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Glucose induces FGF21 mRNA expression through ChREBP activation in rat hepatocytes

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A B S T R A C T

Fibroblast growth factor 21 (FGF21) has beneficial effects of improving the plasma glucose and lipid profiles in diabetic rodents. Here, we investigated carbohydrate response element binding protein (ChREBP) involvement in the regulation of FGF21 mRNA expression in liver. Glucose stimulation and adenoviral overexpression of dominant active ChREBP increased FGF21 mRNA. Consistently, adenoviral expression of dominant negative Mlx inhibited glucose induction of FGF21 mRNA. Furthermore, deletion studies of mouse FGF21 gene promoter (−2000 to +65 bp) revealed a glucose responsive region between −74 and −52 bp. These findings suggest that FGF21 expression is regulated by ChREBP.

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1. Introduction

Fibroblast growth factor 21 (FGF21) belongs to the fibroblast growth factor (FGF) family involved in cell growth, cell differentiation, and embryonic development [1]. FGF21 has been found to have multiple beneficial effects in treatment of metabolic syndrome including obesity as well as diabetes mellitus [2]. Transgenic mice with FGF21 overexpression are resistant to diet-induced obesity and glucose intolerance [3]. Treatment with FGF21 induces energy expenditure and improves glucose intolerance, hypertriglycemia and hepatic steatosis in ob/ob mice [4]. In human, serum FGF21 levels in diabetic or obese individuals are higher than normal, and are significantly correlated with adiposity, plasma fasting insulin and the triglyceride concentration [5]. Furthermore, Akt signaling and PPAR- increases FGF21 mRNA expression in muscle and adipose tissue, respectively [6]. These findings suggest that FGF21 is increased in adaptation to increased body weight and energy intake.

We have reported that ChREBP, a glucose activated transcription factor, regulates lipogenic enzyme gene expression and is involved in the development of metabolic syndrome [7,8]. Transactivity of ChREBP is increased in genetically obese mice, and gene deletion of ChREBP improves metabolic disorders such as fatty liver and glucose intolerance [9]. Recently, some groups have reported that overexpression of dominant negative Mlx, which forms a heterodimer with ChREBP, inhibits glucose mediated FGF21 gene expression in rat hepatocytes [10]. These findings suggest that glucose activation of ChREBP might be involved in FGF21 mRNA expression.

In this study, we tested whether glucose stimulation or activation of ChREBP could increase FGF21 mRNA expression in rat hepatocytes We also performed a deletion study of mouse FGF21 gene promoter and identified the glucose responsive region. Our findings demonstrate that glucose stimulation via ChREBP activation induces FGF21 gene expression in rat primary hepatocytes and that FGF21 has an important role in glucose and lipid homeostasis.

2. Materials and methods

2.1. Materials, tissue materials, hepatocyte isolation, and Taqman PCR analysis

The protocols for all animal experiments were approved by the Institutional Animal Care and Use Committee of Gunma University

Abbreviations: FGF21, fibroblast growth factor 21; ChREBP, cabohydrate response elemnt binding protein; LPK, liver type pyruvate kinase; FASN, fatty acid synthase

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Medical School (code nos. 08-025 and 08-026). Rat primary hepatocytes were isolated and cultured from 6 weeks age male Wistar rats (SLC) as previously described [11,12].

2.2. Construction of plasmid and adenovirus vectors

pcDNA-daChREBP, Ad-daChREBP, Ad-dnMlx, and pGL4 TK RLuc were used previously [11,12]. The series of pGL3-mFGF21 vectors were constructed as follows: a fragment representing −2000, −1205, −1012, −797, −317, −197, −100, −67, and −40 bp (position −2000/+65, −1205/+65, −1012/+65, −797/+65, −317/+65, −197/+65, −100/+65, −67/+65, and −40/+65 relative to the transcription start site of mouse FGF21 of the native 5′ sequence flanking the mouse FGF21 gene were cloned upstream of the luciferase gene in pGL3 basic vector. The series of pGL3 promoter mFGF21 vectors were constructed as follows: a fragment representing −100/−30, −90/−40, −67/−30, −100/−67 bp (position −100/−30, −90/−40, −67/−30, −100/−67 relative to the transcription start site of mouse FGF21 gene, respectively) of the native 5′ sequence flanking the mouse FGF21 gene were cloned upstream of TK promoter in pGL3 promoter vector. A fragment 3XFGF21 E1(−101/−80 bp) or 3XFGF21 E2 (−74/−52 bp) was cloned upstream of TK promoter in pGL3 promoter vector. Mouse FGF21 cDNA expressing adenovirus (Ad-FGF21) was constructed as follows: Mouse FGF21 full-length cDNA was cloned using Prime Star DNA polymerase reagents. PCR fragment was ligated into pENTR vector (Invitrogen). Recombination of adenovirus and pENTR FGF21 vector was performed to produce Ad-FGF21 vectors according to manufacture's protocol. All plasmid and adenovirus vectors were verified by sequencing analysis.

