control the entry into mitosis, and it seems likely that the ratio of their activity is altered by signals that influence the entry into mitosis (*1*). We described this control by a function F, which changed once per cycle, and checked the sensitivity of the period and amplitude of the oscillations to the modulation by F. To allow for a decreasing activity ratio of *cdc25* to *wee1*, we assumed that F decreased, once per cycle, starting from a value of one. Numerical computations, that included multiplying the right side of Eq. 1 alone, or Eqs. 1 and 2, by F, showed a gradual increase in phase, or cell cycle length. However, the pattern of change in MPF and cyclin phases and amplitudes depended on the modulated reaction. When only the rate of change in MPF concentration was modulated through the function F, the amplitudes of MPF oscillations progressively decreased and the amplitudes of cyclin oscillations increased (Fig. 2A), and the minima rapidly reached a value close to zero. The latter effect may be interpreted as a cell cycle arrest. When both processes were modulated, the amplitudes did not change (*12*), but the phase increased and tended to infinity when F tended to zero (Fig. 2B). In both cases the number of cell divisions until a cell cycle arrest was reached depended on the step size of the function F. On the basis of these results, we suggest that the modulation of the MPF and cyclin reaction rates may be responsible for the roughly constant number of cell divisions observed in some cell lineages. When the modulation was in the right side of Eq. 2 alone, or in each one of the accumulation terms alone, or in the degradation terms of each reaction alone, the effect on the increase in cell cycle duration appeared only marginal.

Erratic changes in cell cycle length were obtained when the function F was allowed to vary randomly, once per cycle, staying close to unity. Here too, if F modulated both reactions, the amplitudes of concentrations did not change, and the simulated cell line appeared "immortal" (Fig. 3). In contrast, if only the MPF reaction was modulated, cyclin concentrations occasionally reached zero.

The work presented here shows that the process in which cyclin accumulation activates MPF, which leads to cyclin degradation and hence in turn to MPF inactivation, suffices to explain multiple cell cycles. Our model relies on limited assumptions to yield robust oscillations in MPF and cyclin amounts. Time delays or thresholds postulated earlier were not required. The model mimics various aspects of the observed dynamics, such as cell cycle shortening after cyclin addition, or abrupt MPF inactivation. However, the possibility that other assumptions can generate a similar behavior should not be excluded. More information about the temporal pattern of change in the concentrations of these proteins may be instructive for verifying the role of MPF and cyclin in normal and neoplastic cell cycles.

Further experiments, in young, senescent, and neoplastic cell lines are warranted for quantifying the ratio of *cdc25* to *wee1* expression in subsequent cell divisions. These experiments may verify our prediction that senescence may be associated with a progressive decrease in the activity ratio of the genes that control the rate of change of the MPF and cyclin reactions, and that cancer cells are characterized by erratic changes in this ratio.

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9. The dimensional equations that describe the MPF and cyclin reactions are

$$\frac{dM'}{dt'} = e'C' + f'C'M'^2 - \frac{g'M'}{M' + h'} \quad \text{(A1)}$$

$$\frac{dC'}{dt'} = i' - j'M' \quad \text{(A2)}$$

It is convenient to introduce dimensionless variables. Thus we measure the MPF and cyclin concentrations as multiples of the half saturation concentration h' and measure time as a multiple of the inverse rate constant $1/j'$:

$$M = \frac{M'}{h'}, \; C = \frac{C'}{h'}, \; t = j't' \quad \text{(A3)}$$

Replacing Eq. A3 in Eqs. A1 and A2 and dividing by $j'h'$, we obtain Eqs. 1 and 2, where the dimensionless reaction coefficients become

$$e = \frac{e'}{j'}, \; f = \frac{f'h'^2}{j'}, \; g = \frac{g'}{j'h'}, \; i = \frac{i'}{j'h'} \quad \text{(A4)}$$

($e, f, g, i > 0$).
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12. The multiplication of both equations by the function F may account for a gradual increase in cell volume in senescent cell lines (*10*), which may slow the reactions. It can be shown analytically that multiplications of both equations by the same factor F essentially just modifies the time scale of events but does not alter the amplitudes of the oscillations.
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Cell Cycle–Dependent Coupling of the Calcitonin Receptor to Different G Proteins

