



The Effects of Cenozoic Global Change on Squirrel Phylogeny

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combination transitions would therefore increase the overall J values over those given here by about 10%.

To evaluate the importance of reaction 2 to the atmospheric photochemistry of H_2SO_4 , we calculated the photolysis rate, incorporating both the J value and the abundance of $\text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O}$. Details of the hydrate's abundance calculation are given as supplementary material (22). The resulting SO_3 production rates calculated for both reactions 1 and 2 are displayed in table S4. The overtone pumping photolysis rate of the complex is 2% of the monomer and becomes a smaller percentage as the altitude increases and its concentration decreases.

The results of recent experimental (9, 10) and theoretical (10–12) studies indicate that H_2SO_4 is transparent to vacuum UV (VUV) radiation at wavelengths greater than 140 nm, eliminating solar VUV photodissociation as a mechanism to explain the measured and modeled SO_2 concentrations in the upper stratosphere and mesosphere. However, overtone pumping as proposed here is a viable mechanism for photochemical decomposition of H_2SO_4 . The reverse of the mechanisms proposed by Morokuma and Mugurama (28) and Larson *et al.* (29) for SO_3 hydrolysis to H_2SO_4 provides a low-activation energy reaction path to explain the stratospheric aerosol layer. This mechanism leads to reaction at significantly lower energies than S-O bond dissociation (9). H_2SO_4 is sequestered in aerosols at low altitudes, until above 30 km, where the temperatures rise sufficiently for these aerosols to evaporate. At altitudes higher than about 35 km, the photolysis rate of SO_3 becomes faster than the rate at which it recombines with H_2O to form H_2SO_4 (4, 9). Consequently, solar pumping of vibrational overtone in H_2SO_4 will generate SO_2 at altitudes higher than about 35 km.

In conclusion, we propose a photochemical mechanism for H_2SO_4 decomposition based on vibrational OH stretching cross sections reported here. The estimated J values are sufficiently large to explain SO_2 stratospheric and mesospheric concentration and the related observation of the sulfate layer, solving a long-standing question raised by field measurements. The proposed mechanism is consistent with the fundamental properties of H_2SO_4 and the observed and modeled magnitude and altitude of the stratospheric aerosol layer. The consequences of photochemical decomposition as described here are not limited to Earth's atmosphere, but are expected to be important in planetary atmospheres containing H_2SO_4 —for example, the atmosphere of Venus.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/299/5612/1566/DC1

Materials and Methods

Tables S1 to S5

References

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By modifying habitats and creating bridges and barriers between landmasses, climate change and tectonic events are believed to have important consequences for diversification of terrestrial organisms. Such consequences should be most evident in phylogenetic histories of groups that are ancient, widespread, and diverse. The squirrel family (Sciuridae) is one of very few mammalian families endemic to Eurasia, Africa, and North and South America and is ideal for examining these issues. Through phylogenetic and molecular-clock analyses, we infer that arrival and diversification of squirrels in Africa, on Sunda Shelf islands, across Beringia, and across the Panamanian isthmus coincide in timing and location with multiple well-documented sea-level, tectonic, and paleontological events. These precise correspondences point to an important role for global change in the diversification of a major group of mammals.

Comprising some 50 extant genera and 273 species and endemic to all continents except Australia and Antarctica, the squirrel family, Sciuridae, is both diverse and geographically cosmopolitan (1). Squirrels are morphologically primitive rodents that diverged early within that clade (2, 3), and rodents constitute

nearly half of all extant species of mammals (4). An understanding of squirrel phylogeny, therefore, has important implications for tracing the historical biogeographic relationships among the continents worldwide and the evolutionary history of a key component of mammalian diversity.

