

Control of ovarian primordial follicle activation

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The ovarian follicles develop initially from primordial follicles. The majority of ovarian primordial follicles are maintained quiescently as a reserve for the reproductive life span. Only a few of them are activated and develop to an advanced follicular stage. The maintenance of dormancy and activation of primordial follicles are controlled by coordinated actions of a suppressor/activator with close communications with somatic cells and intra-oocyte signaling pathways. Many growth factors and signaling pathways have been identified and the transforming growth factor-beta superfamily plays important roles in early folliculogenesis. However, the mechanism of maintaining the dormancy and survival of primordial follicles has remained unknown for decades. Recently, since the first finding that all primordial follicles are activated prematurely in mice deficient forkhead box O3a, phosphatidylinositol 3 kinase/phosphatase and tensin homolog (PTEN) signaling pathway was reported to be important in the regulation of dormancy and initial follicular activation. With these informations on early folliculogenesis, clinical application can be expected such as *in vitro* maturation of immature oocytes or *in vitro* activation of follicles by PTEN inhibitor in cryopreserved ovarian cortical tissues for fertility preservation.

Keywords: Ovarian follicle; Folliculogenesis; PI3K; PTEN; *In vitro* activation

Introduction

The mammalian ovary contains follicles at various developmental stages and its function includes the cyclic recruitment, development and regression of the follicles. The pool of primordial follicles is maintained in dormancy and serves as a reserve for cyclic recruitment of follicles and oocytes.

The primordial follicles are maintained in dormancy or activated to grow into antral follicles. Some of the antral follicles reach ovulation under proper gonadotropin stimulation, however, most of the follicles undergo atresia during follicular development or directly from the dormant state [1]. The maintenance of the quiescence and survival of primordial follicles is important as a reserve to provide oocytes throughout the reproductive life. The mechanisms of the main-

tenance of such a long dormancy of the primordial follicles have remained unknown, however the gonadotropin-dependent process of follicular development is otherwise well elucidated [2]. In the control of primordial follicle activation, bidirectional communication between the oocytes and surrounding somatic cells is involved. The oocytes are supported by granulosa cells and oocytes also play an active role in the growth of the granulosa cells [3].

Recently, it was reported that phosphatidylinositol 3 kinase (PI3K) pathway is important for the control of follicular activation. In mouse ovaries deficient in forkhead box O3 (*Foxo3a*), the entire pool of primordial follicles is prematurely activated [4]. Moreover, with the findings from a transgenic mouse model, it has been shown that the PI3K/phosphatase and tensin homolog (PTEN) signaling pathway involves the regulation of dormancy and activation of primordial follicles [5,6].

Maintaining the dormancy of primordial follicles

The pool of primordial follicles is under inhibitory control, therefore, when these inhibitory factors decrease or stimulatory factors increase,

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the follicles are activated [7]. The activated primordial follicles develop into primary, secondary, and antral follicles, however, most of the antral follicles undergo atresia. Only a few of them grow to the pre-ovulatory stage under the stimulation of gonadotropin [1]. Activation of the primordial follicles of bovine cortical tissue was noted in an *in vitro* culture in serum-free medium. This implies that the primordial follicles are under inhibitory control to maintain the dormant state [8]. Primordial follicles activate spontaneously *in vitro* and incubation with exogenous cytokine and growth factors increases the number of activated follicles.

Regulation of activation by multiple activator and repressor pathways

Multiple activator and repressor or signaling pathways are involved in the control of primordial follicle activation. Neurotrophin, nerve growth factor (NGF) and its tyrosine receptor kinase NTRK1 are important in follicular activation [9]. Other neurotrophins, such as neurotrophin 4 (NT4), brain-derived neurotrophic factor (BDNF) and their receptor NTRK2 are also involved in regulating oocyte survival [10].

Some ligands play multiple roles in folliculogenesis. transforming growth factor-beta (TGF- β) ligand, growth differentiation factor 9 (GDF9), and bone morphogenic protein 15 (BMP15) play roles in pre-antral follicular development in addition to their facilitation of primordial follicle activation.

