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Using RNAi to catch *Drosophila* genes in a web of interactions: insights into cancer research

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The completion of whole-genome sequencing of various model organisms and the recent explosion of new technologies in the field of Functional Genomics and Proteomics is poised to revolutionize the way scientists identify and characterize gene function. One of the most significant advances in recent years has been the application of RNA interference (RNAi) as a means of assaying gene function. In the post-genomic era, advances in the field of cancer biology will rely upon the rapid identification and characterization of genes that regulate cell growth, proliferation, and apoptosis. Significant efforts are being directed towards cancer therapy and devising efficient means of selectively delivering drugs to cancerous cells. In this review, we discuss the promise of integrating genome-wide RNAi screens with proteomic approaches and small-molecule chemical genetic screens, towards improving our ability to understand and treat cancer.
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Introduction

Genetic and biochemical analyses in model systems such as the fruitfly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* have successfully identified genes that play key regulatory roles in fundamental cellular and developmental processes. Understanding the normal function of these genes has provided significant insights into what goes awry in abnormal situations, such as tumorigenesis. Recent analyses of the complete genome sequences of model organisms such as *Drosophila* and *C. elegans*, as well as of humans, reveal that traditional genetic and biochemical approaches have ascribed functions for only a fraction of the total number of predicted genes (Venter *et al*., 1998; Adams *et al*., 2000). Thus, the roles for many as yet uncharacterized genes in normal development and cancer remain to be discovered. The full potential of the genome sequence can be realized by devising new technologies that efficiently and systematically bridge the gap between the genomic sequence of a predicted gene and its function. It is also increasingly clear that individual proteins are almost always found in a variety of complexes with numerous other molecules within a cell, such as other proteins, DNAs or RNAs. Thus, it is the coordinate activity of these complex interactions that eventually determine the biological characteristics of a cell (Hartwell *et al*., 1999; Bray, 2003; Hucka *et al*., 2003; Milo *et al*., 2004). The same is also true of interaction/crosstalk between entire signaling pathways that regulate cell growth, proliferation, and differentiation, as common effectors and/or integrators of multiple signaling pathways need to be coordinately regulated to determine cell behavior (Spirin and Mirny, 2003; Barabasi and Oltvai, 2004). A key challenge for the present day biologist is to devise ways of integrating information at the whole-genome scale in order to better understand the regulation and dynamics of complex molecular interactions and their function in determining cell biological and developmental events.

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Functional genomics: from gene sequence to gene function

The challenge presented by the various large-scale genome projects is how to derive biologically relevant information from the raw sequences. In the past few years, a number of approaches to mine this information have emerged, such as Expression Genomics, Proteomics, Computational Genomics, and Functional Genomics. Expression Genomics rely on approaches such as microarray and SAGE technologies, which allow the comparison of expression profiles of genes in various samples at a given time, and correlate the expression of groups of genes with a specific genotype. This can be used to identify target genes of specific signaling pathways in different cell types at different stages of development, and to assign molecular signatures to specific mutant or disease cell types (Sanchez-Carbayo and Cordon-Cardo, 2003; van Duin *et al*., 2003). Proteomic approaches, on the other hand, help to determine when, where, and how proteins interact with other molecules (protein, DNA, RNA) in the cell. This has been made possible by the use of new techniques

that allow semi-automated yeast two-hybrid screens, chromatin immunoprecipitation (ChIP) chips, protein chips (Buckholz *et al*., 1999; Auerbach *et al*., 2002; Bader and Hogue, 2003; Diaz-Camino *et al*., 2003; Giot *et al*., 2003) and high-throughput mass spectrometry using the tandem affinity purification (TAP)-tag technology (Rigaut *et al*., 1999; Puig *et al*., 2001; Gavin *et al*., 2002). Information generated from these approaches gives rise to a web of larger network of interactions including those of protein–protein interactions, signal transduction cascades, and transcription-regulatory networks, and allow researchers to generate testable hypotheses. Computational Genomics, which is a fast expanding field, encompasses every approach based on bioinformatics as the primary 'data-mining' vehicle (Rosamond and Allsop, 2000; Li and Wang, 2003). For example, bioinformatic analysis of the primary sequence generated from the completion of large-scale genome sequencing projects in multiple model organisms has provided a powerful tool to assign putative functions to open reading frames (ORFs). Such analyses of course have to rely on and are limited by previously determined primary experimental data on the function of specific structural domains found in a variety of proteins. As a result, such computational approaches are highly dependent on the quality of the information from primary experimental data.

