Research article

Novel gene expression patterns along the proximo-distal axis of the mouse embryo before gastrulation

Stephen Frankenberg[1,2], Lee Smith[3], Andy Greenfield[3] and
Magdalena Zernicka-Goetz*[1]

Address: [1]The Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, Tennis Court Rd, Cambridge CB2 1QR, UK, [2]Inserm, U384, Clermont-Ferrand, F-63001, France and [3]MRC Mammalian Genetics Unit, Harwell, Didcot OX11 0RD, UK

Email: Stephen Frankenberg - stephen@frankenberg.com.au; Lee Smith - l.smith@har.mrc.ac.uk; Andy Greenfield - a.greenfield@har.mrc.ac.uk; Magdalena Zernicka-Goetz* - mzg@mole.bio.cam.ac.uk

* Corresponding author

Abstract

Background: To date, the earliest stage at which the orientation of the anterior-posterior axis in the mouse embryo is distinguishable by asymmetric gene expression is shortly after E5.5. At E5.5, prospective anterior markers are expressed at the distal tip of the embryo, whereas prospective posterior markers are expressed more proximally, close to the boundary with the extraembryonic region.

Results: To contribute to elucidating the mechanisms underlying the events involved in early patterning of the mouse embryo, we have carried out a microarray screen to identify novel genes that are differentially expressed between the distal and proximal parts of the E5.5 embryo. Secondary screening of resulting candidates by *in situ* hybridisation at E5.5 and E6.5 revealed novel expression patterns for known and previously uncharacterised genes, including *Peg10*, *Ctsz1*, *Cubilin*, *Jarid1b*, *Ndrg1*, *Sfmbt2*, *Gjb5*, *Talia* and *Plet1*. The previously undescribed gene *Talia* and recently identified *Plet1* are expressed specifically in the distal-most part of the extraembryonic ectoderm, adjacent to the epiblast, and are therefore potential candidates for regulating early patterning events. *Talia* and the previously described gene *XE7* define a gene family highly conserved among metazoans and with a predicted protein structure suggestive of a post-transcriptional regulative function, whilst *Plet1* appears to be mammal-specific and of unknown function.

Conclusion: Our approach has allowed us to compare expression between dissected parts of the egg cylinder and has identified multiple genes with novel expression patterns at this developmental stage. These genes are potential candidates for regulating tissue interactions following implantation.

Background

At 5.5 days of development (E5.5) the mouse egg cylinder appears radially symmetrical about its proximo-distal axis with respect to known molecular markers and to the arrangement of its three principle tissue layers – epiblast, extra-embryonic ectoderm and visceral endoderm. However, shortly after E5.5 the first molecular asymmetries that determine the anterior-posterior axis begin to

emerge. These involve movement of a subset of visceral endoderm cells, anterior visceral endoderm (AVE), located at the distal tip of the egg cylinder towards the future anterior side [1-5]. Subsequent to this, molecular markers with a previously radial distribution near the embryonic-extra-embryonic boundary become restricted to the future posterior side at the site of the emerging primitive streak [6]. In this way the proximo-distal signaling anticipates the anterior-posterior patterning [6,7]. Patterning thus occurs through a combination of tissue interactions and cell movements [reviewed [8]].

The stages of mouse development between implantation and the gastrulating egg cylinder have been relatively little studied. This is due partly to the relative inaccessibility of embryos within the uterine deciduae during this time, and partly to their relatively poor development in culture compared with preimplantation and gastrula stages. More recently, much attention has been focused on the events preceding gastrulation and their relation to earlier preimplantation development, providing an incentive to identify novel genes with restricted expression patterns during these stages.

Several recent microarray screens have focused on stage-specific expression in pre-implantation embryos [9-11], whilst other screening strategies have targeted specific tissues of post-implantation embryos [12-15]. In an effort to identify new genes that are differentially expressed along the proximo-distal axis and may have roles in early pre-gastrula patterning events, we employed microarray analysis to compare gene expression between proximal and distal halves of the E5.5 egg cylinder. The proximal half includes extraembryonic ectoderm and the proximal portion of the visceral endoderm, while, the distal half includes the epiblast and the distal portion of the visceral endoderm. After secondary screening by *in situ* hybridisation, we identified both known and novel genes with previously unreported differential expression in the early mouse egg cylinder.

Results
We compared gene expression between the proximal and distal halves of the E5.5 egg cylinder by microarray analysis to identify genes with previously unreported differential expression at this stage of development. A scatter plot of expression levels in proximal and distal segments reveals a large number of genes that putatively show such differential expression (Fig. 1). Several genes with previously reported differential expression in the egg cylinder showed relative hybridisation levels consistent with such expression patterns. These included *Otx2* [16], *Cripto* [17], *Dnmt3b* [18] and *Oct4* [19] distally, and *Gjb3* [20], *Pem* [21], *Igf2* [22] and *H19* [22] proximally. We therefore wished to test whether other previously uncharacterised

genes were also differentially expressed. We selected 40 genes, partly on the basis of differential and absolute levels of hybridisation in the microarray and partly on their likely involvement in developmental pathways, and further screened these genes by *in situ* hybridisation. Of these, we successfully identified 9 genes with previously unreported differential expression at E5.5 and E6.5, while the remainder showed either undetectable or ubiquitous expression. The 9 genes and their expression patterns are shown in Figures 2 and 3 and summarised in Table 1. *In situ* hybridisation was extended to later stages from E7.5 to E9.5 for several genes, including *Cubilin*, *Jarid1b*, *Sfmbt2*, *Ndrg1*, *Talia* and *Plet1*. However no tissue specific-expression within embryonic tissues was identified for any of these gene later than E8.5 (not shown), aside from continued extraembryonic expression for *Cubilin*, *Sfmbt2*, *Ndrg1*, and *Plet1*.

Expression patterns fell into several broad categories. Peg10, Ctsz and Cubilin were expressed in the visceral endoderm mainly within the proximal or "extraembryonic" part of the egg cylinder. The expression of Cubilin also extended into the distal portion of the egg cylinder in the form of two lateral "wings" overlying the epiblast. Tissue sectioning showed that this expression corresponds to the distal extent of cuboidal visceral endoderm cells (Fig. 3a, b).

Jarid1b (also called *Plu-1* or *Rb-Bp2*) was expressed strongly in the epiblast at E5.5 but more weakly and ubiquitously at E6.5 (Fig. 2) and later stages (not shown). RT-PCR suggested a higher level of expression in whole E7.5 embryos than in adult tissues, with the exception of strong expression in the brain (Fig. 4).

Two genes, and *Gjb5* and *Sfmbt2*, were expressed throughout the extraembryonic ectoderm. In sectioned embryos, *Sfmbt2* expression appeared uniform within the chorionic ectoderm and also within the ectoplacental cone until at least E7.5 (Fig. 3c, d). *Sfmbt2* expression was also detected in all adult tissues tested but was substantially stronger in brain, lung and spleen compared with heart, kidney and liver (Fig. 4).

Ndrg1 was expressed uniformly throughout the extraembryonic ectoderm but more strongly within the labyrinth of the ectoplacental cone in all stages examined (Fig. 2, Fig. 3). At E7.5 and E8.0 (Fig. 2, 3f), expression was also present in the node, while becoming weaker within the chorionic ectoderm. No specific expression in other embryonic tissues was detected later at either E8.5 or E9.5 (not shown).

Plet1 was also specifically expressed in the extraembryonic ectoderm, but restricted to its distal-most part as early as
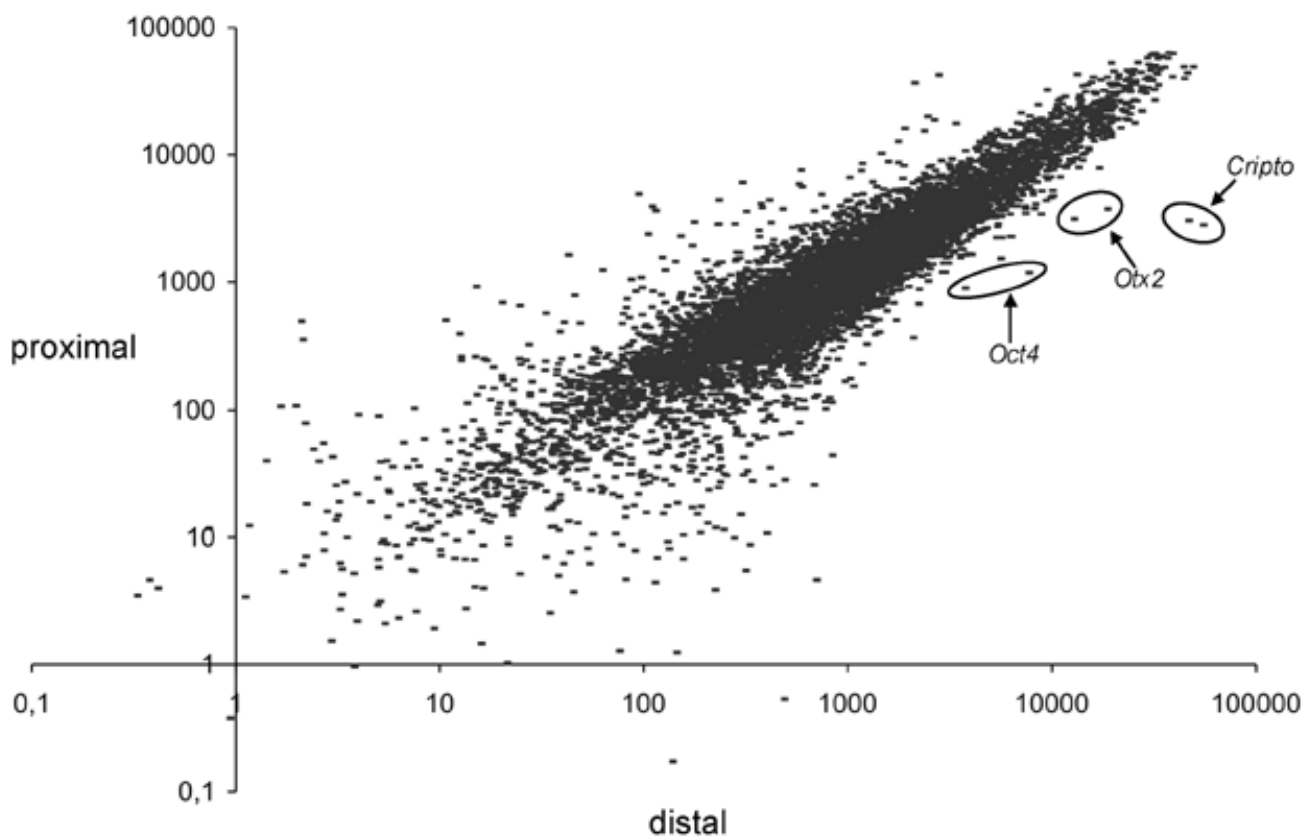
Figure 1
Scatter plot of microarray data comparing relative levels of gene expression for proximal and distal parts of the E5.5 egg cylinder (x – distal, y – proximal). Points representing several previously published genes (*Oct4*, *Otx2*, *Cripto*) that display clear differential expression between proximal and distal parts are circled.

