ER-Golgi Rab protein (Ypt1p) and the Golgi–cell surface Rab protein (Sec4p) can fulfill Rab function at several steps of secretory pathway in the absence of both normal Rab proteins (*7*). This provides compelling evidence that Rab proteins do not accomplish vesicle targeting. Other insights into Rab proteins have also emerged, including the identification of critical proteins that can modulate GTP binding or hydrolysis by Rab proteins or that control attachment of Rab proteins to membranes (*8*).

Meanwhile, the SNARE protein family was discovered, and its central role in compartment-specific docking was established (*2*). With this framework in place, evidence has gradually accumulated during the last few years for a different view of Rab proteins, in which they somehow control the speed (but not the intrinsic specificity) of membrane fusion by regulating the rate of assembly of SNARE complexes. Complexes of v- and t-SNAREs are inherently stable and can assemble spontaneously in vitro in the absence of Rab proteins. Yet, Rab proteins act as upstream regulators of SNARE complex assembly in living cells because mutations in them prevent assembly of SNARE complexes (*9*, *10*). And recently, in an important advance, Zerial and colleagues (*11*) have shown directly that the level of Rab[GTP] determines the overall rate of membrane fusion. GTP hydrolysis is not only independent of fusion but actually is irrelevant to fusion per se, which occurs perfectly well with mutant Rab proteins that cannot hydrolyze GTP, also ruling out various kinetic proofreading models for Rab action (*12*). What is relevant to the rate of fusion is the absolute level of GTP-bound Rab, which is set by a balance that is modulated by various protein regulators and probably many unknown factors. Rab[GTP], produced from Rab[GDP], is the throttle that sets the pace of membrane fusion, nicely in keeping with the general role of GTP-binding proteins (G proteins) in biology, which turn on signaling pathways as they are switched into their GTP-bound states.

The engine of membrane fusion also has dampers in the form of a family of proteins (*13*) related to the yeast *SEC1* gene whose members bind to one or another t-SNARE (*9*, *14*). These proteins are required for vesicular transport but act as negative regulators of SNARE complex assembly, as first shown by Scheller and colleagues (*15*), who found that a Sec1-family protein prevents its t-SNARE from binding a v-SNARE. The physiological importance of damping fusion by this mechanism is clear. Certain mutations in a Sec1-family member (Sly1p) allow cells to live happily without the local Rab protein, Ypt1p (*6*). Rab proteins and Sec1-family proteins are therefore pitted against each other in a tug-of-war, the balance of which must somehow control the rate of fusion at the level of SNAREs.

Lupashin and Waters (*1*) now provide a molecular mechanism that explains how this works. Three new important observations establish the first concrete link between the Rab, Sec1p, and SNARE families of proteins. First, they report that the ER-Golgi Rab protein Ypt1p, normally present on transport vesicles budding from the ER, interacts with the free Golgi t-SNARE (Sed5p), but does not do so when this same t-SNARE is complexed with cognate v-SNAREs. Second, the local Sec1-family member (Sly1p) is bound to the same t-SNARE before, but not after, assembly with cognate v-SNAREs. Third, the amount of Sec1-family member bound to its t-SNARE systematically decreases as the level of the local Rab protein is increased in living cells.

Together, these new findings imply that Rab and Sec1-family proteins are, respectively, throttles and dampers of membrane fusion, directly opposing each other by allowing or preventing v-SNAREs access to t-SNAREs. The fusion engine, burning ATP as SNAREs are activated for fusion by the ATPase NSF, will be throttled up when the level of Rab[GTP] on transport vesicles is increased, displacing the Sec1-family dampers from t-SNAREs. Formally, Rab[GTP] acts as a catalyst of SNARE complex assembly (*9*), because it is neither generated nor consumed as it accelerates the docking reaction (see figure). In addition, Sec1-family proteins may improve the reactivity of cognate t-SNAREs toward Rab[GTP] (*1*).

