which collects, stores, and distributes standard reference microorganisms, cell lines and other materials for research and development

The organization holds a collection of more than 3,000 human and animal cell lines and an additional 1,200 hybridomas. ATCC's microorganism collection includes a collection of more than 18,000 strains of bacteria, as well as 3,000 different types of animal viruses and 1,000 plant viruses. In addition, ATCC maintains collections of protozoans, yeasts and fungi with over 7,500 yeast and fungus species and 1,000 strains of protists.

The Frozen Zoo

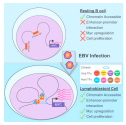
San Diego Zoological Society

It contains over 10,000 living cell cultures, oocytes, sperm, and embryos representing nearly 1,000 taxa, including one extinct species, the po'ouli.

San Diego Zoo Wildlife Alliance

all primary cells, none of them transformed.

Global samples from humans: lymphoblastoid cell lines

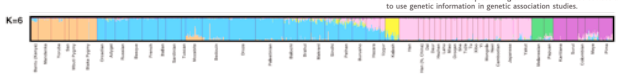


2600 EBV transformed cell lines derived from white blood cells from around the world.

Genetic Structure of Human Populations

Noah A. Rosenberg,[1]* Jonathan K. Pritchard,[2] James L. Weber,[3] Howard M. Cann,[4] Kenneth K. Kidd,[5] Lev A. Zhivotovsky,[6] Marcus W. Feldman[7]

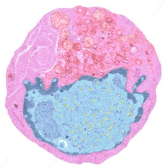
We studied human population structure using genotypes at 377 autosomal microsatellite loci in 1056 individuals from 52 populations. Within-population differences among individuals account for 93 to 95% of genetic variation; differences among major groups constitute only 3 to 5%. Nevertheless, without using prior information about the origins of individuals, we identified six main genetic clusters, five of which correspond to major geographic regions, and subclusters that often correspond to individual populations. General agreement of genetic and predefined populations suggests that self-reported ancestry can facilitate assessments of epidemiological risks but does not obviate the need to use genetic information in genetic association studies.

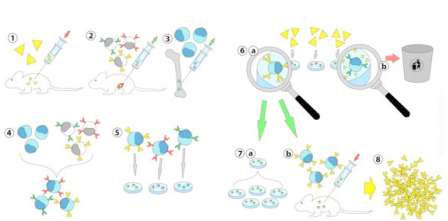


Science 2002

Kenn and Judy Kidd of Yale University have collected white blood cells from thousands of individuals from around the world.

Hybridomas fusing B-cell with bone marrow cells

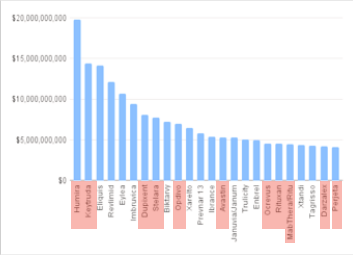




B-cells from spleen of immunized animals fused with cancerous bone marrow cells (myeloma) generate immortal cells that produce monoclonal antibodies.

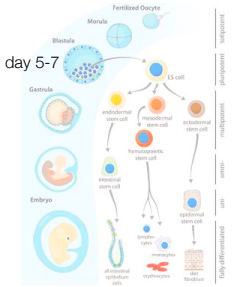
(1) Immunisation of a mouse (2) Isolation of B cells from the spleen (3) Cultivation of myeloma cells (4) Fusion of myeloma and B cells (5) Separation of cell lines (6) Screening of suitable cell lines (7) in vitro (a) or in vivo (b) multiplication (8) Harvesting

Monoclonal antobodies: market value > 100 billion



Humira (adalumimab) **AbbVie** anti-TNF, Crohns, RA, Psoriasis
Keytruda **Merck**: anti PD1 on T-cells, cancers
Dupixent (dupilimab) **Sanofi**: anti-IL4 receptor alpha, allergies, autoimmunity
Stelara (ustekinumab), **Jansen**: IL12 & IL23 Crohns, Ulcer Col, Psoriasis
Opdivo (nivolumab), **Bristol Myers Squib**: anti-PD1, cancer
Avastin (bevacizumab), **Roche**: anti-VEGF A, cancers, AMD
Ocrevus (ocrelizumab), **Roche**: anti-CD20, MS
Rituxan (rituximab) **Roche**: antri-CD20, MS
Darzalex (daratumumab), **Johnson & Johnson**: anti-CD38, myeloma
Perjeta (pertuzumab), **Roche**, anti-HER2 breast cancer

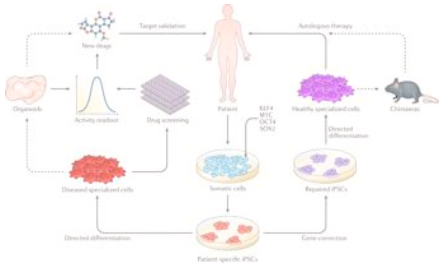
Embryonic Stem Cells



over 620 000 frozen human embryos in the USA.

Tapping the “Germ Line”? The inner cell mass day

iPS, induced pluripotent stem cells



Induced pluripotent stem cells, a way around using embryonal stem cells.

Retrotransposons are a class of Mobile Elements or “Jumping Genes”

Barbara McClintock
Nobel Prize, 1983

Creating genetic mosaics

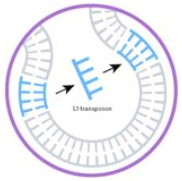
LINE1: Long Interspersed Nuclear Element - Only autonomous mobile elements that are active in humans (comprises 20% of the genome, coding regions are approx. 2%)

McClintock, B. Chromosome organization and genic expression. *Cold Spring Harbor Symp. Quant. Biol.* ***16, 13–47 (1951).***

Retrotransposons are endogenous mobile elements or fragments of DNA that can copy themselves and insert into new chromosomal locations. That is the reason why transposons are also referred to as “jumping genes”. Transposons have been discovered more then 50 years ago in maize by Barbara McClintock that won the Nobel prize for that discovery.
SHE COULD NOT EXPLAIN THE INHERITANCE OF MAIZE KERNEL COLORS BY MENDELIAN LAWS!

