Metabolomics at the Single-cell Level

Peter Nemes
The George Washington University,
Washington, DC

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Acknowledgment

Sally A. Moody

M. Chiara Manzini

S. Choi, E. Corcoran, A. Baxi, C. Lombard, E. Portero
R. Onjiko, R. Al Shabeeb, D. Plotnick

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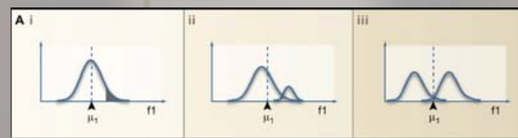
New Frontiers: Single-Cell Analysis

Cell Heterogeneity Matters!

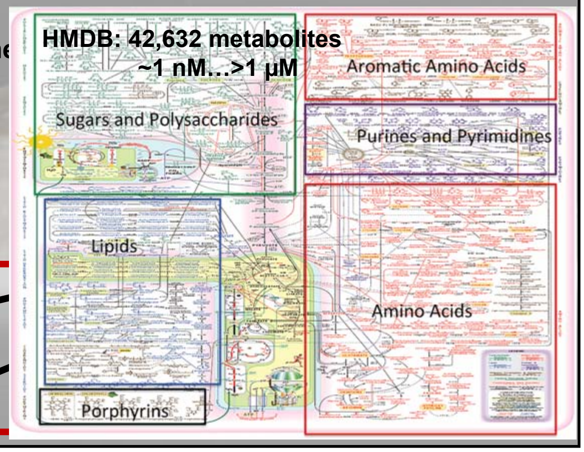
Implicated in:
- Disease: cancer
- Drug resistance
- Normal development:
  - Brain: ~100 billion
  - Embryo developme



Altschuler and Wu, Cell 2010, 141, 559

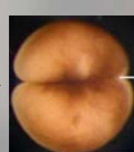
Systems Biology Approach:

Genome → Transcriptome → Proteome → Metabolome

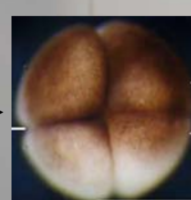


Development: Complex, Tightly Controlled

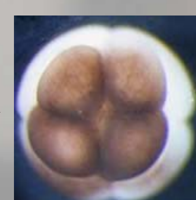
1 cell



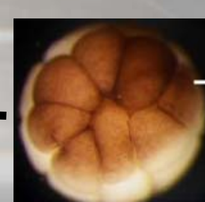
2 cells



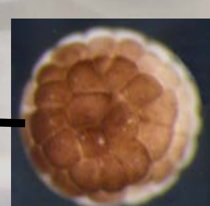
4 cells



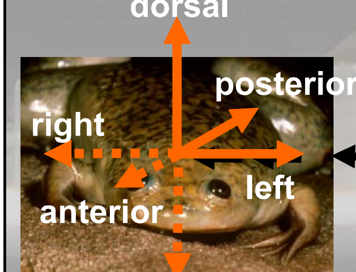
8 cells



16 cells



32 cells



http://wiki.xenbase.org

→Cell Heterogeneity Really Matters

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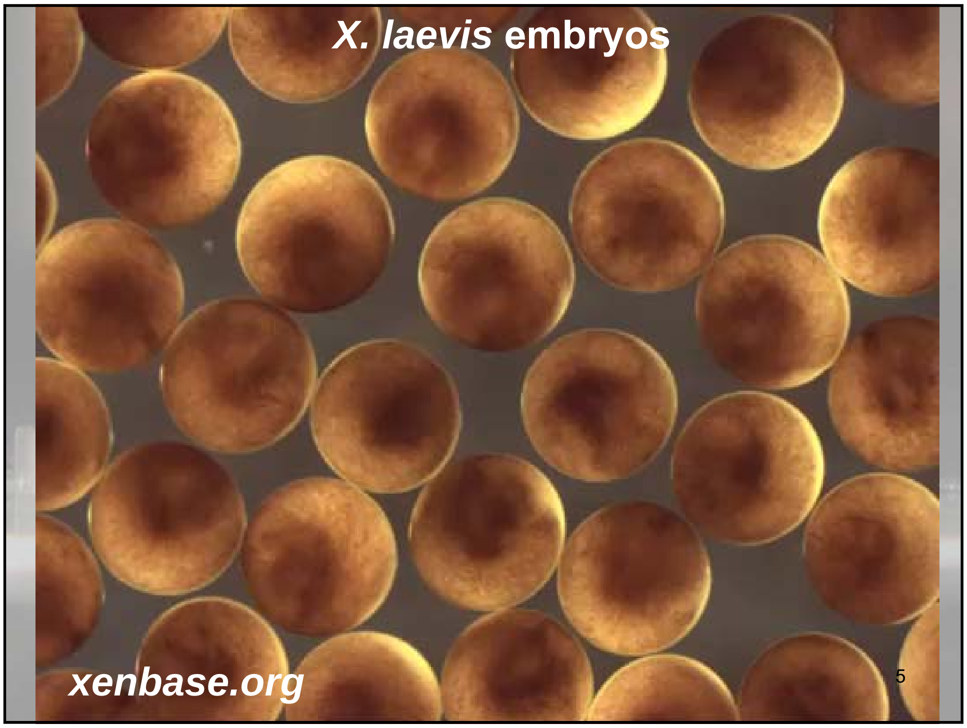

