C. elegans select

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The technical toolkit for Caenorhabditis elegans expands to include experimental selection using antibiotic resistance genes.

Genetic selection systems that use antibiotics in combination with antibiotic resistance genes are the workhorse of any molecular biology laboratory. These flexible tools allow stringent, conditional selection of experimentally manipulated individuals. Until now, however, their broad applicability has been limited to studies in single-cell organisms, cultured cells and plants. New work reported in Semple et al.1 and Giordano-Santini et al.2 in this issue of Nature Methods applies these selection systems to C. elegans and related nematodes, adding this powerful method to the C. elegans molecular biology toolkit1,2.

Introduction of experimental DNA into cells to produce stably transmitted transgenes is an essential tool to incorporate new or modified genes into research organisms or to otherwise manipulate their genomes. In C. elegans, transgenes can be rapidly produced by injection of experimental DNA (such as plasmid DNA) directly into the gonad of a parental worm3. In the offspring, the injected DNA is assembled into large, extrachromosomal arrays by recombination. Conveniently, the transgenic arrays can incorporate representatives of any DNAs included in an injected mixture, bypassing the need to covalently link the experimental DNA to other reporter DNA included as a transformation positive control. These arrays are replicated and transmitted through mitosis and meiosis in a semistable manner in the worm, even without the inclusion of chromosomal features such as telomere or centromere sequences in the injected DNA.

Although the relative ease with which these C. elegans multicopy extrachromosomal transgenes can be produced has led to their widespread use, there are substantial limitations. These include the fact that the multiple copies of the experimental gene will express in unpredictable—frequently excessive—amounts. Additionally, expression from the transgenes can be silenced or induce cosuppression (silencing of homologous gene sequences on the chromosome), especially in the germline4,5. Furthermore, injected marker systems used to identify the transgenes can be identified visually but generally do not provide ‘selection’ strategies in which non–transgene-bearing worms do not survive. When selection is incorporated, the system requires introduction of specific C. elegans mutations into the genetic background of an experimental strain, limiting the use of such strategies in large-scale experiments and in non-C. elegans nematodes.

Some of these shortcomings have been recently addressed by the optimization of methodology for integration of a single-copy transgene into a chromosomal locus, using a transposon excision and repair strategy6. Although this method addresses the problems of transgene expression amount and silencing that arise from multiple gene copies, it is still constrained by the selection marker systems available for C. elegans.

Enter Semple et al.1 and Giordano-Santini et al.2, to provide the missing piece of the puzzle. These groups turned to antibiotic resistance gene systems that are widely used in eukaryotic cells (such as yeast and cultured cells) to expand the transgenic marker selection systems available for C. elegans. They show that worms with constitutive expression of antibiotic resistance genes (PuRoR or NeoR) are healthy and that the gene expression confers resistance to drug concentrations (puromycin or G-418), which will otherwise kill the worms. Notably, Semple et al.1 bypass a problem encountered in the past by other researchers (the relative inaccessibility of cells in intact worms to externally administered antibiotic, necessitating large treatment doses) by including small amounts of detergent when they subjected worms to drug selection. Both groups also show that their selection systems are

Figure 1 | Developing animal transgenesis methods. The selection approach of Semple et al.1 and Giordano-Santini et al.2 combined with identification of species-appropriate DNA transformation strategies yields a general scheme to develop a transgenic system for experimental animals.

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Stem cells feel the difference

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Arrays of microposts of different heights generate substrates with different flexibility, on which cells can be grown.

Fat is softer than bone, and mimicking this difference with flexible substrates has been observed to influence the differentiation of adult stem cells. In this issue of Nature Methods, Chen and co-workers have made dense arrays of various-flexibility pillars for cells to adhere on top of and show that the density and consistency of experiments carried out with these traditional transgenes. They can also be used with other nematode species that lack the full genetic toolbox of C. elegans. Both groups showed the selection system works in the related C. briggsae, and Giordano-Santini et al. demonstrate G-418 sensitivity in various nematode species.

Cells lack eyes to see differences in substrates, but they do possess tactile mechanisms that allow them to feel differences. Using a silicone rubber material commonly used to caulk the gaps between tile and tub and using methods for micro-molding widely adapted from the computer industry for lab-on-a-chip microfluidics, Chen and co-workers made arrays of short or long, micrometer-diameter pillars spaced by a few micrometers so that mesenchymal stem cells (MSCs)—which are about 10 micrometers in diameter in suspension—could adhere to the tops of many pillars all at once.

MSCs are adult stem cells that can be obtained from many human tissues and can be induced to differentiate into cells that express markers for various solid mesenchymal tissues such as fat, muscle, cartilage and bone. Empirically established cocktails of soluble factors, when added to cultured MSCs, will stimulate them to differentiate over days to weeks into select lineages. Adhesion to plastic culture dishes is the usual means by which MSCs are separated from tissues, and these cells must adhere in order to survive and differentiate in culture. However, MSCs will certainly attach to other substrates, and some physical properties of the substrate seem to affect how the cells respond, with wider implications for how cells in general respond to soluble factors, including drugs.

In their work, Chen and colleagues adsorbed the extracellular matrix molecule fibronectin to the flat tops of the pillars so that cell membrane adhesion receptors could engage the pillars, but such cell attachment does not stop at the cell membrane. Receptors that mediate adhesion are quickly linked to the actin cytoskeleton, which also contains highly active myosin similar to that found in muscle. What this means is that cells adhere and pull in a manner similar to someone grabbing and lifting a barbell. If the pillars are long, a cell pulls with relative ease and deflects the tops of posts by many micrometers, whereas if the pillars are short, then the cell cannot deflect the tops of posts very much at all. For insight, grab a plastic ruler with a hand at either end; you will find it rather easy to bend, but if you bring your hands closer together (say by a factor of ten) then the ruler will be harder to bend to the same extent (in fact, 1,000-fold more force is needed). Arrays of long pillars are therefore effectively equivalent to a soft substrate, perhaps as soft as fat, whereas arrays of short pillars might be perceived by cells as effectively stiff or rigid like bone (Fig. 1).

Past work with various gels of different elasticity had indeed shown that matrix elasticity can direct lineage specification of stem cells. Very soft gels that mimic brain tissue tend to be inductive for neuronal genes, whereas an intermediate elasticity...