Expression, Proteomic, and Bioinformatic approaches lead to various degrees of prediction of gene function; however, these hypotheses remain to be tested experimentally. By contrast, Functional Genomics allows a direct test of the function, of genes predicted from the primary sequence. One of the most promising functional genomic approaches that have emerged in the past few years is based on RNA interference (RNAi). In several organisms including *Drosophila*, *C. elegans*, *Arabidopsis*, mouse, and even human cells, introduction of double-stranded RNAs (dsRNAs) has proven to be an effective tool in suppressing gene expression (Sharp, 1999; Dykxhoorn *et al*., 2003). The phenomenon of gene silencing by RNAi was first discovered in plants and in the worm. In *C. elegans*, RNAi can be triggered by the introduction of long $\sim$500 nucleotide dsRNAs, which can be delivered by injection into the animals. Alternatively, the dsRNAs can be delivered by feeding worms with bacteria expressing the desired dsRNAs or simply by soaking the animals into a dsRNA solution (Hannon, 2002; Paddison and Hannon, 2002; Denli and Hannon, 2003). Similarly, in *Drosophila*, long dsRNAs have been shown to be effective at gene-specific silencing. In flies, these dsRNAs can be introduced into the animal by injection into embryos, or delivered following the production of transgenic animals that express RNAi-hairpin constructs. Importantly, in *Drosophila*, the simple addition of dsRNAs to *Drosophila* cells in culture ('dsRNA bathing') was found to efficiently eliminate or reduce the expression of target genes, thus phenocopying loss-of-function mutations (Clemens *et al*., 2000). This methodology allows a variety of cell-based RNAi screens to be conducted at a genome-wide level.

Extensive research into the mechanism of RNAi has revealed that the introduction of dsRNAs into cells or animal models leads to its recognition and eventual degradation by a nuclease (of the RNaseIII family), now known as Dicer (Hammond *et al*., 2000; Bernstein *et al*., 2001; Ketting *et al*., 2001; Hannon, 2002; Bernstein *et al*., 2003; Carmell and Hannon, 2004). The Dicer enzymes are conserved through evolution and have been isolated in *Arabidopsis*, *C. elegans*, *Drosophila*, and mammals. Upon the introduction of dsRNAs into cells, the Dicer enzymes recognize and process the dsRNA into short-interfering RNAs (siRNAs), which are 21–23 nucleotides in length. These siRNAs then form a part of a multicomponent nuclease complex called RISC (RNA-induced silencing complex). It is thought that the activation of RISC leads to the unwinding of siRNAs (contained within the complex), which in turn serve as guides to the corresponding target mRNA and lead to the recognition and eventual degradation of the targeted transcript. The study of the mechanism of RNAi has now enabled the widespread use of this technology in mammalian cells (mouse and human cells), where the efficacy of RNAi was initially stymied since the introduction of long dsRNAs led to activation of an interferon response. This problem has been overcome by making use of synthetic siRNAs – which can essentially mimic Dicer-induced degradation products of dsRNAs (Martinez *et al*., 2002; Xia *et al*., 2002; Scherr *et al*., 2003). Since the effect of synthetic siRNAs is transient, several plasmid-vector-based systems have been designed to produce short-hairpin RNAs (shRNAs) (Paddison *et al*., 2002). Typically expressed under the control of RNA PolIII-dependent promoters such as U6 and H1, shRNAs subsequently undergo Dicer processing into siRNAs, which in turn efficiently silence the target gene.

High-throughput screens (HTSs) in *Drosophila* cells

Drosophila has been a favored organism for genetic studies for over several decades and has proven to be an excellent model system to identify genes involved in developmental and cellular processes (St Johnston, 2002). The contributions made by studies in *Drosophila* are numerous, and many important genes including proto-oncogenes, tumor suppressors, and other crucial players involved in cell proliferation, differentiation, and cell death were first identified in this organism. An important benefit from the completion of the sequence of the *Drosophila* genome is that it provides us with an unprecedented resource, as we can now fully evaluate the degree of conservation of this organism with others (Adams *et al*., 2000). The relevance of *Drosophila* to humans is best illustrated by the fact that more than $\sim$60% of the genes identified in human diseases have counterparts in *Drosophila* (Rubin, 2000; Rubin *et al*., 2000).

