J. Membrane Biol. 82, 207–212 (1984)

The Journal of
Membrane Biology

Intercellular Communication and the Control of Growth: XI. Alteration of Junctional Permeability by the *src* Gene in a Revertant Cell with Normal Cytoskeleton

R. Azarnia and W.R. Loewenstein
Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33101

Summary. To learn whether the reduction of cell-to-cell communication in transformation is a possible primary effect of pp60^{src} phosphorylation or secondary to a cytoskeletal alteration, we examined the junctional permeability in transformed cells with normal cytoskeleton. The permeability to fluorescent-labelled mono- and diglutamate was compared in clones of Faras' vole cells—clones transformed by Rous sarcoma virus and reverted from that transformation. One revertant clone (*partial revertant*), had the high levels of pp60^{src} kinase activity and tumorigenicity of the fully transformed parent clone, but had lost the cytoskeletal alterations of that clone. Another revertant clone (*full revertant*) had lost the tumorigenicity and most of the pp60^{src} kinase activity, in addition (J.F. Nawrocki et al., 1984, *Mol. Cell Biol.* **4**:212). The junctional permeability of the *partial revertant* with normal cytoskeleton was similar to that of the fully transformed parent clone with abnormal cytoskeleton. The permeabilities of both were lower than those of the *full revertant* and the normal uninfected cell, demonstrating that the junctional change by the *src* gene is independent of the cytoskeletal one.

Key Words cell-to-cell communication · cell-to-cell channel · cell junction · communicating junction · gap junction · Rous sarcoma virus · transformation · cancer · growth control · tyrosine phosphorylation · *src* gene · protein kinase · pp60^{src} · cytoskeleton

Introduction

The cellular alterations by the *src* gene that go under the portmanteau of transformation comprise a wide spectrum of changes in cellular state. Among these changes are: phosphorylation of tyrosine residues in certain cytoplasmic proteins (Radke & Martin, 1979; Sefton, Hunter, Ball & Singer, 1981); modifications of cytoskeleton, such as microfilament disorganization, loss of actin and consequent changes in cell shape (Edelman & Yahara, 1976; Ash, Vogt & Singer, 1976; Wang & Goldberg, 1976); loss of fibronectin from extracellular matrix (Hynes & Wyker, 1975); alterations of growth *in vitro*, such as loss of density-dependent inhibition and of anchorage dependence (*cf.* Bishop, 1983); and, as shown in the preceding paper, a loss in the capacity of cell-to-cell communication, namely a reduction in junctional permeability (Azarnia & Loewenstein, 1984*a*). The transformation culminates in decontrol of growth, as manifested by the genesis of tumors.

One cannot piece together as yet a coherent picture of the chain of events leading to the decontrol of growth; many of the foregoing changes may well be merely peripheral to that chain. But to all indications, a tyrosine phosphorylation by pp60^{src}, the *src* gene protein, is a primary event, perhaps the triggering one of the chain (*cf.* Hunter, 1980).

Thus, in terms of the change in cell-to-cell communication, our immediate problem is to identify a link between pp60^{src} and the cell-to-cell channel. Two possible mechanisms come immediately to mind. One is a mechanism where pp60^{src} phosphorylates a critical protein in the regulation of the channel open state, perhaps the channel protein itself. The thought of such a chemical linkage flows naturally from the knowledge that the normal, high junctional permeability is contingent on a cyclic AMP-dependent protein kinase—the isoenzyme form I—that phosphorylates serine and threonine residues (Flagg-Newton, Dahl & Loewenstein, 1981; Wiener & Loewenstein, 1983). This is the guiding thought behind the present work.

In the second mechanism, the effect on the cell-to-cell channel is secondary to a pp60^{src} action on the cytoskeleton. Here the phosphorylation of a cytoskeletal element would lead to cytoskeletal disorganization and hence to mechanical disturbance of the cell contact relation. The idea of such a mechanical linkage between the channel and the target of phosphorylation is a sequel of the hypothesis advanced by Sefton, Hunter, Ball and Singer (1981) to account for the change in cell shape in transformation: tyrosine phosphorylation of vinculin, the protein that anchors the actin microfilaments to the cell

membrane (and the cell substratum), would destabilize this anchorage, leading to a rounding of the cell shape. Vinculin, in this hypothesis, owes its pivotal role to its strategic location in the cytoskeleton and to the fact that it is one of the cytoplasmic proteins that is phosphorylated by pp60^{src} (Chen & Singer, 1982).

