Regulation of a promoter from the mouse insulin like growth factor II gene by glucocorticoids

Ghislane Dell[a], Andrew Ward[a,b], Wilhelm Engström[b,c,*]

[a]*Developmental Biology Programme, School of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK*
[b]*Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*
[c]*Centre for Reproductive Biology and Department of Pathology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden*

Received 7 November 1997

Abstract We have microinjected constructs containing the murine IG II P3 promoter linked to different flanking sequences and a luciferase reporter gene into mouse pronuclei to establish transgenic lines of mice. The offspring was used as a source of embryonic fibroblast cultures and the effect of exogenous addition of glucocorticoids on transgene expression was analysed. It was found that both dexamethasone and hydrocortisone gave rise to a functional stimulation of the IGF II P3 promoter when the construct also contained other elements. This study demonstrates for the first time that there is an effect of glucocorticoids on the activation of an embryonic IGF II promoter, thus providing a molecular rationale for previous findings that glucocorticoids can under certain circumstances give rise to an increased transcriptional activity of the IGF II gene.

Key words: Insulin-like growth factor II; Glucocorticoid; Promoter

1. Introduction

The insulin like growth factor II is one of the key growth regulatory peptide factors in mammalian embryogenesis [1]. Its expression is regulated at many levels in all vertebrates examined, including man [2]. For instance, gene imprinting usually inhibits transcription from the allele inherited from the mother [3], translational control discriminates between mRNA with different leader exons [4–6] and the IGF II/Mannose-6-phosphate receptor acts as a scavenger for circulating IGF II [7]. IGF II levels in tissues and plasma are also influenced by other hormones, by tissue type, by stage of development and when neoplasia develops [8,9]. It has previously been suggested that IGF II transcription is under the control of glucocorticoids. Whereas some studies suggest that the IGF II expression is down-regulated by glucocorticoids [10–12], others suggest the contrary [13,14]. In all IGF II genes hitherto studied, existing data suggest the presence of different promoters. Moreover in higher mammals, the IGF II promoters appear to fall into two categories, adult and embryonic [15–18].

The transcription of the IGF II gene is driven from three distinct promoters in the mouse. All three promoters are exclusively active in embryonic life but their activities differ with respect to stage and tissue. In this study we have focused on the IGF II P3 promoter which is a major contributor of IGF II transcripts in the mouse embryo [4]. In particular we aimed to find out if this promoter could be specifically activated by addition of glucocorticoids. This was studied by pronuclear injection transgenesis which was further developed into an in vitro system which provided us with a possibility to evaluate stably integrated constructs in reporter gene assays.

It has previously been shown that the P3 promoter sequence alone linked to a reporter gene was relatively poorly active in transgenic mice. However when the P3 promoter was coupled with other enhancers from the IGF II/H19 locus it expressed at considerably higher levels [19]. In this study we therefore selected four different cis-acting elements for our construct: the H19 promoter, the H19 enhancer, the DMR1 (5′ differential methylated region) and the intergenic region (central conserved region, CCD). It has been shown that deletion of H19 enhancer severely impaired the expression of both the H19 gene and the neighbouring IGF II gene [20]. In contrast, deletion of the H19 coding region together with about 10 kb of 5′ sequence from the maternal chromosome resulted in activation of the normally silent maternal IGF II allele [21]. These observations are consistent with a model in which the H19 promoter activity limits this enhancers activity on cis IGF II promoters [22].

The DMR1 was chosen because there is indirect evidence that it might control IGF II gene activity. DMR1 lies about 2 kb 5′ to the transcriptional start site of exon 1 of the IGF II gene. This region was more highly methylated when paternally inherited [23–26], and methylation was known to be needed for the onset of IGF II expression in development [27]. DMRs have also been found in all imprinted genes to date [28]. Finally, the intergenic region (CCD) which is located between the IGF II and H19 genes was chosen because it is a highly conserved sequence [29]. It is not associated with any novel transcriptional unit and it can act as a tissue specific enhancer of IGF II P3 in transgenic mice [19].

These elements were variously linked to the IGF II P3 promoter and a luciferase reporter gene. The constructs were injected into pronuclei, and the offspring used for the production of embryonic fibroblast cultures, which could be used as an assay to examine the effect of glucocorticoids on the P3 promoter activity. Four out of five constructs tested were expressed in embryonic fibroblasts and in both cases, their expression was elevated 2–3-fold by glucocorticoid treatment.

