Reeler gene discrepancies

Sir — Hirotsune *et al.*[1] reported in *Nature Genetics* the isolation by positional cloning of a candidate gene for the mouse neurodevelopmental defect reeler. This complements studies published in *Nature*[2] and *Genomics*[3] by our labs on the mapping and characterization of *reelin*, a candidate gene for the reeler defect. Unfortunately, there are several discrepancies between our findings and those reported in *Nature Genetics*. Although all three groups report the independent cloning of the same gene, there are errors in the mapping, nucleotide sequence, mRNA size and the predicted size and amino acid sequence of the protein reported by Hirotsune *et al.*. We have verified each of the points of contention and we wish to clarify the situation.

1. **Concerning the physical map.** There are many minor discrepancies compared to the map published by Bar *et al.*[3]. An important difference concerns the location of the deletion in the reeler Edinburgh (Jackson) strain, which is actually located between *D5Mit61* and *D5Mit72* and does not lie proximal to *D5Mit61* as shown by Hirotsune *et al.* This is also consistent with the analysis of Montgomery *et al.*[4].

2. **The mRNA size.** D'Arcangelo *et al.*[2] report that the *reelin* mRNA is greater than 12 kb. This is evident on several northern blots (see Fig. 3 (ref. 2)) using 10 μg of total cell RNA. Thus, it is rather surprising that Hirotsune *et al.* were unable to detect a signal using as much as 10 μg of poly(A$^+$) RNA. The large size of the *reelin* mRNA has now been confirmed by A.G.

3. **Expression pattern of *reelin*.** The widespread expression pattern of *reelin* reported by Hirotsune *et al.* using RT-PCR is not entirely consistent with the northern blot analysis of D'Arcangelo *et al.* This latter shows that the *reelin* gene is well expressed in adult brain, although at lower levels than in the developing brain. Weak expression was detected in the spleen and thymus, but not in otherorgans.

4. **Sequence of *reelin*.** The sequence reported by Hirotsune *et al.* corresponds to a partial cDNA of the *reelin* sequence by D'Arcangelo *et al.*[2] (GenBank accession #U24703), with several out-of-frame mutations. The initiator methionine codon predicted by Hirotsune *et al.* actually corres-ponds to amino acid 2,581 of Reelin. A stop codon upstream of this amino acid in the Hirotsune *et al.* sequence appears to be due to a sequencing error. A guanine base at position 7,889 in the *reelin* sequence (confirmed in two independent clones on both strands) is missing in the Hirotsune *et al.* sequence. Furthermore, the initiator codon predicted by Hirotsune *et al.* does not fulfill the consensus sequence requirements for translation initiation. Finally, two frame-shift errors and one base substitution in the Hirotsune sequence are present in the region of *reelin* corresponding to nucleotides 9,973–10,027. These alter the consensus of the last EGF-like repeat of Reelin[2].

5. **Predicted Reelin protein.** Although Hirotsune *et al.* indicate that the protein product of the reeler gene should be secreted, their predicted amino acid sequence does not contain a leader peptide. The protein deduced from the sequence of D'Arcangelo *et al.* contains a potential leader peptide and a region of similarity in the N terminus with the secreted protein F-spondin[2].

We hope that our remarks have clarified the status of the *reelin* gene. Reelin is a strong candidate product of the reeler gene. It appears to correspond to an extracellular matrix protein that plays a critical role in controlling brain development. Further studies will hopefully shed light on the function of this interesting protein.

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IN REPLY — Subsequent precise analyses have revealed that there were several sequencing errors in our report[1], including two sites that caused frame shifts in the open reading frame. The corrected sequence is shown in GenBank D63520. Addition of one base at two positions, 920 and 1154, extends the open reading frame farther 5' upstream. Therefore, the resulting length of the cDNA and the molecular weight of the product are longer than those reported. This agrees with the amino acid sequence data deduced from the cDNA sequence reported by D'Arcangelo *et al.*[2]. In the downstream 5 kb region, however, sequence discrepancies remain, including the frame shift at our position 2659 to 2710. Other than this frame shift, there are no large discrepancies except for some single base differences. Unfortunately, we can not explain the remaining discrepancies between Bar *et al.*[3] and our clones, because they reported neither nucleotide sequence nor amino acid sequence of their clone. (One further correction we wish to make with regard to our report[1] is that on page 81, line 25, right column, instead of references 22 and 23, please refer to reference 4.)

Another discrepancy between our data and that of D'Arcangelo *et al.* was that we did not detect a positive signal in northern blot analysis using poly(A$^+$) RNA, while they did using total RNA. Using total RNA, we have now detected a 12 kb reeler transcript in a northern analysis. We are currently trying to determine why we still do not detect a reeler transcript when using poly(A$^+$) RNA.

Our data and that of Bar *et al.* also differ with regard to the relative location of the deletion region of *DSMit61* in the Jackson reeler. We have no conclusive information about this at present. We based our mapping data of the deletion on the following: We focused on two YACs to construct the contig map; YAC 24-9 was isolated from a library we constructed, which carried *Bss*HII-digested DNA fragments; YAC FBEA6 was isolated from the Princeton YAC libraries. YAC 24-9 is the only YAC reported to span from *D5Mit61* to *D5Mit72*. As reported in our paper, cosmid clones were prepared from the insert DNA