polarizing generator potential. The only reservation we have about interpreting Erxleben's data is that the patch experiments were done on the soma or primary dendrite, rather than on secondary processes, and that the distribution of channels might have varied (*2*).

Gustin *et al.* (*7*) demonstrated in yeast that whole-cell currents could be evoked by inflation of the protoplast and that pressure sensitivity increased with the diameter of the protoplast, as expected from Laplace's law. This contradicts the contention of Morris and Horn that stimulation was limited to the region of the pipette attachment, because in that case there should have been no correlation with size.

Without any patch clamping, Sigurdson *et al.* (*8*) showed that in in vitro heart cells, gentle mechanical prodding produced an inward calcium flux. This flux satisfied many of the properties expected from SA channels: the flux was blocked by gadolinium, carried by extracellular calcium, and was sensitive to mechanical stimulation. Without specific blockers whole-cell currents are not convincing evidence of specific transduction and the stimulus is, in most cases, poorly controlled.

Finally, Morris and Horn's *in proof* reference to dissolution of the cytoskeleton in patches (*9*) is inappropriate. The patches in that study were made with giant pipettes 10 to 15 μm in diameter and did not form gigaseals. Gigasealed patches from normal pipettes contain cytoskeleton (*10*) that can change with flexing of the patch, or with time (*11*), and may affect the sensitivity of MS channels.

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Response: Gustin's new data leave little doubt that I_{MS} in yeast spheroplasts are channel-mediated. Three critical questions are no longer at issue. Does I_{MS} saturate? Is it blocked, like the single-channel current, by gadolinium? Are the channels contributing to I_{MS} only those near the pipette rim? Additional support is given to the hypothesis of channel-mediation by the finding that yeast species that seal without vigorous suction also exhibit I_{MS}.

The membranes and SA channels of yeast and snail neurons (*1*) may simply be different. We note that yeast SA channels (*2*) are nonselective between cations and anions, that they have two open levels like a two-barrelled channel, and that they occur in a membrane whose natural state is one of high curvature (*3*). By Laplace's law, the yeast I_{MS} shown in figure 1 above is activated at tensions that would be generated by applying ~130 mmHg to a typical patch of 5 μm^2. In patch recordings, the ubiquitous snail neuron SA K^+ channels were at least this sensitive (*1*). This sensitivity did not, however, translate to macroscopic recordings. In yeast, half-maximal activation occurs at ~5 dyne/cm^{-1}. In our experiments on neuronal membrane, we assume that comparable or greater tensions developed over many square micrometers before stimulus-induced rupturing occurred, but these tensions did not generate the expected currents.

Despite Gustin's results neither the small I_{MS} we observed in some snail neurons (and in GH_3 cells) nor that in yeast spheroplasts can be assumed to represent physiological currents. We detected currents only in cells that were near rupturing or were irreversibly distended, and the currents in yeast cells were seen in wall-free cells with their cytoplasm exchanged for pipette solution. Nevertheless, we concur that patching does not appear to render SA channels artifactually mechanosensitive in yeast spheroplasts. Data from more preparations are needed before the same can be said of animal cells.

Sachs *et al.* describe the behavior of patches during sealing and stretching (*4, 5*). It is clear that patching can traumatize the membrane, and passive (viscoelastic) or active (contractile) effects can interfere with the effects of pressure applied to the pipette (*4*). Furthermore, the different pipette tip geometries that have been used to record SA channel activity affect the nature of mechanostimuli applied to patched membrane (*5*). These uncertainties make it difficult to predict the magnitude of in situ or physiological channel mechanosensitivity on the basis of patch mechanosensitivity.

We disagree with the statement that SA channels "could only be activated by pressures greater than 70 to 100 mmHg" in *Lymnaea* neurons. Sigurdson found that 30 mmHg suction-activated SA K^+ channels in some patches (*6*), and we reported [(*1*), reference 13] that SA K^+ channels turned on with suction at ≥40 mmHg. Moreover, in looking for whole-cell responses, we consistently used stimuli that at high intensity brought the cell to rupture, yet we observed at best minute currents that could be attributed to a handful of channels. Altered reversal potentials during osmotic shock should not have prevented the detection of conductance changes, but we saw none.

We agree with Sachs *et al.* that macroscopic MS currents can be demonstrated; we observed them in GH_3 cells [(*1*), reference 23], but only under conditions that caused irreversible distension. Sachs *et al.* refer to the preliminary report by Davis *et al.* (*7*) of mechanosensitive whole-cell currents in smooth muscle, but it is too early to tell whether these macroscopic currents represent a physiologically relevant expression of single-channel SA currents. Are the currents gadolinium-sensitive? Do they saturate or show any "dose" dependence? What is the state of the cells when currents are obtained?

The other preliminary report to which Sachs *et al.* refer (*8*) was suggestive, but not conclusive, since no control for Ca^{2+} channel blocking by gadolinium was done, no current measurements were made, and mechanostimulation was not necessarily "gentle." The stimulating probe was a patch pipette, used in a manner that sharply indented the plasma membrane, inevitably shearing cortical cytoskeleton against contractile machinery and intracellular organelles. This unusual stimulus may alter the mechanical environment of SA channels, rendering them hypermechanosensitive.

Bear (*9*) did not make whole-cell recordings. Erxleben's work (*10*) is important, but he did not claim to have made an unequivocal connection between single-channel and whole-cell currents. Some of the missing links have been listed in (*11*). If the channels contributing to the whole-cell currents measured by Gustin *et al.* (*12*) were mechanically disturbed near the recording pipette, they would have experienced the curvature of the cell and so would, indeed, have been expected to conform to Laplace's law. Finally, we made no reference to "dissolution of the cytoskeleton." We used the term "disrupt." Sachs *et al.* (*4*) show just how disrupted the cytoskeleton can be beneath a patched membrane.