E5.5 as well as a separate domain of much stronger expression within the ectoplacental cone. The distally-restricted expression persisted, but becoming weaker and restricted to the peripheral chorion, until at least E8.5 (Fig. 2, 3g–h). Expression was also detected within the ventral layer of the node (Fig. 3i).

Talia

A previously undescribed gene corresponding to clone H3001D07-3 was also uniformly expressed in the extraembryonic ectoderm at E5.5, and by E6.5 was also restricted to its more distal part, adjacent to the epiblast. Although levels appeared lower at later stages, expression appeared to be strongest around the perimeter of the distal part of the chorionic ectoderm at E7.5 (Fig. 3j). Expression was detected ubiquitously in all adult tissues examined by RT-PCR (Fig. 4).

A BLAST search of genomic databases identified the gene as mapping to region XA2 of the murine X-chromosome.

A human orthologue was also identified in the syntenic region Xq24 of the human X-chromosome and in the marsupial *Monodelphis domestica* by sequence database searches, indicating that the gene is conserved in mammals. The 5'-most part of the predicted transcript also showed homology to another previously described human gene, *XE7*, which maps to Xp22.3 of the X-chromosome and was originally identified as a pseudoautosomal gene that escapes X inactivation [23,24] and encodes a cell surface glycoprotein expressed in trophoblast and lymphocytes [25]. The murine gene represented by clone H3001D07-3 we thus named *Talia* (a Polish word for "waistline") to reflect its belt-like expression pattern in the distal part of the extraembryonic ectoderm.

Sequence analysis of Talia and XE7

Analysis of genomic sequence data and ESTs revealed 7 exons for murine *Talia* with a predicted mRNA length of 6152 nucleotides (Fig. 5, 6). Promoter prediction software [26] indicated an additional promoter and transcription
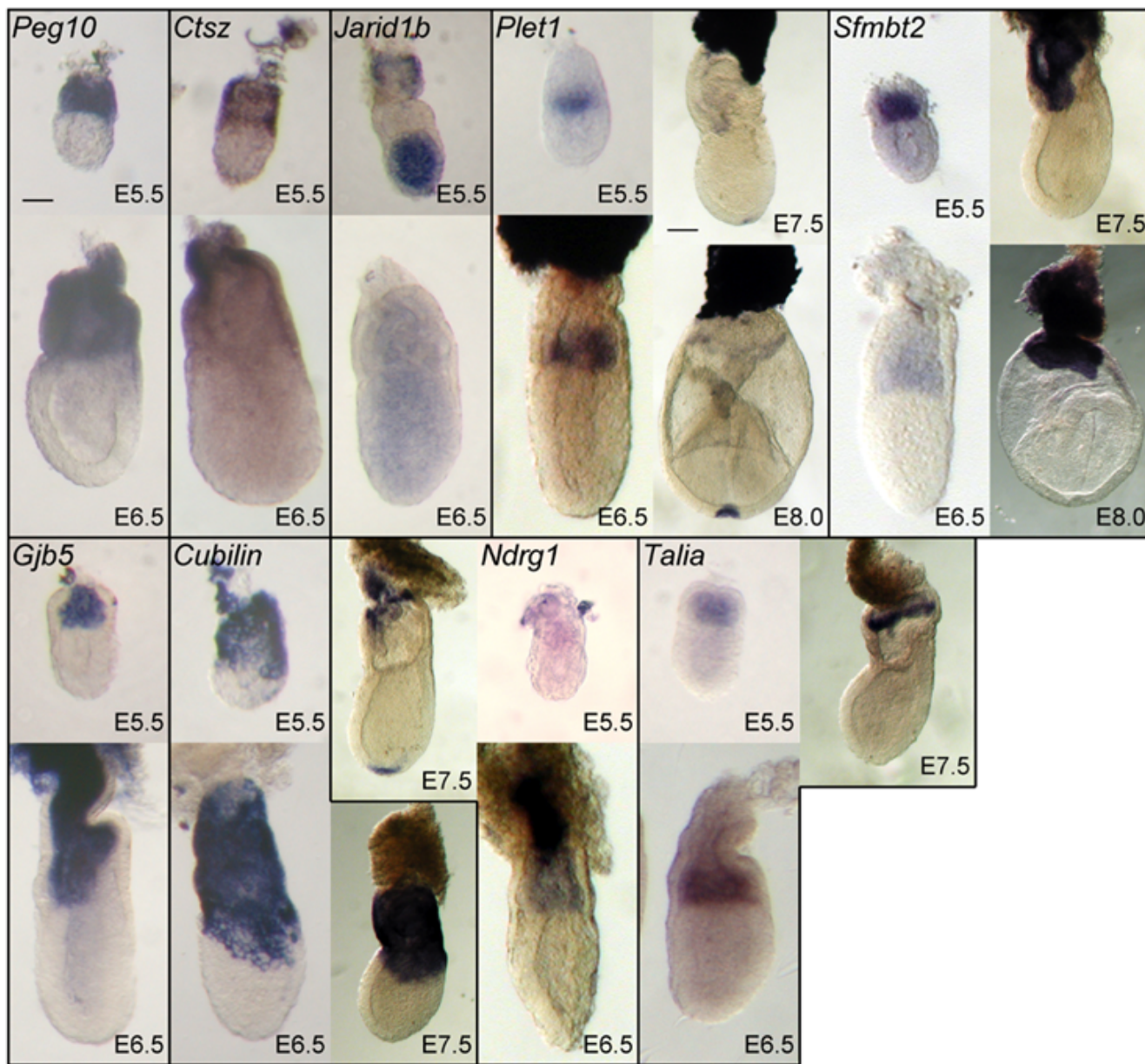
Figure 2
Whole-mount *in situ* hybridisation of genes identified in microarray screen with differential expression patterns at stages as indicated. The two scale bars represent respectively 100 μm for all E5.5 and E6.5 images and 200 μm for all E7.5 and E8.0 images.

initiation site within the 5' part of exon 7, suggesting alternative primary transcripts. However probes specific for sequences either 5' or 3' of this position showed indistinguishable expression patterns by *in situ* hybridisation (not shown), suggesting that either the latter putative promoter is non-functional or that both are functional but share common regulatory mechanisms.

Talia and *XE7* showed homology between exons 3–5 of murine *Talia* and exons 2–4 of human *XE7* (Fig. 6, 7). BLAST searches revealed numerous ESTs derived from various tissue sources that were concluded to represent murine *Xe7*, despite its apparent absence from current genomic databases. BLAST searches also identified ESTs representing genes with homology to this conserved
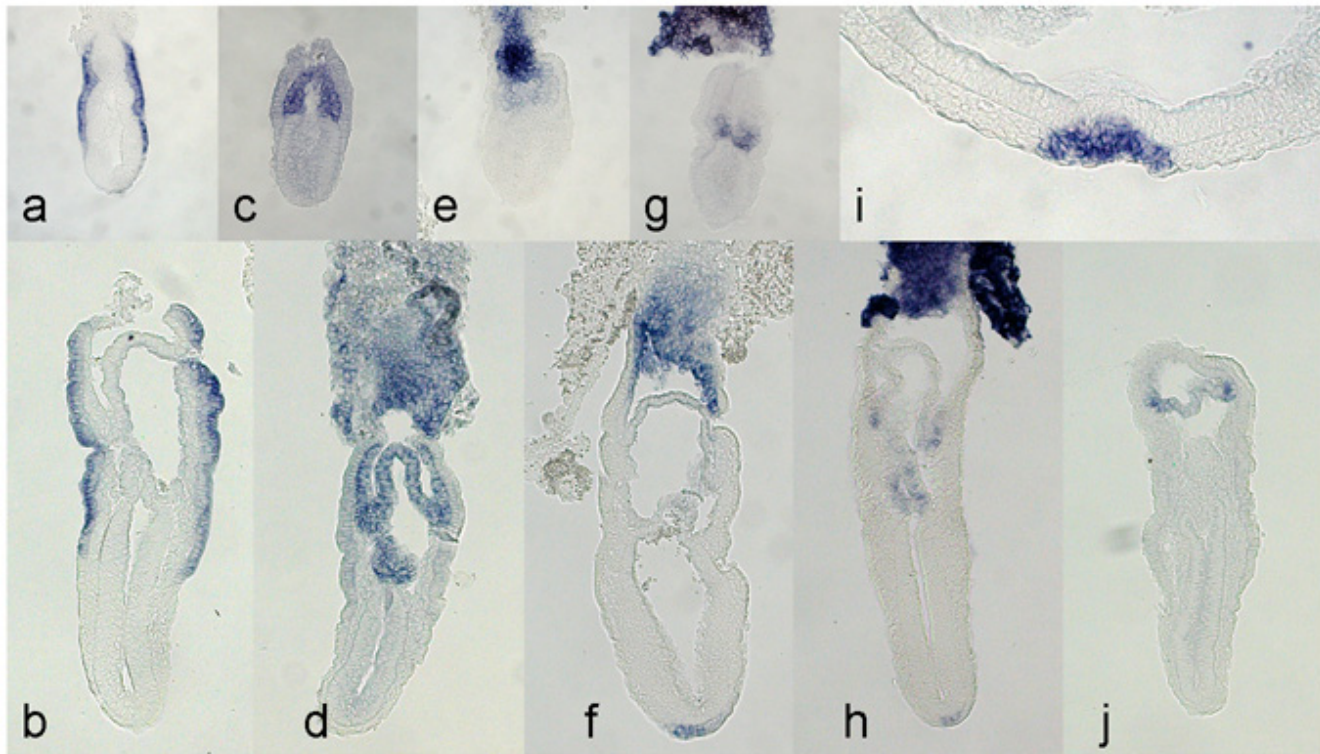
Figure 3
Longitudinal sections of embryos after whole-mount *in situ* hybridisation, showing expression of: *Cubilin* at E6.5 (a) and E7.5 (b); *Sfmbt2* at E6.5 (c) and E7.5 (d) (note that due to the distorted shape of the specimen this section does not fully pass through the lumen of the proamniotic cavity and the distal-most group of *Sfmbt2*-expressing cells in fact forms part of the extraembryonic ectoderm near the anterior amniotic fold); *Ndrg1* at E6.5 (e) and E7.5 (f); *Plet1* at E6.5 (g), E7.5 (h) (note also that the distal-most extraembryonic expression represents extraembryonic ectoderm near the anterior amniotic fold) and in the node at E8.0 (i); and *Talia* at E7.5 (j).