First fossil occurrences on each continent suggest that squirrels originated in the Northern Hemisphere and quickly became widespread. The earliest fossil squirrel, *Douglas-*

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sciurus jeffersoni (5–7), is known from mid-Chadronian (late Eocene), about 36 million years ago (Ma) (8), in western North America. This animal lacked the arrangement of masticatory muscles known as sciuromorphy, which is typical of modern squirrels, but it shares several features of the skeleton with modern New World tree squirrels such as *Sciurus* to the extent that *Sciurus* has been termed a “living fossil” (5). The earliest sciuromorphic squirrel fossils, *Palaeosciurus*, date from early Oligocene in Europe (9). Also known from early Oligocene deposits in Europe and in Pakistan are some isolated teeth attributed to flying squirrels (10, 11). In Africa, the earliest squirrel fossils described are Miocene (12), and in South America there is no evidence of squirrels before the Panamanian land bridge was established between North and South America, about 3.1 Ma (13, 14). An apparent high diversity of fossil flying squirrel teeth early in sciurid history has been taken as evidence that flying squirrels (subfamily Pteromyinae) have an origin independent of tree and ground squirrels (subfamily Sciurinae), justifying the current division of the squirrel family into two distinct subfamilies and even prompting suggestions that flying squirrels belong in a separate family (1, 2, 10). Taxonomic treatments using morphological features of the teeth, the cranium, and the male reproductive system have subdivided modern tree and ground squirrels into between six and eight tribes (2, 15), but relationships among these tribes and between the tribes and flying squirrels have remained unclear.

We applied Bayesian, maximum-likelihood (ML), maximum-parsimony (MP), and minimum-evolution methods to analyze DNA sequences from every modern genus of squirrel currently recognized (1, 16) [except *Biswamyopterus*, an Indian flying squirrel known only from its type specimen (17)], plus the outgroup *Aplodontia rufa* [the only living representative of the Aplodontidae, which is the sister group of Sciuridae (2, 3, 6)] and one member of the Gliridae [sister group to sciurids plus aplodontids (3)]. To mitigate the effects of long-branch attraction, we included two species from some genera that exhibited relatively long, basally emerging branches. To more firmly establish the timing of colonization of South America across the Panamanian isthmus, we sequenced additional North, Central, and South American tree-squirrel species for use in molecular-clock analyses. Our data set comprised 2659 base pairs of DNA sequence, which we obtained from three genes: the nuclear gene coding for interphotoreceptor retinoid-binding protein (IRBP), and mitochondrial 12S and 16S ribosomal DNA (18–20).

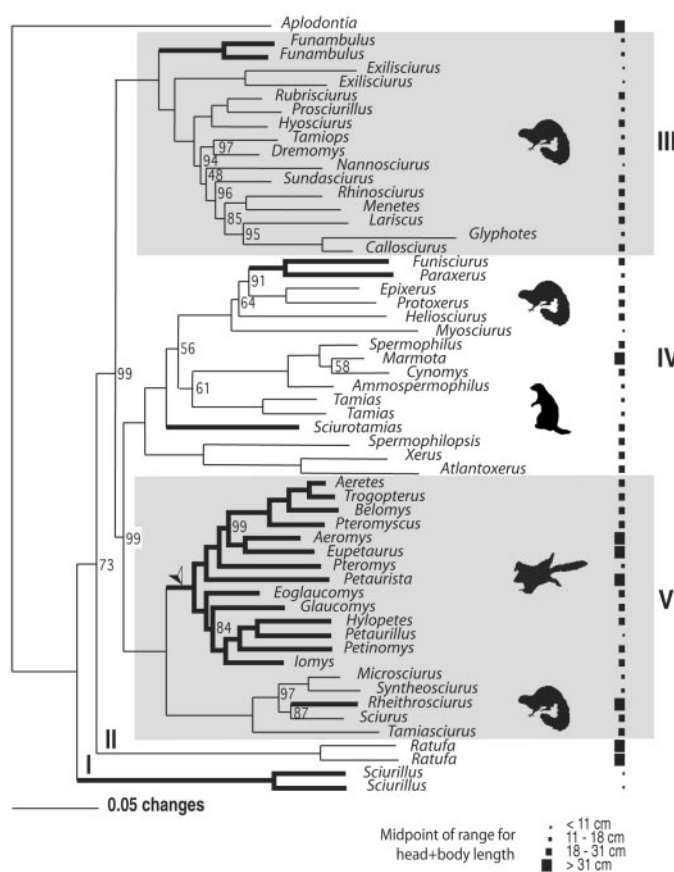
Our results argue against the long-standing practice of segregating all tree and ground squirrels into a group distinct from flying squirrels. Instead, our analyses indicate that

extant squirrels stem from a rapid diversification of the family into two monotypic and three major lineages (clades I to V in Fig. 1).

