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ORIGINAL ARTICLE

Expression and regulation of FAAP in the mouse epididymis

Nai-Zheng Ding · Mei He · Cheng-Qiang He · Jin-Song Hu · Junlin Teng · Jianguo Chen

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Abstract The focal adhesion-associated protein (FAAP), encoded by the murine D10Wsu52e gene, is named as involved in modulating cell adhesion dynamics. It is a highly conserved and ubiquitously expressed protein, and its human homologue HSPC117 has been identified in many protein complexes. However, the expression and regulation of the FAAP gene have not yet been well characterized. Herein, we demonstrate that FAAP mRNA and protein expression are highly regionalized in the mouse epididymis with predominant enrichment in the initial segment. During sexual maturation, FAAP mRNA is always expressed in the caput epididymides. Castration causes rapid and significant decrease of FAAP mRNA abundance within the initial segment, whereas testosterone replacement fails to reverse the regression. Unilateral orchidectomy and efferent duct ligation studies further validate that expression of the FAAP mRNA is highly dependent on the presence of luminal testicular factors rather than testosterone. Furthermore, FAAP expression in the initial segment is not affected by cryptorchism.

Keywords FAAP · Initial segment · Epididymis

Introduction

The epididymis is the crucial link of the mammalian male reproductive tract. Only after transported through the long convoluted epididymal tubule can spermatozoa acquire their functional maturation to be motile and fertile. In general, the epididymis is anatomically and functionally divided into four major regions: initial segment (IS), caput, corpus, and cauda [1, 2]. Each region is also made up of intraregional segments separated by connective tissue septa and has segment-specific gene and protein expression profile that results in unique luminal microenvironment progressively contributing to sperm maturation, protection, and final caudal storage [3–5].

The initial segment, the most proximal region where the extratesticular maturation process commences, is more active in protein secretion than other epididymal regions [6]. It is of great importance in functional modification of spermatozoa as exemplified by the sterile c-ros knockout mice which just lack the IS without affecting the remainder of the male reproductive system [7]. Numerous genes with diverse functions, including enzymes, transporters, receptors, and signal transducers, have been identified to exhibit highly restricted patterns of expression within the segment [1, 8]. Besides those specific to the epididymis, some have broader tissue distributions, such as the transcription factor Etv5 [9].

Expressions of most epididymal genes are under the control of androgens, as evidenced by orchidectomy and testosterone replacement [10]. However, many genes

Authors Nai-Zheng Ding and Mei He contributed equally to this study.

N.-Z. Ding · M. He · J.-S. Hu · J. Teng · J. Chen (✉)
The Key Laboratory of Cell Proliferation and Differentiation of Ministry of Education and The State Key Laboratory of Bio-membrane and Membrane Bio-engineering, College of Life Sciences, Peking University, Beijing 100871, China
e-mail: chenjg@pku.edu.cn

N.-Z. Ding · C.-Q. He
School of Biological Science and Technology, Central South University, Changsha 410013, China

C.-Q. He
College of Life Science, Shandong Normal University, Jinan 250014, China

expressed within the IS, including Etv5, are also dependent on unknown testicular factors that reach the epididymis directly via the efferent duct, referred to as luminal testicular factors (LTFs) [11, 12]. These may include growth factors (e.g. basic fibroblast growth factor [13]) and sperm associated factors [14]. Efferent duct ligation (EDL) establishes the necessity of LTFs in maintaining proper IS structure and function [10].

The focal adhesion associated protein (FAAP), product of the murine *D10Wsu52e* gene, is a highly conserved and ubiquitously expressed protein of 505 amino acids. It is named as involved in modulating cell adhesion dynamics through interaction with vinculin and talin [15]. The FAAP has more than 99% sequence identity to human homologue HSPC117, which has been identified in many protein complexes, such as the spreading initiation center in early stages of cell spreading [16], a large RNA-transporting granule in neuronal dendrites [17], and a protein complex that binds to the AU-rich element of TNF-α mRNA [18]. Recently, it has also been reported that HSPC117 deficiency in cloned embryos causes placental abnormality and fetal death [19].

