The H19 Transcript Is Associated with Polysomes and May Regulate IGF2 Expression in trans*

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The imprinted H19 gene produces a fully processed transcript that does not exhibit any conserved open reading frame between mouse and man. Although transcriptional control elements associated with the mouse H19 locus have been shown to control the neighboring Igf2 gene in cis, the prevailing view is that the cytoplasmic H19 transcript does not display any function. In contrast to earlier reports, we show here that the H19 transcript is associated with polysomes in a variety of cell types, in both mouse and man. A possible trans-function of the H19 gene is suggested by a reciprocal correlation in trans between cytoplasmic H19 and IGF2 mRNA levels, as well as IGF2 mRNA translatability. We discuss these results in terms of their challenge to the prevailing dogma on the function of the enigmatic H19 gene, as well as with respect to the ontogeny of the Beckwith-Wiedemann syndrome, and propose that the human H19 gene is an antagonist of IGF2 expressivity in trans.

The H19 gene, which was first identified a decade ago, has been suggested to belong to the category of polymerase II-driven genes that do not code for a protein product (1). Although the processed and polyadenylated transcript is highly conserved between mouse and man, it does not display a conserved open reading frame (2). Moreover, the mouse H19 transcript, which is localized in the cytoplasm, has been reported to be excluded from the polysomal fraction and to be unable to be translated in vitro (2). The only indication so far that the human H19 RNA may have any role derives from the observation that an H19 expression vector rescues the normal phenotype of rhabdomyosarcoma cells (3). This result would, however, appear to be at odds with the lack of a documented increased incidence of cancer in H19-deficient mice (4). Moreover, H19 expression is maintained at high levels in some human tumors and has been proposed to be selected for during tumor progression (5). No consistent role (if any) has been established, therefore, for H19 in trans.

Both H19 and the insulin-like growth factor gene-2 (IGF2), which are close physical neighbors on chromosome 7 in mouse and on chromosome 11 in humans (6), were among the first genes to be identified as being genomically imprinted (7, 8). The generation of deletion mutants in the mouse has shown that the imprinting status of Igf2 and H19 is coordinated such that the deletion of H19 and a 10-kilobase upstream region up-regulates maternal Igf2 when the deleted region is maternally inherited (4, 9). Conversely, a deletion of the endodermal-specific H19 enhancers has shown that they are vital for the activity of paternally derived Igf2, at least in some cell types, when the deleted enhancer region is inherited paternally (10). It appears, therefore, that the region within and/or flanking the mouse H19 gene can regulate the allele-specific expression of Igf2 in cis.

The observations that many human tumors do not express H19, but express both parental Igf2 alleles, has prompted suggestions that the human H19 gene or its flanking sequences may also control the activity of IGF2 in cis (11, 12). Such notions have not, however, been substantiated at the cellular level. Indeed, it has been shown by examination at the cellular level that H19 can be biallelically expressed in a subpopulation of human placental cells that expresses Igf2 monoallelically (13). It has also been shown that the generation of Wilm’s tumors involves a mosaic Igf2 imprinting status that does not correlate with the expression of H19 at the individual cellular level (14). Collectively, these data, although circumstantial, are not supportive with respect to a cis function of the human H19 gene.

Here we report that, in contrast to previous claims, the H19 transcript is associated with polysomes in a variety of cell types in both mouse and man. H19 expression appears to directly or indirectly modulate the cytoplasmic levels of IGF2 mRNAs without being genetically linked to the expressed Igf2 allele. We submit that the human H19 gene is an antagonist to IGF2 in trans.

EXPERIMENTAL PROCEDURES

Cell Samples and Extraction of Nucleic Acid—The Wilm’s tumor was collected at the Uppsala University Hospital. Routinely processed formalin-fixed, paraffin-embedded tissues were used for in situ hybridization. DNA was extracted from snap-frozen tissues and from peripheral leukocytes, as has been described previously (13). The mouse embryos, at 17 days postconception, were crosses between Mus musculus and Mus mus domesticus. The JEG-3 cell line was maintained as has been described (15). Pectanycin (a kind gift of Pharmacia-Upjohn) was administered to JEG-3 cells for 3.5 h before harvesting and used at a concentration of either 2.0 or 6.9 × 10⁻⁶ M without any detectable difference in the effect. Genomic DNA and total cellular RNA were prepared according to routine protocols (13).

