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Recombination Has Little Effect on the Rate of Sequence Divergence in Pseudoautosomal Boundary 1 Among Humans and Great Apes

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Recent studies indicated that recombination is strongly mutagenic. In particular, data from the mouse pseudoautosomal boundary (PAB) suggested that locally intensive recombination increased the nucleotide substitution rate by more than 100-fold and greatly increased the GC content. Here we study the rates of nucleotide substitution in eight introns of the human and great ape XG gene, which spans the boundary between the pseudoautosomal region 1 (PAR1) and the X-specific region. Contrary to what is expected under the above hypothesis, our sequence data from humans and great apes reveal that the PAR1 introns of XG have actually evolved slightly slower than X-specific introns. Only when a New World monkey was compared with hominoids were the rates slightly increased in the PAR1 introns. In terms of base composition, although the intergenic regions of the human PAR1 show a significant increase of G and C nucleotides, the base composition of the surveyed PAR1 introns is similar to that of the X-specific introns. Direct and indirect evidence indicates that the recombination rate is, indeed, much higher in PAR1 introns than in X-specific introns, and that the present PAB has persisted since the common ancestor of hominoids. Therefore, the mutagenic effect of recombination is far weaker than previously proposed, at least in hominoid PABs.

[Supplemental material is available online at www.genome.org. The following individuals kindly provided reagents, samples, or unpublished information as indicated in the paper: D. Filatov.]
We estimated the effect of recombination on neutral substitution in PAR1 and adjacent regions of the human sex chromosomes by several methods. First, we sequenced 12 intronic segments of XG from diverse catarhines, and compared patterns of nucleotide substitution within PAR1-linked and X-linked regions. We found recombination to have little or no effect on nucleotide substitution rate in great ape XG introns. Second, we took a genomic approach to assess the effects of recombination on base composition across the human X-chromosome; in contrast to the results of the small-scale analysis of XG, recombination appeared to influence sequence evolution at a chromosomal scale. Finally, we inferred evolutionary distances between New World monkey and ape XG sequences and found a weak mutagenic effect of recombination. Within the anthropoid clade, therefore, the mutagenic effect of recombination near the PAB may be less pervasive than has been posited.

RESULTS

Evolutionary Rates in XG Introns in Great Apes

Overall averages of XG intron Tamura-Nei distances from human to bonobo (Pan paniscus), western lowland gorilla (Gorilla gorilla gorilla), and Bornean orangutan (Pongo pygmaeus pygmaeus) were 0.0157 (± 0.007), 0.0189 (± 0.007), and 0.0350 (± 0.010), respectively. These differences slightly exceed published estimates from autosomal intergenic regions in the same taxa (Chen and Li 2001). When repetitive sequences and similar motifs were excluded via the RepeatMasker program, average Tamura-Nei divergences between human and bonobo, gorilla, and orangutan were 0.0129, 0.0159, and 0.0315, respectively, which are in good agreement with earlier estimates (Chen and Li 2001). Excluding repeats did not affect any of the conclusions reported below (data not shown).

We sought to discern any evolutionary rate difference between the great ape PAR1-linked and X-specific introns of XG. Tamura-Nei distances for taxon pairs, estimated from concatenated sequences from these two regions, are shown in Table 1. No tendency toward increased evolutionary rates in PAR1-linked sequences is evident. Unlike results from mouse FXY (Perry and Ashworth 1999; Montoya-Burgos et al. 2003), our findings do not support the hypothesis that recombination is globally mutagenic (i.e., Lercher and Hurst 2002; Hellmann et al. 2003). Filatov and Gerrard (2003) also reported no substantial difference in evolutionary rates between PAR-borne and X-specific sequences within short segments of human and orangutan XG.

In fact, between human and bonobo or orangutan, X-specific XG introns appear to have evolved faster than their pseudautosomal counterparts, although between human and gorilla, the substitution rate appears slightly slower in X-specific sequences (Table 1). These results show no statistically significant trend that distinguishes great ape PAR1 from X-specific regions.

