brief communication

Tandem linkage of *Shaker* K^+ channel subunits does not ensure the stoichiometry of expressed channels

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ABSTRACT *Shaker* K^+ channels are multimeric, probably tetrameric proteins. Substitution of a conserved leucine residue to valine (V2) at position 370 in the *Drosophila Shaker* 29-4 sequence results in large alterations in the voltage dependence of gating in the expressed channels. In order to determine the effects of this mutation in hybrid channels with a fixed stoichiometry of V2 and wild-type (WT) subunits we generated cDNA constructs of two linked-monomeric subunits similar to the tandem constructs previously reported by Isacoff, E. Y., Y. N. Jan, and L. Y. Jan. (1990. *Nature (Lond.)*. 345:530–534). In addition, we constructed a tandem cDNA containing a wild-type subunit and a truncated nonfunctional subunit (Sh^{102}) that suppresses channel expression. We report that the voltage-dependence of the channels produced with WT and V2 subunits varied significantly with the order of the subunits in the construct (WT-V2 or V2-WT), while the WT-Sh^{102} construct yielded currents that were much larger than expected. These results suggest that the tandem linkage of *Shaker* subunits does not guarantee the stoichiometry of the expressed channel proteins.

INTRODUCTION

Most K^+ channel genes that have been isolated belong to the *Shaker* family of voltage-gated channels. The characterization of these channels has generally utilized the ability of *Xenopus* oocytes to express channels in the plasma membrane upon injection of RNA transcripts. Channels with mixed properties have been observed from the coinjection of cRNAs from different *Shaker* (*Sh*) genes or alternative splice products (1–4), implying that these channels are multimeric, and probably tetrameric (5), as would be expected by analogy to the pseudo-tetrameric structure of voltage-gated Na^+ and Ca^{++} channels.

The coinjection of different RNA species is a means of generating channels with a mixed population of subunits, but it does not constrain the subunit stoichiometry. In an alternative approach, Isacoff et al. (2) demonstrated that functional channels arise from fusion proteins translated from constructs containing two *Sh* cDNAs that differ in their inactivation properties. Since the amino and carboxy termini are thought to be cytoplasmic (6), it was assumed that linked subunits would necessarily assemble into the same tetrameric complex, and thus, constrain the stoichiometry of the expressed channels. The resulting channels did show intermediate properties consistent with the formation of hybrid complexes, but the uniformity of the subunit stoichiometry was not specifically assayed. Tandem constructs of potassium channel subunits have subsequently been used to study the binding site for channel blockers (7, 8) and the gating mechanism (9) of potassium channels.

The mutation L370V (V2) in *Drosophila Sh* 29-4 channels substitutes valine for a conserved leucine residue near the end of the S4 domain. It shifts and alters the voltage sensitivity of activation (10), without altering the total amount of charge moved during the conformational transitions leading to channel opening (11). We wished to further investigate the way in which this mutation perturbs channel gating by constructing heterooligomeric channels incorporating an identical number of wild-type and V2 subunits. We generated tandem constructs similar to those of Isacoff et al. (2), but using the *Sh* 29-4 cDNA (12) and linking the last COOH-terminal residue of the first repeat to the first NH_2-terminal residue of the second with the decapeptide sequence Gly-$(Gln)_7$-Ser-Gly. As controls for the symmetrical assembly and stoichiometry of the resulting oligomers, we generated tandems of wild-type (WT) and mutant subunits in both orientations, WT-V2 and V2-WT, as well as generating the constructs WT-WT and V2-V2. As a further control, we constructed a tandem (WT-102) incorporating the truncated subunit Sh^{102} in the second position. The 102 subunit is intact from the amino-terminus through the first five putative transmembrane domains but replaces Trp-422 with a stop codon (13, 14) in the H5 segment (putative pore region; 15–17). The 102 cRNA suppresses the expression of K^+ currents from coinjected WT RNA (10) presumably by combining with wild-type subunits to form nonfunctional complexes. We report here the properties of the channels expressed from these constructs, which suggest that our tandem constructs do not form tetrameric channel complexes with the expected stoichiometries.

