Table 1. Presence of antigen related to M-PMV p27 in human breast carcinomas as determined by competition radioimmunoassays. The detailed histopathology of human breast tumors is as follows: (B-34) poorly differentiated mammary duct cell carcinoma with osteoid metaplasia, left breast; (B-65) infiltrating poorly differentiated duct cell carcinoma, left breast; (B-44) mammary duct cell carcinoma; (B-51) poorly differentiated invasive duct cell carcinoma, right breast; (B-78) poorly differentiated invasive duct cell carcinoma, left breast; (B-84) poorly differentiated mammary duct cell carcinoma, right breast; (B-85) mammary duct cell carcinoma, left breast; (B-87) infiltrating mammary duct cell carcinoma; (B-70) not available; (B-89) not available; (B-91) invasive duct cell carcinoma, left breast with metastasies to lymph nodes; (B-92) invasive mammary duct cell carcinoma with marked desmoplasia, left breast; (B-83) poorly differentiated invasive adenocarcinoma, left breast; (B-63) not available; (B-79) not available; (B-77) moderately differentiated mammary duct cell carcinoma, right breast; (B-86) not available; (B-88) metastatic carcinoma. The 14 human nonbreast tissues that were examined included two normal human placentas, one hepatoma, one carcinoma of lung, two carcinomas of colon, one carcinoma of rectum, one carcinoma of gallbladder, two granulosa tumors of ovary, one adenocarcinoma of stomach, one leiomyosarcoma of uterus, one renal cell cancer of stomach, and one meothelioma of pleura. Ten to 15 g of each tissue was processed, resulting in 1 ml of protein extract containing 55 to 142 μg per milliliter of protein. None of these tissues competed with M-PMV p27 in our RIA's.

Patient	Age (years)	Tumor wet wt (g)	Final extract (ml)	Final protein (mg/ml)	Activity ratio (ng/mg)
B-34	52	8	0.5	27	0.4
B-65	65	8	0.5	31	0.3
B-44	48	5	0.5	33	1.2
B-51	62	8	0.4	15	2.9
B-78	56	13	0.8	55	2.3
B-84	60	10	0.5	63	1.9
B-85	45	4	0.4	13	5.6
B-87	40	17	1.0	260	1.7
B-70		18	1.0	230	*
B-89		5	0.5	36	*
B-91	58	6	0.5	26	*
B-92	71	7	0.5	29	*
B-83	55	8	0.7	18	*
B-63	48	13	1.0	116	*
B-79		16	1.0	52	*
B-77	74	17	1.0	155	*
B-86		8	0.5	27	*
B-88	29	7	0.5	20	*

*Not detectable.

false positive result, the RIA supernatants from the lowest dilutions of tumors B-87, B-51, and B-78 (these competed most effectively for ^{125}I-labeled M-PMV p27) were applied onto sodium dodecyl sulfate–polyacrylamide gels and the positions of the radioactive peaks compared to those of protein standards (*7*). There was no appreciable breakdown of the ^{125}I-labeled p27 since, in each case, more than 80 percent of the released radioactivity was found under the p27 peak (data not shown).

Among the group of eight positive breast tumor specimens (Table 1), the amount of antigens detected ranged from 0.3 to 5.7 ng per milligram of protein extract. Since the extraction and column chromatography procedure achieved from 200- to 1000-fold reduction of cellular material, the amount of p27-like protein in these breast tumors appears to be very small. Experiments were done to approximate the level of recovery of p27 from tumor material by adding 100 ng of purified p27 to 10 g of normal placental tissue before proceeding with the extraction and chromatography purification steps. The partially purified tissue material was quantitatively tested for p27 by RIA. Almost 50 percent of the input p27 was recovered at the end of the purification procedure. This value of 50 percent probably represents the maximum amount recoverable by this procedure because undoubtedly some intracellular antigens would have been trapped with the cell debris and therefore lost during the purification steps. Even assuming a 10 percent recovery of the M-PMV specific antigens from tumor tissues, the amount of antigens in breast tumor tissues is far less than the level of SSV-1 antigens found by Sherr and Todaro in peripheral white blood cells of patients with acute leukemia (*6*). This low level of viruslike antigens in malignant breast tissues may be a partial explanation for our inability to detect such antigens in the other ten breast tumors examined.

