lar fraction is substantially enriched in ribosomal RNA precursors relative to hnRNA, and is also enriched in nucleolar small nuclear RNA's such as U3 (*6*). RNA from this nucleolar fraction is shown in lane 7 of Fig. 2 (cross-links reversed) and lane 8 (cross-links not reversed). The absence of hybridization with U2 DNA shows that U2 RNA is not base-paired with nucleolar RNA. This result and the fact that the RNA used in lanes 5 and 6 was isolated from well-characterized hnRNA-ribonucleoprotein particles (*13, 15*) indicate that U2 RNA is base-paired with hnRNA.

Because both U1 (*6*) and U2 RNA's are base-paired with hnRNA, we have considered the possibility that they might also interact with each other, leading to the formation of a base-paired U1-U2-hnRNA ternary complex. However, the fact that cloned U2 DNA does not hybridize with U1 RNA (Fig. 2, lanes 1, 2, and 4) and that cloned U1 DNA does not hybridize with U2 RNA (*6*) argues against extensive sequence complementarity (or homology) between these two RNA's. In addition, a computer-assisted search has revealed no thermodynamically stable complementarity between rat U1 and U2 RNA's (*16*). Both U1 and U2 RNA's have potential sequence complementarity with intron-exon borders in messenger RNA (mRNA) precursors (*17*), and it is possible that it is these sites which the psoralen cross-linking has detected (*6*) (Fig. 2). Analysis of the roles of U1, U2, and possibly other small nuclear RNA's in mRNA processing may be facilitated by psoralen-mediated RNA-RNA cross-linking.

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Osmotic Swelling of Phospholipid Vesicles Causes Them to Fuse with a Planar Phospholipid Bilayer Membrane

Abstract. *Fusion of phospholipid vesicles with planar bilayer membranes occurs if the vesicles that contact the planar membrane swell osmotically after the replacement in their medium of an impermeant solute by a permeant one. This finding directly demonstrates that osmotic swelling is a driving force for vesicle–planar membrane fusion. The method used to induce vesicle swelling and fusion may have relevance for biological systems.*

The fusion of two biological membranes is a ubiquitous phenomenon. It occurs in such diverse processes as neurotransmitter and hormone release (*1, 2*), insertion of integral membrane proteins into plasma membranes (*3, 4*), and raising of the fertilization membrane (*5*). Although many of the physiological functions mediated by fusion events are understood, the mechanism by which the fusion process itself occurs is not. To elucidate this mechanism, we have been studying the fusion of phospholipid vesicles to planar bilayer membranes as a model for the fusion process (*6–8*). In this system, an osmotic gradient across the planar membrane is required to produce fusion, with the vesicle-containing *cis* side hyperosmotic with respect to the vesicle-free *trans* side (*7*). It was suggested that Ca^{2+}, which greatly augments the rate of fusion, promotes the close association of vesicles to the planar membrane and that the osmotically induced water flow across the planar membrane and into the vesicles results in vesicle swelling and subsequent fusion (*7*). In this view it is not water flow across the planar membrane per se but rather the net movement of water into the vesicle that is required for fusion. This is crucial to the possible biological relevance of vesicle–planar membrane fusion, since in general an osmotic gradient does not exist across the plasma membrane of most animal cells. To substantiate that water flow across the planar membrane is not essential to the fusion mechanism, we performed experiments in which vesicle swelling occurs without water flow across the planar bilayer. We report that osmotic swelling of vesicles is the driving force for the fusion of vesicles to planar membranes.

Vesicles were formed with porin [a voltage-dependent, slightly cation-selective, channel-forming integral membrane protein isolated from the outer membrane of *Escherichia coli* (*9*)] reconstituted in their membranes. Fusion was assayed by monitoring the incorporation of this vesicle membrane marker into the voltage-clamped planar membrane. Entry of porin into the planar membrane occurs by vesicle–planar membrane fusion and not by its transfer, either directly or through the aqueous phase, from one membrane to the other (*6, 7, 10*).