Sucrose Gradient Analysis—Postmitochondrial supernatants of both mouse embryos and cultured tumor cells were prepared according to Brannan et al. (2). Supernatants without added EDTA were layered onto 10.6 ml of 10–50% sucrose gradient containing 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM MgCl₂, and 15 units of RNasin/ml. The supernatant with added EDTA (30 mM final concentration) was layered onto a 10.6-ml 10–50% sucrose gradient containing 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 30 mM EDTA. The gradients were centrifuged in an LKB 2331 ultracentrifuge with a 40T768 rotor at 35,000 rpm for 1.5 or...

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6 h, as indicated in the legend to Fig. 1. Following division into 18–20 fractions, RNA was phenol-extracted from 100-ml aliquots and analyzed by Northern blot, RNase protection assay, and RT-PCR. 1

Probes—DdeI-linearized human H19 cDNA cloned in Bluescript (a kind gift from Dr. Wolf Reik, Babraham, Cambridge, UK) was used to generate an antisense riboprobe (253 bases; T7 polymerase). A 558-base pair HindIII-PstI human IGF2 cDNA insert, cloned in pGem-3 and encompassing exon 7 to the 5'-region of exon 9, was cut with XhoI and used as the template for generation of an antisense riboprobe (145 bases; SP6 polymerase) (16). A 756-base pair antisense H19 RNA probe (17) was used to detect mouse H19 transcripts.

Northern Blot Hybridization and RNase Protection Analysis—Northern blot hybridization analysis on 1% agarose gels was performed as described (18). RNase protection analysis was performed using the RPA II™ ribonuclease protection assay kit (Ambion).

Analysis of Allele-specific IGF2 Transcripts—The overall functional IGF2 imprinting status was determined by thermocyclic amplification of cDNA, produced by priming of IGF2 mRNA, and by diagnostic digestion with Apal as has been described (19, 20) with the following exception. To ensure a linear amplification of low abundance IGF2 cDNAs in the sucrose gradient analyses, the oligo primers were 5'-labeled and the number of cycles were reduced to 25. This approach was verified by mixing experiments (not shown) as has been described (21). The resulting PCR products were analyzed on 8% polyacrylamide-urea sequencing gels, as has been described (21).

Allelic expression patterns at the cellular level were analyzed by allele-specific in situ hybridization as has been reported (13, 14). Regular in situ hybridization analysis of IGF2 and H19 expression was performed as has been described (13). Both approaches used serial 5-mm sections from formaldehyde-fixed and paraffin-embedded tissues according to routine procedures. Following hybridization, the slides were dipped in NTB2 (Kodak) emulsion, developed, and counterstained in Mayer's hematoxylin before mounting.

RESULTS

The H19 Transcript Co-sediments with Polysomes—In initial attempts to identify any ribonucleoprotein particles containing human H19 RNA, we investigated the distribution of H19 RNA in sucrose gradients. Fig. 1A shows an RNase protection analysis which documents that H19 RNA distributes along a 10–50% sucrose gradient, with a peak that appeared to partially overlap with polysomes containing IGF2 mRNAs. In EDTA-treated samples, the collapse of the polysomes to generate ribosomal subunits appears to be accompanied by a similar shift of the H19 RNA-protein complexes to the

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1 The abbreviations used are: RT-PCR, reverse transcription-polymerase chain reaction; BWS, Beckwith-Wiedemann syndrome.
upper portion of the sucrose gradient. To examine the extent of sedimentation overlap of H19 RNA-protein complexes with polysomes containing the different types of IGF2 mRNA transcripts derived from the three major promoters, we repeated the experiment and analyzed the sucrose gradient fractions by Northern blot analysis. Fig. 1B shows that there is an extensive similarity in the distribution of H19 RNA with the promoter 3-derived 6.0-kilobase IGF2 transcript. Because this similarity extends to the samples treated with EDTA, we reasoned that H19 might be associated with polysomes of a size similar to those containing IGF2 mRNA.

Because such a high proportion of the RNA-protein complexes pelleted in our sucrose gradient analyses, we were prompted to resolve the pelleted H19 transcripts. We repeated the sucrose gradient analyses, therefore, using a significantly shorter centrifugation time. Fig. 1C shows that the resolution of the distribution of the H19 transcript in sucrose gradient analyses of JEG-3 cells is improved with such a change in the protocol. The previous notion that the H19 transcript co-sediments with polysomes is reinforced by these results. All of these observations have been reproduced numerous times with very similar or identical results.

