5,000 RNAi experiments on a chip

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Microarrays have revolutionized gene expression studies and are set to do the same for genome-wide RNA interference screens.

In theory, genome-wide RNAi screens offer the potential to identify genes required for any biological process of interest. In practice, because an individual experiment needs to be performed for every gene in a genome, even a single screen can be time-consuming. Hence the system biologist's dream of using the data from hundreds of RNAi screens to provide an unbiased, systematic understanding of metazoan gene function still seems like a rather distant prospect. With a report described on page 127 of this issue, however, Sabatini and co-workers move this dream a step closer to reality[1].

As with so many technologies, one way to ramp up the speed of RNAi screens is to miniaturize the process. This is the approach taken by Sabatini and colleagues, who have adapted their 'reverse-transfection' technology[2] to allow them to perform thousands of RNAi experiments on a single glass slide. In the original reverse-transfection protocol, mammalian cells are cultured on a glass slide that has first been printed at high density with different DNA expression constructs[2]. The printed DNAs are mixed with a lipid-based transfection reagent, so the cells growing on the printed areas very efficiently take up the DNA, creating defined spots of localized transgene expression within a lawn of nontransfected cells. To adapt the method for RNAi screening, the authors print double-stranded RNA instead of DNA onto the slides. Cells that overlay each spot take up the dsRNA, which then enters the RNAi pathway and causes sequence-specific inhibition of gene expression. Each spot therefore defines an area of cells that have a knockdown in expression of a single, defined gene. The entire array can be analyzed for a phenotype of interest, thus allowing genome-wide RNAi screens to be performed on a few slides (**Fig. 1**).

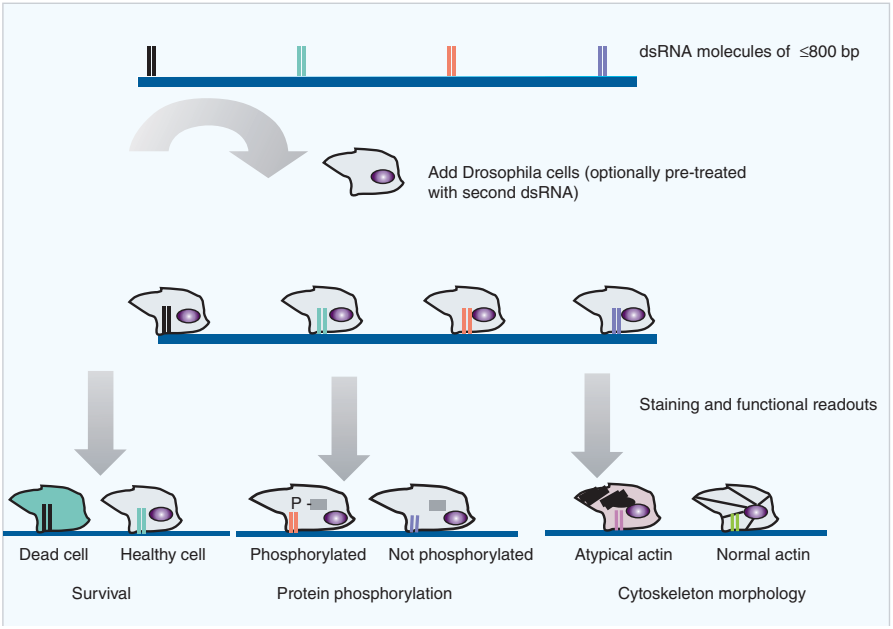

Figure 1 | Schematic of dsRNA microarray for Drosophila cells.

Several other groups have also recently described reverse transfection–based RNAi microarrays[3–6]. However, these groups have all used mammalian cells—which, because of an antiviral response to dsRNA, can only be transfected with ~21–23 nt short interfering RNAs (siRNAs) or short hairpin RNA (shRNA)-expressing vectors. siRNAs and shRNAs are often less effective triggers for RNAi than the long dsRNAs that can be used in invertebrates. In the current work, Sabatini and co-workers developed new slide chemistries and printing methods that allowed them to develop RNAi microarrays for *Drosophila melanogaster* cells, in which RNAi is triggered very effectively by long dsRNAs. Currently the arrays are printed at a density that would allow ~5,600 dsRNAs to be printed on a standard microscope slide, allowing a genome-wide screen to be performed, in sufficient replicates, on ~5–10 slides. Printing RNAi microarrays uses the same robots that are used to produce expression microarrays in hundreds of laboratories worldwide; so, provided the various collections of dsRNAs are made available to researchers, the technology could rapidly be established in many laboratories.