A priori, both mechanisms seem plausible. To distinguish between them, one needs a mutant cell system bereft of the cytoskeletal response to the virus. Such a system has been developed by Faras and his colleagues: a revertant clone of RSV-transformed cells of the vole *Microtus agrestis*. This clone is a *partial revertant* that retained the high level of pp60^{src} kinase activity, the anchorage independence, and the tumorigenicity of the fully transformed cells. But it lost the cytoskeletal alteration: it is normal in respect to cell shape, cytoskeleton organization, and amounts of actin and fibronectin (Lau, Krzyzek, Brugge, Erikson, Schollmeyer & Faras, 1979; Collett, Brugge, Erikson, Lau, Krzyzek & Faras, 1979; Nawrocki, Lau & Faras, 1984). The analytical complement of this system is a subclone that lost 97–98% of the pp60^{src} kinase activity, the anchorage independence and the tumorigenicity, in addition—a *full revertant* (Lau, Krzyzek & Faras, 1981). Dr. Anthony Faras kindly provided us with these revertants together with the fully transformed ancestor. We compare their junctional properties and show that the junctional permeability of both, the partial revertant and the fully transformed clone, is reduced.

Materials and Methods

Cells and Media

Normal, uninfected fibroblasts of *Microtus agrestis*, the European field mouse (vole), a clone of such fibroblasts—1T—transformed by RSV (Schmidt-Ruppin group D), and two subclones of 1T, the *partial revertant* (866-5RC) and the *full revertant* (866-4) (Krzyzek, Lau, Spector & Faras, 1977; Krzyzek, Lau, Vogt & Faras, 1978; Lau et al., 1979) were grown in Dulbecco's MEM supplemented with 10% calf serum in an incubator, 6–8% CO_2 in air, at 37°C. The same batch of serum and the same passaging protocol was used for all cells.

Junctional Probing with Two Molecular Species

The junctions were probed with the fluorescent molecules listed in Table 1. In preliminary tests, in which these molecules were microinjected as single species, LRB-Glu and LRB-Glu-Glu were found to be close to or below the permeation threshold in the least permeable of the four classes of cells. We used these two molecules as our principal probes.

In the main series of probings, each one of these principal probes (red fluorescent) was microinjected together with the smaller Lucifer Yellow (yellow-green fluorescent), the auxiliary probe. The mixtures in the micropipettes were 7 mM LRB-Glu/10 mM Lucifer Yellow and 5 mM LRB-Glu-Glu/23 mM Lucifer Yellow.

Lucifer Yellow was far above threshold of junction permeation in all cells (Table 2) and, hence, unsuited for sensitive detection of permeability differences. But this very same suprathreshold-permeation property made it useful as an indicator of artifactual junctional blockade in co-injection with the close-to-threshold probe (Socolar & Loewenstein, 1979). *The passage of the auxiliary probe to, at least, 50% of the first-order neighbors of the injected (source) cell served as our acceptance criterion of the data of each individual injection, in the case of a negative passage of the principal probe.* Besides, as in the work of the preceding paper, data from cells with visible damage (granularity, blebbings, etc.) were rejected.

The junctional transfers of the principal and auxiliary probes from the source cell were scored in succession by switching the filter optics. *The scoring was done within 3–5 min of the microinjection.* This was the optimal period for the present cell systems, by the test criteria described in the preceding paper. The cells were at room temperature, 30–32°C during the probings.

In our preliminary search for an adequate auxiliary probe, we considered carboxyfluorescein, besides Lucifer Yellow. Carboxyfluorescein gave incidences of permeable interfaces comparable to those of Lucifer Yellow, and so would have been useful as an auxiliary probe, too (it is easily distinguishable from LRB). Besides, as a channel probe, it is probably superior to the more charged and protein-binding Lucifer dye. We chose Lucifer, nonetheless, because it fluoresced more intensely than carboxyfluorescein with our excitation system—the same system we used in the work of the preceding and subsequent papers (both

Table 1. Junctional probes

	Mean molecular diameter[a] (Å)	Charge[b] (−)	Mol wt	Peak fluorescence emission (nm)
Lucifer Yellow	10.4		443	510
Carboxyfluorescein	10.4	1–2	376	520
LRB-Glu	13.4	2	688	590
LRB-Glu-Glu	14.6	3	817	590
LRB-Glu-Glu-Glu[c]	15.6	4	946	590

[a] The geometric mean of the dimensions of the minimum rectangular parallelopiped containing the molecule.
[b] Net charge.
[c] Used in the next paper of this series only (Azarnia & Loewenstein, 1984*b*).
For preparation, purification, and characteristics of the probes, *see* Stewart, 1978 (Lucifer Yellow); Socolar & Loewenstein, 1979 (carboxyfluorescein); Simpson et al., 1977 (LRB-Glutamates).

