FEBS Letters 452 (1999) 100–103 FEBS 22004

Minireview

Lipid signaling in CD95-mediated apoptosis

Florence Malisan*, Roberto Testi

Department of Experimental Medicine and Biochemical Sciences, University of Rome 'Tor Vergata', via di Tor Vergata 135, 00133 Rome, Italy

Received 13 April 1999

Abstract Ceramides play an important role mediating different cell responses such as proliferation, differentiation, growth arrest and apoptosis. They are released upon sphingomyelin hydrolysis which occurs after triggering of a number of cell surface receptors including CD95. Ceramide generation also regulates glycosphingolipid and ganglioside metabolism. In particular, ganglioside GD3 biosynthesis represents an important event for the progression of apoptotic signals generated by CD95 and mediated by ceramide in hematopoietic cells.

Key words: Apoptosis; CD95; Sphingomyelinase; Ceramide; Disialoganglioside GD3; Mitochondrion

1. Introduction

Apoptosis or programmed cell death is a genetically regulated, cellular suicide mechanism that plays a crucial role in development, homeostasis, and in many diseases including cancer, AIDS, and autoimmune and degenerative disorders. The process of apoptosis can be divided into three main phases: (a) the initiation phase in which, depending on the stimulus received by the cell, signal transduction and damage pathways are activated; (b) the effector/decision phase during which the cell commits itself to die; (c) the degradation phase, when the cell acquires the morphological and biochemical hallmarks of apoptosis. Morphologically, cells undergoing apoptosis show membrane blebbing, nuclear/cytoplasmic condensation, fragmentation and packaging of cellular material into apoptotic bodies. Common biochemical events include collapse of the mitochondrial inner transmembrane potential, intracellular acidification, selective proteolysis of cellular proteins, and degradation of DNA into internucleosomal fragments. It has become clear that mitochondria are major players during apoptosis and that disruption of the inner membrane potential which follows the opening of the permeability transition (PT) pore constitutes a decisive event of the cell death program by apoptosis. This is followed by the release of diffusible factors responsible for the activation of caspases. Thus, any agent capable of inducing mitochondrial PT is a potential mediator of apoptosis. This appears to be the case for some lipid and glycolipid effector molecules derived from membrane sphingomyelin metabolism.

2. Ceramide and the sphingomyelinase pathway

Sphingomyelin breakdown initiates an evolutionarily conserved and ubiquitous intracellular signaling pathway [1–4]. Sphingomyelin is a membrane phospholipid composed of a sphingosine backbone and fatty acid joined in an amide bond, and a phosphocholine polar head group. Both sphingosine and the amide-linked fatty acid constitute the lipid component of the sphingomyelin moiety called ceramide. As a major phospholipid, sphingomyelin exists at both the outer and inner leaflets of the plasma membranes and is hydrolyzed by sphingomyelinases, which are sphingomyelin-specific type C phospholipases, in ceramide and phosphocholine. Different pools of sphingomyelin are potentially available to sphingomyelinases during membrane biogenesis and degradation. After being generated in the early Golgi compartments, sphingomyelin is transported along the exocytic pathway to the extracellular membrane where it is exposed.

Currently, based on their cellular localization and activation requirements such as pH optimum and cation dependence, five distinct mammalian sphingomyelinases have been identified.

An acidic sphingomyelinase (ASM) found in the lysosomes, having a pH optimum at about 4.5–5.5, is the product of a single gene, and requires diacylglycerol (DAG) and zinc to be activated [5–7]. Genetic mutations of ASM, impairing the capacity of sphingomyelin degradation, are responsible for some forms of Niemann-Pick disease (NPD), a group of lethal diseases characterized by sphingomyelin accumulation in different tissues. ASM activity has been found in every tissue examined and is localized primarily in caveolae, endosomes and lysosomes [8].

Additionally, a Zn^{2+}-dependent ASM has also been found in serum [9]. It is secreted by diverse cell types and is a product of the ASM gene that occurs independently of alternative splicing.

A neutral membrane-bound Mg^{2+}-dependent sphingomyelinase (NSM), produced by a different gene [10], has been described in several mammalian tissues. It shows a neutral (7–7.5) pH optimum and is activated by arachidonic acid [11]. Another neutral Mg^{2+}-independent NSM has been characterized. This phospholipase is present in the cytosol and is activated by 1,25-dihydroxyvitamin D_3 [12]. The last enzyme has an alkaline pH optimum and was found in the gastrointestinal tract [13].