We are probably only beginning to perceive a sophisticated regulatory and signal-transducing system that controls the speed of what have long been regarded as constitutive transport pathways. Because each transport segment has a distinct set of Rab proteins, any one segment can, in principle, be throttled up or down in speed independently of the others, allowing local flow patterns to be fine-tuned to momentary physiological needs.

References

1. V. V. Lupashin and M. G. Waters, *Science* **276**, 1255 (1997).
2. T. Söllner *et al.*, *Nature* **362**, 318 (1993); J. E. Rothman, *ibid.* **372**, 55 (1994).
3. A. Salminen and P. J. Novick, *Cell* **49**, 527 (1987); B. Goud, A. Salminen, N. C. Walworth, P. J. Novick, *ibid.* **53**, 753 (1988).
4. N. Segev, J. Mulholland, D. Botstein, *Cell* **52**, 915 (1988); H. D. Schmitt, M. Puzicha, D. Gallwitz, *ibid.* **53**, 635 (1988); N. Segev, *Science* **252**, 1553 (1991); M. F. Rexach and R. W. Schekman, *J. Cell Biol.* **114**, 219 (1991).
5. J. P. Lian and S. Ferro-Novick, *Cell* **73**, 735 (1993).
6. C. Dascher, R. Ossig, D. Gallwitz, H. D. Schmitt, *Mol. Cell. Biol.* **11**, 872 (1991); R. Ossig, C. Dascher, H. H. Trepte, H. D. Schmitt, D. Gallwitz, *ibid.*, p. 2980.
7. P. Brennwald and P. Novick, *Nature* **362**, 560 (1993); B. Dunn, T. Stearns, D. Botstein, *ibid.*, p. 563.
8. S. R. Pfeffer, *Curr. Opin. Cell Biol.* **6**, 522 (1994).
9. M. Sogaard *et al.*, *Cell* **78**, 937 (1994).
10. P. Brennwald *et al.*, *ibid.* **79**, 245 (1994).
11. V. Rybin *et al.*, *Nature* **383**, 266 (1996).
12. H. R. Bourne, *Cell* **53**, 669 (1988).
13. M. K. Aalto, S. Keranen, H. Ronne, *ibid.* **68**, 181 (1992).
14. Y. Hata, C. A. Slaughter, T. C. Südhof, *Nature* **366**, 347 (1993); E. P. Garcia, E. Gatti, M. Butler, J. Burton, P. De Camilli, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2003 (1994); J. Pevsner, S. C. Hsu, R. H. Scheller, *ibid.*, p. 1445.
15. J. Pevsner *et al.*, *Neuron* **13**, 353 (1994).

PLANETARY ATMOSPHERES

Warming Early Earth and Mars

James F. Kasting

Sagan and Chyba (*1*), in their article on page 1217 of this issue, have revived an old debate about how liquid water was maintained on early Earth and Mars despite a solar luminosity 25 to 30% lower than that at present. A theory that has been popular for some time (*2*) is that greatly elevated concentrations of atmospheric CO_2, produced by the action of the carbonate-silicate cycle, provided enough of a greenhouse effect to warm early Earth. However, Rye *et al.* (*3*) have placed geochemical constraints on early atmospheric CO_2 abundances that fall well below the levels needed to warm the surface. These constraints are based on the absence of siderite ($FeCO_3$) in ancient soil profiles—a negative and, hence, rather weak form of evidence—and apply to the time period 2.2 to 2.8 billion years ago, when Earth was already middle aged. Nonetheless, the soil data provide some indication that atmospheric CO_2 levels may have been lower than previously thought. An even more serious problem arises if one tries to keep early Mars warm with CO_2. Model calculations predict that CO_2 clouds would form on Mars in the upper troposphere, reducing the lapse rate and severely limiting the amount of surface warming (*4*). A suggestion that CO_2 clouds may have warmed