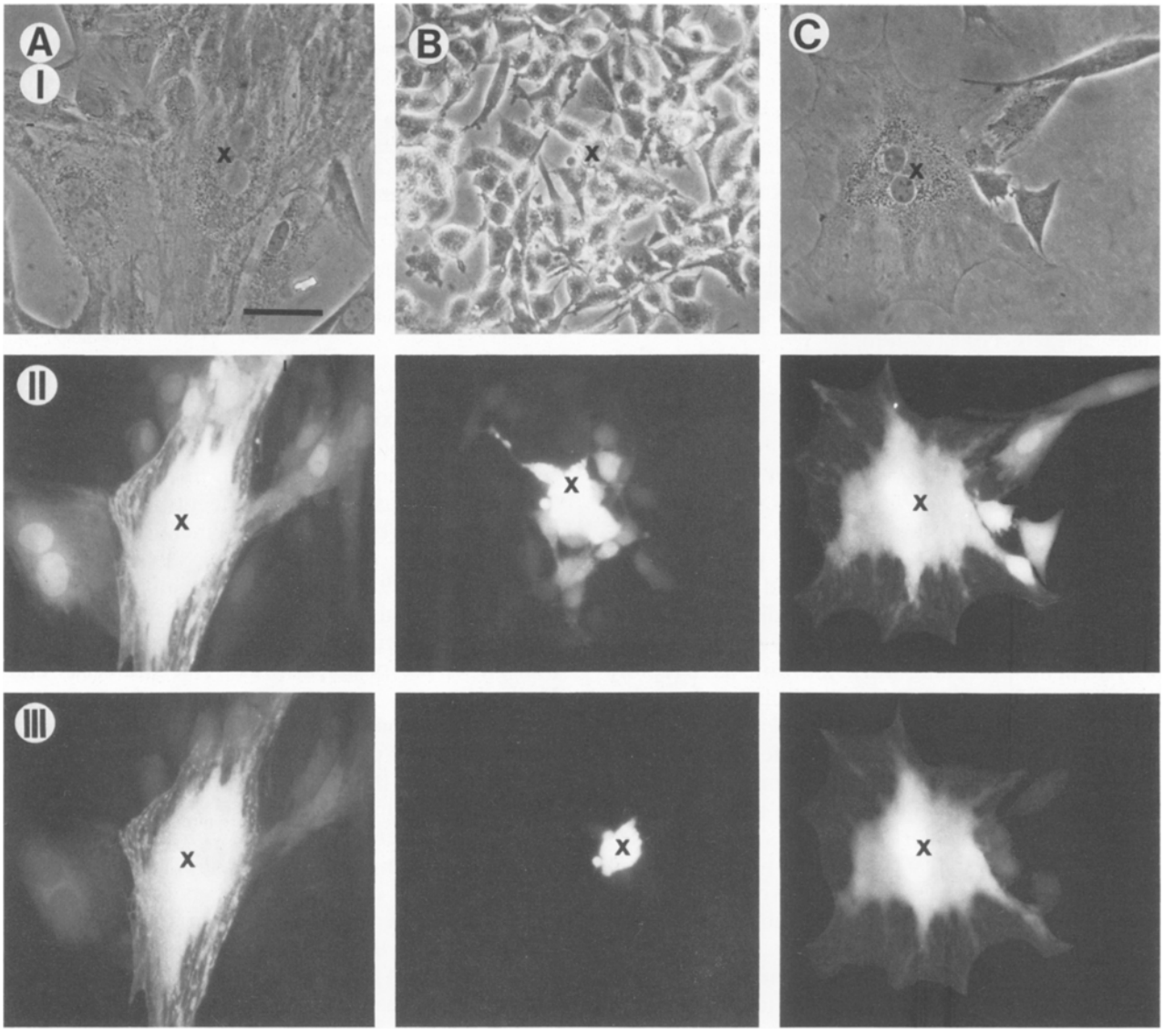

Fig. 1. Dual probings of vole cells. (*A*) *normal*, (*B*) *fully transformed*, and (*C*) *partial revertant*. LRB-Glu_2 and Lucifer Yellow are co-injected into cell marked *x*. Row *II* shows the Lucifer fluorescence; row *III*, the LRB-Glu_2 fluorescence (both in darkfield), and row *I*, the cells in phase contrast. Calibration, 100 μm

done before the present study). By modifying the system for the present work, we could have brought the carboxyfluorescein fluorescence up to the level of Lucifer, and so have gained perhaps a better auxiliary probe, but we would have lost the advantage of comparability throughout the three papers.

