tor present at days 9 and 11. However, this seems unlikely since nuclear receptor increases in this interval whereas the ratio of apo-II to VTG-II responsiveness is the same at days 9 and 11. In addition, we have not detected significant differences in the sensitivity of the apo-II and VTG-II genes to low doses of exogenous estrogens in 2-week-old chicks (*9*).

A further consideration is that acquisition of hormone responsiveness may be associated with modifications in gene or chromatin structure that do not take place in coordinate fashion for all estrogen responsive genes. For example, the methylation status of regions surrounding the apo-II gene changes between days 7 and 9 of development (*10*). Such changes could reflect the transition of the apo-II gene to the responsive state. In contrast, the VTG-II gene remains fully methylated before the administration of hormone and shows only minor changes in methylation status even in the adult (*11, 12*). The VTG-II gene shows some evidence of developmental change as judged by tissue-specific nuclease hypersensitive sites present at day 9 (*12*). However, since these changes also occur in the oviduct, they are not sufficient for estrogen inducibility of the VTG-II gene. These results may indicate that hormone responsiveness for each gene is acquired in a series of independent events. The comparison of apo-II and VTG-II responsiveness (Fig. 2) also shows that genes that are of common function and coordinately regulated in the adult may be on independent developmental programs during embryogenesis.

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Calcium Source for Excitation-Contraction Coupling in Myocardium of Nonhibernating and Hibernating Chipmunks

Abstract. *The amplitude of the early plateau phase of the action potential and the slow action potential of cardiac muscle were much lower in hibernating chipmunks than in nonhibernating chipmunks. The frequency-dependent contraction was decreased in hibernating animals but increased in nonhibernating animals. Caffeine caused a negative inotropic effect in hibernating animals but a positive inotropic effect in nonhibernating animals. Ryanodine caused greater inhibition in hibernating animals than in nonhibernating animals. These results suggest that the respective roles of the sources of calcium for cardiac excitation-contraction coupling are changed during hibernation.*

The hearts of hibernating animals, in vivo and in vitro, continue to function under temperature conditions (below 5°C) at which the hearts of nonhibernating animals cease to function (*1*). Changes in the electrocardiogram and in myocardial performance that occur in animals during hibernation, such as prolongation of various waves and intervals or a marked reduction in heart rate, have been characterized (*2*). However, the physiological mechanism responsible for changes in myocardial performance during hibernation is not well defined. We used microelectrode techniques to investigate the electromechanical characteristics of isolated papillary muscle from hibernating and nonhibernating chipmunks. Our results suggest that hibernation causes marked changes in some fundamental characteristics of cardiac muscle function in this species.

