exists in years with global dust storms and does not exist in years prior to them. Complicating this issue is the occurrence of early and generally less intense fall storms. Such storms were observed before the great storms of 1971 and 1977. The most recent storm listed in Table 2 falls in this category. These storms may also play a role in the observed variability.

Clearly, a longer observational record is needed. This record will be extended by Earth-based observations (the International Planetary Patrol Program, for example) and by viewing Mars from orbit during the forthcoming Mars Observer mission. Until a longer record can be established, however, the only firm conclusion is that enhanced dustiness in the north relative to the south during winter weakens the Hadley circulation and the likelihood of global dust storms.

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23. Helpful discussions with O. B. Toon, J. B. Pollack, D. L. Westphal, R. W. Zurek, P. R. Christensen, J. R. Barnes, C. B. Leovy, and L. J. Martin are gratefully acknowledged. Supported by funds from NASA's Mars data analysis program.

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Gap Junctional Conductance and Permeability Are Linearly Related

V. VERSELIS, R. L. WHITE, D. C. SPRAY, M. V. L. BENNETT

The permeability of gap junctions to tetraethylammonium ions was measured in isolated pairs of blastomeres from *Rana pipiens* L. and compared to the junctional conductance. In this system, the junctional conductance is voltage-dependent and decreases with moderate transjunctional voltage of either sign. The permeability to tetraethylammonium ions was determined by injecting one cell of a pair with tetraethylammonium and monitoring its changing concentration in the prejunctional and postjunctional cells with ion-selective electrodes. Junctional conductance was determined by current-clamp and voltage-clamp techniques. For different cell pairs in which the transjunctional voltage was small and the junctional conductance at its maximum value, the permeability to tetraethylammonium ions was proportional to the junctional conductance. In individual cell pairs, a reduction in the junctional conductance induced by voltage was accompanied by a proportional reduction in the permeability of the gap junction over a wide range. The diameter of the tetraethylammonium ion (8.0 to 8.5 Å, unhydrated) is larger than that of the potassium ion (4.6 Å, hydrated), the predominant current-carrying species. The proportionality between the permeability to tetraethylammonium ions and the junctional conductance, measured here with exceptionally fine time resolution, indicates that a common gap junctional pathway mediates both electrical and chemical fluxes between cells, and that closure of single gap junction channels by voltage is all or none.

GAP JUNCTIONS ARE CLUSTERS OF specialized membrane channels that directly connect the interiors of adjoining cells (*1*). Communication via gap junctions can be quantified by measurement of junctional permeability (*2*) for a specific molecule (P_j) or by junctional conductance (g_j), which reflects the composite junctional permeability to small cytoplasmic ions, dominated in most cells by potassium (*3*). Both g_j and P_j are most easily measured in coupled pairs of cells, where voltage and concentration are uniform in each cell so that all junctions are subject to the same gradient. Determined qualitatively from tracer studies, the upper size limit for permeation of gap junctions is about 1.0 to 1.5 kD or 12 to 14 Å in diameter, which would allow inorganic ions and many metabolites, but not macromolecules, to pass between cells (*4*).

The conductance of gap junctions can be reduced by several physiological and pharmacological agents, most notably applied voltages and intracellular hydrogen and calcium ions (*5*). In some cases these treatments were reported to depress dye transfer to a much greater extent than electrical coupling or to block transfer of larger dyes preferentially (*6*). However, junctional permeability and conductance were not measured, and the apparent selectivity may have reflected a lower sensitivity in the measurement of the larger tracers (*7*). A more recent study reports proportional reductions in dye permeability and g_j for one treatment that decreases coupling in *Chironomus* salivary gland (*8*).

Quantitative correlation of g_j and P_j would help distinguish between two proposed mechanisms for gap junction gating. If closure is all or none and channels exist in open or closed states, a reduction in g_j (producing uncoupling) results from a reduction in the number of channels in the open configuration (Fig. 1). If closure is graded or in multiple steps and each channel behaves in the same way, a reduction in g_j results from decreased conductance and permeability of each channel. These two models of closure imply quite different relative changes in g_j and P_j. For all-or-none closure, g_j and P_j will be decreased proportionately and for graded closure, P_j for larger molecules will be decreased more than g_j, which is dominated by permeability to small ions.

