a calcium-dependent event that is essential for prolactin gene regulation by peptide hormones. We therefore investigated the possibility that cobalt ions might distinguish the effects of cyclic AMP on prolactin and growth hormone gene transcription. Cobalt inhibited the stimulation of prolactin gene transcription by forskolin (Fig. 1) or 8-bromo cyclic AMP, which is comparable to the effects of cobalt on TRH-stimulated prolactin gene transcription. In contrast to the effects on prolactin gene expression, the stimulation of growth hormone gene transcription by forskolin was potentiated by cobalt ions (Fig. 1). Cobalt also decreased the level of prolactin gene transcription in unstimulated cultures by 50 percent (Fig. 1). We (*13*) and others (*18*) have noted that, under usual conditions of culture with 10 percent serum, prolactin gene transcription is not basal but is approximately two times greater than the level observed in serum-free culture conditions, presumably because of low concentrations of hormones in the serum. Thus, prolactin gene transcription was 1.5 to 2 parts per million per kilobase in serum-free conditions, and no further decrease in transcription rate was observed when cobalt was added. The possibility that the regulatory mechanism for cyclic AMP stimulation of prolactin and growth hormone gene expression is the same, but that a calcium-dependent process operates selectively in prolactin but not in growth hormone gene transcription, is therefore unlikely.

Cobalt ions inhibited forskolin-stimulated BRP phosphorylation (Fig. 3A) to the levels observed in cells cultured in serum-free media; however, cobalt ions did not prevent forskolin stimulation of cyclic AMP accumulation (*19*) or the phosphorylation of histone H1 at Ser^{37} (Fig.2B), showing that cobalt does not inhibit cyclic AMP–dependent protein kinase activity. Cobalt ions also potentiated phosphorylation by cyclic AMP of a 19-kD basic nuclear protein (CBP) (Fig. 3A) that was soluble in dilute acid, could be precipitated by perchloric acid, and could be distinguished from previously described histone and high-mobility group proteins. The phosphorylation of CBP was greatly stimulated by addition of cyclic AMP to homogenates of GH_4 cells (Fig. 3B). Phosphorylation of BRP was unaffected by addition of cyclic AMP to identical homogenates (Fig. 3B). Therefore, CBP may be a direct substrate of the cyclic AMP–dependent protein kinase in vivo. A relation between the phosphorylation of BRP and CBP and the mediation of altered prolactin and growth hormone gene transcription is unlikely on the basis of their abundance. However, their analysis together with the transcriptional data shows that increased amounts of intracellular cyclic AMP modulate a second protein kinase that exhibits calcium-dependent activation or activity.

Cyclic AMP exerts various effects on cellular calcium metabolism (*20*) and can modulate many kinase systems either directly or by activation of a protein phosphatase inhibitor (*21*). The identification of discrete regulatory pathways mediating the effects of cyclic AMP on prolactin and growth hormone gene transcription suggests that a single intracellular mediator can simultaneously regulate the transcription of different sets of responsive genes by stimulating independent biochemical events. In this regard, it is important to establish the pathway by which cyclic AMP acts to regulate transcription of each responsive gene.

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Ca^{2+} and Ca^{2+}-Activated K^+ Currents in Mammalian Gastric Smooth Muscle Cells

Abstract. *Inward movement of calcium through voltage-dependent channels in muscle is thought to initiate the action potential and trigger contraction. Calcium-activated potassium channels carry large outward potassium currents that may be responsible for membrane repolarization. Calcium and calcium-activated potassium currents were identified in enzymatically isolated mammalian gastric myocytes. These currents were blocked by cadmium and nifedipine but were not substantially affected by diltiazem or D600. No evidence for a tetrodotoxin-sensitive sodium current or an inwardly rectifying potassium current was found.*