Within one of the three major lineages of sciurids (clade V, Fig. 1), evidence for a single origin of flying squirrels (bold branch indicated with arrowhead) is strong (21), but so is the evidence that this clade of flying squirrels is nested well within the squirrel family and that it forms a sister group to the clade comprising most New World tree squirrels (*Microsciurus*, *Syntheosciurus*, *Sciurus*, and *Tamiasciurus*). The relatively late origin of the flying squirrel clade (Fig. 2) raises questions about dental characters that link modern flying squirrels to early Oligocene fossils and suggests either that these traits are labile and arose more than once in sciurid history or that they are not exclusive to flying squirrels and instead originated in an ancestor that flying squirrels share with typical tree squirrels.

A second major lineage (clade III) embraces a morphologically diverse array of Indo-Malayan tree squirrels that have traditionally been placed together in the tribe Callosciurini (2, 15). Although several morphological characters have suggested that *Funambulus*, which is from the Indian subcontinent, is more closely related to African tree squirrels (2) [especially *Funisciurus* and *Paraxerus* (15)], our data clearly group *Funambulus* with other Asian squirrels, albeit as an early offshoot within the clade.

Fig. 1. A tree for squirrels: Bayesian phylogram of relationships among genera of Sciuridae, with *Aplodontia* as outgroup. Posterior probabilities at all nodes are 100% unless indicated. Phylogenetic positions of branches drawn with heavy lines are discussed in text; an arrowhead marks the base of the flying-squirrel clade. Three basic forms—tree, ground, and flying—are shown as silhouettes opposite each clade or subclade in which they appear. (The ground-squirrel taxa listed vertically as *Spermophilus* to *Atlantoxerus* and tree squirrels *Microsciurus* to *Sciurillus* each represent three distinct subclades.) Body-size categories (column at right), which were calculated from (21) and museum specimen tags, were defined at about logarithmically spaced intervals, with boundaries set to minimize the number of borderline taxa.



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squirrels exclusively shared a single common ancestor that produced a single evolutionary radiation within Africa. In addition, although the affinities of terrestrial *Sciurotamias*, from China, have been interpreted variously by other workers to be with Southeast Asian tree squirrels (2) (in our clade III), North American red and Douglas squirrels (15) (*Tamiasciurus*, in clade V), or *Tamias* (23) (clade IV), its affiliation here with this large clade (IV) of terrestrial and/or African squirrels is unambiguous.

The geographic coherence of modern clades of squirrels suggests that dispersal over large expanses of water or desert is uncommon and that squirrel distributions may therefore be important indicators of geological and environmental history. For example, callosciurine tree squirrels (clade III) reached and diversified considerably throughout the Sunda Shelf islands in Southeast Asia, which were periodically united and linked to the mainland by lowered sea levels. The Caribbean islands, on the other hand, are more isolated and evidently were not colonized by squirrels (1). According to our data, ground squirrels and tree squirrels in Africa (clade IV) each descended from a single common ancestor; hence, all of the modern diversity of African squirrels can be accounted for by just two colonizations by source populations from Eurasia (Fig. 2). Our analyses also indicate that a single lineage of squirrels crossed Wallace's Line to give rise to the three genera (*Rubrisciurus*, *Prosciurillus*, and *Hyosciurus*; clade III) and multiple species of squirrels on Sulawesi. We show elsewhere (24) that, with the exception of *Sciurillus* (see below), the entire radiation of tree squirrels in South America appears to have descended from a single lineage (in clade V) that entered that continent with establishment of the Panamanian isthmus.

