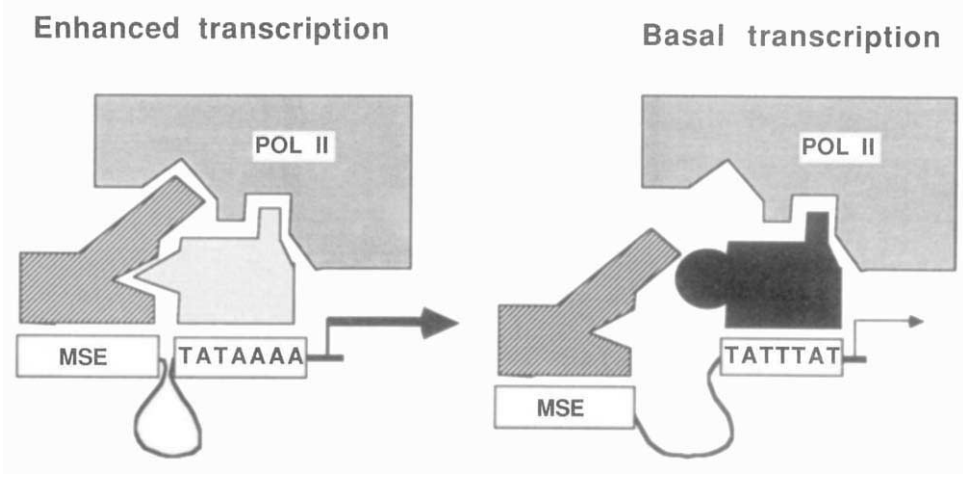

FIG. 4 Schematic model of possible mechanism for functional heterogeneity of the TATAAAA and TATTTAT elements based on differential binding of discrete cognate binding factors that differ in their ability to interact with factors binding the myoglobin MSE. The TATAAAA binding factor includes a domain that forms a complex with MSE-binding factors, resulting in enhanced transcription. By contrast, the TATTTAT-binding factor forms this higher-order complex less efficiently, and only basal levels of transcription are observed. Alternatively, an identical TATA box-binding factor could assume different conformations when bound to TATAAAA versus TATTTAT, only one of which permits effective interaction with the enhancer. Pol II, RNA polymerase II.

TATA-box sequences. Although all proteins of this class are capable of interaction with RNA polymerase II and other general components of the pre-initiation complex, we hypothesize that these proteins differ in their ability to form higher-order complexes through protein–protein interactions with enhancer-binding proteins. An alternative model is that, when bound to varying TATA-box sequences, a single form of TATA-box binding protein can assume different configurations, only a subset of which can be recognized by certain enhancer-binding proteins.

These models must ultimately be tested with purified components in cell-free systems. Roeder and colleagues[10–13] have purified a eukaryotic TATA-box binding factor (TFIID) and studied its interaction with various viral and mammalian promoters. Single-base mutations of TATA-box sequences can alter the affinity for binding of TFIID (ref. 12), with concomitant changes in transcription. In addition, binding of other factors to upstream regulatory sites can result in large changes in the specific nucleotides protected from DNAse I by preparations of TFIID[12–13]. These studies support the concept of protein–protein interactions between TATA-box-binding proteins and other transcription factors, and indicate approaches by which the interactions of proteins binding to the myoglobin upstream enhancer and specific TATA-box binding factors may be explored. □

Received 15 December 1989; accepted 19 January 1990.

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ACKNOWLEDGEMENTS. We thank Ivor Benjamin for assistance in mutagenesis, and Tammie Bernard for valuable technical contributions. Myoglobin genomic sequences were originally cloned by Alec Jeffries and colleagues. Other plasmids were provided by Eric Olson, Gray Crouse, Bryan Cullen and Nadia Rosenthal. The oligonucleotide primer used for start site analyses was provided by Charles Ordahl. This work was supported by the NIH, the American Heart Association, and the North Carolina Biotechnology Center (R.S.W.). F.C.W. is a Merck Fellow of the American College of Cardiology.

