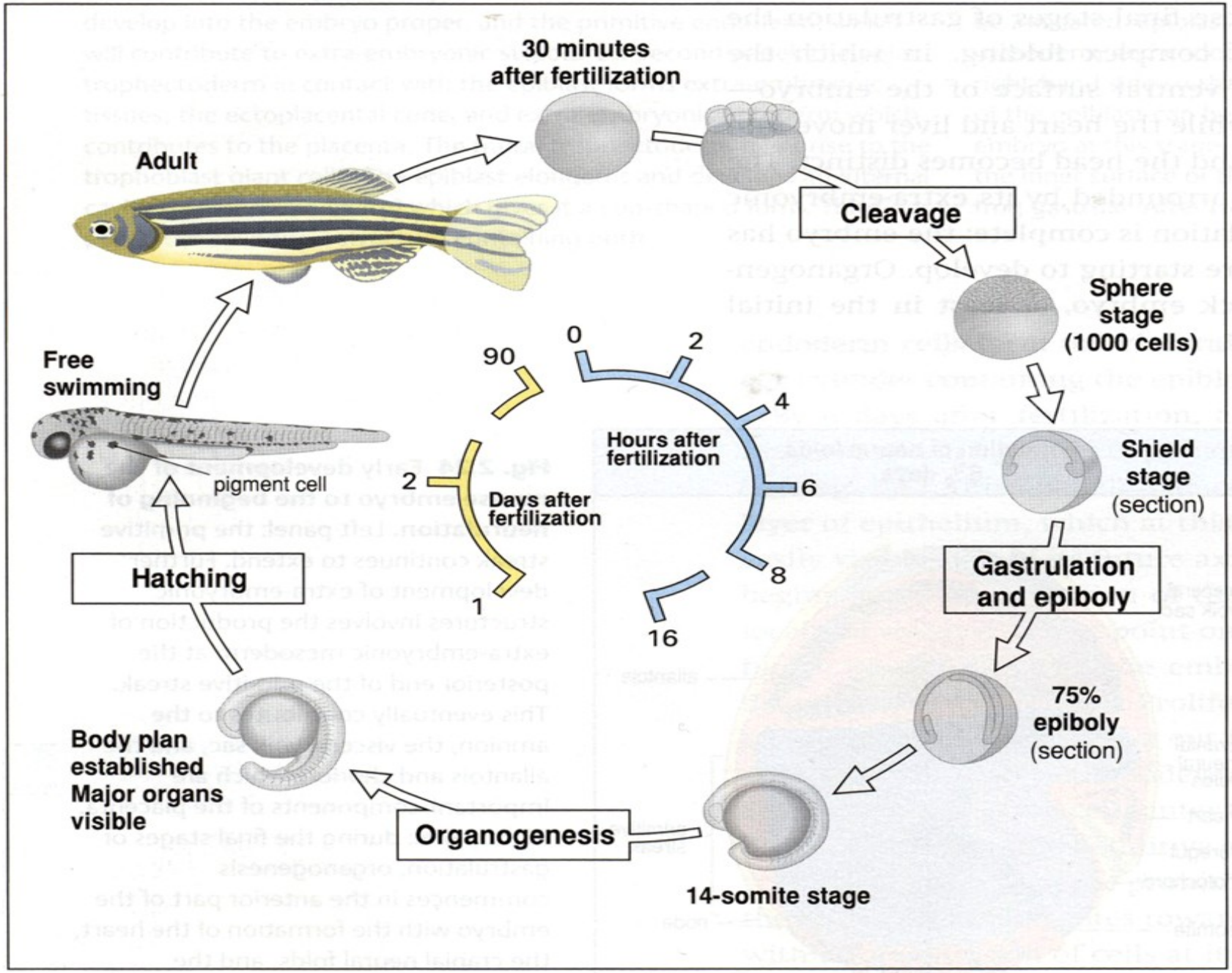
