

SOT Symposium (virtual)
‘Developmental Toxicity Hazard Assessment without Animals: Pathways and Prospects’
Tuesday, March 16, 2021

Synthetic Microsystems, Computational Intelligence, and Artificial Life

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DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

Disclosures

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<https://stemcells.nih.gov/research/registry.htm>

Funding: our research with human pluripotent stem cell lines (hPSCs) was performed under EPA's *Chemical Safety for Sustainability Research Program, Research Area 5 'Virtual Tissue Models' (VTMs)*.

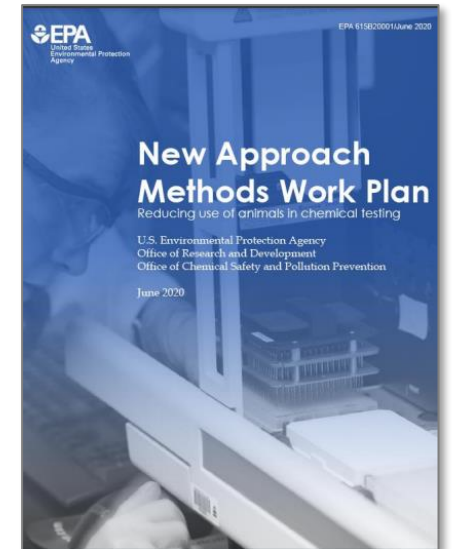
Compliance: work involving established hPSC lines is compliant with Executive Order 13505 (issued 2009) to ensure that is ethically responsible, scientifically worthy, and conducted in accordance with applicable law.

The H9 cell line is registered in the NIH Human Embryonic Stem Cell Registry: WA09 (H9): NIH Approval Number: NIHhESC-10-0062 (EPA contract EP-D-13-055 with Stemina Biomarker Discovery).

Other pluripotent stem cell lines: endodermal hPSC line from Allele Biotech #ABPSC-HDFAIPS (EPA contract EP-D-13-054 with Vala Sciences, Inc.).

Shifting toxicity testing to animal-free alternatives

- **June 2016:** *Frank R. Lautenberg Chemical Safety for the 21st Century Act* enacted to advance chemical safety evaluations with novel methods that reduce testing on vertebrate animals and are translatable to vulnerable populations / lifestages.
- **September 2019:** directive issued by USEPA Administrator Wheeler set a vision to reduce mammalian study requests 30% by the year 2025 and eliminate them by 2035.
- **June 2020:** USEPA work plan to accelerate scientifically valid *New Approach Methods* (NAMs) for assessing toxicity of large numbers of chemicals with less reliance on animal testing.
- **Science challenge:** build confidence in the predictive power of computational models and computer simulation for human-relevant pathways underlying developmental toxicity.



Developmental toxicity

- Observation of fetal outcome in pregnant animal studies, most often in testing rats and/or rabbits, is the accepted means of developmental hazard identification.
- A guideline prenatal developmental toxicity study (e.g., OECD 414) is mechanistically complex, requires many animals, and potentially confounded by maternal effects.
- *In vitro* assays and *in silico* models that reflect key aspects of embryo-fetal development will be indispensable for NAM-based detection of developmental hazard potential.
- *In vitro* profiling of human pluripotent stem cell (hPSC) lines is an active area of investigation and one of the most promising alternatives to pregnant animal testing.

Novel features of PSC lines



- **Self-renewal:** cells replicate themselves indefinitely when cultured under appropriate growth factor conditions.
- **Pluripotency:** cells have the potential to form most of the different cell types comprising the embryo/fetus.
- **Autopoiesis:** capacity to self-organize into rudimentary tissues and more complex organoid structures.

PSC lines established from the embryoblast (mouse, human) can recapitulate **some** of the biology driving embryogenesis during the period covered by guideline prenatal studies (e.g., OECD TG 414).

Conceptual and practical considerations

[illegible]

Chemicals with Market Pharmaceutical Action / Specialty Uses

Chemicals in only a study returned, with 0 compounds that return either generic, specialty use

Market name (hyperlinked)

Study Count

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

Study Count

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[illegible]

- **Detailed literature review:** survey of extant ESC assays used to classify developmental toxicants:
 - chemical domain
 - biological domain
 - standardized protocols
 - reproducibility
 - biomarker readouts
 - predictive power.
- 1,533 records in PubMed reduced to 333 (AI for relevance) and 192 (manual curation).***

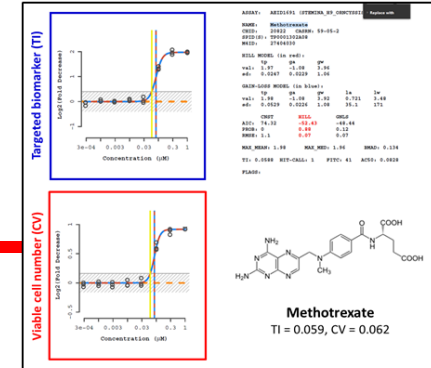
***1,533 records in PubMed
reduced to 333 (AI for
relevance) and 192
(manual curation).***

- **1,250 annotated chemicals (through 2020):**
 - 18 publications tested ≥ 10 compounds (primary)
 - 174 publications tested 1-9 (evidentiary support)
 - most frequently represented: ATRA, 5-FU, MTX.

*Abstract Sifter, SWIFT, MeSH terms, Chemicals
Dashboard, ...*

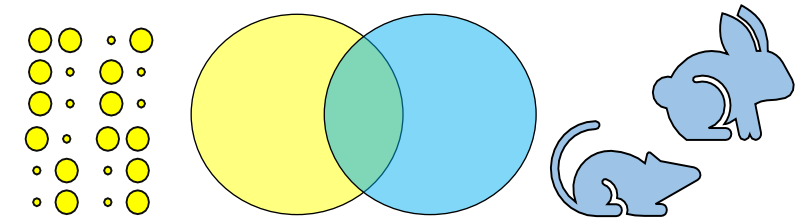
Piersma et al., manuscript in preparation

ToxCast HTS Profiling with the devTOX^{qP} Assay



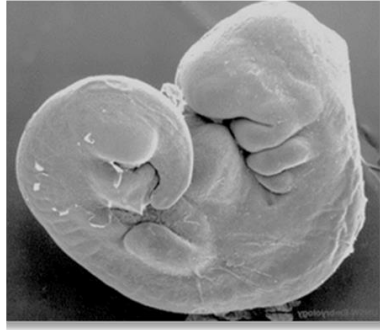
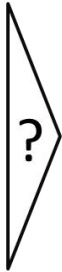
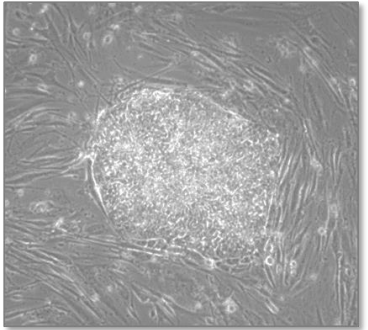
- Pluripotent human (H9) stem cell-based biomarker assay for developmental toxicity screening developed by Stemina Biomarker Discovery [*Palmer et al., 2013, BDRB*] ...
- ... defined developmental toxicity potential by the concentration of a test chemical reducing the ratio of ornithine (secreted) to cystine (utilized) to a critical level (77% accuracy).
- We used DevTOX^{qP} to test 1065 ToxCast chemicals for teratogenicity index (TI) and pipelined the dataset into EPA's CompTox Chemicals Dashboard [*Zurlinden et al., 2020, Toxicol Sci*] ...
- ... and observed a 19.2% positivity rate across the 1065 chemicals tested, with performance reaching 79%–82% balanced accuracy to well-curated teratogens and non-teratogens.

Bridging animal-human studies



- Query of prenatal developmental studies in EPA's ToxRefDB database found adverse fetal outcome for 53 of 283 (18.7%) chemicals tested in pregnant rats and rabbits.
- Profiling 1065 ToxCast chemicals with a pluripotent stem cell (hPSC) assay showed a 19.2% positivity rate for teratogenic potential [Zurlinden et al. 2020].
- Closely matches the 18.7% positivity rate from concordant animal studies, but only a subset of the positives are detected by both *in vitro* and *in vivo* platforms.
- Discordance: (i) biology missed by the hPSC platform; (ii) concurrence of fetal outcomes with maternal toxicity; (iii) mesoscopic properties of complex systems.