Analysis of the *Drosophila* genome has led to the annotation of $\sim$16 000 genes (Adams *et al*., 2000; Hild

et al., 2003; Oliver and Leblanc, 2003), and it is clear that a wealth of information remains to be mined from this model organism as a good functional annotation is only available for approximately 25% of the genes. Although conventional genetic approaches will clearly continue to provide valuable information, new powerful methods are needed to systematically and rapidly analyse the functions of all ~16 000 predicted genes. Recently, the development of RNAi-based HTS methods in tissue culture cells has provided such a methodology. RNAi in *Drosophila* cells have now been successfully used to perform genome-wide or large-scale screens for genes involved in the regulation of cell viability and cell morphology (Kiger *et al*., 2003; Boutros *et al*., 2004), and the technology is currently being applied to address many questions in signal transduction and cell biology (see http//flyrnai.org) (Lum *et al*., 2003; Michelson, 2003).

With regard to studies in signal transduction, the integration of data obtained from multiple RNAi screens for a variety of signaling pathways will enable researchers to potentially identify specific versus common regulators of signal transduction cascades, as well as how they might be involved in the maintenance of general cellular characteristics of cell viability and growth. In addition, it should now be possible to perform synthetic RNAi screens in *Drosophila* cells using multiple dsRNAs to uncover functions of genes that do not display a phenotype when mutated individually (see Kiger *et al*., 2003). Such screens would enable researchers to identify genes that are functionally redundant or act together in large protein complexes in the regulation of cell proliferation, growth, and apoptosis.

Although high-throughput RNAi screens in mammalian cells are now starting to be conducted (Milhavet *et al*., 2003; Berns *et al*., 2004; Grimm, 2004; Paddison *et al*., 2004; Zheng *et al*., 2004), there are numerous advantages of conducting genome-wide RNAi screens in *Drosophila*. First, RNAi is extremely effective (Kennerdell and Carthew, 1998; Clemens *et al*., 2000) and the excellent annotation of the genome allows almost full genome coverage (Adams *et al*., 2000; Oliver and Leblanc, 2003). Second, the high conservation between the *Drosophila* and vertebrate genomes, and organization of important signaling pathways, makes the translation of the findings from flies to vertebrates obvious (Belvin and Anderson, 1996; Bale, 2002; Pandur *et al*., 2002; Evans *et al*., 2003; Wajant and Scheurich, 2004). In fact, it is likely to be more effective to perform such screens in *Drosophila* cells first and then look at the functions of their orthologs in the mammalian system. Such a strategy overcomes the problem associated with the high degree of functional redundancy that exists in higher vertebrates. Third, the powerful genetics and the availability of large numbers of chemically and transposon-induced mutants and deficiency lines in *Drosophila* offers a unique opportunity to quickly validate *in vivo* the targets identified from the RNAi screens (Adams and Sekelsky, 2002; St Johnston, 2002; Nagy *et al*., 2003). Further, a number of methodologies, such as targeted gene knockout and hairpin RNAi constructs, can also be employed to engineer loss-of-function mutations in specific genes and analyse their functions.

Cancer research and RNAi screens in *Drosophila* cells

Cancer usually results from misregulation of the cell-division cycle, resulting in uncontrolled growth and/or proliferation. Cancer cells are also often resistant to cell death as a result of mutations in one or more proapoptotic genes. Inappropriate activation of a number of signaling pathways has been implicated in the generation of tumorigenic state, such as the Wnt/Wingless (Wg), Hedgehog (Hh), TGFβ, and most prominently the Ras gene family of small GTPases (Bos, 1989; Matise and Joyner, 1999; Murone *et al*., 1999; Oldak *et al*., 2001; Bak *et al*., 2003; van Es *et al*., 2003). Research in the last 20 years has led to the identification, cloning, and functional characterization of several proto-oncogenes and tumor suppressors. However, it is still a mystery as to how these proto-oncogenes, which often belong to core signaling pathways that are required for normal animal development, are misregulated and can co-operate to give rise to a cancerous state.

Metastasis, on the other hand, refers to the spreading and migration of cancerous cells from their point of origin to other tissue types. It often involves dramatic changes in cell polarity, cell shape, and cell fate, such as epithelial–mesenchymal transitions (EMTs) in several epithelial cancers (Takai *et al*., 1994; Montell, 1999; Schmitz *et al*., 2000; Mercurio *et al*., 2001; Billadeau, 2002; Pagliarini and Xu, 2003). Recent work from Tian Xu's laboratory has very well shown the cooperation between oncogenic *RasV12* expression (which causes noninvasive overgrowths on its own), and the inactivation of any one of a number of genes affecting cell polarity can lead to a host of metastatic behaviors in *Drosophila* (Pagliarini and Xu, 2003). Interestingly, the inactivation of any of the individual cell polarity genes did not cause metastatic behavior either. These studies strongly suggested that early oncogenic events during tumorigenesis could predispose cells with tumor-initiating mutations to display metastatic behavior.