1. GDF 9

Treatment of immature rats with recombinant GDF9 leads to an increased rate of follicular activation. The addition of recombinant GDF9 to human ovarian cortical tissue *in vitro* increases the activation and improves the survival of follicles [11]. In GDF9 null mice, the follicles do not progress beyond the first stage and granulosa cell proliferation is limited [12]. However, oocyte growth is accelerated. These results suggest that GDF9 plays a role in recruiting granulosa cell while limiting the growth of the oocytes.

2. SMADS

SMADS are downstream intracellular signaling molecules of the TGF- β ligands (BMP 4 and 7). SMADS null mice have impaired folliculogenesis showing increased numbers of primordial follicles and decreased numbers of developed follicles in adult life [13].

3. FOXL2

Forkhead boxL2 (*Foxl2*) is expressed in mammalian eyelids and ovaries. In mice, *Foxl2* is expressed in pre-granulosa cells and the expression decreases in the granulosa cells of pre-antral follicles. In humans, mutation of the *FOXL2* gene causes blepharophimosis-ptosis-epican-

thus-inversus (BPES), an autosomal dominant genetic disease showing eyelid dysplasia and high risk of POF [14]. In *Foxl2* inactivated mice, though the primordial follicles form normally, they do not develop into secondary follicles because pre-granulosa cells do not complete the cuboidal transition. Most of the oocytes in primordial follicles are activated prematurely, and undergo atresia, which implies the depletion of the primordial follicle pool [15]. *FOXL2* in pregranulosa cells plays an important role in the quiescence of primordial follicles by inhibiting the initiation of the growth of oocytes.

4. Sohlh1

Spermatogenesis- and oogenesis-specific basic helix-loop-helix-containing protein 1 (*Sohlh1*) is expressed in the germ cell clusters and in new primordial follicles. *Sohlh1* null mice show a rapid decrease in the number of oocytes by 7 weeks of age and a defect in the primordial to primary transition [16].

5. AMH

AMH and its receptor AMHRII are expressed in granulosa cells. The role of AMH in primordial follicle was investigated an *Amh*^{-/-} mice. *Amh* deficient mice showed premature depletion of primordial follicles, indicating that AMH inhibits the growth of primordial follicles [17]. In mouse ovaries cultured with AMH, growing follicle numbers decreased by 40-50% [18], and human ovarian tissue cultured with recombinant rat AMH showed decreased growth of primordial follicles [19]. Therefore, AMH plays a role in maintaining quiescence by inhibiting the transition from primordial to primary follicles.

Intracellular signaling in oocytes for maintenance of dormancy of primordial follicles

1. PTEN

Phosphatase and tensin homolog deleted on chromosome 10 is a negative regulator of PI3K. In the absence of PTEN, phosphatidylinositol 3,4,5-triphosphate (PIP3) increase leads to protein kinase B (Akt) signaling. The role of PTEN in primordial follicles was elucidated by a study using oocyte-specific *Pten* deleted mice (*OoPten*^{-/-} mice) [6].

Oocyte-specific *Pten* was deleted from oocytes of primordial follicles in mice by crossing *Pten*^{loxP/loxP} mice with transgenic mice expressing *Gdf-9* promoter-mediated Cre recombinase (Gcre mice) [20]. In the *OoPten*^{-/-} mice, the primordial follicles are overactivated and grow to primary and secondary follicles, which results in the early depletion of the primordial follicle pool. Another study with *pten*-deleted primary oocytes in oocyte-specific Gcre transgenic mice showed similar results of follicular overactivation and early depletion [21]. These results suggest that PTEN is essential for the maintenance of dormancy of primordial follicles [22] (Figure 1).

