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Studying cell division by RNAi

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Abstract

A large-scale RNA interference screen has proved an efficient and powerful reverse genetic approach for identifying genes involved in cell division in *Caenorhabditis elegans*.

Significance and context

Gene function can be tested directly through established assays in a given system, or indirectly through the characterization of related genes in other organisms, but it is often difficult, if not impossible, to assign a function to a significant fraction of genes uncovered by sequencing projects. There is therefore a need for approaches that take advantage of an organism's completely sequenced genome to identify gene function rapidly and efficiently.

Although large-scale functional genomic studies have been successful in *Saccharomyces cerevisiae*, such analysis is lacking in multicellular organisms. Gönczy *et al*. have developed a high-throughput assay using RNA interference (RNAi) to assign functions to genes involved in *C. elegans* cell division. RNAi is a method of blocking endogenous gene expression in a sequence-specific way by the introduction of appropriate double-stranded RNA. Because the *C. elegans* genome is completely sequenced, and cell division processes in the nematode embryo can be examined easily with time-lapse microscopy, the authors had a highly tractable system. They made use of the potency and sequence-specificity of RNAi as an inhibitor of gene function and base their entire screen on this tool. After screening almost 2,300 (about 96%) of the predicted open reading frames (ORFs) on chromosome III, they have identified 133 genes necessary for proper cell division in the *C. elegans* embryo. Their results suggest that such large-scale genome-wide screens are both feasible and useful for identifying the functions of genes involved in other biological processes.

Key results

The authors generated double-stranded RNA (dsRNA) corresponding to 2,232 of the 2,315 predicted ORFs (96.4%) on chromosome III and injected them into wild-type animals. Single embryos laid by these RNAi-treated animals were collected and filmed until the four-cell stage to identify any deviations from normal cell division. Some injected animals were also scored for whether they produced progeny, and whether their progeny deviated from wild-type development. Using this screening strategy, Gönczy

et al. discovered that dsRNAs corresponding to 133 genes (6.1% of genes tested) gave rise to a cell-division phenotype detectable under time-lapse microscopy. Another 139 genes, when disrupted, produced a phenotype in which the progeny of the injected animals were mutant for embryogenesis or larval/adult development.

Amazingly, of the 133 genes identified by time-lapse microscopy, only 11 (8.2%) had been previously assigned a function by direct experimentation. The majority of the remaining 122 genes have homologs in other organisms. The authors therefore argue that this screen represents an effective tool for identifying genes involved in embryonic cell division. They describe some of the phenotypes observed and the genes that, when disrupted, produced those phenotypes. For example, RNAi of five genes resulted in a phenotype in which the nuclei of one- and two-cell embryos are either poorly visible or not visible, probably due to a defect in nuclear envelope assembly. Three of these genes turn out to encode the small GTPase Ran and two of its activating proteins, all of which have been shown in *Xenopus* extracts to be crucial for nuclear envelope assembly. The remaining two genes were not previously known to affect nuclear envelope assembly, and the screen provides *in vivo* evidence that they may have a role.

Gönczy *et al*. also note the surprising involvement of certain biochemical pathways in mediating embryonic cell division. They found that 27 of the RNAi-affected genes encoded components of the translational machinery, three encode DNA replication licensing factors, and six encode components of the DNA replication machinery itself. Finally, the authors investigated whether the genes identified in the screen have orthologs in other organisms. They found that over 47% of the *C. elegans* genes that gave an RNAi phenotype in the time-lapse microscopy assay had orthologs in both *Drosophila* and *S. cerevisiae*.

Links

Supplementary data to *Nature* 2000, **408:** 331-336 is freely available. The authors have made additional information available at their website Worm protein database A closely related paper by Fraser *et al.* on an RNAi screen on *C. elegans* chromosome I appeared in the same issue of *Nature*, and is available to subscribers (see related report - *Genome* ***Biology*** **2**(2):reports0002).

Conclusions

The authors conclude that RNAi is an efficient reverse genetic tool for large-scale functional genomic analysis. They predict that they could identify over 1,000 genes essential for early cleavage divisions in the *C. elegans* embryo if they screened the entire genome. They also predict that by using RNAi for other processes with other assays, and by comparing the data with functional data emerging from other organisms, many more eukaryotic genes could be assigned functions.

Reporter's comments

This screen, together with the related RNAi screen on chromosome I performed by the Ahringer laboratory, represent the first attempts to mine the completely sequenced nematode genome for gene function. Gönczy *et al*. provide compelling evidence that a large-scale functional genomic screen in *C. elegans* is not just feasible but is amazingly powerful for assigning functions to novel genes and for identifying new and unexpected roles for known genes.

Table of links

Nature

Supplementary data to *Nature* 2000, **408:** 331-336

Worm protein database

Genome ***Biology*** **2**(2):reports0002

References

1. Gönczy P, Echeverri C, Oegema K, Coulson A, Jones SJM, Copley RR, Duperon J, Oegema J, Brehm M, Cassin E, et al: Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. Nature. 2000, 408: 331-336. 0028-0836

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