2. Material and methods

2.1. Transgenesis and breeding

Transgene constructs (Fig. 1) and derivation of transgenic mouse lines were previously described [19]. The transgenic lines used in this

*Corresponding author. Centre for Reproductive Biology and Department of Pathology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.

0014-5793/97/$17.00
PII S0014-5793(97)01434-8

study were Oat (O construct), Hamish, Harry and Holly (H construct) Elvis (E construct), Azure, Ann and Archy (A construct) and Titus (T construct) selected as representatives of several lines made using each construct [19]. Transgenes were made and maintained on an F1 (C57BL/6×CBA) genetic background and primary embryonic fibroblasts were made using embryos from mating a transgenic male with non-transgenic F1 females. Matings were timed by checking females for the presence of copulation plugs, with the day that a plug was detected assigned embryonic day E0.5.

The presence of transgenes was detected by assaying luciferase expression in yolk sacks and placentas, dissected along with embryos used to make primary fibroblast cultures. The efficacy of using transgene expression to follow transgene transmission had previously been tested extensively, and the methods used for assaying luciferase activity in homogenates of tissue samples were also detailed [19]. In the case of cultured fibroblasts, the cell monolayers were lysed directly using 0.5–1 ml 1×lysis buffer (Promega, UK), and lysates were transferred to microfuge tubes and cleared by centrifugation at 2000×*g* for 20 s. Ten ul of the supernatant was assayed directly for luciferase activity in a luminometer (Berthold model LB953 or Anthos model Lucy 1).

2.2. Primary embryo fibroblasts

On embryonic day E14.5, pregnant mice were dissected and the embryos, placentas and yolk sacks were collected. Parts of the placenta and the yolk sack were used for luciferase determination. A relative light unit (RLU) value significantly above background (buffer only and non-transgenic samples gave approximately 60 RLUs whereas transgenic placenta and yolk sack samples gave >10000 RLUs) was taken as evidence for successful transmission and expression of the transgene. All embryos were numbered, humanely killed and their bodies (following removal of the head and abdominal organs) dissected under sterile conditions in ice cold PBS into small lumps by scalpels. The lumps were then incubated in 0.25% trypsin for 5 min at room temperature. The supernatant was then transferred to a fresh tube containing equal amounts of alpha modified Eagle's medium (α-MEM) supplemented with 10% serum. The cell suspension was spun twice and resuspended in α-MEM with 10% serum and then plated onto a 90 mm Petri dish. After 24 h the medium was changed and 24–48 h later the cells were trypsinised with 0.25% trypsin and the cultures split in 1:8–1:15 depending on the initial density.

After one further round of subcultivation the cultures were exposed to 10^{-7} M hydrocortisone or 10^{-9}–10^{-6} M dexamethasone. After 24 h the cells were rinsed with ice cold PBS, scraped off the dishes and transferred into tubes for luciferase activity measurement. A small aliquot was taken for protein determination by a Biorad protein assay. All RLU values were finally corrected for any differences in soluble protein content.

In a separate set of experiments an expression vector containing the human glucocorticoid receptor gene was added (pRShGRa, [30]), to ensure a constitutive expression of the receptor in the cells. In these experiments, cells were plated and rinsed after 25 h. Fresh medium containing the plasmid was added and the cells was exposed for 48 h. After this period, the medium was changed and different concentrations of dexamethasone or hydrocortisone were added as described in Tables 1 and 2. After 24 h exposure to the glucocorticoid containing medium the cells were rinsed in cold PBS, scraped off the plates and analysed as described above.

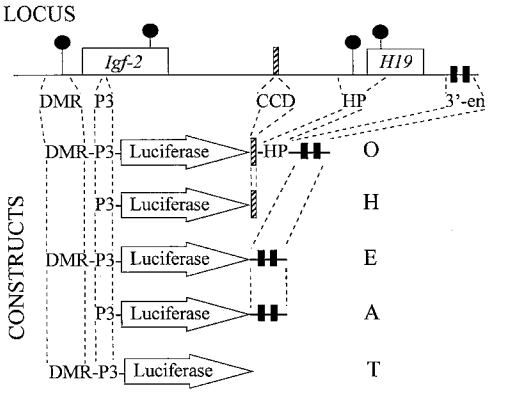

Fig. 1. The murine IGF II/H19 locus and transgene constructs (data from [21,22,25,26,28,36]). A genomic region (top, not drawn to scale) is depicted, showing the relative positions of IGF II and H19 (open boxes), the 5′ differentially methylated region (DMR), IGF II promoter 3 (P3), the central conserved domain (CCD, hatched box), and 3′ enhancer region (filled boxes). Regions containing cytosine residues that are differently methylated on the two parental alleles are marked (lollipops, note that each region contains multiple differentially methylated residues). Transgene constructs O, A, H, T and E containing a firefly luciferase reporter gene (LUC) with different combinations of sequences from the IGF/H19 locus are also shown (bottom).