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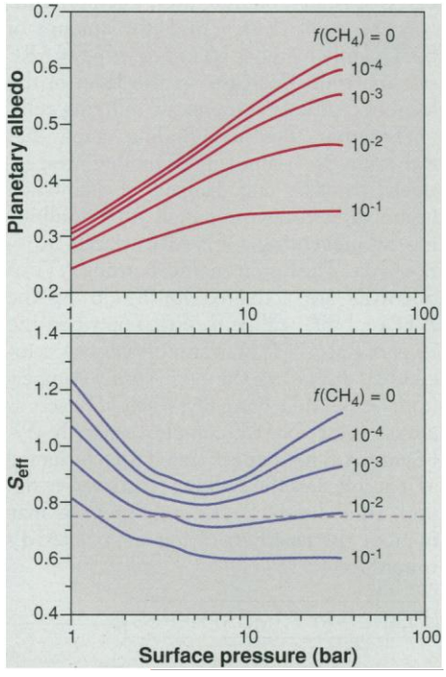
the planet radiatively (5) has yet to be borne out by detailed calculations.

An alternative mechanism for warming planetary climates, originally propounded by Sagan and Mullen (6), is to take advantage of the strong greenhouse effect provided by NH_3. On Earth, atmospheric NH_3 concentrations of 10^{-5} to 10^{-4} could have compensated for reductions in solar luminosity of several tens of percent (6, 7). Keeping NH_3 around in the primitive atmosphere is not easy, though, because it photolyzes rapidly and irreversibly to form N_2 and H_2 (7, 8). Sagan and Chyba (1) suggest that NH_3 may have been shielded from solar ultraviolet (UV) radiation by a layer of photochemically generated organic haze. Such a layer actually exists on Saturn's moon Titan, where it is produced by UV and charged-particle irradiation of the CH_4-N_2 atmosphere.

The difficulty with this hypothesis is that organic haze can only be produced efficiently in atmospheres that are strongly reducing. In weakly reducing atmospheres, CH_4 oxidizes to CO_2 instead of polymerizing to form hydrocarbons. In charged-particle irradiation experiments performed in Sagan's laboratory at Cornell University (9), the key factor was determined to be the local C/O ratio. Ratios of C/O >1 promoted polymerization of CH_4; C/O < 1 led primarily to oxidation. In the anoxic atmosphere of early Earth (or Mars), the primary reservoirs of O would have been H_2O and CO_2. Sagan and Chyba rightly point out that the H_2O concentration should have been low in the upper stratosphere, where CH_4 would have been photolyzed. This low concentration is not likely to have been the case, however, for CO_2. Carbon dioxide should have been a major component of volcanic emissions (10) and would also have been generated by photochemical oxidation of CH_4 and CO. To maintain C/O (or CH_4/CO_2) > 0.1, volcanic CO_2 must either have been rapidly removed from the atmosphere or else prevented from entering the atmosphere in the first place. Rapid weathering of the ejecta from frequent, large impacts could, in principle, have provided a large sink for CO_2 (11). But the objects hitting Earth during this time may also have brought in large amounts of carbon, most of which should have been oxidized on impact (12), so it is not clear that frequent impacts would have resulted in low concentrations of atmospheric CO_2. A more radical idea (13) is that volcanic CO_2 may have been efficiently trapped beneath a globe-encircling layer of ice. This suggestion solves the faint young sun problem by giving in to it, in which case there is no need for a shield of organic haze. But while it is difficult to rule this idea out during the earliest part of Earth's history, the planet cannot have been globally covered by ice after about 3.5 billion years ago, because the presence of sedimentary rocks (6) and life (14) at this time indicates that surface water must have existed.