However, the expression and regulation of the FAAP gene have not yet been well characterized. Studying the expression pattern of the FAAP gene, we detect that both FAAP mRNA and protein are highly expressed in the IS of mouse epididymis. Therefore, we perform further experiments to examine its regulation in the tissue, and find that such regionalized enrichment of FAAP is dependent on LTFs.

Materials and methods

Experimental animals

Mature male BAL/BC mice (about 35 g body weights) were housed under a constant 14 h light:10 h dark cycle and were allowed free access to food and water. Orchiectomy, EDL, and cryptorchism were carried out under anesthesia. In unilateral orchiectomy and EDL, a mimic operation was performed on the contralateral side within the same animal for control. Androgen replacement began on the next day after castration, and mice were daily injected with 0.1 mg/mouse testosterone propionate (TP) (Sigma) in sesame oil [20]. Epididymides were then collected for in situ hybridization, respectively. Each experiment was performed with three mice. All animal procedures were approved by the Institutional Animal Care and Use Committee of Peking University.

Sexual maturation

The male mice were killed by cervical dislocation on Postnatal Days 1, 5, 10, 15, 20, and 30. The caput epididymides including the initial segments were collected for RNA extraction.

In situ hybridization

The 1518-bp full-length encoding sequence of the FAAP gene was used as a probe. In situ hybridization was performed as described previously [15].

Isolation of total RNA from tissues

Isolation of total RNA was performed with Trizol reagent according to the manufacturer's instructions (Invitrogen). Genomic DNA contamination was removed by digestion of RNA samples with RNase-free DNase I (Promega).

RT-PCR and semi-quantitative PCR

1 μg of total RNA from each epididymal region was reverse transcribed with SuperScript III Reverse Transcriptase according to the manufacturer's instructions (Invitrogen). All RT-PCR reactions were carried out only after DNA contamination was ensured to be eliminated in all controls as performed previously [21]. In Semi-quantitative PCR, products of three sequential optimized cycles were used for final analysis. The relative concentrations of FAAP and GAPDH PCR products were determined by densitometric measurement with a UVP laboratory imaging and analysis system. Three independent experiments were carried out for statistical analysis. Statistical analysis was performed using one-way ANOVA to compare the expression levels of the FAAP gene. Mouse FAAP primers were 5′-GTATGCCGCCAAGAAGAT (799-816) and 5′-TCAGTGCCAGTCAGAACG (1326–1309) for a 428-bp fragment (Genebank accession number AY424364). Mouse GADPH primers were 5′-ACCACAGTCCATGCCATCAC (566–585) and 5′-TCCACCACCCTGTTGCTGTA (1017–998) for a 452-bp fragment (Genebank accession number M32599) [22].

Western blotting

Each region of the mouse epididymis was homogenized in ice-cold TNE buffer containing 1× Complete Mini, EDTA-Free Protease Inhibitor Cocktail (Roche). Insoluble material was removed by centrifugation at 15,000×*g* for 10 min. Samples were separated by SDS-PAGE and then transferred to nitrocellulose membranes (Millipore). Membranes were incubated with anti-HSPC117 (kindly provided by Prof. Hirokawa) and anti-actin polyclonal antibodies, followed with secondary goat anti-rabbit IgG conjugated with alkaline phosphatase. Immunoreactive bands were detected by BCIP/NBT. Endogenous alkaline

phosphatase activity was inhibited with 2 mM levamisole (Sigma).