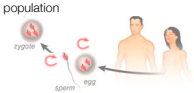
Consequences of Mosaicism after LINE1 Mobility: Mutations, Diversity and Disease

LINE1 (L1) mobility influence chromosome integrity and gene expression upon reinsertion causing genetic diversity that can generate changes in behavior and and potentially diseases.



Linker, Gage and Bedrosian, *the Scientist* 2017

Germline insertions can cause structural variants, deletions and sequence insertions within the human population



Disease:

First Evidence:
Hemophilia A resulting from de novo insertion of LINE1 sequences. Kazazian *et al* ***Nature***, 1988.

To date, over 120 human diseases are associated with LINE1 events.

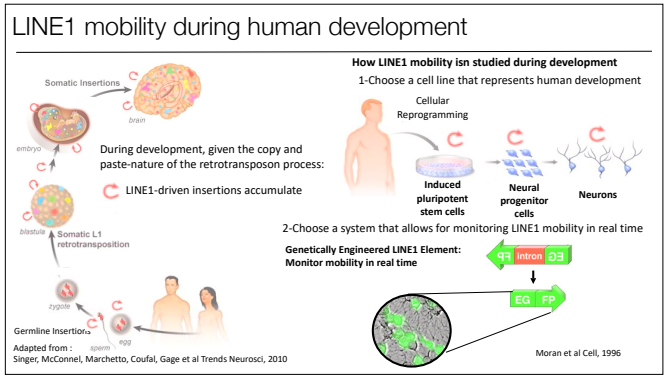
Detecting recent (and relevant) events of LINE1 mobility in humans, prompted the field to look for when during development these insertions were happening and for many years it was thought that the insertions were only happening in the germline.

However, work from us and others have shown that new Line1 insertions happen during embryonic development and adulthood. Hence the idea that we are all walking mosaics.
WE ARE ALL “GENETIC MOSAICS” BUT ONLY TO LIMITD DEGRE AND ESPECILLY IN BRAIN AND TESTES…
JUMPING GENES ARE A VERY DANGEROUS LIABILITY TO GENOMIC INTEGRITY ND SUCCESSFUL MULTICELLULARITY

In the following slides I will show you examples of studies lead by me and others that used reprograming technology to study retrotransposon mobility and we will also speculate on the implications of LINE1 mobility for disease and human evolution.

Germline retrotransposons are a major source of structural variants, deletions and sequence insertions within the human population[11–15]. The vast majority of these germline variants have unknown functional effects. However, some variants are likely to have functional consequences for the individual. For example, although polymorphic insertions of retrotransposon sequences are abundant in the healthy human population, specific *de novo* retrotransposon insertions can cause haemophilia[16], neurofibromatosis[17] and other diseases. In addition to the insertion of the retrotransposon sequence, retrotransposition can mediate the deletion of the host DNA sequence[18]. Furthermore, retrotransposon events can result in the presence of highly homologous sequences in different genomic locations. These sequences can then recombine, through nonallelic homologous recombination, to cause deletions, duplications, inversions

and translocations.



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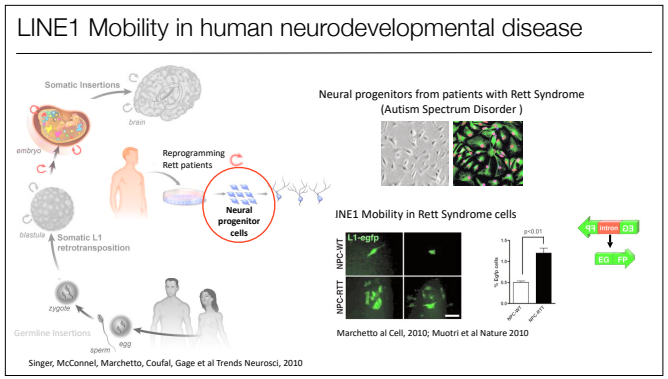
I WOULD STRESS: MOSAICISM MEANS THAT TWO NEIGHBROING NEURONS ARE NOT TOTALLY GENETICALLY IDENTICAL ANY MORE.

Putative implications of L1-mediated somatic mosaicism in the brain In a reversal of the commonly held belief that retrotransposition occurs primarily in the germline [83], it became clear that L1 elements are expressed in many somatic tissues, including the brain [7, 13, 84]. Recent evidence shows that L1 retrotransposition (curved red arrows) does not occur in the parental germline but in the soma during early embryonic development (colored dots), resulting in individuals that are genetically mosaic with respect to L1 composition [33]. It has been suggested, however, that L1 RNA may be transcribed in the parental germline and carried over in both male and female germ cells in the form of RNPs (black line with red dots) and integrated into the genome at the preimplantation stage [33] (colored spots); however, these events are probably rare, since retrotransposons are effectively silenced in the germline through a small RNA induced mechanism [78, 85]. Somatic L1 retrotranspositon events that occur during embryogenesis would result in clonal sectors of cells (colored patches) that carry the same insertion event. The size of clonal sectors depends on the developmental stage when the insertion occurred and the number of subsequent cell divisions. L1 insertion events that happen during embryonic brain development will be found in different brain regions (colored patches and dots), whereas events that happen during adult neurogenesis will be restricted to specific areas, such as the dentate gyrus (insert). According to our hypothesis, L1-induced mosaicism could increase variability in the brain (blue curve), which could have implications for behavioral phenotypes. The environment could influence regulation of somatic L1 retrotransposition in the brain and this influence could be mediated by epigenetic or hormonal mechanisms. Depending on its impact on the brain and the consequences, L1-induced somatic variability could either increase the risk for neurological disease or induce behavioral changes that could help the organism to better adapt to changing environments.



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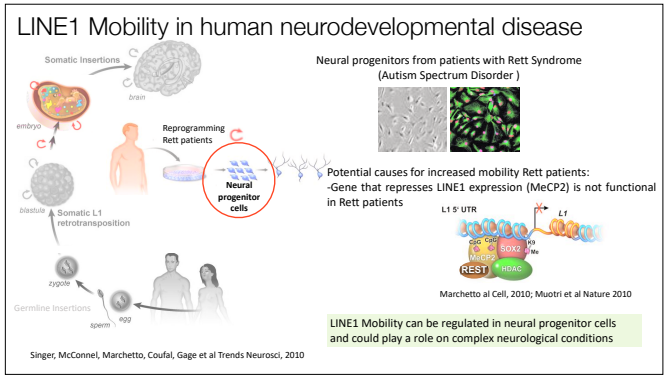
COULD THE BRAIN BENEFIT FROM INCREASED SOMATIC DIVERSITY CREATED BY JUMPING GENES?