Table 1: Summary of genes with restricted expression patterns

| NIA clone ID	Gene name	Tissue with specific expression
H3001E07-3	*Peg10*	proximal VE
H3017E04-3	*Ctsz*	proximal VE
H3004E08-3	*Cubilin*	proximal VE
H3041C04-3	*Jarid1b*	epiblast + ectoplacental cone
H3031C12-3	*Ndrg1*	extraembryonic ectoderm + node
H3001A06-3	*Sfmbt2*	extraembryonic ectoderm
H3016G11-3	*Gjb5*	extraembryonic ectoderm
H3001D07-3	*Talia*	extraembryonic ectoderm
H3011D11-3	*Plet1*	extraembryonic ectoderm + node
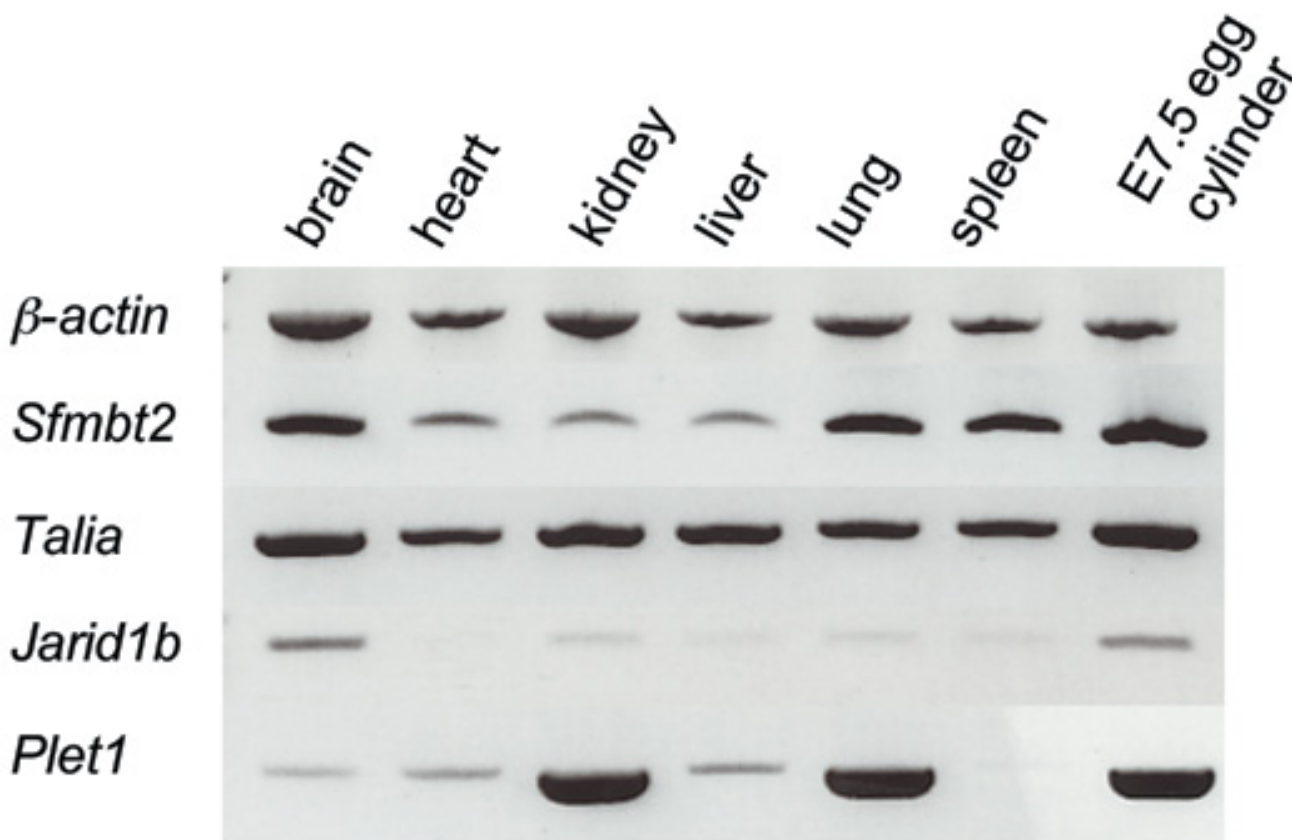
Figure 4
RT-PCR of selected genes showing expression in adult tissues compared with whole E7.5 embryo (negative image). *β-actin*
expression (first row) was used as a control.

region at the amino acid level from each of a broad range
of metazoans, including *Gallus gallus*, *Xenopus spp.*, *Danio
rerio*, *Drosophila melanogaster*, *Caenorhabditis elegans* and
Hydra magnipapillata, suggesting a highly conserved func-
tion for this gene among metazoans. Alignment of
sequences indicated amino acid conservation was maxi-
mal within the region corresponding to exon 3 of *Talia*
(Fig. 6).

Talia/TALIA appears to be specific to mammals, being
more divergent than *XE7* from homologues in other meta-
zoans, and apparently represents the only conserved evo-
lutionary duplication of the ancestral gene detectable in
genomic databases. Functional orthologues of *Talia* in pig
and rat are supported by EST evidence, however human
TALIA is apparently not expressed, as no corresponding
ESTs were identified in existing databases. Furthermore,
the human genomic sequence (LOC139516) contains
two premature in-frame stop codons (corresponding to

positions 234 and 348 in Fig. 6), suggesting that it is non-
functional. Conversely, many more ESTs for human *XE7*
appeared to be present in databases compared with those
for murine *Xe7*, raising the possibility that mammalian
XE7 and *TALIA* have overlapping roles and may variously
substitute for one another in different species.