Results

FAAP mRNA and protein are highly expressed in proximal mouse epididymis

FAAP mRNA expression in adult mouse epididymis was localized by in situ hybridization. As shown in Fig. 1a, FAAP mRNA was highly regionalized within the tissue and was predominantly expressed in the IS with decreasing amount in the proximal caput and undetectable levels in the distal epididymal regions, including whole corpus and cauda. No hybridization signals were observed in the epididymis with the sense probe (Fig. 1B-a). Higher magnification of the IS indicated that FAAP mRNA was concentrated on the basal side of the luminal epithelial cells, most likely the principal cells as was the major cell type present, but the possible expression in the other cell types could not be excluded (Fig. 1B-d).

The region-specific distribution of FAAP mRNA was confirmed by semi-quantitative PCR (Fig. 2a). FAAP mRNA could be detected in all four epididymal regions. However, the relative mRNA abundance in the IS was the highest among the regions. In addition, western blotting analyses were performed to determine the expression pattern of FAAP protein within the epididymis. As could be seen from Fig. 2b, FAAP protein was expressed in similar manner as its mRNA, comparing to the internal control actin.

Expression of FAAP mRNA in the caput during mouse sexual maturation

In order to examine the expression of FAAP mRNA in the caput epididymis during sexual maturation, caput regions from the mice of 1, 5, 10, 20, and 30 days of age were isolated for RNA extraction and submitted to RT-PCR. FAAP cDNA were observed in all samples (Fig. 2c), that is, the FAAP gene was expressed all through the sexual maturation process. According to the results from semi-quantitative PCR, although mRNA of FAAP was higher on day 10 and 15 than on the other days, the difference was small.

Effects of castration and testosterone supplementation on FAAP mRNA expression in the initial segment

After castration, most of epididymides were found not to be necrosis and undergo only the change of morphology after day 2 of surgery. Any mice with necrotic epididymis due to surgery were given up in this study. Castration and testosterone supplementation were carried out to test if enrichment of FAAP mRNA in the IS was regulated by androgens. Castrated mice were treated with either sesame oil or TP for 1, 3, and 7 days beginning on the next day after surgery. After castration, there were no positive signals in the IS. However, neither vehicle nor TP was functional in reversing the regression even been supplied for a week (Fig. 3a). The results indicated that serum testosterone was not responsible for the regionalized expression of FAAP in the epididymis, as confirmed by unilateral orchiectomy which caused severe regression of FAAP