Thus, the proposed organic haze layer on early Earth should only have existed if the primitive atmosphere contained more CH_4 than CO_2. This prediction is substantiated, in part, by photochemical model experiments (15). Simulated UV irradiation of CO_2-rich atmospheres containing up to 1% CH_4 does not form appreciable amounts of organic haze, at least not by the type of polyacetylene photochemistry that is thought to occur on Titan (16). The presence of oxidizing radicals, primarily O and OH, short circuits the process by which CH_4 polymerizes. Note that Titan, where such a smog layer exists, is not a good analog for early Earth because the extremely cold temperatures there keep its atmosphere virtually free of both CO_2 and H_2O.



Warming trends. (**Top**) Planetary albedo as a function of surface pressure and CH_4 mixing ratio $f(CH_4)$ for a CO_2-dominated atmosphere on Mars. The assumed surface temperature is 273 K. Calculations were made with the climate model of (4). (**Bottom**) Effective solar flux $S_{eff} = S/S_0$ required to maintain the surface of Mars at 273 K (S_0 is the current value). The dashed line shows the solar flux at 3.8 billion years ago, when most of the valleys are thought to have formed. A few bars of CO_2 combined with ~1% CH_4 could have kept early Mars warm.

How, then, can one solve the climate problem for early Mars and keep early Earth warm without violating the geochemical constraints on CO_2? Methane may indeed be the answer, but the mechanism by which it warmed the planets' surfaces was probably different from that envisioned by Sagan and Chyba. Methane is itself a greenhouse gas, that is, it absorbs radiation at thermal infrared wavelengths. Kiehl and Dickinson (17) have shown that a CH_4 concentration $f(CH_4)$ of 100 parts per million in the primitive terrestrial atmosphere could have compensated radiatively for roughly a 10-fold reduction in CO_2. This is within a factor of 2 of the amount needed to reconcile the CO_2 constraints from paleosols (3) with the evidence for liquid water and life. A CH_4 concentration of 100 ppm at 2.2 to 2.8 billion years ago could plausibly have been maintained by methanogenic bacteria, provided that the atmosphere at that time was essentially free of O_2 (18, 19).

Methane may have had an even bigger effect on the paleoclimate than has hitherto been realized because it also absorbs visible (red) and near-infrared radiation (20). Indeed, this is why Uranus and Neptune appear blue to the eye: the red wavelengths of sunlight are absorbed by CH_4 (21). The concentration of CH_4 needed to significantly alter planetary albedos is on the order of 0.1 to 1%. One can demonstrate this with a calculation (see figure). The top panel of the figure shows planetary albedo as a function of surface pressure and $f(CH_4)$ for a CO_2-dominated atmosphere on Mars. The procedure followed is identical to the "inverse" climate calculation shown in figure 8 of (4): The surface temperature was fixed at 273 K, and the tropospheric lapse rate was assumed to follow a moist adiabat in which both H_2O and CO_2 were allowed to condense. The results shown here are very crude because the complex band structure of CH_4 in the visible–near infrared (20) has been approximated by a continuum. Thermal infrared absorption by CH_4 has been modeled accurately, however, using exponential sum coefficients derived from laboratory absorption measurements. The implications for the martian paleoclimate are illustrated in the bottom panel of the figure, which shows the "effective" solar flux (that is, the flux normalized to the present value) required to maintain a surface temperature of 273 K for different values of $f(CH_4)$. The horizontal dashed line at $S_{eff} = 0.75$ represents the effective solar flux 3.8 billion years ago, when most of the martian valleys are thought to have formed (22). Evidently, global mean surface temperatures above freezing could have been maintained by several bars of CO_2 combined with ~1% CH_4. The required abundances of these gases might be lower if high-altitude CO_2 clouds contributed some degree of surface warming.