Evolutionary transfer of the chloroplast *tuf*A gene to the nucleus

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EVOLUTIONARY gene transfer is a basic corollary of the now widely accepted endosymbiotic theory, which proposes that mitochondria and chloroplasts originated from once free-living eubacteria[1]. The small organellar chromosomes are remnants of larger bacterial genomes, with most endosymbiont genes having been either transferred to the nucleus[2] soon after endosymbiosis[3,4] or lost entirely, with some being functionally replaced by pre-existing nuclear genes[5,6]. Several lines of evidence indicate that relocation of some organelle genes could have been more recent. These include the abundance of non-functional organelle sequences of recent origin in nuclear DNA[7,8], successful artificial transfer of functional organelle genes to the nucleus[9], and several examples of recently lost organelle genes[4,10], although none of these is known to have been replaced by a nuclear homologue that is clearly of organellar ancestry. We present gene sequence and molecular phylogenetic evidence for the transfer of the chloroplast *tuf*A gene to the nucleus in the green algal ancestor of land plants.

The *tuf*A gene, encoding chloroplast protein synthesis elongation factor Tu (EF-Tu), was first identified as a chloroplast gene in the green alga *Chlamydomonas reinhardtii* by filter hybridization with an *Escherichia coli* gene probe[11] and in *Euglena gracilis* by complete sequencing of the chloroplast gene[12]. Our attempts to hybridize the putative *Chlamydomonas tuf*A to various land plant chloroplast DNAs were unsuccessful, and complete sequencing of three land plant chloroplast chromosomes[10,13] has confirmed the absence of *tuf*A from a nonvascular (*Marchantia polymorpha*) and two flowering (rice and tobacco) land plants.

We looked for *tuf*A in land plant nuclear DNA by Southern blot analysis of *Arabidopsis thaliana* DNA. Figure 1 shows that the putative *Chlamydomonas tuf*A hybridizes to a single fragment in Arabidopsis nuclear DNA digested with either *Eco*RI or *Hin*dIII. There is no hybridization detected with *Arabidopsis* chloroplast DNA (Fig. 1) or mitochondrial DNA (data not shown), despite overloading of these sequences relative to single-copy nuclear sequences (Fig. 1, cp control). Thus, *tuf*A appears to be present in *Arabidopsis* in nuclear DNA exclusively.

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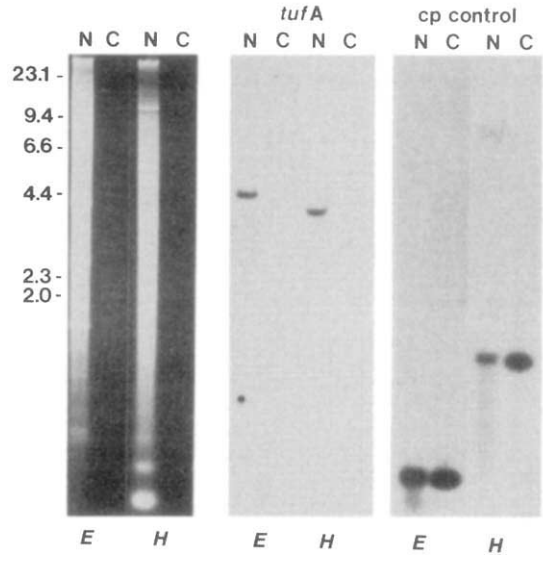