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Lysyl Oxidase and *rrg* Messenger RNA

Several of us recently reported that the *rrg* gene was a putative tumor suppressor gene or anti-oncogene of *ras* (*1*). Mouse NIH 3T3 cell lines transformed by *ras* expressed almost no *rrg*, in contrast with nontransformed NIH 3T3 cell lines and interferon-reverted cell lines. Revertants continued to express *ras* p21 and mRNA in amounts comparable to those expressed by transformed cell lines. A revertant cell line transfected with an *rrg* antisense expression construct became retransformed. These data suggested that the regulated expression of *rrg* product forms a part of the pathway of cell transformation by *ras*.

A search (*2*) of GenBank Release 65.0 and PIR Release 26.0 revealed a match between *rrg* cDNA sequences (*3*) and a 2672-bp cDNA of rat lysyl oxidase (*4*). The two nucleotide sequences were 92% identical, but the protein sequences were only 79% similar with two blocks of nonhomologous sequences interrupting the alignment. Frame shifts at four locations restored identity; each shift location was rich in GC. GC-rich areas are often compressed on sequencing gels. The sequences of *rrg* and rat lysyl oxidase cDNA in these areas were verified with the dGTP analog dITP, which prevents compressions (*5*). Several base insertions in the rat sequence restored exact alignment with *rrg* (*6*). Therefore, *rrg*, a regulator of *ras* expression, encodes lysyl oxidase.

Determinations of lysyl oxidase activity in the culture media of NIH 3T3 and derived cell lines (Table 1) indicated a direct correlation between lysyl oxidase activity and *rrg* mRNA expression (Fig. 1). Nontransformed cell lines exhibited high lysyl oxidase activity and large amounts of mRNA, while the transformed lines had low lysyl oxidase activity and small amounts of mRNA.

The down-regulation of lysyl oxidase expression in transformation and the induction of lysyl oxidase in interferon-mediated reversion of transformed cells suggests that this enzyme plays a role in tumor suppression. Lysyl oxidase is a copper-dependent amine oxidase that catalyzes the oxidative deamination of peptidyl lysine in elastin and collagen; intra- and intermolecular condensations then form covalent cross-linkages that insolubilize these matrix proteins (*7*). The cDNA sequences each predict signal peptide sequences and cleavage sites as well as potential sites for *N*-glycosylation (*4*, *6*). This is consistent with the known secretory fate of lysyl oxidase in cultured cells (Table 1) (*8*). Thus, if catalytic activity is essential to the reversion process, it likely occurs in extracellular space.

There is ample evidence for the modulation of cell phenotype by the extracellular matrix (*9*). Intracellular communication with extracellular cross-linked collagen or elastin may be critical to the present observation. Alternatively, as lysyl oxidase can act on proteins other than elastin and collagen in vitro (*10*), it may oxidize other accessible proteins such as membrane-bound receptors that are capable of transducing signals through *ras*, or it may oxidize other matrix components to modulate matrix-cell communication, which is important to the nontransformed phenotype.

Table 1. Lysyl oxidase activity in cell lines. Lysyl oxidase activity was assayed in both media and cell extract, summed to give total activity, and normalized against cell number and cell protein. In all cell lines, more than 90% of the total lysyl oxidase activity was in the media. ND, not detectable. AS, antisense cell lines, PR4 cells with *rrg* antisense expression construct; S, sense cell lines, PR4 cells with *rrg* sense expression construct; AS3BT1, cell line cultured from tumor induced in a nude mouse by subcutaneous injection of cell line AS-3B. Tumors grew to ~1 cm in diameter within 7 days of first appearance (+++), within 7 to 14 days of first appearance (++), or 14 days or more after first appearance (+); or they did not grow (−).

Cell line	Total lysyl oxidase activity (*11*)		Tumor growth (*1*)
	Cpm/10^6 cells	Cpm/μg protein	
NIH 3T3	11,945	22.1	−
PR4	5,260	20.9	−
RS485	620	2.7	+++
AS-3B	1,486	9.2	+++
AS-3BT1	ND	ND	
AS-30	1,744	11.5	+++
AS-4	4,065	12.3	+
S-10	17,927	38.5	−
S-16	6,267	33.3	−

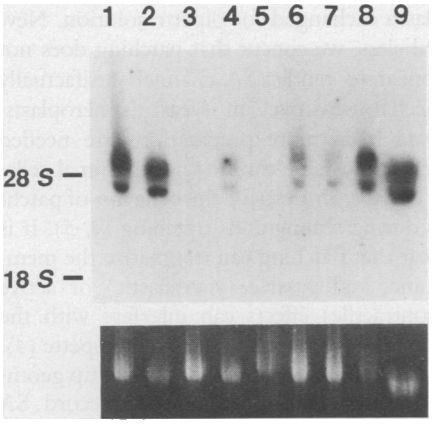

Fig. 1. RNA blot hybridized with rat lysyl oxidase cDNA (*4*). Total cellular RNA (15 μg) was separated by electrophoresis on a 1% agarose-formaldehyde gel and transferred to a nitrocellulose membrane. Cell lines as in Table 1. Lanes: 1, NIH 3T3; 2, PR4 (revertant of RS485); 3, RS485 (*ras*-transformed NIH 3T3); 4, AS-3B; 5, AS-3BT1; 6, AS-30; 7, AS-4; 8, S-10; 9, S-16. Bottom panel shows an ethidium bromide staining of 28*S* ribosomal RNA.

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