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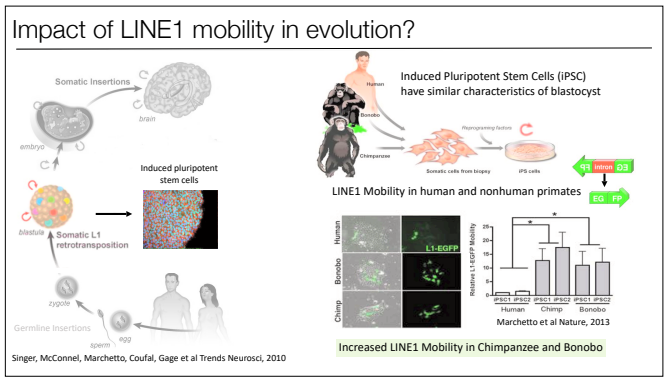
RETT'S DEMONSTRATED THE HUGE DANGER OF UNCRONTROLLED, EXCESSIVE JUMPING



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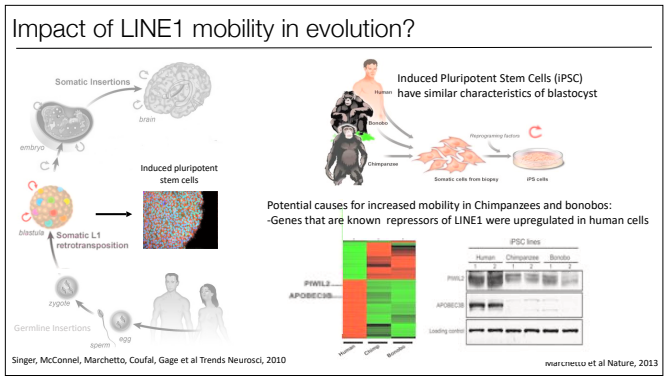

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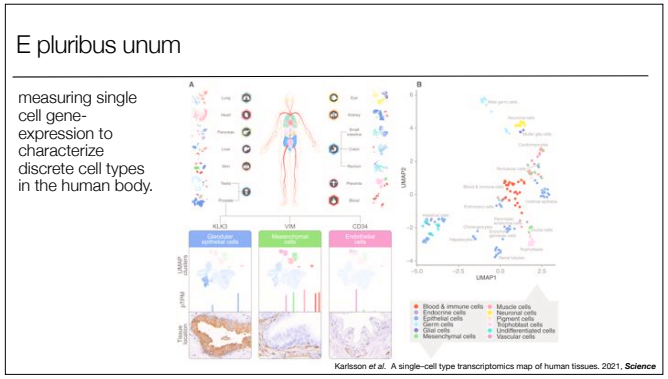
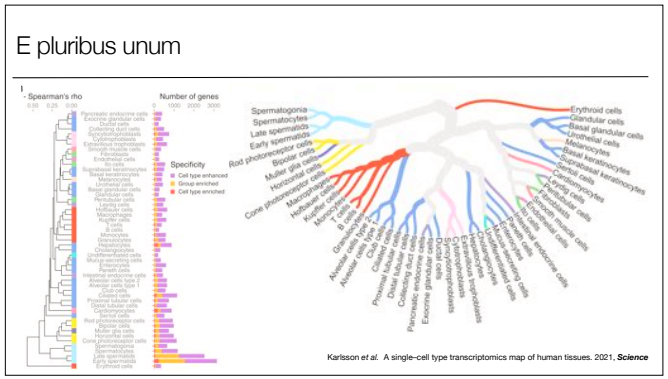
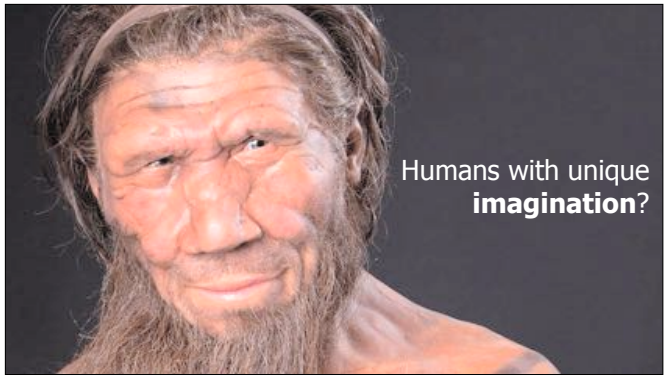
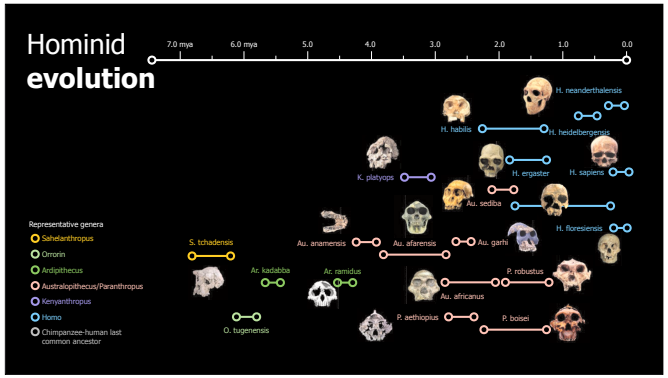
LINE1 ACTVITY (THEIR JUMPING) IS REPRESSED MOREN HUMANS THAN IN APES, IAM SURE THAT APES HAVE SOME LEVE OF REPRESSION AS WELL, OR THEIR GENOMES WOULD MELT….






Humans with unique
imagination?