Measurement of Fluorescence Loss

The fluorescence loss was measured in single cells as in the preceding paper. In addition, and in order to insure that the measures reflected the conditions under which the junctional transfer was determined, we studied the losses in two-cell systems where the acceptance criterion of junctional transfer was applied. In these measurements Lucifer Yellow was co-injected with LRB-Glu or LRB-Glu-Glu. The loss of LRB fluorescence was determined in the injected cell (with the photodiode looking at a spot of that cell), when the system had passed the Lucifer junctional transfer test.

The method for microinjection, scoring of the incidence of permeable interfaces, and statistical treatment of the data are described in the preceding paper (Azarnia & Loewenstein, 1984*a*).

Table 2. Mean incidence of permeable interfaces (%) in the vole cell system

Probing mode	Principal probe	Normal	Fully transformed	Partial revertant	Full revertant
Dual	LRB-Glu	**89 ± 3** /100 ± 0 (17)	**21 ± 4** /83 ± 4 (17)	**0 ± 0** /82 ± 6 (18)	**39 ± 3** /78 ± 5 (19)
	LRB-Glu_2	**50 ± 3** /100 ± 0 (20)	**0 ± 0** /52 ± 4 (9)	**9 ± 3** /64 ± 3 (20)	**47 ± 4** /83 ± 5 (10)
Single	LRB-Glu	**94 ± 2** (28)	**10 ± 2** (49)	**0 ± 0** (26)	**12 ± 3** (19)
	LRB-Glu_2		**0 ± 0** (8)	**0 ± 0** (9)	**22 ± 6** (19)

Mean incidence of permeable (first-order) cell interfaces ± SE. The incidence value for the principal probe (boldface) is followed by that for the auxiliary probe, Lucifer Yellow, in the dual probing mode. In parentheses, the number of injected cells (the *n* for all statistics). The number of first-order interfaces examined ranged 46–348 per datum. Each horizontal line in this table subsumes the strictly comparable sets of data (*see* text).

Results and Discussion

We compared the junctional transfer in four classes of cells: (*i*) *fully transformed* by RSV, (*ii*) *partial revertants* from that transformation, (*iii*) *full revertants*, and (*iv*) *normal*. The culture parallelism between the various classes of cells had to be specially tight for these comparisons, as we did not have the convenience of temperature-sensitive virus mutants we had in the preceding work, allowing us to rapidly reverse the *src*-gene junctional effect. It helped that three of the cells had a common genealogy, but their culture histories may have been different in respect to hormone composition of the serum supplements and to passage protocol, before we received them. Such factors, particularly hormones, can influence junctional permeability (Flagg-Newton & Loewenstein, 1981; Radu, Dahl, & Loewenstein, 1982). Thus, our first endeavor was to establish a common culture base. Only after the cells had been carried over several weeks in matched conditions, did we begin with the testing of their junctions.

A further problem concerned the measurements of junctional transfer themselves. The *partial* revertant and the *full revertant* turned out to be fragile. They were more prone to damage by the micropipette impalement than the *fully transformed* cell (or the cells dealt with in the preceding paper), as indicated by intracellular granularity, cell opacity, blebbing, and even shriveling in some cases. We resorted, therefore, to the use of specially fine-tipped micropipettes to minimize damage. Besides, we used the method of testing junctional transfer simultaneously with two molecules, one large and one small (Simpson, Rose & Loewenstein, 1977). The large molecule, LRB-Glu or LRB-Glu_2, served as the actual probe of junctional permeability; it was close to the junction permeation threshold and, hence, suited for sensing small changes of permeability. The smaller companion, Lucifer Yellow—a molecule high above permeation threshold in all four cells (light-printed data in Table 2; Fig. 1)—served as an auxiliary probe to control against general junctional blockade caused by cell damage. The cell-to-cell channels close up in response to elevations of cytoplasmic Ca^{2+} concentration, such as those produced by injury to cell membrane or cytoplasm (Délèze & Loewenstein, 1976; Rose & Loewenstein, 1976). Thus, the positive junctional transfer of the auxiliary probe provided a useful criterion for accepting the data, in the case of negative transfer of the principal probe (*see* Materials and Methods).