X. laevis embryos
xenbase.org
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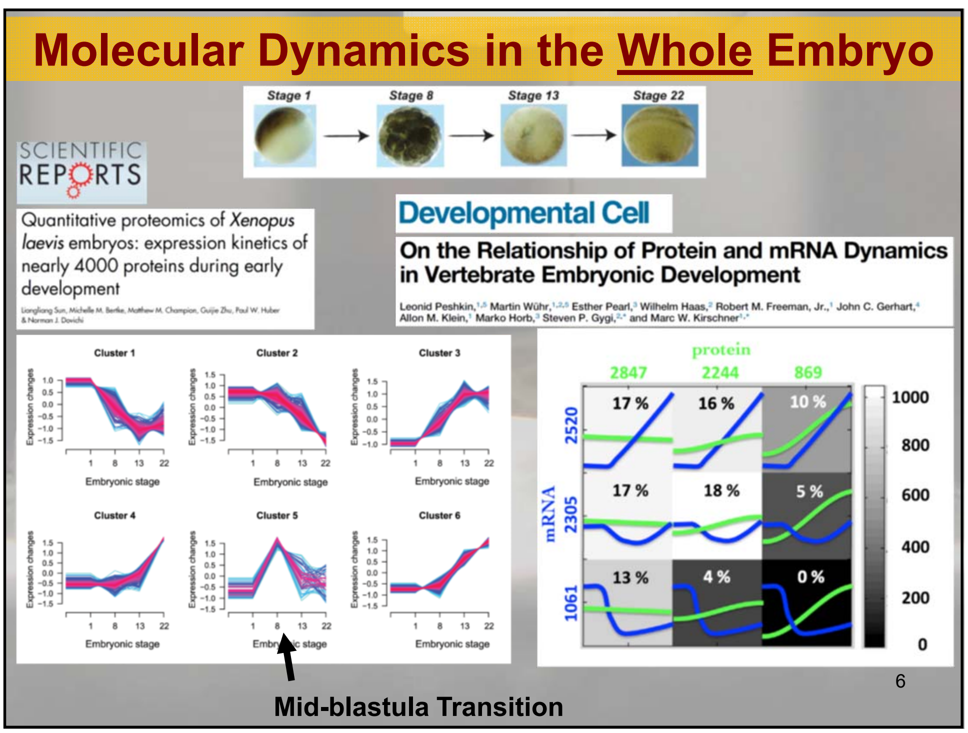


Molecular Dynamics in the Whole Embryo
Stage 1
Stage 8
Stage 13
Stage 22
SCIENTIFIC REPORTS
Quantitative proteomics of *Xenopus laevis* embryos: expression kinetics of nearly 4000 proteins during early development
Liangliang Sun, Michelle M. Bertke, Matthew M. Champion, Guijie Zhu, Paul W. Huber & Norman J. Dovichi
Developmental Cell
On the Relationship of Protein and mRNA Dynamics in Vertebrate Embryonic Development
Leonid Peshkin, Martin Wühr, Esther Pearl, Wilhelm Haas, Robert M. Freeman, Jr., John C. Gerhart, Allon M. Klein, Marko Horb, Steven P. Gygi, and Marc W. Kirschner
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Expression changes
Embryonic stage
protein
2847
2244
869
mRNA
2520
2305
1061
17 %
16 %
10 %
17 %
18 %
5 %
13 %
4 %
0 %
1000
800
600
400
200
0
6
Mid-blastula Transition

How about the Metabolome in the <u>Whole</u> Developing Embryo?

Remodeling of the Metabolome during Early Frog Development

Livia Vastag[1], Paul Jorgensen[2], Leonid Peshkin[2], Ru Wei[3], Joshua D. Rabinowitz[1], Marc W. Kirschner[2]

PLoS one
2011, 6, e16881

GOALS

Better understand **<u>cell molecular mechanisms</u>** governing embryonic development (health *vs.* disease) at the level of **<u>single cells</u>**:

- **Obj. 1: Small molecules: Metabolites <500 Da**
- **Obj. 2: Proteins**

<u>Challenge for singe-cell analysis:</u>

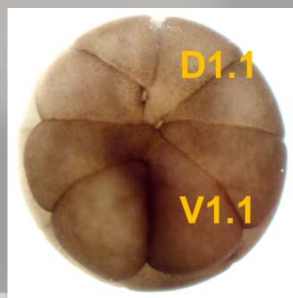
Typically, MS needs

- **~10 µg protein needed**
- **~20 µL volume**

Xenbase.org

"The Samples"