In addition, we estimated genetic distances after excluding all sites of a CpG dinucleotide in any of the sequences examined. Evolutionary distances estimated from such non-CpG sequences are shown in parentheses in Table 1. As expected, sitewise evolutionary distances for non-CpG sequences are significantly lower than for CpG-containing sequences. Overall, we found a 32% reduction in estimated evolutionary distance after removal of CpG sites, which occupy on average only ~4% of all sites in our data. The proportion of CpG sites in XG introns closely approximates that in other introns of several primates, as reported in Subramanian and Kumar (2003).

Montoya-Burgos et al. (2003) showed that the GC content of sequences in mouse FXY increased as a direct consequence of increased recombination. However, our great ape XG data showed no such pattern. In fact, the GC content of the sampled XG regions was consistently lower in PAR-linked sequences than in X-linked sequences (Table 1).

Correlation Between GC Content and Evolutionary Rate

We assessed the relation between GC content and evolutionary distance between human and orangutan XG sequences. We chose this species pair because they are the most divergent among the orthologous sequences compared and therefore may yield the most statistical power for this type of analysis. In addition to the 12 aforementioned segments newly sequenced in this work, we added five segments from PAR1 sequenced by Filatov and Gerrard (2003). They explored the pattern of molecular evolution of nine DNA segments between the human and orangutan. From these we excluded three segments from the XG region that overlap with our data, and one from the SYBL1 locus, which occupies PAR2. We found a strong correlation between GC content and the Tamura-Nei evolutionary distance (p < 0.01; Fig. 2A); this correlation remained significant when we excluded all CpG sites in either lineage (Fig. 2B).

Table 1. The Average Tamura-Nei Evolutionary Distances Between Species and the GC Contents for the PAR1 (PAR)- and X-Linked XG Sequences

<table>
<thead>
<tr>
<th>Species pair</th>
<th>PAR</th>
<th>X-specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of base pairs</td>
<td>4216</td>
<td>7546</td>
</tr>
<tr>
<td>T–N distance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human–bonobo</td>
<td>0.014 (0.010)</td>
<td>0.015 (0.011)</td>
</tr>
<tr>
<td>Human–gorilla</td>
<td>0.019 (0.013)</td>
<td>0.018 (0.012)</td>
</tr>
<tr>
<td>Human–orangutan</td>
<td>0.032 (0.023)</td>
<td>0.041 (0.028)</td>
</tr>
<tr>
<td>Bonobo–gorilla</td>
<td>0.016 (0.011)</td>
<td>0.016 (0.011)</td>
</tr>
<tr>
<td>Bonobo–orangutan</td>
<td>0.031 (0.023)</td>
<td>0.039 (0.027)</td>
</tr>
<tr>
<td>Gorilla–orangutan</td>
<td>0.034 (0.025)</td>
<td>0.036 (0.026)</td>
</tr>
<tr>
<td>% GC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>45.6 (43.9)</td>
<td>48.4 (46.5)</td>
</tr>
<tr>
<td>Bonobo</td>
<td>45.3 (43.9)</td>
<td>48.0 (46.5)</td>
</tr>
<tr>
<td>Gorillas</td>
<td>45.1 (43.5)</td>
<td>48.5 (46.3)</td>
</tr>
<tr>
<td>Orangutan</td>
<td>44.2 (43.5)</td>
<td>47.5 (45.9)</td>
</tr>
</tbody>
</table>

Exons are excluded from the analyses. Within parentheses are the evolutionary distances estimated after excluding the CpG dinucleotides.
GC Content of the Human Pseudoautosomal Regions

From the entire human X-chromosome, we extracted 45,153,479 bp of intergenic DNA (see Methods), of which 467,756 bp, 166,183 bp, and 44,519,539 bp are in PAR1, PAR2, and the X-specific region, respectively. The GC contents of the three regions are 46.5%, 38.9%, and 38.9%, respectively. Thus, intergenic sequences from PAR1 have a much higher GC content than do those from PAR2 or the rest of the X-chromosome (see Table 2; the data when repetitive sequences are excluded are also shown).

To assess the significance of the observed high GC content in PAR1, we first conducted a Monte Carlo analysis. Briefly, we randomly sampled PAR1-sized DNA segments from the X-specific sequences. We computed the GC content of each sampled segment and scored whether it was greater or less than the GC content of PAR1. From several replicates of 1000 randomly chosen segments, only 30 cases per average replicate showed a higher GC content than that of PAR1. That is, the probability of obtaining a DNA segment with a GC content at least as high as that of PAR1, given the sequence context of the X-specific region, was ∼0.03.