Voland *et al*. [25] identified several potential functional
motifs within XE7, including a putative leucine zipper
domain, transmembrane domain and N-glycolsylation
sites. However these motifs are not conserved in either
Talia or *XE7* from different species. Moreover, comparison
of the predicted amino acid sequences of *Talia* and *XE7*
with protein databases revealed a significant similarity
within the conserved region to the first two tandem RNA
recognition motifs (RRMs) of Hu proteins, which bind to
adenosine-uridine-rich elements (AREs) in the 3' untrans-
lated regions of mRNAs [27]. The predicted secondary
structure of this region of Talia is similar to the known sec-

```
GGAGCTCTCGAGGGGAGTCAATTGGTCCGCTGGAGCCCACCAAGGGATTGGAGCAGGCAGGCGTGCAAACCTGCACCCTGTAGCCCCCAG        90
CCAGGAATCAGGAGGCAGCCACCTGGAAGCTCACGGGGCCTGCAGCGGGGCGAGGGGCGCTCGGGGCGTTGGCACGAGCGCGCGCGCCTG       180
CGTCGCCTTCTCGGATGCGGCGAGGACGACTCTGTGTCGCGACCTGAGCCATTCAACAGCTGGCCTCGCCCGCGGCGGTGGAAGAGAGCG       270
ACCAGGAGGGTTGATCCTTGACACTGCGGCGGCTGGAGGGAGGCGGCGGCAGGAGCGGTAGCGGTTCCGCTACCCCAGCGGAGCTAAGCG       360
TGAAGTGTCCCTTGCTTAGGATGCCACAGCACAGTGCTCGCCATGAAGAGTTCCCATGAATCGTGGCCTTTGTGATATTTTCTTCTTTGA       450
CACATTTTCTGCATAAGGTGGTGTGAGGACACCATGACAGTCACAGTGGTTTATGATAATCTTGAGGCAACAGAGCTCTGTGCTGCTCAG       540
                              M  T  V  T  V  V  Y  D  N  S  E  A  T  E  L  C  A  A  Q
CACCTCTACCTCAAGCCTATAGCAAAATTAATGATCAACGTGTTGCTCCCAGAATGTATAGAACCTGTGAGGCCCTTCTCTAACTGGGAA       630
 H  L  Y  L  K  P  I  A  K  L  M  I  N  V  L  L  P  E  C  I  E  P  V  R  P  F  S  N  W  E
GTTCTTGACCAACTGAAAAGTCTGATTTGCCCTGACCAGTTTACCACAGTGCGCCTCTCCAAGAGTACGAAAGACTTCATCCGATTTGAG       720
 V  L  D  Q  L  K  S  L  I  C  P  D  Q  F  T  T  V  R  L  S  K  S  T  K  D  F  I  R  F  E
GGTGAGGCTGAAACACGAAGTTTGGTTCAGATCCTGAAAGCCAAGTTACATGGGAAGATCATCAAGCTTAATGGTTTGAAAACTGACTTA       810
 G  E  A  E  T  R  S  L  V  Q  I  L  K  A  K  L  H  G  K  I  I  K  L  N  G  L  K  T  D  L
AAAGTAGTAGCGACAGATGCCCAGGGAGAGTGGGAACACTTCCCAAAAGAGAAAGAGGCCTCAGTGATTGAGGGAGCTGAAGAACAAGAC       900
 K  V  V  A  T  D  A  Q  G  E  W  E  H  F  P  K  E  K  E  A  S  V  I  E  G  A  E  E  Q  D
CATGATAAGGGCCCTGATTCTATCTACTTTGAAGGCTTGCCCTGTAAGTGGTTTGCACCTAAAGGCTCCAGTGGAGAGAAGCCCTGTGAG       990
 H  D  K  G  P  D  S  I  Y  F  E  G  L  P  C  K  W  F  A  P  K  G  S  S  G  E  K  P  C  E
GAGATCCTCCGAGTAGTCTTTGAGAGTTTTGGGAAGATCAAGAATGTGGATATCCCCATGCTTGACCCCTACCGAGAAGTGATGACAGGT      1080
 E  I  L  R  V  V  F  E  S  F  G  K  I  K  N  V  D  I  P  M  L  D  P  Y  R  E  V  M  T  G
GGGAGCTTTGGGGGTTTGAACTTTGGCTTGCAAACATTCGAAGCCTTTATACAATACCAGGAGTCAACTGACTTTATAAAAGCCATGGAA      1170
 G  S  F  G  G  L  N  F  G  L  Q  T  F  E  A  F  I  Q  Y  Q  E  S  T  D  F  I  K  A  M  E
TCCCTTCGAGGAATGAAGCTGATGCTTAAAGGAGACGATGGCAAAGCTCTGGCATGTAATATTAAGGTTTATGTTTGATACCACCAAACAT      1260
 S  L  R  G  M  K  L  M  L  K  G  D  D  G  K  A  L  A  C  N  I  K  V  M  F  D  T  T  K  H
TTCAGTGAAGGAGCTATACAGAGGAGGAATCAGGAAAGGCTAAAATTACAGGAGCTGGAAGAAGAGAGGAAAAAAGAAAAGAAGAGAGAA      1350
 F  S  E  G  A  I  Q  R  R  N  Q  E  R  L  K  L  Q  E  L  E  E  E  R  K  K  E  K  K  R  E
GAGGAAGTAGCTGAAAGTAAAAAGAAAAGATGAAGTAAGGAAAGCCCAAGAAAAGAGAAAGAAGGCCAGGGACAGGAGGCGAGCCCTGAAG      1440
 E  E  V  A  E  R  K  R  K  D  E  V  R  K  A  Q  E  K  R  K  K  A  R  D  R  R  R  A  L  K
GAAAGAGACAGACACCGTCAAAGAAAGGAAAAAGTGAAAACCAAGCCTGAGGCCCGTCAGGAGCTGGACTCTTCGGGAGAGTGGGAGGAG      1530
 E  R  D  R  H  R  Q  R  K  E  K  V  K  T  K  P  E  A  R  Q  E  L  D  S  S  G  E  W  E  E
CGGAAGTACTTGCTGGCTCGGAGGCGGGTTGAAGCTCTTCGGTTACTCCGGGTACTCCTGAAGAAAATTGCAGTTTGGATGAGAAAAGAG      1620
 R  K  Y  L  L  A  R  R  R  V  E  A  L  R  L  L  R  V  L  L  K  K  I  A  V  W  M  R  K  E
AAGAAACCTCAAGCCTTGTCAAACACACATTAGCCTTCTCTTGAACTGTCTGGATCCACACAGTGTAATCCACAGGAAGTTGGTGTTATG      1710
 K  N  L  K  P  C  Q  T  H  I  S  L  L  L  N  C  L  G  S  T  Q  C  N  P  Q  E  V  G  V  M
AAGTCCGAAGCTCATCATACCCCAGGGACTGCATTGAAATCACCCGAGGAGGAAGAATTAAACACTCGGCATCTTCTAGATTGGGAAGAA      1800
 K  S  E  A  H  H  T  P  G  T  A  L  K  S  P  E  E  E  E  L  N  T  R  H  L  L  D  W  E  E
ATGCCAAGCTACCAGGCTTCCACACTTGATCTTAGTCAAAAGGCAGAGAAGCGATGCTATCAGGCTTCCAAGTCTGACAATAAACAAAAA      1880
 M  P  S  Y  Q  A  S  T  L  D  L  S  Q  K  A  E  K  R  C  Y  Q  A  S  K  S  D  N  K  Q  K
CAAAAAGGGAAGAAAAAAACTAAGGCTCAGTTACAGAAATCTTCACACACTCCTGGGGTGAAACAAATGAGACACTCAGCTAGAAAAGAA      1970
 Q  K  G  K  K  K  T  K  A  Q  L  Q  K  S  S  H  T  P  G  V  K  Q  M  R  H  S  A  R  K  E
GAAACTGGAAAAGTGTTAAGTGATGGGTGTGATTACAGTTTGGTCTCTGACCAGAATTCTTTACAGAGTACAATGATTGCAGATCAGTCC      2020
 E  T  G  K  V  L  S  D  G  C  D  Y  S  L  V  S  D  Q  N  S  L  Q  S  T  M  I  A  D  Q  S
CTTGCAAAGGAAGACCACGCCAGTTCTAATGAATCCCATTCTTCTAGACAAGCTGTGATCAACCGTGGCAGGAAACAGAAGATCTATGAA      2110
 L  A  K  E  D  H  A  S  S  N  E  S  H  S  S  R  Q  A  V  I  N  R  G  R  K  Q  K  I  Y  E
ACAGATGAATTTATTGACTACCTATTAAACTATTATCAGACTCCAGAGTATGCCCGATTCTGTCTTGAAGCAAGTCACCTAACAAGCACA      2200
 T  D  E  F  I  D  Y  L  L  N  Y  Y  Q  T  P  E  Y  A  R  F  C  L  E  A  S  H  L  T  S  T
TGTCAGTGGCAGAGAGATGTCTACGCTAAGGGGGATGGCTTTCAGATCTATTTAAGACAAACAGGGGTACCACTCAAACAGCCTGAGTGAA      2290
 C  Q  W  Q  R  D  V  Y  A  K  G  D  G  F  Q  I  Y  L  R  K  Q  G  Y  H  S  N  S  L  S  E
GAGGAGAGCCTGCAAGGGATAGAGCAGGACGAAGAGCAGGACTGGCCACAAGTGTATATTAAAGATCCTGAGAGTAAGTCGCAAAAGACA      2380
 E  E  S  L  Q  G  I  E  Q  D  E  E  Q  D  W  P  Q  V  Y  I  K  D  P  E  S  K  S  Q  K  T
GGCAAGATCAATTATGCCAAAGAATTTACAAAGAAATTAAAAAACCATTGCAAGGACATAGCAAGTGAAACTGATGAGCACCTGTCTCCA      2470
 G  K  I  N  Y  A  K  E  F  T  K  K  L  K  N  H  C  K  D  I  A  S  E  T  D  E  H  L  S  P
ACTGATGGAGAGAGCTATCCTGTGGAAAAGATTCACAGTCACGGAGTCAAAGATTCTCAACACATAAGAAAAAGCCCAGTTTCCTCATCA      2560
 T  D  G  E  S  Y  P  V  E  K  I  H  S  H  G  V  K  D  S  Q  H  I  R  K  S  P  V  S  S  S
TTAAGGTCAGTAGATGTAGGTTTGCCATTGGCAGACTTCTTGGAGGAAATTAGTAGCGATTCTGAGTGCTTCAGTGAAACTCTTAGCATG      2650
 L  R  S  V  D  V  G  L  P  L  A  D  F  L  E  E  I  S  S  D  S  E  C  F  S  E  T  L  S  M
AACCAAGAGGAGGAGGAAAGGTCTATGGCTGTCTATAATAGCTCCCCGGAAAAAAGACCTCTTGGTACTGACAAAATCAGTACTTGCAAA      2730
 N  Q  E  E  E  R  S  M  A  V  Y  N  S  S  P  E  K  R  P  L  G  T  D  K  I  S  T  C  K
CAGAAAACGAAGTCGAGTCAGCAGACCTTGTGTTCAGAGTGGAAACATGACAATGAGGGAAAATACTCTAAACACAGAGTTAGGGCACCA      2800
 Q  K  T  K  S  S  Q  Q  T  L  C  S  E  W  K  H  D  N  E  G  K  Y  S  K  H  R  V  R  A  P
GGTAAAAGGTCCAAGTCTGAACTGAGATACTCGTGGCTTGAGGAGGAAGGTGATTCCATTAGAGTTGACAAGAACATAAGCAAAACTAGG      2880
 G  K  R  S  K  S  E  L  R  Y  S  W  L  E  E  E  G  D  S  I  R  V  D  K  N  I  S  K  T  R
GGGAAACTAGCCCCGAAACACATGCTTGATGAAGGCCAGTACCATGAGTCCAGTTCCAGTGATGAATTAGAAAGCACCACGAGGAAGAAG      2970
 G  K  L  A  P  K  H  M  L  D  E  G  Q  Y  H  E  S  S  S  D  E  L  E  S  T  T  R  K  K
AGGAGACTTACTAGCGGTATGTTTGACCAGAATGTGAAATACAGGCCCCTTCATATGCCATCAATGCCGCCAAAACGGGCTAGAGCATTC      3060
 R  R  L  T  S  G  M  F  D  Q  N  V  K  Y  R  P  L  H  M  P  S  M  P  P  K  R  A  R  A  F
TATTTGGGATATTTCTCCAGGAAAAGGCAGACTCCGTGGAAGTCAGAATATAACCAGGATGTAAGAAGGTTCAAAAGATATGAAAATTCT      3150
 Y  L  G  Y  F  S  R  K  R  Q  T  P  W  K  S  E  Y  N  Q  D  V  R  R  F  K  R  Y  E  N  S
GAAGATTACACGCTTGATTCTGATAATTACTATGTCAGTCACGATGACAATCAGGAGCCACATTGATGATGAGCTACTTAGGAGACAGAC      3240
 E  D  Y  T  L  D  S  D  N  Y  Y  V  S  H  D  D  N  Q  E  H  I  E  Y  G  S  Y  L  G  D  S
TACTCTAGTTCTTTGTATTTTAAAATGTTCTGACGGCCACTTTGAGCAGGAACACCCTTATTTACAAGAAACAGTGGACGGGAAGAAAAA... 3330
 Y  S  S  S  L  Y  F  K  M  F  *
```