METHODS

The construction and properties of the WT, V2 and 102 *Sh* subunits have been previously described (10). These cDNAs, in the Bluescript vector (Stratagene, Inc., La Jolla, CA), were altered near the 5′ start site or the 3′ translation stop site through the use of polymerase chain reaction (PCR). cDNAs coding for the first subunit of the tandem were

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altered on the 3′ end by introduction of the linker sequence, which replaced the stop codon of 29-4. This linker consisted of a translational reading frame coding for the amino acid sequence Gly-$(Gln)_7$-Ser-Gly as well as BspE I and Kpn I restriction nuclease sites. PCR amplification using an antisense oligo containing the linker sequence and the last eight translated codons, and a sense oligo which overlapped the Spe I site (nucleotide 1,747) were used to join the linker region to the 3′ coding region of the *Sh* cDNAs. The PCR-generated fragments were digested with Spe I and Kpn I and cloned into the corresponding sites of 29-4 and V2 cDNAs in Bluescript. Glycine residues were placed at the ends of the linker to introduce flexibility and the Ser was introduced to obtain a Bspe I restriction site for the splicing together of subunit cDNAs.

For the second position of the tandem constructs, 29-4, V2 and 102 cDNAs were altered at the 5′ end. PCR amplification between a sense oligonucleotide coding for Sac I and Bspe I restriction sites and the first seven amino acids of 29-4, and an antisense oligo that overlapped the Nar I restriction site (nucleotide 366) generated a fragment that was digested with Nar I and Sac I and cloned into the corresponding sites of 29-4, V2, and 102.

The sequences of all the PCR-generated constructs were verified through DNA sequence analysis with the Sequenase kit (United States Biochemical Corp., Cleveland, OH). The 5′ and 3′ modified cDNAs were then digested with Bspe I and Kpn I and ligated into the tandem constructs which were mapped by restriction enzyme digestion to insure proper insertion and orientation. The tandem constructs generated in this manner were WT-WT, WT-V2, WT-102, V2-WT, and V2-V2. RNA transcription reactions, autoradiography, gel electrophoresis and *Xenopus* oocyte injection were performed as described (12) except that all cDNAs were linearized with Kpn I and the 3′ overhang was filled in using 0.16 units/μl T4 DNA polymerase and 80 μM dNTPs. RNA size standards were obtained from GIBCO BRL (Gaithersburg, MD).

Two-microelectrode voltage clamp recordings were obtained 4–15 d after injection with an OC-725 voltage clamp (Warner Instrument Co., New Haven, CT) and the AQUIRE program (Instrutech Corp., Mineola, NY). Data were filtered at 2 KHz and leak subtracted using the P/4 protocol, with a subtraction holding potential of −120 mV.

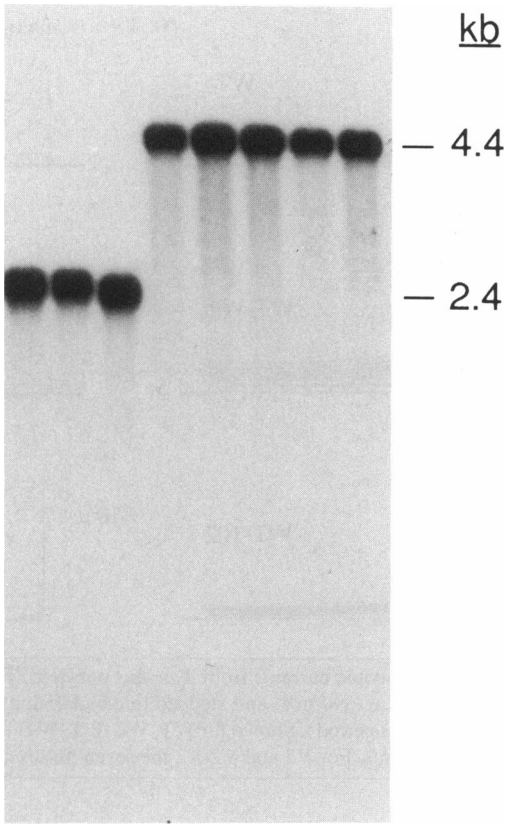

FIGURE 1 Autoradiograph of RNA transcripts. ^{32}P labeled, in vitro transcribed, RNA samples were denatured at 60°C for 15 min in 50% formamide before electrophoresis. The first three lanes (*left to right*) contain samples of in vitro transcribed 102, V2 and WT RNAs (2.5 kb) while the next five lanes contain similar examples of WT-102, WT-WT, WT-V2, V2-WT and V2-V2 RNAs, respectively (4.5 kb). The position of the RNA size standards (in kilobases) are indicated.