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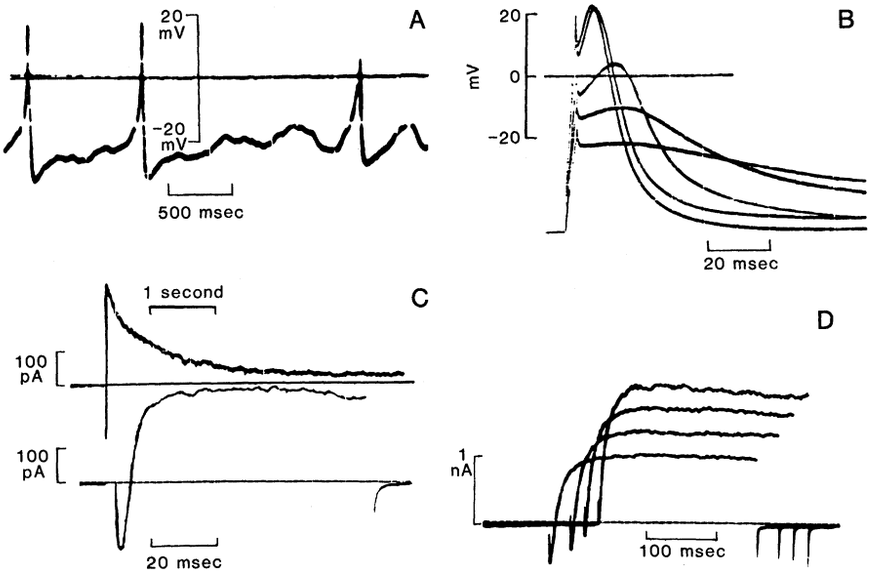
Electrophysiological studies of mammalian gastric muscle strips have shown membrane activity consisting of slow wave depolarizations, spike discharges, plateau phases, or a combination of these activities (*1–3*). The presence of any particular type of activity depends on the species as well as on the anatomical location of the gastric muscle from which it is recorded. These studies have also examined the ionic and pharmacologic dependence of the electrophysiological properties. Voltage clamp studies, however, have been complicated by the syncytial nature of this tissue. Temporal and spatial clamp inhomogeneity and the accumulation and depletion of ions in restricted extracellular spaces have made it difficult to characterize the ionic currents. These problems are largely overcome by studying isolated cells, and such studies have already been performed on toad gastric cells (*4*) and mammalian jejunal cells (*5*). We now describe the electrophysiological properties of isolated single gastric myocytes studied by the whole-cell voltage clamp technique (*6*). A new method was used to isolate gastric cells from the corpus region of guinea pig and rabbit stomach (*7*). We found that gastric myocytes have