2.3. Treatment with recombinant adenovirus in rat hepatocytes

Rat primary hepatocytes were cultured in six-well plates in 2 ml DMEM medium. After 2, 10, and 50 m.o.i. of adenovirus bearing GFP, dominant active ChREBP (daChREBP) dominant negative Mlx (dnMlx), or FGF21 was infected into hepatocytes for 2 h, media were removed and infected hepatocytes were incubated in media with 2.5 or 25 mM glucose concentration for 18 h. Cells were then collected and used for RNA extraction, cDNA synthesis and RT-PCR analysis as previously described [11,12].

2.4. Mammalian transfection and reporter assay

Rat primary hepatocytes were cultured in six-well plates in 2 ml DMEM without antibiotics. The cells were transfected with Lipofectamine2000 (10 μl), the series of pGL3-mFGF21 (3.6 μg), and the pGL4 TK RLuc vector (0.4 μg) [12]. After 24 h of incubation at 2.5 or 25 mM glucose concentration, the cells were collected and used to measure luciferase activity (Dual Luciferase assay system; Promega, Madison, WI) according to manufacture's protocol. To determine glucose dependency on the glucose response region in mouse FGF21 gene promoter, cells were transfected with 3.1 μg of pGL3 promoter 3XFGF21 E1, 3XFGF21 E2, or 3XLPK ChoRE vectors, 0.4 μg of pGL4-RLuc-TK vectors and 0.5 μg of pcDNA6.2 empty vector or daChREBP vector. After 24-h incubation with various glucose concentrations, the cells were collected for measurement of luciferase activity.

2.5. Data presentation and statistical methods

All data are expressed as means ± S.D. The listed *n* values represent the number of single experiments performed (each experiment was duplicated). Comparisons between two groups were performed by student *t*-test and comparison between multiple groups was performed by Tukey–Kramer test. A value of $P < 0.05$ was regarded as significant.

3. Results

3.1. The role of ChREBP in glucose mediated FGF21 gene expression

As FGF21 mRNA is expressed mainly in islets and liver (Fig. S1A), we examined FGF21 mRNA expression in liver of diabetic model mice. FGF21 mRNA levels in 6 weeks age male STZ mice and ob/ob mice were 1.1- and 2.5-fold higher than that in 6 weeks age C57BL/6J mice, respectively (Fig. S1B). To verify that glucose increased FGF21 mRNA expression, we examined the effect of glucose and ChREBP on FGF21 mRNA expression. High glucose stimulation increased FGF21 mRNA expression in a time dependent manner (Fig. 1A). These findings suggest that a metabolite in the glycolytic and pentose pathway induces FGF21 mRNA expression. Adenoviral overexpression of daChREBP increased FGF21 mRNA expression in a dose-dependent manner (Fig. 1B). Furthermore, Mlx is an obligate heterodimer partner of ChREBP that is required for binding and activation of glucose-regulated gene expression [11]. In accord with Fig. 1B, dnMlx successfully inhibited glucose induction of FGF21 mRNA gene expression in rat primary hepatocytes (Fig. 1C). Similarly, siRNA against ChREBP inhibited not only ChREBP mRNA but also glucose mediated FGF21, liver type pyruvate (LPK), and fatty acid synthase (FASN) gene expression in rat primary hepatocytes (Fig. S1C). Thus, transactivation of ChREBP induces FGF21 mRNA expression in hepatocytes.

