transformed with the *prp2*$^+$ gene. These preliminary results indicate that the human U2AF^{65} gene functionally complements the mutant yeast *prp2.1* strain.

U2AF is composed of two polypeptides of 65 and 35 kD. The U2AF 65-kD polypeptide is the active component that binds the characteristic long polypyrimidine stretch between the branchsite sequence and the 3′ end of mammalian introns (*17*). In contrast, the distance between the branchsite and the 3′ end of the intron is short in *S. pombe* (*18*). Some fission yeast introns have short stretches of pyrimidines between the branchsite and the 3′ splice site, whereas others have a balanced purine and pyrimidine ratio. Generally, fission yeast introns are very pyrimidine-rich, with more U-runs present at the 5′ end of the intron as compared with the 3′ end of the intron (*19*). Identification of a homolog of U2AF^{65} in fission yeast suggests at least two possibilities: (i) either U2AF^{65} in fission yeast does not need to interact with a polypyrimidine stretch or it interacts with a polypyrimidine stretch at the 5′ end of the intron, or (ii) U2AF^{65} is essential for the splicing of some fission yeast introns (those containing polypyrimidine stretches) and not others (introns with a balanced purine-pyrimidine ratio). Perhaps fission yeast splicing could be regulated by the differential need for U2AF. It should now be possible to differentiate between these possibilities by studying U2AF genetically in *S. pombe*.

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4 June 1993; accepted 16 August 1993

Potentiation by the β Subunit of the Ratio of the Ionic Current to the Charge Movement in the Cardiac Calcium Channel

Alan Neely,* Xiangyang Wei,† Riccardo Olcese, Lutz Birnbaumer, Enrico Stefani

The voltage-activated rabbit cardiac calcium channel α_1 subunit was expressed in *Xenopus* oocytes. The charge movement of its voltage sensor was measured and related to the opening of the ion-conducting pore. The half-activation potential for charge movement was 35 millivolts more negative than that for pore opening. Coexpression of the cardiac calcium channel β subunit reduced this difference without affecting charge movement. Thus, intramolecular coupling between the voltage sensor and the channel pore opening can be facilitated by a regulatory subunit.

Voltage-activated ion channels are membrane proteins with at least two functional domains: a pore through which ions flow and charged structures that serve as voltage sensors. The understanding of the molecular events coupling the voltage sensor to the pore opening requires not only measurements of ion fluxes through the pore (ionic currents) but also measurements of current associated with the movement of the voltage sensor (gating currents). One difficulty in assessing gating currents is the coexistence of multiple types of ion channels, each with its own set of voltage sensors. In some specialized cells, one particular channel type may be dominant, but even then contamination by other channels restricts the analysis to certain voltages. For example, in studies of Ca^{2+} channel gating currents in cardiac cells, Na^+ channels contribute nearly half the total gating charge (*1*) and voltage protocols designed to immobilize Na^+ channel gating current also alter the voltage dependence and kinetics of charges thought to come from Ca^{2+} channels (*2*). Molecular cloning of channels and expression in *Xenopus* oocytes is one solution to this problem, provided that voltage-clamped currents can be measured within the time immediately before channel opening in which the movement of the voltage sensor occurs. A technique, referred to as the cut-open oocyte voltage clamp (COVG), was developed to measure both the gating and the ionic currents of cloned K^+ channels (*3*, *4*). For these channels, the voltage-sensing structures were found to move with high cooperativity for channel opening (*5*).

We investigated whether the COVG

A. Neely, X. Wei, R. Olcese, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.
L. Birnbaumer, Departments of Cell Biology, Medicine (Endocrinology), and Molecular Physiology and Biophysics, and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.
E. Stefani, Department of Molecular Physiology and Biophysics and Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

*To whom correspondence should be addressed.
†Present address: Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA 30912.

technique could also be applied to more complex channels such as voltage-gated Ca^{2+} channels. These channels appear to exist in the membrane as heteromultimeric proteins composed of a channel-forming subunit (α_1) and up to three more subunits, termed β, γ, and $\alpha_2\delta$, that are thought to have regulatory functions (6). The α_1 subunit has four domains or repeats, each resembling one of the K^+ channel monomers (7). Using an improved COVG technique (8), we were able to measure gating currents of the rabbit cardiac Ca^{2+} channel α_1 subunit ($\alpha_{1C\text{-}a}$) (9, 10). Our results indicate that the stimulatory activity of β subunits (β_{2a}) (9, 11, 12) results from events that occur after most of the voltage sensor charge has moved and before the actual opening of the pore.