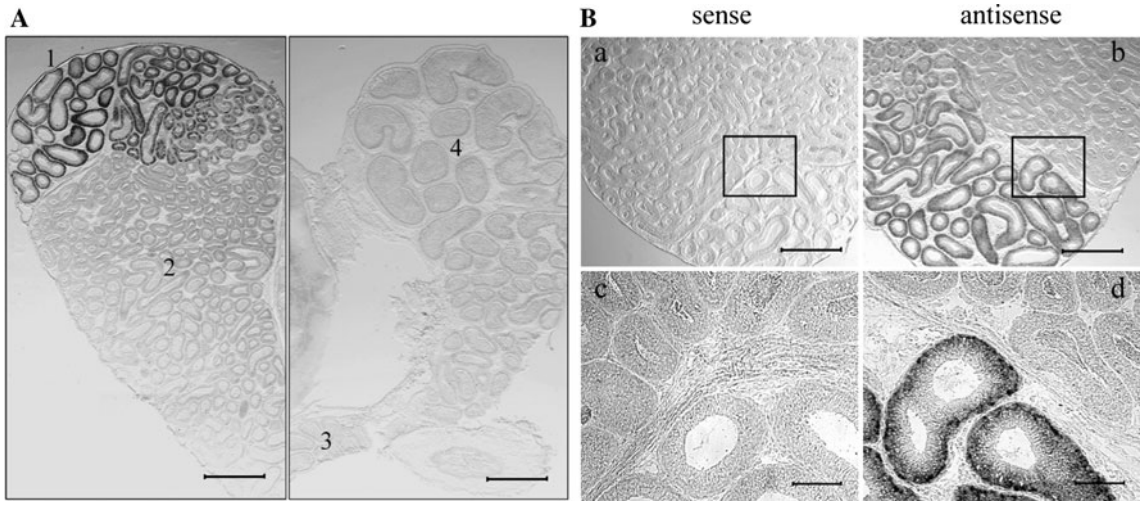

Fig. 1 In situ hybridization of FAAP mRNA in adult mouse epididymis. **A** A longitudinal section of the mouse epididymis was hybridized with a DIG-labeled antisense riboprobe to the FAAP cDNA. *1* initial segment, *2* caput, *3* corpus, *4* cauda. Bar = 0.5 mm. **B**. Sections of the initial segment and proximal caput were hybridized with FAAP sense *a* and antisense, *b* RNA probes. Higher magnifications of the framed regions in *a* and *b* (Bar = 0.5 mm) are presented in *c* and *d* (Bar = 0.1 mm), respectively. Signals representing FAAP mRNA appear as dark under bright field illumination

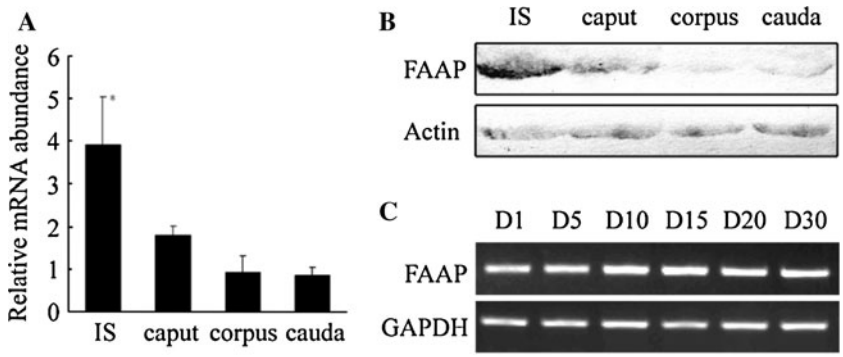

Fig. 2 **a** Relative abundance of FAAP mRNA in the four regions of the mouse epididymis normalized with internal control GAPDH (Mean ± SEM; *, $P < 0.05$, $n = 3$). **b** Western blotting analysis of FAAP from the four regions of the mouse epididymis. The protein was probed with an anti-HSPC117 polyclonal antibody and actin is detected as internal reference. **c** Expression of FAAP mRNA in the caput including initial segment during sexual maturation. FAAP cDNA were detected with GAPDH as internal control (*D* day after birth)

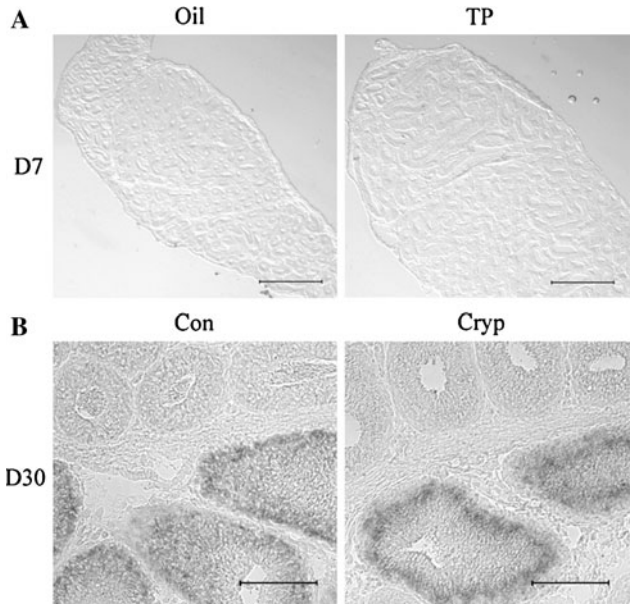

Fig. 3 **a** Hormonal regulation of FAAP mRNA expression in the castrated mice treated with sesame oil and testosterone propionate (TP), respectively. No signals are observed in both treatments seven days after injection (D7). *Bar* = 0.5 mm. **b** In situ hybridization of FAAP mRNA in the epididymis from cryptorchid mouse. Signals are observed in both control and cryptorchid epididymides (*D* day after surgery). *Bar* = 0.1 mm

mRNA expression in the same lateral IS, despite that the contralateral testicle was functional (Fig. 4a).