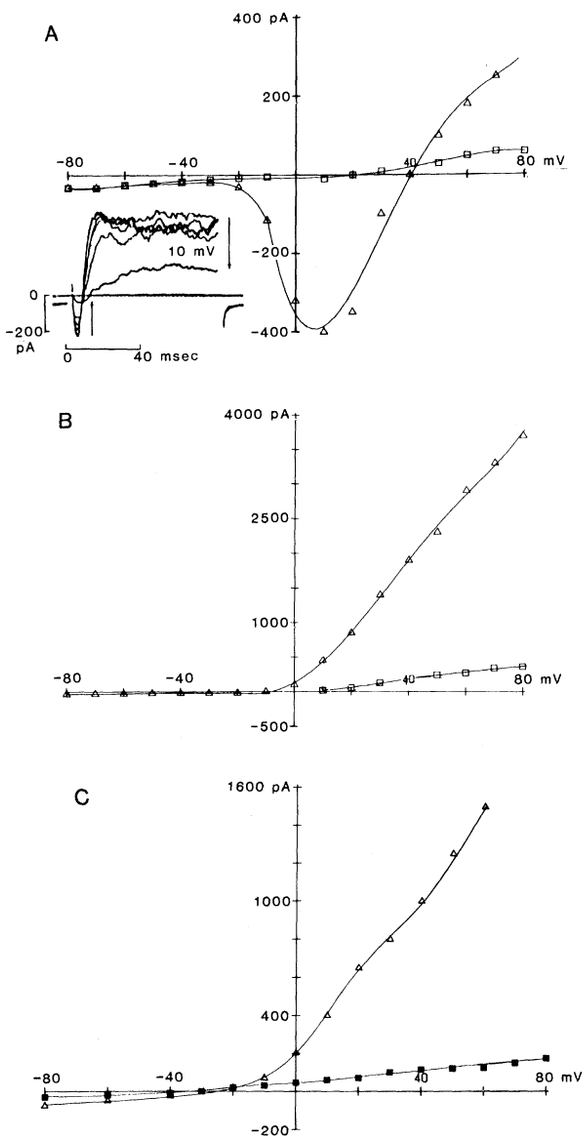


Fig. 1 (above). Action potentials and voltage clamp records from guinea pig gastric cells. Composition of internal pipette solution was as follows (in millimoles per liter): KCl, 70; potassium gluconate, 70; NaCl, 10; $MgCl_2$, 5; dipotassium adenosine triphosphate, 5; Hepes buffer, 10; $CaCl_2$, 1; and K_2EGTA, 11 (*p*H 7.20). (A) Spontaneous activity of a cell from the corpus region. (B) An increase in the strength of depolarizing current pulses resulted in shortening of the action potentials. (C) Top trace: membrane current recorded during a 4-second clamp pulse to +10 mV; holding potential, −80 mV. Lower trace: same pulse, expanded time scale showing time course of inward current. (D) Net membrane currents accompanying clamp steps to +20, +30, +40, and +50 mV (left to right). Holding potential, −80 mV. Traces are horizontally offset for clarity. (B) and (C) are from the same cell; (A) and (D) are from different cells. Temperature, 35°C. Fig. 2 (right). Relation between current and voltage of the inward and outward currents in gastric myocytes from the same rabbit cell (A and B) and from a guinea pig cell (C). Internal pipette solution was as described for Fig. 1. (A) Suppression of peak inward current by 0.5 m*M* Cd^{2+}. (Inset) The suppressive effect of Cd^{2+} on inward and outward current as it washes in. (B) Suppression of isochronal current-voltage relation at 80 msec (peak outward current) by 0.5 m*M* Cd^{2+}. (C) Suppression of current-voltage relation at 350 msec in the absence of Ca^{2+} in the external solution. Symbols: (△) control; (□) 0.5 m*M* $CdCl_2$; and (■) Ca^{2+}-free solution.

Ca^{2+} and Ca^{2+}-activated K^+ currents but have neither a tetrodotoxin (TTX)-sensitive Na^+ current nor an inwardly rectifying K^+ current. These findings are consistent with the low resting potential and the slow rate of rise of the action potential.

Guinea pigs (250 to 350 g in body weight) or rabbits (1 to 2 kg) were injected intraperitoneally with heparin (10 unit/g) 10 minutes before they were killed by cervical dislocation. The stomach was excised, and a PE 22 catheter attached to a Langendorff column (height, 1 m) was inserted into the submucosal space through a small incision. Care was taken not to pierce either the mucosal or muscle layers during insertion. The stomach was perfused for 20 minutes with a Ca^{2+}-free Tyrode's solution (*p*H 7.3, 35°C) of the following composition (in millimoles per liter): NaCl, 136; KCl, 5.4; $MgCl_2$, 1; NaH_2PO_4, 0.5; and Hepes buffer, 10. The perfusate was then switched to an enzyme-containing solution [2 mg of collagenase I (Sigma) and 0.3 mg of Protease XIV (Sigma) per milliliter, dissolved in the Ca^{2+}-free solution] for 25 minutes. The stomach was then washed free of the enzyme with a Tyrode's solution containing 0.2 m*M* Ca^{2+} and was placed in a petri dish. Cells were dispersed after the muscle layer was separated from the mucosa and were viewed through an inverted microscope on a video screen. Yields of nonbeating, spindle-shaped Ca^{2+}-tolerant cells were 30 to 60 percent.

Patching pipettes (tip diameter, 1 to 2 μm; tips not fire-polished) were pulled from WPI Kwik-fil capillaries (outside diameter, 1.5 mm). Pipette resistance ranged from 1 to 10 megohms, and seal resistances ranged from 10 to 15 gigaohms. A clamp amplifier (Dagan model 8900) fitted with a 100-megohm headstage was used for voltage and current clamp experiments. Series resistance compensation was used to the point of ringing. Experiments were done in 5 m*M* Ca^{2+}-containing Tyrode's solution at 35°C.

Gastric cells were typically 3 to 5 μm in diameter, but their length varied from 100 to 300 μm. The nucleus was ellipsoidal in shape (15 μm in length and 1 to 2 μm in width) and was located at the center of the cell. We chose only those cells that had smooth surfaces and were relaxed along their entire length.