The most unexpected results, from a geographic perspective, are the phylogenetic positions of three tree-squirrel genera of unusual body size (Fig. 1). Giant *Rheithrosciurus*, whose single species is found on Borneo (1), is nested within a branch of the clade (V) comprising most of the tree squirrels of the New World (Fig. 2), a position suggested by a few morphological features but also questioned by the authorities who cite them (15). Either the extinction of all relatives distributed across a large expanse of Asia or long-distance dispersal must be invoked to explain how a clade that is largely confined to the Americas and Palearctic could give rise to a single member isolated on Borneo. Two other genera, pygmy *Sciurillus* and giant *Ratufa*, constitute monotypic lineages (I, II) that appear to have diverged early in the history of the Scuridae from other members of the family, before the three major lineages (III to V) described above became established.

In ML, MP, and Bayesian analyses, the Neotropical pygmy squirrel *Sciurillus* (clade

I) emerges at the base of the tree as the sister group to all other squirrels—a position that has never previously been suggested. We used simulation tests (9, 25) to evaluate the possibility that the genus was grouped incorrectly because its lineage occupies a relatively long branch, but we found no evidence that long-branch attraction explains its position; nor does inclusion of a dormouse, *Graphiurus* (3, 20), as an immediate outgroup to both *Aplodontia* and Scuridae change the conclusion. All partitions of the molecular data—protein-coding as well as ribosomal genes, data from nuclear as well as mitochondrial

genomes, and even the relatively neutrally evolving third-position sites of codons—point to *Sciurillus* as an early offshoot within the family. How, when, and from where its ancestors first reached South America; where they were living during South America's isolation (from Paleocene through Pliocene) as an island continent; and whether the lineage experienced unrecognized episodes of diversification (and extinction) remains to be clarified by the fossil record.

The evolutionary history of the other monotypic lineage to branch off early from the rest of the squirrel family, Indo-Malayan ge-