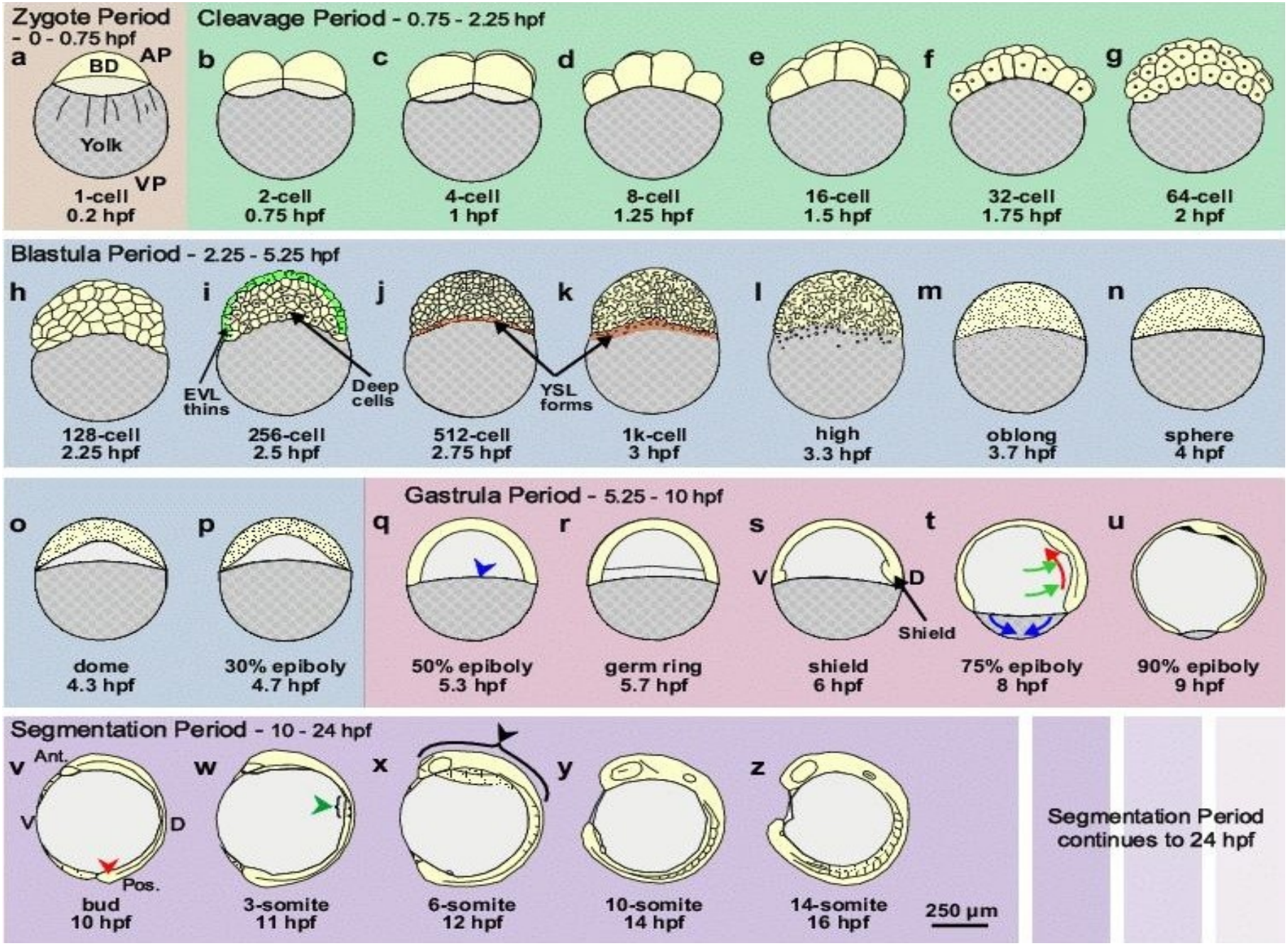
VÝVOJOVÁ FYZIOLOGIE I