Hominid
evolution
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3.0
2.0
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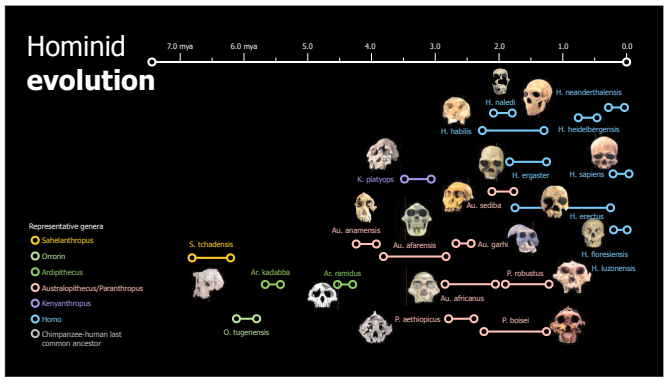
H. neanderthalensis
H. habilis
H. heidelbergensis
K. platyops
H. ergaster
H. sapiens
Au. sediba
H. floresiensis
Au. anamensis
Au. afarensis
Au. garhi
S. tchadensis
Ar. kadabba
Ar. ramidus
P. robustus
Au. africanus
P. aethiopius
P. boisei
O. tugenensis
Representative genera
Sahelanthropus
Orrorin
Ardipithecus
Australopithecus/Paranthropus
Kenyanthropus
Homo
Chimpanzee-human last common ancestor


Hominid
evolution
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6.0 mya
5.0
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3.0
2.0
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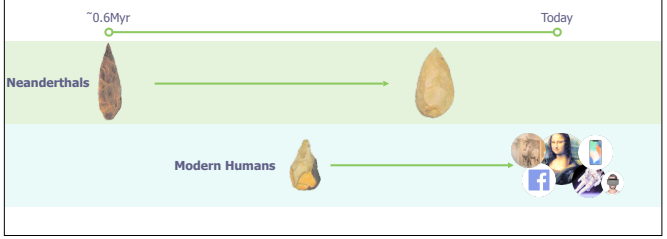
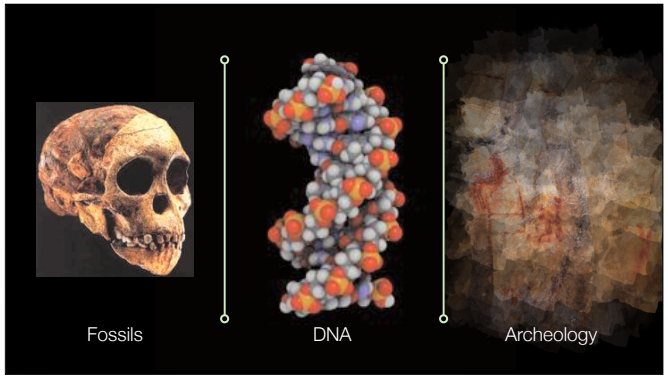
H. naledi
H. neanderthalensis
H. habilis
H. heidelbergensis
K. platyops
H. ergaster
H. sapiens
Au. sediba
H. erectus
Au. anamensis
Au. afarensis
Au. garhi
H. floresiensis
H. luzonensis
S. tchadensis
Ar. kadabba
Ar. ramidus
P. robustus
Au. africanus
P. aethiopicus
P. boisei
O. tugenensis
Representative genera
Sahelanthropus
Orrorin
Ardipithecus
Australopithecus/Paranthropus
Kenyanthropus
Homo
Chimpanzee-human last common ancestor

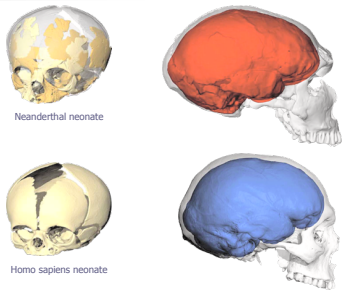
Arts, Technology and Adaptation





Neanderthal and Modern Human

There brains might have had different ways of processing information.

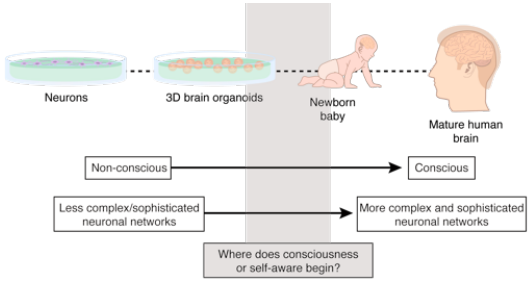


Gunz et al, 2010

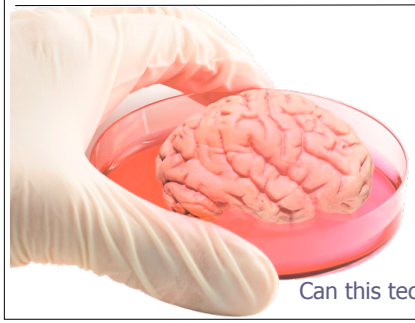
Fossil records used for genome sequencing

Svante Paabo

Where does cognition begin?



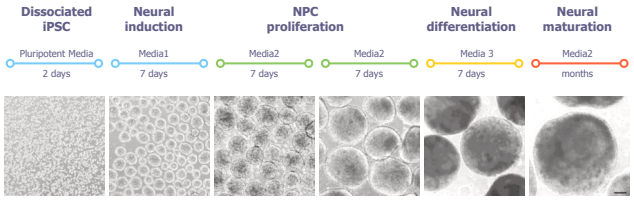
Brain models in a dish?

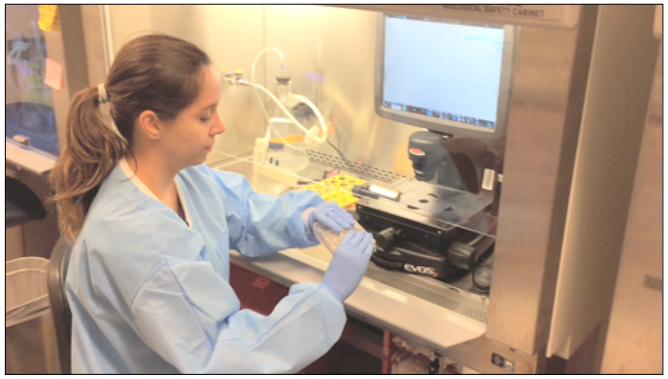


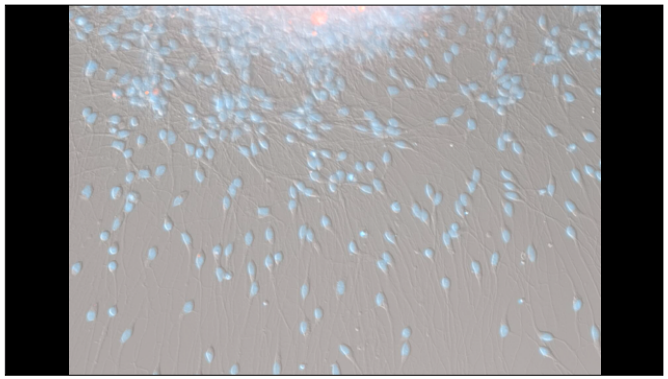
*Immature neurons
*Not vascularized
*Not all cell types
*Not ideal culture
*Translational?
*Etc…

Can this technology be disruptive?