We measured the junctional permeability to tetraethylammonium ions [P_j (TEA)] and g_j simultaneously in pairs of amphibian blastomeres. In these cells g_j is reduced by moderate transjunctional voltage (V_j) of either sign (*9*). We compared P_j (TEA) to g_j

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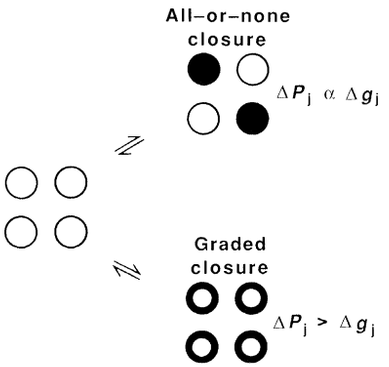


Fig. 1. Models for gap junction channel gating. In all-or-none gating, a reduction in g_j and P_j results from complete closure of a fraction of the channels with properties of the open channels being unaffected. P_j for all permeant species and g_j, which represents permeation by small ions, are equally reduced. Alternatively, if reduction in g_j and P_j is due to partial closure of each channel, P_j to larger permeant species is more reduced than g_j.

in different cell pairs when V_j was small and the channels were maximally open, and in the same cell pair when channel closure was induced by increasingly larger V_j's of either polarity. For several reasons, TEA is a suitable probe. Its concentration is easily determined with ion-sensitive electrodes. It has the same charge as K^+ and is essentially spherical. It is significantly larger than K^+ (8.0 to 8.5 Å, unhydrated, compared to 4.6 Å, hydrated) (*10*), the principal current-carrying ion, yet TEA is small enough so that transfer is sufficient to allow computation of P_j (TEA) at low g_j values. Finally, nonjunctional membranes are relatively impermeable to TEA and, unlike some fluorescent tracers (*8, 11*), TEA gives no indication of binding to cytoplasmic constituents (*12*).

For each experiment, a pair of amphibian blastomeres was mechanically isolated from embryos of *Rana pipiens* L. and placed in Holtfreter's solution containing 75 m*M* NaCl, 2 m*M* KCl, 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 5 m*M* Hepes at *p*H 7.6. Each cell was impaled with a single-barreled electrode for current application and a double-barreled electrode, one barrel filled with K^+ liquid ion-exchange resin 477317 (Corning), which is highly selective for tetraalkylammonium ions over K^+ (*13*), and one filled with 0.5*M* KCl for voltage measurement. The double-barreled ion-selective–voltage electrodes were calibrated before and after each experiment in a series of solutions containing different concentrations of TEA (typically 1 μ*M* to 100 m*M*) in a background solution of 75 m*M* KCl to simulate the intracellular environment. Ionic strength was adjusted to 175 m*M* with NaCl. Junctional (g_j) and nonjunctional (g_{nj}) conductances were obtained in two ways. In the current-clamp experiments, constant current pulses were alternately applied to each cell and, from the resulting input and transfer voltages, g_j and g_{nj} were determined by applying the π-T transform (*14*). In the voltage-clamp experiments, each cell was clamped by independent circuits to a common resting potential, and voltage steps were applied to a single cell or alternately to each cell. During these steps the current in the cell clamped at the resting potential was the transjunctional current from which g_j was determined directly by dividing by the voltage step (*9*). The difference of the clamp currents divided by the voltage step gives g_{nj} of the stepped cell. g_{nj} served only as a monitor of integrity of nonjunctional membranes, and none of those data are presented.

