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New techniques and tools in 2011

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The last year has seen the development and application of a large number of novel techniques and probes, most notably for genome manipulation and imaging. Since technological papers can appear in a number of different journals it is easy to miss some of them. This overview briefly summarizes recent developments while providing links to the original publications. This technology overview will be a regular feature in *Worm* and over time can serve as a compendium describing new techniques and tools. For more techniques, *Methods in Biology* has recently published two lengthy issues devoted solely to *C. elegans* techniques.[1,2] We encourage authors to alert us to novel publications so that we can incorporate them in future editions.

Genome Manipulation

RAD for positional cloning. The last few years have seen a tremendous advancement of DNA sequencing technologies and other technologies interrogating and manipulating the genome. This has dramatically changed strategies to identify phenotype-causing mutations.[3] O'Rourke et al. recently described a new rapid mapping technique based on restriction site associated DNA polymorphism (RAD) markers, which are identified through large-scale sequencing.[4] In combination with selective pull-down and sequencing of the genomic interval containing the region of interest this provides a cost-effective and rapid approach for positional cloning. This approach complements previously published strategies using whole genome sequencing for simultaneous mapping and mutant identification.[5,6]

Single copy gene insertion. Evaluating the effects of transgenes in *C. elegans* is notoriously difficult as most methods either insert an uncontrollable amount of copies of the transgene, creating unwanted dosage effects, or introduce extrachromosomal arrays that are easily silenced, especially in the germline. Transposons circumvent these problems and have emerged as powerful tools to manipulate and modify the *C. elegans* genome.[7] Recently, Zeiser et al. provided a set of vectors designed to insert a single copy of a transgene expressing fluorescently tagged proteins in the germline and early embryo.[8] The authors are able achieve this by exploiting the Mos1 mediated single copy insertion[9] that not only allows for the insertion of a single copy transgene, but also allows for targeted insertion. This strategy should help avoid dosage-related effects of transgenes and problems with germline silencing.

Targeted gene manipulation. Targeted manipulation of the *C. elegans* genome is still challenging. Wood et al. have adopted nucleases targeted to specific sites by engineered zinc-finger containing DNA-binding proteins (ZFN)[10] or transcription activator-like effector domains nucleases (TALENs).[11,12] Both strategies use nucleases fused to DNA-binding proteins, which have been modified to bind specifically to the target sequence. Repair of the double-strand breaks introduced by the nuclease can lead to deletions or insertions at the break-point. This approach has been used to efficiently recover deletions in genes of interest.

Zheng et al. developed tools for special and temporal regulation of MicroRNA activity.[13] They are based on caged antisense oligonucleotides that are released by the application of UV light, allowing the researcher to block MicroRNA activity at specific time points. This study provides valuable tools to examine the requirements for MicroRNA at particular stages or in particular cells.

Proteomics

Stable-isotope labeling for proteomics. Proteomic analysis in *C. elegans* has proven to be a difficult as ^{15}N-labeling, a technique that labels proteins by converting isotope-labeled arginine to proline,[14] does not come without a few complications. However, Fredens et al. and Larance et al. were able to bi-pass some of these problems by using stable-isotope labeling with amino acids in cell culture (SILAC).[15,16] The group were able to avoid the problematic arginine to proline conversion by feeding the worms heavy lysine- and heavy arginine–labeled *Escherichia coli*, ultimately creating new methods for efficient quantitative mass spectrometry-based proteomic strategies.

In vivo degradation assays. The degradation of proteins via the ubiquitin/proteasome system (UPS) is extremely important for maintaining the homeostasis of proteins, or proteostasis. It is unclear what cues the UPS needs in order to maintain this delicate balance of proteins. Segref et al. have designed an assay to uncover these specific cues by identifying ubiquitin-dependent proteolysis pathways. The group did this by engineering a novel substrate, ubiquitin fusion degradation (UFD), tagged with a noncleavable ubiquitin N-terminally fused GFP (UbV-GFP), under the control of the promoter *sur-5*.[17] Using this tagging method the group were able to identify novel endogenous degradation factors in *C. elegans*.