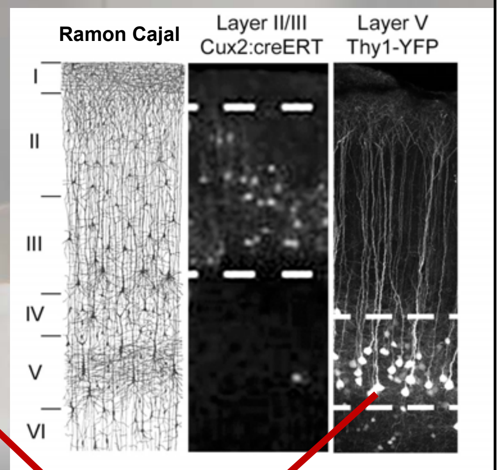
16-cell *Xenopus* Embryo



1 blastomere = 250 µm (~90 nL)

- Complex 3D structure
- Spatially evolving
- Temporally evolving
- Limited sample
- Complex metabolome
- Complex proteome

Mammalian Cortex: Neurons



Microsampling/sorting +
Mass Spectrometry

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Solution

Advance <u>mass spectrometry</u> sensitivity single cells (blastomeres) in the early embryo.

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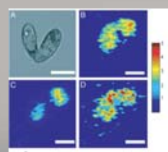
Single-cell MS for Metabolomics

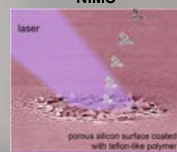
In Vacuo

Tof-SIMS



Ewing *et al.*, *PNAS* **2010**, 107, 2751

NIMS

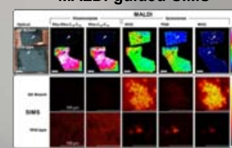


Siuzdak *et al.*, *Anal. Chem.*,**2011**, 83, 2

NAPA

Vertes, A. *et al.*, *Phys., Chem., Chem., Phys.* **2011**,13, 9146

MALDI-guided SIMS

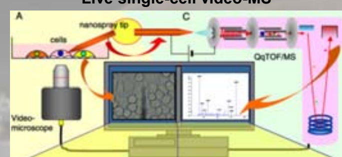


Sweedler *et al.*, Anal. Chem. **2014**, 86, 9139

At Ambient Conditions

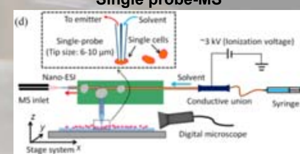
- Fast analysis
- *In situ* studies

Live single-cell video-MS



Masujima *et al.*, *Nat. Protoc.* 2015,10, 1445

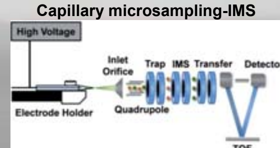
Single probe-MS



Yang *et al.*, *Anal. Chem.* **2014**,86, 9376

- Reduce sample complexity
  - Differentiate isobaric ions
  - Aid identifications

Capillary microsampling-IMS



Vertes *et al*, *Analyst*, **2014**,139, 5079

- **CE-ESI-MS**
  - Minimize matrix effects
  - Aid Identifications

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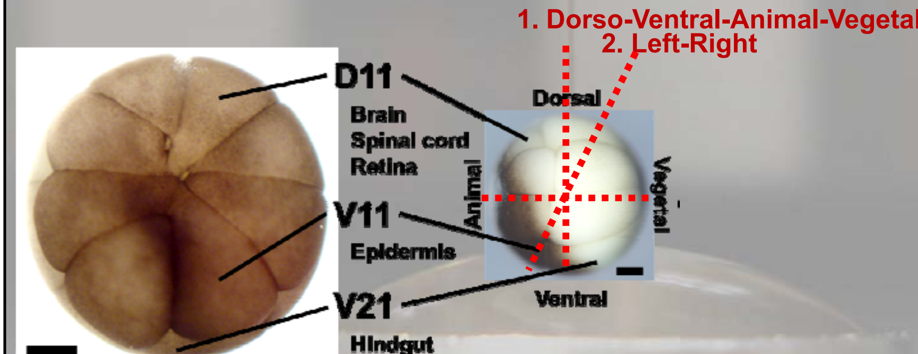
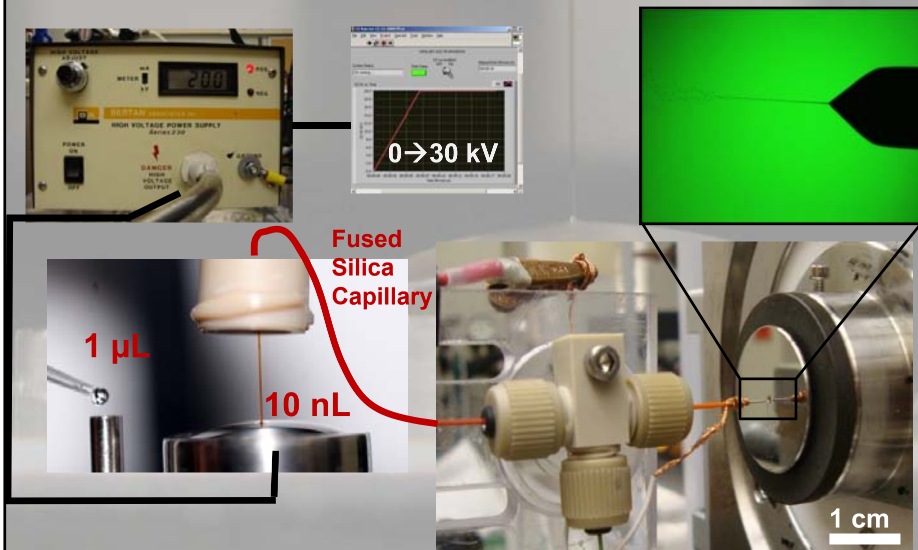
Developed Metabolomics Workflow