In summary, we detected the presence of an antigen similar to the M-PMV major structural protein in 8 out of 18 human malignant breast tumors. Such antigen was not detected in malignant or normal human tissues not of breast origin.

J. YEH
M. AHMED
S. A. MAYYASI
John L. Smith Memorial for Cancer Research, Pfizer Inc., Maywood, New Jersey 07607
A. A. ALESSI
Hackensack Hospital, Hackensack, New Jersey 07601

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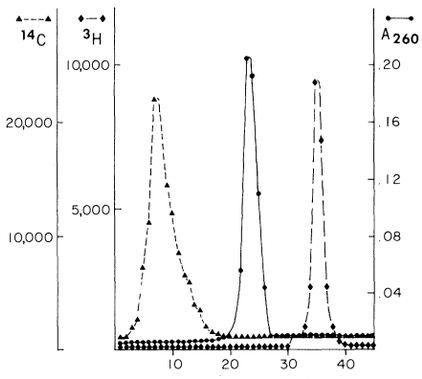
Guanidinium-CsCl Density Gradients for Isopycnic Analysis of Nucleic Acids

Abstract. *The addition of guanidinium chloride to CsCl gradients lowers the apparent density of RNA, DNA, and hybrid polymers in such a way that all three can be banded, fractionated, and analyzed in one gradient with essentially no damage to their chemical integrity or to their biological activity.*

CsCl density gradients (*1*) are widely used for isopycnic analysis of DNA because of their simplicity and flexibility (*2*). However, CsCl, is not useful for the analysis of RNA. Several attempts (*3*) to design density gradients adequate for banding of RNA have met with incomplete success. It would be useful to have available a simple analytical procedure that would permit simultaneous isopycnic analysis of DNA, RNA, and DNA-RNA hybrids. In the course of some experiments with guanidinium chloride to dissociate nucleoproteins into their two moieties, it was noted that, in the presence of guanidinium chloride, DNA had a lower buoyant density in CsCl. Therefore we set out to determine whether this was also true for RNA so that it would band at a density within the range normally produced by CsCl gradients. After some

Table 1. Banding positions of various nucleic acids. The relative densities are expressed as the percentage of the distance from the top of the centrifuge tube, with the top-to-bottom distance equal to 100 units. The various nucleic acids were centrifuged individually and in combinations in 4.5-ml gradients in the SW 50.1 rotor (Beckman) at 35,000 rev/min and 15°C for 65 to 72 hours. The gradients contained 12.88 g of CsCl and 2.4 g of guanidinium chloride per 10 ml of 10 m*M* tris (*p*H 7.4). Sources of the specimens were as follows: f1 single-stranded DNA and T7 double-stranded DNA were obtained from purified virions by extraction with neutralized phenol in 0.1 percent SDS and subsequent ethanol precipitation of the aqueous phase. Poly(rA)·poly(dT), poly(rC)·poly(dG), and poly(rU)·poly(rA) were purchased from Gibco. *Escherichia coli* RNA was extracted from K12 cells that had been labeled with [^{3}H]uracil (100 μc/ml) for 90 minutes and the reaction was halted by the addition of unlabeled uracil (20 minutes) in the presence of rifampin (200 μg/ml) (Calbiochem). The extraction was carried out as described by Blumberg *et al.* (*6*). Abbreviations: rA, riboadenylate; dT, deoxyribothymidylate; rC, ribocytidylate; dG, deoxyriboguanylate; rU, ribouridylate; ss, single-stranded; and ds, double-stranded.