Given that it has been previously argued that mouse H19 RNA is not associated with polysomes (2), we subjected H19 transcripts expressed in mouse fetal liver to a polysome analysis, using the shorter centrifugation periods described above. The results show that the distribution of H19 RNA partially overlapped with the polysomal marker, β-actin mRNA (Fig. 1D). Both types of transcripts appear to exist in polysomal and non-polysomal pools. As was the case for the human H19 RNA, the mouse H19 RNA (and the β-actin mRNA) was shifted to the lighter portion of the sucrose density gradient in the presence of EDTA. We have performed these experiments according to the protocol of Brannan et al. (2) with the exception of the cycloheximide treatment. Because cycloheximide would stabilize the polysomes and potentially yield larger polysomes than in untreated animals, it is possible that such polysomes would pellet quantitatively during centrifugation, perhaps explaining why H19 RNA association to polysomes has gone unnoticed (2).

It was still possible that the sedimentation properties of the H19 transcript were fortuitously similar to the polysome profile of control transcripts, such as GAP and β-actin mRNAs. To resolve this issue, we treated JEG-3 cells with pactamycin, which is an inhibitor of initiation of mRNA translation. Fig. 2 shows that the shift in sedimentation properties of the control GAP mRNA in pactamycin-treated cells is accompanied by a similar, but less dramatic, shift in the sedimentation of both the H19 and IGF2 transcripts. We conclude that H19 is associated with polysomes, at least in human cells. The reason for the less pronounced difference in pactamycin-sensitivity for the IGF2 and H19 transcripts, when compared with the GAP mRNA, is not known. One possible explanation is that the elongation of the IGF2 and H19 transcripts is attenuated. This would be expected to yield polysomes that are less sensitive to pactamycin treatment, as has been demonstrated for polysomes containing the HSP70 mRNA (22).

Absence of H19 Transcripts Correlates with Increased Cytoplasmic Levels of IGF2 mRNA in a Wilms’ Tumor—We have previously documented a Wilms’ tumor that is unique in the sense that it expresses IGF2 and H19 at high levels in almost identical patterns and displays a postneoplastic loss of IGF2 imprinting, where only a subpopulation of the tumor cells express both parental alleles, as determined by allele-specific in situ hybridization (Fig. 3A) (14). Fig. 3B shows a summary of these results: where subpopulations of cells with no H19 expression but monoallelic IGF2 expression are marked red, and cells with no H19 expression and biallelic IGF2 expression are marked green. Magnified views of these areas can be seen in Cui et al. (14). A closer look at the IGF2 expression levels revealed that the hybridization signal was 2–3-fold higher in the H19-negative cells when compared with the neighboring H19-positive cells (Fig. 3B). This observation could be documented in each of the H19-negative areas, suggesting an inverse correlation between H19 expression and cytoplasmic levels of IGF2 mRNA.

H19 Expression Correlates Inversely with Translatability of IGF2 mRNAs in a Wilms’ Tumor—We next addressed whether or not the pattern of H19 expression correlates with a difference in the polysome profile of IGF2 mRNAs. This approach

![Fig. 2. The sedimentation property of the H19 transcript is pactamycin-sensitive. Postmitochondrial lysates of JEG-3 cells, control, and pactamycin-treated were analyzed on sucrose gradients as described under “Experimental Procedures.” RNA was extracted from every second fraction and subjected to RNase protection (A). The analysis of the sedimentation properties of the H19 transcript was performed separate from the analysis of the GAP and IGF2 mRNAs. The H19- (B) and GAP- (C) specific protected bands were quantitated by PhosphorImager analysis. The filled and unfilled staples display relative levels of transcripts in control and pactamycin-treated cells, respectively.](image-url)
was made possible by the strategy outlined in Fig. 4, where the parental alleles (A and B) are distinguishable by a sequence polymorphism. The generally silent allele B of IGF2 was only expressed in H19-negative cells, whereas the majority of the tumor cells expressed only the normally active allele A in H19-positive cells. If H19 expression modifies the translatability of IGF2 mRNAs, we would predict that the over-all sedimentation properties of transcripts derived from alleles A and B would differ in a sucrose gradient analysis.