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Calcitonin is a calcium regulating peptide hormone with binding sites in kidney and bone as well as in the central nervous system. The mechanisms of signal transduction by calcitonin receptors were studied in a pig kidney cell line where the hormone was found to regulate sodium pumps. Calcitonin receptors activated the cyclic adenosine monophosphate (cAMP) or the protein kinase C (PKC) pathways. The two transduction pathways required guanosine triphosphate (GTP)–binding proteins (G proteins) (the choleratoxin sensitive G_s and the pertussis toxin sensitive G_i, respectively) and led to opposite biological responses. Moreover, selective activation of one or the other pathway was cell cycle–dependent. Therefore, calcitonin may induce different biological responses in target cells depending on their positions in the cell cycle. Such a modulation of ligand-induced responses could be of importance in rapidly growing cell populations such as during embryogenesis, growth, and tumor formation.

CALCITONIN IS A 32–AMINO ACID peptide hormone of neural crest origin and is secreted by the parafollicular cells of the thyroid in response to elevations in serum calcium. In addition to its effects on bone and kidney to regulate calcium homeostasis (*1*), this hormone also has distinct binding sites in the central nervous system (*2*) where it exerts a modulatory role on nociception and food and water intake (*4*). Calcitonin also has a potent natriuretic effect on the kidney (*5*) and a rapid and pronounced effect on the osteoclast volume (*6*), a process that is known in

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other cells to involve changes in sodium and potassium transport (*7*). The main target cells are characterized by active ion transport and a high concentration of Na^+,K^+–adenosine triphosphatase (ATPase) in their membranes (*8*). Both calcitonin and inhibition of sodium pumps by ouabain decrease bone resorption in vitro (*9*). The cellular mechanisms by which calcitonin mediates its hypocalcemic effects in kidney and bone (*1*) or its antinociceptive and anorexic effects in brain (*4*) are not fully understood. Calcitonin induces an increase in cAMP in the osteoclast and the kidney tubule (*1*), but not in brain (*4*); in both cases, the effector molecules regulated by this hormone have not yet been identified.

To test whether the sodium pump is an effector molecule that mediates the biological effects of calcitonin, we measured the influence of this hormone on ouabain binding (*10*) in LLC-PK1 cells [clone 4 (*11*)], a kidney tubule cell line known to express both calcitonin receptors and Na^+,K^+-ATPase (*12*). When the cells were synchronized in their cell cycle (*13*) (Fig. 1, A and B), calcitonin had striking and opposite effects on sodium pump activity at different points in the cell cycle (Fig. 1C). During G2, incubation of LLC-PK1 cells in the presence of calcitonin (12 nM) for 30 min, led to a two- to threefold increase in ouabain binding. In contrast, a four- to fivefold decrease in ouabain binding was observed during S phase (Fig. 1C). Both effects were dependent on the dose of calcitonin ($P < 0.001$) (Fig. 1D) and were specific [parathyroid hormone and vasopressin had no effect (see Fig. 2C)]. In contrast, calcitonin gene related peptide (CGRP), a neuropeptide that binds to and activates calcitonin receptors, had effects similar to those produced by calcitonin (*14*). These results indicate that the sodium pump is affected by calcitonin and that the hormone has opposite effects on the activity of the pump in LLC-PK1 cells at two phases of the cell cycle.

To further elucidate the mechanisms underlying these reponses, we first considered the possibility that LLC-PK1 cells expressed two subtypes of calcitonin receptors, linked to different signal transduction pathways or effectors. Scatchard analysis of ^{125}I-labeled–calcitonin (*15*) (Fig. 1E) and ^{125}I-labeled–CGRP binding (*14*) failed to demonstrate two receptor classes at either phase of the cell cycle. The maximal binding values were almost identical at G2 and S phases (40 fmol versus 39 fmol per 10^5 cells; that is, 2.5×10^5 receptor sites per cell). However, we observed a slight but significant ($P < 0.01$) decrease in calcitonin affinity during S phase. The dissociation constant (K_d) increased from 1.5 to 3.1 nM (Fig. 1E). The differences in biological response and in binding affinity could be due to the existence of two receptor subtypes or to changes in the environment of the receptor. Because the Scatchard plots were linear and the change in affinity was only about twofold, we favor the second interpretation. Receptor subtypes usually have much larger differences in affinity (from 10- to 200-fold).