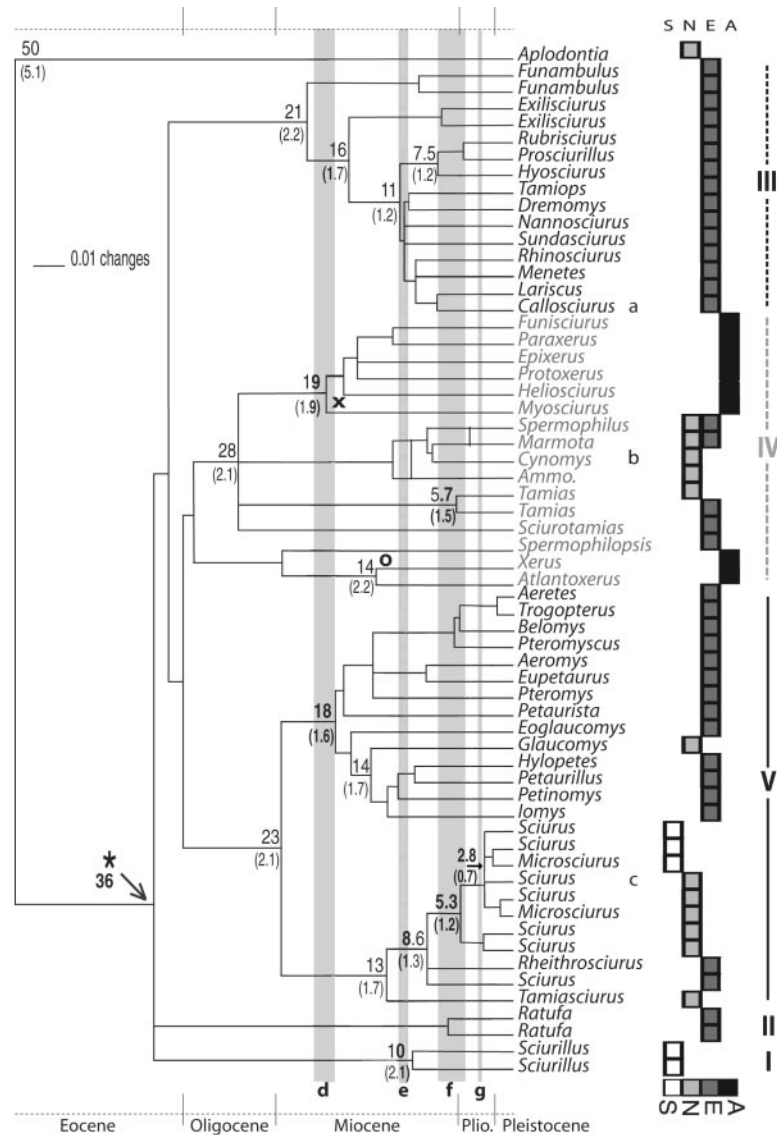


Fig. 2. ML, molecular-clock tree, with branch lengths (some so short they appear to be zero) set by changes in the third nucleotide-position of codons in IRBP. Geographic distributions [from (7)] at right: South America, S; North America, N; Eurasia, E; Africa, A. Time calibration at asterisk is set to 36 Ma (8). Dates inferred for key divergence events are shown (in Ma) beside nodes, with standard deviations produced by repeated simulations in parentheses. Southeast Asian *Glyphotes*, sister taxon to *Callosciurus* (marked "a"), and Central American *Syntheosciurus*, sister taxon to the *Sciurus* species marked "c," were omitted from this analysis because their sequences are partial. Nonclock ML analysis indicates accelerated evolution in *Cynomys* IRBP (marked "b"); vertical lines indicate divergences among *Spermophilus*, *Marmota*, and *Ammospermophilus* if *Cynomys* is omitted. Gray shading, intervals d to g, X, and O are explained in the text.

nus *Ratufa* (clade II), is also unclear, although some Miocene specimens from Europe have been assigned to this genus (26). Although an alternative branching sequence of the main lineages is suggested by minimum-evolution distance analyses, all methods point to *Ratufa* and *Sciurillus* as the earliest divergences within the family, to a rapid succession of major branching events, and to a tree with fundamentally the same structure and substructure as that shown in Fig. 1 [Supporting Online Material (SOM) Text].

Because squirrels are widespread and their clades are geographically highly coherent, it is of interest to determine the timing of major geological events that could have influenced their migration between landmasses. The tree shown in Fig. 2 was inferred with the use of the assumption of a molecular clock and calibrated by assigning the age of *Douglassciurus* (36 million years) to the base of the sciurid crown radiation (8). This calibration also yields a molecular-clock date of 50 million years for the

node joining *Aplodontia* and the Sciuridae, which is an excellent match for the Bridgerian age of the ischyromyid rodent that has been proposed as the common ancestor of these taxa (6, 8). This molecular-clock tree also indicates that the divergence of the five major clades of squirrels took place during the latest Eocene and earliest Oligocene, “the most significant episode of climatic change and extinction” (27) since early Eocene.

Superimposed on this tree are vertical gray bars showing the timing of other major geological events with potential effects on sciurid vicariance and dispersal. The ages of the earliest known arboreal (×) and ground (○) squirrel fossils in Africa (13) are plotted on this tree within clade IV. These fossil dates fall very close in timing to the base of the evolutionary radiations producing, respectively, the modern genera of tree and ground squirrels of that continent. Moreover, the interval labeled “d,” 20 to 18 Ma, which is when geological and faunal evidence point to establishment of a land bridge between Asia and Africa (28), coincides both with the first known squirrel fossil in Africa (a tree squirrel) and with the base of the African tree-squirrel radiation.

