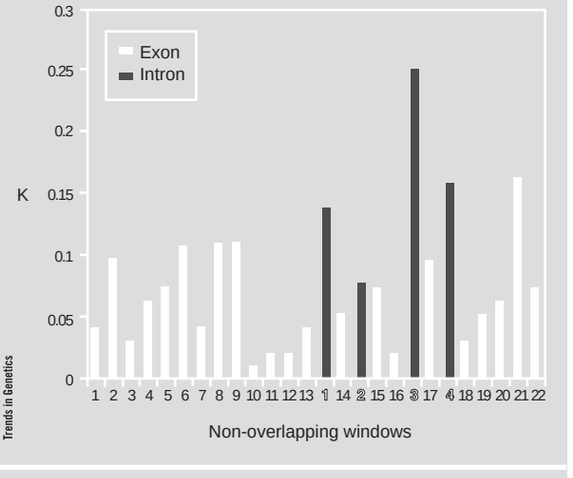
Molecular evolutionary evidence that *H19* mRNA is functional

A few mammalian genes are known to have expression dependent upon the sex of the parent from which they were inherited[1]. While most of these imprinted genes described in human and mouse code for proteins, a few [*H19* (Ref. 2), *Xist* (Ref. 3) and *IPW* (Ref. 4)] code only for RNA. Here we investigate the molecular evolution of one of these, *H19*, so as to determine whether the spliced RNA might have function.

The prevailing view[5] is that the cytoplasmic *H19* transcript does not have any function, although the situation is by no means resolved. The murine knockout has a growth phenotype[6] but it is uncertain whether this is related to the activity of the transcript, given that over-expression of the gene has no growth phenotype[7]. Furthermore, while it was speculated that the RNA may function in regulating *Igf2* imprinting[2], the replacement of the *H19* structural gene with a protein-coding gene shows this not to be so[8]. Similarly, a tumour suppressing role has been claimed[9], but these results could not be reproduced[10]. Most recently[5], it has been shown that the *H19* transcript is associated with polysomes and may be an antagonist of *Igf2* expressivity *in trans*. Here we show that molecular evolutionary comparison of the mouse and rat versions of the gene indicate that the RNA is under stabilizing selection and hence is most likely functional.

To show that a protein coding region is functional and under stabilizing selection, one would typically show that the rate at which the protein evolves is very much lower than the rate of evolution of the DNA at degenerate sites, that is, those sites where changes do not affect the protein. For noncoding RNAs this analysis is not possible. Instead we have performed two tests. First, we have compared the rate of evolution per base pair within the exons of *H19* with the rate of evolution at fourfold degenerate sites (i.e. those where all four nucleotides at the third site specify the same amino acid) in a large number of orthologous mouse–rat genes (orthology has been ensured through checking in Hovergen[11]). Assuming that the substitution rate at fourfold degenerate sites is a measure of the rate of evolution on exon sites that are free from selection, we can then ask whether the rate of evolution in *H19* is what one would expect were the sequence randomly selected from a sample of neutrally evolving genes. From a sample of 432 orthologous genes, we find that only three have a lower fourfold site substitution rate than *H19* has across all the bases in its exons ($P = 0.007$). Some of the variance in the distribution of fourfold site evolution is the result of differences in the sizes of the genes concerned (inclusion of small genes increases the variance). Restricting our analysis to the half of our data set with the longest complete coding sequence, we find now that no genes have lower rates of evolution ($P < 0.005$). The mean total exonic size in this large set is comparable to that in *H19* (although note that in coding sequences less than a third of all sites are fourfold degenerate ones, whereas we examine all sites in the exons of *H19*) (Fig. 1).

FIGURE 1. Substitution rates across *H19*



The exonic parts of the gene are split into non-overlapping windows 100 bp long (numbered 1 to 22). The introns are analysed whole and numbered in order. We used ClustalW[15] to align the intronic and exonic sequences of *H19* reported for rat (X59864) and mice (AF049091). The Y axis is the rate of substitution (K) using Tamura92[16] as the method of adjusting for multiple hits. Note that all introns have higher substitution rates than their flanking exons.