→10 nL: ~0.1% of the total metabolite content of the blastomere

R. Onjiko, S.A. Moody, P. Nemes*, PNAS 2015, 112, 6545

Developed Single-cell CE-ESI-MS



R. Onjiko, S.A. Moody, P. Nemes*, PNAS 2015, 112, 6545

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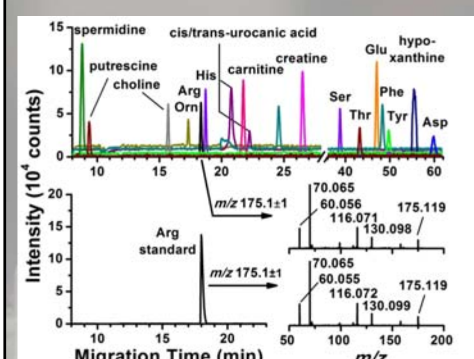
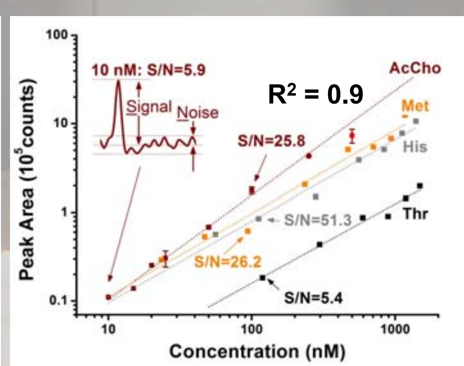
Single-cell CE-ESI-MS

Identifies Small Molecules



Quantifies Small Molecules



→Limit of Detection: ~10 nM, viz. ~50 amol

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R. Onjiko, S.A. Moody, P. Nemes*, PNAS 2015, 112, 6545

Metabolites ID'd in Single Blastomeres

ID	Compound	Formula	t_m (min)	*m/z* measured	*m/z* theor.	Δ (mDa)	Δ (ppm)
1	histamine	$C_5H_9N_3$ (H^+)	8.57	112.0875	112.0875	-0.10	-0.89
2	thiamine	$C_{12}H_{17}N_4OS$ (+)	12.19	265.1115	265.1123	0.40	3.84
3	choline	$C_5H_{14}NO$ (+)	13.08	104.1078	104.1075	0.80	3.02
4	ornithine*	$C_5H_{12}N_2O_2$ (H^+)	14.05	133.0983	133.0977	-0.60	4.51
5	lysine*	$C_6H_{14}N_2O_2$ (H^+)	14.19	147.1136	147.1133	-0.60	-4.51
6	β-alanine	$C_3H_7NO_2$ (H^+)	14.34	90.0558	90.0555	-0.30	-2.04
7	nicotinamide	$C_6H_6N_2O$ (H^+)	14.64	123.0588	123.0558	-0.30	-3.33
8	arginine*	$C_6H_{14}N_4O_2$ (H^+)	14.75	175.1191	175.1195	0.40	2.28
9	acetylcholine*	$C_7H_{16}NO_2$ (+)	14.77	146.1180	146.1181	0.10	0.68
10	GABA	$C_4H_9NO_2$ (H^+)	15.04	104.0710	104.0711	0.10	0.96
11	histidine*	$C_6H_9N_3O_2$ (H^+)	15.08	156.0775	156.0773	-0.20	-1.28
12	carnitine*	$C_7H_{15}NO_3$ (H^+)	17.17	162.1129	162.1130	0.10	0.62
13	serotonin	$C_{10}H_{12}N_2O$ (H^+)	17.52	177.1020	177.1028	0.80	4.52
14	acetylcarnitine*	$C_9H_{17}NO_4$ (H^+)	18.71	204.1233	204.1236	0.30	1.47
15	glycine	$C_2H_5NO_2$ (H^+)	19.42	76.0400	76.0399	-0.10	-1.32
16	cytidine	$C_9H_{13}N_3O_5$ (H^+)	20.07	244.0930	244.0933	0.30	1.23
17	adenosine*	$C_{10}H_{13}N_5O_4$ (H^+)	20.74	268.1045	268.1046	0.10	0.37
18	alanine	$C_3H_7NO_2$ (H^+)	21.51	90.0553	90.0555	0.20	2.22