DEVELOPMENTAL BIOLOGY

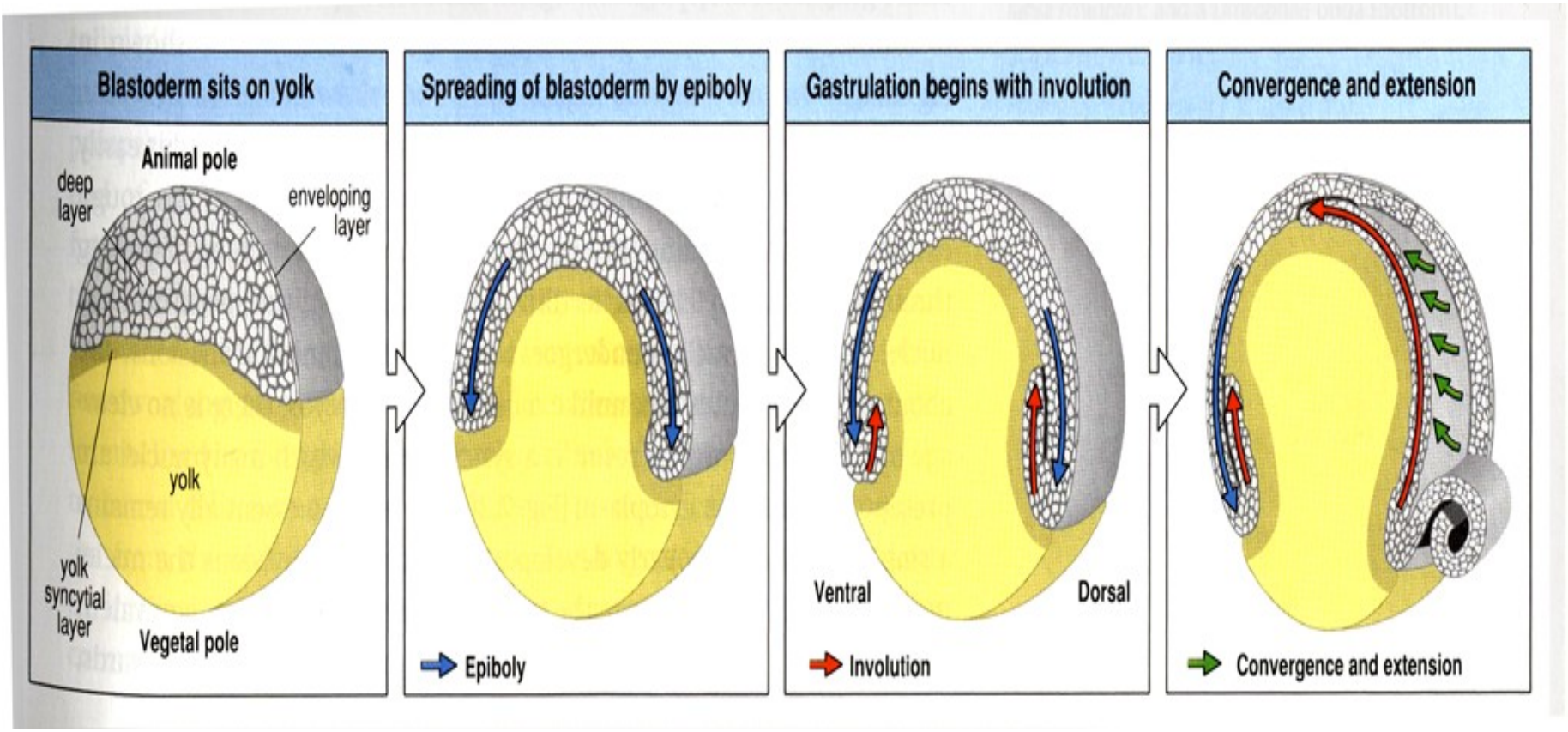
- central to all other areas of biology – unites cell biology, genetics and morphology
- in last 25 years grew into the most exciting and dynamic field of biology…..due to the advances in three main traditions: the experimental embryology, developmental genetics and molecular biology. All animals develop similarly, that including the worm, fly, fish or mammal.
- significant impact on society, in vitro fertilization, teratology, birth defects.
- future impact: functional genomics and functional proteomics, disease therapy, prenatal screening, transplantation, embryonal stem cells, therapeutic cloning.