The next possibility we considered was that the same receptor led to the activation of two different signal transduction pathways depending on the phase of the cell cycle. Analysis of the signal transduction events that occurred during G2 indicated that intracellular cAMP increased sixfold upon incubation of LLC-PK1 cells with calcitonin (Fig. 1F). Consistent with the

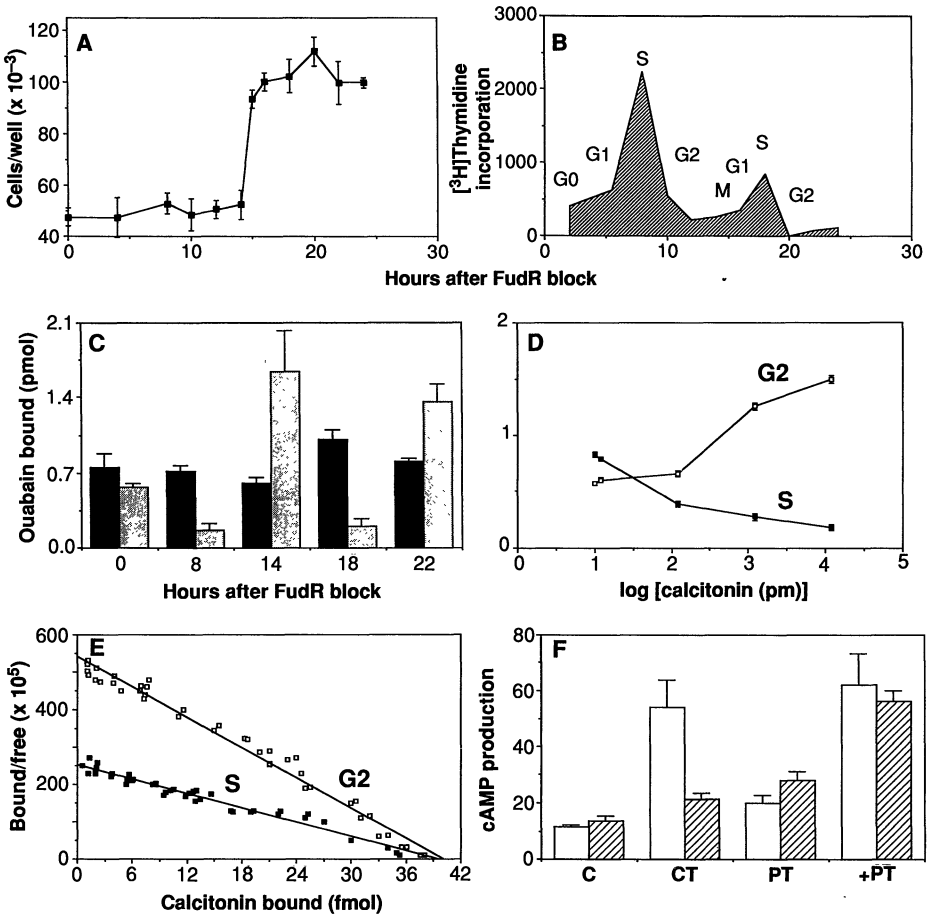

Fig. 1. Calcitonin effects on ouabain binding at G2 and S phases of the cell cycle in LLC-PK1 cells. (**A**) and (**B**) Cell cycle of LLC-PK1 (clone 4) cells. The cells were synchronized by incubation in the presence of the phase specific blocker 5-fluorouridine deoxyriboside [FudR] as described (*13*) (A) The position of the cells in the cell cycle was determined by cell count [Mean ± standard deviation (SD) of three experiments] and (B) [^{3}H]thymidine (15 Ci per mmol, Amersham) incorporation (cpm per 10^6 cells per hour) (2 μCi per well per 0.5 ml of media) (**C**) Effect of calcitonin (CT) on [^{3}H]ouabain binding at different phases of the cell cycle. [^{3}H]ouabain binding was measured as described (*13*). Black filled bars, −CT; dotted bars, +CT. Identical responses were observed at G2 and S phase of the first or second cycle (mean ± SD; for 0 hours, $n = 8$; 8 hours $n = 4$; 14 and 18 hours, $n = 12$; and 22 hours, $n = 4$). Similar effects were observed when the cells were synchronized according to two other methods (serum deprivation and vinblastin sulfate) even though the timing of the different phases of the cell cycle was different. (**D**) Dose dependency of CT action on [^{3}H]ouabain binding at G2 and S phase of the cell cycle. Measurement of [^{3}H]ouabain binding was carried out at the G2 and S phases in the presence of increasing concentrations of CT (from 40 pg/ml to 40 ng/ml); both the stimulatory effect at G2 and the inhibitory effect at S phases were dose dependent ($P < 0.001$) (mean ± SD of six observations). (**E**) Scatchard analysis of ^{125}I-labeled CT binding to LLC-PK1 cells at G2 and S phases. CT was ^{125}I-labeled as described (*14*). To determine nonspecific binding, control sets were incubated in presence of 1000-fold excess unlabeled CT along with ^{125}I-labeled CT. Each point represents the average of six independent measurements. (**F**) Effect of CT and pertussis toxin (PT) on intracellular cAMP (picomoles per milligram of total cell protein) at G2 and S phases. Cells at G2 (open bars) and S (hatched bars) phases were incubated in the absence (C) or presence (CT) of CT (12 nM final concentration) for 30 min and washed thoroughly in HBSS buffer (*13*). Cells were then extracted with 6% trichloroacetic acid and cAMP was measured (Amersham cAMP kit). No difference was observed whether the experiments were performed in the presence or absence of IBMX (3-isobutyl-1-methylxanthine) (1 mM). PT (50 ng/ml) was added alone (PT) or 10 min before CT (+PT) and was present during the additional 30 min incubation (mean ± SD from six experiments).