The data from such dual probings constitute the backbone of the present results. For corroboration, we also include the data from the probings with the single principal molecular species. As it turned out, they are in general agreement with the more stringent data of the dual probings. For each principal probe, the data of the four classes of cells can be directly compared (the data are horizontally aligned in Table 2, for this purpose). However, comparisons should not be made between the data from different principal probes (vertically in the table); the proportions of the two principal probes in the probe mixtures were different (*see* Materials and Methods).

Table 2 summarizes the results. The junctional transfer of the principal probes was much lower in the *fully transformed* and the *partial revertant* cells, compared with that in the *normal* and *fully revertant* cells. The differences between the mean incidences of both LRB-Glu and LRB-Glu_2-permeable interfaces were statistically highly significant (Table 2*A*).

The cell densities at which the junctional transfer was studied ranged from 2 to 12 (10^4 cells/cm^2). Over this range (and wider), the vole cells, including the *normal* cell and *partial revertant*, showed no dependence on density in their junctional transfer. Figure 2 illustrates this for the *normal*- and the *fully transformed* cell.

The lower junctional transfer in the *partial revertant* and *fully transformed* cell is not attributable

Table 2A. Statistical confidence levels of the difference between the mean incidence values

Mode		Normal	Fully transf.	Partial revertant	Full revertant
Dual	Normal		<0.00009 <0.00009	<0.00009 <0.00009	<0.00009 0.21
	Full revertant	<0.00009 0.21	0.0007 <0.00009	<0.00009 <0.00009	
Single	Normal		<0.00009 —	<0.00009 —	<0.00009 —
	Full revertant	<0.00009 —	<0.32 <0.0004	0.001 0.0004	

Confidence levels (P) by t test of the differences between the means of Table 3. In each comparison the upper P value corresponds to the probings with LRB-Glu and the lower value, to the probings with LRB-Glu_2.

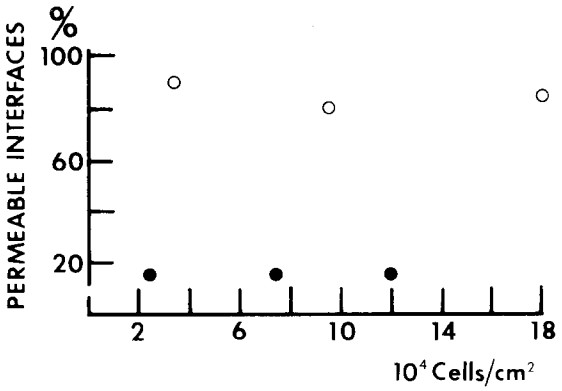

Fig. 2. Density independence of junctional transfer in the vole cell system. First-order permeable interfaces *vs.* cell density in the normal (○) and the fully transformed (●) cells

to a higher nonjunctional membrane permeability in these cells. In the *fully transformed* cell (and normal cell), the curves of LRB-Glu and LRB-Glu_2 cell fluorescence *vs.* time were virtually flat over 5 min, the maximum period of our tests of junctional transfer (*see* Materials and Methods); and in the *partial revertant*, the loss rate constants were 1.5–3%/min, the same as in the *full revertant* (the respective patterns were like those of the curves in Fig. 5*A* and *B* of the preceding paper). So, once again the combined results on junctional transfer and nonjunctional membrane permeance demonstrate a reduction in junctional permeability associated with the *src*-transformed state.

The reduction of junctional permeability in the *partial revertant* is comparable (for both probes) to that in the *fully transformed* cells (Table 2). Thus, the answer to the main question posed in this paper is clear: *the reduction of junctional permeability caused by* $pp60^{src}$ *is independent of cytoskeletal alterations.*

Table 3. Cell generation times

Cell	Doubling time[a] (hr)
Normal	34
Fully transformed, 1T	14.5
Partial revertant, 866-R5C	14.5
Full revertant, 866-4	24

[a] Doubling time of the number of cells in the logarithmic growth phase.