Figure 5
Predicted coding region of mouse *Talia*. The start ATG codon is underlined, immediately following a Kozak translation initiation motif (bold). Exon-exon boundaries are indicated by I-shaped separators.
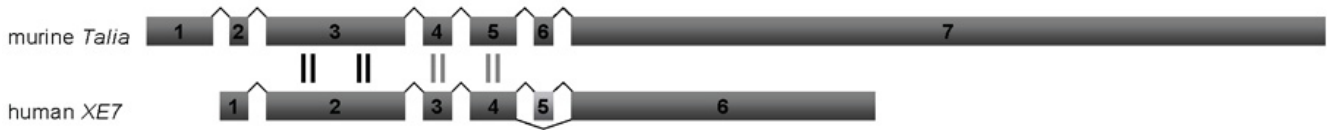
Figure 6
Comparison of the exon structure and homology of mouse *Talia* with human *XE7*. Homology between exons is indicated by dashed vertical bars, with the highest sequence conservation between exon 3 of *Talia* and exon 2 of *XE7* (darker bars). Two splice variants have been demonstrated for *XE7*, as indicated. The inclusion of exon 5, which contains two in-frame stop codons, is predicted to result in a truncated protein [23]. (Relative scaling of exon sizes are only approximate.)
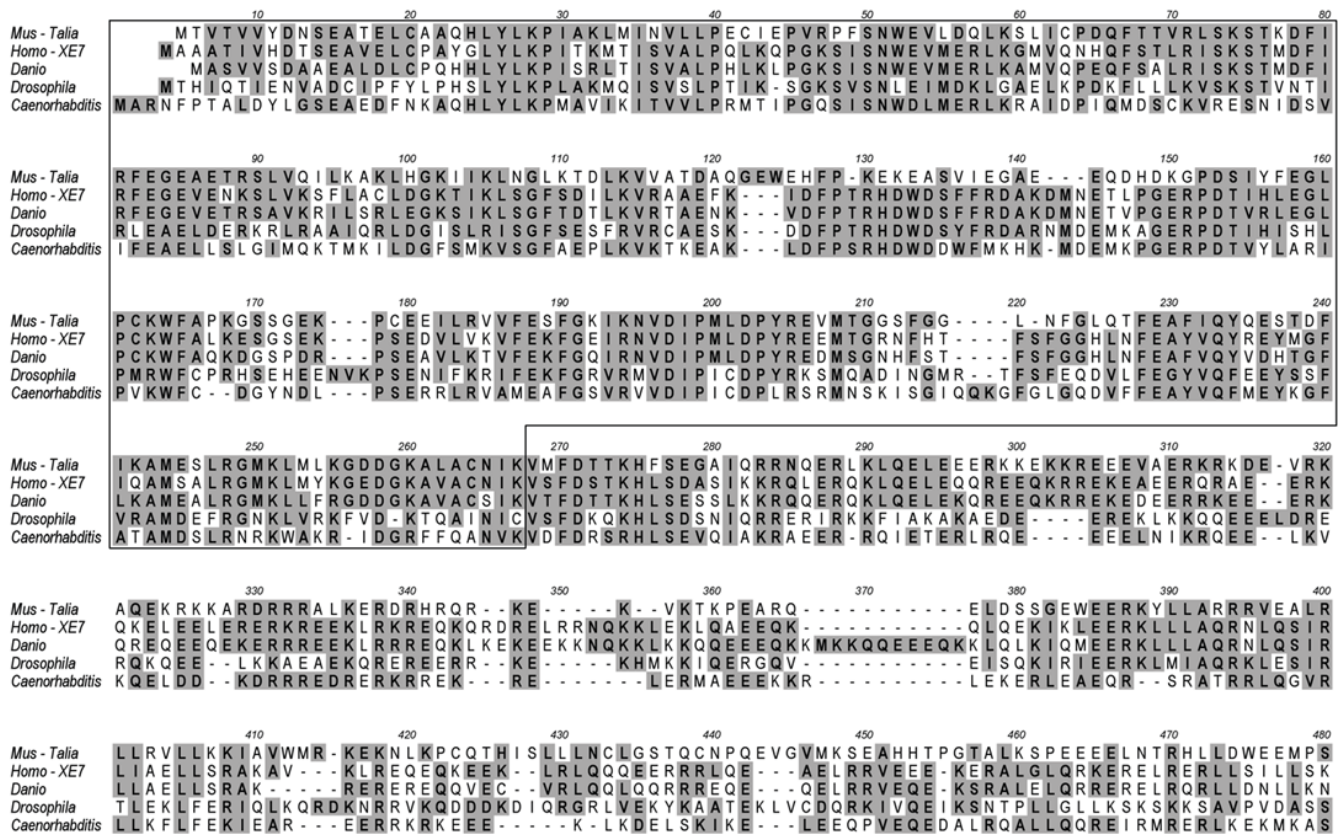

Figure 7
(a) Alignment of predicted amino acid sequences from *Talia*, *XE7* and homologues from other metazoans within the region of homology. The most highly conserved region, corresponding to exon 3 of *Talia*, is boxed. Highly conserved amino acids (in > 50% of sequences) are highlighted. Human *TALIA* (not shown) includes two "stop" codons at the equivalent of positions 234 and 248 in the alignment. Accession numbers of nucleotide sequences, from which protein sequences were predicted, were: *Talia* (*Mus musculus*) – AW261571; *XE7* (*Homo sapiens*) – NM_005088; *Danio rerio* – NM_200682; *Drosophila melanogaster* – NM_169093; *Caenorhabditis elegans* – NM_066250.

ondary structure of HuD (Fig. 8), which consists of a β1-α1-β2-β3-α2-β4 topology within each RRM [28]. However, the residues of HuD that were shown to interact with ARE sequences are not conserved in Talia or XE7, suggesting that they interact with different target sequences.

Discussion

This study identified a number of genes with previously unreported differential expression in the early postimplantation mouse embryo. Several represent new candidates for genes involved in tissue interactions controlling early events upon implantation. Three genes identified in the screen – *Peg10*, *Ctsz* and *Cubilin* – showed specific expression in the visceral endoderm of the egg cylinder. The human orthologue of *Peg10* was originally identified as a paternally expressed imprinted gene with homologies in two open reading frames to gag and pol proteins of some vertebrate retrotransposons [29]. It forms part of a novel imprinted gene cluster on human chromosome 7 [30] and mouse chromosome 6 [31] and has been shown to have oncogenic activity in hepatoma cells, suggesting a role in cell proliferation [32]. Cathepsin Z, encoded by *Ctsz*, is a member of the C1 family of cysteine proteases of unknown function. The gene lies proximal to a cluster of imprinted genes but is not itself imprinted [33]. Previously, *Ctsz* was reported as ubiquitously expressed in adult tissues [34]. This study, however, shows that *Ctsz* has tissue-specific expression at least in the early postimplantation embryo.

Cubilin encodes a multiligand endocytic receptor involved in uptake of low density lipoproteins and is present in absorptive epithelia of the ileum, kidney and visceral yolk sac [reviewed in: [35,36]]. Its lateral endodermal expression overlying the epiblast at both E6.5 and E7.5 is of interest as it may reflect the movements of this tissue during early gastrulation (Thomas et al, 1998; Perea-Gomez et al, 2001). While no antero-posterior asymmetry was evident in the expression of *Cubilin* alone, it would be interesting to investigate the degree to which it overlaps with AVE markers such as *Lefty1* [37,38], *Cer1* [39] and particularly *Dkk1*, which is expressed in the more proximal part of the embryonic VE from E5.25 and of the later AVE [40].

Jarid1b showed expression in epiblast and the outer part of the ectoplacental cone at E5.5, however by E6.5 epiblast expression was weak or undetectable. *Jarid1b* encodes a nuclear protein that was originally identified in human breast cancer cell lines [41]. In normal adult tissues of human and mouse, expression is largely restricted to testis and ovary [41,42]. In E12.5-15.5 mouse embryos, expression occurs in a spatially restricted pattern that overlaps with those of Bf-1 and Pax-9 [42,43], with which PLU-1 has been shown to interact [44].