Much more work needs to be carried out to better understand the molecular mechanisms underlying the dysregulation of these signaling pathways and tumor-initiating oncogenes and how they may interact with the environment (or the 'cellular context') to generate a cancerous state and metastasis. In addition to the identification and characterization of novel regulators of oncogenesis and metastasis, significant effort needs to be directed into the identification of targets whose activity can be modulated through the use of new drugs. The availability of whole-genome sequences from multiple animal model systems and the surge of new functional genomic/proteomic methodologies provide us with a unique opportunity to pursue these goals. Not only can we now begin to probe the function of each and every gene in a variety of signaling pathways, but we can

also devise ways of systematically identifying proteins that could serve as efficient drug targets.

The RNAi technology could be used to rapidly and systematically identify the function of every predicted gene in the genome in the regulation of the delicate balance between cell proliferation, growth, survival, as well as cell morphology. Moreover, the synergy between RNAi screens and small-molecule chemical genetic screens of specific pathways could help identify important drug targets more efficiently. Through the use of RNAi, one could envisage how the selective depletion of one or more gene products could prevent or slow down the progression to a disease state. In order to realize this goal, several laboratories have already generated either whole-genome or large sets of dsRNA libraries from *Drosophila*, *C. elegans*, mouse, and human cells (Kamath and Ahringer, 2003; Kiger *et al*., 2003; Miyagishi and Taira, 2003; Boutros *et al*., 2004). Such dsRNA libraries are now being widely used in *Drosophila* cells for screening whole genomes to identify new regulators of a variety of different signaling pathways and factors that affect basic cell biological processes, such as cell shape changes, cell division, growth, and apoptosis (Kiger *et al*., 2003; Lum *et al*., 2003; Boutros *et al*., 2004). The limiting step in performing such screens in *Drosophila* cells is the design and optimization of specific assays that can be implemented in a high-throughput fashion. Although the nature of the screen designs is such that it would lead to the identification of only cell-type-specific factors, the primary screens are typically followed by specific secondary screens in multiple cell types. This enables researchers to identify both cell-type-specific and core regulators. Additionally, the secondary screens allow researchers to group subsets of regulators in a particular pathway or cellular process into specific functional categories. In the future, numerous such screens will be performed. The comparison of the screen data between the different assays will allow us to not only identify pathway-specific or cell-type-specific regulators, but also genes that may play multiple roles in many cell biological or signaling pathways, which could be acting as important integrators of the multitude of signals received by a cell. For example, recent work has already identified both GSK3β as well as CK1α as important regulators for both the Wg and Hh signaling pathways in *Drosophila* (Lum *et al*., 2003) and it has been speculated that the number of such common regulators is likely to grow. Genes shared between different pathways/processes may be important in regulating how a cell reacts upon multiple signals.

Cross-referencing RNAi screens with protein–protein interaction screens/databases and small-molecule chemical genetic screens

Most signal transduction pathways are carried out by multiprotein complexes. The identification and analysis of their components provide important insights into how the ensemble of expressed proteins (proteome) is organized into functional units (Hartwell *et al*., 1999; Bray, 2003; Spirin and Mirny, 2003; Barabasi and Oltvai, 2004). How then do these functional units coordinately regulate signaling pathways? This is where a systematic comparison of candidate genes (in a specific cell based assay) obtained from RNAi screens to that of known protein interaction databases would be immensely useful in understanding the 'molecular context' of their activity. Moreover, mapping the RNAi functional network to that of the protein interaction networks could help identify important new regulators that were missed in RNAi screens and therefore generate some testable hypothesis regarding gene function. This strategy has already been implemented in *C. elegans* by Tewari *et al*. (2004), who used a systematic interactome mapping of the TGFβ signaling network in conjunction with functional analysis of the proteins found in the complex using RNAi.