* Also confirmed by tandem MS; migration time, t_m

Metabolites ID'd in Single Blastomeres

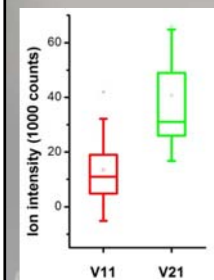
ID	Compound	Formula	t_m (min)	*m/z* measured	*m/z* theor.	Δ (mDa)	Δ (ppm)
19	valine*	$C_5H_{11}NO_2$ (H^+)	24.92	118.0864	118.0868	0.40	3.39
20	isoleucine*	$C_6H_{13}NO_2$ (H^+)	25.27	132.1026	132.1024	-0.20	-1.51
21	serine	$C_3H_7NO_3$ (H^+)	25.47	106.0506	106.0504	-0.20	-1.89
22	leucine*	$C_6H_{13}NO_2$ (H^+)	25.62	132.1025	132.1024	-0.10	-0.76
23	threonine	$C_4H_9NO_3$ (H^+)	27.26	120.0657	120.0661	0.40	3.33
24	indoleacrylic acid*	$C_{11}H_9NO_2$ (H^+)	27.80	188.0710	188.0711	0.10	0.53
25	tryptophan	$C_{11}H_{12}N_2O_2$ (H^+)	27.80	205.0974	205.0977	0.30	1.46
26	glutamine*	$C_5H_{10}N_2O_3$ (H^+)	28.08	147.0768	147.0770	-0.20	-1.36
27	glutamic acid*	$C_5H_9NO_4$ (H^+)	28.71	148.0611	148.0610	-0.10	-0.68
28	phenylalanine*	$C_9H_{11}NO_2$ (H^+)	29.08	166.0871	166.0868	-0.30	-1.81
29	tyrosine*	$C_9H_{11}NO_3$ (H^+)	29.62	182.0814	182.0817	0.30	1.65
30	proline*	$C_5H_9NO_2$ (H^+)	30.06	116.0714	116.0711	-0.30	-2.58
31	aspartic acid*	$C_4H_7NO_4$ (H^+)	32.70	134.0454	134.0453	-0.10	-0.75
32	glycine betaine	$C_5H_{11}NO_2$ (H^+)	32.75	118.0872	118.0868	-0.40	-3.39
33	proline betaine*	$C_7H_{13}NO_2$ (H^+)	33.55	144.1021	144.1024	0.30	2.08
34	β-alanine betaine	$C_6H_{13}NO_2$ (H^+)	37.00	132.1026	132.1024	-0.20	-1.51
35	glutathione	$C_{10}H_{17}N_3O_6S$ (H^+)	37.88	308.0913	308.0916	0.30	0.97
36	taurine	$C_2H_7NO_3S$ (H^+)	50.20	126.0226	126.0225	-0.10	-0.79

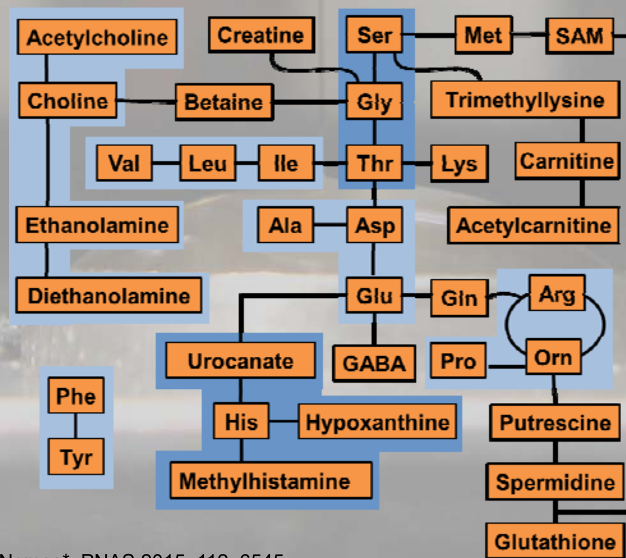
* Also confirmed by tandem MS; migration time, t_m