Spontaneous activity could be recorded from some of the cells (Fig. 1A). The resting potential was generally unstable and irregular, fluctuating at about −30 mV. Repolarization was followed by a

pacemaker-type afterpotential, which at times generated a second action potential. The duration, amplitude, and morphology of the action potentials were similar to those recorded in multicellular preparations from guinea pig stomach (*2, 8*). The resting and overshoot potentials, however, deviated by about 20 mV toward more positive potentials in the isolated cells compared to the multicellular preparation (*2, 8*). This discrepancy probably arises from the junction potential caused by the presence of potassium gluconate in the pipette (*9*). Consistent with this idea, we found that in cardiac cells such pipette solutions resulted in an underestimation of the resting potential by 10 to 15 mV. These findings suggest that the resting, and therefore the overshoot, potentials of gastric myocytes are shifted positive by 10 to 15 mV. Thus, our results are in good agreement with those obtained in multicellular preparations.

Action potentials elicited by increasing stimulus current pulses (4 msec in duration, Fig. 1B) revealed that larger current pulses increased the peak of the action potential and enhanced the rate of repolarization, causing shorter action potentials. This observation was confirmed by the finding that activation of a rapid inward current with a clamp pulse to +10 mV was followed by a large outward current that gradually decayed within 2 seconds (Fig. 1C). Clamps to more positive potentials activated further outward current while decreasing the apparent peak inward current (Fig. 1D).

The relation between voltage and current measured at 6 msec (peak inward) showed that the inward current activated at −30 mV, had a maximum around +10 mV, and reversed at +40 mV (Fig. 2A). We identified the inward current as Ca^{2+} current primarily on the basis of its absence in Ca^{2+}-free solution; its reduction in Cd^{2+}-, Co^{2+}-, and nifedipine-containing solutions; and its voltage and time dependence. This current was relatively insensitive, however, to two other organic Ca^{2+} antagonists, D600 and diltiazem (1 μM). The difference in effectiveness of Ca^{2+} channel blockers in isolated myocytes is consistent with the observation that these agents vary in their effect on different types of smooth muscle (*10*). Among organic Ca^{2+} antagonists, nifedipine appears to be the most effective in suppressing phasic tension and the associated activation of Ca^{2+} current (*10*).

Because epinephrine increases the Ca^{2+} current in cardiac preparations, we examined its effects on gastric myocytes

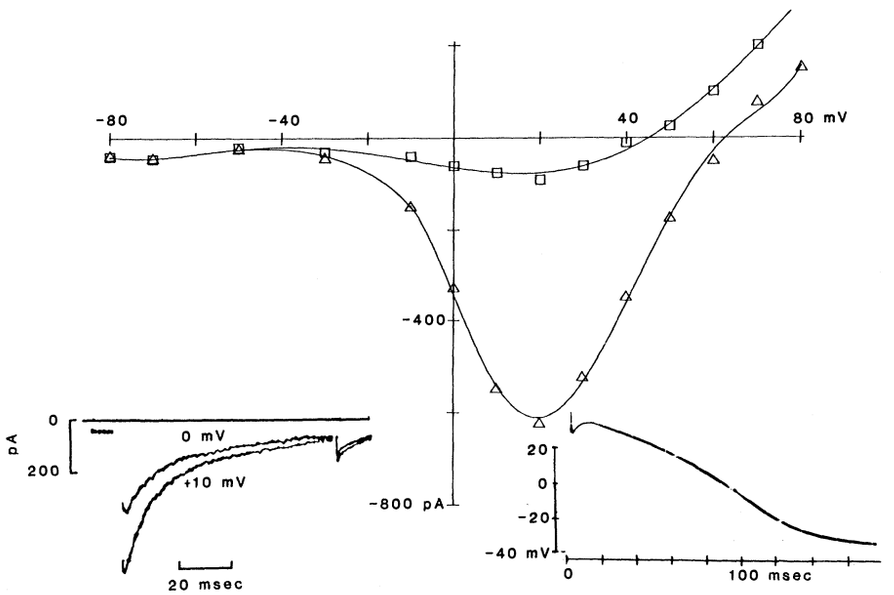


Fig. 3. Replacement of internal K^+ with Cs^+ in guinea pig gastric myocytes. Internal solution was as follows (in millimoles per liter): CsCl, 140; NaCl, 10; $MgCl_2$, 5; dipotassium adenosine triphosphate, 5; Hepes buffer, 10; $CaCl_2$, 1; and K_2EGTA, 11 (*p*H 7.20). The relation between current and voltage shows maintained inward current at 80 msec. (Insets) Two current traces to the indicated potentials and the action potential from the same cell. Holding potential, −80 mV. Symbols: (△) peak inward current and (□) inward current at 80 msec.