We recorded currents in 10 mM external Ba^{2+} (13) from uninjected oocytes (Fig. 1A) and oocytes injected either with α_1 complementary RNA (cRNA) alone (Fig. 1B) or with both α_1 and β cRNAs (Fig. 1C). The α_1 cRNA induced not only inward Ba^{2+} currents but also transient outward currents at the beginning of the depolarizing steps. When ionic currents were blocked by replacing 10 mM Ba^{2+} with 2 mM Co^{2+}, transient currents were recorded at both the beginning and the end of the depolarizing pulses (Fig. 2). These transient currents have the properties of gating currents: (i) they did not reverse with membrane potential (Fig. 2A); (ii) they always followed the direction of the voltage step (Fig. 2A); (iii) the integral of these currents, or charge moved, approached limiting values at both positive (+40 mV) and negative (−90 mV) potentials (Fig. 2B); and (iv) the charge moved by a voltage step (Q_{on}) matched that moved when returning to the holding potential (Q_{off}) (Fig. 2C). Also, the voltage dependence of the charge movement followed that predicted by a simple Boltzmann distribution (Fig. 2B). Under our recording conditions (14), these gating currents were observed only in oocytes injected with α_1 or with α_1 plus β subunits; we therefore conclude that these gating currents pertain to the movement of charged elements encoded in the ion channel protein.

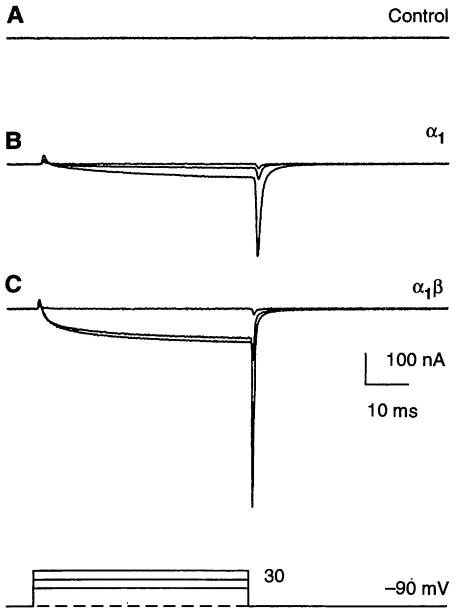

Fig. 1. Ionic and gating currents induced by cardiac α_1 subunits and potentiation by the cardiac isoform of the β subunit. Voltage clamp traces were recorded during 50-ms depolarizing steps to −30, 0, and +30 mV from a holding potential of −90 mV recorded in an extracellular solution containing 10 mM Ba^{2+}. (**A**) Uninjected oocyte. (**B**) Oocyte injected with cRNA encoding the α_1 subunit. (**C**) Oocyte coinjected with cRNA encoding the α_1 and β subunits. Traces shown are averages of 4, 10, and 7 runs for traces in (A), (B), and (C), respectively. Currents were filtered at 1 kHz and digitized at 10 kHz.