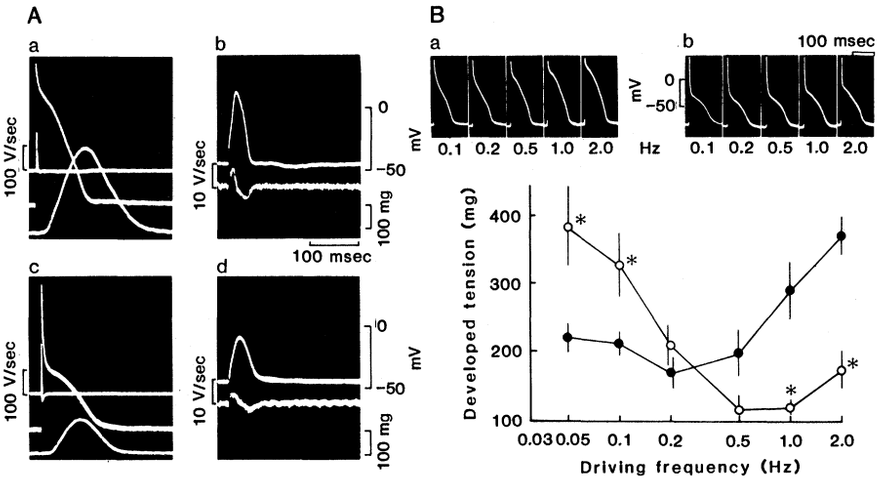

Fig. 1. (A) Transmembrane action potentials and slow action potentials in preparations from nonhibernating (a and b) and hibernating (c and d) animals. Both preparations in normal medium (a and c) and in the high K^+ medium (b and d) were driven at 1.0 Hz and 0.5 Hz, respectively. The first derivative of the action potential is shown in the top trace in (a) and (c) and in the lower trace in (b) and (d). A membrane action potential is shown by the middle trace in (a) and (c) and by the upper trace in (b) and (d). The bottom trace in (a) and (c) shows a developed tension. (B) Frequency-dependent changes in the membrane action potential (a and b) and contractile activity (graph) on preparations from nonhibernating [(a) and (●) on graph] and hibernating animals [(b) and (○) on graph]. The driving frequency is shown under each panel. Values plotted on the graph represent the mean ± standard error of the mean of six preparations. The asterisk indicates that the difference between the preparations from hibernating and nonhibernating animals is significant. The probabilities were adjusted by Bonferroni type statement.

Table 1. The transmembrane action potential was measured in isolated papillary muscles from hibernating and nonhibernating animals. Values are means ± standard error of the mean. Abbreviations: *n*, number of preparations; APA, action potential amplitude; MDP, maximum diastolic potential; dV/dt_{max}, maximum upstroke velocity of action potential; APD 30 and APD 80, action potential duration from the upstroke to 30 percent and 80 percent repolarization, respectively.

	n	APA (mV)	MDP (mV)	dV/dt_{max} (V/sec)	APD 30† (msec)	APD 80 (msec)
Nonhibernating	6	102 ± 2.6	78.9 ± 0.7	151 ± 5.6	27.8 ± 5.6	78.3 ± 5.6
Hibernating	10	107 ± 3.3	77.8 ± 1.3	186 ± 11*	4.4 ± 0.6*	71.1 ± 5.0

*Significant difference between the two preparations at $P < 0.05$. †The Welch procedure was used for statistical analysis in the APD 30 data.

Asian chipmunks (*Tamias sibiricus*) of either sex were killed by a blow on the head. The heart was quickly excised, and a papillary muscle was isolated from the right ventricle. All preparations were fixed and equilibrated for 2 hours in a tissue bath superfused with aerated (95 percent O_2 and 5 percent CO_2) Krebs-Ringer solution. The composition of the Krebs-Ringer solution in millimoles per liter was: NaCl, 120; KCl, 4.8; $CaCl_2$, 1.2; $MgSO_4 \cdot 7H_2O$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 24.2; and glucose, 5.5 (*p*H 7.4). In some experiments the K^+ concentration of the superfusate was raised to 26 m*M* by substituting KCl for NaCl on an equimolar basis to inactivate the fast Na^+ channels. The temperature of the superfusate was maintained at 30°C. Isometric tension was measured with a force displacement transducer. The preparations were constantly stimulated at 1 Hz with pulses 1 msec in duration and twice the diastolic threshold, unless otherwise specified. In the high K^+ solution, a frequency of 0.5 Hz and voltage five times that of the diastolic threshold in normal solution were applied to elicit the slow action potential. Membrane action potentials were recorded through glass microelectrodes filled with 3*M* KCl. The action potential, the first derivative of the action potential, and the mechanical tension were displayed simultaneously on a storage oscilloscope (Tektronix 7613). The mechanical tension was also recorded on a polygraph (Nihon Kohden TB612T). The action potential and contraction were not affected by several hours of incubation at 30°C. Student's *t*-test was used for statistical analysis, and significance was established at $P < 0.05$.