and found that it had no appreciable effect on the Ca^{2+} current at 1 μM. Although epinephrine has been reported to increase the K^+ conductance in taenia coli (*11*), we noted no change in either the holding current or the Ca^{2+} and K^+ currents in the corpus cells. We also found that acetylcholine (ACh) had no effect on these currents in the corpus cells, which is contradictory to the reported effect of ACh on the antral cells of guinea pig stomach (*2*). We considered the possibility that cell dialysis might mask any effect of ACh; however, addition of ACh ($10^{-6} M$) to intact isolated cells did not elicit contraction. In mixed populations of isolated guinea pig gastric myocytes, however, ACh has been reported to decrease the average cell length (*12*). In that study a wide span in the dose-response relation that could be attributed to the heterogeneous distribution of the ACh receptor in antrum, corpus, or fundus cells was also noted. The lack of response to ACh in our cells may be due in part to an alteration of the ACh receptor by the isolation procedure. However, as judged from the low sensitivity of the intact tissue to ACh and the variability of response of single cells (*2, 12*), further experimental evidence is required to substantiate the hypothesis of receptor modification.

In experiments where K^+ was the primary cation in the patch pipette, the transient outward current was one order of magnitude larger than the Ca^{2+} current. Ca^{2+}-free or Cd^{2+}-containing solutions not only blocked the Ca^{2+} current but also strongly suppressed the outward current (Fig. 2, B and C). These findings suggest that the peak outward current may be activated by Ca^{2+}. Since cytosolic Ca^{2+} was buffered with 10 mM EGTA ($[Ca^{2+}]_i$, $\leq 10^{-8} M$), it is likely that activation of this current by Ca^{2+} occurs either near the channel or at a site close to the inner surface of the membrane. The outward current remaining in the presence of Cd^{2+} was five to ten times smaller than the Ca^{2+}-activated K^+ current and is likely to be carried by K^+.

To examine the magnitude and duration of the Ca^{2+} current, we replaced most of the K^+ in the pipette with Cs^+ or *N*-methylglucamine. Under such conditions, the magnitude of the outward current, which was normally greater than 1 nA, was reduced to less than 0.2 nA at +80 mV (Fig. 3). Since most of the outward current was suppressed by Cs^+ (Fig. 3), the net membrane current remained inward for at least 80 msec (up to potentials of +40 mV). When K^+ was the intracellular cation, however, the current at 80 msec was always outward at potentials positive to −20 mV (Fig. 2B). The duration of the action potential was also markedly increased when K^+ was replaced by Cs^+ (Fig. 3, inset). Depolarizing current pulses produced action potentials lasting 200 msec compared to 50 msec in the absence of Cs^+ (Fig. 1B).

The steady-state relation between current and voltage showed no indication of inward rectification regardless of whether Ca^{2+} current was present or whether it was suppressed in the presence of Cd^{2+}. The membrane resistance of gastric myocytes, at potentials between −80 and −20 mV, was 1.5 to 2.0 gigaohms compared to 10 to 15 megohms in guinea pig cardiac myocytes, where the inwardly rectifying K^+ current is well developed (*13*). Hyperpolarizing conditioning clamp pulses also did not activate an inward Na^+ current or reveal a TTX-sensitive component of the Ca^{2+} current. We conclude, therefore, that gastric cells lack a TTX-sensitive Na^+ channel.

Our findings reveal Ca^{2+} and Ca^{2+}-activated K^+ channels in isolated single smooth muscle cells of the stomach. The Ca^{2+}-activated K^+ current was present even though Ca^{2+} was buffered with CaEGTA in the nanomolar range, which may indicate that the activation of this current occurs at a site that excludes EGTA. Ca^{2+} channels in mammalian gastric smooth muscle were effectively blocked by Cd^{2+} and Co^{2+} but appeared to be relatively insensitive to the Ca^{2+} antagonists D600 and diltiazem. This behavior of gastric Ca^{2+} channels is different from that in cardiac ventricular myocytes, in which organic and inorganic Ca^{2+} antagonists are equally effective in blocking the channel (*14*).