Next, we derived a sliding-window frequency distribution of X-specific GC content. A roughly PAR1-sized window was advanced across the X-specific published human genome sequence by 1% of the window size per step. Figure 3A summarizes this analysis. The GC content of PAR1 lies within the upper 0.7% of this distribution.

From these analyses, we conclude that the GC content of PAR1 significantly exceeds the expected value for a random segment from the X-specific region. This conclusion holds whether we consider the intergenic or the intronic regions, and regardless of whether repetitive sequences are included (Table 2). In contrast, the GC content of PAR2 lies near the middle of the X-derived distribution in all such analyses.

We also checked for any significant difference in the GC content between PAR1 and the adjacent X-specific segment of the same size. Whereas the GC content in human PAR1 is 46.5%, that of the adjacent X-specific segment is 41.3%. These values differ significantly (p < 0.01). In contrast, the GC content of PAR2 is statistically equivalent to that of the adjacent same-sized X-linked region.

To estimate the effect of CpG methylation on the heterogeneity of the base composition of the X-chromosome, we performed the same analyses after excluding all CpG, TpG, and CpA dinucleotides. Directional hypermutability of CpG dinucleotides to TpG and CpA is well-known (Bird 1980; Hendrich et al. 1999). By excluding such dinucleotides, we may remove the effect of CpG methylation during the recent evolution of the human X-chromosome. We excluded ∼30% of our DNA sequence in this procedure. Figure 3B (see also Table 2) shows the resulting frequency distribution of GC proportion. The GC content of human PAR1 still lies within the upper 1% of the distribution. Therefore, the GC-richness of PAR1 is not entirely due to the accumulation of CpG or CpG-derived dinucleotides.

Evolutionary Rates From a New World Monkey Outgroup

We obtained XG sequences from a New World monkey, the spider monkey, *Ateles geoffroyi*, for 1200 bp from PAR1, as well as two X-specific DNA segments totaling 2400 bp. After excluding exon sequences, we estimated taxon-pairwise Tamura-Nei distances (Table 3). The genetic distances among hominoids showed the same pattern as in the overall data set: Evolutionary rates were slightly higher in X-specific regions. But when the distance from the New World monkey sequence was estimated, the hominoid PAR1 sequence appeared to have evolved on average ∼19% faster than the X-specific sequence. This disparity remained after we excluded dinucleotides in CpG contexts as above.

DISCUSSION

Is Recombination Mutagenic in Hominoid PAB?

The most striking finding of this study, unexpected under the widely accepted hypothesis that recombination is mutagenic (Perry and Ashworth 1999; Fullerton et al. 2001; Lercher and Hurst 2002; Montoya-Burgos et al. 2003), is the lack of accele-
tion of sequence evolution in PAR1-linked segments of the human XG gene. When evolutionary rates of human and great ape sequences were compared, PAR1-linked and X-specific regions showed no clear-cut rate difference, contrary to what has been observed surrounding the house mouse PAB. Nor did the PAR1 and X-specific regions differ significantly in GC content, unlike the case of the PAR-linked portions of the FXY locus in the mouse (Montoya-Burgos et al. 2003).

What might explain these discrepancies? Under the view that recombination is mutagenic and that XG intronic sequence is evolving neutrally, there are at least two alternative hypotheses. First, recombination rates in human PAR1 versus X-specific regions may not differ significantly. We show below that this is unlikely, in view of existing literature and our unpublished data. Second, human PAB1 may be too young to show a significant effect of differential recombination. This too is highly unlikely; we show below that the catarrhine PAB appears to have a long history.

Recombination Rate in Human PAR1
Lien et al. (2000) estimated subregional male meiotic recombination rates in human PAR1 by genotyping more than 1900 single sperm for 25 PAR1 markers. They inferred overall recombination frequencies in PAR1 to be 13- to 38-fold higher than the genome average. Similar estimates were reported in studies using long-range restriction mapping (Brown 1988; Petit et al. 1988). More importantly, according to Lien et al. (2000), the PAB1 region is one of the most recombinant regions in PAR1. They suggested that the male meiotic recombination frequency in this region is 26- to 38-fold higher than the genome average.