Can an hPSC assay live up to the NAM challenge?

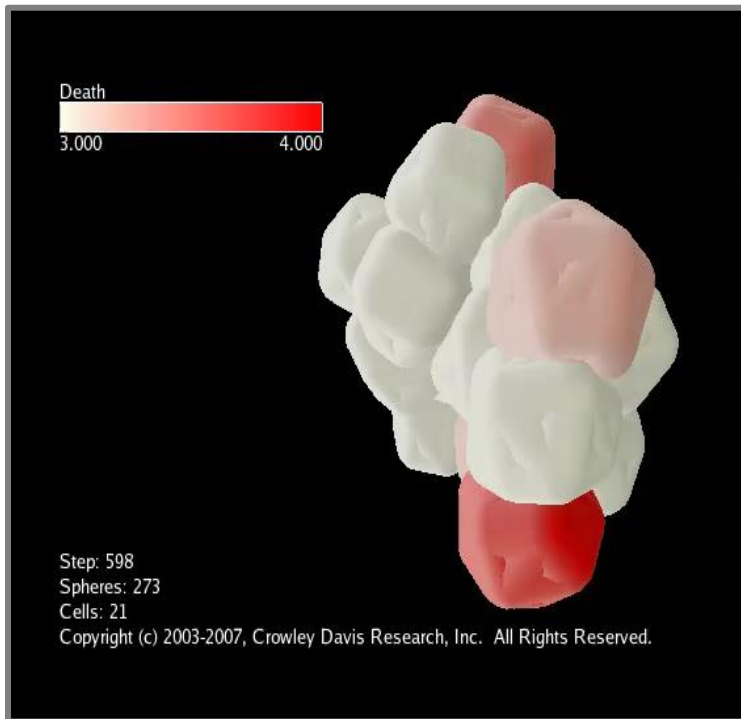


Motivation for building a more synoptic view to improve mechanistic understanding of developmental processes and toxicities around hPSCs.

- does not encompass the full complexity of anatomical development;
- blind to the precise spatial-temporal control of cell-cell interactions *in vivo* ;
- misses developmental effects secondary to maternal or placental toxicity;
- uncertainty of post-organogenesis vulnerability and post-natal manifestations;
- cross-species extrapolation (mESC to human, hESC to animals);
- limited xenobiotic metabolism and other ADME considerations (toxicokinetics);
- uncertainties in translatability to the intact embryo (toxicodynamics).

A more synoptic view ...

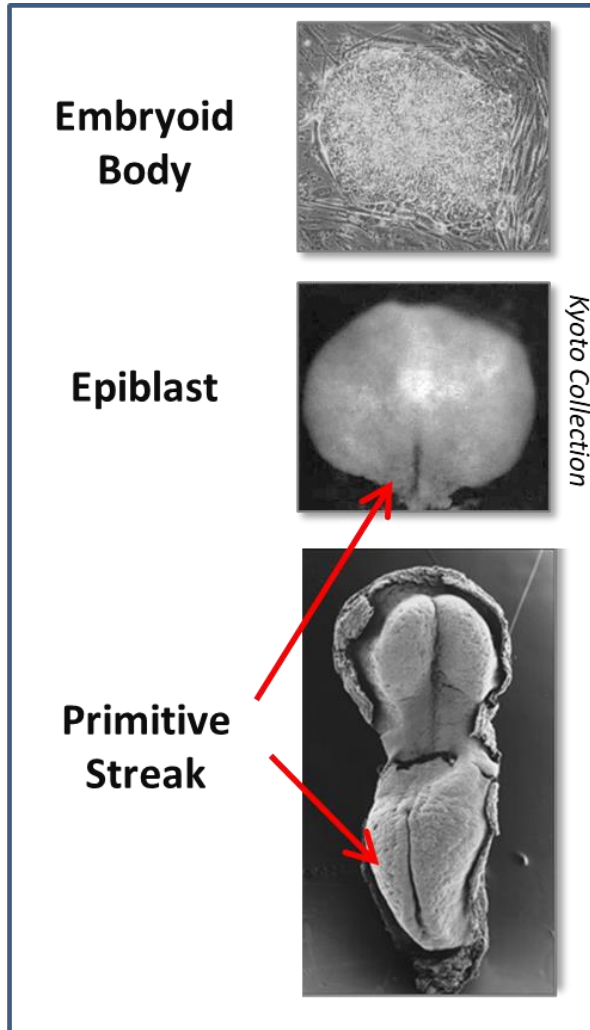
Anatomical homeostasis in a self-regulating 'Virtual Embryo'



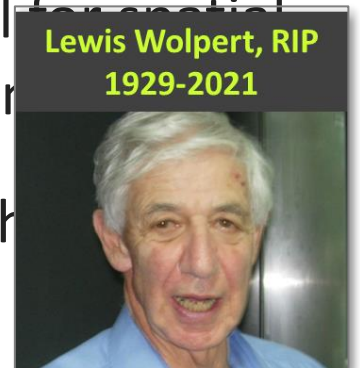
- **synthetic microsystems:** recapitulate the microphysiology and cellular behaviors of a physical system.
- **computational intelligence:** biological-inspired algorithms use fuzzy logic to fill in missing or incomplete information.
- **artificial life:** computer simulation of biological processes evolved through automation, control networks.

*SOURCE: Andersen, Newman and Otter
(2006) Am. Assoc. Artif. Intel.*

Gastrulating embryo: *remarkable example of a self-organizing system*



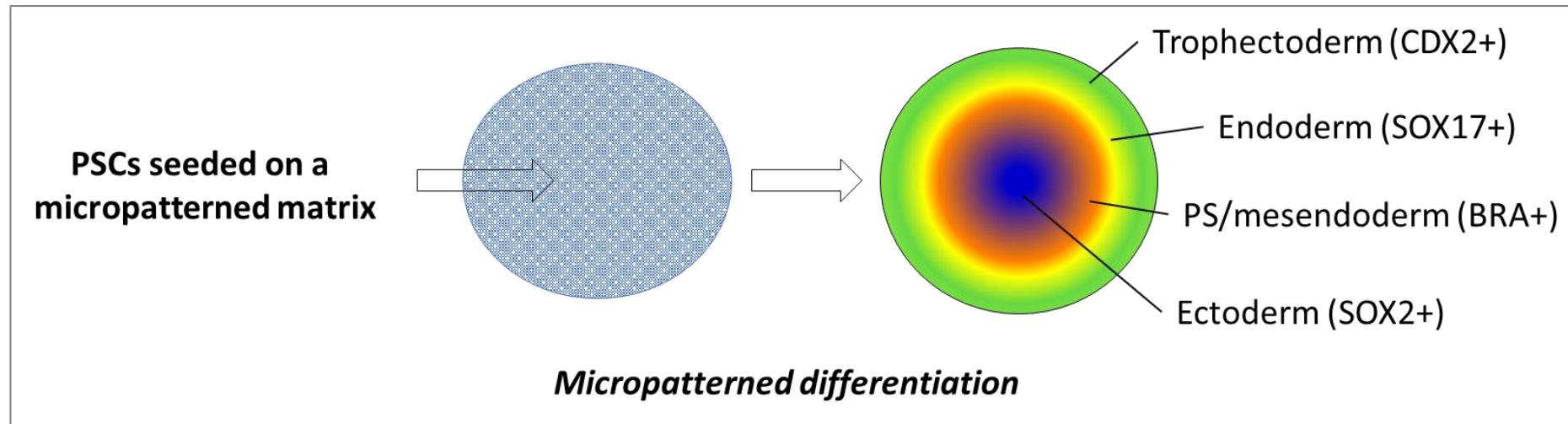
- The molecular biology and behavior of hESCs in culture most closely resembles the **epiblast** of an early embryo during 'gastrulation'.
- The hallmark of gastrulation in *Vertebrata* is **primitive streak** formation through which the genomic body plan is set up.
- Cell migration through the primitive streak is essential for germ cell organization, regional specification, and lineage determination.
- Although cultured hESCs can form most cell types in the body, they lack **positional information** of an intact epiblast.