The above result may either be because *H19* is under stronger stabilizing selection than fourfold degenerate sites or has a low mutation rate. The latter may be quite reasonable given that, as a class, imprinted genes tend to have low synonymous substitution rates[12]. Indeed, 2 of 15 imprinted genes have lower fourfold degenerate site substitution rates than *H19* (*Igf2* and *Neuronatin*), but both of these are very small sequences so the significance of this is unclear. More importantly, analysis of the evolution in the introns indicates that a low mutation rate across the gene does not alone explain why *H19* has such a very low substitution rate. We find that the introns of *H19* on the average evolve at 2.5 times the rate of the exons ($K_{exon} = 0.06$ per site, $K_{intron} = 0.15$) and that every intron has a higher substitution rate than both of its flanking exons (Fig. 1). Compared with a sample of 41 mouse–rat orthologous genes for which intronic data is available[13] only two have a higher ratio of intronic to exonic fourfold site rate ($P < 0.05$). Both of these have small total exonic content. This evidence, along with suggestive evidence of secondary structure conservation[14], is indicative of stabilizing selection acting on the exons. This in turn is consistent with *H19* mRNA having a function.

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An ethical collaboration

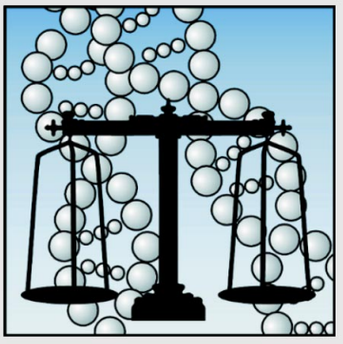
Ethics in Human Procreation, Genetic Diagnosis and Therapy, Sheffield, UK, 7–10 January 1999

The European Network for Biomedical Ethics convened earlier this year for the final symposium in a series of three, concerned with fundamental ethical questions and the social implications of human genetics and assisted reproduction. The symposium, organized by Deryck Beyleveld and Hille Haker, marked a collaboration between the Center for Ethics in the Sciences and Humanities, Tübingen, Germany (ZEW), and Sheffield Institute for Biotechnological Law and Ethics, UK (SIBLE). It brought together some 80 scientists and academics.

The symposium was opened at dinner on Thursday evening by Richard Cabourn MP (Sheffield Central, UK). He indicated that the British Government was considering setting up a committee to take direct action to review and revise the structure of advisory committees in the field of biotechnology. After dinner, Dietmar Mieth (ZEW), spoke, outlining the very real need for bringing social and ethical debate into the realm of science.

The symposium dealt with such varied subjects as procreation and parenthood; the moral protection of the human embryo and fetus; autonomy and recognition; the social implications of the new technologies; moral reasoning in applied ethics; and the legal regulations of assisted procreation, genetic diagnosis and therapy. In addition to the usual practice of inviting audience response to the papers, experts distinguished in their fields had been asked to prepare more detailed critiques of the main presentations, with a view to ensuring the highest quality of comment and criticism.

SIBLE



ZEW

All of the papers given were fascinating and insightful, but the contributions generating the most debate were those of Deryck Beyleveld (SIBLE); Marcus Düwell (ZEW); and the collaboration of Shaun Pattinson (SIBLE), and Deryck Beyleveld. Beyleveld, employing the rights theory of Alan Gewirth, attempted to clarify the nature and extent of rights had by the embryo/fetus, giving them as unassailable and concrete a basis as possible. Gewirth is unique among modern philosophers, although akin to Kant, in that he claims that right answers to moral questions are possible: rationality requires that a moral point of view be adopted, and rationality dictates the determinate content of moral behaviour, and the identity of moral beings. Beyleveld, although agreeing that the fundamental principle of Gewirth's theory is sound, has substantially modified the application of the theory. A claim to

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