Other data on recombination rate come from within-population haplotype distributions. We are in the process of collecting nucleotide polymorphism data from >100 chromosomes from the regions surveyed in this paper (N.M. Pearson, P.R. Obara, S.Yi, B. Nikbin, P.A. Underhill, L.L. Cavalli-Sforza, M. Kreitman, and B.T. Lahn, unpubl.). The SNPs from the X-specific regions exhibit linkage disequilibria over significantly longer physical distances than those on PAR1, indicating that the PAR1 region has a significantly higher level of recombination than the X-specific region. Therefore, the first hypothesis above is not supported.

The Age of the Present-Day PAB in Hominoids
We now consider the hypothesis that the effect of recombination on XG neutral substitution rates is not yet conspicuous because
human PAB1 has arisen only recently. The structure of the PAB in nonhuman anthropoids (homologous to human PAB1) has been well studied (see Fig. 1; Ellis et al. 1989, 1990). On the X-chromosome, the present-day PAB is conventionally marked by an Alu element, flanked centromerically by ~220 bp of X-Y-homologous sequence (Ellis et al. 1990). Farther toward the centromere, the sex-chromosome-specific sequence, which shows no clear X-Y homology, begins. In particular, the sex-determining locus, SRY, follows on the Y-chromosome.

Although the age of the present-day PAB is unknown, available sequence data from the PAB of the X- and the Y-chromosomes of diverse catarrhines (Ellis et al. 1990) provide much insight when we consider the phylogenies of the Alu-proximal and the Alu-distal regions separately. The Alu-proximal region shows, throughout catarrhines, ~10% X-Y sequence divergence, roughly the same as that observed in genes comprising “stratum 4” of the human sex chromosomes (Lahn and Page 1999; Iwase et al. 2003). The phylogenetic tree of catarrhine Alu-proximal regions shows distinct clades of X- versus Y-derived sequences, well supported by high bootstrap values (Fig. 4A), even though only 234 bp were aligned. In sharp contrast, the phylogenetic tree of the Alu-distal region, although comparable in length to that of the Alu-proximal region, shows a mixture of the X- and Y-derived sequences (Fig. 4B), implying local recombination events between the two chromosomes.

Moreover, the total branch length of the Alu-proximal regions greatly exceeds that of the Alu-distal region (Fig. 4). In the former, the branch leading to the ancestor of the X- and Y-chromosomes is notably extended, and substitution rates are clearly higher among Y-derived than X-derived branches. These observations are in excellent accord with the hypothesis of male-driven evolution (Makova and Li 2002). Clearly, Figure 4 shows that the Y-sequences have been evolving much faster, implying that the Y-sequences have had male-limited transmission for a substantially long period of time.

Thus, a boundary between Alu-proximal and the Alu-distal regions appears to have arisen before the divergence of hominoids from Old World monkeys. For this reason, the lack of rate difference between the PAR-linked and X-specific introns of the XG gene cannot be explained by the hypothesis of late emergence of the PAB1 in the human and ape lineages.

As noted, the rate of recombination in PAB1 exceeds that of the X-linked region in our data set, and the emergence of PAB1 appears to predate the last common ancestor of hominoids. The lack of a noticeable mutagenic effect of recombination on the sequence evolution of PAB1 is therefore puzzling. One possible explanation is that recombination and mutation do correlate in the regions surveyed, but are localized to a scale undetected in our survey. We note that May et al. (2002) posited highly patchy recombination in the SHOX gene region of human PAR1. However, it is rather unlikely that we have sampled more than 30% of XG intron sequences without hitting such a hotspot.

**Substitution Rates in Mouse PAB and the New World Monkey**

The glaring disparity between the house mouse PAB and the human PAB1 in the inferred mutagenic effect of recombination is quite difficult to explain, especially as house mouse FXY likely moved to a PAB less than 3 million years ago, according to molecular divergence. Even if we take into account the differences in generation times between primates and rodents, the contrast between the analogous human and the mouse PABs is hard to reconcile.