"It is not birth, marriage, or death, but **gastrulation** which is truly the most important time in your life." - *Lewis Wolpert*

Geometric confinement

- Randomly seeded PSCs readily generate primary germ layers in culture; however, patterns of differentiation are heterogeneous and spatially disordered.
- PSCs differentiated on a micropatterned surface express lineage-specific markers and self-organize in symmetrical domains.



Cellular dynamics and signaling in the epiblast

Key regulatory signals

STATE 1 - Naïve Pluripotency (self-renewal)

LIF/STAT3

OCT4, SOX2, NANOG

PI3K/AKT/MEK/ERK

ESC pluripotency signal

pluripotency core triad

signal transduction

STATE 2 - Primed Pluripotency (patterning)

FGF4

BMP4

WNT3 *

NODAL

LEFTY1/CER1

maintains *Bmp4* in ExE (GD 5.5)

primes posterior cell fate

pinpoints PS & propels pAVE

induces primitive streak (GD 6.25)

NODAL antagonists in AVE

STATE 3 – Determination (gastrulation)

ACTIVIN A

FGF8

ATRA

...

mesoendoderm formation

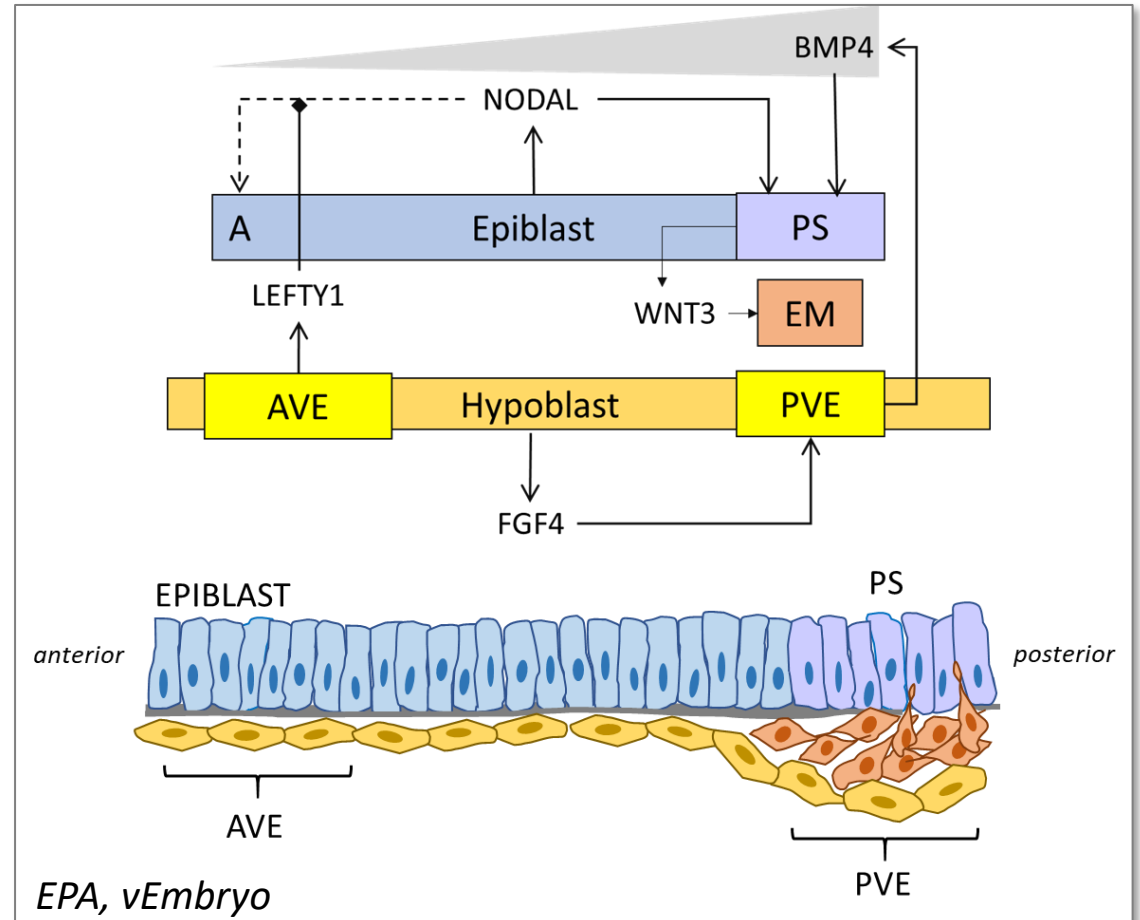
Hox clock

mutual antagonism with Fgf8

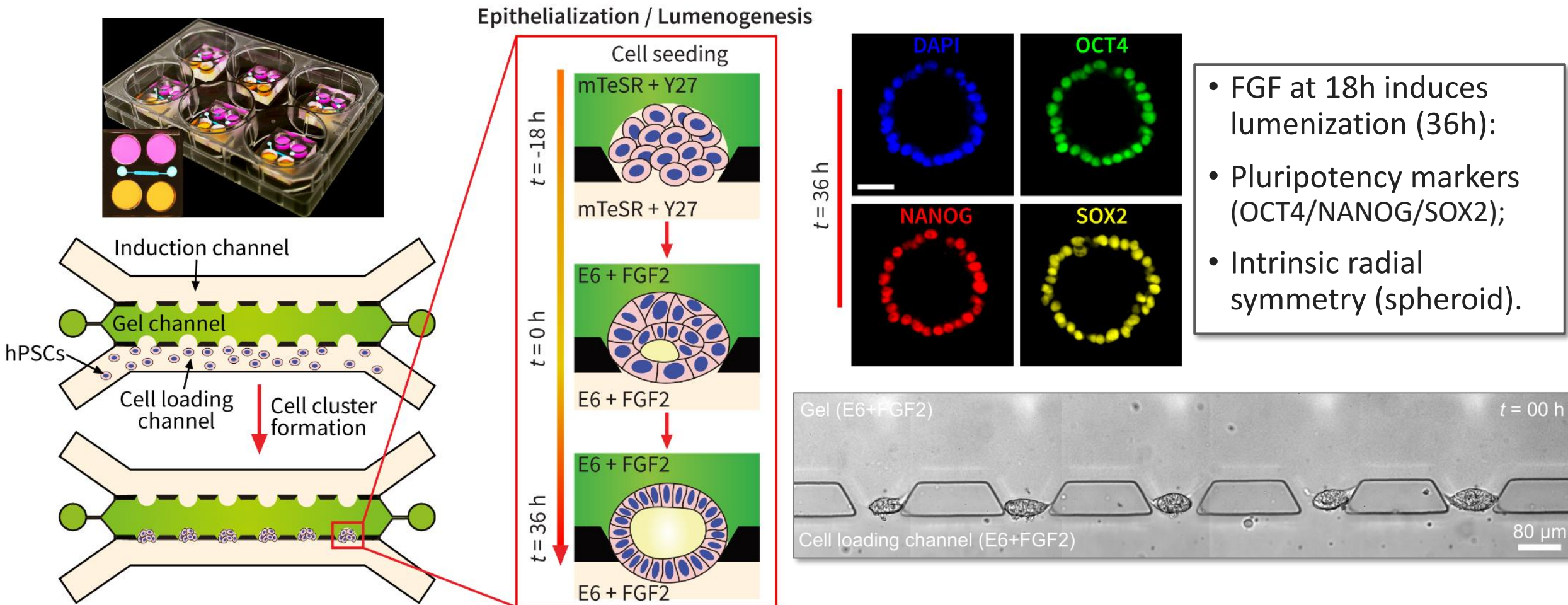
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SOURCE: Tam et al. (2006) Curr Opin Gen Dev