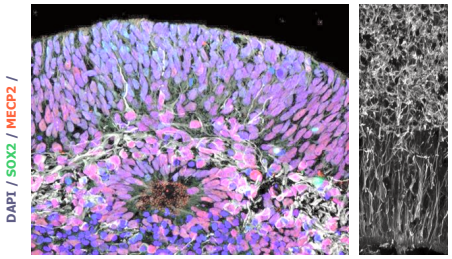
Muotri lab brain organoid recipe







Radial and tangential pattern

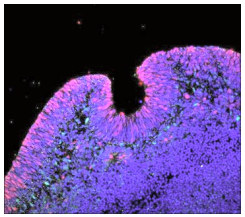


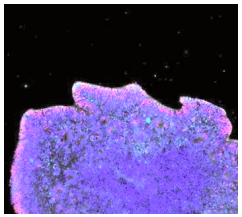
DAPI stains cell nuclei.

Staining for SOX2 in green: SOX2, is a transcription factor that is essential for maintaining self-renewal, or pluripotency, of undifferentiated embryonic stem cells. Sox2 has a critical role in maintenance of embryonic and neural stem cells.

MECP2 (methyl CpG binding protein 2) is a gene that encodes the protein MECP2. MECP2 appears to be essential for the normal function of nerve cells.

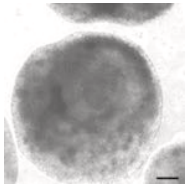
They fold

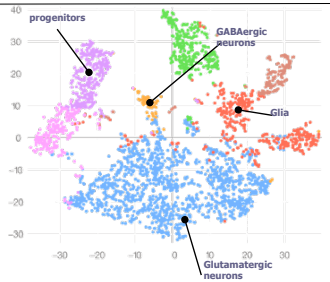




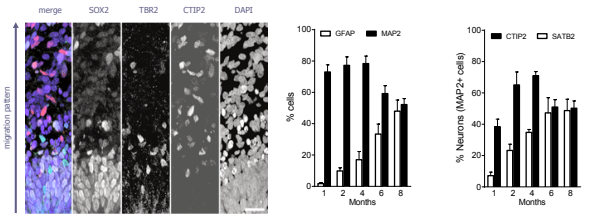
NeuN also non as Fox 3 is a neuronal nuclear antigen that is commonly used as a biomarker for neurons.
Antigen KI-67 is a nuclear protein that is associated with cellular proliferation.
Syn1 (Syngap1) is aprotein that is critical for the development of cognition and proper synapse function. Mutations in humans can cause intellectual disability, epilepsy, autism and sensory processing deficits.

Brain organoid at 4 months





Population analyses



MAP2, microtubule also cited protein 2. This gene encodes a protein that belongs to the microtubule-associated protein family.

CTIP2 transcription factor expressed by subconical projecting neurons

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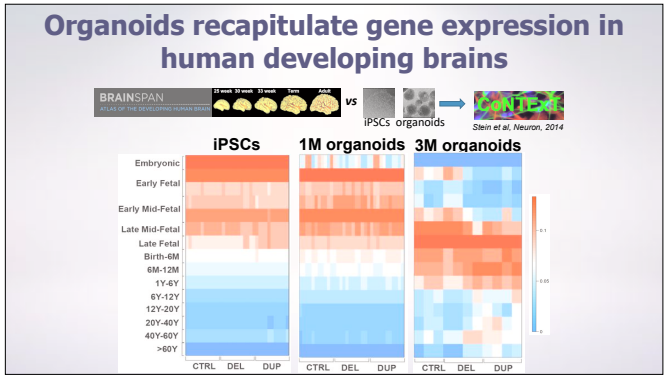
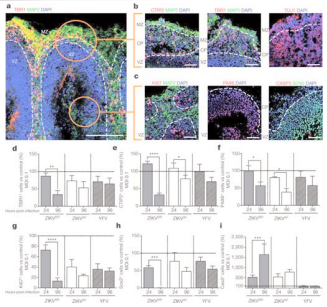


ZIKV^{BR} affects cortical layers in organoids



reconstruction of Neanderthal burial at La Chapelle aux Saints, Southern France (~50 000 year old)

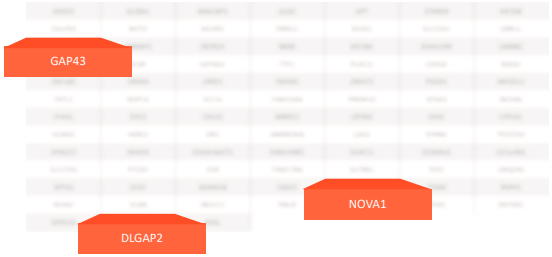
Reconstruction of a Neanderthal by Kennis brothers admired by modern human in London's Natural History museum.s

Catalog of human-specific variant

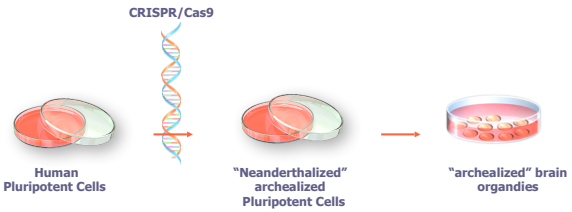
DNA differences with effects on protein sequence