Vesicles were added to the *cis* side of a planar membrane (Fig. 1A). Although, as reported previously (*7*), Ca^{2+} alone did not induce fusion, it did cause the close apposition of the vesicles to the planar membrane. This apposition was irreversible on a time scale of minutes (*10*). Thus, when the *cis* side was perfused, the vesicles in solution were washed out but the vesicles in close association with

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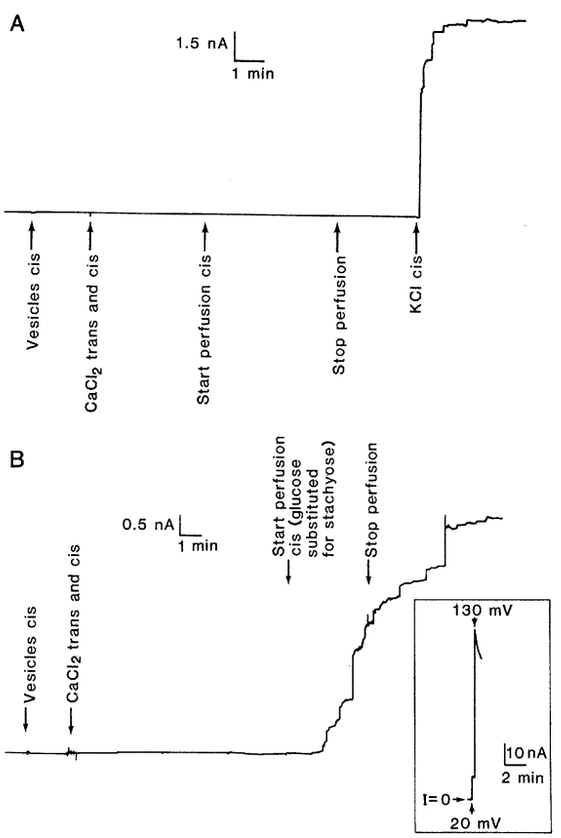
the planar membrane remained. If an osmotic gradient was then established across the planar membrane, with the *cis* side hyperosmotic with respect to the *trans* side, a burst of fusion occurred rather than the continuous fusion reported previously, because only vesicles osculating the planar membrane were present and available to fuse (*10*).

In the next experiment, stachyose-loaded vesicles were added to the *cis* side (Fig. 1B). Because the *cis* and *trans* sides of the planar membrane contained the same concentration of impermeant solute as the vesicle interior, net water flow did not occur across either the planar or vesicle membranes. Again, Ca^{2+} alone did not induce fusion. When the stachyose-containing *cis* buffer was replaced by isosmotic glucose-containing buffer, no osmotic gradient was created and no net water flow occurred across the planar membrane. However, since the vesicle membranes were permeable to glucose but not to stachyose [because of the porin channels (*11*)], glucose entered the vesicles and water followed (Fig. 2). As a result the vesicles swelled and, as seen in Fig. 1B, a burst of fusion occurred. (Because of the multilamellar nature of the vesicles and the relatively slow entry rate of glucose through the porin channels, vesicle swelling and subsequent fusion lasted for several minutes.) Thus, classical osmotic swelling of the vesicles in contact with the planar membrane led to fusion.

The above experiment shows that osmotic swelling is a driving force for vesicle–planar membrane fusion. [The importance of vesicle swelling has also been implicated in the fusion of fragmented sarcoplasmic reticulum vesicles to planar bilayers (*12*).] This same mechanism might also apply to fusion in bio-