Nucleic acid	Relative density
f1 DNA (ss)	20.2
T7 DNA (ds)	22.5
Poly(rA)·poly(dT)	57.0
Poly(rC)·poly(dG)	61.1
Poly(rU)·poly(rA)	83.1
E. coli RNA	85.0



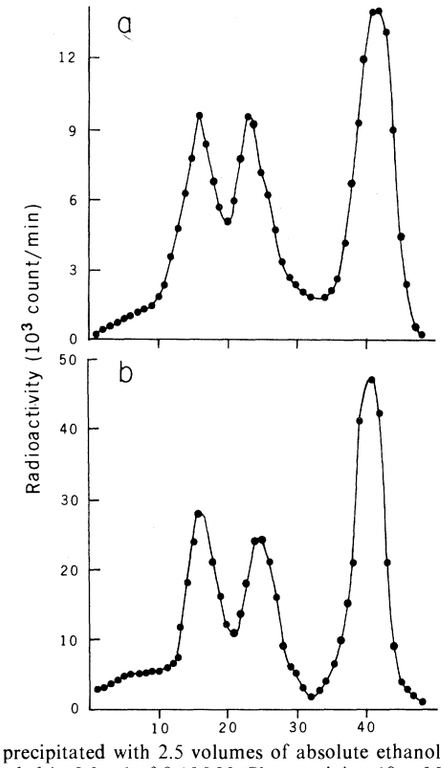


Fig. 1 (left). ^{14}C-Labeled total RNA of *Escherichia coli*, ^{3}H-labeled f1 DNA, and unlabeled poly(rA)·poly(dT) were centrifuged in 4.8-ml gradients containing 12.88 g of CsCl, 2.4 g of guanidinium chloride per 10 ml of 10 m*M* tris-HCl, *p*H 7.4; centrifugation was performed at 38,000 rev/min and at 15°C in the SW 50.1 rotor (Spinco) for 72 hours. Fractions were collected from the bottom, their absorbance at 260 nm was recorded, and their radioactivity was measured by drying 20-μl portions on filter paper and counting in a toluene-based scintillation fluid. Fig. 2. (a) Long-term labeled [^{14}C] RNA of *Escherichia coli* was centrifuged in a guanidinium-CsCl gradient as for Fig. 1. Fractions were collected from the bottom. The fractions containing the radioactivity were pooled, diluted threefold with 10 m*M* tris, *p*H 7.0, and precipitated with 2.5 volumes of absolute ethanol overnight at –20°C. The precipitate was resuspended in 0.2 ml of 0.1*M* NaCl, containing 10 m*M* tris, *p*H 7.2, and 0.1 percent sodium dodecyl sulfate (SDS), and layered on the top of a sucrose gradient (5 to 20 percent) in 0.1*M* NaCl, 10 m*M* tris, *p*H 7.2, and 0.1 percent SDS. Centrifugation was performed at 45,000 rev/min and 15°C for 150 minutes in the SW 50.1 rotor (Beckman). (b) The control was a sample of the same RNA preparation not banded in guanidinium-CsCl gradients. Fractions were collected from the bottom on filter paper, dried, and counted in a toluene-based scintillation fluid.

trials, we found that a solution containing 12.88 g of CsCl, and 2.4 g of guanidinium chloride, 10 ml of 10 m*M* tris, *p*H 7.4, was satisfactory.

The relative banding position of DNA, RNA, and DNA-RNA hybrids expressed as percentage of the distance from the top of the gradient is shown in Table 1. All these components band in the same gradient and at well-separated positions. This provides a convenient method for the total analysis of nucleic acid mixtures. In particular it should prove useful for DNA-RNA hybridization studies, in that it provides a simple way to evaluate three critical parameters: (i) extent of hybridization, (ii) recovery, and (iii) class distribution of various hybrids in terms of DNA : RNA ratios.

Despite the denaturing properties of guanidinium chloride, no denaturation of the nucleic acids occurred (*4*). This fact has been proved directly with poly(rA)·poly(dT) duplexes, which band as a unique component between DNA and RNA (Fig. 1). Denaturation would have given rise to two peaks banding at the positions of the ribo- and deoxyribo- polymers. The absence of denaturation is probably due to the protective effect of the high concentration of CsCl. On the other hand, these conditions still promote stripping of proteins from nucleoprotein complexes (*5*). Therefore the method can be used advantageously to prepare pure nucleic acids from nucleoproteins without the need for extensive phenol or chloroform extractions, thereby potentially increasing the yields and the integrity of the nucleic acids. The integrity of nucleic acids in such gradients was studied as follows. The DNA of coliphage f1, both in its viral single-stranded state and in its double-stranded covalently closed replicative form (RFI), were run in guanidinium-CsCl density gradients and then checked for biological activity in a transfection system. Both samples were found to be nearly as infectious as the controls (Table 2). Similarly, the chemical integrity of RNA subjected to guanidinium-CsCl density gradient was investigated. Long-term labeled RNA from *Escherichia coli* was run for 65 hours at 18°C, collected from the gradient, and analyzed in a 5 to 20 percent sucrose gradient. Little degradation of 23*S*, 16*S*, and 4*S* materials occurred (Fig. 2).