To measure permeability, TEA was pressure-injected rapidly into the "prejunctional" cell through a third micropipette. The transjunctional flux (J_j) of TEA (through all the junctions in parallel) was determined by measuring the change in TEA concentration in the postjunctional cell (ΔC_2) over a given time interval (Δt) so that

$$J_j = \text{vol}_2 \Delta C_2 / \Delta t \quad (1)$$

where vol_2 is the volume of the postjunctional cell (*15*). Permeability is measured as the ratio of the flux to the driving force, which for a charged species involves both the concentration gradient and the electric field. Assuming that the Goldman-Hodgkin-Katz equation applies (*16*), the permeability is given by

$$P_j = \frac{J_j\,(1 - \exp(-zFV_j/RT))}{zFV_j/RT\,(C_1 - C_2 \exp(-zFV_j/RT))} \quad (2)$$

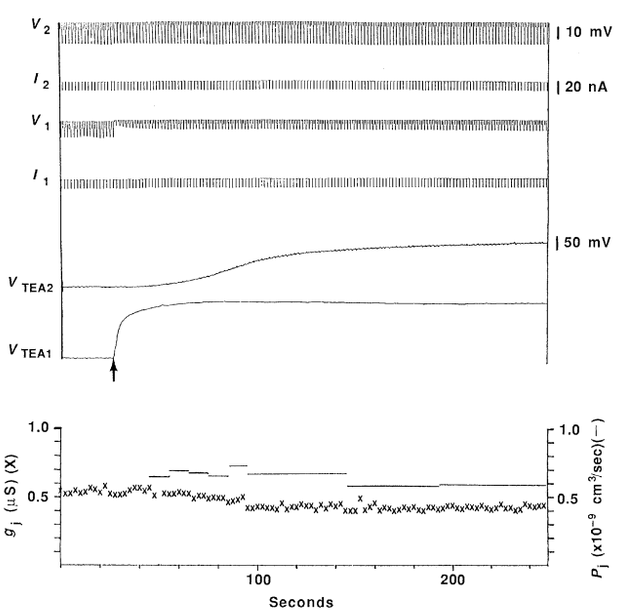
where V_j is the transjunctional voltage and C_1 and C_2 are the concentrations of TEA in the prejunctional and postjunctional cells, respectively (*17*). In the absence of an electric field Eq. 2 reduces to

$$P_j = J_j/(C_1 - C_2) \quad (3)$$

A record from an experiment on an isolated cell pair is shown in Fig. 2. The upper four traces represent current and voltage records in current clamp from which g_j was determined. The next two traces are the corresponding ion-sensitive electrode responses in the postjunctional (V_{TEA2}) and injected (V_{TEA1}) cells; P_j (TEA) was calculated from these records. Pressure injection of TEA into cell 1 (arrow) caused an abrupt rise in V_{TEA1}. After a brief delay, V_{TEA2} rose more slowly as a result of TEA entering cell 2 via the gap junctions. P_j (TEA) was determined by measuring flux over the time intervals indicated in Fig. 2, whereas g_j was calculated for each pair of test pulses. Both P_j (TEA) and g_j remained relatively constant throughout the experiment at 0.65×10^{-10} cm^3/sec and 0.45 μS, respectively (Fig. 2).

To evaluate how electrical and chemical coupling are related, P_j (TEA) was compared to g_j among different cell pairs under voltage clamp with markedly different g_j values and within individual pairs where g_j was reduced by application of V_j. In all of the cell pairs, both cells were initially clamped to a common holding potential to keep V_j at or close to zero. Voltage test pulses too small to affect electrical coupling were applied to measure g_j. After stable

Fig. 2. Simultaneous recording of g_j and P_j (TEA) between a pair of blastomeres from *R. pipiens*. V_1, V_2, I_1, and I_2 are the measured membrane potentials and applied currents in each cell of the pair. V_{TEA1} and V_{TEA2} are the voltage responses from the ion-sensitive electrode in each cell (upward indicates an increase in TEA activity). The abrupt rise in V_{TEA1} indicates a brief injection of TEA. g_j was determined from the test currents and resulting voltages by means of the π-T transform (*14*). Because the resting potentials were equal and voltage pulses small, the electric field can be neglected and Eq. 3 was used to determine P_j. Graph at bottom: P_j (TEA) for the time intervals indicated (——) and g_j for each pair of test pulses (x) are plotted as a function of time.



coupling was established TEA was pressure-injected into one cell and P_j (TEA) was evaluated with Eq. 3 to obtain P_j for the maximum g_j (that is, when V_j is zero). Thirty-second hyperpolarizing steps were then applied to one of the cells to reduce g_j to a new steady-state value. The corresponding P_j (TEA) was evaluated with Eq. 2. Successively larger voltage steps were applied to compare changes in P_j (TEA) over a wide range of g_j values.