The ramifications of this outcome concerning growth control are discussed in the following paper (Azarnia & Loewenstein, 1984*b*). For the present, it is sufficient and encouraging to note that the junctional change in transformation does not follow the cytoskeletal change to the heap of epiphenomena. The junctional alteration, even in its subtle form, is a factor to be reckoned with in the chain leading to decontrol of growth.

A side result was a difference between the *full revertant* and the *normal* cell. As probed with LRB-Glu (dual- and single mode), the incidence of permeable interfaces was lower in the *full revertant* (P <0.00009; Table 2*A*). Perhaps, there was still enough $pp60^{src}$ kinase activity in the *revertant* to affect junctional permeability; the analysis of that clone by Lau et al. (1981) puts the remnant kinase activity at 2–3% and, in the cultures in our laboratory, the generation cycle of the *full revertants* was shorter than that of the *normal* cells (Table 3). An explanation of this sort implies that the junctional sensitivity to the kinase is high. This does not seem implausible, considering that $pp60^{src}$ is closely associated with cell membrane (Courtneidge, Levinson,

& Bishop, 1980; Krueger, Wang & Goldberg, 1980), including the membrane at junctions (Willingham, Jay & Pastan, 1979; Schriver & Rohrschneider, 1981; Nigg, Sefton, Hunter, Walter & Singer, 1982). However, the difference between the *full revertant* and *normal cell* did not show up in the probings with LRB-Glu_2 (Table 2). This negative finding beclouds the side result.

We thank Dr. Anthony Faras for providing us with the cells and Dr. Joan Brugge for invaluable suggestions. The work was supported by Research Grant No. CA14464 from the National Institutes of Health.

References

Ash, J.F., Vogt, P.K., Singer, S.J. 1976. Reversion from transformed to normal phenotype by inhibition of protein synthesis in rat kidney cells infected with a temperature-sensitive mutant of Rous sarcoma virus. *Proc. Natl. Acad. Sci. USA* **73**:3603–3607

Azarnia, R., Loewenstein, W.R. 1984*a*. Intercellular communication and the control of growth: X. Alteration of junctional permeability by the *src* gene. A study with temperature-sensitive mutant Rous sarcoma virus. *J. Membrane Biol.* **82**:191–205

Azarnia, R., Loewenstein, W.R. 1984*b*. Intercellular communication and the control of growth: XII. Alteration of junctional permeability by simian virus 40. Roles of the large and small T antigens. *J. Membrane Biol.* **82**:213–220

Bishop, J.M. 1983. Cellular oncogenes and retroviruses. *Annu. Rev. Biochem.* **52**:301–354

Chen, W.-T., Singer, S.J. 1982. Immunoelectron microscopic studies of the sites of cell-substratum and cell-cell contacts in cultured fibroblasts. *J. Cell Biol.* **95**:205–222

Collett, M.S., Brugge, J.S., Erikson, R.L., Lau, A.F., Krzyzek, R.A., Faras, A.J. 1979. The *src* gene product of transformed and morphologically reverted ASV-infected mammalian cells. *Nature (London)* **281**:195–198

Courtneidge, S.A., Levinson, A.D., Bishop, J.M. 1980. The protein encoded by the transforming gene of avian sarcoma virus ($pp60^{src}$) and a homologous protein in normal cells ($pp60^{proto\text{-}src}$) are associated with the plasma membrane. *Proc. Natl. Acad. Sci. USA* **77**:3783–3787

Délèze, J., Loewenstein, W.R. 1976. Permeability of a cell junction during intracellular injection of divalent cations. *J. Membrane Biol.* **28**:71–86

Edelman, G.M., Yahara, I. 1976. Temperature-sensitive changes in surface modulating assemblies of fibroblasts transformed by mutants of Rous sarcoma virus. *Proc. Natl. Acad Sci. USA* **73**:2047–2051

Flagg-Newton, J.L., Dahl, G., Loewenstein, W.R. 1981. Cell junction and cyclic AMP: I. Upregulation of junctional membrane permeability and junctional membrane particles by administration of cyclic nucleotide or phosphodiesterase inhibitor. *J. Membrane Biol.* **63**:105–121

Flagg-Newton, J.L., Loewenstein, W.R. 1981. Cell junction and cyclic AMP. II. Modulations of junctional membrane permeability, dependent on serum and cell density. *J. Membrane Biol.* **63**:123–131

Hunter, T. 1980. Proteins phosphorylated by the RSV transforming function. *Cell* **22**:647–648