However, the mouse PAR is shorter than human PAR1 (720 kb vs. 2.6 Mb; Perry et al. 2001). Therefore, the effect of condensed obligatory male meiotic recombination may be stronger, and perhaps more homogeneous, in the mouse PAR than in human PAR1. It is also possible that recombination-induced mutagenesis may depend on other biological factors such as a certain chromatin structure, which may differ between human and mouse PABs.

In addition, in the mouse PAR, the GC content at the third codon positions of the FXY was on average 30.1% higher than in XG, no such difference was found. Unlike the case of mouse FXY, we do not know the GC landscape of the ancestral XG locus, which presumably resided in a relatively uniform recombination environment. Therefore, we cannot quantify the rate of GC change of XG compared with its ancestral situation. However, although we do not know the GC content beyond orangutan, we can see clearly the GC content in the common ancestor of orangutan, chimpanzee, bonobos, and humans, which has little changed. Thus, there is little evidence that the GC content of the XG has substantially changed in hominoids. As exon sequences tend to accumulate more CpG dinucleotides than do noncoding sequences (Subramanian and Kumar 2003), assessing the evolutionary rates from intron sequences of FXY may offer an interesting and informative contrast in this respect.

We are further intrigued by the increased evolutionary rate in PAR1 among the larger anthropoid clade, at least for the smaller portion of XG sequenced in New World monkeys. This may indicate that in some primates recombination is indeed mutagenic in PAB1; however, such an increased rate cannot be ascribed with certainty to recombination rate, because XG may have come to straddle the pseudoautosomal boundary after the divergence of catarrhines from the New World monkeys. As noted above, the GC content of human PAR1 significantly exceeds that of the rest of the X-chromosome, consistent with the mutagenic effect of recombination. Moreover, Filatov and Gerhard (2003) reported faster evolutionary rates in PAR1-linked se-

![Figure 4](https://example.com/figure4.png)
quences than in X-specific regions, as expected if recombination is mutagenic; however, we note, again, that in their analyses the partial $X_G$ region stood out as an outlier as having no difference in evolutionary rates between PAR-borne and the X-specific segments. In view of these observations, it is a great puzzle why human PAB1 shows no obvious mutagenic effect of recombination on evolutionary rate.

Another issue that might complicate the analyses of evolutionary rates in the $X_G$ has to do with the fact that the $X_G$ gene was duplicated in some catarrhines (Weller et al. 1998; Gläser et al. 1997). Briefly, Weller et al. (1995) described several male-specific transcripts of $X_G$-related sequences. They concluded that those sequences were the results of alternative splicing of a gene mapped to Yq11.21. All of the transcripts encoded premature stop codons; hence, the duplicated copy is named $X_{GPY}$ ($X_G$ pseudogene on the Y-chromosome). The $X_{GPY}$ resides within a larger segmental duplication, which encompasses at least the entire $X$ region (S. Yi, unpubl.). This could have originated from the X-chromosome copy or from the ancestral copy before the differentiation between the X-chromosome and Y-chromosome $X_G$ loci. If gene conversion occurs between the $X_G$ and $X_{GPY}$ similar to what has been reported to occur between paired palindromes on the human Y-chromosome (Rozen et al. 2003), then only the PAR-borne $X_G$ portion is affected, because the second half of the $X_G$ locus is truncated on the Y-chromosome (Fig. 1). However, if such gene conversion between the PAR-borne $X_G$ and the Y-specific copy does occur, it is most likely to increase divergence between species in this region, which is the opposite of what we observed. Also, whether such gene conversion events occur in non-palindromic sequences such as $X_G$ and $X_{GPY}$ remains an open question at this point.

Recombination and GC Content in Human PAR1

It is informative to note the differences in GC contents of human PAR1 and PAR2. Although PAR1 has a significantly higher GC content than the rest of the X-chromosome, PAR2 is on the whole indistinguishable from the rest of the X-chromosome in this respect (Fig. 3; Table 3). Recombination frequencies in PAR2 have been estimated to be about an order of magnitude lower than that of PAR1 (Li and Hamer 1995; Lien et al. 2000). Also, PAR2 is not implicated in mediating meiosis (Charuchar et al. 2003). Furthermore, PAR2 has a short history, as it is human-specific. Therefore, the elevated GC content of PAR1 appears correlated with substantially intensified recombination in PAR1, whereas the GC content of PAR2 is not affected.