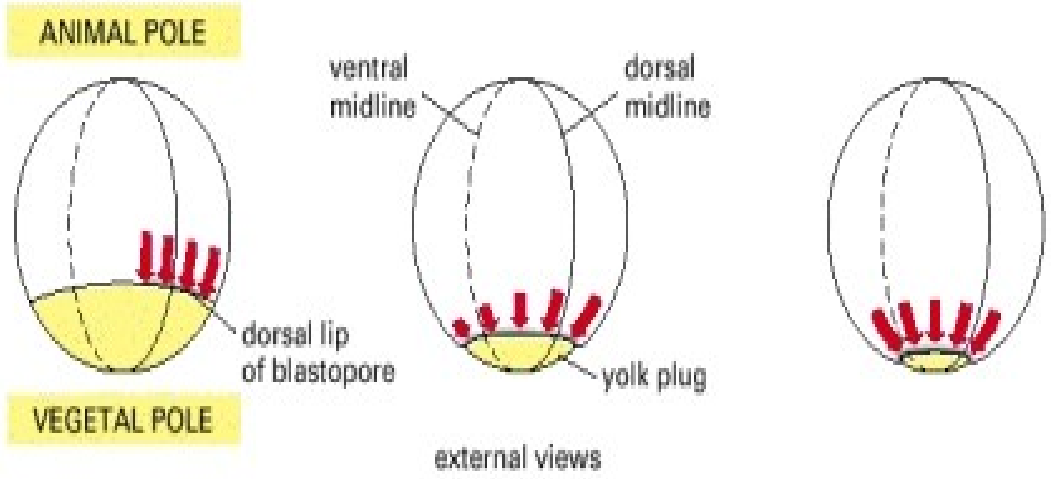
AMAZING NATURAL SPECTACLE AND A SOURCE OF INSPIRATION FOR ALL OTHER AREAS OF BIOLOGY

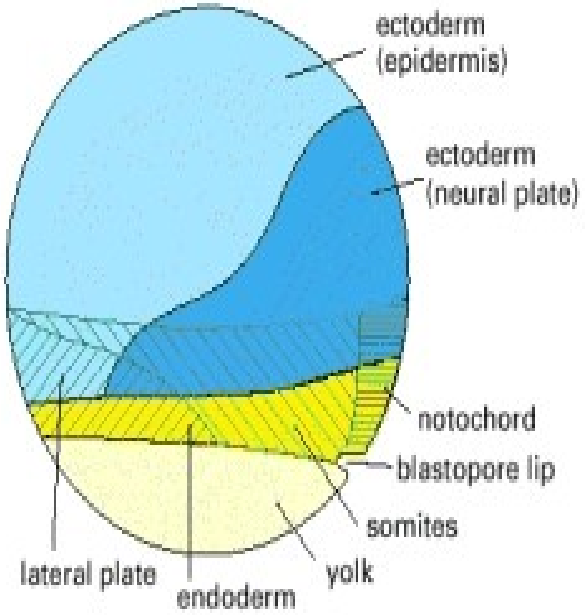

30 minutes
after fertilization
Cleavage
Sphere
stage
(1000 cells)
Shield
stage
(section)
Gastrulation
and epiboly
75%
epiboly
(section)
14-somite stage
Organogenesis
Body plan
established
Major organs
visible
Hatching
Free
swimming
pigment cell
Adult
0
2
4
6
8
16
90
2
1
Hours after
fertilization
Days after
fertilization