3. Sphingomyelin breakdown in signal transduction

Sphingomyelin breakdown represents the most relevant source of ceramide involved in signal transduction. Different stimuli have been shown to induce sphingomyelin degradation and ceramide generation. Among these are vitamin D_3 [6], dexamethasone [14], ionizing radiation [15], as well as ligation of different membrane receptors, such as the tumor necrosis

*Corresponding author.

PII: S0014-5793(99)00543-8

factor receptor-1 [16,17], CD95 [18], CD40 [19], the nerve growth factor receptor [20], CD28 [21] and the interleukin-1 receptor [22]. Moreover, sphingomyelin hydrolysis is associated with the cellular response to stresses such as nutrient withdrawal and radiations [15,23].

Ceramide has been shown to activate multiple pathways. A sequential activation of different kinases, initiated by a ceramide-activated protein kinase (CAPK), involves phosphorylation of Raf-1, and subsequently of the mitogen-activated protein kinase cascade [24]. Importantly, ceramide has been proved to act as a catalyst for the stress response kinase cascade through the consecutive involvement of MEKK1, SEK1, SAPK and c-Jun [25]. A ceramide-induced PKCζ phosphorylation may contribute to NF-κB translocation [26]. Finally, along with protein kinase activation, ceramide has been shown to activate a heterotrimeric protein phosphatase of the PP2A family [27], which might be responsible for ceramide-induced downregulation of c-myc and cell cycle arrest [28].

4. Early apoptotic signaling through the CD95 receptor

CD95 is a widely distributed surface receptor whose crosslinking by its specific ligand triggers apoptosis. Crosslinking of CD95 results in the activation of two main pathways involved in the early propagation of apoptotic signals, both requiring integrity of the receptor death domain: a proteolytic cascade mediated by caspases and the activation of the lipid pathway including the activation of the acidic sphingomyelinase.

Upon ligand-induced receptor oligomerization, the adaptor molecule FADD/MORT1 binds directly to CD95 via its death domain. Concomitantly, the 'effector domain' of FADD/MORT1 interacts with a homologous domain of caspase-8 (FLICE/MACH) resulting in the activation not only of caspase-8 itself but of the downstream caspase cascade [29,30].

The activation of sphingomyelin turnover has been suggested to play an important role in initiating the biochemical pathway leading to active cell death, since the synthetic ceramide analogue C_2-ceramide has been shown to be directly responsible for apoptosis induction in different cell lines. In human cutaneous T cell lymphoma HuT78 cells, an early ceramide accumulation mediated by both neutral (NSM) and acidic (ASM) sphingomyelinases occurs transiently within the first 30 min after CD95 crosslinking [18,31]. Analysis of Fas-resistant cellular mutants revealed that only ceramide released by ASM is relevant for apoptosis [31]. Moreover, cells from individuals affected with NPD, genetically deficient in ASM activity, or from mice in which the gene encoding ASM has been targeted, show remarkable defects in executing the apoptotic program in response to radiations [32]. Accordingly, in NPD lymphoblasts, in which CD95 signaling is impaired, an efficient CD95-mediated apoptotic program can be reconstituted by direct replacement of ASM [33]. These data strongly support the involvement of ASM in generating ceramide as a mediator of apoptotic cell death.

A later and prolonged ceramide accumulation, dependent on cytosolic NSM activation, occurs several hours after CD95 crosslinking. This increase in ceramide intracellular concentration is prolonged and persistent and is also important for the later progression of apoptotic signals [34].

5. The mitochondrial route to caspases

The induction of mitochondrial PT is a critical event in apoptosis [35,36]. This event is due to the opening of a dynamic multiprotein complex, the mitochondrial megachannel, also called the PT pore. It causes the dissipation of the inner mitochondrial transmembrane potential γ ($\Delta\psi m$) leading to an expansion of the cellular matrix, organellar swelling, and disruption of the outer membrane. This latter event thereby permits the release of apoptogenic factors, including cytochrome *c* and apoptosis-inducing factor (AIF), and intramitochondrial caspases such as caspase-9 and -2. AIF can induce nuclear apoptosis. Cytochrome *c*, once in the cytosol, in association with dATP, binds to the CED-4 homologue, Apaf-1, triggering caspase-9. Upon activation of caspase-9, a cascade of caspase activation events is initiated [37]. Caspase-9 recruits caspase-3 which, in turn, triggers CAD, a caspase-activated DNase responsible for DNA fragmentation. Bcl-2 prevents, via a direct effect on mitochondrial membranes, all hallmarks of the early stage of apoptosis including disruption of the inner mitochondrial transmembrane potential and the release of apoptogenic factors such as cytochrome *c*, AIF, and caspase-2 and -9.