participation of cAMP in eliciting the G2 response were the findings that forskolin, dibutyril cAMP, isoproterenol, and cholera toxin (Fig. 2A) produced effects similar to calcitonin on ouabain binding. In contrast, pertussis toxin had no effect on the cells whether stimulated with calcitonin or not (Fig. 2A). The twofold increase in ouabain binding induced in LLC-PK1 cells by calcitonin during the G2 phase of their cell cycle may therefore be mediated by a cholera toxin–sensitive stimulatory G protein (G_s) that activates the cAMP pathway. Furthermore, this response was not affected by activators or inhibitors of PKC (Fig. 2B), suggesting that it did not participate in this signaling pathway. These observations are consistent with the classical concept that calcitonin receptors are coupled to adenylate cyclase (*1, 16*).

In contrast, incubation of LLC-PK1 cells with calcitonin during S not only induced the opposite effect (a decrease in ouabain binding), but the response involved a different signal transduction pathway (Fig. 3). Unlike the observations made in G2, calcitonin induced only a modest (1.5-fold) increase in cAMP during S (Fig. 1F), and the decrease in ouabain binding could not be mimicked by isoproterenol or forskolin (Fig. 3A). These results suggest that in S phase binding of calcitonin to its receptor was no longer activating G_s and the cAMP pathway. However, both forskolin and isoproterenol partially opposed the inhibitory effects of calcitonin on ouabain binding, thereby suggesting that, if generated, cAMP could still induce a response. In order to determine whether the lack of cAMP generation in S was due to the inhibitory G protein (G_i), the assay was performed in the presence of pertussis toxin. Addition of pertussis toxin to the assay modified the response to calcitonin; in pertussis toxin–treated cells, we observed a sixfold increase in cAMP (Fig. 1F) and a twofold increase in ouabain binding (Fig. 3C). These effects were dose-dependent (*14*). Inhibition of G_i in S phase therefore mimicked the G2 response. Further evidence that calcitonin receptors are coupled to the G_i protein during S phase was provided by the fact that addition of exogenous dibutyril cAMP above a threshold concentration of 100 to 200 nM also restored, in a dose dependent fashion, the G2 response (Fig. 3B), thereby demonstrating that the inhibition lies upstream of cAMP generation. The lack of cAMP and ouabain binding stimulation during S phase may therefore be due, at least in part, to activation of the inhibitory G_i protein, which may suppress the calcitonin-induced activation of adenylate cyclase. The association of G_i with the receptor might also explain the observed change in affinity during S phase (*17*).

