



Functional Approaches to Gene Isolation in Mammalian Cells

Andrei V. Gudkov, *et al.*

Science **285**, 299a (1999);

DOI: 10.1126/science.285.5426.299a

The following resources related to this article are available online at www.sciencemag.org (this information is current as of June 1, 2008):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/285/5426/299a>

This article **cites 35 articles**, 21 of which can be accessed for free:

<http://www.sciencemag.org/cgi/content/full/285/5426/299a#otherarticles>

This article appears in the following **subject collections**:

Genetics

<http://www.sciencemag.org/cgi/collection/genetics>

Technical Comments

http://www.sciencemag.org/cgi/collection/tech_comment

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:

<http://www.sciencemag.org/about/permissions.dtl>

Functional Approaches to Gene Isolation in Mammalian Cells

A Techview by Gregory J. Hannon *et al.* (1) provides an overview of procedures described as a new methodology for function-based gene isolation in mammalian cells and termed "MaRX." The basic concepts and most of the technical aspects of the MaRX methodology described therein seem indistinguishable from the principles and methods that our laboratories and others have published over many years. Hannon *et al.* cite only a few earlier publications (2–7), which they describe as "sporadic attempts" to apply molecular genetics to cultured mammalian cells. In fact, not only specific methods used in the MaRX procedure, but even some of the results described by Hannon *et al.* as their unpublished data have already been published by others, but were not credited by Hannon *et al.* (1).

The two concepts attributed to MaRX methodology by Hannon *et al.* (1), are "the use of nucleic acid as a 'virtual mutagen'" and a set of methods for "efficient introduction of cDNA libraries into target cells" and "efficient recovery of either individual genes or complex sublibraries from cell populations that have been enriched on the basis of a specific biological characteristic." Both of these concepts, however, have been well established and extensively utilized in many publications. Specifically, antisense-oriented cDNA sequences and sense-oriented sequences encoding dominant mutants have been used as tools for gene cloning and characterization in mammalian cells. For example, gene cloning through the selection of cDNA sequences cloned in antisense orientation relative to the promoter has been used not only by Deiss *et al.* (2), but also in other studies (8–15) that describe the isolation of different genes involved in apoptosis and tumor suppression. Furthermore, the specific components of the MaRX methodology are not novel, but involve the procedures that have been previously developed for the isolation of genetic suppressor elements (GSEs), which are short sense- or antisense-oriented cDNA fragments with biological activity (16–18). These procedures include the use of retroviral expression vectors for constructing cDNA fragment libraries (7, 19–24), normalization of cDNA prior to cloning (19), and the use of multiple rounds of phenotypic selection with the recovery of the selected sublibraries or individual genes after each round (7, 19–24). A procedure that has been used in MaRX but not the GSE technique is the use of *Cre* recombinase for the recovery of retroviral inserts from the selected cells (25). This approach, however, has been used by others for similar purposes (26), and there is no

evidence that it is more efficient than alternative rapid methods for insert recovery used in GSE selection (18, 19, 27).

Hannon *et al.* (1) chose to illustrate the utility of the MaRX methodology by describing unpublished experiments in which they have selected fragments of p53 cDNA that inhibit biological functions of this tumor suppressor. In fact, selection of inhibitory p53 fragments has been the subject of papers published by our laboratories in 1996 and 1997 (21, 22). Similar selection procedures were also used to isolate inhibitory fragments from the cDNAs of topoisomerase II (7) and kinesin heavy chain (23) genes. Hannon *et al.* (1) also mention their unpublished data on the identification of new genes involved in resistance to widely used pharmaceuticals. Identification of genes with such function, however, was the subject of a 1994 paper of ours, which utilized a normalized cDNA library in a retroviral vector for GSE selection and resulted in the identification of kinesin as a determinant of cellular drug sensitivity and senescence (19). Other published examples of GSE selection from retroviral libraries include the cloning of ING1 candidate tumor suppressor gene (20) and the isolation of GSEs from the genome of HIV-1 that protect CD4+ T cells from HIV infection (24). Aside from the examples of GSE selection, many other groups have utilized retroviral libraries for cloning full-length or nearly full-length cDNAs with functional activities (3, 4, 28–34). Thus, the MaRX technology for function-based gene isolation in mammalian cells is not a novel approach, as presented by Hannon *et al.* (1), but rather a well-established methodology that has already yielded valuable information in a broad range of biological problems in mammalian cells.