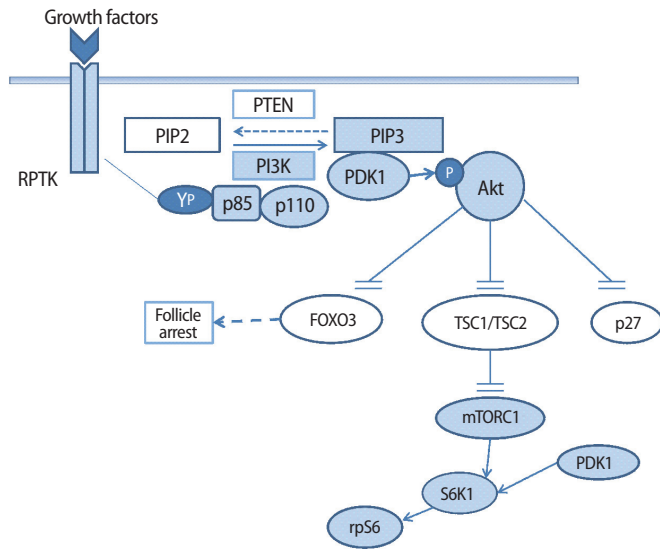


Figure 1. Illustration of the PI3K pathway signaling pathway. Molecules that maintain the dormancy of the primordial follicles are PTEN, Foxo3a, p27, and TSC1/TSC2. Molecules that maintain the survival of primordial follicles include PI3K, PDK1, mTORC1, S6K1, and rpS6. RPTK, receptor protein tyrosine kinase; PTEN, phosphatase and tensin homolog; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)- triphosphate; PI3K, phosphatidylinositol 3-kinase; PDK1, 3-phosphoinositide-dependent protein kinase-1; Akt, protein kinase B; FOXO3a, forkhead box O3a; mTORC1,C2, mammalian target of rapamycin complex 1,2; TSC1/2, tuberlin/tuberous sclerosis complex 1,2; S6K1, p70 ribosomal protein S6 kinase 1; rpS6, S6K1-ribosomal protein S6; p27, cyclin-dependent kinase inhibitor p27^{Kip1} (Modified from Reddy et al. [22]).

2. TSC (tuberlin/tuberous sclerosis complex)

Mammalian target of rapamycin (mTOR) complex 1 (mTORC1) is negatively controlled by a heterodimeric complex consisting of two molecules: Tsc1 (*hamartin*) and Tsc2 (tuberin).

TSC1 and TSC2 are products of *TSC1* and *TSC2* genes, respectively. The *TSC1* and *TSC2* gene mutations occur in autosomal dominant tumor syndrome, tuberous sclerosis [23]. Tsc 1 is considered to be important for maintenance of the dormancy of primordial follicles. In mice with Tsc1 deleted in oocytes (OoTsc1^{-/-} mice), primordial follicles were prematurely activated, which also occurs in OoTsc2^{-/-} mice [24]. These results imply that Tsc1/Tsc2 complex plays a role in regulating the quiescence and activation of follicles. In Tsc1 or Tsc2 deletion, mTORC1 increases and activates S6K1- rpS6 signaling (p70 S6 kinase-ribosomal protein), which promotes protein translation and ribosome synthesis (Figure 1) [21]. The phenotypes of overactivation of primordial follicles in OoTsc1^{-/-} mice and OoPten^{-/-} mice are similar. Although both Tsc and PTEN regulate follicular activation by inhibiting S6K1-rpS6, Tsc inhibits phosphorylation of S6K1 at threonine 389, and PTEN inhibits the phosphorylation at threonine 229 [21]. Therefore, for the maintenance of the dormancy of primordial follicles, S6K1

should be suppressed by Tsc and PTEN, respectively.

3. Foxo3a

Foxo3a is expressed in the nucleus of oocytes of primordial follicles, and its expression decreases in primary or growing follicles. The decrease of *Foxo3a* initiates the activation of follicles and growth of oocytes. In *Foxo3a*-deleted mice (*Foxo3a*^{-/-}), follicles are depleted, which results in infertility, indicating that *Foxo3a* is important for maintaining the quiescence of primordial follicles [4]. *Foxo3a*^{-/-} mice show a phenotype similar to that of *Pten*^{-/-} mice. In the *Pten* deleted mice, constant Akt activation causes the suppression of *Foxo3a*.