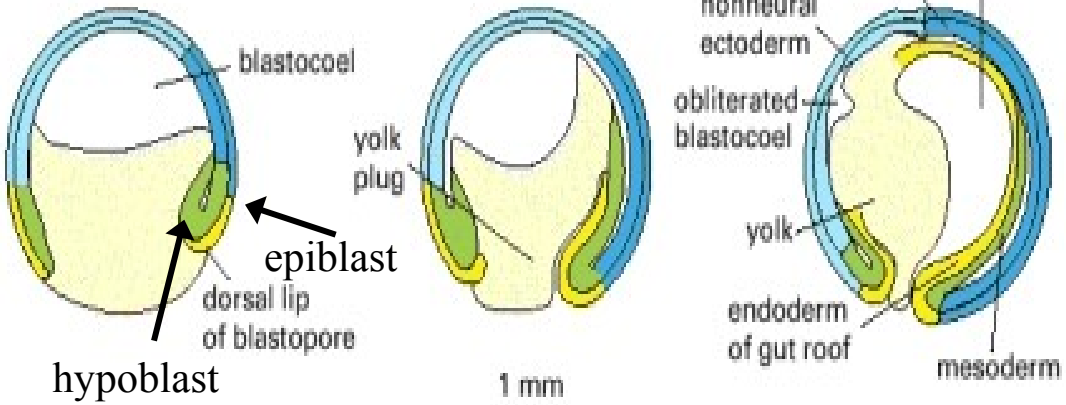
YSL – yolk syncytial layer


Blastoderm sits on yolk
Animal pole
deep layer
enveloping layer
yolk
yolk syncytial layer
Vegetal pole
Spreading of blastoderm by epiboly
Epiboly
Gastrulation begins with involution
Ventral
Dorsal
Involution
Convergence and extension
Convergence and extension

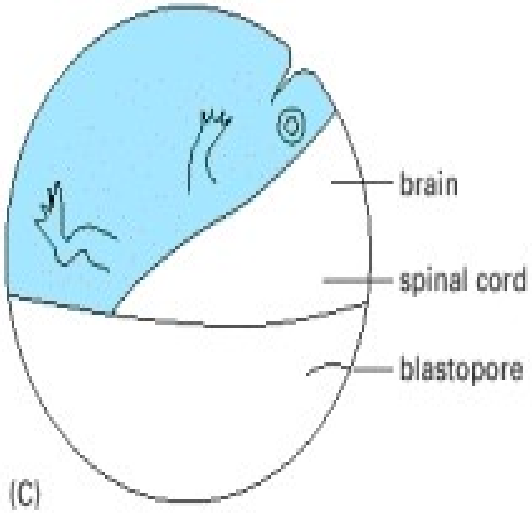

ANIMAL POLE
ventral midline
dorsal midline
dorsal lip of blastopore
yolk plug
VEGETAL POLE
external views


ectoderm (epidermis)
ectoderm (neural plate)
notochord
blastopore lip
somites
lateral plate
endoderm
yolk

(B)


blastocoel
epiblast
dorsal lip of blastopore
hypoblast
yolk plug
1 mm
cross-sections
neural ectoderm
cavity of gut
nonneural ectoderm
obliterated blastocoel
yolk
endoderm of gut roof
mesoderm

(A)


brain
spinal cord
blastopore

(C)

Reconstruction of Zebrafish Early Embryonic Development by Scanned Light Sheet Microscopy

Philipp J. Keller,[1,2]* Annette D. Schmidt,[2] Joachim Wittbrodt,[1,2,3,4]* Ernst H.K. Stelzer[1]

A long-standing goal of biology is to map the behavior of all cells during vertebrate embryogenesis. We developed digital scanned laser light sheet fluorescence microscopy and recorded nuclei localization and movement in entire wild-type and mutant zebrafish embryos over the first 24 hours of development. Multiview in vivo imaging at 1.5 billion voxels per minute provides "digital embryos" that is, comprehensive databases of cell positions, divisions, and

apertures and by focusing a collimated beam with a cylindrical lens. This arrangement provides three-dimensional optical sectioning and reduces the energy load on the specimen (*9–11*).

Digital scanned laser light sheet fluorescence microscopy. To achieve the imaging speed and quality for recording entire embryos, we developed digital scanned laser light sheet fluorescence microscopy (DSLM) (fig. S1). The idea behind DSLM is to generate a "plane of light" with a laser scanner that rapidly moves a micrometer-thin beam of laser light vertically and horizontally through the specimen (Fig. 1 and movie S1).

This approach has several advantages over standard light sheet microscopy. First, DSLM illuminates each line in the specimen with the

- cell nuclei visualized using GFP-tagged histone-2B injection
- 400000 images per embryo taken
-app.18000 cells individually tracked in the 18-hours-old embryo