Andrei V. Gudkov

Igor B. Roninson

Department of Molecular Genetics,

University of Illinois at Chicago,

Chicago, IL 60607-7170, USA

E-mail: roninson@uic.edu

Robert Brown

CRC Department of Medical Oncology,

University of Glasgow,

CRC Beatson Labs,

Garscube Estate, Switchback Road,

Glasgow G61 1BD, United Kingdom

References

1. G. J. Hannon *et al.*, *Science* **283**, 1129 (1999).
2. L. P. Deiss; E. Feinstein, H. Berissi, O. Cohen, A. Kimchi, *Genes Dev.* **9**, 15 (1995).
3. I. Whitehead and H. Kirk, R. Kay, *Mol. Cell Biol.* **15**, 704 (1995).

4. J. R. Rayner and T. J. Gonda, *ibid.* **14**, 880 (1994).
5. M. Goldfarb, K. Shimizu, M. Peruchio, M. Wigler, *Nature* **296**, 404 (1982).
6. M. L. Slater and H. L. Ozer, *Cell* **7**, 289 (1976).
7. A. V. Gudkov *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3231 (1993).
8. L. P. Deiss and A. Kimchi, *Science* **252**, 117 (1991).
9. T. G. Gabig, P. L. Mantel, R. Rosli, C. D. Crean, *J. Biol. Chem.* **269**, 29515 (1994).
10. J. L. Kissil *et al.*, *ibid.* **270**, 27932 (1995).
11. L. P. Deiss, H. Galinka, H. Berissi, O. Cohen, A. Kimchi, *EMBO J.* **15**, 3861 (1996).
12. O. Cohen, E. Feinstein, A. Kimchi, *ibid.* **16**, 998 (1997).
13. N. Levy-Strumpf, L. P. Deiss, H. Berissi, A. Kimchi, *Mol. Cell Biol.* **17**, 1615 (1997).
14. E. R. Hofman *et al.*, *ibid.* **18**, 6493 (1998).
15. N. Levy-Strumpf and A. Kimchi, *Oncogene* **24**, 3331 (1998).
16. T. A. Holzmayr, D. G. Pestov, I. B. Roninson, *Nucleic Acids Res.* **20**, 711 (1992).
17. I. B. Roninson *et al.*, *Cancer Res.* **55**, 4023 (1995).
18. A. V. Gudkov and I. B. Roninson, in *Methods in Molecular Biology*, vol. 69, *cDNA Library Protocols*, I. G. Cowell and C. A. Austin, Eds. (Humana Press, Totowa, NJ, 1996), pp. 221–240.
19. A. V. Gudkov *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3744 (1994).
20. I. Garkavtsev, A. Kazarov, A. V. Gudkov, K. Riabowol, *Nature Genet.* **14**, 415 (1996).
21. V. S. Ossovskaya *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10309 (1996).
22. W. M. Gallagher, M. Cairney, B. Schott, I. B. Roninson, R. Brown, *Oncogene* **14**, 185 (1997).
23. S. A. Axenovich *et al.*, *Cancer Res.* **58**, 3423 (1998).
24. S. J. Dunn *et al.*, *Gene Therapy* **6**, 130 (1999).
25. P. Sun *et al.*, *Science* **282**, 2270 (1998).
26. L. Li and S. N. Cohen, *Cell* **85**, 319 (1996).
27. B. Schott, E. S. Kandel, I. B. Roninson, *Nucleic Acids Res.* **25**, 2940 (1997).
28. A. J. Murphy, A. Efstratiadis, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8277 (1987).
29. B. Y. Wong, H. Chen, S. W. Chung, P. M. Wong, *J. Virol.* **68**, 5523 (1994).
30. T. Kitamura *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9146 (1995).
31. I. Whitehead, H. Kirk, C. Tognon, G. Trigo-Gonzalez, R. Kay, *J. Biol. Chem.* **270**, 18388 (1995).
32. A. C. Zannettino, J. R. Rayner, L. K. Ashman, T. J. Gonda, P. J. Simmons, *J. Immunol.* **15**, 611 (1996).
33. R. F. Wang *et al.*, *Cancer Res.* **58**, 3519 (1998).
34. C. S. Tailor, A. Nouri, C. G. Lee, C. Kozak, D. Kabat, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 927 (1999).