DDX53	SLC8A1	IRAK1BP1	GLDC	GPT	STARD9	KIF26B
CXorf59	NOTO	MCHR2	FRRS1L	DCHS1	SLC12A1	GRB1L
GAP43	ANKMY1	ZBTB24	NEK6	KIF18A	KIAA1199	LMNB2
FRMD7	SCAP	KATNA1	TTF1	PLAC1L	CDH16	RASA1
ZNF185	ORSK4	LRRD1	FBXW5	ZNHIT2	PIE201	MFSD12
TKTL1	NOP14	KLF14	FAM166A	PRDM10	SPAG5	NCOA6
IFI44L	EVC2	CALD1	ARRDC1	LRTM2	SSH2	LYPLA1
VCAM1	HERC5	ERI1	ANKRD30A	LAG3	SYNRG	TP53TG5
SPAG17	DHX29	CSGALNACT1	FAM149B1	SCAF11	CD300LG	C21orf62
SLC27A3	PTCD2	GSR	FAM178A	SLITRK1	TEX2	UBQLN3
SPTA1	SV2C	ADAM18	CASC5	NOVA1	ITGB4	RSPH1
NFASC	VCAN	RB1CC1	PNLIP	TTLL5	RFNG	ENTHD1
GPR132	DLGAP2	ADSL				

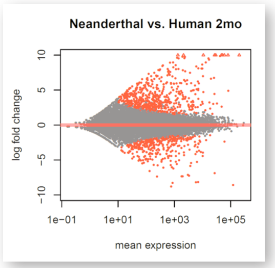
3 key genes highly expressed in neurodevelopment and mental disorders

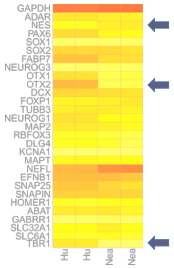


Re-constructing the Neanderthal brain

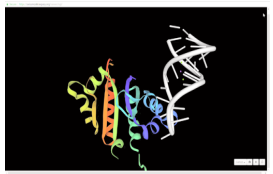


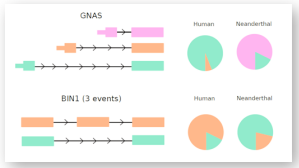
Altered gene expression



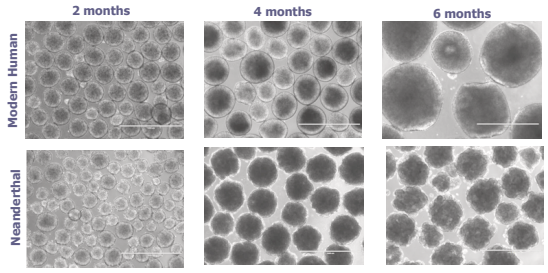


166 differential splicing rates

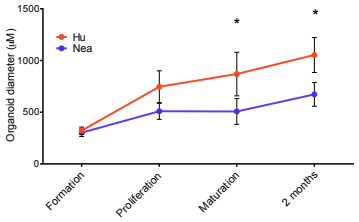




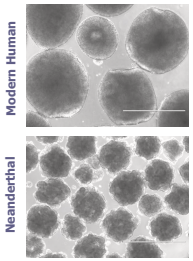
Distinct Neurodevelopment

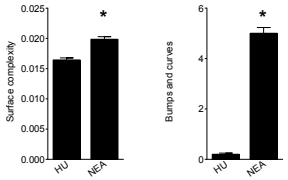


Distinct Neurodevelopment

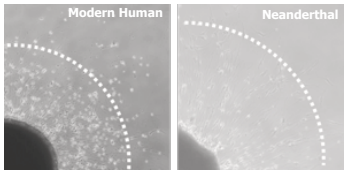


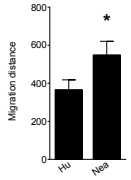
Surface dynamics



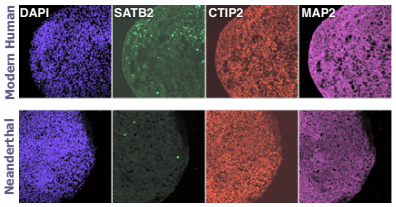


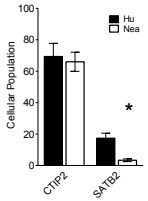
Differential neuronal migration

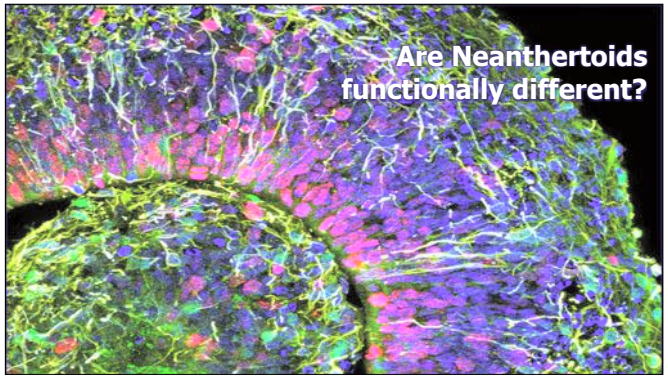




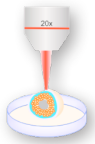
Cortical formation

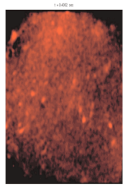


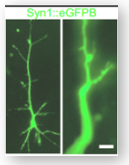




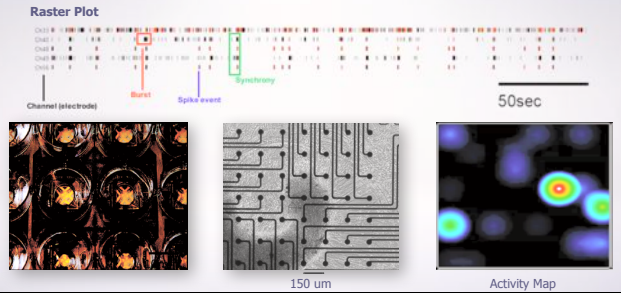
Organoid single-cell level activity (Voltage-dependent dye)