RESULTS

The cDNA tandem constructs when mapped with restriction enzymes are found to be of the appropriate size and orientation, and the resulting RNA transcripts are also of the expected size (Fig. 1). Injection of the RNA into *Xenopus* oocytes results in large outward A-type currents (Fig. 2), which activate at depolarizing potentials and subsequently inactivate in a double-exponential process, as was previously seen with 29-4 channels (12). Also as described previously, V2 currents retain a larger steady-state current at depolarizing potentials, although the inactivation rates are similar to wild type after taking into account the voltage shift of activation (10).

The currents produced by the tandem constructs, with the exception of WT-102, inactivate more slowly and have larger sustained currents at the end of 100 ms depolarizing pulses than currents from the corresponding monomeric constructs. A comparison of the time constants of inactivation for the WT and WT-WT currents at high potentials (Table 1) shows that the tandem linkage increases the shorter time constant τ_1 from 5 ms to ~8 ms. In addition, the relative amplitude of the fast inactivating component, A_1, is decreased in the linked channels relative to the monomeric channels. These effects on the rate and extent of inactivation might be expected since the NH_2-terminus has been shown to be an important determinant in the fast inactivation process of *Sh* channels (12, 18, 19) and the NH_2-terminal region of the second subunit is probably constrained through its covalent link to the first subunit. The alterations in inactivation kinetics are thus consistent with the idea that at least some of the subunits in the second position of the tandem are incorporated into functional channels and that the covalently-linked subunits might be incorporated into the same channel.

The voltage dependences of peak conductance and prepulse inactivation are shown in Fig. 3. Both WT-WT and V2-V2 constructs produce currents with voltage dependences very similar to those of the respective monomeric subunits. Thus, the linkage of the subunits does not appear to affect the voltage dependence of activation or inactivation. The currents produced by WT-V2 and V2-WT constructs have voltage dependences intermediate between WT and V2 currents, like those seen when monomeric WT and V2 cRNAs are coinjected (3). The

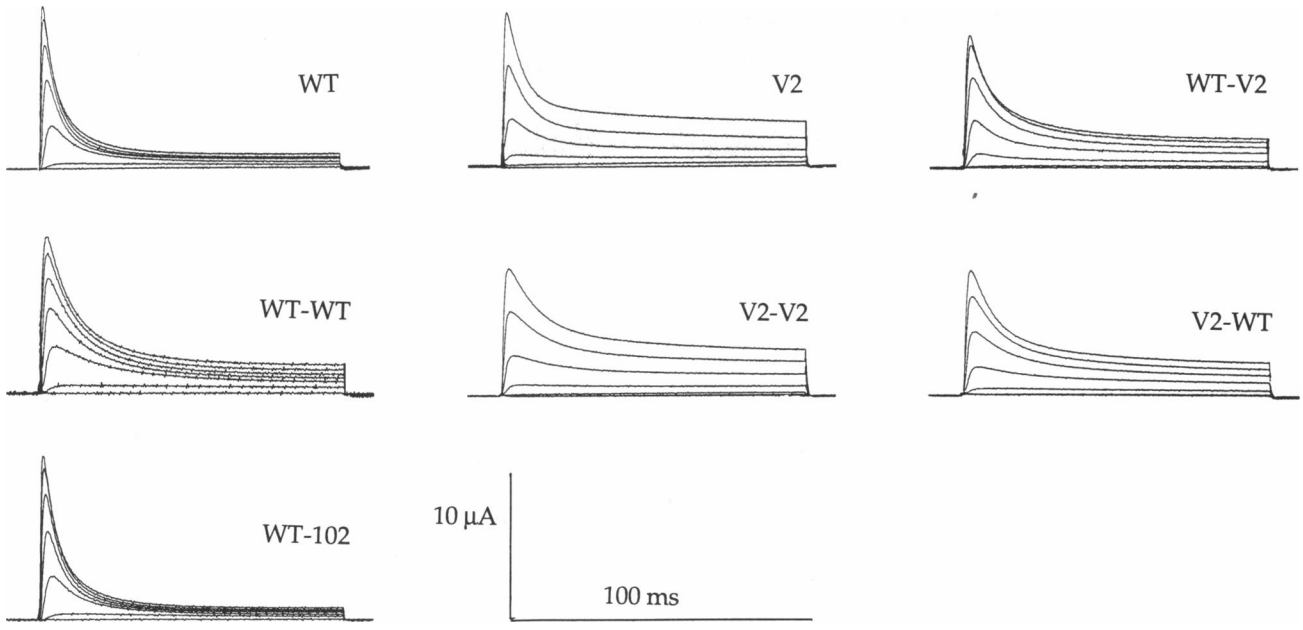

FIGURE 2 Macroscopic currents from *Xenopus* oocytes injected with the indicated cRNA as determined by two microelectrode voltage clamp. Oocytes were held at −90 mV, and stepped to depolarizing potentials for 100 ms. The interval between pulses was 7 s (all currents showed full recovery within this period). Shown for WT, WT-WT, WT-102, WT-V2, and V2-WT channels are seven depolarizing pulses from −50 to +70 mV in 20 mV increments. For V2 and V2-V2 the seven pulses ranged from −30 to +90 mV in 20 mV increments.