12 March 1999; accepted 14 June 1999

As discussed by Hannon *et al.* in their Techview (1), functional approaches to gene cloning in mammalian cells grown in culture are based on two concepts, both of which are directed at overcoming impediments to the use of genetic methods in cultured cells. One concept is the use of nucleic acids as "virtual mutagens" for trapping genes on the basis of their function. The other is the development of efficient strategies for introduction and recovery of complex cDNA libraries to allow multiple rounds of phenotypic selection and rapid screening for identification of the functionally relevant genes. Hannon *et al.* do not discuss how these two concepts have been raised and thoroughly processed over the past decade, serving as a basis for the design of several successful genetic strategies, such as the technical knock out (TKO) (2), the use of genetic suppressor elements (GSE) (3), the "death trap" (4), and others (5). These well-established methods were designed according

TECHNICAL COMMENTS

to the same principles as those applied by Hannon *et al.*, including: (i) use of cDNA libraries (anti-sense or sense fragments, or both) to randomly reduce or inactivate gene expression, (ii) development of different expression vectors that provide an efficient gene transfer system and thus the capability to represent a complex library in a single transfection event, (iii) isolation of the functional cDNA fragments by applying strong positive phenotypic selections, and (iv) easy and rapid recovery approaches for the rescue of the relevant functional genetic elements, approaches which also enable multiple rounds of phenotypic selection. Every step derived from these principles has been thoroughly worked out in different methodologies (6–8). Moreover, an early instance of system validation (that is, the ability to isolate from a complex cDNA library a rate-limiting gene by effectively inhibiting its expression in mammalian cells through an anti-sense cDNA fragment) was achieved in 1991 with the TKO strategy (2).

Previous use of these methodologies led to the successful isolation of novel genes, which have been studied in depth over the past years. The TKO system led to the identification of five

novel cell death-promoting genes (named *DAP* genes) (6, 9–16). These genes were shown to display a wide involvement in apoptotic processes and, in the case of *DAP*-kinase, to possess tumor-suppressive activity (13, 14). The GSE methodology resulted in the cloning of a novel tumor suppressor gene, *ING 1* (17). The “death trap” resulted in the isolation of *ALG-2* and *ALG-3* genes, which play a role in neuronal cell death (4). These approaches were applied in a wide range of biological processes, such as apoptosis triggered by cytokines, cytotoxic drugs, growth factor deprivation, or T cell receptor activation, as well as tumor formation in nude mice.

The power which resides in these function-based gene cloning approaches has already proved itself.

We would like to address in greater detail the TKO system, and provide some additional technical information that may be critical in planning functional approaches to gene cloning in general. This approach was applied in apoptotic cell systems. Detailed structure and function work, done over the past years on several *DAP* proteins, indicates that the TKO method targeted different rate-limiting genes that operate at various junctions within the mo-

lecular networks controlling apoptosis (6, 7, 9–16). This early demonstration showed that functional gene cloning approaches, performed in mammalian cell cultures, could lead to the genetic dissection of a biological process at a resolution comparable to that provided by the classical genetic tools used in yeasts and low invertebrates.

The four basic principles mentioned above were fulfilled in the case of the TKO methodology by choosing an Epstein-Barr virus (EBV)-based, self-replicating, episomal shuttle vector to express a directional anti-sense cDNA library (Fig. 1). The unidirectional strategy increased the probability of acquiring “recessive mutations” resulting from loss of expression. The episomal vector had several advantages over vectors which integrate into the genome. (i) It reduced the background of non relevant phenotypic alterations occurring as a result of random integrations into DNA. (ii) The episomes were easily rescued from the transfected cells by a simple DNA extraction procedure with no need of other genetic manipulations. This yielded a rapid and convenient way to perform multiple rounds of phenotypic selection and also solved the issue of plasticity of tissue culture cells, because only the individual cDNA fragments that transduced the phenotypic change, in subsequent transfections, were scored as real positives (6, 12). (iii) The episomal vector accumulated at multiple copies in the stable transfectants, resulting in high expression levels (6, 12). (iv) The promoter cassette of the vector had been manipulated to confer much stronger expression of anti-sense RNAs during the phenotype selection. Because interferon- γ was the trigger that induced apoptosis, an interferon-responsive enhancer element was introduced into the vector to increase expression during the selection by the killing cytokine (2). Other transcription enhancer elements can be used in other types of selections. In retrospect, the latter manipulation was found to be critical for the success of the functional cloning, because high expression of anti-sense RNA during the phenotypic selection was essential to the efficient reduction of protein concentrations (6, 9, 10). (v) Moreover, the chances of cloning genes from the basic cell death machinery, instead of genes that function proximal to the receptors, were increased by making the selection dependent on this specific enhancer element. All these technical “tips” can be adapted in one way or another to other types of phenotype selections in cell cultures for the rescue of genes that control replicative senescence, cell cycle arrest, or suppressors of tumorigenicity.