As mitochondrial damage is a crucial step of apoptosis, it became obvious to ask whether ceramide could disrupt the mitochondrial membrane potential. According to several authors, ceramide induces PT when added to the cell but there is controversy about the capacity of ceramide to induce PT in isolated mitochondria. Some reports say ceramide can [38], others that it cannot [36]. Another group found that cytosols

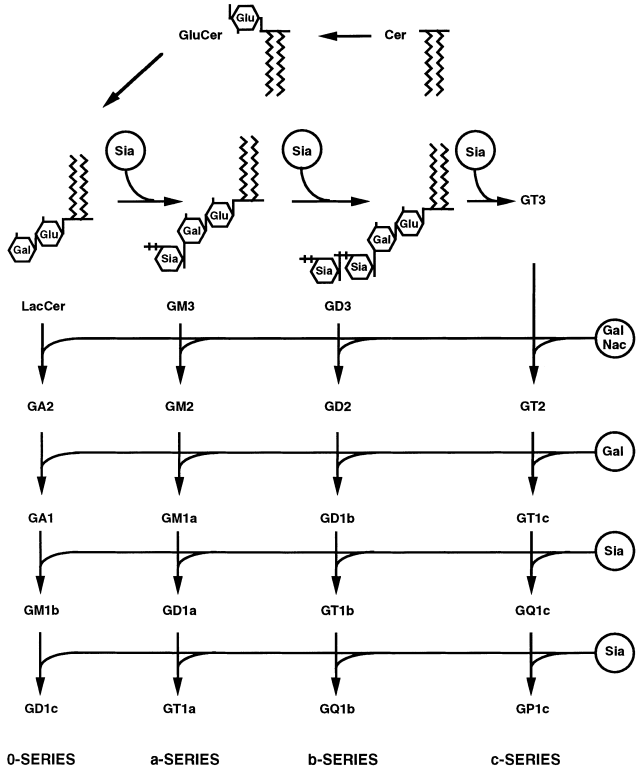

Fig. 1. General scheme for ganglioside biosynthesis. Cer, ceramide; GluCer, glucosylceramide; LacCer, lactosylceramide; Glu, glucose; Gal, galactose; Sia, sialic acid; GalNac, *N*-acetylgalactosamine; GM, monosialogangliosides; GD, disialogangliosides; GT, trisialogangliosides.

from ceramide-treated cells could induce PT on isolated mitochondria from untreated control cells. However, the addition of ceramide to cytosols does not generate PT [39]. Taken together, these latter data suggest that in intact cells, ceramide triggers the generation of another second messenger able to disrupt the mitochondrial membrane potential.

6. Ceramide as a source of disialoganglioside GD3

Newly synthesized or released ceramides are targeted to the Golgi apparatus and regulate sphingolipid and glycosphingolipid metabolism, including the rate of ganglioside biosynthesis within the Golgi [40,41].

Gangliosides are amphipathic constituents of the outer leaflet of cellular membranes, composed of a common hydrophobic ceramide moiety, which acts as a membrane anchor, and a hydrophilic oligosaccharide chain. Ganglioside neosynthesis is initiated within the biogenic membranes of the early Golgi by stepwise glycosylation of ceramide. New-formed ceramides coming from the endoplasmic reticulum, or free ceramides coming from degradative subcellular compartments, enter the early Golgi via vesicular membrane flow to initiate ganglioside synthesis. The addition of glucose by the action of a cytosolic glucosyltransferase turns ceramide into glucosylceramide (Fig. 1). Subsequently, a cytosolic galactosyltransferase mediates the attachment of a galactose residue to the glucose, forming lactosylceramide. Lactosylceramide then flips into the luminal side of early Golgi cisternae, where the attachment of a sialic acid residue over the galactose, mediated by α-2,3-sialyltransferase, generates GM3, the first sialylated ganglioside. GM3 may give rise to either the monosialylated ganglioside series (GM2, GM1, etc.), or to GD3, GT3, GQ3, etc., by the sequential addition of more sialic acid residues.