3.2. Identification of glucose response region in mouse FGF21 gene promoter

Deletion studies of mouse FGF21 gene promoter showed that a region between −100 and −67 bp is critical for the glucose response to FGF21 mRNA induction (Fig. 2A). We tested in detail putative glucose response elements in mouse FGF21 promoter (between −200 and +0 bp) (Fig. 2B). Fig. 2B shows that a region between −90 and −40 bp is critical for the glucose response to FGF21 mRNA expression. We also determined which putative E-Box (E1: −101/−80 bp and E2: −74/−52 bp) functions as the glucose response element (Supplementary Fig. S2). In accord with Fig. 2A and B, the E2 element (−74/−52 bp) is shown to possesses glucose responsiveness or ChREBP mediated activation of FGF21 mRNA expression (Fig. 2C). We also tested the glucose response to luciferase activity in pGL3-3XFGF21 E2 vector. Compared with pGL3 FGF21 −2 kbp vector, luciferase activities were similarly dependent on the glucose concentration (Fig. 2D).

3.3. Effect of FGF21 overexpression on ChREBP and expression of its target genes in rat hepatocytes

FGF21 lowers plasma glucose and increases glucose uptake in muscle and adipose tissue, but its role in liver is unclear. Because the FGF21 signaling cascade requires both FGF receptors (FGFRs) and beta-Klotho [13,14], we confirmed that all of the FGFRs were present in both mouse liver and rat hepatocytes and beta-Klotho was detected in mouse livers (Fig. S3A and B) [14]. We then tested whether FGF21 affects glucose mediated LPK and FASN mRNA expression in rat hepatocytes. Glucose induction of LPK and FASN mRNA expression in rat hepatocytes infected in a dose-dependent manner with Ad-FGF21 were unchanged as compared with those in untreated hepatocytes (Fig. 3).