The amplitude of the early plateau phase of the action potential of isolated papillary muscles from hibernating animals was much less than that from nonhibernating animals (Fig. 1A). No statistically significant difference in action potential duration (APD) at the late phase of repolarization (APD 80) was observed between the two preparations (Table 1). In mammalian ventricular muscle, activation of the slow inward current is of prime importance in the genesis of the early plateau phase of the action potential (*3, 4*). The preparations from nonhibernating and hibernating animals were depolarized to −45.7 ± 0.9 mV and −45.0 ± 1.7 mV ($n = 4$), respectively, by increasing the extracellular potassium concentration (26 m*M*) to inactivate the fast Na^+ channels (Fig. 1A). Under this condition, the slow action potential induced by strong electrical stimulation as a measure of the slow inward current was much less in preparations from hibernating animals (33.3 ± 1.3 mV in amplitude and 3.2 ± 0.2 V/sec in dV/dt_{max}; $n = 4$) than in preparations from nonhibernating animals (55.3 ± 2.2 mV in amplitude and 8.7 ± 1.4 V/sec in dV/dt_{max}; $n = 4$). The depression in the amplitude of the action potential plateau in hibernating animal preparations may be attributed to the reduced activation of the slow inward current resulting in less calcium influx across the cell membrane. The force-frequency relation was also different in the two preparations (Fig. 1B). The stepwise increase in the driving frequency (0.05 to 2.0 Hz) increased the cardiac contractile force of the nonhibernating animals but reduced that of the hibernating animals. This difference in the force-frequency relation in the two preparations is similar to that seen in the ventricular muscles of the rat as opposed to that occurring in these muscles in other species. In rat ventricular muscle, which shows a frequency-dependent decrease in the contractile force, intracellularly derived calcium plays a major role in activation of contraction (*5, 6*). In the ventricular muscle of other species (guinea pig and rabbit), a frequency-dependent increase in contraction may ultimately be attributed to an increased transsarcolemmal calcium influx (*5, 6*). Therefore, in the hibernating animal, the difference in the force-frequency relation may be explained by the change in the respective roles of intra- and extracellularly derived calcium for activation of contraction. Furthermore, the frequency-dependent change in the cardiac contractile force of nonhibernating animals was closely correlated with the change in the early plateau phase of the action potential; this was not the case in the hibernating animals (Fig. 1B). These results suggest that, in hibernating animals, intracellularly derived calcium may play a more important role in contraction than does transsarcolemmal calcium influx during the action potential. However, the smaller amount of calcium influx across the cell membrane may be involved in the hibernating animal preparations in that the lower calcium influx is sufficient to trigger the release of intracellular calcium.

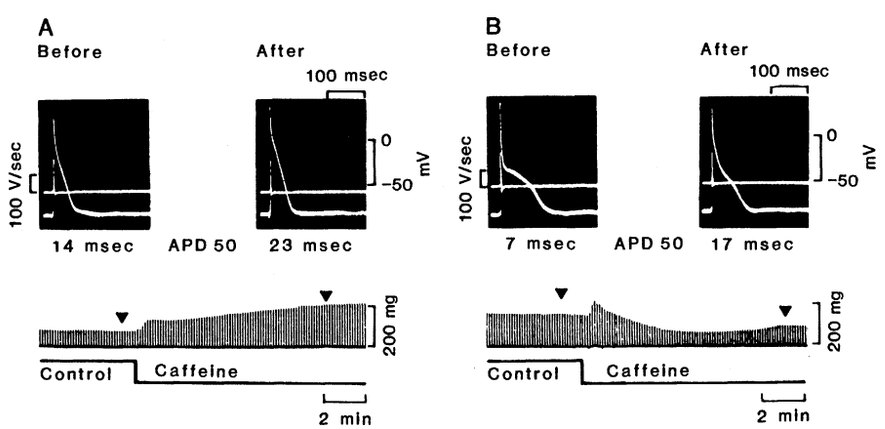

Fig. 2. Electromechanical effects of caffeine on preparations from hibernating and nonhibernating animals. Both preparations were driven at 0.2 Hz. (A) Nonhibernating animals. (B) Hibernating animals. "Before," control; "After," after addition of caffeine (5 m*M*). Arrowheads indicate recording points of a membrane action potential.

In hibernating animals, the maximum contractile force of ventricular muscle was obtained at the lowest driving frequency (0.05 Hz), an indication that this is favorable for hibernating animals which have slow heart beats. In addition, dV/dt_{max} was significantly greater in hibernating animals than in nonhibernating animals (Table 1); the physiological significance of this difference is unclear.

Caffeine augments calcium influx during the action potential and simultaneously reduces the contribution of intracellular calcium stores to myocardial contraction (*6, 7*). Treatment with caffeine (5 m*M*) caused the positive inotropic effect (210 ± 38 percent of control; $n = 5$) with an increase in APD 50 in nonhibernating animal preparations driven by low frequency (0.2 Hz), whereas it caused the negative inotropic effect in hibernating animals (56.5 ± 6.5 percent of control; $n = 5$) in spite of an increase in APD 50 (Fig. 2, A and B). In addition, ryanodine (2×10^{-6} *M*), an inhibitor of the release of calcium from sarcoplasmic reticulum (*8*), abolished the developed tension of ventricular muscle from the hibernating animals but partly inhibited cardiac contraction in the nonhibernating animal preparation (55.8 ± 4.6 percent of control; $n = 6$). These differences in the effects of caffeine and ryanodine on the two preparations further suggest that intracellularly derived calcium makes a greater contribution to the activation of contraction in the myocardium of hibernating animals.