Therefore, both PTEN and *Foxo3a* play important roles in maintaining the dormancy of primordial follicles and PTEN acts upstream of *Foxo3a* [6]. Akt inactivates *Foxo3a* through an extracellular signaling pathway such as insulin signaling, so the effect of insulin for follicular activation is by the inhibition of FOXO3 [25,26].

The signals that maintain the survival and activation of primordial follicles

1. PI3K signaling

PI3K activation is maintained by extracellular signals, and it maintains the survival of primordial follicles [27]. The survival of primordial follicles is maintained through the PI3K pathway [28]. The communications between oocytes and the surrounding granulosa cells depend on their interactive signaling, and the main ligand-receptor is the receptor protein tyrosine kinase (RPTK) Kit and its ligand, Kit ligand (KL) [29]. Kit exists on the surface of oocytes, and KL is produced in the granulosa cells. In early stage oocytes and follicles in which FSH receptors are not expressed yet, follicular growth is dependent on Kit-KL signaling [30-32].

PI3K signaling is one of the important pathways activated by Kit-KL [33]. KL activates the PI3K pathway in primary oocytes, followed by phosphorylation of Akt (protein kinase B) and *Foxo3a*. In mammals, the PI3K pathway is activated by insulin and growth factors and it regulates cell proliferation and survival [33]. PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3), and PIP3 acts as a secondary messenger and provides the docking sites for 3-phosphoinositide dependent kinase-1 (PDK1) and Akt. Akt enhances cell survival, growth, and oocyte activation. Otherwise, PTEN inhibits PI3K by converting PIP3 to PIP2. The FOXO family is a main target of Akt and it suppresses the activation of primordial follicles and maintains dormancy by promoting pro-apoptotic genes (Figure 1) [27]. PI3K signaling is mediated by PDK1, and PDK1 activates Akt signaling. PDK1 phosphorylates Akt at its threonine residue 308, and also phosphorylates S6K1, the kinase of rpS6 [34]. In mice with an oocyte-specific *Pdk1* deletion, most of

the primordial follicles are depleted directly from the dormant state and cause premature ovarian failure. Therefore, it is suggested that the suppression of PDK1-Akt-S6K1-rpS6 signaling leads to the depletion of primordial follicles [35]. In double knock-out mice with deletion of both *Pten* and *Pdk1*, the premature activation does not occur, suggesting that the premature activation of follicles in *Pten* deletion is mediated by PDK1 [36]. PI3K-PDK1 signaling plays an important role in the survival and activation of primordial follicles.

2. mTOR

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase, and regulates protein synthesis and cell growth. mTOR exists in the two forms of mTORC1 and mTORC2. S6K1 is the main substrate of mTORC1 and is phosphorylated by mTORC1 [37]. In *Tsc1*-deleted (*OoTsc1^{-/-}*) mice treated with mTORC1 inhibitor, the overactivation of primordial follicles is suppressed, which implies that mTORC1 is involved in the activation of primordial follicles. These results suggest that the mTORC1-S6K1-rpS6 cascade is also important in the survival of primordial follicles [21]. mTOR deletion is expected to accelerate the loss of primordial follicles [21].

In vitro activation of ovarian follicles

In a mouse model, *Pten* deletion in granulosa cells reduced apoptosis and increased cellular proliferation but not tumors [38]. These results suggest that PTEN inhibitor can activate the primordial follicles. Recently, Li et al. [39] reported that short-term treatment of mouse ovaries with PTEN inhibitor and PI3K-activating peptide increased the activation of primordial follicles with increased nuclear extrusion of Foxo3. The size of the ovarian tissue and the number of follicles increased in PTEN inhibitor-treated ovaries. In addition, in the human ovarian cortical tissues cultured with PTEN inhibitor and PI3K activator, primordial follicles developed to the preovulatory stage, after xeno-transplantation. Thus follicular activation with PTEN inhibitor can be applied to generate more germ cells from cyro-preserved ovarian tissue for fertility preservation in cancer patients.