intermediate voltage dependence is consistent with the idea that linked subunits are incorporated into the same functional channels. However, in both the peak conductance and inactivation protocols the voltage dependence of the two kinds of hybrid channels differ significantly. The voltage dependence of WT-V2 channels is closer to that of WT channels, while V2-WT channels are more similar to V2 channels. The differences suggest that the expressed channels have a broken symmetry, or a different stoichiometry than would be expected for the association of pairs of heterodimers to form channels. Consistent with the idea that the stoichiometry may differ are results from experiments in which unequal amounts of V2 and WT RNA were injected: the resulting currents had voltage dependences closer to that of the subunit RNA injected in larger amounts (data not shown, cf. reference 3).

The WT-102 tandem construct gives rise to currents whose kinetics and voltage dependence are indistinguishable from WT channels (Fig. 1, Table 1). The amplitude of the current is not dramatically suppressed, as would be expected if the 102 subunits were assembled stoichiometrically with WT subunits to produce defective channels. The current from these tandems is approximately half that found for WT-WT channels: the average peak current at 60 mV for WT-WT channels was 9.4 μA in nine experiments, while WT-102 channels produced an average of 5.4 μA in ten experiments. Channel expres-

TABLE 1 Properties of the expressed channels

Sh cDNA	$V_m^{1/2}$	*n*	$V_h^{1/2}$	*z*	*n*	τ_1	τ_2	A1	A2	*n*
WT	−2 ± 6.6	4	−30 ± 1.1	6.5 ± 0.9	3	5.2 ± 0.45	21 ± 4.1	.71 ± .07	.18 ± .01	6
V2	59 ± 2.5	4	23 ± 2.3	2.9 ± 0.2	4	4.7 ± 0.72	33 ± 16	.57 ± .05	.18 ± .06	6
WT-WT	−7 ± 3.9	6	−28 ± 1.0	6.3 ± 0.4	3	7.9 ± 1.2	24 ± 5.7	.45 ± .12	.39 ± .10	9
WT-102	−8 ± 3.2	7	−33 ± 3.7	6.6 ± 0.6	6	5.0 ± 0.3	19 ± 5.1	.64 ± .09	.25 ± .09	9
WT-V2	11 ± 4.4	6	−13 ± 3.9	2.5 ± 0.2	6	7.5 ± 12	30 ± 2.3	.48 ± .05	.34 ± .05	10
V2-WT	26 ± 4.6	7	3 ± 4.1	2.6 ± 0.3	6	7.6 ± 1.3	39 ± 1.2	.46 ± .06	.28 ± .07	11
V2-V2	55 ± 2.0	6	24 ± 2.6	2.8 ± 0.1	7	6.6 ± 0.7	27 ± 1.5	.42 ± .03	.23 ± .04	6

The conductance-voltage and prepulse-inactivation data from individual oocytes injected with the indicated constructs were fitted to Boltzmann functions to obtain the $V_m^{1/2}$ (voltage at half-maximal conductance), $V_h^{1/2}$ (voltage at half-maximal inactivation) and *z* (effective valence) values. Time constants of inactivation (τ_1 and τ_2) and their relative amplitudes (A_1 and A_2) were obtained by fitting the decay of the currents $I(t)$ to the function $I(t) = I_0[A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_s]$ where I_0 is the extrapolated value of the peak current at $t = 0$ and $A_1 + A_2 + A_S = 1$. A_S was taken to be the current remaining at the end of the 100 ms test pulse. Test pulses for obtaining the time constants were chosen to be near saturation of the conductance curve: +40 mV for WT, WT-102 and WT-WT; +60 mV for WT-V2; +80 mV for V2-WT; and +100 mV for V2 and V2-V2. Means ± SD of the fitted parameters are given.