Successful delivery of dsRNA is, however, only half of a successful RNAi experiment—you also need an informative phenotype readout. In the current study the authors assessed three cellular phenotypes: cell number, cell viability, and phosphorylation of the antiapoptotic signaling molecule dAkt. These assays are scored using automated image analysis of cells stained with dyes or fluorescently-labeled antibodies. By screening a collection of 384 dsRNAs (selected to include most Drosophila tyrosine kinases and all serine-threonine phosphatases), the authors were able to identify both known and previously unidentified regulators of cell growth

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and dAkt phosphorylation. Previous RNAi screens have used reporter gene assays[7,8], and these too could be easily adapted to microarray format. However, a cell is only a cell, and for systematically studying gene function by RNAi in the context of a whole organism, *Caenorhabditis elegans* will remain the system of choice at present, because of the ease of systemic RNAi delivery[9].

So why would a researcher choose to perform a genome-wide RNAi screen in Drosophila cells, when the same screen could be performed in mammalian cells using arrayed siRNAs or shRNAs? First, triggering RNAi using long dsRNAs in Drosophila cells is a very robust procedure. Second, genome-scale RNAi libraries are already available for Drosophila [7,8,10], whereas only smaller libraries are currently available for mammalian cells[11–13]. Third, Drosophila has a less redundant genome than mammals, which should allow functions to be identified for a greater proportion of genes by RNAi screens. Finally, and perhaps most excitingly, two distinct dsRNAs can be used in combination to effectively silence two genes at once in Drosophila cells. It is likely that reproducibility targeting any two mammalian genes simultaneously by RNAi will prove a more difficult challenge.

Targeting two genes at once by RNAi opens up the ability to perform large-scale screens for synthetic or epistatic genetic relationships. Sabatini and co-workers demonstrate the power of this approach by identifying genes that affect the phosphorylation levels of the antiapoptotic protein dAkt when the major dAkt phosphatase dPTEN is simultaneously inactivated by RNAi. Understanding the phenotypic consequences of interactions between two or more genes is one of the great challenges for many biologists, whether they work on model organisms or complex genetic diseases in humans. Indeed, recent work using the gene-deletion collection in yeast has suggested that the extent of synthetic interactions between genes is probably much larger than previously imagined[14]. It seems likely that the RNAi microarrays described by Sabatini and co-workers may provide a powerful platform with which to start systematically testing the vast number of gene combinations required to dissect synthetic genetic interactions in metazoans.

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SNPs made routine

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With the sequencing of the human genome, millions of single-nucleotide polymorphisms, or SNPs, have been discovered and can be used as markers to identify genes contributing to common human diseases. Two large sets of SNPs have now been organized in panels for high-throughput genotyping.

Two new platforms for high-throughput genotyping of single-nucleotide polymorphisms (SNPs) are presented in this issue of *Nature Methods*. These new technologies promise very fast, high-quality data, and have the potential to improve our ability to map genes for common human diseases by either linkage or association. Murray *et al.*[1] report on a panel of approximately 4,700 SNPs for use in human genetic linkage studies, in which researchers seek to identify an association *within families* between a disease trait and the pattern of inheritance of DNA at marker loci. Matsuzaki *et al.*[2] report on a complementary pair of chips for the simultaneous genotyping of over 100,000 SNPs that could potentially be useful for genome-wide association studies, in which researchers seek to establish an association *across a population* between individuals' disease status and their marker genotypes. Genetic linkage provides lower-resolution mapping than does association analysis, as the extent of association along a chromosome is broader within a family than across a population, but for the same reason, several orders of magnitude fewer markers are needed for linkage than for association mapping.

Short tandem repeat polymorphisms (STRPs, also known as microsatellites) have been the most commonly used genetic marker for human genetic linkage studies over the last decade. STRPs are differences in the lengths of repeats of motifs such as CA or GATA, and typically have many alleles; SNPs are single base differences, and generally have just two alleles. As a result, an individual STRP marker is generally more informative than a SNP, and so linkage analysis with STRPs may be done with fewer markers; a typical genome scan with STRPs includes just 400 markers.

Murray *et al.* genotyped nearly 500 individuals in 28 large families with their 4,700-SNP assay, and used these data to estimate a genetic map for the markers. Although the map itself provides little new biological information, because it is of lower resolution than the earlier map produced by deCODE Genetics Inc.[3], these data allow the identification of genotyping errors that can not be identified by other means, and provide relatively precise estimates of genotyping error rates. Using this information, Murray *et al.* report extremely low rates of genotyping errors, on the order of 1/10,000, and low rates of missing data. In comparison, the genotyping error and missing data rates for STRPs are 50–100 times higher[4].

Matsuzaki *et al.* report similarly low error

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