FIG. 1 A *Chlamydomonas* chloroplast *tuf*A probe hybridizes to *Arabidopsis* nuclear DNA. Nuclear (N) and chloroplast (C) DNAs from *Arabidopsis thaliana* were digested separately with the restriction enzymes *Eco*RI (*E*) and *Hin*dIII (*H*) and fractionated on a 0.9% agarose gel (left panel). A nylon filter replica of the gel shown was hybridized sequentially with a *Chlamydomonas tuf*A gene-specific probe (400 bp *Pst*I-*Eco*RI) and a *Brassica campestris* chloroplast DNA probe (a 600-bp *Eco*RI fragment of a 9.0-kb *Sac*I clone). The latter shows overloading of the chloroplast lane relative to chloroplast contamination in the nuclear DNA. Sizes in kb are indicated on the left.
METHODS. 5′ and 3′ fragments of the *Chlamydomonas tuf*A gene[11] (1.7 kb *Bam*HI-*Eco*RI and 1.3 kb *Eco*RI fragments, respectively) were subcloned from a chloroplast DNA library (gift from J. Boynton and N. Gillham). A 400-bp *Pst*I-*Eco*RI fragment, shown to be internal to the gene by hybridization with *Euglena tuf*A[12] (gift from R. Helling), was subcloned from the *Bam*HI-*Eco*RI fragment. Nuclear DNA was extracted from Percoll-gradient-purified nuclei of *Arabidopsis thaliana*, strain Columbia (gift from C. Somerville) as described[24], omitting pretreatment of leaves with ether. Crude leaf extract was concentrated at 1,000*g* and layered over 30%/60% Percoll step-gradients. Nuclei were collected from the 30%/60% interface and then pelleted through 45% Percoll. Chloroplast DNA was extracted from sucrose gradient-purified chloroplasts[25]. Restriction enzyme digestion, agarose gel electrophoresis, Southern transfer (without liquid reservoir), preparation of ^{32}P-labelled probes, and hybridization were as described[26]. Nylon filters (Zetabind; AMF Cuno Inc.) were prepared as recommended by the manufacturer, apart from baking at 80 °C under vacuum for one hour. Hybridizations were at 60 °C, and after hybridization filters were washed once at room temperature and three times at 60 °C in 2×SSC, 0.5% SDS. Probes for hybridization were fractionated in 1.0% low-gelling-temperature agarose and nick-translated directly in agarose slices.

The *Arabidopsis tuf*A-hybridizing fragment was isolated from a genomic DNA library and sequenced together with the *Chlamydomonas tuf*A. Both loci contain a single, uninterrupted open reading frame of 476 (*Arabidopsis*) and 418 (*Chlamydomonas*) codons (Fig. 2). There are an extra 201 nucleotides at the 5′ end of the *Arabidopsis* open reading frame which are absent in all other known eubacterial and chloroplast *tuf*As. This sequence seems to encode a typical chloroplast transit peptide, which functions in subcellular targeting of nuclear-encoded chloroplast proteins[14], in that it is rich in serine and threonine, lacks acidic residues and has a net positive charge. The rest of the *Arabidopsis* sequence aligns throughout with the entire *Chlamydomonas* sequence, except for a 27-nucleotide insertion (nucleotides 1080–1106) which, by comparison with other EF-Tus[12,15,16], is unique to *Chlamydomonas*. Overall sequence similarity between the two genes is 77% for the amino acids and 67% for nucleotides. No similarity is apparent between the putative transit peptide sequence of *Arabidopsis* and the 5′ flanking DNA of the *Chlamydomonas tuf*A.

We used northern blotting to show that the *Arabidopsis tuf*A gene is actively expressed. A single *tuf*A transcript of ~2.0 kilobases (kb) is enriched in poly(A)$^+$ RNA and depleted in poly(A)$^-$ RNA, a pattern similar to that found for *cab*4, a well characterized nuclear gene encoding a chloroplast protein synthesized in the cytoplasm[17] (Fig. 3).

The evolutionary relationship between the *Arabidopsis* nuclear *tuf*A and known chloroplast *tuf*A genes was investigated by phylogenetic analysis using amino-acid sequences of EF-Tu and EF-1α, the eukaryotic and archaebacterial homologue of EF-Tu. In the resulting tree (Fig. 4) the *Arabidopsis* EF-Tu is found nested within a clade of chloroplast-encoded EF-Tus. This group is in turn the sister group to a clade containing the EF-Tu of the cyanobacterium *Anacystis*, which is consistent with the proposed origin of chloroplasts from endosymbiotic cyanobacteria. Thus the *Arabidopsis* nuclear *tuf*A seems to be derived from a green algal chloroplast gene.