Reconstructed Metabolic Networks

Single embryonic cells

Network data from: KEGG





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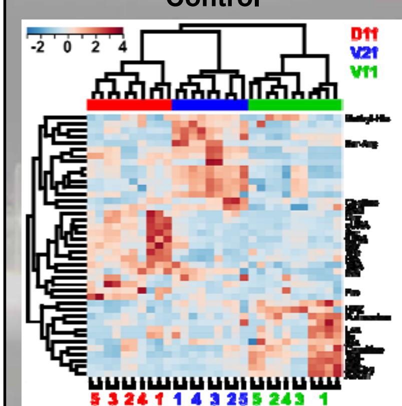
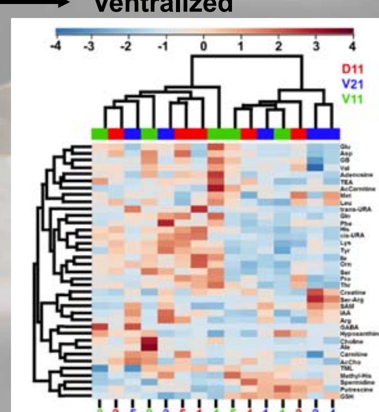
Found Metabolic Cell Heterogeneity

NO Brain
NO Spinal cord
Epidermis

Control —UV→ Ventralized





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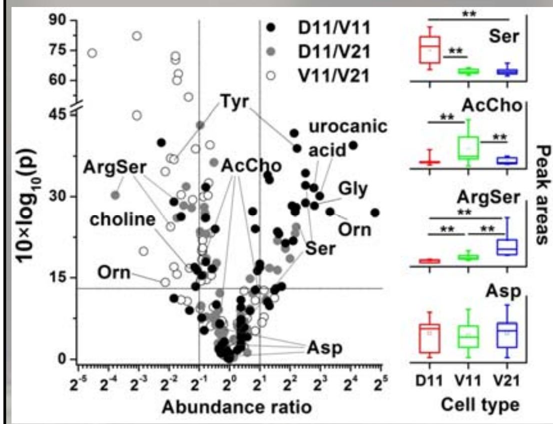
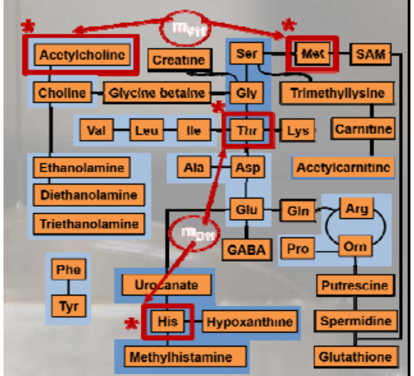
Cell-to-cell Differences





→ **Suggests metabolic asymmetry along:**
- **Dorsal-ventral axis (D11/V11)**
- **Animal-vegetal axis (V11/V21)**

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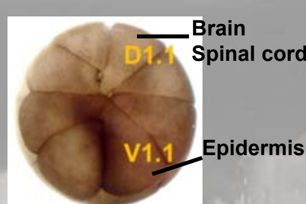
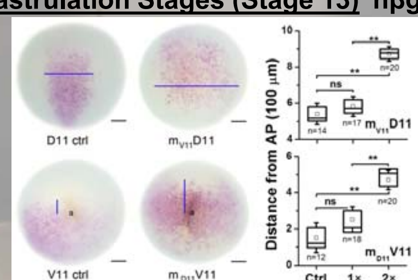
Discovered: Metabolites Alter Cell Fates

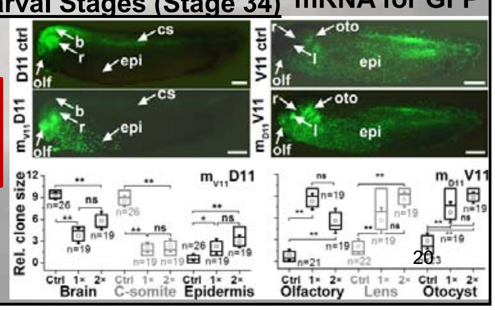
Metabolite	pmol/blastomere				
	$D11_6$	$D11_7$	$D11_8$	Average	RSD
His	15.1	9.1	5.5	9.9	4.9
Thr	17.6	10.5	6.2	11.5	5.8
	$V11_6$	$V11_7$	$V11_8$	Average	RSD
AcCho	1.3	0.9	12.	1.2	0.2
Met	21.7	10.2	38.5	23.5	14.2
Ala	n/d	25.8	39.8	32.8	9.9



Gastrulation Stages (Stage 13) nβgal



Larval Stages (Stage 34) mRNA for GFP



→ **Discovered small molecules capable of altering normal cell fates.**

R. Onjiko, S. A. Moody, P. Nemes*, PNAS 2015, 112, 6545

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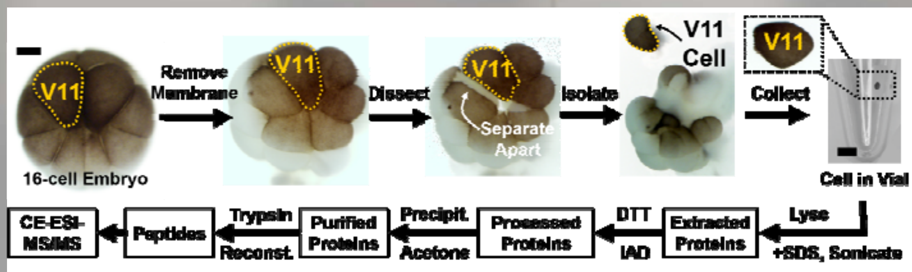
How to Handle Smaller Cells?