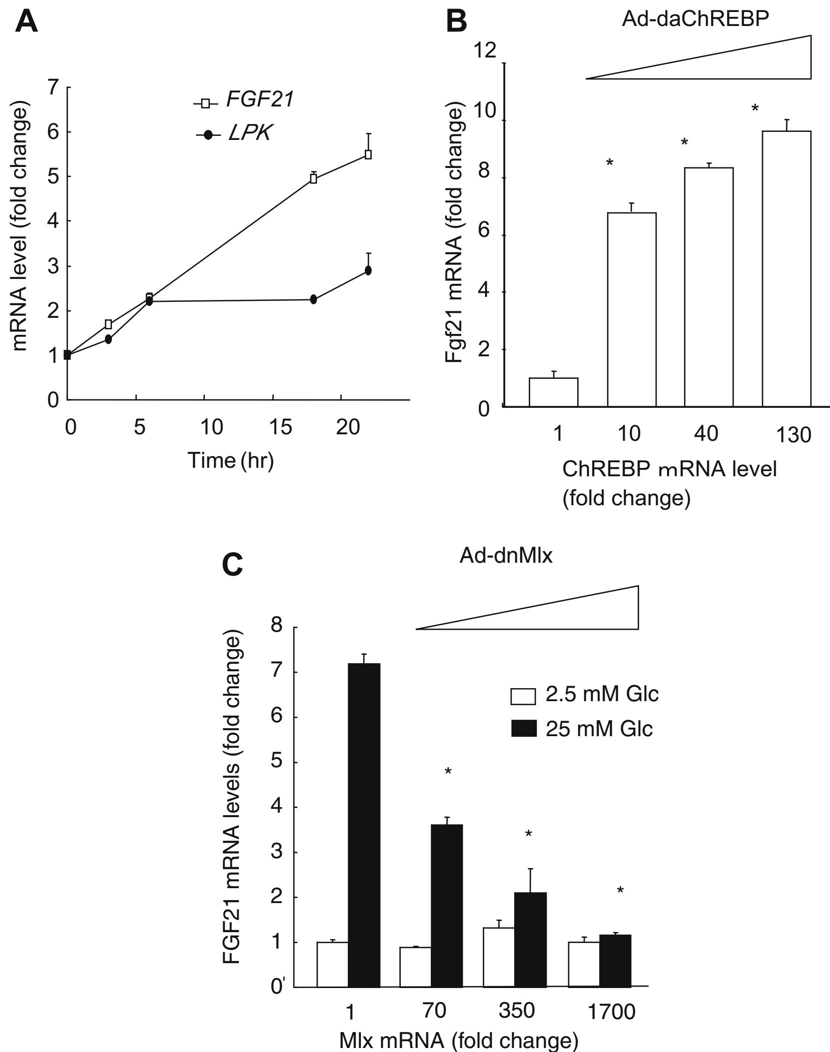

Fig. 1. (A) Glucose stimulates increased FGF21 mRNA expression in rat hepatocytes. Isolated hepatocytes were cultured in Dulbecco's modified Eagle's medium (DMEM) including several concentrations of glucose for 24 h and collected at the indicated hours for Taqman RT-PCR analysis. (B) Adenoviral overexpression of dominant active ChREBP increased FGF21 mRNA expression in rat hepatocytes. Isolated hepatocytes were infected with 2, 10, and 50 m.o.i. of Ad-daChREBP for 2 h. After culture in DMEM with 2.5 mM glucose concentration for 18 h, the cells were collected for Taqman RT-PCR analysis. Data represent means ± S.D. (C) Adenoviral overexpression of dominant negative Mlx increased FGF21 mRNA expression in rat hepatocytes. Isolated hepatocytes were infected with 2, 10, and 50 m.o.i. of Ad-dnMlx for 2 h. After culture in DMEM with 2.5 or 25 mM glucose concentration for 18 h, the cells were collected for Taqman RT-PCR analysis. Values represent means ± S.D. At most points, the error bars are too small to be shown.

4. Discussion

In this study, we show that FGF21 is a target gene of ChREBP in rat primary hepatocytes. Moreover, we identified a glucose response region located between −74 and −52 bp in the FGF21 promoter. Adenoviral delivery of FGF21 cDNA into rat primary hepatocytes was found not to directly affect glucose induction of LPK and FASN mRNA expression in rat hepatocytes. Thus, FGF21 is directly regulated by the glucose activated transcription factor ChREBP, and ChREBP is not directly regulated by FGF21.

As found in a previous study, FGF21 mRNA is most abundant in mouse islets and liver, in which ChREBP is also highly expressed (Fig. S1A) [2,15]. FGF21 mRNA is more highly upregulated in ob/ob mice, but is only slightly induced in STZ mice (Fig. S1B). In addition, ob/ob mice show hyperinsulinemia and hyperglycemia, while STZ mice show hypoinsulinemia and hyperglycemia (Fig. S1B). Considered together with our previous finding that ChREBP activation requires insulin action, glucose-stimulated FGF21 mRNA expression may well require insulin action [12,16]. In accord with the data shown in Fig. 1A, overexpression of daChREBP induced FGF21 mRNA expression and overexpression of dnMlx inhibited glucose-induced FGF21 mRNA expression (Fig. 1B and C). Moreover, siRNA against ChREBP suppressed glucose induction of FGF21 mRNA expression in rat primary hepatocytes (Fig. S1C). These findings indicate that glucose activation of ChREBP induces hepatic FGF21 mRNA expression. In fact, some groups have reported that plasma FGF21 concentrations and hepatic FGF21 mRNA expression in obese and diabetic animal models are much higher than those in control lean animals [5]. ChREBP is remarkably activated in livers from these diabetic animals, further suggesting that glucose activation of ChREBP induces FGF21 mRNA expression. Moreover, satiety signaling such as PPAR- and Akt signaling induces FGF21 mRNA expression [6,16–18]. Thus, FGF21 gene expression is regulated by satiety signals such as glucose and insulin.