The postmitochondrial cell lysate of the Wilms’ tumor was subjected, therefore, to a sucrose density gradient fractionation. Fig. 5A shows that, in contrast to the specimens analyzed in Fig. 1, the sedimentation properties of the various IGF2 mRNA complexes in the sucrose gradients were refractory to EDTA treatment, as examined by Northern blot hybridization. This result suggests that the bulk of IGF2 mRNAs was poorly translated in this Wilms’ tumor. We next examined whether or not the presence or absence of H19 transcripts correlated with the sedimentation properties of IGF2 transcripts, as outlined above. To this end, IGF2 mRNA, which was extracted from every second sucrose gradient fraction, was subjected to reverse transcription. Using labeled primers to thermocyclically amplify a fragment encompassing a diagnostic ApaI polymorphic site, the cDNA was digested with ApaI to allow discrimination of allelic origin. Fig. 5B shows that the IGF2 transcripts derived from allele A, which is predominantly expressed in H19-positive cells, are associated with gradient fractions, which suggest that they are poorly translated. This result agrees well with the conclusion from the Northern blot hybridization analysis of the samples from the same sucrose gradient. Conversely, the IGF2 transcripts derived from the allele B (exclusively expressed in H19-negative cells) generally sediment as larger complexes than transcripts derived from allele A. Upon the addition of EDTA, the relative distribution of these transcripts is shifted to fractions containing nontranslated IGF2 mRNA.

Because it has been hypothesized that mitotic crossing-overs can switch the parental epigenotype during Wilms’ tumorigenesis (23), we could not formally exclude the possibility that the epigenotype of allele B would be maternal in the tumor and paternal in the normal kidney. If so, the suggested overrepresentation of allele B-derived IGF2 transcripts in the polysome fraction might simply reflect contamination of stroma cells expressing allele B at low or undetectable levels (see Fig. 3). A thermocyclic amplification analysis of reverse transcribed...
mRNA, extracted from both normal and tumor compartments, revealed that allele A can be found to be preferentially active in both instances (Fig. 6). Given the more than 50-fold lower expression of IGF2 in the normal cells, it appears clear that IGF2 transcripts derived from allele B are highly unlikely to result from contamination by stroma and are, therefore, expressed only in tumor cells.

DISCUSSION
A function for H19 in trans has been disputed because a previous study claims that the H19 transcript is not associated with polysomes and does not produce a protein in the mouse (2). In addition, the lack of a reported increased incidence of cancer in H19-deficient mice (4) would appear to question a link to the suggested tumor suppressor function of human H19 in trans. On the other hand, the H19 knock out data does not rule out that an H19 trans-function is redundant and/or that a removal of an H19 function in trans contributes to the overgrowth phenotype of H19-deficient conceptuses. In addition, it is possible that human H19 has acquired a trans-function that is absent or has been lost in the mouse during evolution. Moreover, a recent transgenic study has strongly argued against the possibility that the mouse H19 gene represses the transcription of Igf2 in cis, by means of its own expression (24). We have, therefore, reexamined a putative role for the H19 transcript in humans.

First, we document that in contrast to previous reports, H19 mRNA co-sediments with polysomes in a variety of cell types, in both mouse and man. In cell cultures, this sedimentation is sensitive to pactamycin, which is an inhibitor of initiation of mRNA translation. This observation would seem to support a previous observation that the human H19 transcript is translatable in vitro and has an open reading frame that could formally encode a protein of 26 kDa (25). Second, by both direct and indirect lines of evidence, we were able to document that the cytoplasmic levels of H19 transcripts inversely correlated with cytoplasmic levels of IGF2 in a Wilms’ tumor. Because the parental H19 alleles were identical with respect to a number of polymorphic markers, we have been unable to trace the parental origin of the expressed allele. On the other hand, because

FIG. 5. Parent of origin-dependent sedimentation properties of Wilms’ tumor-derived IGF2 mRNAs in sucrose gradients. A, Northern blot analysis of IGF2 mRNA species extracted from every second fraction. B, selected fractions of the sucrose gradient of panel A were subjected to RT-PCR analysis to examine allelic origin of IGF2 transcripts. Panels A and B denote the ApaI noncutting and cutting alleles, respectively. – and + denote minus and plus reverse transcriptase, respectively.