Conclusion

Primordial follicle activation is regulated by close interactive communication with somatic cells and oocytes. The dormancy of primordial follicles is maintained by multiple factors such as PTEN, *Tsc*, *Foxo3a*, *Foxl2*, and AMH. With the deletion of these factors, follicles are activated prematurely leading to early depletion of the primordial follicles. To maintain the survival and activation, PI3K signaling mediated by PDK1 and mTORC1-S6K1-rpS6 are involved. With this knowledge on the regulatory mechanism for dormancy, survival, and activation

of primordial follicles, possible clinical applications could include *in vitro* maturation of immature oocytes or *in vitro* activation of follicles by PTEN inhibitor in cryopreserved ovarian cortical tissues for fertility preservation in cancer patients or diminished ovarian reserve.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

References

1. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocr Rev* 2000;21:200-14.
2. Elvin JA, Matzuk MM. Mouse models of ovarian failure. *Rev Reprod* 1998;3:183-95.
3. Eppig JJ, Wigglesworth K, Pendola FL. The mammalian oocyte orchestrates the rate of ovarian follicular development. *Proc Natl Acad Sci U S A* 2002;99:2890-4.
4. Castrillon DH, Miao L, Kollipara R, Horner JW, DePinho RA. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* 2003;301:215-8.
5. John GB, Gallardo TD, Shirley LJ, Castrillon DH. Foxo3 is a PI3K-dependent molecular switch controlling the initiation of oocyte growth. *Dev Biol* 2008;321:197-204.
6. Reddy P, Liu L, Adhikari D, Jagarlamudi K, Rajareddy S, Shen Y, et al. Oocyte-specific deletion of *Pten* causes premature activation of the primordial follicle pool. *Science* 2008;319:611-3.
7. Wandji SA, Srsen V, Voss AK, Eppig JJ, Fortune JE. Initiation in vitro of growth of bovine primordial follicles. *Biol Reprod* 1996;55:942-8.
8. Wandji SA, Srsen V, Nathanielsz PW, Eppig JJ, Fortune JE. Initiation of growth of baboon primordial follicles in vitro. *Hum Reprod* 1997;12:1993-2001.
9. Dissen GA, Romero C, Paredes A, Ojeda SR. Neurotrophic control of ovarian development. *Microsc Res Tech* 2002;59:509-15.
10. Spears N, Molinek MD, Robinson LL, Fulton N, Cameron H, Shimoda K, et al. The role of neurotrophin receptors in female germ-cell survival in mouse and human. *Development* 2003;130:5481-91.
11. Hreinnsson JG, Scott JE, Rasmussen C, Swahn ML, Hsueh AJ, Hovatta O. Growth differentiation factor-9 promotes the growth, development, and survival of human ovarian follicles in organ culture. *J Clin Endocrinol Metab* 2002;87:316-21.
12. Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 1996;383:531-5.
13. Kaivo-oja N, Jeffery LA, Ritvos O, Mottershead DG. Smad signal-