The advantage of functional approaches to gene cloning is that they select the relevant rate-limiting genes controlling a biological process in an unbiased manner. As a consequence,

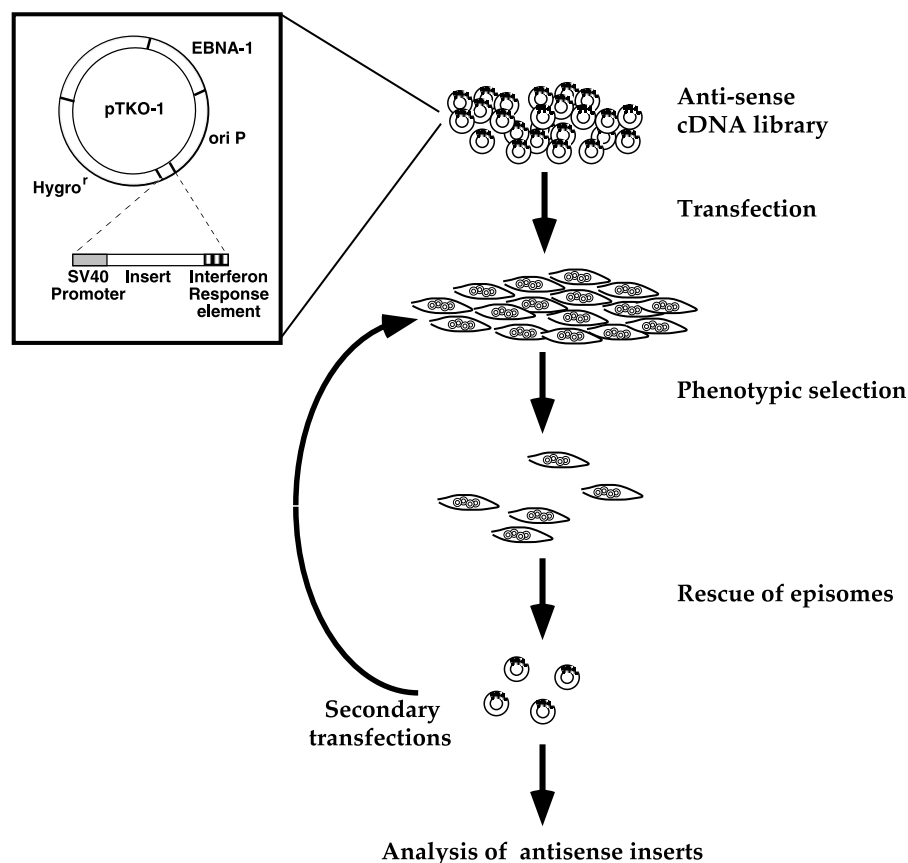


Fig. 1. Technical knock out (TKO) strategy. Cells are transfected with an anti-sense cDNA expression library carried by an Epstein-Barr virus (EBV)-based episomal expression vector (pTKO-1). Cultures are then subjected to selection by a killing cytokine. Surviving cells are pooled, and episomes are extracted. Pools of rescued episomes can be subjected to subsequent rounds of screening. Inserts carried by individual episomes are sequenced and analyzed.

