PublisherInfo		
PublisherName	:	BioMed Central
PublisherLocation	:	London
PublisherImprintName	:	BioMed Central

Comprehensive interference

ArticleInfo		
ArticleID	:	4842
ArticleDOI	:	10.1186/gb-spotlight-20030918-01
ArticleCitationID	:	spotlight-20030915-01
ArticleSequenceNumber	:	194
ArticleCategory	:	Research news
ArticleFirstPage	:	1
ArticleLastPage	:	3
ArticleHistory	:	RegistrationDate : 2003–9–18 OnlineDate : 2003–9–18
ArticleCopyright	:	BioMed Central Ltd2003
ArticleGrants	:	
ArticleContext	:	130594411

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RNA interference (RNAi) is a molecular biological technique that has been extensively used to deplete specific components in cells and has been invaluable in determining the requirements of a given protein in a particular cellular process. One such system is the *Drosophila* S2 cell system, which is very sensitive to double-stranded RNA (dsRNA)-mediated gene silencing, but comprehensive study of the molecular components required for cellular processes in higher eukaryotes - such as mitosis and cell migration - has been more difficult to examine. Two papers from the University of California, San Francisco in the September 15 Journal of Cell Biology report comprehensive studies elucidating the molecular requirements for actin-binding proteins in lamella formation and motor proteins in mitosis.

In the first paper, Steven L. Rogers and colleagues used RNAi to investigate the requirements of approximately 90 actin-binding proteins in lamella formation in the *Drosophila* S2 cell line. S2 cells are normally spherical, nonmotile, and have no polarity, but when plated on concanavalin A (con-A)-covered glass coverslips, they undergo substantial morphological changes. The authors plated GFP-actin=expressing S2 cells on con-A coverslips, examined them by fluorescence microscopy, and observed that 90% of the cells formed lamella. This process of lamella formation was used as an assay to test the actin-binding proteins involved, and 19 of the proteins tested were shown to result in aberrant lamella formations when depleted from cells. These included Arp2/3, SCAR, cofilin, Aip1, profilin, and cyclase-associated protein (*Journal of Cell Biology* 2003, **162:**1079-1088).

The authors also observed that the SCAR inhibitors kette, Sra-1, and Abi had the same phenotype as RNAi-depleted SCAR cells; one would expect to observe opposite phenotypes in these cells. This contradiction was resolved when it was determined that kette, Sra-1, and Abi RNAi treatment caused reductions in SCAR levels, suggesting that these proteins were required for SCAR stability

"[We] have developed a novel system of the study of actin cytoskeletal dynamics that is amenable to in vivo imaging and targeted inhibition of proteins via RNAi," conclude Rogers et al.

In the second paper, Gohta Goshima and Ronald D. Vale preformed RNAi to all 25 *Drosophila* kinesins and to cytoplasmic dynein and screened the RNAi-depleted cells for mitotic defects. Cell division - and particularly the mitotic spindle - in S2 cells was examined by antitubulin staining or GFP-tubulin expression, followed by time-lapse observations using spinning-disk confocal microscopy. The S2 cells were then treated with dsRNA, cultured for 3 to 4 days, and reexamined for defects. Eight of the 25 kinesins and cytoplasmic dynein caused mitotic defects without causing changes to the interphase microtubule pattern. Four of the kinesins (Klp61F, Klp10A, Klp67A, and Ncd) showed distinct mitotic spindle abnormalities, including monopolar spindles, spindles with unfused poles, and multiple γ-tubulin foci. The authors then showed that the depletion of three kinesins - CENP-meta, Klp3A, and Nod - caused chromosome misalignment at the metaphase plate, suggesting that chromosome congression is mediated by these three kinesins (*Journal of Cell Biology* 2003, **162:**1003-1016).

"Our work indicates that the *Drosophila* S2 cell line provides a powerful model system for investigating the mechanisms of mitosis [and lamella formation] through a combination of gene inactivation and high-resolution fixed- and live-cell imaging," conclude Goshima and Vale.

With the issue of functional redundancy always pertinent, the systematic use of such RNAi techniques may prove useful in future determinations of the involvement (or not) of a particular protein in a specific cellular process.

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