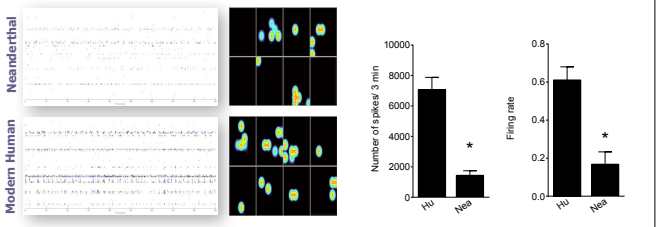




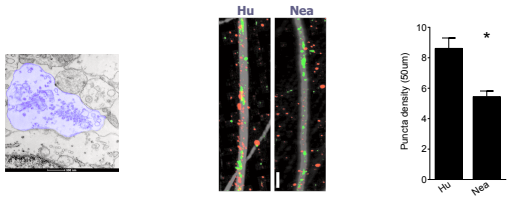
Organoid network activity (MEA)

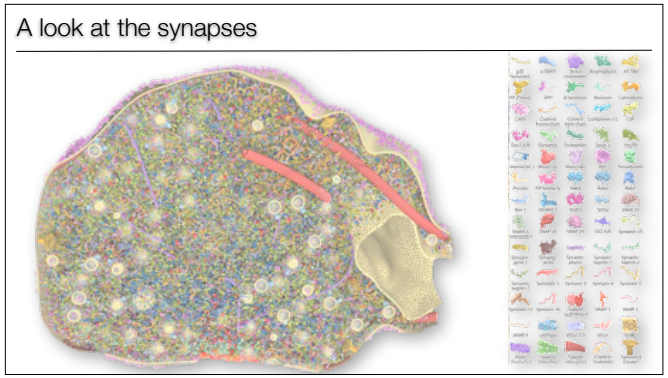


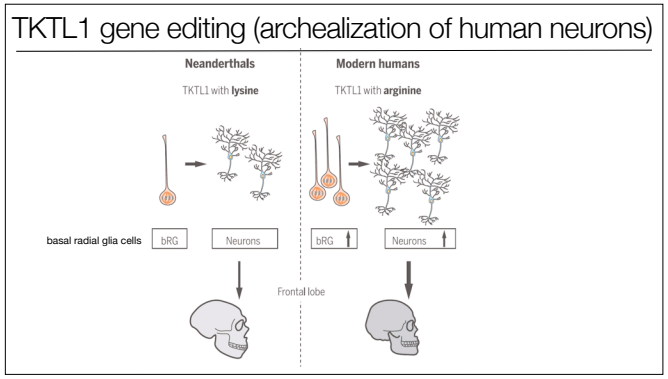
Reduced network neuronal connectivity



Differences in synaptogenesis







TKTL1 and hominin cortical neurogenesis. The single lysine-to arginine substitution in modern human TKTL1 leads to greater bRG numbers than in Neanderthals. These bRG in turn generate more neocortical neurons in modern humans. Because TKTL1 expression in fetal human neocortex is **particularly high in the developing frontal lobe**, these findings imply that the frontal lobe of modern humans contains more neurons than that of Neanderthals.

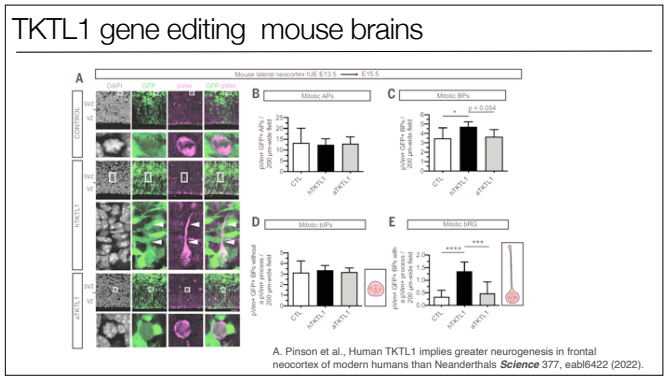


Fig. 1. Modern human TKTL1, but not archaic TKTL1, when expressed in embryonic mouse neocortex,
increases mitotic bRG abundance. Mouse neocortex E13.5 IUE with GFP plasmid, together with either
empty (control, CTL), hTKTL1, or aTKTL1 plasmid; analyses: E15.5. (A) GFP/pVim (green/magenta)
immunofluorescence plus DAPI staining (gray). Bottom rows, white boxed areas at higher magnification;
dashed boxed areas: GFP+/pVim+/BP without pVim+ process (mitotic bIP);
solid boxed area: GFP+/

pVim+/BP with pVim+ basal process (arrowheads) (mitotic bRG). Scale bar, 40 mm. (B to E) Quantifications
in 200-mm-wide fields. Means of 8 embryos. Error bars, SD. (B) pVim+ GFP+ mitotic APs. One-way analysis
of variance (ANOVA). (C) Total pVim+/GFP+/BPs. One-way ANOVA with Tukey post hoc test, *P < 0.05.
(D) pVim+/GFP+/BPs without pVim+ process (mitotic bIPs). One-way ANOVA. (E) pVim+/GFP+/BPs with
pVim+ process (mitotic bRG). One-way ANOVA with Tukey post hoc test, ****P < 0.0001, ***P < 0.001. Here
and in the remaining figures, data reported without P values are not significant.

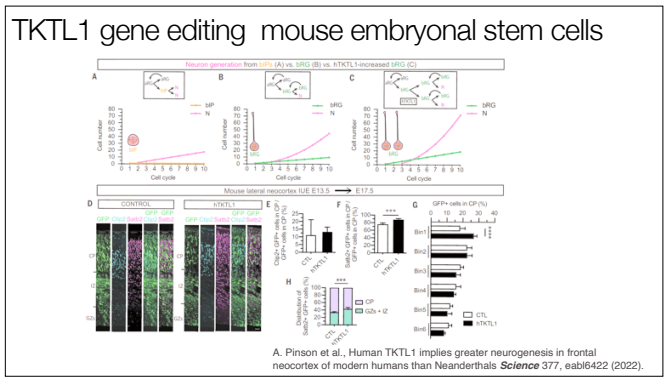


Fig. 2. The hTKTL1-induced increase in bRG in embryonic mouse neocortex results in increased production of cortical neurons, notably upper-layer neurons, at late neurogenesis. (A to C) Modeling of the number of neurons generated, over 10 cell cycles, by the aRG → bIP → neuron lineage (A), the aRG → bRG → neuron lineage (B), or the aRG → bRG → neuron lineage with one round of hTKTL1-induced symmetric proliferative bRG division (C). Curved
arrows denote self-renewal. (A) and (B) are adapted from figure S4 of (12). (D to
H) Mouse neocortex E13.5 IUE with GFP plasmid, together with either empty