Three genes – *Ndrg1*, *Sfmbt2* and *Gjb5* – identified in the screen showed specific expression throughout the extraembryonic ectoderm at both E5.5 and E6.5. *Ndrg1* was originally identified by its upregulation in *N-myc* deficient mice [45]. While its precise function remains obscure, it has been reported to be involved in cell growth and differentiation [45-48]. Sfmbt2 is related to the *Drosophila* polycomb group of transcriptional repressors, which regulate
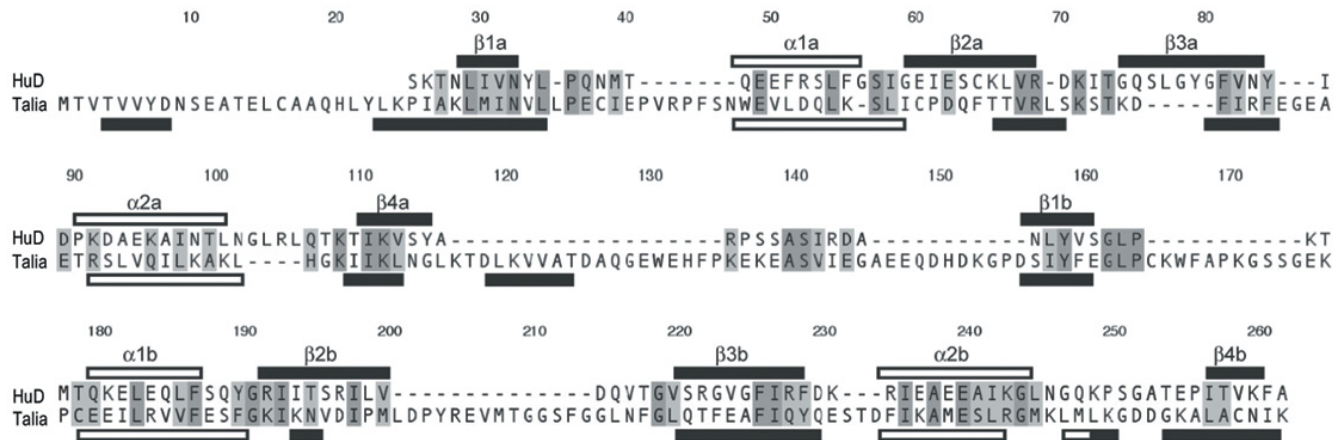

Figure 8
Predicted primary and secondary structures of Talia aligned with the known structure of HuD protein [28]. Dashes are introduced for the purpose of alignment. Talia shows a similar arrangement of predicted α-helices (open rods) and β-strands (closed rods) to that of HuD, which contains two repeated β1-α1-β2-β3-α2-β4 motifs separated by a linker region, and strongly suggests a similar function to HuD in binding to the 3' UTRs of mRNAs. High divergence in primary structure, however, suggests they differ in their target sequences.

homeotic and other genes [49-51]. The closely related murine gene *Sfmbt1* (= *Sfmbt*) was shown to be highly expressed in adult testis, with a much lower expression in other adult and late embryonic tissues [52]. *Sfmbt2* has not been characterised, however database searches of matching ESTs indicate expression in testis and germ cells, suggesting that Sfmbt1 and Sfmbt2 may have related roles. *Gjb5*, which showed very strong specific expression in the extra-embryonic ectoderm, encodes one of a large family of gap junction proteins. A number of other gap junction proteins also show spatially restricted expression during early post-implantation development, indicative of a role in establishing communication compartments [53,54], whilst *Gjb5* expression has previously been shown in preimplantation embryos [55]. While the roles of gap junctions during early development remain unclear, it is possible they may help to facilitate the transduction of signalling molecules within tissues.

Two genes – *Plet1* and *Talia* – showed localised expression in the extraembryonic ectoderm that may suggest a role in the interactions between epiblast and extraembryonic ectoderm. Signals from this region of the egg cylinder are believed to regulate proximo-distal patterning of the epiblast via such factors as Nodal, Cripto and Otx2 [5,17,56-62]. Proximo-distal signaling contributes to anterior-posterior patterning via asymmetric cell movements that position the AVE opposite the site of primitive streak formation. The specific identity of extraembryonic ectoderm adjacent to the epiblast is thought to be regulated by Fgf4 signaling via activation of the Erk pathway (reviewed [63]). Thus it is likely that expression of *Plet1* and *Talia* is regulated downstream of this signaling, and may have roles in specifying this identity. In particular, the restricted expression of *Plet1* from E5.5 appears to be earlier than has been reported for other genes such as *Eomes* [64] and *Bmp4* [65], suggesting that *Plet1* expression may be directly regulated by this pathway. Recently identified by its trophoblast-specific expression at later stages of mouse, pig and human [66,67], no functional motifs are evident to suggest a role for the protein.

By contrast, we have shown from its predicted tertiary structure that Talia is likely to function as a post-transcriptional regulator due to its similarities with HuD, an RNA-binding protein shown to have a role in specifying neural cell identity [68]. Interestingly the role of Talia is possibly substituted by a homologue, XE7, in humans. The high conservation of orthologues of *Talia/XE7* amongst metazoans further supports an essential role in development.

Conclusion
This study demonstrated for the first time the application of a microarray strategy for identifying genes that are differentially expressed between dissected parts of the early post-implantation mouse embryo. It successfully identified several genes, both known and previously uncharacterised, with novel expression patterns in the early mouse post-implantation embryo. Some of these, such as *Talia* and *Plet1*, will be of particular interest for further analysis, particularly with respect to possible roles in specifying the identity of extraembryonic ectoderm adjacent to the epiblast or in signaling to the proximal epiblast.

Methods
Tissue collection
Fifty E5.5 embryos from F1 (C57/BL6 × CBA) females crossed with F1 males were collected into M2 medium. After removal of Reichert's membrane, embryos were cut at the embryonic-extraembryonic boundary into proximal and distal halves, using a finely drawn glass needle, respectively pooled, frozen on dry ice and stored at -80C until RNA extraction.

Preparation of labelled target cDNA
Total RNA was extracted using an RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Target cDNA synthesis and single primer amplification (SPA) followed by labelling with Cy5- or Cy3-dCTP were performed as previously described [69]. Labelled target cDNA was hybridised to an array of plates 3001–3048 of the NIA 15 K mouse cDNA set [70] spotted in duplicate (4608 ESTs, 9216 spots) on CMT-GAPS-coated slides (Corning) and analysed as previously described [69]. Candidate genes were selected on the basis of both ratio and absolute difference in hybridisation level of each of the target cDNAs. Clone names used below can be identified via the NIA/NIH Mouse Genomics website [71].

Whole mount *in situ* hybridisation
E5.5 and E6.5 embryos were collected as above and, after removal of Reichert's membrane, fixed in 4% paraformaldehyde in phosphate buffered saline overnight at 4°C. DNA templates were prepared by PCR from plasmid DNA using T3, SP6 and T7 promoter-specific primers. Plasmid templates for each probe were either transcribed directly from NIA clones (corresponding to those used in the array) or were cloned by RT-PCR from E6.5 embryo mRNA into the *Eco*RI and *Sal*I sites of pBluescript II KS(+). Respective forward and reverse primers for the latter were: *Jarid1b*, 5'-GGAATTCGGGTTGCTTGCTTCTGCTTCTTC and 5'-GCGTCGACATCAGGGGAAACTGGTATCGGC; *Gjb5*, 5'-GGAATTCCTACCTCTTCCACGCATTCTATCC and 5'-GCGTCGACAGGCATTTGCTCATCGGTGC; *Sfmbt2*, 5'-GGAATTCGTCTCTGGGGACATCTACTGCTTG and 5'-GCGTCGACTGCTCTGCCTCGGTTCTGTG; *Ndrg1*, 5'-GGAATTCGAGAGAGAGAGGCAGGAAAGTTGG and 5'-GCGTCGACTACAAACCCAGTCAGCAGGAGG; *Cubilin*, 5'-GGAATTCAACCTTGCCCGTGTTCTATTCC and 5'-GCGTCGACTGAAGACCCGATTTGATGAAGC; *Talia*

(exon 7), 5'-GGAATTCATCCTGGCACATCAATAATGGC and 5'-GCGTCGACAAGTAACCCCACAGACTGACATCC; *Talia* (exons 3–4), CATTTTCTGCATAAGGTGGTGTGAG-GAC and GCCTGATAGCATCGCTTCTCTGCC;*Plet1*, 5'-GGAATTCCTGAAAGCAGTGAAGGAGGACG and 5'-GCGTCGACCACGCAGGATGGATGGACTAAG.

Digoxygenin-labelled antisense RNA probes were prepared using an Ambion MegaScript transcription kit (SP6, T3 or T7) according to the manufacturer's instructions. *In situ* hybridisation was performed as described by Wilkinson and Nieto [72].

Sequence analysis
Genomic structure was analysed using the Genomatix web-based sequence analysis software[26]. ESTs were searched using BLAST and sequence alignments performed using MacVector. Protein secondary structure prediction and similarity searches were performed using the 3D-PSSM web-based software [73,74].

Analysis of expression by RT-PCR
Total RNA was extracted from adult female mouse tissues and pooled E7.5 egg cylinders using the RNeasy Mini Kit (QIAGEN) according to the manufacturers instructions. Oligo-dT(20)-primed first strand cDNA was prepared from 0.5 μg of total RNA in a 10-μl reaction volume using SuperScript III (Invitrogen) at 50°C for 1 hour according to the manufacturer's instructions. 0.5 μL of template was then used for each 15-μL PCR reaction mixture containing 0.05 Units/μL GoTaq polymerase (Promega), 1× supplied PCR buffer, 0.25 mM dNTPs and 0.5 μM each of forward and reverse gene-specific primers. 35 cycles of PCR were performed comprising 15 seconds denaturation (94°C), 15 seconds annealing (55°C) and 30 seconds extension (72°C). 8 μL of each PCR product was separated by electrophoresis in a 1.5% agarose gel containing ethidium bromide.