Metabolite profiling. Analysis of metabolites in the tissues of experimentally altered *C. elegans* strains (altered via your method

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of choice) is an extremely valuable source for phenotypical information. Not surprisingly, how the tissue is harvested and processed is important to obtain high-quality, reproducible studies. Geier et al. have tested and evaluated 12 combinations using either the solvent chloroform/methanol or aqueous methanol along with six types of tissue disruption. They concluded that using 80% methanol as a solvent along with bead-beating obtains the best results.[18] Their optimization for tissue harvesting and processing should provide others with more time for actual experiments.

Imaging Probes and Techniques

Expanded palette for optogenetic control. Optogenetic methods are increasingly popular to manipulate and study behavior in *C. elegans*. Two reports early last year describe systems that use light-activated channels to stimulate or inhibit neurons in freely moving animals.[20] Both systems can track a freely moving animal in real-time and are able to illuminate targeted regions with laser light while the animal is moving. This allows optogenetic studies to be performed in unrestrained animals. The optogenetic tool set for use in *C. elegans* has been expanded to include a photo-activated adenylyl cyclase α (PACα), which can be used to increase cAMP levels.[21] This allows the in vivo manipulation of yet another important intercellular signaling molecule. Recently, Zhao et al. have gone even further by expanding the palette of genetically encoded Ca^{2+} indicators.[22] New indicators include an improved green indicator as well as novel blue and red indicators. This allows improved single-color imaging and also simultaneous imaging of Ca^{2+} in different subcellular compartments using different color indicators.

Microfluidic chambers for drug screening and imaging. The small size of *C. elegans* and its ability to live in liquid culture media has made microfluidic devices a method of choice to observe and manipulate these animals. Novel developments in this area include a new drug screening microfluidic platform, for observing and recording multiple response parameters at high resolution after drug application,[23] a microfluidic chip to study neuronal responses after multiple sequential chemical stimuli in immobilized animals[24] and a new device to study subcellular processes like axonal transport in unanaesthetized animals.[25] These high-throughput devices are changing how experiments are designed and executed. In return, voluminous amounts of data have already been collected with much more to come.

Lensfree imaging. As seen above, high-throughout screening of *C.elegans* on a microfluidic platform is becoming standard practice. One notable setback, however, is that a relatively large field of view (FOV) is required for imaging many worms at one time, and this is problematic. Standard microscopy techniques can only image small FOVs at a time and require tedious mechanical scanning, along with a bulky apparatus. One way to increase the FOV is by using lensfree on-chip imaging, and Coskun et al. did just that. Using a prism interface, they were able to capture the emitted fluorescent signal on a large opto-electronic sensor-array. The divergent nature of the fluorescent signal results in a blurry image; however, using a compressive sampling algorithm they were able to achieve a resolution of around 10 microns.[26] Imaging a large FOV, without time-wasting scanning, should greatly speed up current research reliant on large, worm-on-a-chip screenings.

3D live imaging of dynamic processes. In vivo imaging is especially useful for the study of dynamic biological processes. In the past, confocal epi-fluorescent microscopy[27] and single plane illumination microscopy (SPIM)[28] have been able to reconstruct 3D images of small organisms in vivo. However, these images lack high-resolution and the ability to observe ongoing biological processes mainly because the epi-fluorescence required for visualization restricts the ability to image intrinsic or extrinsic absorption. Riecker et al. have overcome this problem by adapting the existing technology of optical projection tomography (OPT) to resolve in vivo microscopic organisms at high-resolution using both fluorescence and absorption/brightfield imaging. Using this technology, the authors were able to resolve specific neuronal structures in live *C. elegans,* making this system ideal for the study of dynamic neuronal processes; such as degeneration, regeneration and migration.[29] Ultimately, this technology could be combined with microfluidics for high-throughput, dynamic, in vivo imaging.