Effect of EDL on FAAP mRNA expression in the initial segment

In order to further validate that it was LTFs that might contribute to the regulation of FAAP expression in the IS, as in the cases of many other genes, unilateral efferent duct ligation was then used to prevent testicular fluid from flowing into the epididymis at the same side. One day after operation, FAAP mRNA levels in the IS of ligated epididymis were already significantly declined, similar to that in the unilateral orchiectomy (Fig. 4b).

Effect of cryptorchism on FAAP mRNA expression in the initial segment

Furthermore, to test whether the expression of FAAP mRNA in the epididymis was affected by sperm-associated factor(s) and potential component of LTFs, cryptorchism was done to matured mice for a month. It came out that there was no visible difference in hybridization signal between the IS from cryptorchid mouse and the normal one (Fig. 3b).

Discussion

FAAP mRNA is widely expressed in many mouse tissues, demonstrated by Northern blotting (data not shown), and the UniGene and SOURCE databases. However, in the present study, we show that both FAAP mRNA and protein expression are highly regionalized within the epididymis. The expression level is highest in the IS, but is rapidly decreased in the proximal caput and low in the distal epididymal regions.

Androgens are the main factors that regulate development of epididymis and maintenance of the adult structure and function. We then check hormonal regulation of FAAP expression in the tissue. FAAP mRNA abundance in the IS is drastically declined to undetectable level 1 day after castration. However, the hybridization signals can still not be observed after 7 days of TP administration, suggesting that the FAAP gene is not directly controlled by circulating testosterone. Since the IS, where the testicular fluid first

