phin to different cell lines is summarized in Table 2. Some cell lines do not bind the peptide while other cells only exhibit the low-affinity sites [concentration producing 50 percent inhibition (IC_{50}) between 100 and 1000 nM]. Only transformed lymphocytes appear to have both high-affinity (apparent K_d, 3 nM) and low-affinity (IC_{50} = 100 nM) sites. The high-affinity sites can be destroyed by digestion with trypsin but are resistant to digestion by phospholipase A_2 (data not shown).

Studies of the interaction of iodinated β-endorphin with putative opiate receptors in the brain demonstrate that the binding is inhibited by very low concentrations of enkephalin analogs as well as by opiate agonists and antagonists; this indicates that β-endorphin can bind well to opiate receptors (*11*). However, the nature of the binding interaction is different from that of enkephalins, since various cations affect the binding of β-endorphin and enkephalins markedly differently (*11*). In the present studies β-endorphin binding is not blocked by opiates or enkephalins, which strongly suggests that the β-endorphin binding sites in cultured lymphocytes and in the cell lines exhibiting only the low-affinity sites (Table 2 and data not shown) are quite different from the opiate receptors present in the brain.

β-Endorphin, but not enkephalins, is found mainly in the pituitary gland (*16*) and has a broad range of pharmacological activities. The presence of β-endorphin in serum, the increases induced by stress (*6*), and the existence of specific receptors that do not have the characteristics of opiate receptors suggest that β-endorphin may mediate some central and peripheral physiological functions by mechanisms distinct from those associated with traditional opiate receptors. It will be important to examine other tissues for β-endorphin receptors, and to identify possible pharmacological and physiological effects that are not suppressed by opiate antagonists.

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13. β_H-[D-Ala^2]Endorphin was iodinated by chloramine-T method in the presence of 2M urea and purified on Sephadex G-15 column previously equilibrated and eluted with 0.1M acetic acid (*11*).
14. Amino acid abbreviations: Ala, alanine; Phe, phenylalanine; Met, methionine; Leu, leucine; Lys, lysine; Asn, Asparagine; Ile, isoleucine; Tyr, tyrosine; Gly, glycine; and Glu, glutamic acid.
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Chromosome 13 Long Arm Interstitial Deletion May Result from Maternal Inverted Insertion

Sparkes *et al.* (*1*) reported on a child with an interstitial deletion of chromosome 13 and retinoblastoma. They reported the child's karyotype as 46,XX, del(13)(q14q22) and suggested that the abnormal chromosome was derived from the mother's normal chromosome 13. The maternal homolog was reported to carry a simple paracentric inversion with break points in bands q12 and q22. Sparkes *et al.* hypothesized that the derived chromosome present in the daughter may have resulted from the formation of two breaks followed by rehealing in a prophase I inversion loop.

I suggest that the mother may instead have an inverted insertion with a karyotype of 46,XX,inv ins(13)(pter→q12; :q22→q14::q12→q14::q22→qter). The inverted insertion chromosome would appear indistinguishable from the inversion suggested by Sparkes *et al.* when stained by the G and Q banding techniques that these workers used (Fig. 1).

Such a chromosome, when paired with its normal homolog, would form a double loop during prophase I similar to the loops resulting from overlapping inversions in *Drosophila* (*2*). A single crossover within the noninverted interstitial segment would then produce a chromosome with an interstitial deletion of the type found in the child (Fig. 2A).

It is generally recognized that in humans inverted insertions will also produce duplication and deletion chromosomes by crossing over. Palmer *et al.* (*3*) studied a proband with a duplicated segment of chromosome 1. Her father carried an inverted insertion of that chromo-

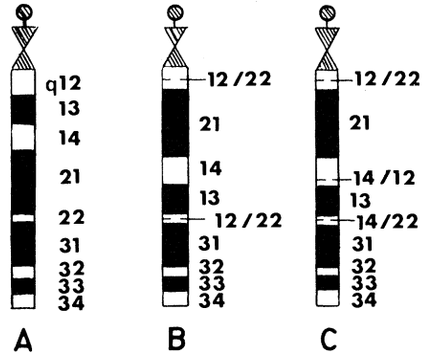
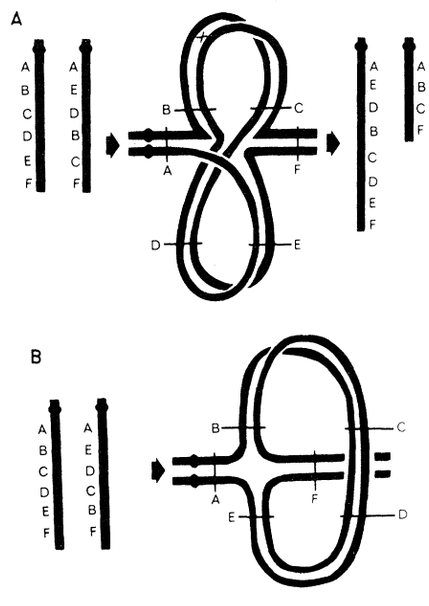


Fig. 1 (left). Chromosome 13. (A) Normal 13. (B) Inversion of bands q12 to q22. (C) Inverted insertion with break points at q12, q14, and q22. Fig. 2 (right). Pachytene inversion loops. Two of four strands are shown. (A) An inverted insertion. A single crossover within the inserted portion will produce deleted and duplication products. (B) Inversion loop from a paracentric inversion drawn in three-dimensional perspective.



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some. The proband's abnormal chromosome 1 was therefore somewhat similar to the duplication chromosome shown in Fig. 2A.