Ultra quick, damage-free cryoprocessing. Preserving the ultrastructure in a tissue sample during the fixation process is a frustrating, long and perilous task. Typically, ice crystals that form during the freezing process destroy the delicate structures of a sample. One of the best ways to prevent ice crystals from forming during the freezing process is to use freeze- substitution (FS). FS circumvents damage by replacing the cellular fluid in the sample with an organic solvent. However, getting good results with FS requires quite a bit of time, taking anywhere from 3 h[30] to 4 d,[31] depending on the amount of tissue and cell type involved. McDonald and Webb have vastly improved the FS protocol by reducing this time to between 90 min and 3 h, otherwise known as the quick-freeze substitution (QFS).[32] Using this method, it is now possible to freeze cells in the morning and be off to image them by the afternoon.

Fluorescence on a nano scale. To get a full picture of the molecular topography of a cell, we have to be able to visualize proteins along with the organelles they are associated with. This poses a problem as current microscopy of imaged fluorescent proteins only gives a resolution around 200 nm. Not nearly enough to resolve tiny proteins and organelles that fit in a much smaller, even single digit, range. Combining the high resolution obtained from electron microscopy (EM), with fluorophore detection, would be the best of both worlds. Immunocyto-chemical electron microscopy (immune-EM) is one answer to this dilemma, but this method comes with its own technical difficulties; including a lack of antibodies, low resolution and a processing protocol that squelches much of the fluorescent signal. Watanabe et al. have found an answer to this dilemma by combining two microscopy techniques: stimulated emission depletion microscopy (STED) and photo-activated localization microscopy (PALM). This combo not only retains fluorescence, but also can image at a nanoscale resolution.[33] Using this method, the authors were not only able to pinpoint a protein's location, but also to see its associated structure. Imaging cells in this way

will provide us with a new level of detail, providing an increasingly more descriptive molecular topography.

Automatic cell identification for genetic and phenotypic assays. Identifying individual cells in *C. elegans* is not always easy, even when automatic single-cell tracking and identification techniques are applied. These techniques rely on a two-stage process: first segment, then recognize. Needless to say, any inaccuracies in the segmentation process results in inaccurate cell identification. This process is also limited by how many color channels are available during imaging and also by the availability of transgenic animals. Qu et al. have bypassed these setbacks by developing a new method for automatic cell identification called simultaneous recognition and segmentation (SRS). This method uses a direct atlas-to-image approach by aligning a 3D atlas image to a 3D image stack. Using this approach, they were able to correctly identify 97.7% of cells with accuracy rising to 99.1% when heavily rotated images, around 90°, were removed from the calculation.[34] This new method not only improves the reliability of existing protocols, it is also applicable to many other protocols, including those for other animal models.

Behavioral Analysis

A new tracking system, called Multi-Worm-Tracker (MWT), can simultaneously record the movement parameters of a large number of animals moving on an agar plate in real time.[35] Large scale screens are now feasible for behavioral studies such as chemotaxis assays or habituation assays. A new microfluidic system with microstructured areas allows researchers to evaluate the crawling behavior in response to defined spatio-temporal chemical stimuli.[36] The automated analysis helps to dissect behavior into specific components and allows a more reproducible and flexible experimental design. *C. elegans* move along straight lines at a fairly constant speed in an electric field. Taking advantage of this peculiar behavior, Maniere et al. manufactured a simple sorting device to separate populations according to their speed of movement, a gel electrophoresis chamber.[37] This technique was able to successfully separate animals moving at different speeds.

Genome Analysis, Bioinformatics and Databases

The identification of homologous genes is not always trivial. Recently She et al.[38] used a new approach based on the Basic Local Alignment Search Tool (BLAST).[39] BLAST provides short local alignments from pair-wise sequence comparisons, which by themselves do not necessarily identify homologous genes. She et al. took advantage of the speed of BLAST and used the local alignments produced by BLAST as input for an algorithm (genBlastG) to build gene models by exploiting sequence signals to identify introns as well as the beginning and end of a gene. genBlastG runs faster than commonly used algorithms while at the same time performing better. With more and more genome sequences becoming available genBlastG should speed up analysis and gene identification.