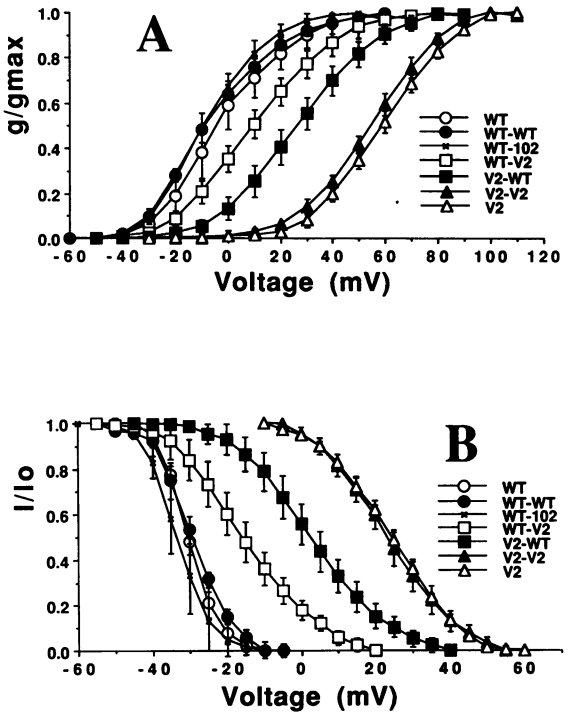

FIGURE 3 (*A*) Normalized conductance-voltage relations of the indicated channel constructs. The oocytes were held at a resting potential of −90 mV and depolarized every 7 s to the indicated potential in 10 mV increments. Peak current amplitudes were measured and used to calculate the normalized conductance-voltage relationships assuming a reversal potential of −90 mV. Data are plotted as mean ± SD where n = 4, 4, 6, 7, 6, 7, and 6 for WT, V2, WT-WT, WT-102, WT-V2, V2-WT and V2-V2, respectively. (*B*) Normalized prepulse-inactivation curves for the indicated channel types. Oocytes were held at −100 mV, stepped for 500 ms to the indicated prepulse voltages, and then depolarized to a test pulse of +80 mV. A recovery period of 10 s was given between episodes. For each prepulse potential, the peak current amplitude during the test pulse were divided by the peak current amplitude obtained after a prepulse to −80 mV to obtain the normalized curve. Data are mean ± SD, where n = 3, 4, 3, 6, 6, 6, 7, for WT, V2, WT-WT, WT-102, WT-V2, V2-WT and V2-V2, respectively.

sion in *Xenopus* oocytes is notoriously variable, but histograms of peak current levels (Fig. 4) are also consistent with only a moderate reduction in current in WT-102 channels.

DISCUSSION

A simple explanation for the differences we observe in the voltage dependences of the expressed WT-V2 and V2-WT constructs, as well as for the high expression of the WT-102 construct, would be that the stoichiometry of the expressed channels is not constrained as expected from the composition of the tandem constructs. When two different subunits are contained in a tandem construct, the phenotype of the first subunit seems to predominate, as if it is more likely to be incorporated into the oligomeric channel protein than the second subunit. Assuming that *Sh* channels are tetrameric and have a fourfold symmetry, the order of the subunits in the tandem constructs should have little effect on channel properties unless the symmetry of the channel is broken in a way important for channel function. For example, if the linker is the wrong length or otherwise unfavorably disposed, the covalent linkage might strongly distort the conformation of one or both of the subunits. The resulting asymmetry could have two effects. First, alterations in the conformational states of some of the subunits might alter the gating properties of the channel and this could explain the different voltage dependences of the WT-V2 and V2-WT channels. However, it is difficult to explain how this type of symmetry-breaking could result in the high expression of the WT-102 construct. Second, although we expected that covalently-linked subunits would be much more likely to assemble together in the endoplasmic reticulum than separately-translated subunits, it is possible that structural constraints reduce the likelihood of assembly of covalently-linked subunits, making it no more favorable than the diffusional association of subunits from other tandem proteins. This could result in channel complexes containing various subunit stoichiometries.