These observations suggest that such gradients could be used routinely for purification of phenol-extracted RNA, usually heavily contaminated with DNA, instead of a somewhat risky deoxyribonuclease digestion. For this purpose the use of the Beckman type-40 rotor is most satisfactory with regard to both the separation of RNA from DNA and loading capacity. The following formula: 14.82 g of CsCl, 2.73 g of guanidinium chloride, 10 ml of 10 m*M* tris, *p*H 7.4 (10-ml gradients), produces a shallow gradient in which DNA bands near the top and RNA near the bottom of the tube. Milligram quantities of nucleic acids can be loaded and banded per tube. Similar gradients may be effective for the fractionation of single-stranded and double-stranded RNA mixtures.

Table 2. Biological activity of bacteriophage f1 DNA's before and after banding in guanidinium-CsCl gradients. Viral, single-stranded DNA (ss DNA) and the double-stranded replicative form of f1 DNA (RFI) were tested for their infectivity in a transfection assay (*7*) before and after centrifugation to equilibrium in guanidinium-CsCl gradients. The infectivity is expressed as plaque-forming units (PFU) per 0.05 μg of DNA.

Sample	Infectivity (PFU)	
	Control	Gradient
ss DNA	1.3×10^3	1.0×10^3
RFI	1.2×10^4	1.1×10^4
No DNA	0	0

VINCENZO ENEA
NORTON D. ZINDER
Rockefeller University, New York 10021

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Ionophore-Mediated Calcium Entry Induces Mussel Gill Ciliary Arrest

Abstract. *Lateral cilia of freshwater mussel gills, which normally beat with metachronal rhythm, are arrested pointing frontally by perfusion with 6.25 to 12.5 millimolar calcium and 10^{-5} molar A23187, a calcium ionophore. Arrest does not occur in either calcium or ionophore and monovalent cations alone. Activity returns with continued perfusion in potassium chloride or calcium chloride, and more slowly in sodium chloride, after removal of ionophore. These results support the hypothesis that a local rise in internal calcium causes ciliary arrest.*

Although once beating the ciliary axoneme is an autonomous motor organelle that operates via a sliding microtubule mechanism (*1*), cells can often turn their ciliary beat on and off. The cellular control of ciliary activity is perhaps best analyzed in the protozoan *Paramecium* where Ca^{2+} entry into the cell causes beat stoppage and reversal (*2*). In metazoan epithelia, such as the gill tissue of lamellibranch mollusks, mechanical or nervous stimuli can sometimes be shown to cause ciliary arrest (*3*).

Satir *et al.* (*4*) have found that local lesions 30 μm in diameter produced by laser irradiation of lateral (L) cells of freshwater mussel gill (*Elliptio* and related genera) placed in 5 to 15 m*M* $CaCl_2$ or NaCl, but not in KCl or NH_4Cl, initiate ciliary arrest, which spreads for distances greater than 1 mm to either side of the lesion. Motokawa and Satir (*5*) have shown that for *Mytilus* gill similar arrest responses occur in normal, but not in Ca^{2+}-free, seawater. We postulate that in such cases, just as in *Paramecium*, the cause of arrest is a local rise in Ca^{2+} concentration in the cytoplasm which inhibits ciliary beat at sensitive points in the stroke cycle. The L-cells are only a few micrometers wide and intracellular microelectrode recording is difficult with this system (*6*), so that my test of this hypothesis relies on an alternate method. The calcium ionophore A23187 (*7*) offers an experimental tool for controlling Ca^{2+} entry into the cell cytoplasm. For example, in concentrations of 10^{-6} or 10^{-5} *M*, this ionophore induces secretion of ATP in platelets, histamine release from mast cells, contraction of frog eggs and muscle, and so forth (*8*)—phenomena that depend on Ca^{2+} entry from either external or internal stores into the cytoplasm proper. In the experiments described here we were able to show that in the presence of Ca^{2+} and ionophore, beat of mussel gill L-cilia is arrested, but that arrest does not occur in either Ca^{2+} or ionophore alone.