An additional database, NEMBASE (link: www.nematodes.org/nembase4/), allows for the analysis of nematode transcriptomes. This database has recently been updated to version NEMBASE4 and now contains data from 62 different nematode species.[40] As sequencing costs keep dropping, the sequencing of new genomes no longer requires the resources of large genome sequencing centers. Consequently, even small labs are able to sequence nematode genomes. Kumar et al. established a wiki to track sequencing efforts of nematode genomes with the ultimate goal of having the genomes of 959 nematodes sequenced.[41,42] The wiki is community-based and should allow researchers to connect more easily to establish collaborations or to share data.

References

1. Andew JH, Singson A, eds. [Elsevier Inc.]. Methods Cell Biol2011; 106:1-488.
2. Rothman JH, Singson A, eds. Methods Cell Biol 2012; 107:1-458; PMID:22226519
3. Doitsidou M, Poole RJ, Sarin S, Bigelow H, Hobert O. C. elegans mutant identification with a one-step whole-genome-sequencing and SNP mapping strategy. PLoS One 2010; 5:e15435; PMID:21079745; http://dx.doi.org/10.1371/journal.pone.0015435
4. O'Rourke SM, Yochem J, Connolly AA, Price MH, Carter L, Lowry JB, et al. Rapid Mapping and Identification of Mutations in Caenorhabditis elegans by RAD Mapping and Genomic Interval Pull-down Sequencing. Genetics 2011; 189:767-78; PMID:21900274; http://dx.doi.org/10.1534/genetics.111.134031
5. Zuryn S, Le Gras S, Jamet K, Jarriault S. A strategy for direct mapping and identification of mutations by whole-genome sequencing. Genetics 2010; 186:427-30; PMID:20610404; http://dx.doi.org/10.1534/genetics.110.119230
6. Doitsidou M, Poole RJ, Sarin S, Bigelow H, Hobert O. C. elegans mutant identification with a one-step whole-genome-sequencing and SNP mapping strategy. PLoS One 2010; 5:e15435; PMID:21079745; http://dx.doi.org/10.1371/journal.pone.0015435
7. Bessereau JL. Transposons in C. elegans. WormBook 2006; 18:1-13; PMID:18023126
8. Zeiser E, Frøkjær-Jensen C, Jorgensen E, Ahringer J. MosSCI and gateway compatible plasmid toolkit for constitutive and inducible expression of transgenes in the C. elegans germline. PLoS One 2011; 6:e20082; PMID:21637852; http://dx.doi.org/10.1371/journal.pone.0020082
9. Frøkjaer-Jensen C, Davis MW, Hopkins CE, Newman BJ, Thummel JM, Olesen SP, et al. Single-copy insertion of transgenes in Caenorhabditis elegans. Nat Genet 2008; 40:1375-83; PMID:18953339; http://dx.doi.org/10.1038/ng.248
10. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nat Rev Genet 2010; 11:636-46; PMID:20717154; http://dx.doi.org/10.1038/nrg2842
11. Sanjana NE, Cong L, Zhou Y, Cunniff MM, Feng G, Zhang F. A transcription activator-like effector toolbox for genome engineering. Nat Protoc 2012; 7:171-92; PMID:22222791; http://dx.doi.org/10.1038/nprot.2011.431
12. Wood AJ, Lo TW, Zeitler B, Pickle CS, Ralston EJ, Lee AH, et al. Targeted genome editing across species using ZFNs and TALENs. Science 2011; 333:307; PMID:21700836; http://dx.doi.org/10.1126/science.1207773
13. Zheng G, Cochella L, Liu J, Hobert O, Li WH. Temporal and spatial regulation of microRNA activity with photoactivatable cantimirs. ACS Chem Biol 2011; 6:1332-8; PMID:21977972; http://dx.doi.org/10.1021/cb200290e
14. Krijgsveld J, Ketting RF, Mahmoudi T, Johansen J, Artal-Sanz M, Verrijzer CP, et al. Metabolic labeling of C. elegans and D. melanogaster for quantitative proteomics. Nat Biotechnol 2003; 21:927-31; PMID:12858183; http://dx.doi.org/10.1038/nbt848
15. Fredens J, Engholm-Keller K, Giessing A, Pultz D, Larsen MR, Højrup P, Møller-Jensen J, Færgeman NJ. Quantitative proteomics by amino acid labeling in C. elegans. Nat Methods 2011 Aug; 28(10):845-7; PMID:21874006; http://dx.doi.org/10.1038/nmeth.1675
16. Larance M, Bailly AP, Pourkarimi E, Hay RT, Buchanan G, Coulthurst S, et al. Stable-isotope labeling with amino acids in nematodes. Nat Methods 2011; 8:849-51; PMID:21874007; http://dx.doi.org/10.1038/nmeth.1679
17. Segref A, Torres S, Hoppe T. A screenable in vivo assay to study proteostasis networks in Caenorhabditis elegans. Genetics 2011; 187:1235-40; PMID:21288877; http://dx.doi.org/10.1534/genetics.111.126797