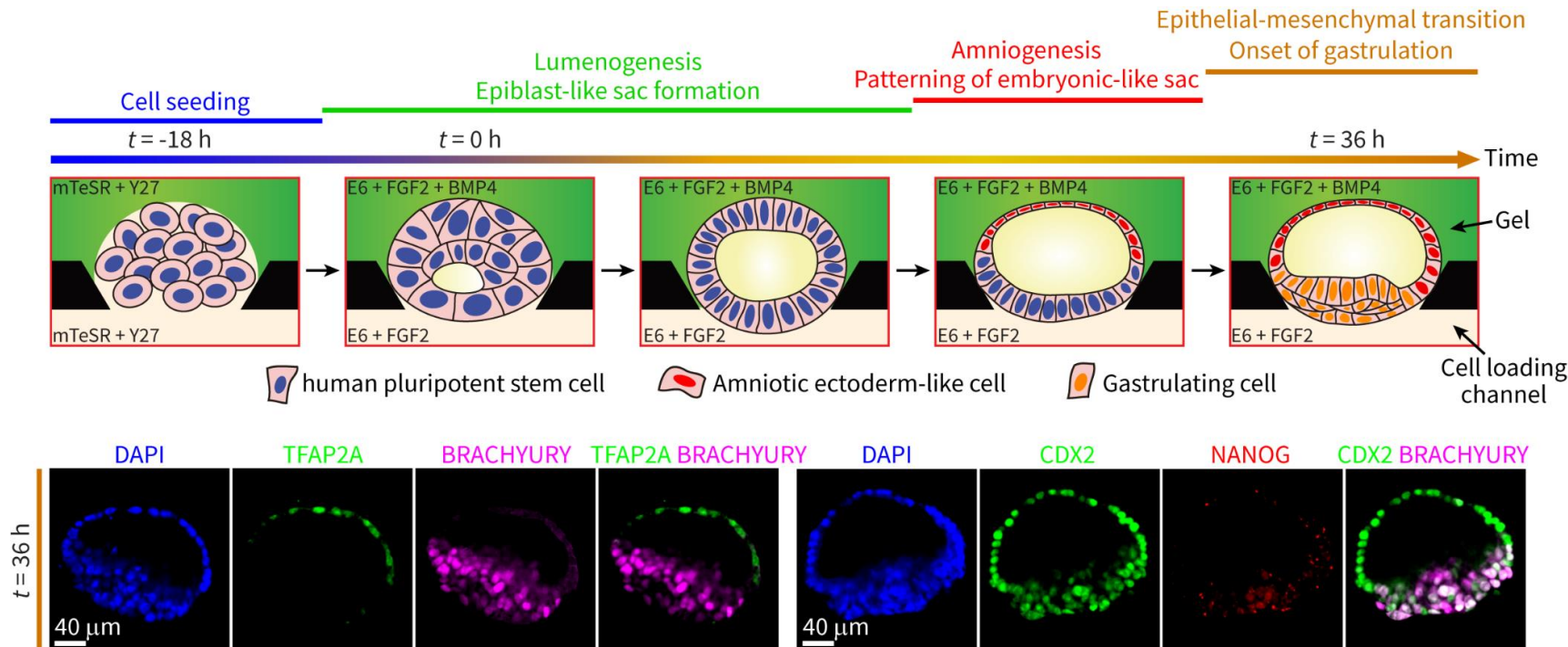
Morphological programming logic



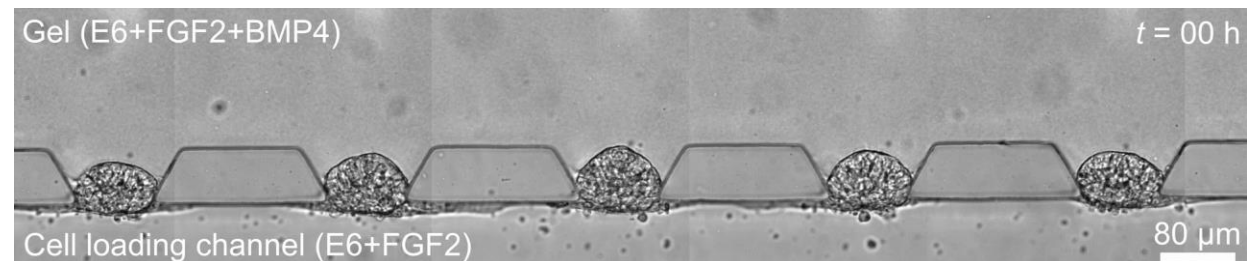
Synthetic epiblast: *microphysiological system*



Breaking the symmetry with BMP4

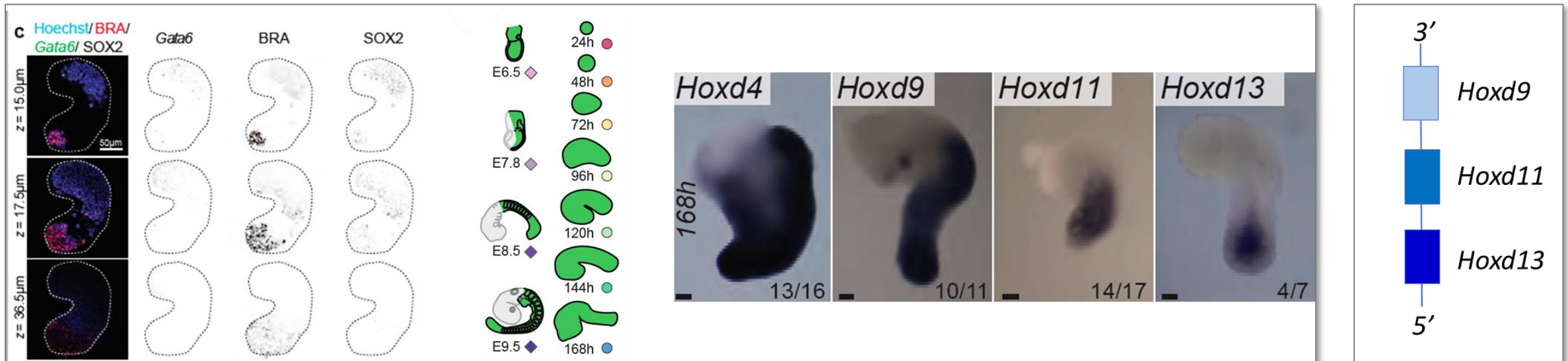


- BMP4 gradient breaks intrinsic symmetry.
- BMP4 primes posterior cell fate.
- Distinct axial domains emerge.
- Pluripotency advances to a determined state.
- But a bona fide primitive streak has not formed.



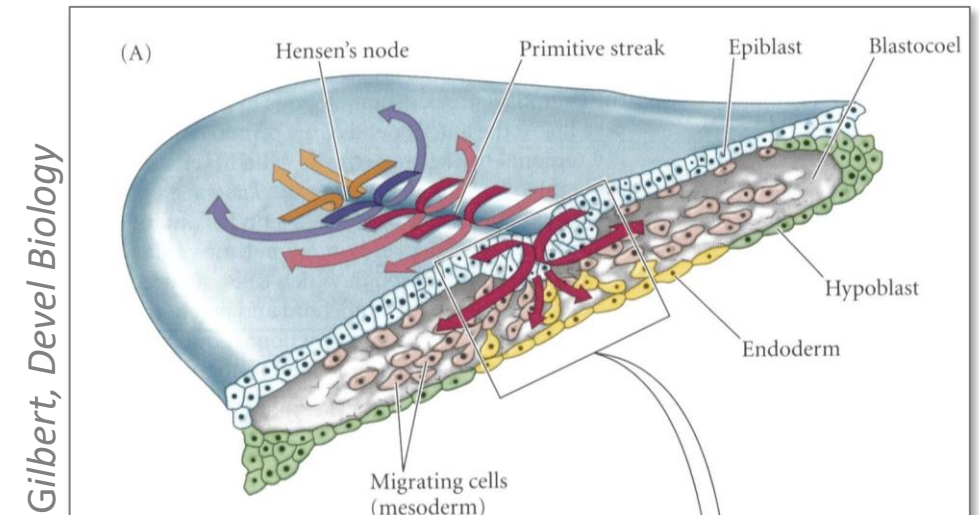
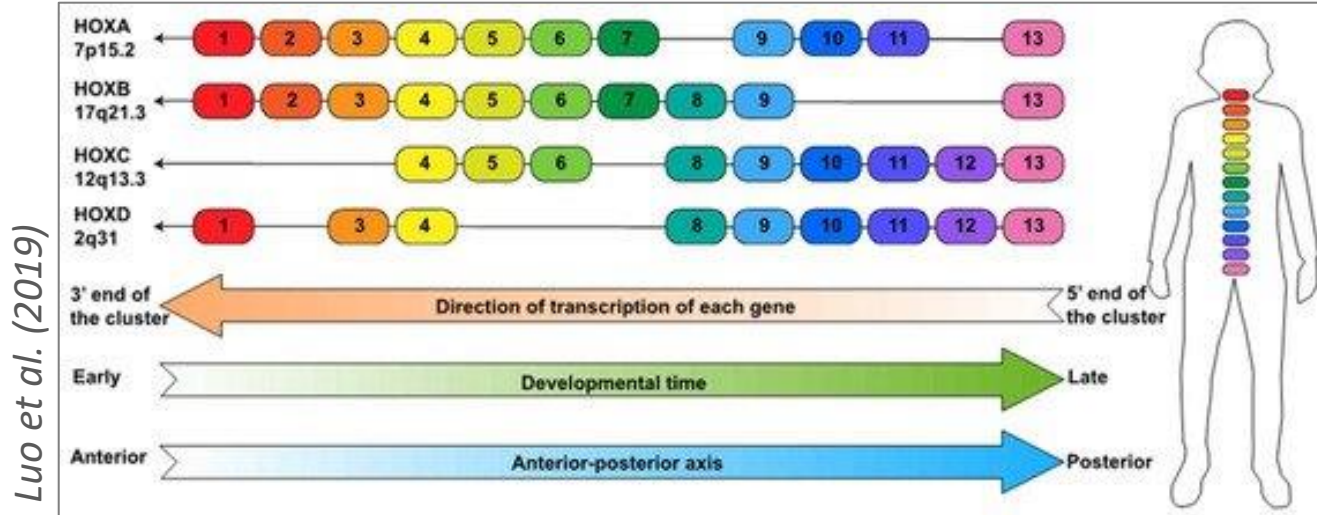
Gastruloids