2.3. Statistics

All differences between means were analysed by the Student's paired *t*-test. The level of significance was set at 0.025.

3. Results and discussion

Five different constructs were injected into pronuclei (Fig. 1). The first and largest construct (O) contained the IGF II P3 promoter coupled to a luciferase (LUC) reporter gene. These core elements were flanked by the DMR1, the intergenic region, the H19 promoter and the H19 enhancer. The second construct contained the P3 promoter LUC linked with the intergenic region only (H). The third construct consisted of the P3 promoter LUC fused with the H19 enhancer (A). The fourth construct consisted of the 5′ DMR linked to the P3 promoter LUC (T). The fifth construct contained the P3 LUC flanked by the 5′ DMR and the H19 enhancer (E). At gestational time E14.5 transgenic fetuses and their extraembryonic organs were collected. Primary embryonic fibro-

Table 1
Luciferase activity

Construct	Placenta	Yolk sack	Cells	
			P1	P10
5′ DMR P3 LUC IGR H19 promoter H19 enhancer (O)	+	+	+	+
P3 LUC IGR (H)	+	+	+	+
P3 LUC H19 enhancer (A)*	+	+	−	−
5′ DMR P3 LUC (T)	+	+	+	+
5′ DMR P3 LUC H19 enhancer (E)	+	+	+	+

The transmission of different constructs in extraembryonic organs surrounding F1 transgenic embryos as well as fibroblast cultures produced from such embryos. The activity was assayed both in primary cultures (P1) and after 10 passages in culture (P10). RLU values <100 were taken as negative.

Transgene expression was detected in 50% of all placentae and yolk sacks and in most cases fibroblasts derived from the corresponding embryos indicated by (+).

However this experiment (*) was repeated using three different transgenic lines containing the A construct. In two out of three cases the transgene did not express in the primary embryo fibroblasts.

blast cultures were established from the embryos and the luciferase activity determined in the cells as well as in samples from the corresponding placenta and yolk sack. The results are summarised in Table 1. It was found that all five constructs were transmitted to approximately 50% of the offspring from matings of transgenic males with non-transgenic females and were properly expressed in placenta and yolk sack. Moreover four of the constructs (O, H, T and E) were expressed in the fibroblast cultures whereas the P3 LUC H19 enhancer construct (A) was only expressed in one out of three lines (Archy). The expression of O, H, T and E appeared to be stable and occurred at comparable levels both in the primary cultures and after ten passages. The frequent non-expression of construct A in embryonic fibroblasts is consistent with their derivation from predominantly mesodermal cell lineages, since the H19 enhancer has previously been shown to operate mostly (but not exclusively) in endodermal tissues [20,21,31] whereas the expression of e.g. construct H is consistent with the proposed role of the CCD as an enhancer in mesodermal tissues [19]. The unexpected finding that one out of three A lines (Archy) expressed the transgene raises the possibility that it was inserted in the vicinity of an enhancer element.

This report, therefore, forms the basis for elucidating the molecular mechanism of glucocorticoid regulation of IGF II expression. Certainly, we believe that these studies should be conducted using constructs with endogenous enhancers which address the IGF II gene; firstly because of the relatively poor activity of enhancer-less constructs in transgenic mice [19] and

Table 2
The expression of transgenes in fibroblast cell cultures derived from F1 embryos