One important goal of genome-wide RNAi screens and the systematic documentation of protein–protein interaction is to combine them with small-molecule chemical genetic screens in order to identify chemical inhibitors of different signaling pathways involved in development and disease. Forward chemical genetics involves identifying a phenotype in an organism or cell caused by a small molecule and then identifying the target affected. In principle, this is analogous to a classical genetics screen, in which one screens for a mutation that has a desired phenotype and then identifies the mutant gene that is responsible. For example, Mayer *et al*. (1999) identified a small molecule, monastrol, that causes inhibition of mitosis by collapsing the mitotic spindle. The target of this small molecule was shown to be Eg5, a kinesin involved in maintaining the spindle structure (Mayer *et al*., 1999; Kapoor *et al*., 2000). However, one of the major limitations of chemical genetic screens is the efficient identification of targets. In fact, in the case of Mayer *et al*., the previous knowledge of the Eg5 mutant phenotype was instrumental in its identification as a target of monastrol. In other words, for small-molecule screens to be useful, both economically and biologically, the targets must be known. This is where RNAi screens will be extremely useful. RNAi screens will allow researchers to determine which proteins to target in the cell using small molecules, in order to regulate cell signaling or morphology. One important advantage of cataloguing the RNAi phenotypes in various cell-based assays is that they could be directly compared with those of the phenotypes observed in cells treated with specific small molecules. The comparison of chemical genetic screens with whole-genome RNAi screens could lead to rapid identification of specific drug targets for genes involved in tumorigenesis. For example, the identification of the targets of small molecules and their comparison with the ones obtained from the RNAi screens could be very powerful as a first step in identifying potential drug targets for a variety of oncogenes/tumor suppressors such as Wnts, TGFβ, Hedgehog, and the Ras proteins. One could also envision applications whereby the RNAi and chemical genetic screens could be used to identify small molecules

that act as 'cell killers' in cells that have undergone oncogenic transformation due to mutations in oncogenes or tumor-suppressor genes. This would be equivalent to a developing a 'smart bomb', which would specifically target the cell carrying an oncogene but not the normal wild-type cells. For instance, it should be possible to screen for dsRNAs or molecules that kill cells specifically expressing the *RasV12* oncogene but not wild-type cells. Moreover, similar screens could be designed to identify genes or small molecules that would target cells containing mutation in tumor suppressor genes such as *p53*, *APC* or *patched*. Finally, chemical genetic screens could also be employed to screen for suppressors, and hence targets of specific RNAi mutant phenotypes in cells. At present, most of the high-throughput RNAi or chemical genetic screens are being performed in a 384-well plate format. However, dsRNA libraries are now be printed on glass slides using microarrayers with each spot representing a different dsRNA or small molecule (Stewart *et al*., 2003; Carpenter and Sabatini, 2004). Cells are then plated on the slides and assayed for different phenotypes. The use of such array formats for cell-based assays would greatly enhance the speed and efficacy of RNAi and small molecule screens.

Conclusion

In the future, the breakthroughs in cancer biology will rely on the efficient identification and functional characterization of a multitude of as yet uncharacterized, but important genes involved in oncogenesis. This information is essential to the generation of new therapeutic measures to prevent and/or treat cancer. The RNAi technology has now been established in several model organisms and research laboratories all over the world. Together with the mushrooming protein interaction databases and powerful forward chemical genetic screens, RNAi screens have the potential to revolutionize the field of signal transduction and cancer biology, since it provides an efficient method to identify and characterize all genes involved in specific pathways that have been implicated in the generation of cancer. Additionally, it also makes inroads into the field of drug discovery through basic science.

Abbreviations

RNAi, RNA interference; dsRNA, double-stranded RNA; siRNA, short-interfering RNA; shRNA, short-hairpin RNA; HTS, high-throughput screen.

References

Adams MD and Sekelsky JJ. (2002). *Nat. Rev. Genet.*, **3,** 189–198.

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, George RA, Lewis SE, Richards S, Ashburner M, Henderson SN, Sutton GG, Wortman JR, Yandell MD, Zhang Q, Chen LX, Brandon RC, Rogers YH, Blazej RG, Champe M, Pfeiffer BD, Wan KH, Doyle C, Baxter EG, Helt G, Nelson CR, Gabor GL, Abril JF, Agbayani A, An HJ, Andrews-Pfannkoch C, Baldwin D, Ballew RM, Basu A, Baxendale J, Bayraktaroglu L, Beasley EM, Beeson KY, Benos PV, Berman BP, Bhandari D, Bolshakov S, Borkova D, Botchan MR, Bouck J, Brokstein P, Brottier P, Burtis KC, Busam DA, Butler H, Cadieu E, Center A, Chandra I, Cherry JM, Cawley S, Dahlke C, Davenport LB, Davies P, de Pablos B, Delcher A, Deng Z, Mays AD, Dew I, Dietz SM, Dodson K, Doup LE, Downes M, Dugan-Rocha S, Dunkov BC, Dunn P, Durbin KJ, Evangelista CC, Ferraz C, Ferriera S, Fleischmann W, Fosler C, Gabrielian AE, Garg NS, Gelbart WM, Glasser K, Glodek A, Gong F, Gorrell JH, Gu Z, Guan P, Harris M, Harris NL, Harvey D, Heiman TJ, Hernandez JR, Houck J, Hostin D, Houston KA, Howland TJ, Wei MH, Ibegwam C, Jalali M, Kalush F, Karpen GH, Ke Z, Kennison JA, Ketchum KA, Kimmel BE, Kodira CD, Kraft C, Kravitz S, Kulp D, Lai Z, Lasko P, Lei Y, Levitsky AA, Li J, Li Z, Liang Y, Lin X, Liu X, Mattei B, McIntosh TC, McLeod MP, McPherson D, Merkulov G, Milshina NV, Mobarry C, Morris J, Moshrefi A, Mount SM, Moy M, Murphy B, Murphy L, Muzny DM, Nelson DL, Nelson DR, Nelson KA, Nixon K, Nusskern DR, Pacleb JM, Palazzolo M, Pittman GS, Pan S, Pollard J, Puri V, Reese MG, Reinert K, Remington K, Saunders RD, Scheeler F, Shen H, Shue BC, Siden-Kiamos I, Simpson M, Skupski MP, Smith T, Spier E, Spradling AC, Stapleton M, Strong R, Sun E, Svirskas R, Tector C, Turner R, Venter E, Wang AH, Wang X, Wang ZY, Wassarman DA, Weinstock GM, Weissenbach J, Williams SM, Woodage T, Worley KC, Wu D, Yang S, Yao QA, Ye J, Yeh RF, Zaveri JS, Zhan M, Zhang G, Zhao Q, Zheng L, Zheng XH, Zhong FN, Zhong W, Zhou X, Zhu S, Zhu X, Smith HO, Gibbs RA, Myers EW, Rubin GM and Venter JC. (2000). *Science*, **287,** 2185–2195.