- mESCs aggregated with defined numbers of cells and induced with extrinsic BMP4 may under certain conditions spontaneously organize axial structures (gastruloids).
- These display hallmarks of postcranial axial gene regulatory systems such as colinear Hox expression along an extending antero-posterior axis.



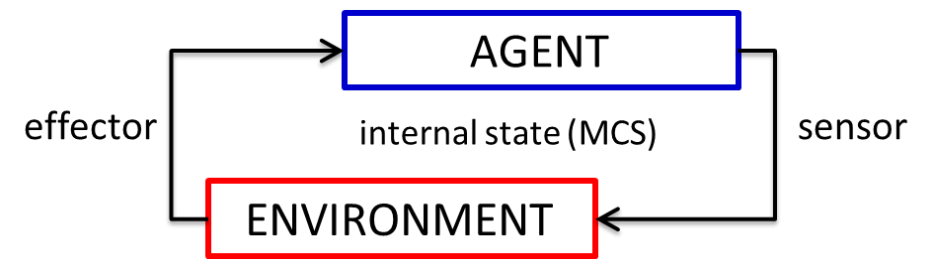
Positional Information and mesoderm formation

- HOX pattern is determined as epiblast cells pass through the primitive streak; still other extrinsic signals needed to position a primitive streak (e.g., NODAL, LEFTY1, WNT3).



- A→P fate of a cell is based on epiblast position, which determines when and where it ingresses through the primitive streak and into the endomesodermal population.

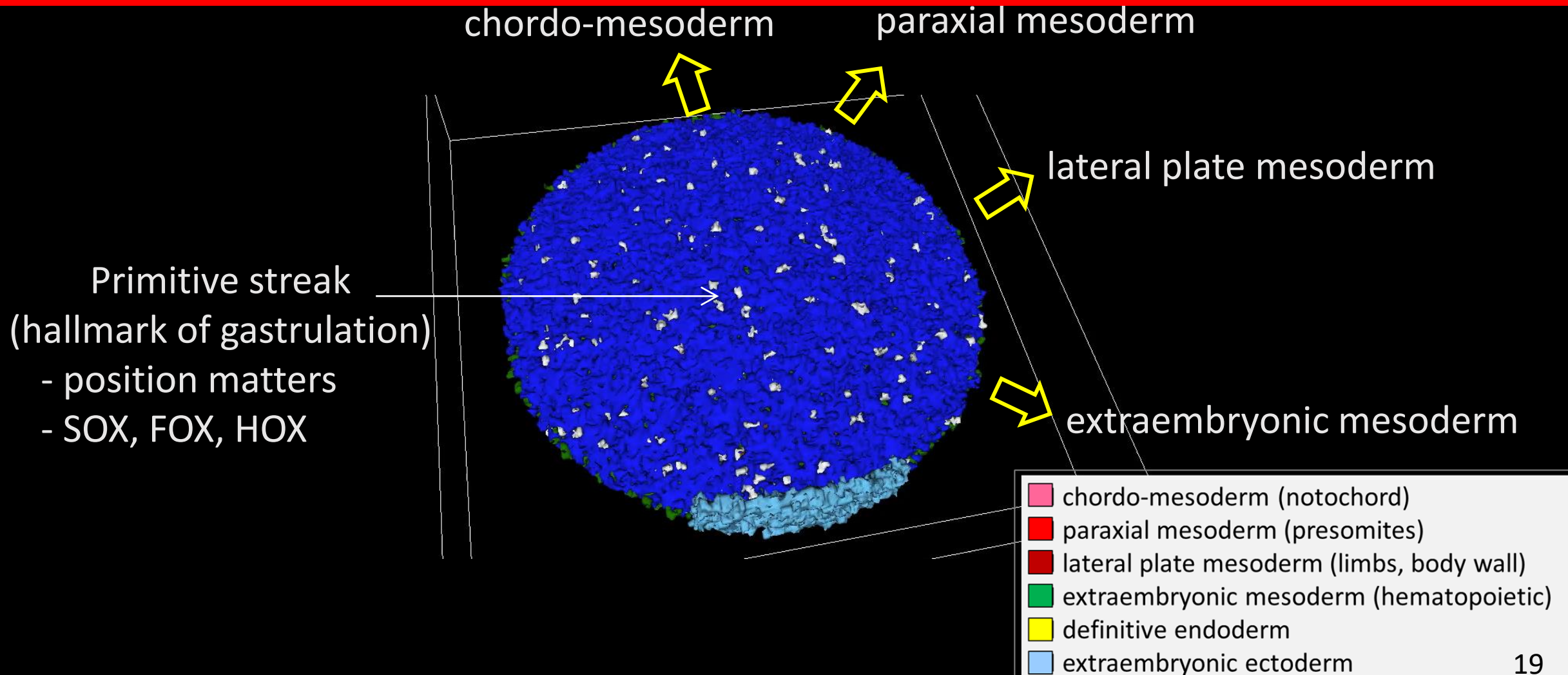
Agent-Based Models (ABMs)



- Nature-inspired *agents* (cells) and *rules* (behaviors) are set into motion as a self-organizing virtual system, using an open-source modeling environment ([CompuCell3d.org](https://compuCell3d.org)).
- Soft-computing uses ‘fuzzy logic’ to simulate forces or properties governing cell fate and behavior where rules are inexact or knowledge incomplete ([computational intelligence](#)).
- Can change course in response to a particular situation or stimulus, such as genetic errors or biomolecular lesions fed to the model from real world data ([dynamic translation](#)).
- Probabilistic rendering of where, when and how a particular condition might lead to an adverse developmental outcome ([cybermorphs](#)).