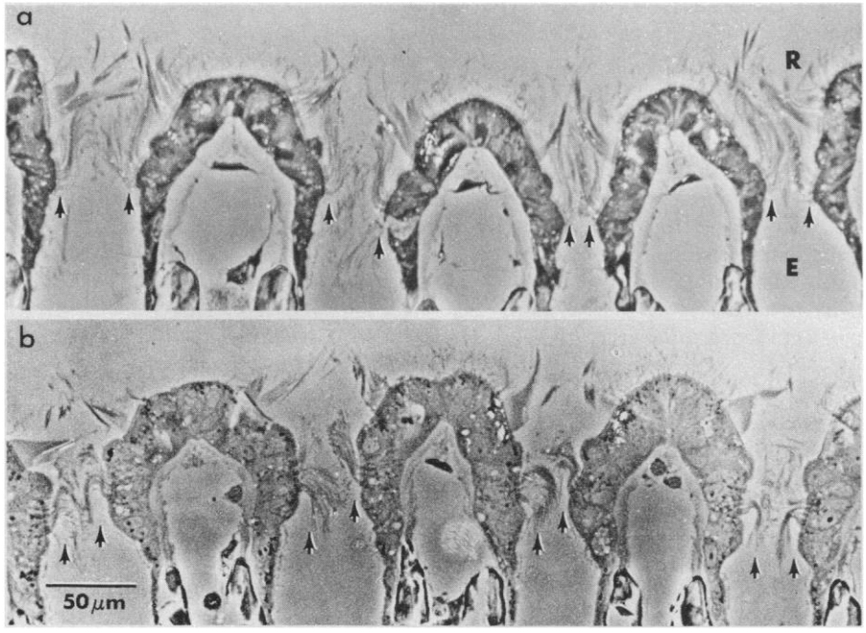

Fig. 1. Phase contrast micrographs of gill filaments embedded in Epon, showing positions of L-cilia (arrowheads) after quick fixation (*9*). (a) Tissue in 12.5 m*M* Ca^{2+} and 10^{-5} *M* A23187; L-cilia are arrested. Nearly all L-cilia have a basal bend. The distal parts of the axonemes lie straight, parallel to the epithelium, with the tips pointing in a frontal (*R*) direction. Abfrontal direction is indicated by *E*. (b) Portion of the same gill. The arrested cilia were placed in 20 m*M* KCl so that metachronism was resumed before fixation. The L-cilia are fixed in various metachronal wave positions, pointing in both abfrontal and frontal directions.

Pieces of gill tissue, excised from healthy mussels usually into 12.5 m*M* $CaCl_2$ or 20 m*M* NaCl, are stripped to give an undamaged piece of gill lamella and placed in a small perfusion chamber, as described previously (*9*). Flow through the chamber is continuous except for brief intervals (< 30 seconds) when solutions are changed. Low-power fields containing about 15 filaments whose L-cilia are beating with well-defined metachronism are selected for subsequent experimentation. Each filament bears two rows of L-cells that are measured independently with respect to metachronal activity; the number of active rows that pass metachronal waves across the entire field is used to determine percentage of metachronism in the tissue at appropriate intervals. In rows that are not active, L-cilia are usually stopped in a characteristic position shown in Fig. 1. The laterofrontal cirri and terminal and frontal cilia generally continue to beat, even when the L-cilia are stopped.

The gill is initially perfused with $CaCl_2$, NaCl, or a mixture of the two salts adjusted to approximately equal osmotic strengths. Greater than 60 percent metachronism (Fig. 2) is normally obtained in these solutions; serotonin (5-hydroxytryptamine) can initially be used to increase metachronal activity if necessary, but is always removed by perfusion for > 5 minutes before experimentation is begun. Figure 2, a and b, compares the effect of adding 10^{-5} *M* A23187 to initial perfusates of Ca^{2+} against Na^+. In the former instance (Fig. 2a) metachronism is affected almost immediately and completely abolished within less than 10 minutes of addition; in the latter case (Fig. 2b) addition