Where might atmospheric CH_4 have come from on early Mars? On Earth, as noted earlier, methanogenic bacteria could have generated copious quantities of CH_4 once surface life had become established. Such bacteria could have existed on early Mars as

well, and there is controversial evidence from the martian meteorite ALH84001 that supports this view (*23*). But there are possible abiotic sources for CH_4, too, on both Mars and early Earth. The one that was probably most important is that identified by Sagan and Chyba, namely, submarine outgassing at mid-ocean ridges. Submarine volcanism favors CH_4 over CO_2 by shifting the chemical equilibrium to the right in the reaction $CO_2 + 2\,H_2O \leftrightarrow CH_4 + 2\,O_2$. Today, the CH_4/CO_2 ratio in hydrothermal vent fluids is only ~1% (*24*), but it could have been much higher in the past if the primitive mantle were more reduced. Evidence for a reduced early mantle is provided by thermodynamic analyses of diamond inclusions (*25*) and by recent studies of metal-silicate partition coefficients of siderophile elements (*26*), which indicate that the upper mantle could originally have been in equilibrium with metallic iron. Applying these same arguments to early Mars is speculative but not outside the realm of possibility.

I note, parenthetically, that maintaining $f(CH_4) = 0.01$ in the primitive martian atmosphere would require a CH_4 source comparable to that on modern Earth, which would almost certainly have to be biological. It would also require that hydrogen be bottled up at the exobase so that it escaped at less than the diffusion-limited rate (*18*). This latter constraint seems physically plausible in light of the cold temperatures expected in a CO_2-rich upper atmosphere, but it has not been demonstrated that the escape of H would have been slow. So, it is far from obvious that high CH_4 concentrations are the solution to the early Mars climate problem. It does seem likely that Sagan and Chyba are on the right track, however: Reduced gases probably were present in significant concentrations on both early Earth and Mars, and they played an important role in climate evolution on both planets. Sagan himself had been fond of this idea for many years. It seems likely that his excellent scientific intuition will once again be found to be correct.

References

1. C. Sagan and C. Chyba, *Science* **276**, 1217 (1997).
2. T. Owen, R. D. Cess, V. Ramanathan, *Nature* **277**, 640 (1979); J. C. G. Walker, P. B. Hays, J. F. Kasting, *J. Geophys. Res.* **86**, 9776 (1981).
3. R. Rye, P. H. Kuo H. D. Holl, *Nature* **378**, 603 (1995).
4. J. F. Kasting, *Icarus* **94**, 1 (1991).
5. R. T. Pierrehumbert and C. Erlick, in preparation.
6. C. Sagan and G. Mullen, *Science* **177**, 52 (1972).
7. W. R. Kuhn and S. K. Atreya, *Icarus* **37**, 207 (1979).
8. J. F. Kasting, *J. Geophys. Res.* **87**, 3091 (1982).
9. B. N. Khare, C. Sagan, J. E. Zumberge, D. S. Sklarew, B. Nagy, *Icarus* **48**, 290 (1981).
10. H. D. Holland, *The Chemical Evolution of the Atmosphere and Oceans* (Princeton Univ. Press, Princeton, NJ, 1984).
11. K. J. Zahnle, private communication.
12. J. F. Kasting, *Origins Life* **20**, 199 (1990).
13. J. L. Bada, C. Bigham, S. L. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1248 (1994).
14. J. W. Schopf, *Earth's Earliest Biosphere: Its Origin and Evolution* (Princeton Univ. Press, Princeton, NJ, 1983).
15. L. Brown, thesis, Pennsylvania State University, in preparation.
16. Y. L. Yung, M. Allen, J. P. Pinto, *Astrophys. J. Suppl. Ser.* **55**, 465 (1984); C. P. McKay, J. B. Pollack, R. Courtin, *Icarus* **80**, 23 (1989).
17. J. T. Kiehl and R. E. Dickinson, *J. Geophys. Res.* **92**, 2991 (1987).
18. J. C. G. Walker, *Evolution of the Atmosphere* (Macmillan, New York, 1977).
19. J. F. Kasting, K. J. Zahnle, J. C. G. Walker, *Precambrian Res.* **20**, 121 (1983).
20. U. Fink, D. C. Benner, K. A. Dick, *J. Quant. Spectrosc. Radiat. Transfer* **18**, 447 (1977); L. P. Giver, *ibid.* **19**, 311 (1978).
21. J. B. Pollack *et al.*, *Icarus* **65**, 442 (1986).
22. J. B. Pollack, J. F. Kasting, S. M. Richardson, K. Poliakoff, *ibid.* **71**, 203 (1987); M. H. Carr, *J. Geophys. Res.* **100**, 7479 (1995).
23. D. S. McKay *et al.*, *Science* **273**, 924 (1996).
24. J. A. Welhan, *Chem. Geol.* **71**, 183 (1988).
25. J. F. Kasting, D. H. Eggler, S. P. Raeburn, *J. Geol.* **101**, 245 (1993).
26. M. J. Walter and Y. Thibault, *Science* **270**, 1186 (1995); K. Righter and M. J. Drake, *Earth Planet. Sci. Lett.* **146**, 541 (1997).