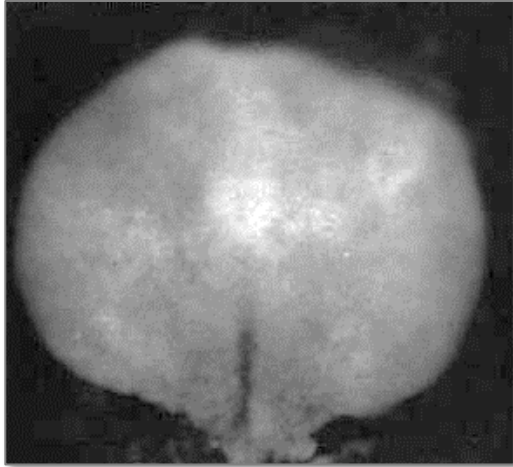
“Molecular biology took Humpty Dumpty apart ... mathematical modeling is required to put him back together again.” – Schnell et al. (2007) Amer Scientist

Quasi-gastrulation: *recoding the genomic blueprint of the fetal body plan?*

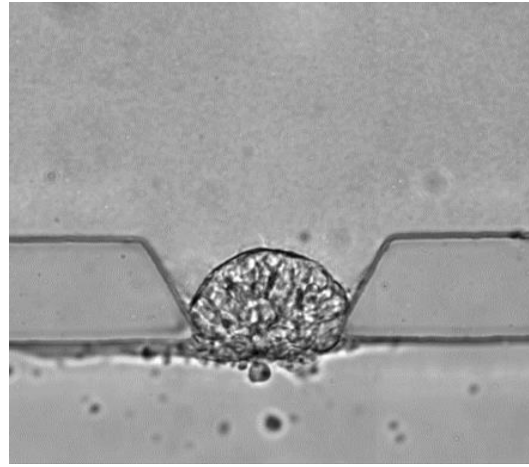


Practical use of a synoptic manifold

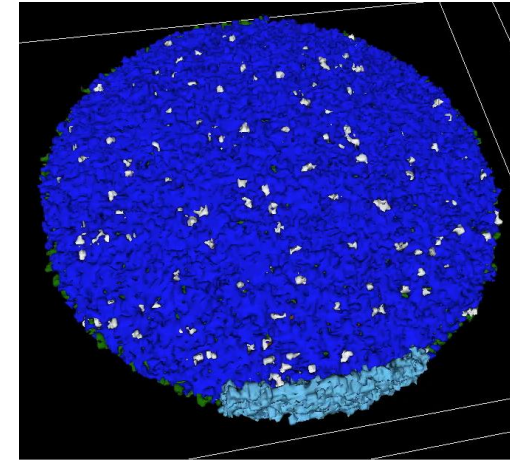
In vivo



In vitro

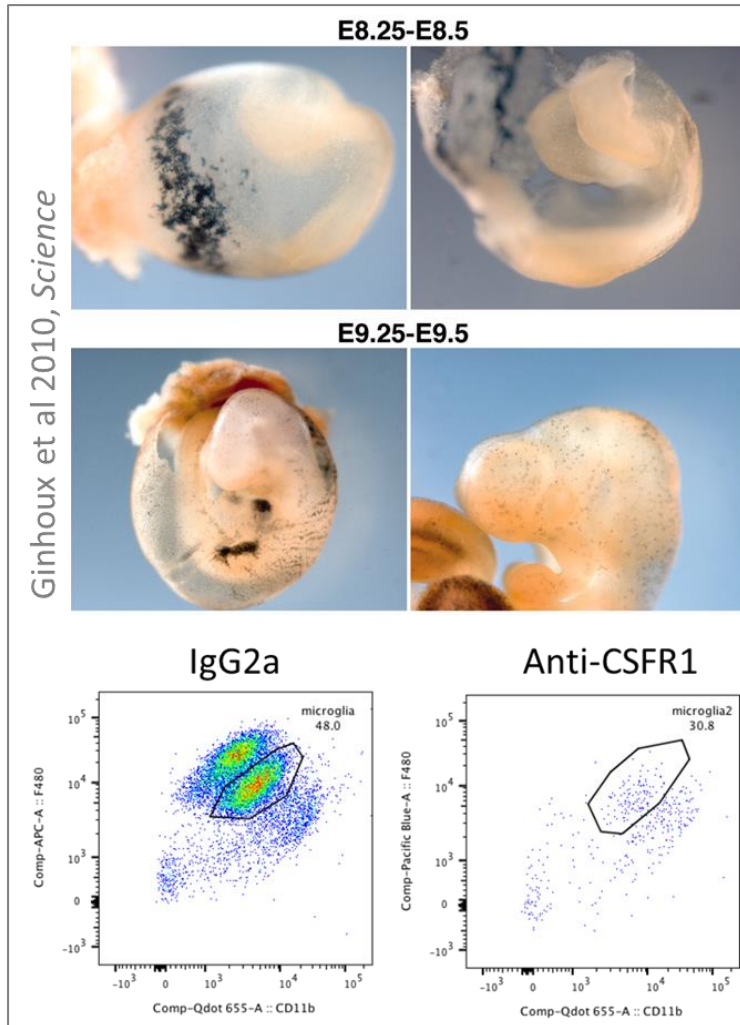


In silico



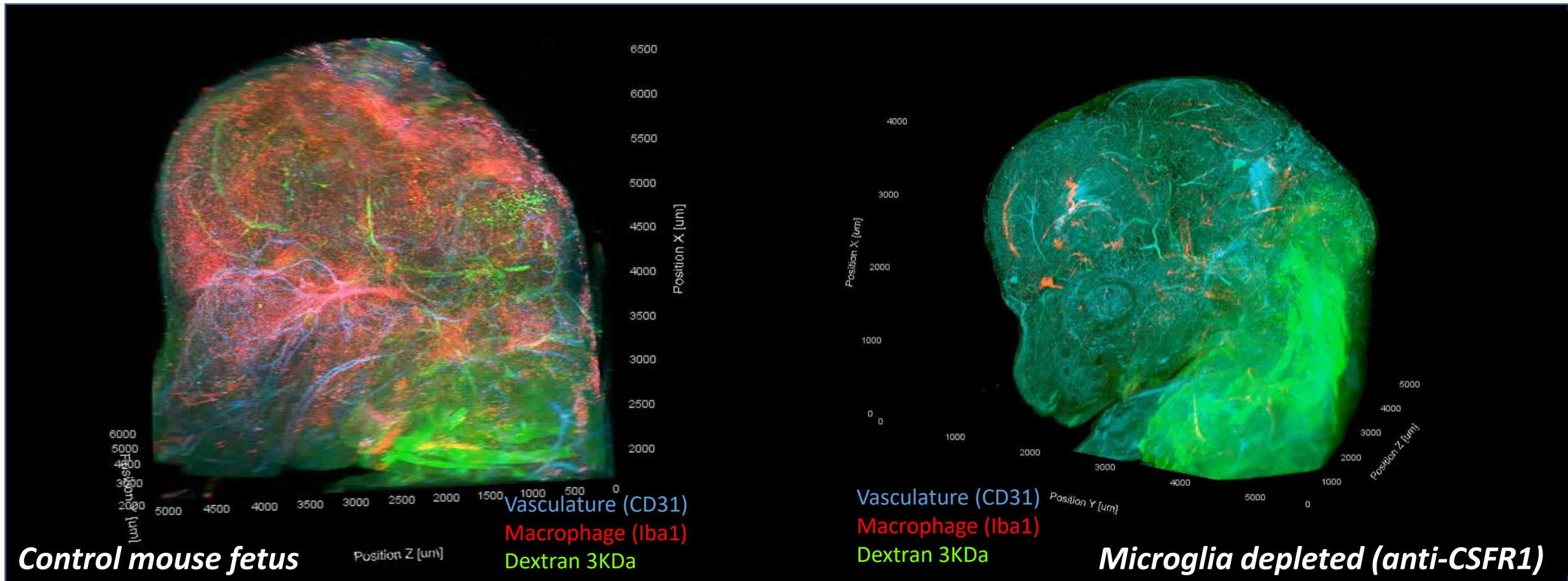
- MPS models can probe the interaction of physical geometry and cell signaling;
- FGF2 and BMP4 is a start, but still other signals needed to position a primitive streak;
- ABM adds positional information and tracks individual cell behaviors;
- computational intelligence can fill in for missing or incomplete knowledge;
- quantitatively simulate what chemical exposures could actuate at the cellular level;
- provide inferences on developmental effects in a human-relevant manner.