Activation of G_i in S phase could explain the lack of cAMP response and, thereby, the lack of stimulation of the Na^+,K^+-ATPase.

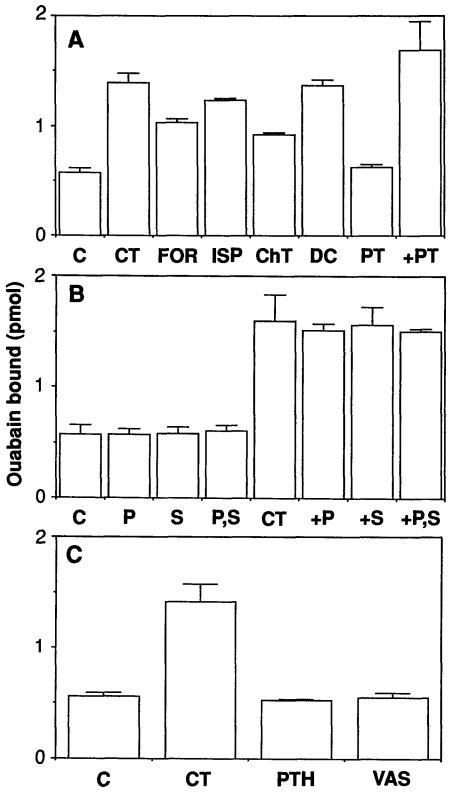

Fig. 2. Response to calcitonin in G2 is cAMP mediated, choleratoxin sensitive, and PKC independent. (**A**) Effects of different adenylate cyclase stimulating agents on [^{3}H]ouabain binding during G2. Synchronized cells in G2 phase were incubated for 30 min at 37°C with no additions (C, control), with calcitonin (CT) (12 nM), forskolin (FOR) (50 μM), isoproteronol (ISP) (1 μM), cholera toxin (ChT) (0.5 μg/ml), dibutyryl cAMP (DC) (0.2 mM), pertussis toxin (PT) (50 ng/ml), or CT and PT (+PT), and [^{3}H]ouabain binding was measured (mean ± SD of 3 experiments). (**B**) Effects of activators and inhibitors of PKC on [^{3}H]ouabain binding during G2. Synchronized cells in G2 phase were incubated as above (C, control) with CT (12 nM), phorbol ester (P) (phorbol 12, 13 dibutyrate, PdBu (10^{-6}M), or sphingosine (S) (10^{-5} M), individually or in combination, and [^{3}H]ouabain binding was measured (mean ± SD; for C and CT, n = 3; n = 6 for others). (**C**) Effects of parathyroid hormone (PTH) and vasopressin (VAS) on [^{3}H]ouabain binding during G2. Cells were incubated in G2 phase with PTH (4.2 nM), VAS (37 nM), and CT (12 nM), and ouabain binding measured (mean ± SD from three experiments).