FIG. 6. Analysis of allelic expression pattern of IGF2 in the normal kidney tissue and Wilms’ tumor of the same patient. The analysis was carried out by ApaI digestion of genomic PCR products (DNA) and RT-PCR products (RNA). The molecular weight (MW) marker lane displays Sau3A I/TaqI-restricted pUC 19 DNA (Stratagene). The extra band, migrating as a 140-base pair fragment, represents a PCR artifact.

FIG. 7. A model of the postulated H19-specific control loop. Whereas the H19-specific control would be coordinated with IGF2 activity, other levels of controls, represented by IGF-binding proteins for example, may be uncoordinated. In the normal context with cells displaying low levels of IGF2 mRNAs, the uncoordinated controls would be expected to successfully prevent production of high levels of IGF-II peptide. The coordinated control, represented by H19 transcripts, will be of minor importance because the levels and pattern of H19 expression closely follows those of IGF2 (27). By the same token, increased activity of the IGF2 gene will be followed by increased activity of the H19 gene. In this scenario, the coordinated H19 control would gain importance in direct proportion to the inability of the uncoordinated controls to deal with abnormal levels of IGF2 activity. If the H19 function is abnormally silenced in a situation with high levels of IGF2 transcripts, as has been documented in numerous contexts (31), this report suggests that production of the IGF-II peptide will increase abnormally to contribute to overgrowth syndrome syndromes, such as the Beckwith-Wiedemann syndrome, and neoplasia (outlined in the right-most panel). This model may be valid only in the absence of prominent translational suppression of IGF2 mRNAs.
The H19 Transcript Is Polysome-associated

absence of H19 transcripts correlates both with increased levels of cytoplasmic transcripts derived from one allele and with increased translatability of transcripts derived from the other allele, we argue that one or both of these effects can be attributed to an H19 function in trans. Collectively, the data support the notion that H19 modifies IGF2 mRNA cytoplasmic levels and, potentially, polysome association in trans.

The observations of this report provide yet another glimpse into the complex regulation of the function of IGF2. This involves multiple steps of controls from gene dosage, differential promoter usage, splicing patterns, translational control(s) to postsecretory attenuation of IGF2 function by IGF-binding proteins (26). Whereas some of the cytoplasmic and extra-cellular levels of control appear to be uncoordinated with IGF2 expression levels, the expression of H19, and hence the antagonistic function of H19 in trans, is expected to be coordinated (27). This allows us to formulate a model in which H19 serves to prevent overshoot of IGF2 expression in trans. In this model, an increase in H19 expression would accompany an increase in IGF2 expression because their expression patterns are coordinated (10, 27) (Fig. 7). This coordination would be of particular importance in cases where the high levels of IGF2 expression could be expected to saturate the uncoordinated types of negative cytoplasmic and/or extracellular controls, such as IGF-binding proteins. In practice, this would mean that the higher the levels of IGF2 expression, the more important the H19 regulatory pathway would become. According to this model, a loss of the H19 function in trans would be expected to be a key event in cells expressing high levels of IGF2 mRNAs, resulting in a significant increase of free IGF-II ligand. Our model would highlight the consequences of losing the H19 function in trans when IGF2 is overexpressed. In a parallel study, we have been able to show that this loss of H19 expression is an early event which may predispose for Wilms’ tumors (14). Another interesting case is the previous documentation that IGF2 could not be genetically linked with a familial form of BWS (28), despite the close link between BWS and IGF2 (29, 30). The possibility that H19 can be the direct culprit in this cancer-predisposing disease, at least in some instances, and that the role of IGF2 may be more indirect would appear to be compatible with the proposed model.

Our observations are consistent with the possibility that the human H19 gene modifies the expression of IGF2 in trans. These data may have an impact in our understanding of human diseases, such as BWS, and allows us to further penetrate the nature of disease-associated (epi)genetic abnormalities.