Microglia and neurovascular patterning

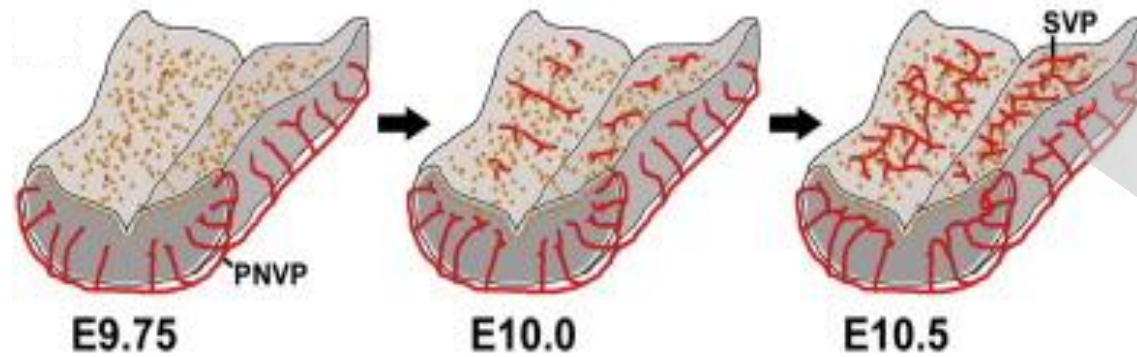


- Vascularization of the neural tube commences on E9-10 (mouse) with formation of blood-brain barrier by E11.
- Microglia from yolk sac blood islands form on E8 and circulate to colonize the neuroepithelium by E9.
- Anti-CSFR1 treatment on E6.5 -7.5 depletes 95-99% of the microglial population in the brain by E14.5.
- Microglia have 3 phenotypic states: M0 (resting), M1 (activated), M2 (protective).
- Microglia orchestrate neurovascular patterning, but when stress-activated → neuroinflammatory response.

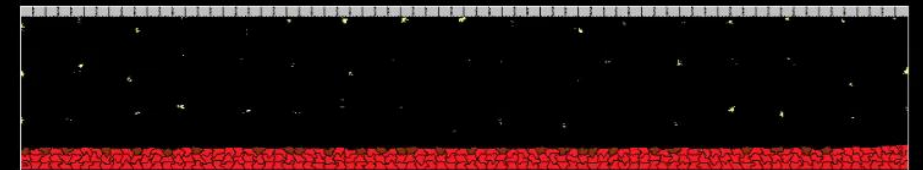
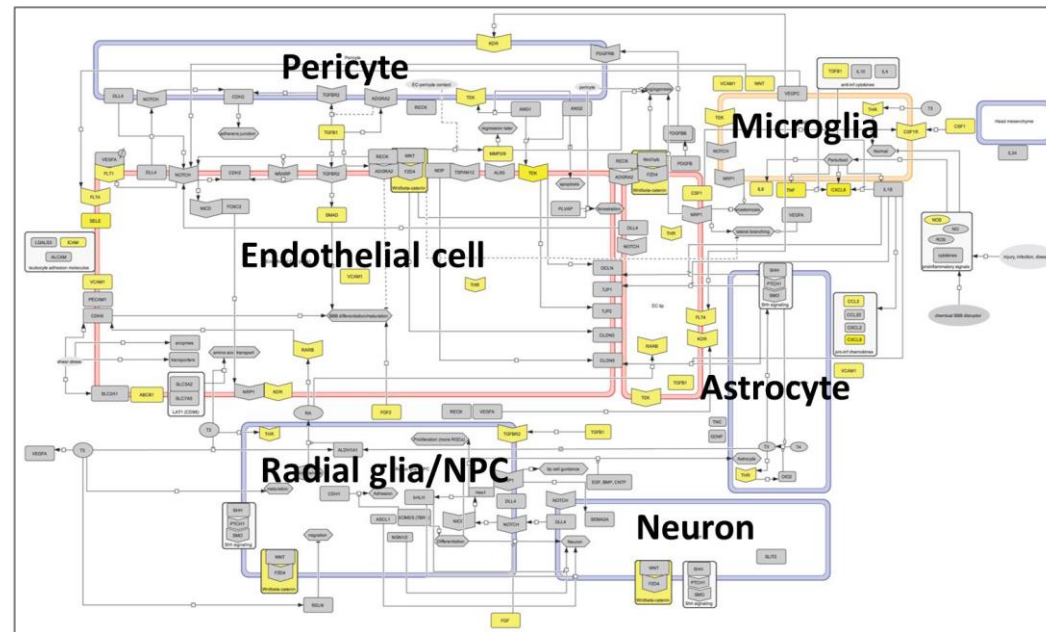
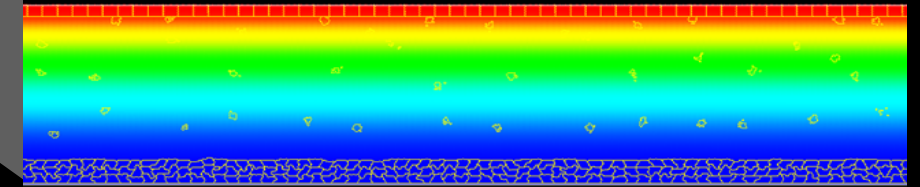
Microglia depletion: *reduces angiogenesis of the fetal brain and impairs or delays the development of barrier function of the microvasculature.*



Computational Systems Model



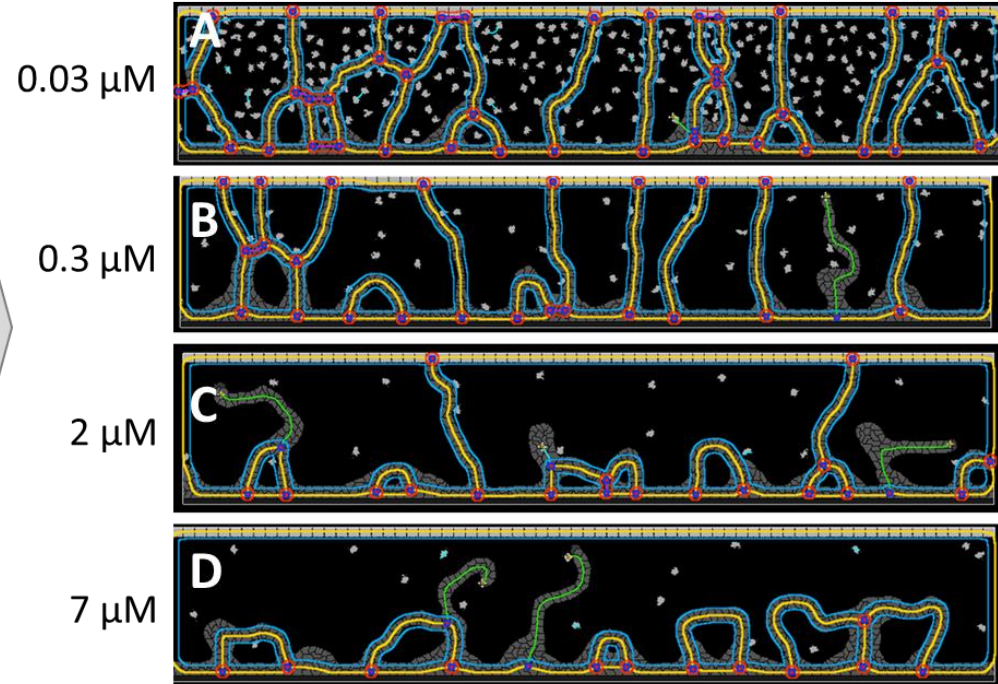
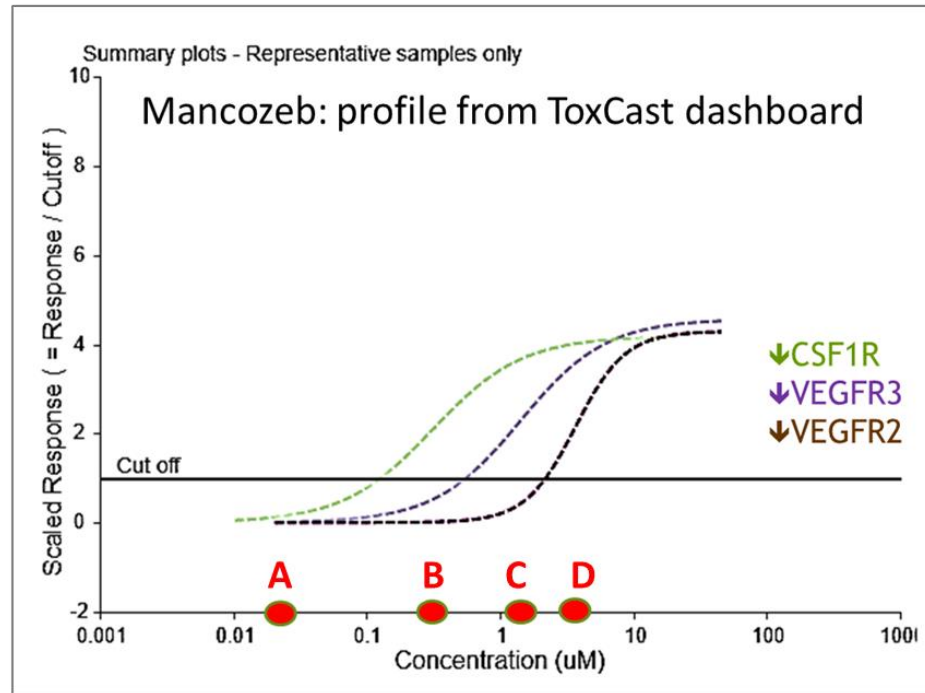
VEGF-A gradient: NPCs in subventricular zone