Representative flux records for V_j's of equal magnitude, but opposite polarity, are shown in Fig. 3A. The configuration of the cells is diagramed at the top of Fig. 3A (the injected cell indicated in black). Hyperpolarizing either cell by 20 mV reduced both g_j and TEA flux to new steady-state values. After termination of the voltage step, TEA flux recovered, as did g_j. Although the changes in g_j occurred in less than 1 second (*18*), there was a delay of 5 to 10 seconds in the onset of TEA flux reduction and recovery. The lag in TEA flux can be explained by redistribution of TEA in the cytoplasm of the postjunctional cell upon opening and closing of the gap junction channels.

For a given pulse amplitude, the reduction in TEA flux depended markedly on the sign of V_j even though g_j was reduced equally. When V_j was applied in the direction that favored movement down the TEA concentration gradient (hyperpolarizing the postjunctional cell) (Fig. 3A, left traces), the rate of change of V_{TEA} and calculated flux were greater than when V_j opposed movement down the gradient (hyperpolarizing the injected cell) (Fig. 3A, right traces). When permeability was calculated with Eq. 3, which takes into consideration both concentration and voltage gradients, P_j (TEA) was found to be equal for both polarities of V_j. The data are summarized for three different cell pairs in Fig. 3B. In the preparation denoted by the circles, each cell of a pair was alternately hyperpolarized so that both polarities of V_j that assisted and impeded flux are shown. In the other two preparations, only the TEA-injected cell was hyperpolarized, giving the P_j (TEA) and g_j values for one polarity of V_j. There is a linear relationship between P_j (TEA) and g_j for cell pairs with different intrinsic g_j's and for each cell pair when g_j was reduced by V_j. The proportionality constant of the P_j (TEA) to g_j relation is 1.02×10^{-3} cm^{-3} sec^{-1} S^{-1}. The effect of V_j on both P_j (TEA) and g_j is better illustrated in Fig. 3C where P_j (TEA) and g_j, each normalized to the value obtained with V_j at or near zero, are plotted as a function of V_j. The data are from the cell pair denoted by the circles in Fig. 3B. The solid line is a Boltzmann relation describing normalized junctional conductance (G_j) as a function of V_j (*9*). P_j (TEA) decreases symmetrically about $V_j = 0$ and parallels the changes in g_j for both polarities of V_j.

The proportionality between P_j (TEA) and g_j, which represent permeation by molecules of different sizes, indicates that voltage gating of gap junction channels is all or none. The simplest model is that all channels are alike with respect to voltage sensitivity and permeability and, therefore, a change in g_j and P_j results from a change in the number of open channels. From our data we cannot exclude the possibility that permeability and voltage sensitivity vary among channels, but that the variation of the two parameters is independent. Moreover, the rapid and reversible quantitative correlation between electrical and chemical coupling is evidence for a common diffusional pathway for all permeant molecules. Further evidence for all-or-none closure is the exponential time course of changes in g_j during application of voltage steps (*18*). These data indicate that conductance changes as a reversible first-order process that would be difficult to reconcile with graded channel closure. Also,