Auerbach D, Thaminy S, Hottiger MO and Stagljar I. (2002). *Proteomics*, **2,** 611–623.

Bader GD and Hogue CW. (2003). *BMC Bioinform.*, **4,** 2.

Bak M, Hansen C, Tommerup N and Larsen LA. (2003). *Pharmacogenomics*, **4,** 411–429.

Bale AE. (2002). *Annu. Rev. Genomics Hum. Genet.*, **3,** 47–65.

Barabasi AL and Oltvai ZN. (2004). *Nat. Rev. Genet.*, **5,** 101–113.

Belvin MP and Anderson KV. (1996). *Annu. Rev. Cell Dev. Biol.*, **12,** 393–416.

Berns K, Hijmans EM, Mullenders J, Brummelkamp TR, Velds A, Heimerikx M, Kerkhoven RM, Madiredjo M, Nijkamp W, Weigelt B, Agami R, Ge W, Cavet G, Linsley PS, Beijersbergen RL and Bernards R. (2004). *Nature*, **428,** 431–437.

Bernstein E, Caudy AA, Hammond SM and Hannon GJ. (2001). *Nature*, **409,** 363–366.

Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV and Hannon GJ. (2003). *Nat. Genet.*, **35,** 215–217.

Billadeau DD. (2002). *Int. J. Gastrointest. Cancer*, **31,** 5–13.

Bos JL. (1989). *Cancer Res.*, **49,** 4682–4689.

Boutros M, Kiger AA, Armknecht S, Kerr K, Hild M, Koch B, Haas SA, Consortium HF, Paro R and Perrimon N. (2004). *Science*, **303,** 832–835.

Bray D. (2003). *Science*, **301,** 1864–1865.

Buckholz RG, Simmons CA, Stuart JM and Weiner MP. (1999). *J. Mol. Microbiol. Biotechnol.*, **1,** 135–140.

Carmell MA and Hannon GJ. (2004). *Nat. Struct. Mol. Biol.*, **11,** 214–218.