Oat (O)			
	Line 2 (+)	Line 4 (−)	Line 6 (+)
Control	38 (100)	< 0.1	48 (100)
10^{-9} M dexamethasone	133* (350)	< 0.1	64 (133)
10^{-8} M dexamethasone	144* (379)	< 0.1	179* (373)
10^{-7} M dexamethasone	202* (532)	< 0.1	74* (154)
10^{-7} M hydrocortisone	128* (337)	< 0.1	58 (121)
Harry (H)			
	Line 1 (+)	Line 2 (−)	Line 5 (+)
Control	46 (100)	< 0.1	54 (100)
10^{-9} M dexamethasone	272* (591)	< 0.1	139* (257)
10^{-8} M dexamethasone	104* (226)	< 0.1	160* (296)
10^{-7} M dexamethasone	124* (269)	< 0.1	172* (318)
10^{-7} M hydrocortisone	88* (191)	< 0.1	64 (118)
Hamish (H)			
	Line 2 (+)	Line 1 (−)	Line 3 (+)
Control	0.119 (100)	< 0.1	0.033 (100)
10^{-7} M dexamethasone	0.119 (100)	n.d.	0.025 (76)
10^{-7} M hydrocortisone	0.131 (110)	n.d.	0.040 (121)
10^{-6} M hydrocortisone	0.123 (103)	n.d.	0.046 (139)
Holly (H)			
	Line 2 (+)	Line 3 (−)	
Control	0.860 (100)	< 0.1	
10^{-7} M dexamethasone	1.625* (188)	n.d.	
10^{-7} M hydrocortisone	1.400* (162)	n.d.	
Archy (A)			
	Line 3 (+)	Line 5 (+)	
Control	1.817 (100)	1.275 (100)	
10^{-7} M dexamethasone	1.799 (99)	1.515 (119)	
10^{-7} M hydrocortisone	1.505 (83)	1.389 (109)	
10^{-6} M hydrocortisone	1.789 (98)	1.512 (119)	
Titus (T)			
	Line 3 (+)	Line 4 (−)	Line 5 (+)
Control	1.209 (100)	< 0.1	0.721 (100)
10^{-7} M dexamethasone	1.672 (138)	n.d.	1.148 (159)
10^{-7} M hydrocortisone	1.777 (147) n.d.	0.954 (132)	
Elvis (E)			
	Line 5 (+)	Line 7 (−)	Line 6 (+)
Control	2.538 (100)	< 0.1	2.191 (100)
10^{-7} M dexamethasone	5.613* (221)	n.d.	8.265* (377)
10^{-7} M hydrocortisone	4.864* (192)	n.d.	4.182* (198)

*, significant difference between means, $P < 0.025$.
n.d., not determined.
Primary cultures were established from all embryos and initially screened for transgene expression. For each of the transgenes, one or two expressing and one non-expressing line was used. The cultures were exposed to different concentrations of glucocorticoids for 24 h whereafter the cells were harvested and subjected to luciferase activity as described in Section 2.
To facilitate comparison of the data, in each case experimental values were converted to percentage of controls (figures within brackets).

Table 3
The expression of transgenes in fibroblast cell cultures derived from F1 embryos, subsequently transfected with an expression vector containing the human glucocorticoid receptor gene

Hamish (H)		
	Line 2 (+)	Line 3 (+)
Control	0.036 (100)	0.017 (100)
10^{-7} M dexamethasone	0.046 (128)	0.026 (153)
10^{-7} M hydrocortisone	0.041 (114)	0.200 (118)
10^{-6} M hydrocortisone	0.037 (103)	0.019 (112)
Holly (H)		
	Line 2 (+)	
Control	0.457 (100)	
10^{-7} M dexamethasone	0.665 (146)	
10^{-6} hydrocortisone	0.269 (58)	
Archy (A)		
	Line 3 (+)	Line 5 (+)
Control	0.180 (100)	0.193 (100)
10^{-7} M dexamethasone	0.272 (151)	0.172 (89)
10^{-7} M hydrocortisone	0.269 (149)	0.151 (78)
10^{-6} M hydrocortisone	0.278 (154)	0.199 (103)
Titus (T)		
	Line 3 (+)	Line 5 (+)
Control	1.138 (100)	0.536
10^{-7} M dexamethasone	1.965* (173)	1.199* (224)
10^{-7} M hydrocortisone	1.560 (137)	0.865* (161)
Elvis (E)		
	Line 5 (+)	Line 6 (+)
Control	0.358 (100)	0.601 (100)
10^{-7} M dexamethasone	1.982* (554)	1.705* (282)
10^{-7} M hydrocortisone	1.804* (504)	(0.986* (164)

The cultures were exposed to different concentrations of glucocorticoids for 24 h whereafter the cells were harvested and subjected to luciferase activity as described in Section 2.
To facilitate comparison of the data, in each case experimental values were converted to percentage of controls (figures within brackets).
*, significant difference between means, $P < 0.025$.

secondly because previous experiments using enhancer-less constructs in transient transfection assays were uninformative (Caricasole and Ward, unpublished observations).