In CD95-mediated apoptosis, we demonstrated that ceramide synthesis induces the accumulation of the disialoganglioside GD3 [42]. GD3 results from the addition of a second sialic acid to the one present on GM3, mediated by the action of GD3 synthase (α-2,8-sialyltransferase), a transmembrane type II protein of about 40 kDa resident in the early Golgi.

GD3 transiently accumulates after apoptotic stimulation via CD95 in HuT78 and U937 tumor cells, but not in cell variants which are resistant to CD95 crosslinking. C2-ceramide, but not C2-dihydroceramide which is ineffective in inducing apoptosis, is sufficient to induce GD3 accumulation. CD95-induced GD3 accumulation is prevented by specific GD3 synthase antisense oligonucleotides. Moreover, NPD lymphoblasts have an impaired CD95-mediated apoptosis and cannot accumulate ganglioside GD3 whereas ASM-reconstituted NPD lymphoblasts can accumulate GD3 and undergo efficient CD95-mediated apoptosis. Therefore, ASM is required for GD3 ganglioside accumulation in response to CD95 [33] and triggering the apoptotic program accelerates GD3 synthase function and GD3 neosynthesis. Similarly to ceramides, GD3 can efficiently induce cell death in both HuT78 and U937 cells, while other gangliosides such as GD1a, GT1b or GM1 do not, when added exogenously. Interestingly, CD95-resistant HuT78 and U937 cell lines, which failed to accumulate GD3 after CD95 crosslinking, are as sensitive as their respective wild type cell lines to GD3, suggesting that exogenous GD3 restores the ability to trigger the apoptotic program in cells with upstream signaling defects which result in lack of GD3 accumulation. Furthermore, the transient overexpression of the GD3 synthase induces apoptosis in HuT78 cells. CD95-induced cell death is substantially prevented using antisense oligonucleotides which block GD3 synthase expression. Thus, the ganglioside GD3 mediates cell death induced by CD95 crosslinking and ceramide.

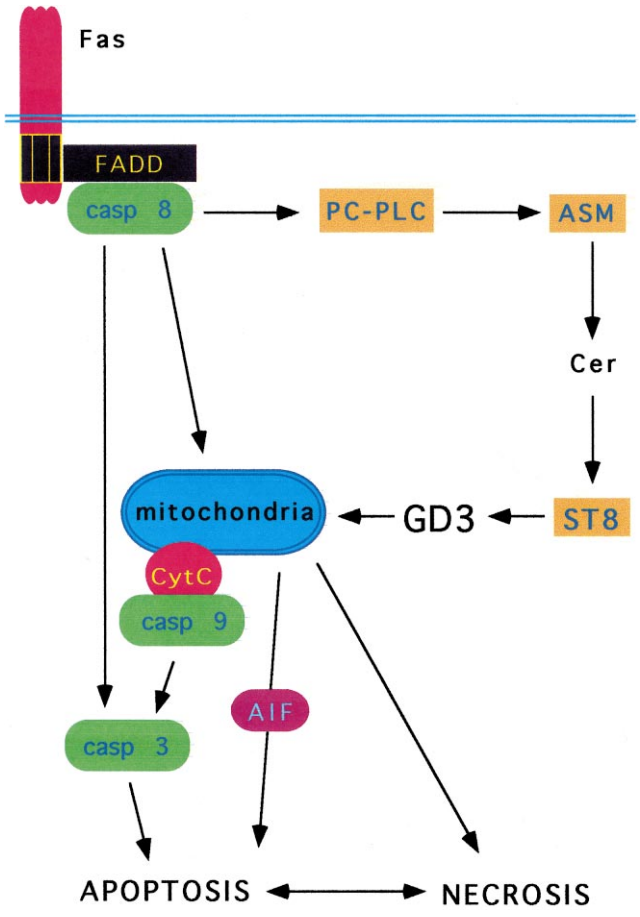

Fig. 2. Schematic representation of CD95-induced signaling. ASM, acidic sphingomyelinase; PC-PLC, phosphatidylcholine-specific phospholipase C; CytC, cytochrome *c*; casp, caspase; Cer, ceramide; AIF, apoptosis-inducing factor; ST8, α-2,8-sialyltransferase.