The *Chlamydomonas* and *Arabidopsis tuf*As seem to be the sole functional *tuf*A in their respective organisms. Each is the only detectable cellular copy of the gene (Fig. 1; ref. 18) and is actively transcribed (Fig. 3; ref. 19). The simplest explanation for the subcellular distribution of the gene is that *tuf*A was transferred from the chloroplast to the nucleus. This conclusion is also further supported by phylogenetic inference (Fig. 4). Alternatively, it could be argued that ancestral *tuf*A was present in the nucleus and then transferred to the chloroplast, but this would necessitate at least four independent nuclear-to-chloroplast gene transfers. Each of these transfers would also entail the precise removal of the transit peptide sequence, because the predicted amino termini of all eubacterial and chloroplast-encoded EF-Tus align precisely. The origination of the *Arabidopsis* nuclear *tuf*A gene as a result of substitution of a pre-existing nuclear *tuf*A-like gene of non-chloroplast origin, such as one encoding a cytoplasmic EF-1α or mitochondrial EF-Tu, is ruled out by overall sequence comparison and by the gene phylogeny (note the disparate positions of the *Arabidopsis* EF-1α and EF-Tu proteins in Fig. 4).

We have found a chloroplast *tuf*A in representative ulvophycean, chlorophycean and charophycean green algae (Fig. 2; S.L.B., J. R. Manhart, M. Kuhsel and J.D.P., unpublished results), and its absence from the chloroplast chromosome of a liverwort has been established by complete sequencing of that genome[13]. The generally accepted phylogeny of green plants places the Charophyceae as the algal lineage giving rise to land plants, and liverworts as the earliest branch within land plants[20]. We therefore propose that *tuf*A was ancestrally present in the green algal chloroplast, retained in each of the main lineages after their separation, and then transferred to the nucleus within the charophycean lineage before the emergence of land plants. This places the *tuf*A transfer at 450–500 million years ago[21].

The two other cases that are indicative of modern evolutionary gene transfer are, in fact, both ambiguous. The evolutionary history of *rbc*S, which is nuclear-encoded in land plants and green algae but chloroplast-encoded in all other algae[22], is confused by uncertainty regarding common or separate endosymbiotic ancestry for the chloroplasts of the different algal groups. The *atp*9 gene is encoded in the mitochondrion in yeast but by the nucleus in *Neurospora*, with *Neurospora* also carrying an apparently silent *atp*9 gene in its mitochondrion[23]. However, sequence similarity between the two *Neurospora* genes is low, indicating that these are probably not orthologous, and the low similarity between *Neurospora* genes and the yeast *atp*9, combined with the small size of the gene, all make it difficult to trace its evolutionary history[23] (S.L.B., unpublished data). Further differences in gene content have also been noted among the three completely sequenced chloroplast genomes[10,13], and among mitochondrial genomes of the principal groups of