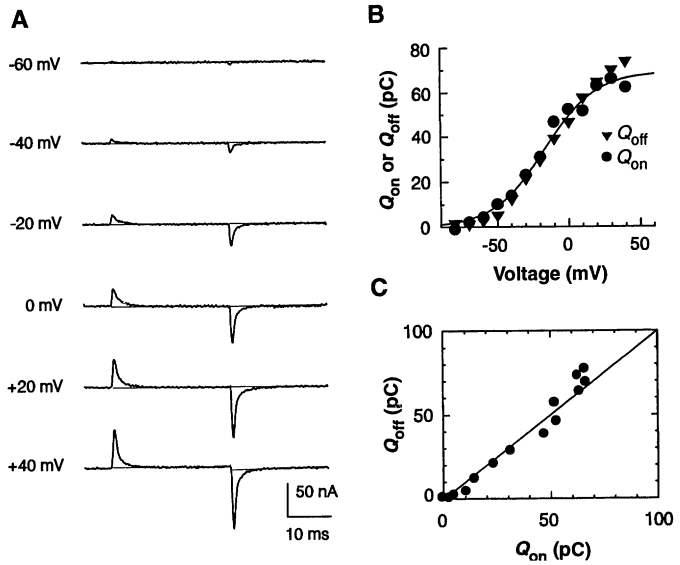

Fig. 2. Gating currents from an oocyte coexpressing both α_1 and β subunits recorded in an extracellular solution containing 2 mM Co^{2+}. (**A**) Voltage clamp traces during 25-ms depolarizing steps to the values shown on the left. Each trace is the average of 14 runs sampled at 10 kHz and filtered at 4 kHz. (**B**) Values for Q_{on} and Q_{off} versus voltage plots obtained by integrating the gating currents during and after the pulse after baseline correction [straight lines in (A)]. The data points of the last 10 ms before the end of the pulse and of the final 10 ms in the recorded trace after the end of the pulse were used to obtain the baseline. Q_{on} was fitted by a single Boltzmann distribution of the form

$$Q_{on} = Q_{max}/\{1 + \exp[z_Q F(V_{1/2Q} - V_m)/RT]\}$$

where Q_{max} = 2.3 pC/nF; $V_{1/2Q}$ = 16.7 mV; z_Q = 1.36 [F is the Faraday constant; R, the gas constant; T, the absolute temperature (295 K); V_m, the pulse potential; $V_{1/2Q}$, the half activation potential; and z_Q, the effective valence for the charge movement]. (**C**) A Q_{on} versus Q_{off} plot shows charge conservation. A straight line with a slope of 1 is shown as a reference. Regression between Q_{on} and Q_{off} gives a slope of 0.99, with a regression coefficient of 0.99.

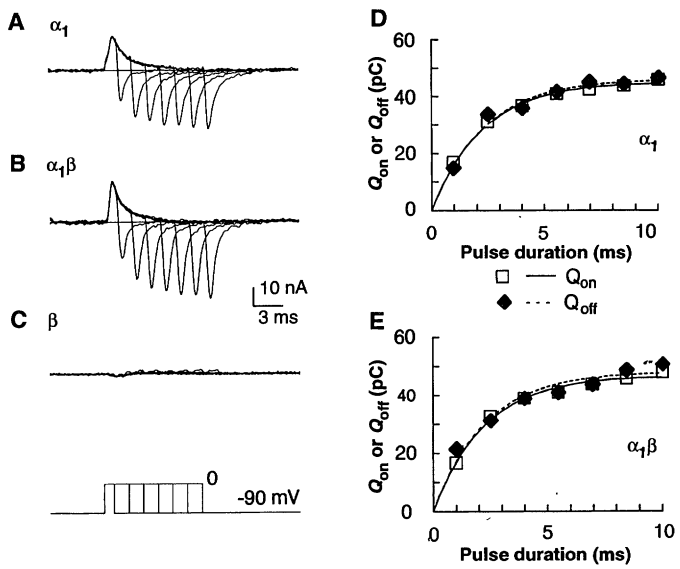

Fig. 3. Gating currents from three oocytes expressing α_1 (**A**), or α_1 plus β (**B**), or β (**C**) subunits recorded in 2 mM external Co^{2+} during depolarizing steps of variable duration to 0 mV from a holding potential of −90 mV. Charge movements were measured by integrating the gating currents during (Q_{on}) and after (Q_{off}) each pulse and plotted versus pulse duration. (**D** and **E**) The lines are single exponential fits to Q_{on} (continuous lines) and Q_{off} (dashed lines) with the following parameters: in (D), Q_{on} = 44.9 pC [1 − exp(−t/2.24 ms)]; and Q_{off} = 46.0 pC [1 − exp(−t/2.31 ms)]; and in (E), Q_{on} = 46.8 pC [1 − exp(−t/2.24 ms)]; and Q_{off} = 49.2 pC [1 − exp(−t/2.26 ms)].