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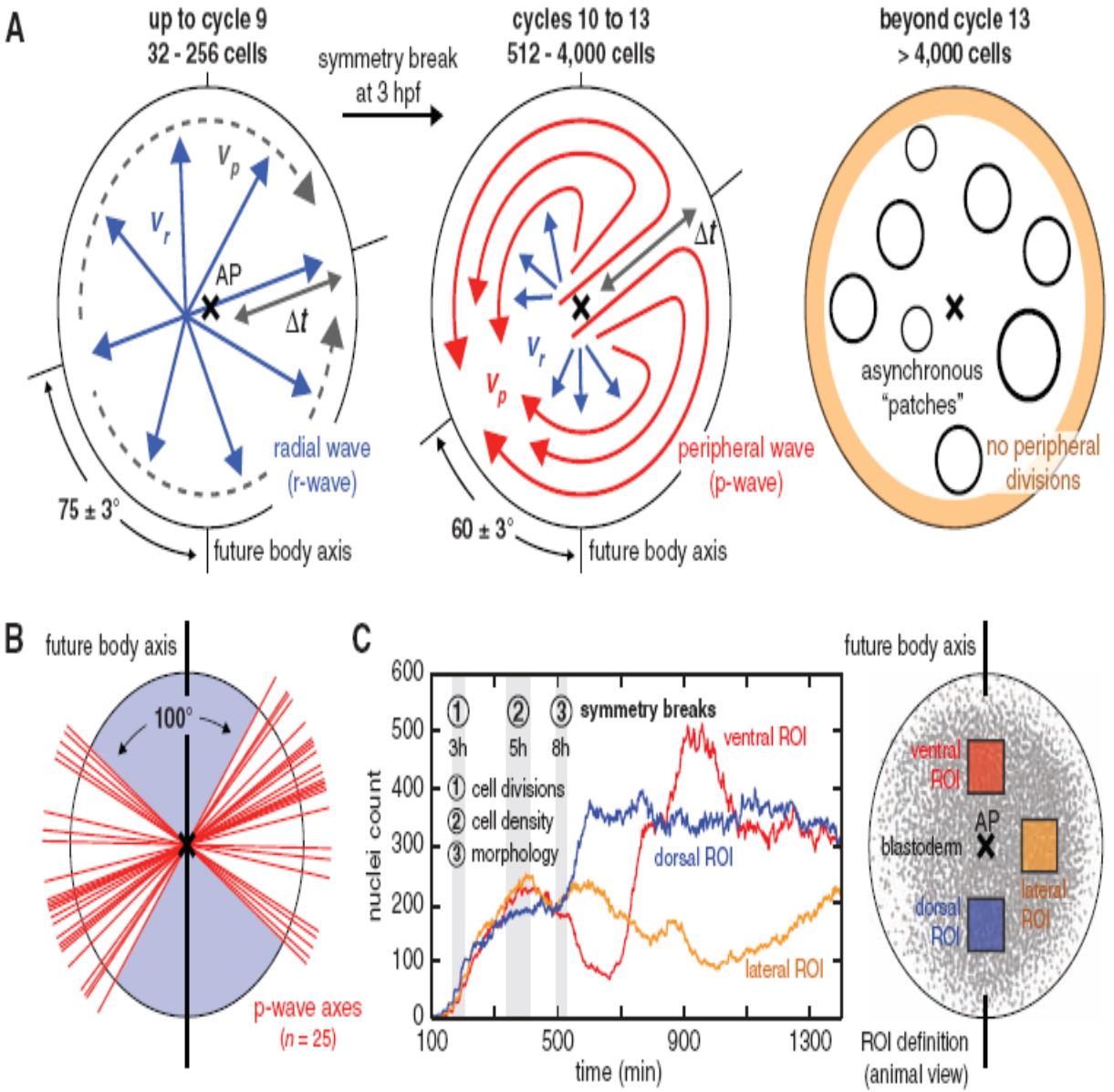

Fig. 4. Symmetry-breaking of the global cell division pattern. (**A**) Illustration of the cell division patterns during early zebrafish embryogenesis: fast radial waves (cycles 1 to 9, progression speed v_r), slow circular peripheral waves (cycles 10 to 13, progression speed v_p), and asynchronous cell-division patches (cycles 14+). Errors are indicated as SEM. A quantification of the parameters v_r, v_p and the time shift Δt between radial and peripheral waves is provided in fig. S8. (**B**) Symmetry axes of 25 peripheral waves (20 slow waves in cycles 10 to 13 and 5 fast waves in cycle 9; n = 5 embryos). Of the 25 waves, 92% occurred at an angle of 45 to 90° to the future body axis. (**C**) Nuclei counts in three 110 by 110 μm^2 domains (future dorsal, ventral, and lateral) reveal the first symmetry break in cell densities at 5 hpf. The symmetry break in the cell division pattern (3 hpf) precedes the symmetry break in cell densities (5 hpf) and the first morphogenetic symmetry break (onset of convergence, 8 hpf).