***Vs.* Average Cell Size**

16-cell ~250 μm → 32-cell ~200 μm → 64-cell ~160 μm → 128-cell ~125 μm

C. Lombard-Banek, Sally A. Moody, P. Nemes*,
Frontiers in Cell and Dev. Biol. 2016, 4, no. 100

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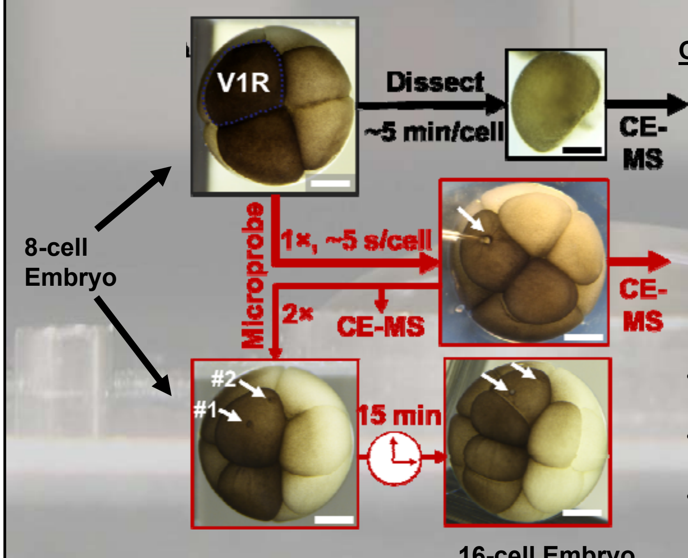
Developed *In situ* Microprobe Sampling



GSH/GSSG:
- 45 ± 27 Diss
- 1,045 ± 76 μP
- p = 2.8 x 10^{-5}

→Dissection Is stressful!

- Visually: Normal Cell Division!
- Live Embryonic Development
- In vivo MS-based proteomics?

R. M. Onjiko, E. P. Portero,
S. A. Moody, P. Nemes*, 2017 Anal. Chem., in print

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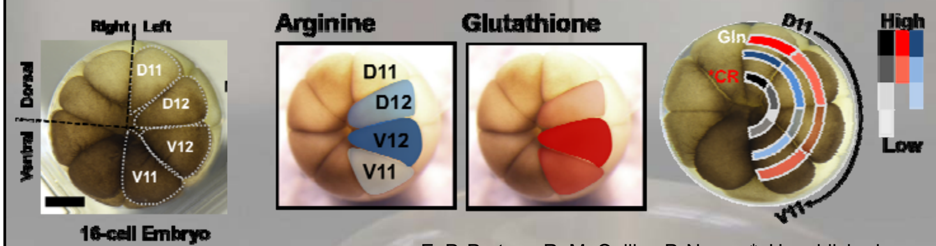
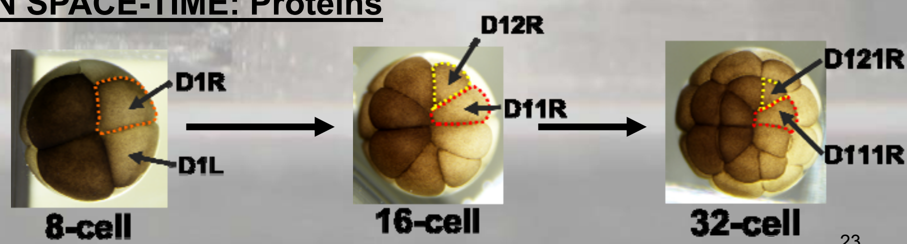
Found Molecular Cell Heterogeneity