Carpenter AE and Sabatini DM. (2004). *Nat. Rev. Genet.*, **5,** 11–22.
Clemens JC, Worby CA, Simonson-Leff N, Muda M, Maehama T, Hemmings BA and Dixon JE. (2000). *Proc. Natl. Acad. Sci. USA*, **97,** 6499–6503.
Denli AM and Hannon GJ. (2003). *Trends Biochem. Sci.*, **28,** 196–201.
Diaz-Camino C, Risseeuw EP, Liu E and Crosby WL. (2003). *Anal. Biochem.*, **316,** 171–174.
Dykxhoorn DM, Novina CD and Sharp PA. (2003). *Nat. Rev. Mol. Cell Biol.*, **4,** 457–467.
Evans CJ, Hartenstein V and Banerjee U. (2003). *Dev. Cell*, **5,** 673–690.
Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G and Superti-Furga G. (2002). *Nature*, **415,** 141–147.
Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E, Vijayadamodar G, Pochart P, Machineni H, Welsh M, Kong Y, Zerhusen B, Malcolm R, Varrone Z, Collis A, Minto M, Burgess S, McDaniel L, Stimpson E, Spriggs F, Williams J, Neurath K, Ioime N, Agee M, Voss E, Furtak K, Renzulli R, Aanensen N, Carrolla S, Bickelhaupt E, Lazovatsky Y, DaSilva A, Zhong J, Stanyon CA, Finley Jr RL, White KP, Braverman M, Jarvie T, Gold S, Leach M, Knight J, Shimkets RA, McKenna MP, Chant J and Rothberg JM. (2003). *Science*, **302,** 1727–1736.
Grimm S. (2004). *Nat. Rev. Genet.*, **5,** 179–189.
Hammond SM, Bernstein E, Beach D and Hannon GJ. (2000). *Nature*, **404,** 293–296.
Hannon GJ. (2002). *Nature*, **418,** 244–251.
Hartwell LH, Hopfield JJ, Leibler S and Murray AW. (1999). *Nature*, **402,** C47–52.
Hild M, Beckmann B, Haas SA, Koch B, Solovyev V, Busold C, Fellenberg K, Boutros M, Vingron M, Sauer F, Hoheisel JD and Paro R. (2003). *Genome Biol.*, **5,** R3.
Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, Arkin AP, Bornstein BJ, Bray D, Cornish-Bowden A, Cuellar AA, Dronov S, Gilles ED, Ginkel M, Gor V, Goryanin II Hedley WJ, Hodgman TC, Hofmeyr JH, Hunter PJ, Juty NS, Kasberger JL, Kremling A, Kummer U, Le Novere N, Loew LM, Lucio D, Mendes P, Minch E, Mjolsness ED, Nakayama Y, Nelson MR, Nielsen PF, Sakurada T, Schaff JC, Shapiro BE, Shimizu TS, Spence HD, Stelling J, Takahashi K, Tomita M, Wagner J and Wang J. (2003). *Bioinformatics*, **19,** 524–531.
Kamath RS and Ahringer J. (2003). *Methods*, **30,** 313–321.
Kapoor TM, Mayer TU, Coughlin ML and Mitchison TJ. (2000). *J. Cell Biol.*, **150,** 975–988.
Kennerdell JR and Carthew RW. (1998). *Cell*, **95,** 1017–1026.
Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ and Plasterk RH. (2001). *Genes Dev.*, **15,** 2654–2659.
Kiger A, Baum B, Jones S, Jones M, Coulson A, Echeverri C and Perrimon N. (2003). *J. Biol.*, **2,** 27.
Li H and Wang W. (2003). *Curr. Opin. Genet. Dev.*, **13,** 611–616.
Lum L, Yao S, Mozer B, Rovescalli A, Von Kessler D, Nirenberg M and Beachy PA. (2003). *Science*, **299,** 2039–2045.
Martinez LA, Naguibneva I, Lehrmann H, Vervisch A, Tchenio T, Lozano G and Harel-Bellan A. (2002). *Proc. Natl. Acad. Sci. USA*, **99,** 14849–14854.
Matise MP and Joyner AL. (1999). *Oncogene*, **18,** 7852–7859.
Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL and Mitchison TJ. (1999). *Science*, **286,** 971–974.
Mercurio AM, Bachelder RE, Rabinovitz I, O'Connor KL, Tani T and Shaw LM. (2001). *Surg. Oncol. Clin. N. Am.*, **10,** 313–328 , viii–ix..
Michelson AM. (2003). *Sci. STKE*, **2003,** PE30.
Milhavet O, Gary DS and Mattson MP. (2003). *Pharmacol. Rev.*, **55,** 629–648.
Milo R, Itzkovitz S, Kashtan N, Levitt R, Shen-Orr S, Ayzenshtat I, Sheffer M and Alon U. (2004). *Science*, **303,** 1538–1542.
Miyagishi M and Taira K. (2003). *Oligonucleotides*, **13,** 325–333.
Montell DJ. (1999). *Cell Biochem. Biophys.*, **31,** 219–229.
Murone M, Rosenthal A and de Sauvage FJ. (1999). *Exp. Cell Res.*, **253,** 25–33.
Nagy A, Sandmeyer S and Plasterk R. (2003). *Nat. Genet.*, **33,** 276–284.
Oldak M, Grzela T, Lazarczyk M, Malejczyk J and Skopinski P. (2001). *Int. J. Mol. Med.*, **8,** 445–452.
Oliver B and Leblanc B. (2003). *Genome Biol.*, **5,** 204.
Paddison PJ and Hannon GJ. (2002). *Cancer Cell*, **2,** 17–23.
Paddison PJ, Caudy AA, Bernstein E, Hannon GJ and Conklin DS. (2002). *Genes Dev.*, **16,** 948–958.
Paddison PJ, Silva JM, Conklin DS, Schlabach M, Li M, Aruleba S, Balija V, O'Shaughnessy A, Gnoj L, Scobie K, Chang K, Westbrook T, Cleary M, Sachidanandam R, McCombie WR, Elledge SJ and Hannon GJ. (2004). *Nature*, **428,** 427–431.
Pagliarini RA and Xu T. (2003). *Science*, **302,** 1227–1231.
Pandur P, Maurus D and Kuhl M. (2002). *BioEssays*, **24,** 881–884.
Puig O, Caspary F, Rigaut G, Rutz B, Bouveret E, Bragado-Nilsson E, Wilm M and Seraphin B. (2001). *Methods*, **24,** 218–229.
Rigaut G, Shevchenko A, Rutz B, Wilm M, Mann M and Seraphin B. (1999). *Nat. Biotechnol.*, **17,** 1030–1032.
Rosamond J and Allsop A. (2000). *Science*, **287,** 1973–1976.
Rubin GM. (2000). *Novartis Found. Symp.*, **229,** 79–82 , discussion 82–83.
Rubin GM, Yandell MD, Wortman JR, Gabor Miklos GL, Nelson CR, Hariharan IK, Fortini ME, Li PW, Apweiler R, Fleischmann W, Cherry JM, Henikoff S, Skupski MP, Misra S, Ashburner M, Birney E, Boguski MS, Brody T, Brokstein P, Celniker SE, Chervitz SA, Coates D, Cravchik A, Gabrielian A, Galle RF, Gelbart WM, George RA, Goldstein LS, Gong F, Guan P, Harris NL, Hay BA, Hoskins RA, Li J, Li Z, Hynes RO, Jones SJ, Kuehl PM, Lemaitre B, Littleton JT, Morrison DK, Mungall C, O'Farrell PH, Pickeral OK, Shue C, Vosshall LB, Zhang J, Zhao Q, Zheng XH and Lewis S. (2000). *Science*, **287,** 2204–2215.
Sanchez-Carbayo M and Cordon-Cardo C. (2003). *Br. J. Cancer*, **89,** 2172–2177.
Scherr M, Morgan MA and Eder M. (2003). *Curr. Med. Chem.*, **10,** 245–256.
Schmitz AA, Govek EE, Bottner B and Van Aelst L. (2000). *Exp. Cell Res.*, **261,** 1–12.
Sharp PA. (1999). *Genes Dev.*, **13,** 139–141.
Spirin V and Mirny LA. (2003). *Proc. Natl. Acad. Sci. USA*, **100,** 12123–12128.
St Johnston D. (2002). *Nat. Rev. Genet.*, **3,** 176–188.