Inhibitors of caspases, involved in CD95-induced apoptosis, prevent GD3 accumulation upon CD95 crosslinking indicating that upstream caspase activation is required for GD3 accumulation and that one or more caspases control GD3 synthesis. Attempts to identify potential targets in the GD3 apoptotic pathway revealed that $\Delta\Psi_m$ disruption is a rapid event that precedes all the major apoptotic features induced by GD3 when added to the cells. Moreover, our preliminary results indicate that GD3 accumulates in close proximity to mitochondria of cells undergoing apoptosis, and that GD3, but not other gangliosides such as GD1a, GM1 or GM3, can directly induce PT on isolated mitochondria, with consequent release of the apoptogenic factors cytochrome *c* and AIF. These data corroborate the hypothesis that ceramide acts on mitochondria after conversion to GD3. Thus, mitochondria may represent the site where GD3 meets its targets (Fig. 2).

7. Conclusions and perspectives

The acidic sphingomyelinase-mediated pathway therefore

contributes to apoptotic signals originating from CD95 death domains. The analysis of genetic models of ASM deficiency in humans and targeted disruption of the ASM gene in mice has established the role of ASM in the progression of apoptotic signals. The involvement of GD3 in the apoptotic program is consistent with the notion that only ceramides produced by ASM in the endocytic degradative compartments come into close functional and topological proximity to the membranogenic organelles, and engage the biosynthetic pathways. In keeping with the notion of the importance of signal compartmentalization, GD3 synthase mutants, lacking the transmembrane domain and therefore not addressed to the Golgi, cannot induce apoptosis of transfected cells as the wild type enzyme does (our unpublished observation). Thus, one of the roles of ASM-released ceramide in apoptosis would be to serve as a precursor of ganglioside GD3, also indicating the early Golgi, where GD3 is generated by GD3 synthase, as a critical subcellular compartment for the progression of apoptotic signals. Some of the neosynthesized GD3 could then be targeted to the mitochondria. Thus, this lipid messenger participates in the optimal completion of the apoptotic process in hematopoietic cells by recruiting mitochondria to the program. Additional studies are needed to identify GD3 targets on mitochondria.

Acknowledgements: This work was supported by a grant from the Associazione Italiana Ricerca sul Cancro. F. Malisan is the recipient of a fellowship from the Fondazione Italiana Ricerca sul Cancro.