- ling in the ovary. *Reprod Biol Endocrinol* 2006;4:21.
14. De Baere E, Beysen D, Oley C, Lorenz B, Cocquet J, De Sutter P, et al. FOXL2 and BPES: mutational hotspots, phenotypic variability, and revision of the genotype-phenotype correlation. *Am J Hum Genet* 2003;72:478-87.
 15. Schmidt D, Ovitt CE, Anlag K, Fehsenfeld S, Gredsted L, Treier AC, et al. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. *Development* 2004;131:933-42.
 16. Pangas SA, Choi Y, Ballow DJ, Zhao Y, Westphal H, Matzuk MM, et al. Oogenesis requires germ cell-specific transcriptional regulators Sohlh1 and Lhx8. *Proc Natl Acad Sci U S A* 2006;103:8090-5.
 17. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegeod JA, et al. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. *Endocrinology* 1999;140:5789-96.
 18. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-Mullerian hormone. *Reproduction* 2002;124:601-9.
 19. Carlsson IB, Scott JE, Visser JA, Ritvos O, Themmen AP, Hovatta O. Anti-Mullerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro. *Hum Reprod* 2006;21:2223-7.
 20. Lan ZJ, Xu X, Cooney AJ. Differential oocyte-specific expression of Cre recombinase activity in GDF-9-iCre, Zp3cre, and Msx2Cre transgenic mice. *Biol Reprod* 2004;71:1469-74.
 21. Adhikari D, Zheng W, Shen Y, Gorre N, Hamalainen T, Cooney AJ, et al. Tsc/mTORC1 signaling in oocytes governs the quiescence and activation of primordial follicles. *Hum Mol Genet* 2010;19:397-410.
 22. Reddy P, Zheng W, Liu K. Mechanisms maintaining the dormancy and survival of mammalian primordial follicles. *Trends Endocrinol Metab* 2010;21:96-103.
 23. Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. *N Engl J Med* 2006;355:1345-56.
 24. Adhikari D, Flohr G, Gorre N, Shen Y, Yang H, Lundin E, et al. Disruption of Tsc2 in oocytes leads to overactivation of the entire pool of primordial follicles. *Mol Hum Reprod* 2009;15:765-70.
 25. Junger MA, Rintelen F, Stocker H, Wasserman JD, Vegh M, Radimerski T, et al. The *Drosophila* forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. *J Biol* 2003;2:20.
 26. Kezele PR, Nilsson EE, Skinner MK. Insulin but not insulin-like growth factor-1 promotes the primordial to primary follicle transition. *Mol Cell Endocrinol* 2002;192:37-43.
 27. Reddy P, Shen L, Ren C, Boman K, Lundin E, Ottander U, et al. Activation of Akt (PKB) and suppression of FKHL1 in mouse and rat oocytes by stem cell factor during follicular activation and development. *Dev Biol* 2005;281:160-70.
 28. Liu K, Rajareddy S, Liu L, Jagarlamudi K, Boman K, Selstam G, et al. Control of mammalian oocyte growth and early follicular development by the oocyte PI3 kinase pathway: new roles for an old timer. *Dev Biol* 2006;299:1-11.
 29. Driancourt MA, Reynaud K, Cortvrindt R, Smits J. Roles of KIT and KIT LIGAND in ovarian function. *Rev Reprod* 2000;5:143-52.
 30. Albertini DF, Barrett SL. Oocyte-somatic cell communication. *Reprod Suppl* 2003;61:49-54.
 31. Packer AI, Hsu YC, Besmer P, Bachvarova RF. The ligand of the c-kit receptor promotes oocyte growth. *Dev Biol* 1994;161:194-205.
 32. Parrott JA, Skinner MK. Kit-ligand/stem cell factor induces primordial follicle development and initiates folliculogenesis. *Endocrinology* 1999;140:4262-71.
 33. Blume-Jensen P, Jiang G, Hyman R, Lee KF, O'Gorman S, Hunter T. Kit/stem cell factor receptor-induced activation of phosphatidylinositol 3'-kinase is essential for male fertility. *Nat Genet* 2000;24:157-62.
 34. Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002;296:1655-7.
 35. Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J* 2000;346 Pt 3:561-76.
 36. Reddy P, Adhikari D, Zheng W, Liang S, Hamalainen T, Tohonen V, et al. PDK1 signaling in oocytes controls reproductive aging and lifespan by manipulating the survival of primordial follicles. *Hum Mol Genet* 2009;18:2813-24.
 37. Wullschlegel S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006;124:471-84.
 38. Fan HY, Liu Z, Cahill N, Richards JS. Targeted disruption of Pten in ovarian granulosa cells enhances ovulation and extends the life span of luteal cells. *Mol Endocrinol* 2008;22:2128-40.
 39. Li J, Kawamura K, Cheng Y, Liu S, Klein C, Duan EK, et al. Activation of dormant ovarian follicles to generate mature eggs. *Proc Natl Acad Sci U S A* 2010;107:10280-4.