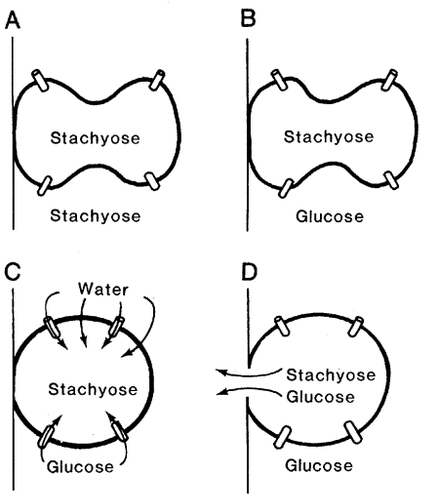


Fig. 1 (left). (A) Fusion induced by an osmotic gradient across a planar bilayer membrane. The membrane was formed at room temperature by the union of two monolayers (*17*) of crude soybean phospholipid [lecithin type II (Sigma) from which neutral lipid was removed (*18*)] across a 0.36-mm-diameter hole in a Teflon partition coated with squalene (*19*). The membrane separated symmetrical solutions containing 100 m*M* KCl, 10 m*M* 2-(*N*-morpholino)ethanesulfonic acid, 2 m*M* $MgCl_2$, and 0.1 m*M* EDTA (*p*H 6.0); the potential across the membrane is clamped at 20 mV, with the *cis* compartment positive. Porin-containing multilamellar vesicles were added to the *cis* compartment to a concentration of 125 μg of lipid per milliliter (arrow 1). Two minutes later, $CaCl_2$ was added to both the *cis* and *trans* compartments to a concentration of 15 m*M* (arrow 2). Four minutes after this, the *cis* compartment was perfused for about 5 minutes with several volumes of a vesicle-free solution of the same composition as that already present in the compartment (arrows 3 and 4). This removed all vesicles from the *cis* compartment except those attached to the planar membrane. Note that there was no fusion during the 11-minute period following the addition of Ca^{2+}. An osmotic gradient was then created across the planar membrane by the addition of KCl to the *cis* compartment to a concentration of 225 m*M* (arrow 5). A burst of fusion then occurred, as manifested by the jumps in the current record, which represent the simultaneous insertion of numerous ion-conducting channels (porin) into the planar membrane. (The large initial jump contains several fusion events unresolved at the time scale of the record.) The vesicles were prepared by suspending 10.05 mg of a porin and lipid mixture (8 mg of egg phosphatidylcholine, 2 mg of bovine phosphatidylserine, and 50 μg of porin) in hexane in a round-bottomed flask, drying the mixture by rotary evaporation at room temperature, adding (along with three glass beads) 1 ml of a solution of the same composition as that bathing the planar membrane, and then shaking the mixture for a few minutes. The vesicles were allowed to swell for about 1 hour and were then extruded through a Nuclepore filter (0.2-μm mesh) (*20*). Egg phosphatidylcholine and bovine phosphatidylserine were from Avanti Biochemicals; porin was prepared by a modification of the procedure of Rosenbusch (*21*). (B) Fusion induced in the absence of an osmotic gradient across the planar bilayer membrane. A membrane of the same composition as that described in (A) separates the same salt solutions. In addition, the *cis* compartment contains 200 m*M* stachyose and the *trans* compartment contains 200 m*M* glucose. (We used glucose, rather than stachyose, in the *trans* compartment purely for financial reasons.) Vesicles were prepared as described in (A), except that the solution in which they were made also contained 200 m*M* stachyose; that is, the vesicles were made in a solution of the same composition as that in the *cis* compartment. The vesicles were added to the *cis* compartment to a concentration of 125 μg of lipid per milliliter (arrow 1), and 2 minutes later $CaCl_2$ was added to both the *cis* and *trans* compartments to a concentration of 15 m*M* (arrow 2). As before, no fusion occurred during the next 10 minutes. The *cis* compartment was then perfused with a vesicle-free solution of the same composition as that already present in the compartment, except that 200 m*M* glucose replaced the 200 m*M* stachyose (arrow 3). After about 30 seconds (by which time the volume of the *cis* compartment had been exchanged about two times) there was a burst of fusion lasting about 7 minutes. [The perfusion of the *cis* compartment was terminated about 4 minutes after it was begun (arrow 4).] (Inset) After the fusion burst terminated, the voltage across the membrane was stepped from 20 to 130 mV. The decay of current (which was aborted in this case because the membrane broke) is characteristic of the closing of porin channels at high voltages (*9*), and thus confirms that the current jumps resulted from the insertion of the vesicular membrane marker (porin) into the planar membrane. Fig. 2 (right). Schematic drawings showing the osmotic swelling of vesicles and their subsequent fusion to the planar membrane. (A) A stachyose-containing vesicle, in an isosmotic stachyose medium, associates tightly with the planar membrane as a result of the addition of $CaCl_2$. (B) The external stachyose solution is replaced by an isosmotic glucose solution. The vesicle remains tightly associated with the planar membrane. (C) Glucose diffuses through the porin channels (*11*) into the vesicle, and water follows (maintaining isosmolality). As a result, the vesicle swells. (D) The vesicle and planar membranes rupture in the region of contact, producing fusion.

logical systems. Osmotic swelling of cytoplasmic vesicles could, in principle, occur through a number of mechanisms, including mobilization of osmotically inactive constituents in the vesicle, alteration of vesicle membrane permeability to ions, or stimulation of ion pumping into the vesicles. In the experiment represented in Fig. 1B, osmotic swelling of the vesicle was accomplished by substituting a permeant solute (glucose) for a nonpermeant one (stachyose). This is formally equivalent to a biological vesicle osmotically swelling because of an increase in the permeability of its membrane to cytosolic constituents, such as ions.