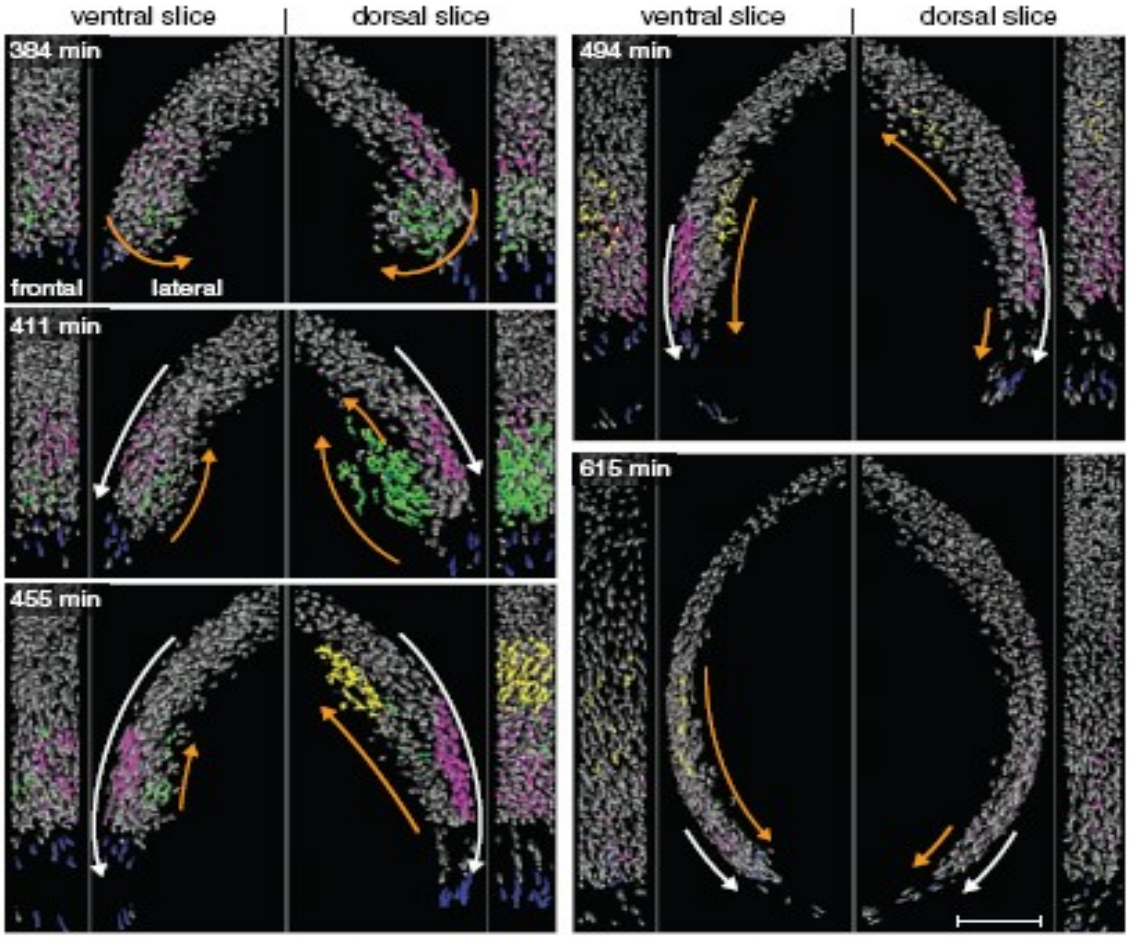

Fig. 5. Mesendoderm internalization and migration in dorsal and ventral hemispheres. Frontal and lateral views of slices on dorsal (shield region, right) and ventral hemispheres (opposite of shield, left). Four cell populations were tracked (movie S16): green or yellow nuclei in the early or late embolic wave, blue nuclei at the leading edge of epiboly, and noninternalizing pink nuclei. Orange and white arrows indicate hypoblast and epiblast cell movements. Scale bar, 100 μm.