Table 1. Average of best-fit parameters describing the Boltzmann distributions for *G-V* and *Q-V* curves. The same data set was used for Fig. 4. Variables are defined in Figs. 2 and 4.

Channel	G_{max} (nS/nF)*	$V_{1/2Q}$ (mV)*	z_G*	Q_{max} (pC/nF)	$V_{1/2Q}$ (mV)	z_Q	G_{max}/Q_{max} (nS/pC)
α_1	31 ± 9	15.1 ± 2.4	2.8 ± 0.1	1.42 ± 0.34	−19.8 ± 3.7	1.5 ± 0.1	22.1
$\alpha_1\beta$	168 ± 30	−1.5 ± 0.7	3.4 ± 0.1	1.78 ± 0.23	−18.1 ± 3.1	1.7 ± 0.1	94.5†

*$P < 0.01$. †Cardiac α_1 subunits expressed in myotubules of dysgenic mice had G_{max}/Q_{max} values in the same range (*20*).

Charge conservation ($Q_{on} = Q_{off}$) was studied further in experiments in which the duration of the voltage steps was increased (Fig. 3). Values for Q_{on} and Q_{off} were plotted against pulse duration and fitted by a single exponential function. The time constants and amplitudes obtained from these fits were virtually identical for Q_{on} and Q_{off} (Fig. 3, D and E). Therefore, expressed Ca^{2+} channels do not undergo charge immobilization as seen with rapidly inactivating Na^+ channels (*15*) and in transient K^+ channels (*3*). Coexpression of the cardiac β subunit with the α_1 subunit did not result in any change in the time course of charge movement despite the fact that the Ba^{2+} currents were larger and faster than in oocytes injected with the α_1 subunit alone. For the oocytes used for Fig. 3, at 0 mV the half time to peak for the ionic currents was 9.6 ms for the oocyte expressing α_1 and 3.3 ms for the oocyte expressing both the α_1 and β subunits. The fact that coexpression of the β subunit did not change the charge movement while it increased the rate of channel opening indicates that the closed-to-open transitions modulated by the β subunit are relatively uncharged and must occur after most of the charge has moved. Oocytes injected with the β subunit alone did not have detectable gating currents (Fig. 3C).

In oocytes that express α_1 alone or α_1 plus β, the charge-voltage (*Q-V*) curves started at more negative potentials than the conductance-voltage (*G-V*) curves and the effective valence for the voltage dependence of ionic conductance was approximately two times that for charge movement. Thus, expressed Ca^{2+} channels go through multiple closed states before opening, and at least two voltage-sensing elements or gates control the activation. Our ability to measure directly the amount of charge associated with the channel protein allowed us to evaluate to what extent potentiation of ionic current by the β subunit might be due to an increase in the channel probability of opening as opposed to an increase in expression or assembly of channels. We obtained a relative estimate of the charge density by integrating outward transients over the first 2 ms of voltage steps from −90 to −30 mV (*16*) and found no statistically significant difference between oocytes injected either with α_1 alone or with α_1 plus β [0.22 ± 0.02 pC/nF (n = 17) and 0.24 ± 0.02 pC/nF (n = 30), respectively; mean ± SEM]. Similarly, the values for the charge movement measured at saturating potentials were similar in oocytes injected with either α_1 or α_1 plus β subunits (see Table 1). In contrast, inward currents were increased severalfold by the β subunit (at +30 mV, −0.9 ± 0.1 nA/nF for α_1 and −3.8 ± 0.5 nA/nF for α_1 plus β) (*17*). Thus, potentiation of ionic currents by the β subunit is likely a result of an increase in the open probability of the channel. This conclusion agrees with the finding that the β subunit increased the maximum conductance (G_{max}) and shifted the *G-V* curves by 16 mV toward more negative potentials without any changes in the *Q-V* curves (Fig. 4 and Table 1).