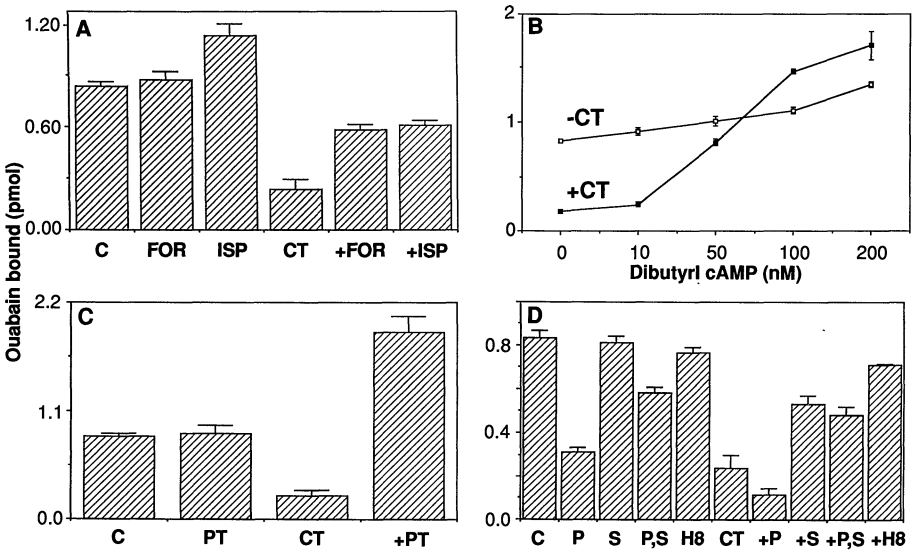

Fig. 3. Response to calcitonin in S phase is pertussis toxin sensitive, cAMP independent, and mediated by PKC. (**A**) Adenylate cyclase–stimulating agents increased [^{3}H]ouabain binding and inhibited calcitonin (CT) effects during S phase. Synchronized cells in S phase were incubated for 30 min at 37°C with FOR and ISP (as in Fig. 2A) and in the presence (+FOR, +ISP) or absence of CT, and [^{3}H]ouabain binding was measured (C, control) (mean ± SD; for C, n = 13; FOR, n = 8; ISP, n = 10; CT, n = 14; +FOR, n = 6; and +ISP n = 6). (**B**) Dose-dependent reversal of the CT effects on [^{3}H]ouabain binding during S by dibutyril cAMP. Cells in S phase were incubated with increasing concentrations of dibutyryl cAMP (0 to 200 nM) in the presence or absence of CT (12 nM), and [^{3}H]ouabain binding was measured (mean ± SD of six observations). (**C**) Inhibition of S phase–specific effects of CT by pertussis toxin. Cells in S phase were incubated under various conditions: C, control; PT, plus PT (50 ng/ml 40 min); CT, plus CT (12 nM, added 10 min after PT); or +PT, CT plus PT. [^{3}H]ouabain binding was measured (Mean ± SD of 3 experiments). (**D**) PKC activators mimic and inhibitors block the effects of CT on [^{3}H]ouabain binding during S phase. Synchronized cells in S phase were incubated with CT, phorbol ester (P), sphingosine (S) (same concentrations as in Fig. 2B), and *N*-[2-Methylaminoethyl]-5 isoquinoline sulfonamide dihydrochloride (H8), (Calbiochem, San Diego, California) (60 μM) individually or in combination under the same experimental conditions described in Fig. 1C for [^{3}H]ouabain binding. (C, control; + indicates that CT was also added) (mean ± SD; n = 12 for P,S,+S,+P,S; n = 13 for C; n = 6 for P,S; n = 4 for H8; n = 14 for CT).

However, this could not explain the calcitonin-induced fourfold decrease in ouabain binding. To test the hypothesis that this inhibitory response was the result of activation of the PKC pathway, we incubated LLC-PK1 cells during S phase with phorbol ester [phorbol 12,13-dibutyrate (PdBu)], an activator of PKC. A decrease in ouabain binding was observed (Fig. 3D) and was dose-dependent (*14*), confirming that the Na^+,K^+-ATPase can be inhibited by direct activation of PKC (*18*). This was further demonstrated by the fact that each of three inhibitors of PKC activity, sphingosine, H8, and staurosporine (*19*), prevented the effects of either calcitonin or PdBu on ouabain binding (Fig. 3D). In contrast, these agents had no influence on the responses observed in G2 (Fig. 2B), further establishing that the effects observed at that phase of the cell cycle were not a result of activation of the PKC pathway. Finally, PKC activity was measured by incorporation of ^{32}P and demonstrated a pertussis toxin–sensitive five- to sixfold increase upon binding of calcitonin, confirming the participation of PKC and G_i in the response to calcitonin in S.