Numerous examples already exist of vesicle swelling being associated with exocytosis (although it is not yet clear that the swelling precedes fusion). Among these are mucocyst discharge in *Tetrahymena* (*13*), serotonin release by mast cell granules (*14*), and granular discharge by *Limulus* amoebocytes (*15*). It has also been shown that antidiuretic hormone–stimulated fusion of cytoplasmic tubular vesicles with the luminal plasma membrane of toad urinary bladder can be regulated by osmotic forces in a manner consistent with that of vesicle–planar membrane fusion (*16*). Although in our system Ca^{2+} stimulates fusion by promoting the close association of vesicle and planar membranes, this need not be its role (or its only role) in biological exocytosis. The possibility that increased levels of Ca^{2+} trigger fusion by stimulating osmotic swelling of vesicles (by any of the mechanisms mentioned above) merits serious consideration.

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Rotating Shift Work Schedules That Disrupt Sleep Are Improved by Applying Circadian Principles

Abstract. *Workers on rotating shifts dislike those aspects of their work schedules that violate circadian sleep-wake cycle physiology. Work schedule satisfaction, subjective health estimates, personnel turnover, and worker productivity improve when schedules are introduced that are designed to incorporate circadian principles.*

The human sleep-wake cycle has evolved on a rotating planet with a regular 24-hour alternation between day and night. Yet within the past 50 years, the need for round-the-clock operations in many industrial plants and emergency services has led to major changes in the day-night schedules to which 26.8 percent of the U.S. work force is exposed, many of whom work shifts which rotate between night, evening, and daytime duties (*1*).

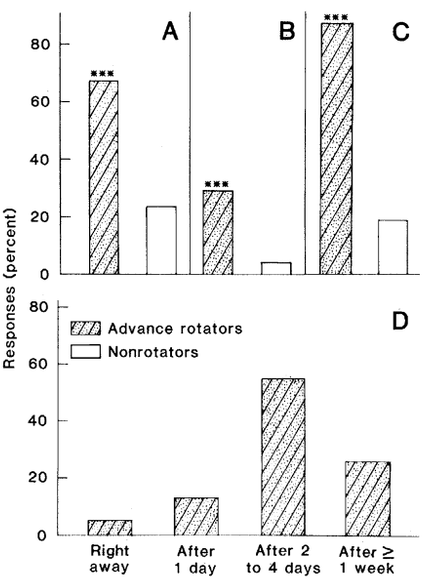

Fig. 1. (A to C) Comparison of sleep-wake cycle questionnaire responses from workers on weekly phase advance rotating shifts and nonrotating day and swing shift workers. The rotating shift workers reported greater problems with (A), poor quality sleep, $\chi^2(1) = 26.4$, $P < .001$; (B), falling asleep at work, $\chi^2(1) = 15.6$, $P < .001$; and (C), the schedule changing too often, $\chi^2(1) = 55.0$, $P < .001$. (D) The number of days taken for the sleep time of the weekly phase advance rotating shift workers to adjust after each shift rotation. ***$P < .001$.

Numerous medical and psychosocial problems associated with rotating shift work schedules have been reported (*2*), and several different approaches to the problems have been suggested (*3*, *4*). Because research findings (*5*–*7*) indicated to us that most rotating work schedules are outside the range of entrainment of the pacemaker timing the human circadian sleep-wake cycle, we postulated that a practical and effective intervention would be to resolve this aspect of the shift work problem. We report that rotating shift workers are often dissatisfied with the features of their schedules that violate circadian principles, and that when schedules are introduced which take into account the properties of the human circadian system, subjective estimates of work schedule satisfaction and health improve, personnel turnover decreases, and worker productivity increases.

We compared 85 male rotating shift workers, aged 19 to 68 (mean ± standard deviation, 31.4 ± 10.0), with a control group of 68 male nonrotating day and swing shift workers with comparable jobs, aged 19 to 56 (mean, 27.3 ± 8.2), at the Great Salt Lake Minerals and Chemicals Corporation in Ogden, Utah (*8*). For 10 years at this plant, weekly shifts were rotated with each crew working a given 8-hour shift for 7 days before rotating to the preceding 8-hour shift. Hence the scheduled work time rotated in a phase advancing direction from night (midnight to 8 a.m.) to swing (4 p.m. to midnight) to day (8 a.m. to 4 p.m.) shift (*9*).

Each worker was given the job de-

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