18. Geier FM, Want EJ, Leroi AM, Bundy JG. Cross-platform comparison of Caenorhabditis elegans tissue extraction strategies for comprehensive metabolome coverage. Anal Chem 2011; 201:3730-6.
19. Leifer AM, Fang-Yen C, Gershow M, Alkema MJ, Samuel AD. Optogenetic manipulation of neural activity in freely moving Caenorhabditis elegans. Nat Methods 2011; 8:147-52; PMID:21240279; http://dx.doi.org/10.1038/nmeth.1554
20. Stirman JN, Crane MM, Husson SJ, Wabnig S, Schultheis C, Gottschalk A, et al. Real-time multimodal optical control of neurons and muscles in freely behaving Caenorhabditis elegans. Nat Methods 2011; 8:153-8; PMID:21240278; http://dx.doi.org/10.1038/nmeth.1555
21. Weissenberger S, Schultheis C, Liewald JF, Erbguth K, Nagel G, Gottschalk A. PACα–an optogenetic tool for in vivo manipulation of cellular cAMP levels, neurotransmitter release, and behavior in Caenorhabditis elegans. J Neurochem 2011; 116:616-25; PMID:21166803; http://dx.doi.org/10.1111/j.1471-4159.2010.07148.x
22. Zhao Y, Araki S, Wu J, Teramoto T, Chang YF, Nakano M, et al. An expanded palette of genetically encoded Ca^{2+} indicators. Science 2011; 333:1888-91; PMID:21903779; http://dx.doi.org/10.1126/science.1208592
23. Wang J, Feng X, Du W, Liu BF. Microfluidic worm-chip for in vivo analysis of neuronal activity upon dynamic chemical stimulations. Anal Chim Acta 2011; 701:23-8; PMID:21763804; http://dx.doi.org/10.1016/j.aca.2011.06.007
24. Carr JA, Parashar A, Gibson R, Robertson AP, Martin RJ, Pandey S. A microfluidic platform for high-sensitivity, real-time drug screening on C. elegans and parasitic nematodes. Lab Chip 2011; 11:2385-96; PMID:21647497; http://dx.doi.org/10.1039/c1lc20170k
25. Mondal S, Ahlawat S, Rau K, Venkataraman V, Koushika SP. Imaging in vivo neuronal transport in genetic model organisms using microfluidic devices. Traffic 2011; 12:372-85; PMID:21199219; http://dx.doi.org/10.1111/j.1600-0854.2010.01157.x
26. Coskun AF, Sencan I, Su TW, Ozcan A. Lensfree fluorescent on-chip imaging of transgenic Caenorhabditis elegans over an ultra-wide field-of-view. PLoS One 2011; 6:e15955; PMID:21253611; http://dx.doi.org/10.1371/journal.pone.0015955
27. Liu Z, Nukazuka A, Takagi S. Improved method for visualizing cells revealed dynamic morphological changes of ventral neuroblasts during ventral cleft closure of *Caenorhabditis elegans*. Dev Growth Differ 2007; 49:49-59; PMID:17227344; http://dx.doi.org/10.1111/j.1440-169X.2007.00906.x
28. Huisken J, Swoger J, Del Bene F, Wittbrodt J, Stelzer EH. Optical sectioning deep inside live embryos by selective plane illumination microscopy. Science 2004; 305:1007-9; PMID:15310904; http://dx.doi.org/10.1126/science.1100035