Fibroblasts containing constructs O, H, T and E and also the one line expressing the A construct were used to examine the effect of exogenously added glucocorticoids on this promoter. The results are summarised in Table 2. It was found that both hydrocortisone and dexamethasone exerted a significant stimulatory effect on cells derived from mice carrying the O and E and in two out of three lines the H transgene. By contrast cells harbouring the T and in some cases the H transgene did not increase their expression after exposure to any of the glucocorticoids. The single A line which expressed the transgene did not respond to glucocorticoid exposure. This is consistent with the notion that in this case the transgene has fallen under the control of an enhancer element at the insertion site. To exclude the possibility that these differences in response to exogenous glucocorticoids were due to lack of endogenous expression of the glucocorticoid receptor, the non-responsive cell lines were transfected with a vector containing the human glucocorticoid receptor. The results of dexamethasone or glucocorticoid addition to the transfected cell lines are shown in Table 3. With one exception (Titus exposed to dexamethasone) the addition of endogenous glucocorticoid receptor expression did not result in a significantly increased glucocorticoid induced expression of the transgene. In the case of the Holly line, the exogenous receptor abrogates glucocorticoid stimulation of transgene expression and we suggest this might be due to a dose dependent effect on receptor concentration specific to this transgene insertion site. Taken together our data indicate that steroid hormones indeed enhance the usage of the insulin like growth factor II P3 promoter if some other control elements are present.

It was initially believed that dexamethasone or hydrocortisone down-regulates the IGF II expression in rodents by binding to the glucocorticoid receptor and that the glucocorticoid surge may be responsible for the postnatal decline in IGF II expression in rodents [10,11]. Furthermore, a similar postnatal decline in expression occurs in many of the transgenic mice from which cell lines were derived for use in this study [19].

However, even though some putative glucocorticoid responsive elements have been identified in the rodent IGF II gene, their functional role remains to be characterised [10] and moreover none of these putative glucocorticoid receptor binding elements were part of our construct. The hormone receptor complex binds to a conserved element in the regulatory region of the target gene. Such glucocorticoid receptor responsive elements (GREs) are extremely well conserved and can either be up-regulating or down-regulating depending on other nuclear factors [32]. Recently it has been reported that an increase in circulating glucocorticoid levels leads to decreased IGF II transcript levels in adult mink liver [12], the opposite situation has been reported after long term treatment exposure to glucocorticoids [13] or in pigs treated with dexamethasone [33]. In different species IGF II gene promoters are differently used and in the case of higher mammals, such promoters are part of the developmental control machinery [15,18,34,35]. Consequently it is plausible that the IGF II gene promoters in different species might respond differently to glucocorticoid exposure. It should also be noted that our study was restricted to embryonic fibroblasts and the response of the IGF II P3 promoter to glucocorticoids might vary in other cell types. Although no putative GRE box has been traced in the murine IGF II P3 promoter, the binding site could be diverged from the consensus, alternatively this region might contain some motif that binds some factor which is synthesised in response to the steroid exposure. The other regulatory elements used in this study, and most particularly the CCD, have not been fully sequenced. It is therefore an alternative possibility that the action of glucocorticoids is indirect the up-regulation of IGF II P3 being effected by intermediate gene products. Binding of these putative intermediaries could be targeted to the enhancing elements rather than the actual P3 promoter. However, binding to the P3 region seems most likely since this is the only regulatory element present in all of the glucocorticoid responsive transgene constructs.

Acknowledgements: The authors wish to acknowledge the support of the Cancer Research Campaign of Great Britain and of Cancerfonden and Barncancerfonden, Sweden. W.E. was the recipient of a UICC technology transfer fellowship.

References

[1] Schofield, P.N., Ed. (1992) The Insulin Like Growth Factors, Oxford University Press, Oxford.