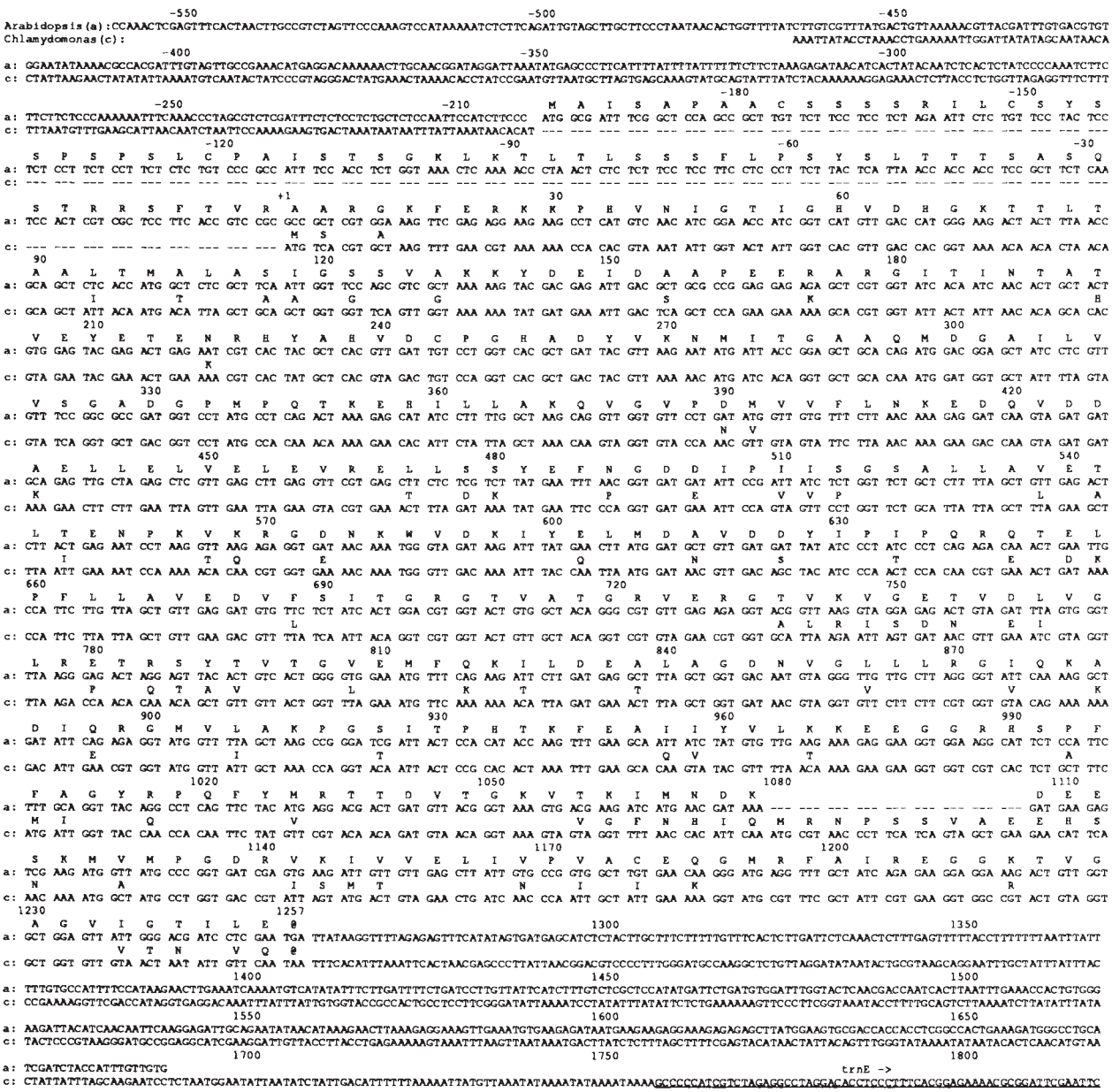

FIG. 2 *Arabidopsis* and *Chlamydomonas tuf*A nucleotide and deduced amino-acid sequences. Nucleotide sequences of the *Arabidopsis* (a) and *Chlamydomonas* (c) *tuf*A coding regions and flanking DNA are shown below the deduced amino-acid sequence of the *Arabidopsis* EF-Tu. Only variable amino acids are indicated for the *Chlamydomonas* sequence. Gaps are indicated by a dash. A *trn*E gene located downstream from the *Chlamydomonas tuf*A is underlined.