IN SPACE: Metabolites



E. P. Portero, R. M. Onjiko, P. Nemes*, Unpublished

IN SPACE-TIME: Proteins



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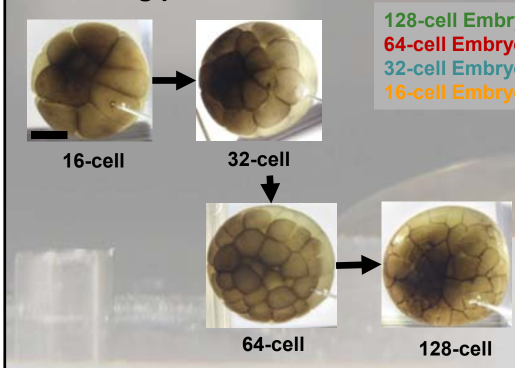
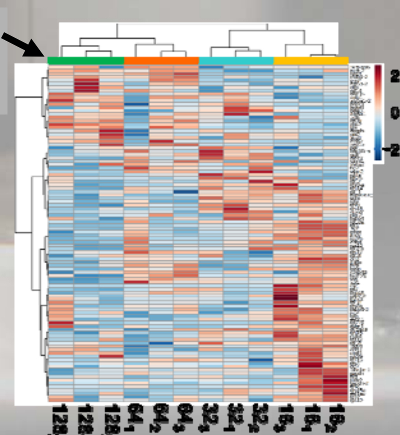
C. Lombard-Banek, A. Baxi, S. A. Moody, P. Nemes*, Unpublished

Tracked Molecular Changes in Dorsal Cell Lineage

Extending proteomics to metabolomics





- Measured ~6 ng digest (~0.06% of the total cell proteome)
- Identified 470 protein groups
- Quantified ~175 protein groups in all replicates

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C. Lombard-Banek, A. Baxi, S. A. Moody, P. Nemes*, 2017, submitted

Conclusions

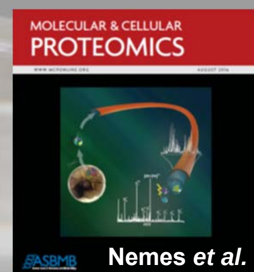
→ <u>(Single-cell) MS:</u>
- Basic and translational research
- Cell and developmental biology
- Discovery metabolomics and proteomics

→<u>New types of questions the life sciences</u>:

PNAS
Single-cell mass spectrometry reveals small molecules that affect cell fates in the 16-cell embryo
Rosemary M. Onjiko, Sally A. Moody, and Peter Nemes

Proteomics
Single-Cell Mass Spectrometry for Discovery Proteomics: Quantifying Translational Cell Heterogeneity in the 16-Cell Frog (*Xenopus*) Embryo
*Camille Lombard-Banek, Sally A. Moody, and Peter Nemes**

Analyst
PAPER
Single-cell mass spectrometry with multi-solvent extraction identifies metabolic differences between left and right blastomeres in the 8-cell frog (*Xenopus*) embryo†
Rosemary M. Onjiko, Sydney E. Morris, Sally A. Moody and Peter Nemes



→<u>New Research Opportunities</u>
- Fundamental Cell/Dev. Biology
- Neuroscience
- Heath vs. disease

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Sally A. Moody

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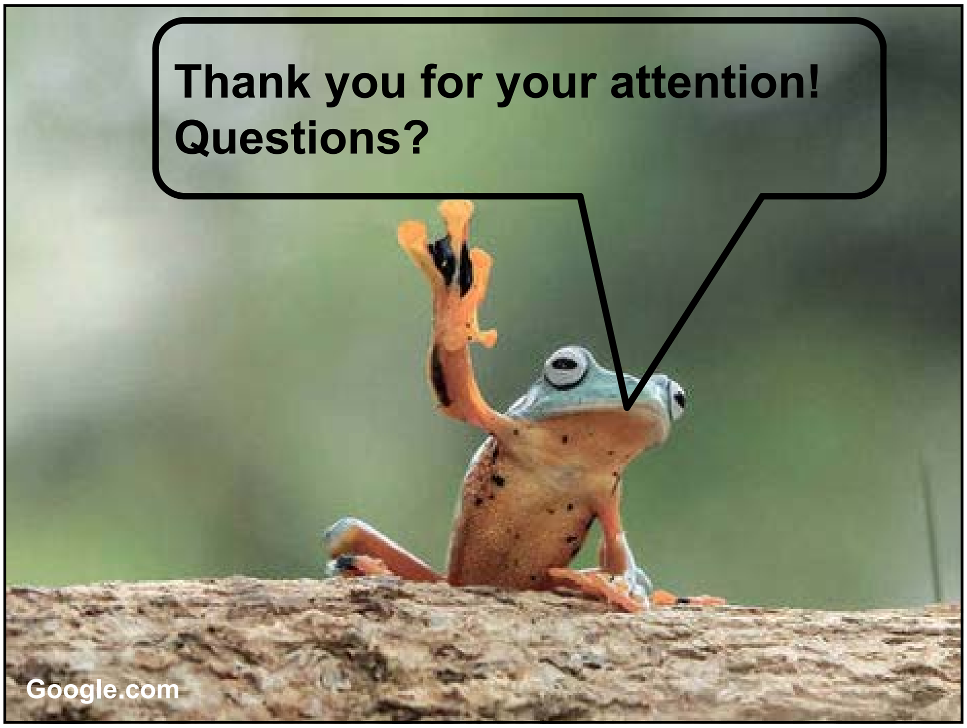
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Questions?
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