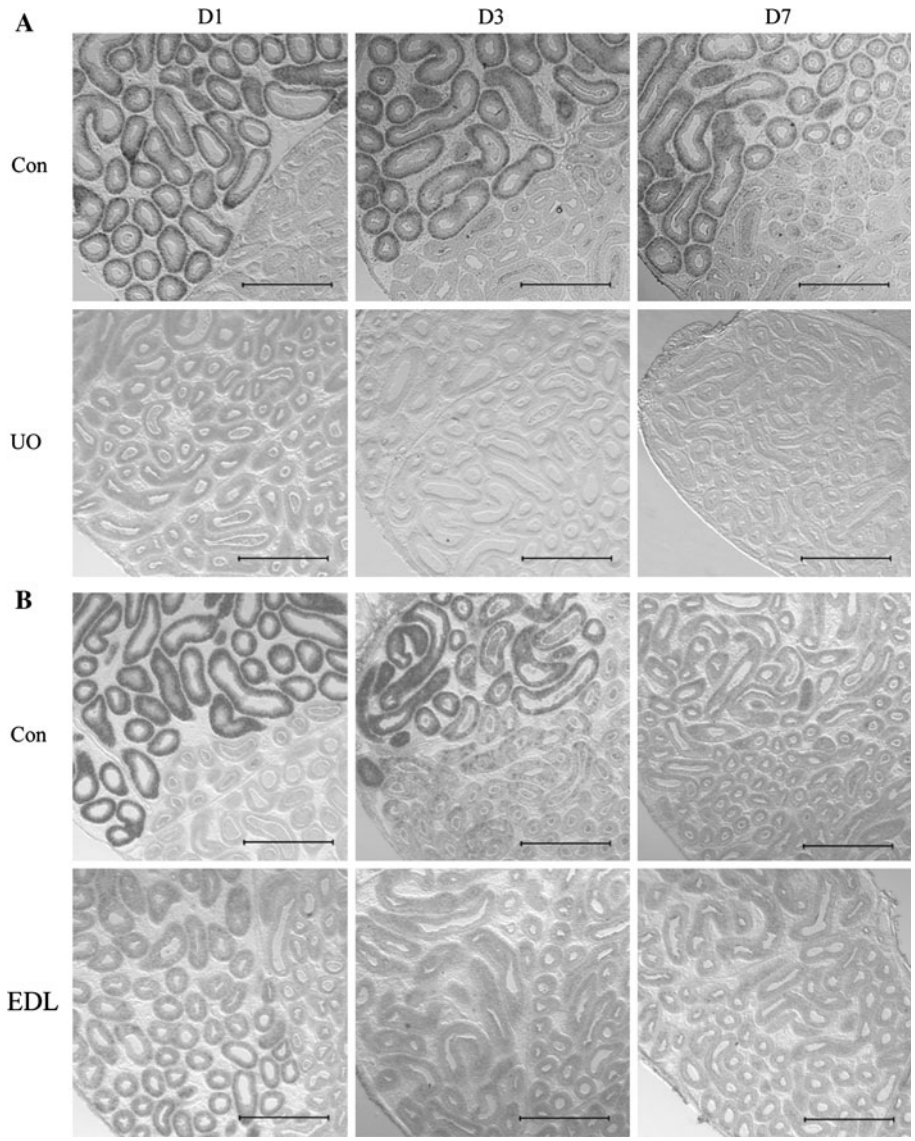

Fig. 4 In situ hybridization of FAAP mRNA to epididymides after unilateral orchiectomy (UO) (**a**) and EDL (**b**). Hybridization signals disappear after both surgeries (*D* day after surgery). *Bar* = 0.5 mm

enters into the epididymal lumen, is particularly sensitive to unknown factors presenting in the fluid, it may be LTFs that are critical for FAAP gene expression. The hypothesis is supported by further surgical studies as unilateral castration and EDL. In both cases, despite the circulating androgen is functional, FAAP mRNA levels are significantly reduced because the epididymis is no longer connected to the testis, producer of LTFs.

Indeed, previous to our observations, there have been many studies postulating that LTFs, in combination with androgens, stipulate the epithelial morphology and gene expression in the IS as well as the proximal caput. The severe atrophy, which the castrated epididymis suffers from, can be sufficiently prevented by testosterone replacement in the caput, corpus, and cauda regions except the IS [10]. Furthermore, several genes that have similar distributions as FAAP mRNA in the epididymis, such as Cres [23], B-myc [24], Etv4 family members [9], and BCL2 family kin (Bfk)[25], have been reported to require LTFs exclusively or together with androgens for their normal expression. Herein, FAAP falls into the former category.

A variety of soluble molecules contained in the testicular fluid, such as luminal androgens, growth factors [13], and sperm-associated factors [14], may be involved in modulating epididymal gene expression. Cryptorchism forces the testis into the abdominal cavity of higher temperature and thus causes sperm loss in the operated epididymis. However, it seemed that FAAP mRNA expression in the IS is unaffected, i.e., FAAP expression may not be regulated by sperm-associated factors or affected by temperature increase that has deleterious effects on male reproduction.

During sexual maturation of mice, FAAP mRNA is always expressed in the caput epididymides, suggesting

that it may play important roles in male reproduction. Structural integrity of the specialized tubular tissue and formation of the blood-epididymal barrier involves the interactions of cell adhesion proteins [26]. FAAP may participate in the processes through modulating the interaction between paxillin and focal adhesion kinase (FAK) [15]. Likewise, it may interfere with the receptor tyrosine kinase (RTK) signal pathway stimulated by growth factors through affecting phosphorylation of extracellular signal-regulated kinases (ERKs) [15]. In addition, protein synthesis is active in the polar IS principal cells, and FAAP may be involved in RNA manipulation as its homology HSPC117 has been identified in some RNA–protein complexes [17, 18]. Indeed, further studies are required to ultimately determine the roles of FAAP in the epididymis.