Stewart SA, Dykxhoorn DM, Palliser D, Mizuno H, Yu EY, An DS, Sabatini DM, Chen IS, Hahn WC, Sharp PA, Weinberg RA and Novina CD. (2003). *RNA*, **9,** 493–501.

Takai Y, Kaibuchi K, Sasaki T, Tanaka K, Shirataki H and Nakanishi H. (1994). *Princess Takamatsu Symp.*, **24,** 338–350.

Tewari M, Hu PJ, Ahn JS, Ayivi-Guedehoussou N, Vidalain PO, Li S, Milstein S, Armstrong CM, Boxem M, Butler MD, Busiguina S, Rual JF, Ibarrola N, Chaklos ST, Bertin N, Vaglio P, Edgley ML, King KV, Albert PS, Vandenhaute J, Pandey A, Riddle DL, Ruvkun G and Vidal M. (2004). *Mol. Cell*, **13,** 469–482.

van Duin M, Woolson H, Mallinson D and Black D. (2003). *Biochem. Soc. Trans.*, **31,** 429–432.

van Es JH, Barker N and Clevers H. (2003). *Curr. Opin. Genet. Dev.*, **13,** 28–33.

Venter JC, Adams MD, Sutton GG, Kerlavage AR, Smith HO and Hunkapiller M. (1998). *Science*, **280,** 1540–1542.

Wajant H and Scheurich P. (2004). *Prog. Mol. Subcell. Biol.*, **34,** 47–72.

Xia H, Mao Q, Paulson HL and Davidson BL. (2002). *Nat. Biotechnol.*, **20,** 1006–1010.

Zheng L, Liu J, Batalov S, Zhou D, Orth A, Ding S and Schultz PG. (2004). *Proc. Natl. Acad. Sci. USA*, **101,** 135–140.