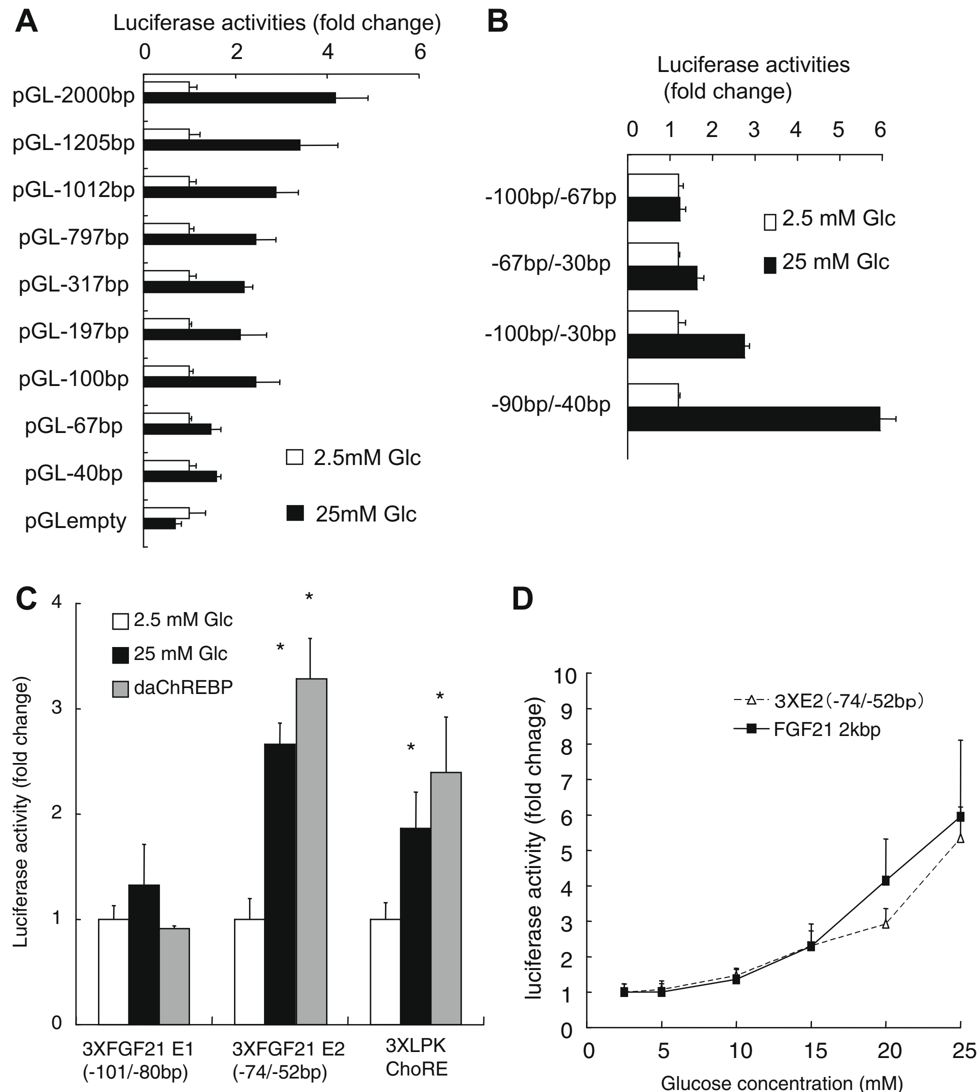

Fig. 2. (A) Deletion studies of mouse FGF21 gene promoter. Isolated hepatocytes were cultured in six-well dishes and transfected with the series of pGL3-mFGF21 vector (3.6 µg) and pGL4 TK RLuc (0.4 µg) using Lipofectamine2000 (10 µl). After 24 h incubation in DMEM including 2.5 or 25 mM glucose concentrations, luciferase activities were measured using Dual Luciferase assay kit. (B) Detailed analysis of the carbohydrate response region in mouse FGF21 promoter. Cells were transfected with the series of pGL3 promoter mFGF mutants (3.6 µg), and pGL4 TK RLuc (0.4 µg) using Lipofectamine2000 (10 µl). After 24 h incubation in DMEM including 2.5 or 25 mM glucose concentrations, luciferase activities were measured using Dual Luciferase assay kit. (C) Reporter analysis of pGL3 promoter 3XFGF21 E2 (−74/−52 bp). Rat primary hepatocytes were transfected with 3.1 µg of pGL3 promoter 3XFGF21 E2 (−74/52p), 3XFgf21 E1 (−101/−80 bp), or 3XLPK ChoRE vectors, 0.4 µg of pGL4-RLuc-TK vectors and 0.5 µg of pcDNA6.2 empty vector or pcDNA6.2 daChREBP vector. Cells were incubated at 2.5 or 25 mM glucose for 24 h and collected for the measurement of luciferase activities. (D) Glucose dependent activation of pGL3 promoter 3XFGF21 E2 (−74/−52 bp) vector and pGL3-mFGF21 −2 kbp. Cells were transfected with 3.6 µg of pGL3 promoter 3XFGF21 E2 (−74/−52 bp) vector or pGL3-mFGF21 −2 kbp and 0.4 µg of pGL4-RLuc-TK vector. Cells were incubated at several glucose concentrations for 24 h and collected for measurement of luciferase activities. Values represent means ± S.D. At most points, the error bars are too small to be shown. $^{*}P < 0.05$ vs. Ad-GFP.