Hynes, R.O., Wyker, J.A. 1975. Alterations in surface proteins in chicken cells transformed by temperature-sensitive mutants of Rous sarcoma virus. *Virology* **64**:492–504

Krueger, J.G., Wang, E., Goldberg, A.R. 1980. Evidence that the *src* gene product of Rous sarcoma virus is membrane associated. *Virology* **101**:25–40

Krzyzek, R., Lau, A.F., Spector, D., Faras, A. 1977. Post-transcriptional control of avian oncornavirus transforming gene sequences in mammalian cells. *Nature (London)* **269**:175–179

Krzyzek, R., Lau, A.F., Vogt, P.K., Faras, A.J. 1978. Quantitation and localization of Rous sarcoma virus-specific RNA in transformed and revertant field vole cells. *J. Virology* **25**:518–526

Lau, A.F., Krzyzek, R.A., Brugge, J.S., Erikson, R.L., Schollmeyer, J., Faras, A.J. 1979. Morphological revertants of an avian sarcoma virus-transformed mammalian cell line exhibit tumorigenicity and contain $pp60^{src}$. *Proc. Natl. Acad. Sci. USA* **76**:3904–3908

Lau, A.F., Krzyzek, R.A., Faras, A.J. 1981. Loss of tumorigenicity correlates with a reduction in $pp60^{src}$ kinase activity in a revertant subclone of avian sarcoma virus-infected field vole cells. *Cell* **23**:815–823

Nawrocki, J.F., Lau, A.F., Faras, A.J. 1984. Correlation between phosphorylation of a 34,000-molecular weight protein, $pp60^{src}$-associated kinase activity, and tumorigenicity in transformed and revertant vole cells. *Mol. Cell Biol.* **4**:212–215

Nigg, E.A., Sefton, B.M., Hunter, T., Walter, G., Singer, S.J. 1982. Immunofluorescent localization of the transforming protein of Rous sarcoma virus with antibodies against a synthetic *src* peptide. *Proc. Natl. Acad. Sci. USA* **79**:5322–5326

Radke, K., Martin, G.S. 1979. Transformation by Rous sarcoma virus: Effects of *src* gene expression on the synthesis and phosphorylation of cellular polypeptides. *Proc. Natl. Acad. Sci. USA* **76**:5212–5216

Radu, A., Dahl, G., Loewenstein, W.R. 1982. Hormonal regulation of cell junction permeability: Upregulation by catecholamine and prostaglandin E_1. *J. Membrane Biol.* **70**:239–251

Rose, B., Loewenstein, W.R. 1976. Permeability of a cell junction and the local cytoplasmic free ionized calcium concentration. A study with aequorin. *J. Membrane Biol.* **28**:87–119

Schriver, K., Rohrschneider, L. 1981. Organization of $pp60^{src}$ and selected cytoskeletal proteins within adhesion plaques and junctions of Rous sarcoma virus-transformed cells. *J. Cell Biol.* **89**:525–535

Sefton, B.M., Hunter, T., Ball, E.H., Singer, S.J. 1981. Vinculin: A cytoskeletal target of the transforming protein of Rous sarcoma virus. *Cell* **24**:165–174

Simpson, I., Rose, B., Loewenstein, W.R. 1977. Size limit of molecules permeating the junctional membrane channels. *Science* **195**:294–296

Socolar, S.J., Loewenstein, W.R. 1979. Methods for studying transmission through permeable cell-to-cell junctions. *In:* Methods In Membrane Biology. E. Korn, editor. Vol. 10, pp. 123–179. Plenum, New York

Stewart, W.W. 1978. Functional connections between cells as revealed by dye-coupling with a highly fluorescent naphthalamide tracer. *Cell* **14**:741–759

Wang, E., Goldberg, A.R. 1976. Changes in microfilament organization and surface topography upon transformation of chick embryo fibroblasts with Rous sarcoma virus. *Proc. Natl. Acad. Sci USA* **73**:4065–4069

Wiener, E.C., Loewenstein, W.R. 1983. Correction of cell-cell communication defect by introduction of a protein kinase into mutant cells. *Nature (London)* **305**:433–435

Willingham, M.C., Jay, G., Pastan, I. 1979. Localization of the ASV *src* gene product to the plasma membrane of transformed cells by electron microscopic immunocytochemistry. *Cell* **18**:125–134

Received 30 August 1984