Interval e, 11.4 to 10.5 Ma (see also Fig. 3), marks the lowest pre-Pleistocene sea stand of the Cenozoic (29, 30) and coincides with the explosive diversification among tree squirrel genera on Sunda Shelf islands (clade III) as well as the divergence of the sole clade (comprising *Rubrisciurus*, *Prosciurillus*, and *Hyosciurus*) to cross Wallace’s Line to Sulawesi. The tectonic history of Sulawesi has challenged geographers with its complexity (31), and its high endemism and oceanic character indicate that it has not easily been colonized. This timing for squirrels is also congruent with minimum age estimates of initial divergence of flightless grasshoppers on the island (32). For Southeast Asia more generally, we hypothesize that sea-level fluctuations promoted diversification of tree squirrels through the alternation of dispersal across the shelf during low stands with allopatric divergence on islands during higher stands. We predict that more detailed phylogeographic study of squirrels at the species level will clarify the complex tectonic history of this region.

Sometime during interval f (7.4 to 4.8 Ma), a marine barrier first opened to separate North America and Asia at Beringea (33). Movement between these continents by flying and tree squirrels is likely to have required not only land but also a forested corridor. For tree squirrels in clade V it is not clear from the phylogeny whether two lineages (giving rise, respectively, to *Tamiasciurus* and American *Sciurus*+*Microsciurus*+*Syntheosciurus*) crossed into America from Eurasia, or the lineage(s) giving rise to

Eurasian *Sciurus* and *Rheithrosciurus* crossed Beringea in the opposite direction. In either case, corresponding dates of divergence (13 and 8.6 Ma) precede establishment of the marine barrier and represent a time during which the paleobotanical record suggests that Beringea was forested (34). The same is true for divergence of North American flying squirrels (*Glaucomys*) from Asian relatives at 14 Ma. By contrast, members of each of the ground squirrel genera *Spermophilus*, *Marmota*, and *Tamias* (in clade IV) may have made the crossing later (Fig. 2), after a land bridge was reestablished but when the terrain was unforested.

Lastly, the divergence within clade V between tree squirrels of Central (part of North) and those of South America closely corresponds in time to the formation of a land bridge between North and South America at the Panamanian isthmus (interval g, 3.4 to 3.1 Ma) (14). [Polyphyly of *Microsciurus* and paraphyly of *Sciurus* are discussed elsewhere (24).]

At present, enhanced scientific attention to global change coincides with growth of comparative information from organismal genomes. Our phylogenetic and molecular-clock analyses of DNA from squirrels reveal striking chronological and geographical correspondence between evolutionary divergence and multiple events that are documented in the geological record. Our results indicate that global change has driven large-scale diversification within this mammalian family and, reciprocally, that sciurid diversification studied on a smaller scale can yield inferences about tectonics, sea level, and climate. Thus, phylogenetic analyses provide a framework for tracing not only organismal evolution but also transformations on scales ranging from evolutionary substitutions of single nucleotides to geological movements of entire continents.

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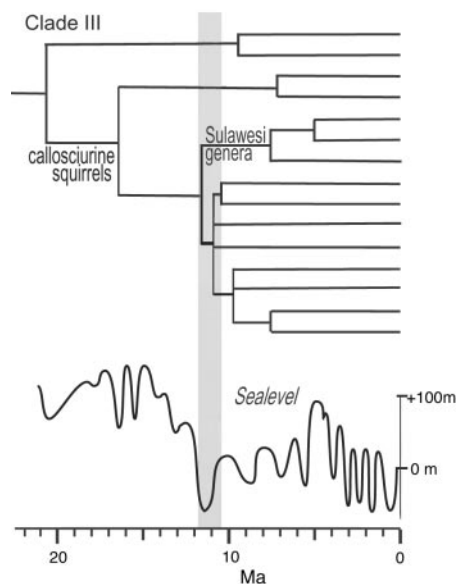


Fig. 3. Detail of molecular-clock tree showing radiation of Southeast Asian tree-squirrel genera in clade III (*Funambulus* through *Callosciurus*, as listed vertically in Fig. 2) with eustatic sea-level curve of de Graciansky et al. (29) and Haq et al. (30). Rapid diversification of many genera and divergence of the Sulawesi clade coincide with the lowest pre-Pleistocene sea-level stand of the Cenozoic (gray shading; this is band e of Fig. 2). Low sea level may have permitted dispersal of squirrels across the Sunda Shelf, where populations then became isolated during higher sea stand and ultimately produced what are now recognized as distinct genera. Divergence and diversification among species within these genera (not treated in this analysis) may have been associated with subsequent sea-level oscillations, which allowed repeated isolation and dispersal, distributing members of different genera among multiple islands and the mainland.