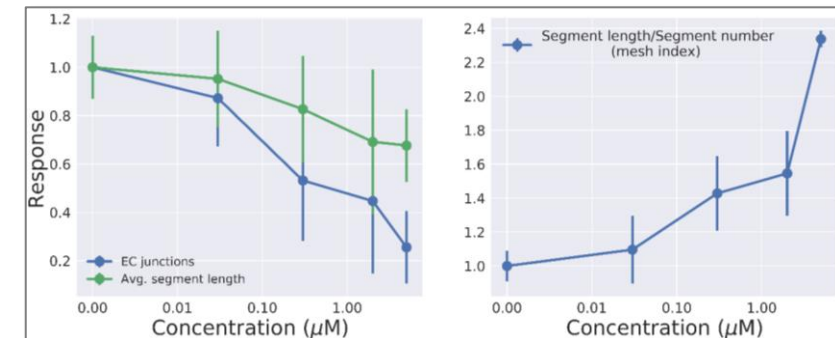
- endothelial tip cell
- endothelial stalk cell
- microglial cell



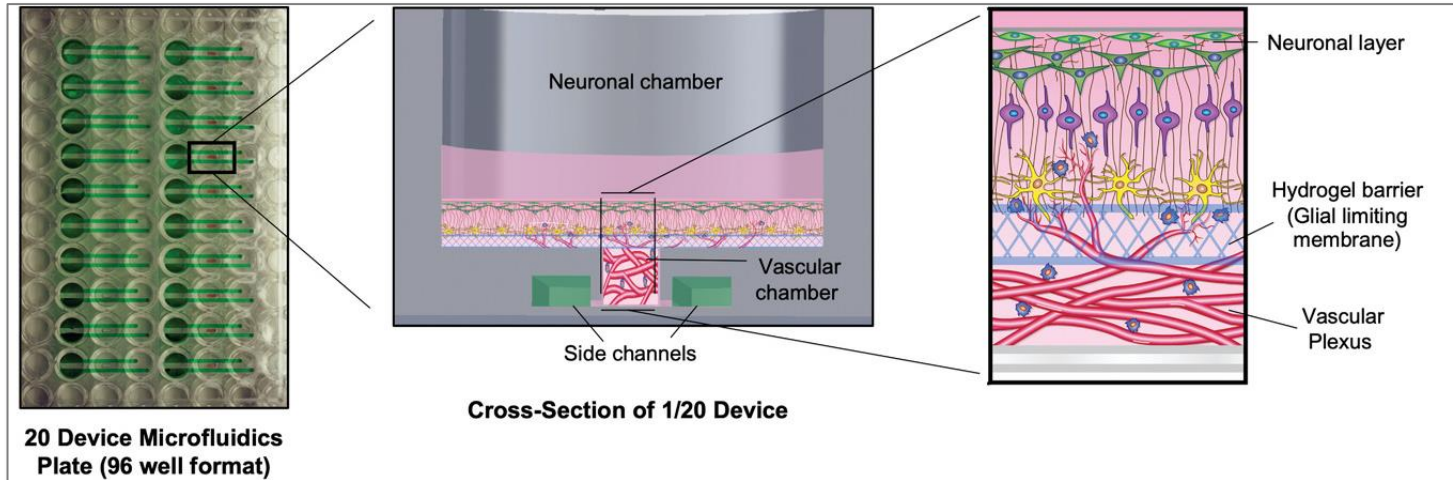
Executing a simulated concentration-response



- Prediction: affects microglial-endothelial interaction (reduced tortuosity \rightarrow deficiency of SVZ).
- Quantitative microvascular 'cybermorphs' predicts an AC50 for Mancozeb disruption at 0.5 μM .



Checking the prediction: *microglial integration in a synthetic microsystem*



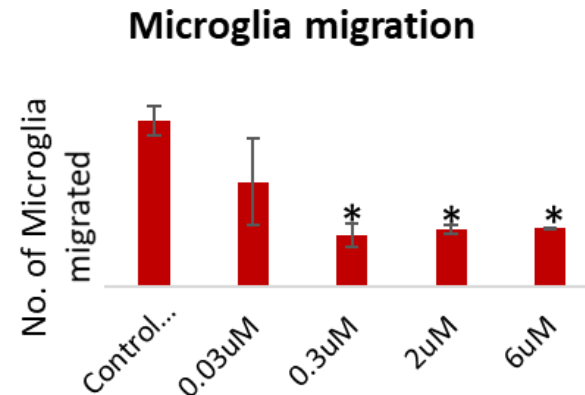
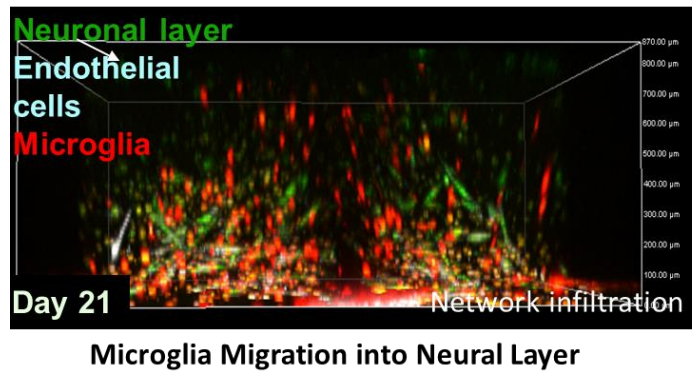
Engineered Perineural Vascular Plexus for Modeling Developmental Toxicity

Gaurav Kaushik, Kartik Gupta, Victoria Harms, Elizabeth Torr, Jonathan Evans, Hunter J. Johnson, Cheryl Soref, Suehelay Acevedo-Acevedo, Jessica Antosiewicz-Bourget, Daniel Mamott, Peyton Uhl, Brian P. Johnson, Sean P. Palecek, David J. Beebe, James A. Thomson, William T. Daly,* and William L. Murphy*

Kaushik et al. (2020), Adv Hlthc Materials

Critical concentration (PoD) for Mancozeb on neural tube vascularization:

- **predicted** by *in silico* cNVU = $0.5 \mu\text{M}$
- **observed** in organotypic culture = $0.3 \mu\text{M}$.



Microglial states may be an important sentinel for neurodevelopmental toxicity.

EPA STAR Center Co-operative grant #835737, Univ Wisconsin (W Murphy)

Looking ahead ...

Translational: what do synthetic models of human development - both computational and organoids - bring to future of DART testing?

Investigational: how smart must these models be (A.I.) to support decision-making in the animal-free (3Rs) zone?

Operational: what best practices are needed to implement synthetic models into an integrative decision framework (eg, AOP-based IATAs)?

Communication: what are the practical considerations for science, engineering, and stakeholder engagement (academics, government, industry, NGOs, policy, ...)?



Acknowledgements



<http://www2.epa.gov/sites/production/files/2015->

Virtual Tissues Team (USEPA/CCTE)

Nancy Baker (Leidos)

Chad Deisenroth

John Gamble (ORISE)

Sid Hunter

Thomas Knudsen

Kate Saili

Imran Shah

Richard Spencer (General Dynamics)

Rusty Thomas

Douglas Young

Todd Zurlinden

Special Thanks

Jianping Fu (Univ Michigan)

Florent Ginhoux (A*STAR Singapore)

James Glazier (Indiana Univ)

Shane Hutson (Vanderbilt Univ)

Guarav Kaushik (Univ Wisconsin)

William Murphy (Univ Wisconsin)

Jessica Palmer (Stemina)

Aymeric Silvin (A*STAR Singapore)

Katya Tsouin (Johns Hopkins Univ)