UPDATE: CELL BIOLOGY

Sticky Endings: Separating Telomeres

R. Scott Hawley

The ends of eukaryotic chromosomes are capped by telomeres, which facilitate the replication of the ends of the DNA molecule. The telomeres of both homologous and nonhomologous chromosomes can easily self-associate, perhaps as a simple consequence of their structure—repeated arrays of a sequence at the ends of all of an organism's chromosomes. Indeed, the clustering of telomeres at one point in the nucleus, creating a so-called "bouquet" of chromosomes, has been noted in a variety of organisms (*1*). As discussed in a recent Perspective (*2*), these associations are sufficiently strong that the separation of telomeres presents a special problem for the meiotic and mitotic segregational systems.

Evidence that cis- and trans-acting functions are required for the separation of telomeres at cell division has been recently obtained in *Tetrahymena* and *Drosophila* (*3*, *4*). In this issue (page 1252), Conrad *et al.* (*5*) report a gene (*NDJ1*) that encodes a telomere-associated protein required for meiotic chromosome segregation in a third organism, the yeast *Saccharomyces cerevisiae*. This protein accumulates at the telomeres of chromosomes during meiotic prophase, and its absence results in high levels of failed meiotic chromosome segregation (meiotic nondisjunction). The failure of homolog separation at meiosis is observed whether or not the homologs have undergone genetic recombination. However, there is no effect of the absence of the Ndj1 protein on the segregation of telomere-less ring chromosomes, arguing that Ndj1 protein is not required for meiotic chromosome separation per se, but rather that the Ndj1 protein is essential to separate segregational partners that have telomeres.

The Ndj1 protein is also required for the completion of homologous synapsis. Loss of the Ndj1 protein delays the formation of the axial elements of the synaptonemal complex, a structure that connects homologous meiotic chromosomes along their length, without affecting recombination. The mechanism by which the Ndj1 protein facilitates synapsis remains unclear, but the normal clustering of telomeres into a bouquet may create three-dimensional chromosome arrangements, such as interlocked bivalents, that would impede proper synapsis. An inability to dissolve telomere-telomere interactions, especially those between nonhomologous telomeres, might prevent the chromosomal movements required to resolve those problems and facilitate homologous alignments. Such a model nicely explains both the effects of Ndj1 deficiency on synapsis and segregation, and the rather curious lack of effect of this deficiency on ring chromosomes.

Thus, even as cells have used telomeres to neatly solve the problem of replicating chromosomal ends, they have introduced difficulties for chromosomal movement because of the inherent stickiness of telomeres. Perhaps not surprisingly, cells have evolved ways to cope.

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References

1. A. F. Dernburg *et al.*, in *Telomeres*, E. H. Blackburn and C. W. Greider, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1996), pp. 295–338.
2. R. S. Hawley, *Science* **275**, 1441 (1997).
3. K. E. Kirk *et al.*, *ibid.*, p. 1478.
4. G. Cenci *et al.*, *Genes Dev.* **11**, 863 (1997).
5. M. N. Conrad *et al.*, *Science* **276**, 1252 (1997).