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18. Materials and methods are available as supporting material on *Science* Online.
19. Species names and GenBank accession numbers are given in table S1.
20. In order to retain more data for resolving relationships within the Scuriidae and because *Graphiurus* is ambiguous in its sequence alignment with *Aplodontia* and Scuriidae at a greater number of sites, analyses illustrated here used *Aplodontia* as the sole outgroup.
21. Our DNA sequence data concur with morphological characters of the wrist (35) in supporting flying squirrel monophyly, which earlier morphological and immunological evidence had contested.
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Role of Chloroplast Protein Kinase Stt7 in LHCII Phosphorylation and State Transition in *Chlamydomonas*

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Photosynthetic organisms adapt to changes in light quality by redistributing light excitation energy between two photosystems through state transition. This reorganization of antenna systems leads to an enhanced photosynthetic yield. Using a genetic approach in *Chlamydomonas reinhardtii* to dissect the signal transduction pathway of state transition, we identified a chloroplast thylakoid-associated serine-threonine protein kinase, Stt7, that has homologs in land plants. Stt7 is required for the phosphorylation of the major light-harvesting protein (LHCII) and for state transition.

Oxygenic photosynthetic organisms have the ability to adapt to changes in light quality and quantity. They balance energy input and consumption in the short term through dissipation of excess energy and regulate energy flow between the two photosystems through state transition. This reversible redistribution leads to an overall increase in photosynthetic quantum yield. State transition has been correlated with the reversible phosphorylation of the polypeptides of the light-harvesting complex II (LHCII) antenna complex (1). We used mutants of *Chlamydomonas reinhardtii* that were deficient in state transition to identify components of the signal transduction chain involved in this process. We found that two of these mutants are affected in the same nuclear gene encoding a chloroplast Ser-Thr kinase that is implicated in LHCII phosphorylation.

The kinetics of state transition match the pattern of LHCII phosphorylation-dephosphorylation: LHCII is phosphorylated under state II and dephosphorylated under state I conditions (1, 2). Numerous thylakoid proteins undergo phosphorylation-dephosphorylation cycles during state transition, although their functions are not clear (1). The activation of the thylakoid-bound kinase responsible for phosphorylation of light-harvesting chlorophyll-binding protein

(LHCP) under state II depends on the redox state of the plastoquinone pool (1), which is part of the photosynthetic electron transport chain and located between photosystem II (PSII) and photosystem I (PSI). Activation of the LHCII kinase is regulated specifically by binding of plastoquinol in the Q_o pocket of the cytochrome b₆f complex (3).

In plants induced to undergo state transition, a portion of LHCII becomes associated with the stromal arrays that are enriched in PSI (1). During transition to state II, the mobile part of LHCII becomes connected to PSI in a cytochrome b₆f-controlled process (4). This connection requires the PsaH subunit (5). The lateral displacement of LHCII from the PSII-rich grana to the PSI-rich lamellar thylakoid regions results in transfer to PSI of about 80% of the excitation energy absorbed by LHCII in *C. reinhardtii* (4), a considerably higher amount than in land plants, in which only 15 to 20% of LHCII is mobile (1). The transition from state I to state II induces a switch from linear to cyclic electron flow in *C. reinhardtii* and reveals a correlation between the redistribution of antenna complexes during state transition and the onset of cyclic electron flow (6, 7). *C. reinhardtii* is advantageous for analyzing state transition because its transition from state I to state II is accompanied by a large fluorescence decrease (8). Screens using fluorescence videomaging have identified several mutants of *C. reinhardtii* that are unable to perform state transition, in particular *stt7-1* (9, 10).

Another allelic mutant, *stt7-2*, was isolated during this study (11). The fluorescence

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