TECHNICAL COMMENTS

University of Illinois at Chicago,
Chicago, IL 60607-7170, USA

novel targets and unpredicted mechanisms may emerge, as became evident from the study of DAP proteins. For example, the calcium/calmodulin-dependent DAP-kinase, which was found to be localized to the actin microfilaments, may provide a molecular handle to study the collapse of the microfilament system in apoptosis (11). Most important, DAP-kinase was found to possess tumor suppressive activities, as analyzed initially in mouse model systems, where DAP-kinase displayed strong anti-metastatic effects (14) and later, in other in vitro systems that tested suppression of oncogenic transformation (18). In human carcinoma cell lines and B cell lymphomas, DAP-kinase expression was lost at high frequency (13). Thus, screening systems for genes that are rate limiting in apoptosis may target tumor suppressor genes. Another breakthrough step related to the discovery of DAP-5. The structure and function features of this novel translation regulator resemble the proteolytically cleaved eIF4G initiation factor, which appears in cells on infection with some RNA viruses, and which directs cap-independent translation (12). The rescue of DAP-5 revealed the existence of a strong link between apoptosis and the control of protein synthesis, which seems to be critical in certain apoptotic systems, and focused some of the mechanistic studies in this direction. Another example is the isolation of cathepsin D by the TKO

method, which suggests that lysosomal proteases are recruited during apoptosis, in addition to the well-known caspase family of proteases. A unique pattern of regulation affecting the processing of this protease was subsequently shown to occur during apoptosis (10), in a study that would not have been initiated otherwise. Altogether, the discovery of DAP genes adds a significant weight to the molecular information available on apoptosis.

In conclusion, the TKO led to a long-term systematic study of several important genes, each of which controls a different rate limiting step in apoptosis. This history shows the power of these strategies to dissect a complex molecular network underlying a biological process.

Adi Kimchi
Ofer Cohen
Joseph Kissil
Tal Raveh
Boaz Inbal

Naomi Levy-Strumpf
Hanna Berissi

*Department of Molecular Genetics,
Weizmann Institute of Science,
Rehovot 76100, Israel*

E-mail: lvkimchi@weizmann.weizmann.ac.il

Louis Deiss

Department of Molecular Genetics,

References

1. G. J. Hannon *et al.*, *Science* **283**, 1129 (1999).
2. L. P. Deiss and A. Kimchi, *ibid.* **252**, 117 (1991).
3. T. A. Holzmayer, D. G. Pestov, I. B. Roninson, *Nucleic Acids Res.* **20**, 711 (1992); A. V. Gudkov and I. Roninson, *Methods Mol. Biol.* **69**, 221 (1997).
4. P. Vito, E. Lacana, L. D'Adamo, *Science* **271**, 521 (1996); P. Vito *et al.*, *J. Biol. Chem.* **271**, 31025 (1996); E. Lacana, J. K. Ganjei, P. Vito, L. D'Adamo, *J. Immunol.* **158**, 5129 (1997).
5. T. G. Gabig, P. L. Mantel, R. Rosli, C. D. Crean, *J. Biol. Chem.* **269**, 29515 (1994); E. R. Hofman *et al.*, *Mol. Cell. Biol.* **18**, 6493 (1998).
6. L. P. Deiss, E. Feinstein, H. Berissi, O. Cohen, A. Kimchi, *Genes Dev.* **9**, 15 (1995).
7. A. Kimchi, *B.B.A. Rev. Cancer* **1377**, F13 (1998); N. Levy-Strumpf and A. Kimchi, *Oncogene* **24**, 3331 (1998).
8. A. V. Gudkov *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3231 (1993); I. B. Roninson *et al.*, *Cancer Res.* **55**, 4023 (1995).
9. J. L. Kissil *et al.*, *J. Biol. Chem.* **270**, 27932 (1995).
10. L. P. Deiss, H. Galinka, H. Berissi, O. Cohen, A. Kimchi, *EMBO J.* **15**, 3861 (1996).
11. O. Cohen, E. Feinstein, A. Kimchi, *ibid.* **16**, 998 (1997).
12. N. Levy-Strumpf, L. P. Deiss, H. Berissi, A. Kimchi, *Mol. Cell. Biol.* **17**, 1615 (1997).
13. J. L. Kissil *et al.*, *Oncogene* **15**, 403 (1997).
14. B. Inbal *et al.*, *Nature* **390**, 180 (1997).
15. J. L. Kissil, O. Cohen, T. Raveh, A. Kimchi, *EMBO J.* **18**, 353 (1999).
16. O. Cohen *et al.*, *J. Cell Biol.*, in press.
17. I. Garkavtsev, A. Kazarov, A. V. Gudkov, K. Riabowol, *Nature Genet.* **14**, 415 (1996); I. Garkavtsev *et al.*, *Nature* **391**, 295 (1998).
18. T. Raveh *et al.*, in preparation.

12 March 1999; accepted 14 June 1999