The fact that the increase in G_{max}, the negative shift in the *G-V* curve, and the acceleration of the macroscopic current occurred without modifications in the charge movement suggests that transitions related to the movement of the voltage sensor are not modulated by the β subunit and that an energy barrier for the opening of the pore is significantly reduced by the interaction between the β and α_1 subunits. It remains to be explained how a cytoplasmic protein can modulate the interaction between intramembrane domains of the channel protein such as the voltage sensor and the pore region.

Our studies stand in apparent contrast to previous studies in which coexpression of the β subunit with α_1 in L cells increased the number of high-affinity dihydropyridine (DHP) binding sites by four- to fivefold (*11*, *18*). Increased binding could have been due to either an increase of α_1 protein expression or to a β-induced shift in the equilibrium of α_1 between two conformations, one with low (experimentally undetectable) affinity for DHP, the other with high affinity for DHP. Nishimura *et al.* (*19*) tested the effect of the β subunit on the expression of α_1 by immunoblotting and failed to detect β-induced increases in α_1. These findings support the idea that the β subunit regulates the equilibrium between two DHP-binding states of α_1 and are in agreement with our findings that the β subunit does not significantly alter expression of α_1.

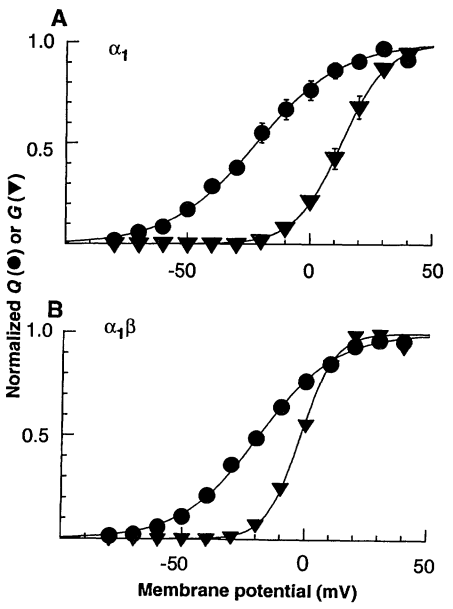

Fig. 4. Voltage dependence of charge movement (*Q*) and membrane conductance (*G*) in oocytes expressing α_1 (**A**) or α_1 plus β (**B**) subunits. For charge versus voltage plots (*Q*), gating currents were recorded in 2 mM external Co^{2+}, and charge movement was measured as described in Fig. 2. Data from individual oocytes were fitted by

$$Q_{on} = Q_{max}/\{1 + \exp[z_Q F(V_{1/2Q} - V_m)/RT]\}$$

and normalized by Q_{max}. The average values after normalization from nine oocytes injected with α_1 cRNA and from 14 oocytes injected with α_1 plus β messages are shown as filled circles in (A) and (B), respectively. For values of *G*, inward currents (I_m) were evoked by 125-ms depolarization steps, filtered at 0.5 kHz and sampled at 4 kHz, and measured at the end of the pulse. For individual oocytes (19 with α_1 alone and 23 with α_1 plus β), normalized membrane conductances were calculated from the fit

$$I_m = G_{max}(V_m - E_{rev})/\{1 + \exp[z_G F(V_{1/2G} - V_m)/RT]\}$$

where E_{rev} is the current reversal potential; $V_{1/2G}$, the conductance half activation potential; and z_G, the effective valence. Continuous lines show Boltzmann distributions that best fitted the averaged data. Error bars correspond to the standard error of the mean; if not visible, they are within the symbol. Variables in equations are as in Fig. 2.