The inverted insertion hypothesis is somewhat more parsimonious than the hypotheses advanced by Sparkes *et al.* While three breaks would be required to produce an inverted inversion as opposed to two for a paracentric inversion, two additional breaks would be required to produce the deleted chromosome from the chromosomes in an inversion loop (their figure 2, B and C). In addition, it is unlikely that the deleted chromosome was produced by the mechanism proposed by Sparkes *et al.* in their figure 2C. That figure shows two breaks occurring in the loop at the point where the "inner" chromosome folds back over itself. In the most probable three-dimensional pachytene configuration of an inversion loop (Fig. 2B), that point does not exist (*4*). It may be only an artifact produced when the loop is rendered in two dimensions.

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4. Of 15 surveyed genetics and cytogenetics texts, only Dobzhansky (*2*) shows inversion loops in three-dimensional perspective. J. Sybenga [*Meiotic Configurations* (Springer-Verlag, New York, 1975), p. 63] differentiates between diagrammatic representation and microscopic appearance of inversion loops.

4 April 1979

Protein and Nucleic Acid Sequence Data and Phylogeny

Having examined the data and methodology on which Schwartz and Dayhoff's (*1*) proposals on the phylogeny of pro- and eukaryotes are based, I find the credibility of their conclusions somewhat limited.

Schwartz and Dayhoff present three evolutionary trees based on, respectively, ferredoxins, 5*S* ribosomal RNA (rRNA), and c-type cytochromes sequences. They then combine data from the three individual trees into a composite tree.

The major objection to phylogenetic conclusions drawn from the ferredoxin and cytochrome trees is that they are based on a set of probably homologous but certainly not entirely orthologous proteins. Schwartz and Dayhoff recognize this for the *Chlorobium* ferredoxins and *Pseudomonas* cytochromes; but they do not allow the fact that while some microorganisms, such as *Chlorobium limicola*, possess two closely related ferredoxins, others possess very different ones; for example, both 8Fe-8S and 4Fe-4S occur in *Rhodospirillum*, and 8Fe-8S and 2Fe-2S occur in *Azotobacter* (*2*). Schwartz and Dayhoff's ferredoxin tree may thus represent a gene phylogeny without significance in the interrelationship of organisms.

Similarly, with the cytochrome tree one cannot draw conclusions from the finding that blue-green algal and chloroplast cytochrome c_6 stand on a different section of the tree from the mitochondrial cytochrome c and the cytochrome c_2 of purple nonsulfur bacteria.

The differences in properties and sequences between c_6 and c_2 cytochromes, and the finding that prokaryotes may possess several c-type cytochromes [such as the c_{551}, c_5 as partially illustrated in figure 5 of (*1*)] are first indications that c_6 cytochromes may not be orthologous with c_2. This possibility becomes a quasi certitude when one considers that c_6 is only one among three soluble c-type cytochromes of blue-green algae (*3*). Of those, the most likely candidate for orthology with c-c_2 is the not yet sequenced cytochrome c_{552}, which has a basic isoelectric point and an α band at a lower wavelength than c_{554} (c_6).

The importance of using only orthologous genes when one wants to infer evolutionary relationships between organisms has been emphasized (*4*). Who would consider seriously a phylogeny of vertebrates drawn from a comparison of myoglobin of some species and hemoglobin from others? The species for which myoglobin is used will cluster together far away from related species for which hemoglobin is selected (*5*). Similarly, given the doubts on the orthology of those cytochromes, a comparison based on the use of cytochrome c_6 to represent blue-green algae and chloroplasts, and cytochromes c_2 and c for purple nonsulfur bacteria and mitochondria, should not be used to demonstrate a separate symbiotic origin of eukaryotic organelles.

From their cytochrome tree Schwartz and Dayhoff concluded that since separate branches leading to the two blue-green algae intermix with eukaryotic algae, chloroplasts must have a polyphyletic origin. This appears to be another hasty conclusion.

The main problem here is the reliability of evolutionary reconstructions based on sequence data. The model in (*1*) is likely to give a false impression of the reliability of the matrix method used. It does indeed show that for distant sequences, the matrix method is more accurate than the ancestral sequence method, but how accurate it is when applied to real data cannot be inferred. There is still no absolute way to define the accuracy of such techniques and even if one succeeds in finding the most parsimonious tree for a set of sequences (*6*), this only represents a probabilistic estimate of evolutionary history for which the confidence limit is unknown.

In the approaches used until now, that confidence limit can only be estimated if, in a model of the type used to compare the relative accuracy of different techniques, the data are closely comparable to those under study (*7*). But the Dayhoff model does not fit many of the data treated, especially the c_6 cytochromes. The reason is that the model deals with two clear-cut pairs of sequences: a member of each pair has a distance, measured along the tree, of 3 L with any member of the other pair, whereas the distance between members of the same pair is only 2 L. In other words, the distance between the two nodes of the network is equal to the distances from the tips to the closest node. In such a situation, it is logical that the dendrogram derived from the comparison of extant sequences gives an incorrect representation (erroneous cladogram) of phylogenetic relationship only in extreme cases; that is, either when an exceptional number of convergent mutations occurred or, as illustrated by Schwartz and Dayhoff, when the distance between the sequences is such (high value of L) that the residues of phylogenetic significance are so few in number that they are overshadowed by random similarities.

With the c_6 cytochromes, however, the extant sequences are more or less equally distant one from another (they are mostly 42 to 56 percent similar), which probably implies short internodal distances in comparison to the distances from the tips to the closest node. This is exactly what appears in the Schwartz and Dayhoff tree where, with the exception of the *Porphyra-Alaria* pair, the distances from the tips to the closest node are about two to six times greater than the internodal distances.

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