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U1 Small Nuclear RNA Genes Are Subject to Dosage Compensation in Mouse Cells

Abstract. *Multiple copies of a gene that encodes human U1 small nuclear RNA were introduced into mouse C127 cells with bovine papilloma virus as the vector. For some recombinant constructions, the human U1 gene copies were maintained extrachromosomally on the viral episome in an unrearranged fashion. The relative abundance of human and mouse U1 small nuclear RNA varied from one cell line to another, but in some lines human U1 RNA accounted for as much as one-third of the total U1. Regardless of the level of human U1 expression, the total amount of U1 RNA (both mouse and human) in each cell line was nearly the same relative to endogenous mouse 5S or U2 RNA. This result was obtained whether measurements were made of total cellular U1 or of only the U1 in small nuclear ribonucleoprotein particles that could be precipitated with antibody directed against the Sm antigen. The data suggest that the multigene families encoding mammalian U1 RNA are subject to some form of dosage compensation.*

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The U class of small nuclear RNA's (U snRNA's) is found in all eukaryotic cells and has been highly conserved during evolution. The U snRNA's range in size from 60 to 216 nucleotides, have a trimethylguanosine cap structure at the 5′ end (with the exception of U6), have highly modified nucleotides, and are not polyadenylated. Most are rich in uridine. The sensitivity of U1 and U2 synthesis to α-amanitin and to DRB (5, 6-dichloro-1-β-D-ribofuranosyl benzimidazole) suggests that these snRNA's are products of RNA polymerase II (*1*). Most, if not all, snRNA's are associated with seven or more proteins in the form of distinct small nuclear ribonucleoprotein particles (snRNP's) (*2*). The study of snRNP structure and function has advanced dramatically since the discovery that autoimmune antibodies in disorders such as systemic lupus erythematosus and polymyositis are often directed against specific snRNP proteins. The U1 snRNP recognizes the 5′ splice junction in mRNA precursors, and the other species of snRNP may also be important in nuclear RNA processing (*3*). The genes for the two most abundant snRNA species, U1 and U2, have been isolated from many eukaryotes including humans, rats, mice, chickens, frogs, fruit flies, and sea urchins (*4*). Pseudogenes are especially abundant in the large multigene families encoding human U1 and U2 RNA (*5*). The human U2 genes are organized in a large tandem array with a 6-kilobase (kb) repeat unit and are clustered at a single site on chromosome 17 (*6*). The organization of the U1 genes may be very similar (*6*). The upstream sequences that promote transcription of U1 and U2 snRNA by RNA polymerase II appear to be different from elements responsible for the initiation of most mRNA species (*7*).

To better understand the expression and regulation of human U1 genes in vivo, we cloned human U1 genes into a vector derived from bovine papilloma virus and introduced the recombinant constructions into C127 mouse cells (*8*). Lund *et al.* (*6*) has shown that human U1 genes can be expressed in mouse cells. Bovine papilloma virus (BPV-1), or a fragment (69 percent) of the viral genome, can transform various susceptible rodent cells that are nonpermissive for viral packaging. Transformed cell lines can be propagated indefinitely and contain multiple copies of the viral DNA as extrachromosomal circular molecules (*9*). When the BPV genome is fused with a pBR322-derived bacterial plasmid lacking the "poison" sequences that inhibit transformation of eukaryotic cells (*10*), the resulting hybrid replicon is capable of shuttling back and forth between bacterial and mammalian cells.

Two human U1 genes (HSD2 and HSD4) isolated by Manser and Gesteland (*4*) and two U2 full-length pseudogenes (U2-18 and U2-19) previously described (*5*) were inserted into the BPV vector 240-6 (Fig. 1). This vector is a derivative of vector 142-6 (*9*), containing the complete BPV genome inserted in a pBR322 derivative, pML2d (*10*). The DNA's were transfected into mouse C127 cells by the calcium phosphate precipitation method (*11*); 3 weeks later, transformed foci were picked, cloned, and thereafter maintained as cell lines. The copy number of the episomal BPV recombinants was determined by blotting appropriate restriction enzyme digests of low molecular weight extrachromosomal DNA (*12*) and was characteris-