29. Rieckher M, Birk UJ, Meyer H, Ripoll J, Tavernarakis N. Microscopic optical projection tomography in vivo. PLoS One 201; 6(4):e18963.
30. Buser C, Walther P. Freeze-substitution: the addition of water to polar solvents enhances the retention of structure and acts at temperatures around -60 °C. J Microsc 2008; 230:268-77; PMID:18445157; http://dx.doi.org/10.1111/j.1365-2818.2008.01984.x
31. Humbel B. Handbook of Cryo-Preparation Methods for Electron Microscopy. Eds. A.Cavalier, D.Spehner& B.M.Humbel. 2009; 319–341. CRC Press.
32. McDonald KL, Webb RI. Freeze substitution in 3 hours or less. J Microsc 2011; 243:227-33; PMID:21827481; http://dx.doi.org/10.1111/j.1365-2818.2011.03526.x
33. Watanabe S, Punge A, Hollopeter G, Willig KI, Hobson RJ, Davis MW, et al. Protein localization in electron micrographs using fluorescence nanoscopy. Nat Methods 2011; 8:80-4; PMID:21102453; http://dx.doi.org/10.1038/nmeth.1537
34. Qu L, Long F, Liu X, Kim S, Myers E, Peng H. Simultaneous recognition and segmentation of cells: application in C.elegans. Bioinformatics 2011; 27:2895-902; PMID:21849395; http://dx.doi.org/10.1093/bioinformatics/btr480
35. Swierczek NA, Giles AC, Rankin CH, Kerr RA. High-throughput behavioral analysis in C. elegans. Nat Methods 2011; 8:592-8; PMID:21642964; http://dx.doi.org/10.1038/nmeth.1625
36. Albrecht DR, Bargmann CI. High-content behavioral analysis of Caenorhabditis elegans in precise spatio-temporal chemical environments. Nat Methods 2011; 8:599-605; PMID:21666667; http://dx.doi.org/10.1038/nmeth.1630
37. Manière X, Lebois F, Matic I, Ladoux B, Di Meglio JM, Hersen P. Running worms: C. elegans self-sorting by electrotaxis. PLoS One 2011; 6:e16637; PMID:21326598; http://dx.doi.org/10.1371/journal.pone.0016637
38. She R, Chu JS, Uyar B, Wang J, Wang K, Chen N. genBlastG: using BLAST searches to build homologous gene models. Bioinformatics 2011; 27:2141-3; PMID:21653517; http://dx.doi.org/10.1093/bioinformatics/btr342
39. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990; 215:403-10; PMID:2231712
40. Elsworth B, Wasmuth J, Blaxter M. NEMBASE4: the nematode transcriptome resource. Int J Parasitol 2011; 41:881-94; PMID:21550347; http://dx.doi.org/10.1016/j.ijpara.2011.03.009
41. Kumar S, Schiffer PH, Blaxter M. 959 Nematode Genomes: a semantic wiki for coordinating sequencing projects. Nucleic Acids Res 2012; 40(Database issue): D1295-300; PMID:22058131; http://dx.doi.org/10.1093/nar/gkr826
42. Kumar S, Koutsovoulos G, Kaur G, Blaxter M. Toward 959 nematode genomes. Worm 2012; 1:41-9; http://dx.doi.org/10.4161/worm.19575