Authors' contributions
SF performed all collection and experimental work on mouse embryos and writing of the manuscript. SF, with the crucial assistance of LS, performed the microarray screen and data analysis. AG was involved in facilitating and coordinating the microarray experiments. MZG was involved in the conception and coordination of this project. All authors approved the final manuscript.

Acknowledgements

References
1. Thomas PQ, Brown A, Beddington RS: **Hex: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors.** *Development* 1998, **125(1):**85-94.
2. Weber RJ, Pedersen RA, Wianny F, Evans MJ, Zernicka-Goetz M: **Polarity of the mouse embryo is anticipated before implantation.** *Development* 1999, **126(24):**5591-5598.
3. Rivera-Perez JA, Mager J, Magnuson T: **Dynamic morphogenetic events characterize the mouse visceral endoderm.** *Dev Biol* 2003, **261(2):**470-487.
4. Srinivas S, Rodriguez T, Clements M, Smith JC, Beddington RS: **Active cell migration drives the unilateral movements of the anterior visceral endoderm.** *Development* 2004, **131(5):**1157-1164.
5. Yamamoto M, Saijoh Y, Perea-Gomez A, Shawlot W, Behringer RR, Ang SL, Hamada H, Meno C: **Nodal antagonists regulate formation of the anteroposterior axis of the mouse embryo.** *Nature* 2004, **428(6981):**387-392.
6. Beddington RS, Robertson EJ: **Axis development and early asymmetry in mammals.** *Cell* 1999, **96(2):**195-209.
7. Liu P, Wakamiya M, Shea MJ, Albrecht U, Behringer RR, Bradley A: **Requirement for Wnt3 in vertebrate axis formation.** *Nat Genet* 1999, **22(4):**361-365.
8. Zernicka-Goetz M: **Patterning of the embryo: the first spatial decisions in the life of a mouse.** *Development* 2002, **129(4):**815-829.
9. Wang QT, Piotrowska K, Ciemerych MA, Milenkovic L, Scott MP, Davis RW, Zernicka-Goetz M: **A genome-wide study of gene activity reveals developmental signaling pathways in the pre-implantation mouse embryo.** *Dev Cell* 2004, **6(1):**133-144.
10. Sharov AA, Piao Y, Matoba R, Dudekula DB, Qian Y, VanBuren V, Falco G, Martin PR, Stagg CA, Bassey UC, Wang Y, Carter MG, Hamatani T, Aiba K, Akutsu H, Sharova L, Tanaka TS, Kimber WL, Yoshikawa T, Jaradat SA, Pantano S, Nagaraja R, Boheler KR, Taub D, Hodes RJ, Longo DL, Schlessinger D, Keller J, Klotz E, Kelsoe G, Umezawa A, Vescovi AL, Rossant J, Kunath T, Hogan BL, Curci A, D'Urso M, Kelso J, Hide W, Ko MS: **Transcriptome analysis of mouse stem cells and early embryos.** *PLoS Biol* 2003, **1(3):**E74.
11. Hamatani T, Carter MG, Sharov AA, Ko MS: **Dynamics of global gene expression changes during mouse preimplantation development.** *Dev Cell* 2004, **6(1):**117-131.
12. Sousa-Nunes R, Rana AA, Kettleborough R, Brickman JM, Clements M, Forrest A, Grimmond S, Avner P, Smith JC, Dunwoodie SL, Beddington RS: **Characterizing embryonic gene expression patterns in the mouse using nonredundant sequence-based selection.** *Genome Res* 2003, **13(12):**2609-2620.
13. Shimono A, Behringer RR: **Differential screens with subtracted PCR-generated cDNA libraries from subregions of single mouse embryos.** *Methods Mol Biol* 2000, **136:**333-344.
14. Ko MS, Threat TA, Wang X, Horton JH, Cui Y, Pryor E, Paris J, Wells-Smith J, Kitchen JR, Rowe LB, Eppig J, Satoh T, Brant L, Fujiwara H, Yotsumoto S, Nakashima H: **Genome-wide mapping of unselected transcripts from extraembryonic tissue of 7.5-day mouse embryos reveals enrichment in the t-complex and under-representation on the X chromosome.** *Hum Mol Genet* 1998, **7(12):**1967-1978.
15. Harrison SM, Dunwoodie SL, Arkell RM, Lehrach H, Beddington RS: **Isolation of novel tissue-specific genes from cDNA libraries representing the individual tissue constituents of the gastrulating mouse embryo.** *Development* 1995, **121(8):**2479-2489.
16. Simeone A, Acampora D, Mallamaci A, Stornaiuolo A, D'Apice MR, Nigro V, Boncinelli E: **A vertebrate gene related to orthodenticle contains a homeodomain of the bicoid class and demarcates anterior neuroectoderm in the gastrulating mouse embryo.** *Embo J* 1993, **12(7):**2735-2747.
17. Ding J, Yang L, Yan YT, Chen A, Desai N, Wynshaw-Boris A, Shen MM: **Cripto is required for correct orientation of the anterior-posterior axis in the mouse embryo.** *Nature* 1998, **395(6703):**702-707.
18. Watanabe D, Suetake I, Tada T, Tajima S: **Stage- and cell-specific expression of Dnmt3a and Dnmt3b during embryogenesis.** *Mech Dev* 2002, **118(1-2):**187-190.
19. Rosner MH, Vigano MA, Ozato K, Timmons PM, Poirier F, Rigby PW, Staudt LM: **A POU-domain transcription factor in early stem**

cells and germ cells of the mammalian embryo. *Nature* 1990, **345(6277):**686-692.

20. Grummer R, Reuss B, Winterhager E: **Expression pattern of different gap junction connexins is related to embryo implantation.** *Int J Dev Biol* 1996, **40(1):**361-367.

21. Lin TP, Labosky PA, Grabel LB, Kozak CA, Pitman JL, Kleeman J, MacLeod CL: **The Pem homeobox gene is X-linked and exclusively expressed in extraembryonic tissues during early murine development.** *Dev Biol* 1994, **166(1):**170-179.

22. Lee JE, Pintar J, Efstratiadis A: **Pattern of the insulin-like growth factor II gene expression during early mouse embryogenesis.** *Development* 1990, **110(1):**151-159.

23. Ellison JW, Ramos C, Yen PH, Shapiro LJ: **Structure and expression of the human pseudoautosomal gene XE7.** *Hum Mol Genet* 1992, **1(9):**691-696.

24. Ellison J, Passage M, Yu LC, Yen P, Mohandas TK, Shapiro L: **Directed isolation of human genes that escape X inactivation.** *Somat Cell Mol Genet* 1992, **18(3):**259-268.

25. Voland JR, Wyzykowski RJ, Huang M, Dutton RW: **Cloning and sequencing of a trophoblast-endothelial-activated lymphocyte surface protein: cDNA sequence and genomic structure.** *Proc Natl Acad Sci U S A* 1992, **89(21):**10425-10429.

26. **Genomatix** [http://www.genomatix.de/]

27. Chung S, Jiang L, Cheng S, Furneaux H: **Purification and properties of HuD, a neuronal RNA-binding protein.** *J Biol Chem* 1996, **271(19):**11518-11524.

28. Wang X, Tanaka Hall TM: **Structural basis for recognition of AU-rich element RNA by the HuD protein.** *Nat Struct Biol* 2001, **8(2):**141-145.

29. Ono R, Kobayashi S, Wagatsuma H, Aisaka K, Kohda T, Kaneko-Ishino T, Ishino F: **A retrotransposon-derived gene, PEG10, is a novel imprinted gene located on human chromosome 7q21.** *Genomics* 2001, **73(2):**232-237.

30. Okita C, Meguro M, Hoshiya H, Haruta M, Sakamoto YK, Oshimura M: **A new imprinted cluster on the human chromosome 7q21-q31, identified by human-mouse monochromosomal hybrids.** *Genomics* 2003, **81(6):**556-559.

31. Ono R, Shiura H, Aburatani H, Kohda T, Kaneko-Ishino T, Ishino F: **Identification of a large novel imprinted gene cluster on mouse proximal chromosome 6.** *Genome Res* 2003, **13(7):**1696-1705.

32. Okabe H, Satoh S, Furukawa Y, Kato T, Hasegawa S, Nakajima Y, Yamaoka Y, Nakamura Y: **Involvement of PEG10 in human hepatocellular carcinogenesis through interaction with SIAH1.** *Cancer Res* 2003, **63(12):**3043-3048.

33. Bonthron DT, Hayward BE, Moran V, Strain L: **Characterization of TH1 and CTSZ, two non-imprinted genes downstream of GNAS1 in chromosome 20q13.** *Hum Genet* 2000, **107(2):**165-175.

34. Deussing J, von Olshausen I, Peters C: **Murine and human cathepsin Z: cDNA-cloning, characterization of the genes and chromosomal localization.** *Biochim Biophys Acta* 2000, **1491(1-3):**93-106.

35. Verroust PJ, Christensen EI: **Megalin and cubilin--the story of two multipurpose receptors unfolds.** *Nephrol Dial Transplant* 2002, **17(11):**1867-1871.

36. Kozyraki R: **Cubilin, a multifunctional epithelial receptor: an overview.** *J Mol Med* 2001, **79(4):**161-167.

37. Meno C, Gritsman K, Ohishi S, Ohfuji Y, Heckscher E, Mochida K, Shimono A, Kondoh H, Talbot WS, Robertson EJ, Schier AF, Hamada H: **Mouse Lefty2 and zebrafish antivin are feedback inhibitors of nodal signaling during vertebrate gastrulation.** *Mol Cell* 1999, **4(3):**287-298.