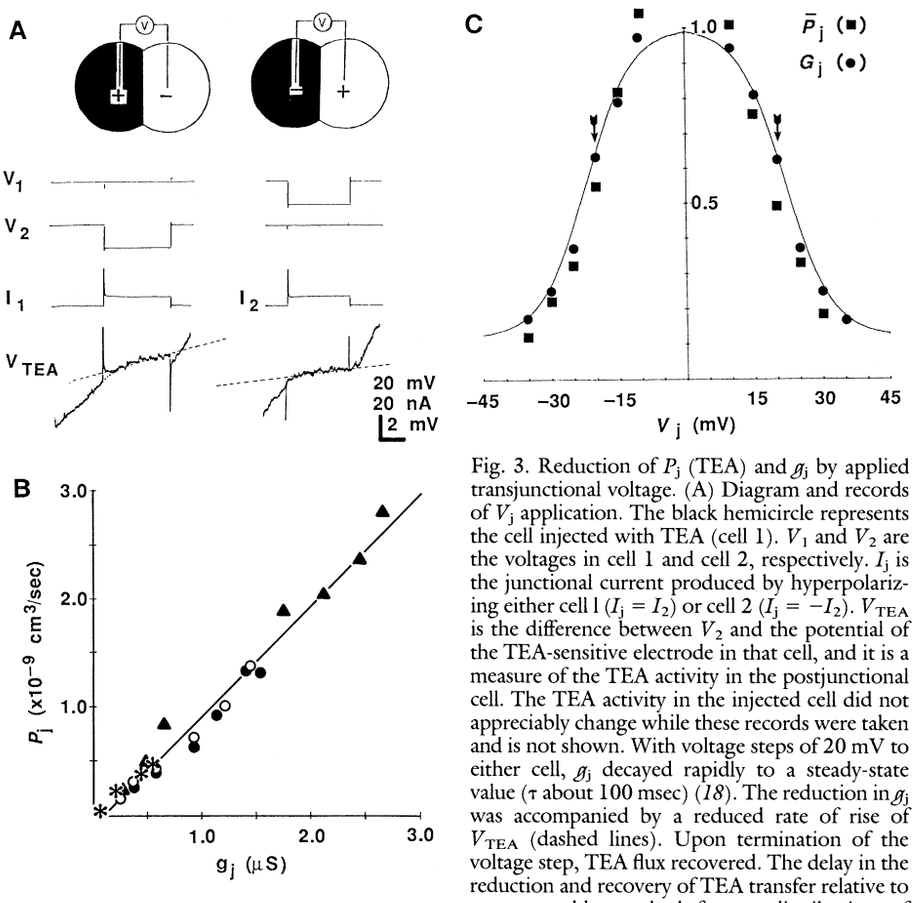


Fig. 3. Reduction of P_j (TEA) and g_j by applied transjunctional voltage. (A) Diagram and records of V_j application. The black hemicircle represents the cell injected with TEA (cell 1). V_1 and V_2 are the voltages in cell 1 and cell 2, respectively. I_j is the junctional current produced by hyperpolarizing either cell 1 ($I_j = I_2$) or cell 2 ($I_j = -I_2$). V_{TEA} is the difference between V_2 and the potential of the TEA-sensitive electrode in that cell, and it is a measure of the TEA activity in the postjunctional cell. The TEA activity in the injected cell did not appreciably change while these records were taken and is not shown. With voltage steps of 20 mV to either cell, g_j decayed rapidly to a steady-state value (τ about 100 msec) (*18*). The reduction in g_j was accompanied by a reduced rate of rise of V_{TEA} (dashed lines). Upon termination of the voltage step, TEA flux recovered. The delay in the reduction and recovery of TEA transfer relative to g_j presumably resulted from redistribution of TEA in the postjunctional cell. When V_j assisted the flux down the concentration gradient (left traces), V_{TEA} increased more rapidly than when V_j opposed the flux (right traces), although g_j was the same in both cases. This difference was observed, although V_{TEA} rose more quickly with no V_j applied in the earlier record on the left. This latter more rapid rise resulted from nonlinearity in the TEA electrode, response and did not represent a more rapid flux. The computed permeabilities for the left- and right-hand records at $V_j = 0$ were 1.32 and 1.29×10^{-9} cm^3/sec, respectively. (B) Plot of P_j (TEA) as a function of g_j in three pairs of blastomeres from *R. pipiens*. The cells were independently voltage-clamped as in (A). The maximum g_j's (at small V_j's) were 0.55, 1.56, and 2.8 μS for the three cell pairs. g_j in each pair was reduced by application of V_j via hyperpolarization of the injected cell (▲ and ∗) or by alternately hyperpolarizing the injected cell (●) and recipient cell (○). The relationship between P_j (TEA) and g_j was linear among different cell pairs and in any individual cell pair when g_j was reduced by V_j. Slope, 1.02×10^{-3} cm^{-3} sec^{-1} S^{-1}; regression coefficient, 0.987; P_j intercept, -0.1×10^{-9} cm^3/sec. (C) Relation between steady-state g_j and P_j (TEA) as a function of V_j from one cell pair [circles in (B)]. G_j and $\bar{P}_j$ are normalized values that represent fractions of the values obtained at very small V_j's. Successive hyperpolarizing voltage steps were alternately applied to each cell; V_j is considered positive when the injected cell is made positive relative to the postjunctional cell. Arrows indicate data in (A). The solid line represents a fit to the Boltzmann relation