In order to further establish that the measured effects of calcitonin on ouabain binding at G2 and S involved G proteins, we studied the effects of GTP and GDP (guanosine diphosphate) analogs on calcitonin-induced transduction mechanisms in semipermeabilized cells (*20*). As expected when GTP binding proteins are involved, GTP(γ)S augmented and GDP(β)S blocked the effects of calcitonin in both G2 and S, whether the response was an increase or a decrease in ouabain binding [G2 : control = 0.45 ± 0.03, +calcitonin (CT) = 1.14 ± 0.07, + GTP(γ)S = 1.33 ± 0.13, + GDP(β)S = 0.47 ± 0.04; S: control = 0.71 ± 0.06, + CT = 0.29 ± 0.04, + GTP(γ)S = 0.25 ± 0.02, + GDP(β)S = 0.68 ± 0.06 pmol of ouabain bound per 10^5 cells, mean ± standard deviation of five experiments]. These results, together with the cholera-toxin and pertussis toxin sensitivities, establish the fact that G proteins, most likely G_s and G_i, respectively, are involved in the response to CT at both G2 and S phases of the cell cycle.

Our results therefore suggest that the calcitonin receptor can be coupled to different G proteins, G_s, G_i, and possibly Gp (a G protein coupled to PKC) in a cell cycle dependent fashion. Although numerous receptors for peptide hormones are coupled to multiple G proteins that activate multiple signal transduction pathways (*21, 22*), our observations are unique in two respects. First, the coupling of the calcitonin receptor to one pathway led to opposite biological effects from that induced by the activation of the other. Second, the factors that determined the predominance of one or the other pathway are linked to events taking place during the cell cycle.

Regarding the first point, it is known that different hormones, when bound to different receptors, can utilize multiple signaling pathways to induce opposite responses in the same cells. Epinephrine and somatostatin use cAMP-independent pathways to alter intracellular pH in opposite directions in enteric endocrine cells (*21, 23*). The β adrenergic response is cAMP independent and pertussis toxin–insensitive, while the somatostatin response, also cAMP independent, involves a pertussin toxin–sensitive G_i. This latter configuration is similar to what we observed for calcitonin during the S phase of the cell cycle. Although it is not possible to entirely rule out the possibility of two receptor subtypes that are similar in number and affinity for calcitonin, the uniqueness of our observation is that the same hormone can use alternate pathways to induce opposite biological responses in the same target cells.

The basis for cell cycle–regulated coupling of different G proteins to the calcitonin receptor (or expression of different receptor subtypes) remains to be determined. Regulation of the cell cycle involves a number of proteins that control entry into S or M phase (*24*). Of particular interest is the fact that these cell cycle regulating proteins are protein kinases (p34-cdc2) and proteins that regulate the activity of these kinases (cyclins). In addition to protein kinases, protein phosphatases and their specific inhibitors also vary in a cell cycle–specific manner (*25*). Because phosphorylation and dephosphorylation are an essential part of regulation of the cell cycle, signal transduction, and other biological responses, we speculate that these cell cycle–related changes in phosphorylating and dephosphorylating enzyme activities could affect the signal transduction pathways and the biological responses associated with the activation of receptors.

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1.2 mM NaH_2PO_4, 10 mM glucose, 20 mM Hepes and 0.5% fatty acid–free BSA, pH 7.4) and were permeabilized for 6 min by addition of 20 μM digitonin in potassium glutamate (139 mM), ATP (2 mM), $MgCl_2$ (2 mM) EGTA (5 mM), 10.5% fatty acid–free BSA, Pipes (20 mM), pH 6.5 in the presence or absence of the analogs GTP(γ)S (100 μM) or GDP(β)S (750 mM). The cells were then washed twice in HBSS buffer (pH 7.4) containing 0.2% BSA and incubated with or without calcitonin for 30 min before measuring ouabain binding as described (*13*).

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Molecular Transfer of a Species-Specific Behavior from *Drosophila simulans* to *Drosophila melanogaster*

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***Drosophila* males modulate the interpulse intervals produced during their courtship songs. These song cycles, which are altered by mutations in the clock gene *period*, exhibit a species-specific variation that facilitates mating. We have used chimeric *period* gene constructs from *Drosophila melanogaster* and *Drosophila simulans* in germline transformation experiments to map the genetic control of their song rhythm difference to a small segment of the amino acid encoding information within this gene.**

THE MOLECULAR AND GENETIC analysis of species-specific behavior may enhance the understanding of the evolution of closely related species. Species-specific acoustic components of the courtship behavior of drosophilids provide an excellent model system for the study of molecular ethology.