(control, CTL) or hTKTL1 plasmid; analyses: E17.5. (E) to (H) are means of 5 embryos. Error bars, SD. (D) GFP/Ctip2/Satb2 (green/cyan/magenta) immunofluorescence.
Scale bar, 30 mm. [(E) and (F)] Percentages of GFP+ cells in CP that are Ctip2+ (E) and Satb2+ (F). Unpaired Student's t test, (F) ***P < 0.001. (G) Percentages of the GFP+ cells in CP that are in bins 1 to 6 (bin 1, uppermost layer;
bin 6, deepest layer) of CP. Two-way ANOVA with Bonferroni post hoc test, ****P < 0.0001. (H) Distribution of GFP+/Satb2+ cells in germinal zones (GZs) plus
intermediate zone (IZ) versus CP. Student's t test, ***P < 0.001.

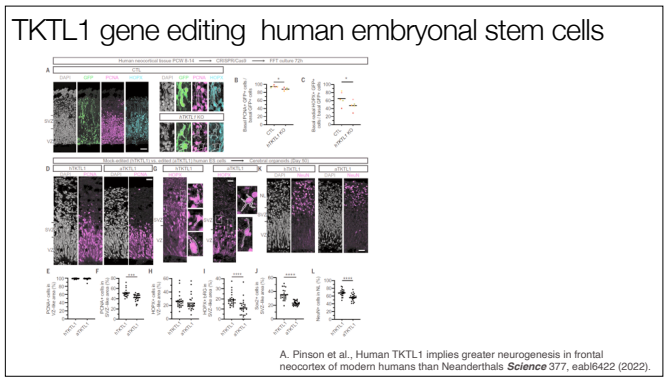


Fig. 4. TKTL1 KO in fetal human neocortical tissue and "Neanderthalized" TKTL1 in human ESC–derived cerebral organoids reveal that modern human TKTL1 is essential to maintain the full level of bRG and neurons.
(A to C) CRISPR-Cas9–mediated disruption of hTKTL1 expression in PCW 8 to
14 fetal human neocortical tissue. Ex vivo electroporation with GFP plasmid plus complexes of recombinant Cas9 protein and gRNAs targeting LacZ (control, CTL) or hTKTL1 (hTKTL1 KO), followed by 72 hours free-floating tissue (FFT) culture. (A) GFP/PCNA/HOPX (green/magenta/cyan) immunofluorescence

plus DAPI staining (gray). Left: CTL electroporation. Right: abventricular cells for CTL (top) and hTKTL1 KO (bottom). White dashed lines: cell morphology. CTL: GFP+/PCNA+/HOPX+ radial cell (bRG), GFP+/PCNA+/weakly HOPX+ cell (multipolar in different optical section, bIP). hTKTL1-KO: multipolar GFP+/PCNA+/HOPX– cell (bIP, top cell), multipolar GFP+/PCNA–/HOPX– cell (neuron, bottom cell). Scale bar, 50 mm. [(B) and (C)] Percentages of basal GFP+ cells that are PCNA+ (B) and HOPX+ with radial morphology (C). Means of 5 different fetal samples. Paired Student's t test, *$P < 0.05$. (D to L) Human embryonic stem cells (ESCs, H9 line) were CRISPR-Cas9–mediated genome-edited to convert hTKTL1 (Arg) to aTKTL1 (Lys). Organoids grown from two mock-edited (hTKTL1-1, hTKTL1-2) and two edited (aTKTL1-1, aTKTL1-2) lines; analyses: day 50. (D) PCNA (magenta) immunofluorescence plus DAPI-staining (gray). Scale bar, 25 mm. [(E) and (F)] Percentages of PCNA+ cells in VZ-like (E) and SVZ-like (F) areas. Means of 21 hTKTL1 (9 hTKTL1-1, 12 hTKTL1-2) and 23 aTKTL1 (12 aTKTL1-1, 11 aTKTL1-2) organoids. Mann-Whitney U test, ***$P < 0.001$. (G) HOPX (magenta) immunofluorescence. Right sides: white boxes at higher magnification; dashed lines: radial cells; solid line: multipolar cell. Scale bar, 25 mm. [(H) and (I)] Percentages of HOPX+ cells in VZ-like area (H) and HOPX+ radial cells in SVZ-like area (I). Means of 24 hTKTL1 (11 hTKTL1-1, 13 hTKTL1-2) and 27 aTKTL1 (19 aTKTL1-1, 8 aTKTL1-2) organoids. Mann-Whitney U test, (I) ****$P < 0.0001$. (J) Percentages of Sox2+ cells in the VZ-like area. Means of 21 hTKTL1 (9 hTKTL1-1, 12 hTKTL1-2) and 23 aTKTL1 (12 aTKTL1-1, 11 aTKTL1-2) organoids. Mann-Whitney U test, ****$P < 0.0001$. (K) NeuN (magenta) immunofluorescence plus DAPI-staining (gray). Scale bar, 25 mm. (L) Percentage of NeuN+ cells in neuronal layer (NL). Means of 23 hTKTL1 (9 hTKTL1-1, 14 hTKTL1-2) and 26 aTKTL1 (12 aTKTL1-1, 14 aTKTL1-2) organoids. Unpaired Student's t test, ****$P < 0.0001$.

Summary

Cells are the units of animal bodies.
Keeping animal cells alive in culture has been a huge challenge for biologists.
From the very beginning, cell and tissue culture was linked to the production of vaccines.
Before the development of cell culture of immortalized cells, huge numbers of animals were required (millions of macaques from India for the Salk Polio vaccine.
Cancerous and virus transformed mammalian cells were developed in the 1950s and revolutionized research and vaccine production.
They also came with the risk of contamination, by both, cells (HeLA) and viruses (SV40).
Stem cell research allowed to follow how stem cells differentiate into most different cell types and tissues.
Induced pluripotent stem cells (IPS) derived from non- or minimally invasive samples now allow to study these processes.
Genetic manipulation of such cells allows to test for the effect of genetic variants in the context of human origins and human health and disease.