38. Perea-Gomez A, Shawlot W, Sasaki H, Behringer RR, Ang S: **HNF3beta and Lim1 interact in the visceral endoderm to regulate primitive streak formation and anterior-posterior polarity in the mouse embryo.** *Development* 1999, **126(20):**4499-4511.

39. Belo JA, Bouwmeester T, Leyns L, Kertesz N, Gallo M, Follettie M, De Robertis EM: **Cerberus-like is a secreted factor with neutralizing activity expressed in the anterior primitive endoderm of the mouse gastrula.** *Mech Dev* 1997, **68(1-2):**45-57.

40. Kimura-Yoshida C, Nakano H, Okamura D, Nakao K, Yonemura S, Belo JA, Aizawa S, Matsui Y, Matsuo I: **Canonical Wnt signaling and its antagonist regulate anterior-posterior axis polariza-

tion by guiding cell migration in mouse visceral endoderm.** *Dev Cell* 2005, **9(5):**639-650.

41. Lu PJ, Sundquist K, Baeckstrom D, Poulsom R, Hanby A, Meier-Ewert S, Jones T, Mitchell M, Pitha-Rowe P, Freemont P, Taylor-Papadimitriou J: **A novel gene (PLU-1) containing highly conserved putative DNA/chromatin binding motifs is specifically upregulated in breast cancer.** *J Biol Chem* 1999, **274(22):**15633-15645.

42. Barrett A, Madsen B, Copier J, Lu PJ, Cooper L, Scibetta AG, Burchell J, Taylor-Papadimitriou J: **PLU-1 nuclear protein, which is upregulated in breast cancer, shows restricted expression in normal human adult tissues: a new cancer/testis antigen?** *Int J Cancer* 2002, **101(6):**581-588.

43. Madsen B, Spencer-Dene B, Poulsom R, Hall D, Lu PJ, Scott K, Shaw AT, Burchell JM, Freemont P, Taylor-Papadimitriou J: **Characterisation and developmental expression of mouse Plu-1, a homologue of a human nuclear protein (PLU-1) which is specifically up-regulated in breast cancer.** *Gene Expr Patterns* 2002, **2(3-4):**275-282.

44. Tan K, Shaw AL, Madsen B, Jensen K, Taylor-Papadimitriou J, Freemont PS: **Human PLU-1 Has transcriptional repression properties and interacts with the developmental transcription factors BF-1 and PAX9.** *J Biol Chem* 2003, **278(23):**20507-20513.

45. Shimono A, Okuda T, Kondoh H: **N-myc-dependent repression of ndr1, a gene identified by direct subtraction of whole mouse embryo cDNAs between wild type and N-myc mutant.** *Mech Dev* 1999, **83(1-2):**39-52.

46. van Belzen N, Dinjens WN, Diesveld MP, Groen NA, van der Made AC, Nozawa Y, Vlietstra R, Trapman J, Bosman FT: **A novel gene which is up-regulated during colon epithelial cell differentiation and down-regulated in colorectal neoplasms.** *Lab Invest* 1997, **77(1):**85-92.

47. Piquemal D, Joulia D, Balaguer P, Basset A, Marti J, Commes T: **Differential expression of the RTP/Drg1/Ndr1 gene product in proliferating and growth arrested cells.** *Biochim Biophys Acta* 1999, **1450(3):**364-373.

48. Gomez-Casero E, Navarro M, Rodriguez-Puebla ML, Larcher F, Paramio JM, Conti CJ, Jorcano JL: **Regulation of the differentiation-related gene Drg-1 during mouse skin carcinogenesis.** *Mol Carcinog* 2001, **32(2):**100-109.

49. van Lohuizen M: **Functional analysis of mouse Polycomb group genes.** *Cell Mol Life Sci* 1998, **54(1):**71-79.

50. Schumacher A, Magnuson T: **Murine Polycomb- and trithorax-group genes regulate homeotic pathways and beyond.** *Trends Genet* 1997, **13(5):**167-170.

51. Gould A: **Functions of mammalian Polycomb group and trithorax group related genes.** *Curr Opin Genet Dev* 1997, **7(4):**488-494.

52. Usui H, Ichikawa T, Kobayashi K, Kumanishi T: **Cloning of a novel murine gene Sfmbt, Scm-related gene containing four mbt domains, structurally belonging to the Polycomb group of genes.** *Gene* 2000, **248(1-2):**127-135.

53. Liptau H, Viebahn C: **Expression patterns of gap junctional proteins connexin 32 and 43 suggest new communication compartments in the gastrulating rabbit embryo.** *Differentiation* 1999, **65(4):**209-219.

54. Dahl E, Winterhager E, Reuss B, Traub O, Butterweck A, Willecke K: **Expression of the gap junction proteins connexin31 and connexin43 correlates with communication compartments in extraembryonic tissues and in the gastrulating mouse embryo, respectively.** *J Cell Sci* 1996, **109 (Pt 1):**191-197.

55. Davies TC, Barr KJ, Jones DH, Zhu D, Kidder GM: **Multiple members of the connexin gene family participate in preimplantation development of the mouse.** *Dev Genet* 1996, **18(3):**234-243.

56. Kimura C, Shen MM, Takeda N, Aizawa S, Matsuo I: **Complementary functions of Otx2 and Cripto in initial patterning of mouse epiblast.** *Dev Biol* 2001, **235(1):**12-32.

57. Perea-Gomez A, Lawson KA, Rhinn M, Zakin L, Brulet P, Mazan S, Ang SL: **Otx2 is required for visceral endoderm movement and for the restriction of posterior signals in the epiblast of the mouse embryo.** *Development* 2001, **128(5):**753-765.

58. Perea-Gomez A, Vella FD, Shawlot W, Oulad-Abdelghani M, Chazaud C, Meno C, Pfister V, Chen L, Robertson E, Hamada H, Behringer RR, Ang SL: **Nodal antagonists in the anterior visceral endoderm prevent the formation of multiple primitive streaks.** *Dev Cell* 2002, **3(5):**745-756.

59. Kimura C, Yoshinaga K, Tian E, Suzuki M, Aizawa S, Matsuo I: **Visceral endoderm mediates forebrain development by suppressing posteriorizing signals.** *Dev Biol* 2000, **225(2):**304-321.
60. Varlet I, Collignon J, Robertson EJ: **nodal expression in the primitive endoderm is required for specification of the anterior axis during mouse gastrulation.** *Development* 1997, **124(5):**1033-1044.
61. Brennan J, Lu CC, Norris DP, Rodriguez TA, Beddington RS, Robertson EJ: **Nodal signalling in the epiblast patterns the early mouse embryo.** *Nature* 2001, **411(6840):**965-969.
62. Perea-Gomez A, Vella FD, Shawlot W, Oulad-Abdelghani M, Chazaud C, Meno C, Pfister V, Chen L, Robertson E, Hamada H, *et al.*: **Nodal antagonists in the anterior visceral endoderm prevent the formation of multiple primitive streaks.** *Dev Cell* 2002, **3:**745-756.
63. Ralston A, Rossant J: **How signaling promotes stem cell survival: trophoblast stem cells and Shp2.** *Dev Cell* 2006, **10(3):**275-276.
64. Ciruna BG, Rossant J: **Expression of the T-box gene Eomesodermin during early mouse development.** *Mech Dev* 1999, **81(1-2):**199-203.
65. Coucouvanis E, Martin GR: **BMP signaling plays a role in visceral endoderm differentiation and cavitation in the early mouse embryo.** *Development* 1999, **126(3):**535-546.
66. Zhao SH, Simmons DG, Cross JC, Scheetz TE, Casavant TL, Soares MB, Tuggle CK: **PLET1 (C11orf34), a highly expressed and processed novel gene in pig and mouse placenta, is transcribed but poorly spliced in human.** *Genomics* 2004, **84(1):**114-125.
67. Zhao SH, Tuggle CK: **Linkage mapping and expression analyses of a novel gene, placentally expressed transcript 1 (PLET1) in the pig.** *Anim Genet* 2004, **35(1):**72-74.
68. Akamatsu W, Fujihara H, Mitsuhashi T, Yano M, Shibata S, Hayakawa Y, Okano HJ, Sakakibara S, Takano H, Takano T, Takahashi T, Noda T, Okano H: **The RNA-binding protein HuD regulates neuronal cell identity and maturation.** *Proc Natl Acad Sci U S A* 2005, **102(12):**4625-4630.
69. Smith L, Underhill P, Pritchard C, Tymowska-Lalanne Z, Abdul-Hussein S, Hilton H, Winchester L, Williams D, Freeman T, Webb S, Greenfield A: **Single primer amplification (SPA) of cDNA for microarray expression analysis.** *Nucleic Acids Res* 2003, **31(3):**e9.
70. Tanaka TS, Jaradat SA, Lim MK, Kargul GJ, Wang X, Grahovac MJ, Pantano S, Sano Y, Piao Y, Nagaraja R, Doi H, Wood WH 3rd, Becker KG, Ko MS: **Genome-wide expression profiling of mid-gestation placenta and embryo using a 15,000 mouse developmental cDNA microarray.** *Proc Natl Acad Sci U S A* 2000, **97(16):**9127-9132.
71. **Laboratory of Genetics, NIA/NIH Mouse Genomics** [http://lgsun.grc.nia.nih.gov]
72. Wilkinson DG, Nieto MA: **Detection of messenger RNA by in situ hybridization to tissue sections and whole mounts.** *Methods Enzymol* 1993, **225:**361-373.
73. Kelley LA, Maccallum R, Sternberg MJE: **RECOMB 99, Proceedings of the Third Annual Conference on Computational Molecular Biology.** Edited by: Istrail S, Pevzner P, Waterman M. The Association for Computing Machinery, New York, New York 10036; 1999:218-225.
74. **3D-PSSM** [http://www.sbg.bio.ic.ac.uk/servers/3dpssm/]