The courtship song of *Drosophila* is generated by extension and vibration of the males' wings. The most prominent feature of these sounds in the *D. melanogaster* subgroup is intermittent bursts of tone pulses and hums (*1–4*). The pulses carry information that enhances the females' mating behavior (*2, 3*). Species-specific pulse information can be conveyed to the female in at least two ways. One way involves the interpulse interval (IPI), which averages about 30 to 40 ms in *D. melanogaster* and 45 to 55 ms in *D. simulans* (*1, 4*). The second way is the modulation of the mean IPI, which occurs with periods ranging from 50 to 65 s in *D. melanogaster* and 30 to 40 s in *D. simulans* (*3, 5*).

The *period* gene (*per*) in *D. melanogaster* influences several temporally dependent phenotypes (*6*). The gene was first defined by the effects of *per* mutations on circadian rhythms. Mutant alleles shorten (per^S), lengthen (per^{L1}), or abolish (per^{01}) the circadian rhythms of eclosion and locomotor activity (*7*). These mutations were shown to have parallel effects on the IPI modulation rhythm in the courtship song: per^S males sing with 35- to 45-s periods, per^{L1}, 75- to 95-s periods, and per^{01} males appear to be arrhythmic (*3, 5, 8–10*).

Behavioral studies of *D. simulans–D. melanogaster* interspecies hybrids have mapped the species difference in song rhythm periodicity to the X chromosome (*3*), on which *per* is located (*7*). Furthermore, deletion of the repeated Thr-Gly–encoding region within *per* by in vitro mutagenesis (*11, 12*) results in a *D. simulans*–like shortening of the song rhythm in *D. melanogaster* transformants (*13*). These findings suggest circumstantially that *per* may be responsible for the species-specific components of the courtship song rhythms.

We tested the hypothesis that *per* contains species-specific song instructions by introducing a cloned copy of *D. simulans per* into the genome of a *D. melanogaster* per^{01} mutant. DNA from the *per* locus of *D. simulans* (Kenscoff strain) was cloned (*14*) (see Fig. 1A), and a P-element construct was generated (*15*) that included a *D. simulans*–derived genomic fragment corresponding to the 13.2-kb *D. melanogaster per* DNA used in the restoration of rhythms to *D. melanogaster* flies carrying per^{01} (*12, 13, 16, 17*).

Because the Thr-Gly repeat of *per* in *D. melanogaster* is implicated in control of song rhythmicity, and because this region of the gene is notable for its interspecific variability (*18, 19*), further transformation experiments focused on the vicinity of the Thr-Gly repeat in *D. simulans* and *D. melanogaster*. Thus, the transformations involved four different *per* constructs (*15*): the 13.2-kb fragment from *D. melanogaster* [13.2m-TGm (*12, 13*)], the corresponding *D. simulans* fragment (13.2s-TGs), the chimeric construct with the *D. melanogaster* Sst I–Bam HI fragment [carrying the Thr-Gly repeat (Fig. 1A)] substituted into *D. simulans per* (13.2s-TGm), and the reciprocal chimeric construct with the *D. simulans* Sst I–Bam HI material inserted within the flanking regions of *D. melanogaster per* (13.2m-TGs).

The biological activity of the transduced *per* constructs was confirmed by testing the locomotor-activity circadian rhythms of the recipient flies. The 13.2s-TGs *D. simulans per* and the two chimeric *per* genes efficiently rescued per^{01}-associated arrhythmicity (*20*). Resultant circadian periods (about 24.5 to 25 hours) were comparable to those obtained by transduction of per^{01} with the *D. melanogaster* 13.2m-TGm gene (*12, 13, 16, 17*). Therefore, the 13.2s-TGs fragment and the two chimeric constructs can provide basic *per* function. In this context we also noted that *D. simulans* wild-type adults showed activity rhythms indistinguishable from those of wild-type *D. melanogaster* (*20*).

Courtship songs were recorded and analyzed blind (*21, 22*) for males of each of the four transformant types. The mean song cycle of the 13.2m-TGm transformant males (Table 1, Experiment 1, and Fig. 2), gave the expected *D. melanogaster* periods of about 55 s (*3, 5, 9, 10*). A dramatic effect

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