METHODS. The *Arabidopsis tuf*A was identified by plaque hybridization and isolated by standard techniques[26] from a genomic DNA library constructed from an *Mbo*I partial digest of total DNA ligated into the *Bam*HI site of the lambda replacement vector EMBL4 (gift from E. M. Meyerowitz). Three positively hybridizing plaques were isolated from two separate screenings of four genome equivalents each with the *Chlamydomonas* 400-bp *Pst*I-*Eco*RI fragment, and were found, by restriction digest analysis, to be identical. A 5.6-kb *Hin*dIII fragment was subcloned, and this then further subcloned for sequencing by dideoxy-chain termination using a combination of forced deletion clones[27] and synthetic primers. Sequences were determined at least once for each strand, overlapping all restriction sites, with the exception of the *Eco*RI site internal to the *Chlamydomonas* gene, which was used in the original cloning.

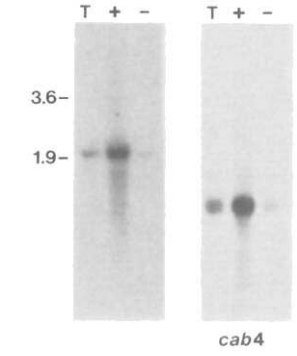

FIG. 3 The *Arabidopsis tuf*A is transcribed into poly(A)$^+$ RNA. Total (T), poly(A)$^+$ (+), and poly(A)$^-$ (−) RNAs were extracted from light-grown *Arabidopsis* plants and fractionated on a 1.5% agarose/formaldehyde gel[26]. RNAs were transferred to a nylon filter and sequentially hybridized with an internal fragment of the *Arabidopsis tuf*A gene (1.2 kb *Eco*RI–*Stu*I) and a *cab*4 gene probe. Sizes of cytoplasmic ribosomal RNA bands are indicated.

METHODS. Total RNA was extracted from whole leaves frozen in liquid nitrogen by homogenization in guanidinium isothiocyanate buffer followed by pelleting through RNAse-free 5.66 M CsCl[28]. Poly(A)$^+$ and poly(A)$^-$ RNA fractions were prepared by poly(U) Sepharose chromatography[29]. Prehybridization and hybridization were in 1% SDS, 10 × SSC, 5% dextran sulphate, 1 mg ml^{-1} carrier DNA. Preparation of ^{32}P-labelled probe and post-hybridization washes were as in Figs 1 and 2. The *cab*4 gene probe was provided by E. Pichersky.