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References

1. B. Robaire, N.A. Henderson, Mol. Cell. Endocrinol. **250**, 190–195 (2006)
2. B,K.C. Reid, Aust J Zool **5**, 223–246 (1957)
3. A. Abu-Haila, M.A. Fain-Maurel, Anat. Rec. **209**, 197–208 (1984)
4. C. Kirchhoff, Int. Rev. Cytol. **188**, 133–202 (1999)
5. T.T. Turner, D. Bomgardner, Mol. Reprod. Dev. **61**, 437–444 (2002)
6. R.C. Jones, Int. J. Androl. **22**, 57–67 (1999)
7. E. Sonnenberg-Riethmacher, B. Walter, D. Riethmacher, S. Godecke, C. Birchmeier, Genes Dev. **10**, 1184–1193 (1996)
8. N. Hsia, G.A. Cornwall, Biol. Rep. **70**, 448–457 (2004)
9. L. Yang, S.A. Fox, J.L. Kirby, B.V. Troan, B.T. Hinton, Biol. Rep. **74**, 714–720 (2006)
10. N. Ezer, B. Robaire, Endocrinology **144**, 975–988 (2003)
11. J.L. Kirby, L. Yang, J.C. Labus, R.J. Lye, N. Hsia, R. Day, G.A. Cornwall, B.T. Hinton, Biol. Rep. **71**, 613–619 (2004)
12. B.T. Hinton, T.T. Turner, News Physiol. Sci. **3**, 28–31 (1988)
13. Z.J. Lan, J.C. Labus, B.T. Hinton, Biol. Rep. **58**, 197–206 (1998)
14. S. FouchéCourt, J. Lareyre, P. Chaurand, B.B. Dague, K. Suzuki, D.E. Ong, G.E. Olson, R.J. Matusik, R.M. Caprioli, M. Orgebin-Crist, Endocrinology **144**, 887–900 (2003)
15. J. Hu, J. Teng, N. Ding, M. He, Y. Sun, A.C. Yu, J. Chen, Front. Biosci. **13**, 7123–7131 (2008)
16. C.L. de Hoog, L.J. Foster, M. Mann, Cell **117**, 649–662 (2004)
17. Y. Kanai, N. Dohmae, N. Hirokawa, Neuron **43**, 513–525 (2004)
18. S. Rousseau, N. Morrice, M. Peggie, D.G. Campbell, M. Gaestel, P. Cohen, EMBO J. **21**, 6505–6514 (2002)
19. Y. Wang, T. Hai, Z. Liu, S. Zhou, Z. Lv, C. Ding, L. Liu, Y. Niu, X. Zhao, M. Tong, L. Wang, A. Jouneau, X. Zhang, W. Ji, Q. Zhou, Biochem. Biophys. Res. Commun. (2010). doi:10.1016/j.bbrc.2010.1005.1105
20. D. Rengaraj, F. Gao, X.H. Liang, Z.M. Yang, Endocrine **31**, 193–201 (2007)
21. N.Z. Ding, C.Q. He, Z.M. Yang, Zygote **10**, 239–243 (2002)
22. S.S. Sidhu, S.J. Kimber, Biol. Reprod. **60**, 147–157 (1999)
23. G.A. Cornwall, M.C. Orgebin-Crist, S.R. Hann, Mol. Endocrinol. **6**, 1653–1664 (1992)
24. G.A. Cornwall, R. Collis, Q. Xiao, N. Hsia, S.R. Hann, Biol. Rep. **64**, 1600–1607 (2001)
25. D.A. Pujianto, A.E. Damdimopoulos, P. Sipilä, J. Jalkanen, I. Huhtaniemi, M. Poutanen, Endocrinology **148**, 3196–3204 (2007)
26. D.G. Cyr, B. Robaire, L. Hermo, Microsc. Res. Tech. **30**, 54–66 (2005)