[2] Ward, A. and Ellis, C. (1992) in: The Insulin Like Growth Factors (Schofield, P.N., Ed.), pp. 45–79, Oxford University Press, Oxford.
[3] De Chiara, T., Robertson, E. and Efstratiadis, A. (1991) Cell 64, 849–859.
[4] Newell, S., Ward, A. and Graham, C.F. (1994) Mol. Reprod. Dev. 39, 249–258.
[5] Nielsen, F.C., Gammeltoft, S. and Christiansen, J. (1990) J. Biol. Chem. 265, 13431–13434.
[6] Nielsen, F.C., Ostergaard, L., Nielsen, J. and Christiansen, J. Nature 377, 358–362.
[7] Lau, M., Stewart, C., Liu, Z., Bhatt, H., Rotwin, P. and Stewart, C.L. (1994) Genes Dev. 8, 2953–2963.
[8] Schofield, P.N. and Engström, W. (1992) in: The Insulin Like Growth Factors (Schofield, P.N., Ed.), pp. 240–257, Oxford University Press, Oxford.
[9] Ward, A., Bierke, P., Pettersson, E. and Engström, W. (1994) Zool. Sci. 11, 167–174.
[10] Beck, F., Samani, N.J., Senior, P., Byrne, S., Morgan, K., Gebhard, R. and Bramma, J. (1988) Mol. Endocrinol. 1, R5–8.
[11] Levinowitz, A. and Norstedt, G. (1989) Mol. Endocrinol. 3, 797–804.
[12] Bäcklin, B.M., Gessbo, Å., Forsberg, M., Schofield, P.N., Rozell, B. and Engström, W. (1997) Clin. Mol. Pathol., in press.
[13] Price, W., Stiles, A., Moats-Staats, B. and dErcole, J. (1992) Endocrinology 130, 1424–1432.
[14] Mouheddine, O.B., Cazals, V., Kuto, E., Lebouc, Y. and Clement, A. (1996) Endocrinology 137, 287–295.
[15] Schofield, P. and Tate, V. (1987) Development 100, 693–704.
[16] Hedley, P.E., Dalin, A.M. and Engström, W. (1989) Cell Biol. Int. 13, 857–862.
[17] Ekström, T.J., Bäcklin, B.M., Lindqvist, Y. and Engström, W. (1993) Gen. Comp. Endocrinol. 90, 243–250.
[18] Joujou-Sisic, K., Granerus, M., Wetterling, H., Wikström, K., Engström, W., Jeffcott, L., Schofield, P.N. and Welin, A. (1993) Cell Biol. Int. 17, 603–608.
[19] Ward, A., Fisher, R., Richardson, L., Pooler, J., Squire, S., Bates, P., Shaposhnikov, R., Hayward, N., Thurston, M. and Graham, C.F. (1997) Genes Funct. 1, 25–36.
[20] Leighton, J.E., Saam, J.R., Ingram, R.S., Stewart, C.L. and Tilghman, S.M. (1995) Genes Dev. 9, 2079–2089.
[21] Leighton, P.A., Ingram, R.S., Eggenschweiler, J., Efstratiadis, A. and Tilghman, S.M. (1995) Nature 375, 34–39.
[22] Bartholomei, M.S., Webber, A.L., Brunkow, M.E. and Tilghman, S.M. (1993) Genes Dev. 7, 1663–1673.
[23] Sasaki, H., Jones, P.A., Chaillet, R.J., Ferguson-Smith, A.C., Barton, S.C., Reik, W. and Surani, M.A. (1992) Genes Dev. 6, 1843–1856.
[24] Brandeis, M., Kafri, T., Ariel, M., Chaillet, I.R., McCarrey, I., Razin, A. and Cedar, H. (1993) EMBO J. 12, 3669–3677.
[25] Feil, R., Walther, J., Allen, N.D. and Reik, W. (1994) Development 120, 2933–2943.
[26] Yoo-Warren, H., Pachnis, V., Ingram, R.S. and Tilghman, S.M. (1995) Mol. Cell. Biol. 8, 4707–4715.
[27] Li, E., Beard, C. and Jaenisch, R. (1993) Nature 366, 362–365.
[28] Razin, A. and Cedar, H. (1994) Cell 77, 473–476.
[29] Koide, T., Ainscough, J., Wijgerde, M. and Surani, M.A. (1994) Genomics 24, 1–8.
[30] Hollenberg, S.M., Weinberger, C., Ong, E.S., Cerelli, G., Lebo, R., Thompson, E.B., Rosenfeld, M.G. and Evans, R.M. (1985) Nature 318, 635–641.
[31] Brunkow, M.E. and Tilghman, S.M. (1991) Genes Dev. 5, 1092–1101.
[32] Dahlman-Wright, K., Wright, A., Gustafson, J.Å. and Carlstedt Duke, J. (1991) J. Biol. Chem. 266, 3107–3112.
[33] Madej, A., Einarsson, S., Romanowicz, K., Forsberg, M., Tsuma, V.T., Engström, W. and Barcikowski, B. (1996) Reprod. Dom. Anim. 31, 565–569.
[34] Otte, K. and Engström, W. (1994) Gen. Comp. Endocrinol. 96, 270–275.
[35] Ferguson-Smith, A.C., Sasaki, H., Cattanagh, B.M. and Surani, M.A. (1993) Nature 362, 751–755.
[36] Zemel, S., Bartolomei, M.S. and Tilghman, S.M. (1992) Nature Genet. 2, 61–65.