References

[1] Testi, R. (1996) Trends Biochem. Sci. 21, 468–471.
[2] Kolesnick, R. and Kronke, M. (1998) Annu. Rev. Physiol. 60, 643–665.
[3] Mathias, S., Pena, L. and Kolesnick, R. (1998) Biochem. J. 335, 465–480.
[4] Perry, D. and Hannun, Y. (1998) Biochim. Biophys. Acta 1436, 233–243.
[5] Schissel, S.L., Keesler, G.A., Schuchman, E.H., Williams, K.J. and Tabas, I. (1998) J. Biol. Chem. 273, 18250–18259.
[6] Kolesnick, R.N. (1987) J. Biol. Chem. 262, 16759–16762.
[7] Schuchman, E.H., Suchi, M., Takahashi, T., Sandhoff, K. and Desnick, R. (1991) J. Biol. Chem. 266, 8531–8539.
[8] Liu, P. and Anderson, R.G.W. (1995) J. Biol. Chem. 270, 27179–27185.
[9] Spence, M., Byers, D., Palmer, F. and Cook, H. (1989) J. Biol. Chem. 264, 5358–5363.
[10] Tomiuk, S., Hofman, K., Nix, M., Zumbansen, M. and Stoffel, W. (1998) Proc. Natl. Acad. Sci. USA 95, 3638–3643.
[11] Jayadev, S., Linardic, C.M. and Hannun, Y.A. (1994) J. Biol. Chem. 269, 5757–5763.
[12] Okazaki, T., Bielawska, A., Domae, N., Bell, R. and Hannun, Y.A. (1994) J. Biol. Chem. 269, 4070–4077.
[13] Duan, R., Nyberg, L. and Nilsson, A. (1995) Biochim. Biophys. Acta 1259, 49–55.
[14] Nelson, D.H., Murray, D.K. and Brady, R.O. (1982) J. Clin. Endocrinol. Metab. 54, 292–295.
[15] Haimovitz-Friedman, A., Kan, C.-C., Ehleiter, D., Persaud, R.S., McLoughlin, M., Fuks, Z. and Kolesnick, R.N. (1994) J. Exp. Med. 180, 525–535.
[16] Dressler, K.A., Mathias, S. and Kolesnick, R.N. (1992) Science 255, 1715–1718.
[17] Schütze, S., Potthof, K., Machleidt, T., Berkovic, D., Wiegmann, K. and Krönke, M. (1992) Cell 71, 765–776.
[18] Cifone, M.G., De Maria, R., Roncaioli, P., Rippo, M.R., Azuma, M., Lanier, L.L., Santoni, A. and Testi, R. (1994) J. Exp. Med. 180, 1547–1552.
[19] Sallusto, F., Nicolo', C., De Maria, R., Corinti, S. and Testi, R. (1996) J. Exp. Med. 184, 2411–2416.
[20] Dobrowsky, R.T., Werner, M.H., Castellino, A.M., Chao, M.V. and Hannun, Y.A. (1994) Science 265, 1596–1599.
[21] Boucher, L.-M., Wiegman, K., Fütterer, A., Pfeffer, K., Machleidt, T., Schültze, S., Mak, T.W. and Krönke, M. (1995) J. Exp. Med. 181, 2059–2068.
[22] Wiegmann, K., Schütze, S., Machleidt, T., Witte, D. and Krönke, M. (1994) Cell 78, 1005–1015.
[23] Jayadev, S., Liu, B., Bielawska, A.E., Lee, J.Y., Nazaire, F., Pushkareva, M.Y., Obeid, L.M. and Hannun, Y.A. (1995) J. Biol. Chem. 270, 2047–2052.
[24] Yao, B., Zhang, Y., Delikat, S., Mathias, S., Basu, S. and Kolesnick, R. (1995) Nature 378, 307–310.
[25] Verheij, M. et al. (1996) Nature 380, 75–79.
[26] Lozano, J., Berra, E., Municio, M.M., Diaz-Meco, M., Dominguez, I., Sanz, L. and Moscat, J. (1994) J. Biol. Chem. 269, 19200–19202.
[27] Dobrowsky, R.T., Kamibayashi, C., Mumby, M.C. and Hannun, Y.A. (1993) J. Biol. Chem. 268, 15523–15530.
[28] Nickels, J.T. and Broach, J.R. (1996) Genes Dev. 10, 382–394.
[29] Thornberry, N. and Lazebnik, Y. (1998) Science 281, 1312–1316.
[30] Villa, P., Kaufman, S. and Earnshaw, W. (1997) Trends Biochem. Sci. 22, 388–393.
[31] Cifone, M.G., Roncaioli, P., De Maria, R., Camarda, G., Santoni, A., Ruberti, G. and Testi, R. (1995) EMBO J. 14, 5859–5868.
[32] Santana, P. et al. (1996) Cell 86, 189–199.
[33] De Maria, R., Rippo, M., Schuchman, E. and Testi, R. (1998) J. Exp. Med. 187, 897–902.
[34] Hannun, Y.A. (1996) Science 274, 1855–1859.
[35] Green, D. and Reed, J. (1998) Science 281, 1309–1312.
[36] Susin, S., Zamzami, N. and Kroemer, G. (1998) Biochim. Biophys. Acta 1366, 151–165.
[37] Slee, E. et al. (1999) J. Cell Biol. 144, 281–292.
[38] Ghafouribar, P., Klein, S., Schucht, O., Schenk, U., Pruschy, M., Rocha, S. and Richter, C. (1999) J. Biol. Chem. 274, 6080–6084.
[39] Susin, S. et al. (1997) Exp. Cell Res. 236, 397–403.
[40] Allan, D. and Kallen, K.-J. (1993) Progr. Lipid Res. 32, 195–219.
[41] van Echten, G. and Sandhoff, K. (1993) J. Biol. Chem. 268, 5341–5344.
[42] De Maria, R., Lenti, L., Malisan, F., d'Agostino, F., Tomassini, B., Zeuner, A., Rippo, M.R. and Testi, R. (1997) Science 277, 1652–1655.