$$g_j = ((g_{max} - g_{min})/(1 - \exp(A(V_j - V_0))) + g_{min}$$

where g_j is the steady-state conductance, g_{max} and g_{min} are the maximum and minimum conductances, V_0 is the voltage at which conductance is halfway between g_{max} and g_{min}, and A is a constant expressing voltage sensitivity (*9*).

discrete conductances of 100 to 160 pS occur between isolated pairs of lacrimal or embryonic cardiac cells (*19*) and in isolated gap junctions incorporated onto lipid membranes (*20*).

Direct intercellular pathways that are shared by ions and other solutes, and their regulation in an all-or-none fashion, have important consequences for the role of gap junctions in physiological and pathological processes. In this situation all permeant molecules, regardless of size or charge, are similarly affected.

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Transformation of *Arabidopsis thaliana* with *Agrobacterium tumefaciens*

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Transformed *Arabidopsis thaliana* plants have been produced by a modified leaf disk transformation-regeneration method. Leaf pieces from sterilely grown plants were precultured for 2 days and inoculated with an *Agrobacterium tumefaciens* strain containing an avirulent Ti (tumor-inducing) plasmid with a chimeric gene encoding hygromycin resistance. After cocultivation for 2 days, the leaf pieces were placed on a medium that selects for hygromycin resistance. Shoots regenerated within 3 months and were excised, rooted, and transferred to soil. Transformation was confirmed by opine production, hygromycin resistance, and DNA blot hybridization of both primary transformants and progeny. This process for producing transgenic *Arabidopsis* plants should enhance the usefulness of the species for experimental biology.

THE VIRTUES OF *Arabidopsis thaliana* as an experimental model plant for genetic, biochemical, and molecular biological studies have been extensively reviewed (*1*). The useful features of this remarkable plant include the following. It is small, prolific, and easy to grow and has a generation time as short as 5 weeks. Many mutations have been identified, studied, and mapped and are readily available. Its five chromosomes correspond to five linkage groups and contain only about 7×10^7 bp, the smallest genome known in the angiosperms (*2*). One experimentally useful technique that has been needed is a facile method to introduce natural or modified genes into *A. thaliana* to obtain complementation of mutants or to study factors involved in gene expression. By applying the tumor-inducing (Ti) transformation system developed for other dicots, we have developed a simple procedure to obtain transgenic *A. thaliana* plants.

Agrobacterium tumefaciens provides a natural gene-transfer mechanism that can be utilized to transfer a defined DNA sequence into the genome of cells of many dicotyledonous plants (*3*). Transgenic plants can be created in a simple process that involves a useful selectable marker and the capacity of somatic cells to regenerate new meristems, as illustrated by the leaf disk–transformation technique with tobacco, petunia, and tomato (*4*). Adaptation of the leaf disk system to *A. thaliana* required a new selectable marker since direct selection for kanamycin resistance conferred by our pMON200 vector (*5*) was not efficient in this species. The inefficiency in selection was characterized by growth of callus from uninfected control tissue and by poor recovery of genuinely transformed tissue on medium containing kanamycin.

Hygromycin B is an aminocyclitol antibiotic that inhibits protein synthesis in prokaryotic and eukaryotic cells (*6*). A gene from a bacterial resistance (R) factor that encodes a hygromycin phosphotransferase (hph) has been used to construct chimeric genes that act as dominant selectable markers for transformation of yeast, mammalian cells, and plants (*7, 8*). These results led us to construct and test an hph marker for transformation of *Arabidopsis*. The hph coding sequence was inserted into the

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