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8. Speed and space clamps were improved by actively clamping the guard compartment. The signal-to-noise ratio was enhanced with the use of low-resistance microelectrodes (0.1 to 0.5 megohm) and inclusion of a high resistor (10 megohms) in the feedback loop in the current-voltage converter. The series resistance that originated from the distance between the tip of the voltage-recording microelectrode and the interior of the upper oocyte membrane was 0.5 to 1 kilohm and thus had negligible influence (<1 mV) on the clamp voltage because the membrane currents were smaller than 200 nA.
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14. In some cases, we observed asymmetric charge movement in uninjected oocytes. This movement was blocked by removal of external Na^+ or by addition of ouabain. This appears to be the charge movement associated with the activity of the Na^+, K^+ adenosine triphosphatase described by R. F. Rakowsky [*J. Gen. Physiol.* **101**, 117 (1993)]. To avoid any potential contamination in our records, we included 100 μM ouabain in all external solutions.
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17. Calcium currents arising from an endogenous α_1-like subunit whose activity can be stimulated in oocytes injected with only the β subunit could be differentiated from Ca^{2+} currents induced by the cardiac α_1 plus β subunits. Currents induced by β cRNA alone had faster kinetics and were insensitive to DHPs. Oocytes injected with cRNA encoding both cardiac α_1 and β subunits were blocked at a holding potential of −20 mV by (+) PN200-100 (2 μM), nifedipine (10 μM), and nisoldipine (1 μM).
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2 June 1993; accepted 26 August 1993

Abnormal Behavior Associated with a Point Mutation in the Structural Gene for Monoamine Oxidase A

H. G. Brunner,* M. Nelen, X. O. Breakefield, H. H. Ropers, B. A. van Oost

Genetic and metabolic studies have been done on a large kindred in which several males are affected by a syndrome of borderline mental retardation and abnormal behavior. The types of behavior that occurred include impulsive aggression, arson, attempted rape, and exhibitionism. Analysis of 24-hour urine samples indicated markedly disturbed monoamine metabolism. This syndrome was associated with a complete and selective deficiency of enzymatic activity of monoamine oxidase A (MAOA). In each of five affected males, a point mutation was identified in the eighth exon of the MAOA structural gene, which changes a glutamine to a termination codon. Thus, isolated complete MAOA deficiency in this family is associated with a recognizable behavioral phenotype that includes disturbed regulation of impulsive aggression.

Studies of aggressive behavior in animals and humans have implicated altered metabolism of serotonin (*1–7*), and to a lesser extent dopamine (*4, 8, 9*), and noradrenaline (*3–5, 10–12*). These observations suggest that genetic defects in the metabolism of these neurotransmitters may affect aggressive behavior, but such mutations have not yet been reported.

We have described a large kindred in which several males are affected by a syndrome of borderline mental retardation and exhibit abnormal behavior, including disturbed regulation of impulsive aggression (*13*). Obligate female carriers in this family have normal intelligence and behavior. The genetic defect for this condition was assigned to the p11-p21 region of the X chromosome, in the vicinity of the genes for MAOA and monoamine oxidase B (MAOB). Because MAOA and MAOB are known to metabolize serotonin, dopamine, and noradrenaline, we evaluated these patients for MAO deficiency. The MAOB activity is normal in affected males from this family (*13*).

To test the hypothesis that affected males in this family have selective MAOA deficiency, we established skin fibroblast cultures from three clinically affected males, two carrier females, and one noncarrier female (*14*). Cultured human skin fibroblasts from normal individuals express both MAOA and MAOB activity in a ratio of about 80 to 90% to 10 to 20%, respectively, and the amounts of activity are stable from passage to passage during the proliferative growth phase (*15*). Treatment of fibroblast cultures with dexamethasone produces a 6- to 14-fold increase in MAOA activity and a 2- to 3-fold increase in MAOB activity (*16*). We assessed MAO activity in homogenates from skin fibroblast strains with a common substrate, tryptamine, at a concentration that favors MAOA mea-

H. G. Brunner, Department of Human Genetics, University Hospital Nijmegen, Geert Grooteplein 20, 6525 GA Nijmegen, the Netherlands.
M. Nelen, H. H. Ropers, B. A. van Oost, Department of Human Genetics, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands.
X. O. Breakefield, Neuroscience Center, Massachusetts General Hospital, Charlestown, MA 02129.

*To whom correspondence should be addressed.