In summary, the electrical and mechanical characteristics of heart muscle are different in hibernating and nonhibernating animals; hibernation may cause a change in the excitation-contraction coupling mechanisms of myocardium. The possible mechanism of contraction during hibernation by which a major part of activator calcium is intracellularly derived is considered to be suitable for effective myocardial function at low temperatures. One might ask whether this change is a direct or an indirect result of hibernation. However, since the intracellular *p*H of the heart is not changed during hibernation (*9*), and prolonged cold exposure does not affect the electrical characteristics of mammalian myocardium (*10*), some possibilities for an indirect influence of hibernation can be eliminated. Although the precise explanation of this mechanism is not clear, it is interesting that in one animal myocardial properties can be exchanged in accordance with environmental changes. These findings can provide a model in which the physiology of the heart or drug effects on different calcium pools can be studied in otherwise similar preparations and may open a new pathway to understanding the mechanism of cold tolerance in hibernating animal myocardium.

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An Activated *ras*^{N} Gene: Detected in Late But Not Early Passage Human PA1 Teratocarcinoma Cells

Abstract. *Early passages of the human teratocarcinoma cell line PA1 are not tumorigenic in nude mice, while late passages are. A transforming gene present in late passages of PA1 cells was isolated as a biologically active molecular clone and is a new isolate of the human* ras^N *locus. Its transforming activity is due to a single* $G \rightarrow A$ *(G, guanine; A, adenine) point mutation at the codon for amino acid 12 which changes the codon for glycine so that an aspartic acid residue is expressed. In contrast to late passage PA1 cells (passages 106, 330, and 338), DNA from the PA1 cell line at early passages (passage 36) does not yield* ras^N *foci in DNA transfection assays. Thus, the presence of an activated* ras^N *in PA1 cells correlates with enhanced tumorigenicity of the cell line and, more importantly, may have arisen during cell culture in vitro.*

Experiments with viral DNA preparations in which gene transfer techniques were used (*1*) were the first to demonstrate DNA-mediated cell transformation in cultures of animal cells. Shih *et al.* (*2*) used this methodology to show that DNA preparations from transformed mouse cells could transfer that phenotype to recipient cultures of NIH 3T3 mouse cells. These findings have led investigators to invoke a genetic theory for cell transformation based on one or more genetic changes, giving rise to a transforming gene that can be assayed in the DNA-mediated transformation of NIH 3T3 cells. It may be that these transforming genes are involved in the multistep progression which ultimately gives rise to the tumorigenic phenotype.

Transforming genes have been detected in the NIH 3T3 cell assay in DNA preparations from various tumors and tumor cell lines (*3*). A number of the transforming genes molecularly cloned from human tumors and tumor cell lines have been shown to be cellular homologs of the oncogenes of the Harvey (*ras*^{H}) and Kirsten (*ras*^{K}) sarcoma viruses (*4–9*). An additional *ras*-related cellular genetic locus, *ras*^{N}, has been identified by transfer to NIH 3T3 cells with the use of DNA from the SK-N-SH neuroblastoma cell line (*10*). Uniformly, the difference responsible for activation of *ras* genes between normal or tumor sources has been found to be point mutations resulting in coding sequence changes for the *ras* protein (p21) (*11–13*).

We have described (*14*) the detection of a transforming gene in DNA isolated from a human teratocarcinoma cell line, PA1 (*15*), at passage 330. The cell line was derived from the culturing of ascites fluid cells from a patient with a metastatic ovarian germ-line tumor and has properties of embryonal carcinoma cell lines. The cells are capable of differentiation in culture under adherent or nonadherent conditions. The cell line shows significant alterations in various malignancy-related phenotypes with extended passage in culture (*15*). Early passage PA1 cells grow slowly in culture and form tumors in athymic nude mice only after