It has been reported that administration of FGF21 in livers of diet-induced obese mice reduces hepatic ChREBP-target gene expression [4,19]. The FGF21 signaling cascade requires the FGF21 receptor complex, FGFR, and an adapter-like molecule, beta-Klotho [14]. Beta-Klotho and FGFR1-4 are abundantly expressed in liver, adipose tissues, and pancreas [14,15,20–22]. In our experiments, all FGFRs were detectable in mouse liver and rat hepatocytes and beta-Klotho was detected only in mouse liver (Fig. S3A and B). The sequence of rat beta-Klotho cDNA remains unknown and we could not check beta-Klotho mRNA expression in rat primary hepatocytes. We then tested whether FGF21 directly affects glucose activation of ChREBP-target gene expressions such as LPK and FASN. We found that FGF21 had no significant effects on the expression of these genes in rat hepatocytes (Fig. 3). GLUT1 gene expression, an FGF21 target, was also unchanged in rat hepatocytes infected by Ad-FGF21 (data not shown). Consistent with these data, some groups have reported that FGF21 does not act on mouse primary hepatocytes and rat hepatoma cell lines, even in the presence of beta-Klotho and FGFRs [14,23,24]. In contrast, some groups reported the opposite evidences that FGF21 is active in cells of hepatic origin [25]. The presence of beta-Klotho in rat primary hepatocytes remains unknown and it will be needed to further investigate the direct action of FGF21 in liver. In conclusion, these results indicate that FGF21 suppresses ChREBP transactivity at least indirectly by lowering plasma glucose concentrations in the whole body.

In conclusion, glucose induces FGF21 mRNA expression via transactivation of ChREBP, indicating that both FGF21 and ChREBP are involved in the regulation of glucose and lipid homeostasis in liver. Thus, FGF21 may well be a useful drug in the treatment of diabetes and obesity.

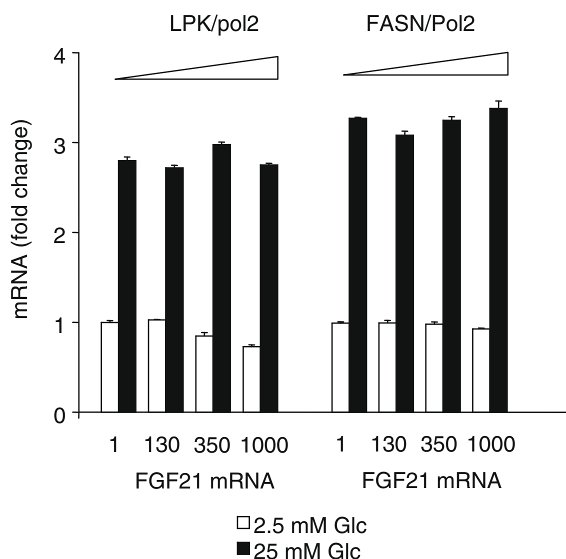

Fig. 3. Adenoviral overexpression of FGF21 did not inhibit LPK and FASN mRNA. Adenovirus expressing either GFP or FGF21 was transduced into rat hepatocytes at m.o.i. of 2, 10, and 50. As control, recombinant adenovirus expressing GFP was used at m.o.i. of 50. Two h after infection, the cells were cultured in DMEM including 2.5 or 25 mM glucose for an additional 18 h. Total RNA was extracted from hepatocytes, and RT-PCR analysis was performed. Values represent means ± S.D. At most points, the error bars are too small to be shown.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.07.053.

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