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REFERENCES
1. Tilghman, S., Bartolomei, M., Webber, A., Brunkow, M., Saam, J., Leighton, P., Pfeifer, K., and Zemel, S. (1993) Cold Spring Harbor Symp. Quant. Biol. 58, 287–295
2. Brannan, C., Dees, E., Ingram, R., and Tilghman, S. (1990) Mol. Cell. Biol. 10, 28–36
3. Hao, Y., Crenshaw, T., Moulton, T., Newcomb, R., and Tycko, B. (1993) Nature 365, 764–767
4. Leighton, P. A., Ingram, R. S., Eggeschweiler, J., Efratiadis, A., and Tilghman, S. M. (1995) Nature 375, 34–39
5. Rachmilewitz, E., Elkin, M., Rosensart, J., Gelman-Kohan, Z., Ariel, I., Lustig, O., Schneider, T., Goshen, R., Biran, H., De Groot, N., and Hobach, A. (1995) Oncogene 11, 863–870
6. Zemel, S., Bartolomei, M., and Tilghman, S. (1992) Nat. Genet. 2, 61–65
7. Bartolomei, M., Zemel, S., and Tilghman, S. (1991) Nature 351, 153–155
8. DeChiara, T., Robertson, E., and Efratiadis, A. (1991) Cell 64, 849–859
9. Ripoche, M., Krese, C., Poirier, P., and Dandolo, L. (1997) Genes Dev. 11, 1596–1604
10. Leighton, P., Saam, J., Ingram, R., Steward, C., and Tilghman, S. (1995) Genes Dev. 9, 2079–2089
11. Tycko, B. (1994) Am. J. Path. 144, 431–443
12. Feinberg, A., Kalikin, L., Johnson, L., and Thompson, J. (1994) Cold Spring Harbor Symp. Quant. Biol. 59, 357–364
13. Adam, O., Cui, H., Miller, S., Flam, F., and Ohlsson, R. (1996) Development 122, 839–847
14. Cui, H., Hedborg, F., He, L., Nordenskjold, A., Sandstedt, P.-O. S., and Ohlsson, R. (1997) Cancer Res. 57, 4469–4473
15. Franklin, G., Donovan, M., Adam, G., Holmgren, L., Specht, A., Pfeifer-Ohlsson, S., and Ohlsson, R. (1991) EMBO J. 10, 1365–1373
16. Ekstrom, T. J., Cui, H., Li, X., and Ohlsson, R. (1985) Development 121, 177–183
17. Walsh, C., Glaser, A., Fundele, R., Ferguson-Smith, A., Barton, S., Surani, M. A., and Ohlsson, R. (1994) Mech. Dev. 46, 55–62
18. Ohlsson, R., Holmgren, L., Glaser, A., Especht, A., and Pfeifer-Ohlsson, S. (1989) EMBO J. 8, 1993–1999
19. Ogawa, O., Eccles, M., Szeto, J., McNoe, L., Yun, K., Maw, M., Smith, P., and Reeve, A. (1993) Nature 362, 749–751
20. Ekstrom, T. J., Cui, H., Nyström, A., Rutanen, E.-M., and Ohlsson, R. (1995) Mol. Reprod. Dev. 41, 177–183
21. He, L., Cui, H., Walsh, C., Mattisson, R., Lin, W., Anneren, G., Pfeifer-Ohlsson, S., and Ohlsson, R. (1995) Oncogene 16, 113–119
22. Theodorakos, N., Banerji, S., and Morimoto, R. (1988) J. Biol. Chem. 263, 14579–14585
23. Cote, G. B. (1995) in Genomic Imprinting: Causes and Consequences (Ohlsson, R., Hall, K., and Ritzen, M., eds) pp. 252–263, Cambridge University Press, Cambridge
24. Webber, A., Ingram, R., Levorse, J., and Tilghman, S. (1998) Nature 391, 711–715
25. Jouvel, A., Curty, J.-J., Pelczar, H., Bego, A., Lagrou, C., Stehelin, D., and Coll, J. (1996) Cell. Mol. Biol. 42, 1159–1172
26. Stewart, C. E., and Rotwein, P. (1996) Physiol. Rev. 76, 1005–1026
27. Ohlsson, R., Hedborg, F., Holmgren, L., Walsh, C., and Ekstrom, T. J. (1994) Development 120, 361–368
28. Nyström, A., Hedborg, F., and Ohlsson, R. (1994) Eur. J. Pediatr. 153, 574–580
29. Reeve, A. E. (1996) Pediatr. Oncol. 27, 470–475
30. Hedborg, F., Holmgren, L., Sandstedt, B., and Ohlsson, R. (1994) Am. J. Pathol. 145, 892–917
31. Tycko, B. (1998) in Genomic Impprinting: An Interdisciplinary Approach (Ohlsson, R., ed), Springer, Heidelberg, in press