In addition, we found significant correlation between the GC content and the evolutionary rate for the human and orangutan $X_G$ comparison (Fig. 2). GC content explained ~58% of the relationship observed (that figure decreased to 45% when we excluded all CpG sites from our data set). In fact, for historically CpG sites only (sequences that code CpG dinucleotides in any species), Tamura-Nei distances between human and bonobo or between human and orangutan were greater in PAR1 $X_G$ introns than in X-specific $X_G$ introns. In other words, at least over short divergence times, evolutionary rates in highly recombinating regions are inflated, partially reflecting fast-evolving CpG contexts.

METHODS

PCR, Cloning, and Sequencing

Genomic DNA samples of male and female bonobo (Pan paniscus), western lowland gorilla (Gorilla gorilla gorilla), and Bornean orangutan (Pongo pygmaeus pygmaeus) were used separately as templates in polymerase chain reaction (PCR). We used the Expand High Fidelity PCR system (Roche) to minimize errors in amplification. We designed PCR primer pairs to amplify ~1 kb of DNA sequences from each of 13 roughly evenly spaced regions spanning the ~64-kb $X_G$ region. Several of the original primer pairs did not amplify in some taxa (data not shown). Additionally, we obtained larger sequence segments spanning the proposed PAB1. As a result, we amplified and sequenced a total of ~14 kb throughout the $X_G$ region, including ~55 kb flanking the PAB1, from male and female hominoids. The most disparate nucleotides sequenced were 57,930 bp apart (Fig. 1). Additionally, we sampled 4 kb from the spider monkey (Ateles geoffroyi), a New World monkey.

The primers used in this work are shown in the Supplemental material, available online at www.genome.org. The regions covered in our collecting scheme are depicted in Figure 1. Some segments were cloned using the TA cloning kit (Invitrogen). We used the BigDye v3 sequencing kit (ABI), run on an ABI 3737 for both strands.

Sequence Analyses

Sequence data were curated using the Sequencher 4.0 program (GeneCode) to make and visually confirm or correct base calls. Sequences were then exported and aligned using CLUSTAL W (Thompson et al. 1994) with default parameters. All exons were excluded from the alignments before further analyses. Repeat elements in these sequences were identified using the RepeatMasker program (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker) and removed for some analyses. To infer the CpG context of sequences, we first identified CpG dinucleotides in the multiple alignments; if any sequence within the alignment showed a CpG dinucleotide, all orthologous dinucleotides were designated as CpG sites for removal from further analysis. We used the Tamura-Nei method (Tamura and Nei 1993) to correct for multiple hits. This method takes into account GC bias. We concatenated sequences from the PAR1 versus X-specific regions to estimate average genetic distances.

X-Chromosome Data Analyses

We downloaded the entire X-chromosome from the UCSC Genome Browser (http://genome.ucsc.edu) using the April 2003 release of the human genome. In this release, repetitive sequences are identified as lower-case letters, using the RepeatMasker program (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker). We used the available data tables to set up a relational database of the coordinates and sequences of all known and predicted regulatory regions, exons, and introns. For most of our analysis, we excluded all annotated/predicted gene regions, including regulatory regions, exons, and introns from the KnownGenes and GeneScan annotations.

Phylogenetic Analyses of the PAB in Higher Primates

The sequences for the PAB boundary from several higher primate species were downloaded from GenBank (http://ncbi.nih.gov/GenBank, accession numbers M34450–M34462). They were aligned using CLUSTAL W (Thompson et al. 1994). We estimated sequence diversifications using Kimura’s two-parameter method (Kimura 1980). The neighbor-joining (NJ) method implemented in MEGA2 (http://www.megasoftware.net; Kumar et al. 2000) was used to draw phylogenetic trees.

ACKNOWLEDGMENTS

Genomic DNA samples of bonobos, gorillas, and orangutans were purchased from the San Diego Zoological Society. We thank Melissa McMahill for her rotation work in the Li lab, during which she generated some sequence data; Dmitry Filatov for sharing unpublished results; an anonymous reviewer for an interesting suggestion; and Peter Bouman for discussions. This study was supported by NIH grants GM65499 and GM30998.

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