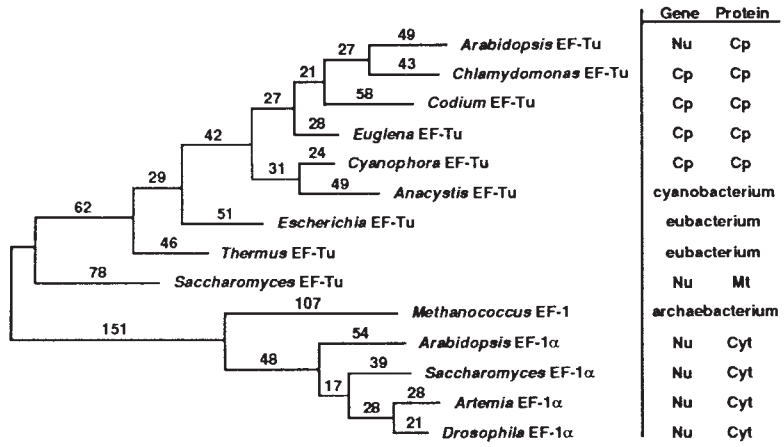

FIG. 4 The *Arabidopsis* nuclear *tuf* A encodes a chloroplast-type EF-Tu. The tree shown is the single shortest tree found by cladistic analysis of fourteen DNA-derived EF-Tu and EF-1α amino acid sequences and has a total length of 1,158 steps and a consistency index of 0.80 excluding autapomorphies. Rooting is at the midpoint, and horizontal branches are drawn proportional to their lengths, which are indicated numerically above the branches. The table on the right shows the coding compartment (Gene) and cellular site of activity (Protein) for each protein included in the tree. Eukaryotic cellular compartments are indicated as chloroplastic (cp), mitochondrial (mt), nuclear (nc) or cytoplasmic (cyt), whereas non-eukaryotic proteins are identified as such. Sources of EF-Tu/EF-1α sequences in addition to the two reported in this paper: *Anacystis nidulans*[15], *Arabidopsis thaliana* nuclear-cytoplasmic[30], *Artemia salina*[31], *Codium fragile* (M. Kuhsel, unpublished results), *Cyanophora paradoxa* (M. Kraus and W. Loeffelhardt, unpublished results), *Drosophila melanogaster*[32], *E. coli*[16], *Euglena gracilis*[12], *Methanococcus vanellii*[33], *Saccharomyces cerevisiae* mitochondrion[34], *Saccharomyces cerevisiae* nuclear-cytoplasmic[35], and *Thermus thermophilus*[36]. METHODS. Deduced amino-acid sequences were aligned[37] with the Multialign program of the Molecular Biological Information Resource package EuGene Release 3.2 (Lark Sequencing Technologies Ltd, Houston, Texas) on a SUN computer running Unix 4.0.3. Default penalties were used for gaps, with minor adjustments made by eye. Cladistic analysis was performed using the branch and bound option of PAUP version 2.4.2 (D. Swofford, Illinois Natural History Survey, Champaign, Illinois) on the SUN computer. Amino acids were scored for all informative sites as equally weighted, unordered, multistate characters. Gaps were scored as missing data and were not used as characters in the analysis.

eukaryotes[4]. Further study will determine which of these coding differences involve gene transfer, gene substitution, or gene loss.

The movement of functional genes must require the fortuitous combination of physical relocation of a complete gene, insertion into a favourable nuclear locus, and acquisition of expression signals and an in-frame transit sequence, all within a relatively short time to prevent mutational loss of function. This could explain why functional transfer is so rare compared with the physical transfer of non-functional organelle sequences to the nucleus[7,8], and would also predict that physical transfer of a gene will not occur simultaneously with the loss of its organellar copy. Characterization of other physical and functional gene transfers may elucidate the mechanisms involved in intracellular DNA movement and in the acquisition of nuclear-expression and organelle-targeting signals. □

Received 27 September 1989; accepted 18 January 1990.

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ACKNOWLEDGEMENTS. We thank M. Kraus, M. Kuhsel, B. Lescure, W. Loeffelhardt, J. R. Manhart, K. H. Schleifer and M. Sugiura for sharing unpublished data; R. G. Olmstead for help with the phylogenetic analysis; D. Swofford for providing prerelease versions of PAUP 3.0; A. M. Murphy for analysis of nucleotide substitution rates; C. dePamphilis, M. Kuhsel, C. Makaroff, H. Michaels, J. Nugent, R. G. Olmstead and M. E. Zolan for critically reading the manuscript; J. Boynton, N. Gillman, R. Helling, J. R. Manhart, E. M. Meyerowitz, E. Pichersky and C. R. Somerville for various DNAs, clones and seeds; and the NIH for financial support.

Peptide mimetics of an actin-binding site on myosin span two functional domains on actin

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THE sites on the myosin heavy chain that interact with actin and are responsible for force generation are ill-defined: crosslinking[1,2] and experiments with isolated domains of the myosin head[3,4] implicate regions in both the 50K and 20K (molecular weights in thousands) domains of the myosin head (subfragment 1, S1) in this process. We have synthesized peptides from the sequence around the fast-reacting SH_1 thiol residue in the 20K domain of S1 in order to delineate precisely an actin-binding site. We used a combination of ^{1}H-NMR and enzyme inhibition assay and also assessed the effects of peptides on skinned rabbit psoas muscle fibres to show that the region of amino acids 690–725 contains an actin-binding site. Peptides from this region bind to actin, act as mixed inhibitors of the actin-stimulated S1 Mg^{2+}-ATPase, and influence the contractile force developed in skinned fibres, whereas peptides flanking this sequence are without effect in our test

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