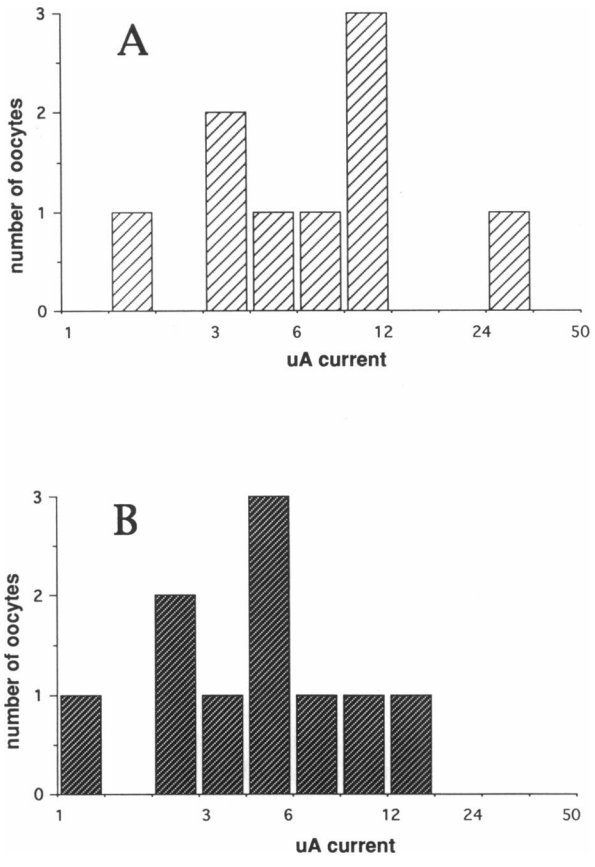

FIGURE 4 Histograms of peak currents, plotted with a logarithmic abscissa, in oocytes expressing *A*, WT-WT or *B*, WT-102 RNA. Oocytes were injected with 0.6 ng of the indicated RNA. Currents were elicited with a depolarizing pulse to +60 mV from a holding potential of −90 mV.

The linking sequence used here was Gly-$(Gln)_7$-Ser-Gly, very similar to the glutamine decamer linker used by Isacoff et al. (2) and to the linkers used in other tandem constructs (7–9), all of which were 9–12 residues in length. However, in contrast to our results, Heginbotham and MacKinnon (7) report controls that suggest a well-constrained stoichiometry in their dimer constructs: dimers containing a single subunit with deletions in the pore (H5) region yielded very low expression levels. Their constructs contain *Sh* H-4 subunits (20) with a deletion (residues 6–46) in the NH_2-terminal region which removes inactivation. One wonders if this deletion better disposes the subunits for linkage in comparison to the full-length *Sh* 29-4 construct used here.

A second explanation for the nonstoichiometric properties of our tandem constructs, independent of the properties of the linker, would be that the preferential degradation of the 3′ end of the injected *Sh* mRNAs results in the translation of a significant fraction of polypeptides that are truncated in the second repeat. We show in Fig. 1 that the RNA transcription reactions produced only a small proportion of transcripts that were not full length. The degradation of microinjected mRNA has been demonstrated for several types of transcripts in *Xenopus* oocytes (21, 22), but it is unclear whether a substantial fraction of the translation products can result from partially degraded RNA transcripts. The 5′ cap and consensus translation sites are probably necessary for translation of the exogenous mRNA (22), so any degradation which removes these regions would result in the loss of translation of that transcript. Having intact 3′ regions is less critical for successful translation, and the *Sh* cDNAs used here are not polyadenylated. Thus, translation products from partially degraded tandem cRNAs would tend to be composed of first subunits with incomplete or absent second subunits, while intact second subunits with incomplete or absent first subunits would probably not occur.

Our experiments do not discriminate between the possibilities of improper assembly or RNA degradation or a combination thereof. The relatively high expression of WT-102 raises the question of whether these channels might actually be octameric, being composed of four normally-arranged WT subunits, with the tethered 102 subunits remaining outside the channel complex; alternatively, it might be imagined that during assembly, the 102 subunits of the tandem are preferentially degraded or cleaved and the WT subunits which assemble into channels are no longer linked. This question would best be resolved through immunoprecipation or other biochemical studies of the cell-surface channels. Whatever the case, the relatively high channel expression observed from the WT-102 construct implies that functional channels can arise from transcripts truncated in the second repeat. If RNA degradation is in fact affecting the stoichiometry of the channels produced by the other tandem constructs, steps could be taken to stabilize full-length RNA transcripts. The use of 3′ untranslated sequences that increase the half-life of transcripts would be expected to increase the proportion of full-length transcripts. For transcripts which are not polyadenylated it might be expected that recording the oocytes after shorter incubation periods could reduce the proportion of degraded translation products. For *Sh* 29-4 cRNA the first measurable currents appear within 2–4 d after injection, but in the experiments reported here we waited 4–15 d after injection because maximal expression occurs in this time period.

In view of our results with the WT-102 construct, it is interesting to consider the nature of the interaction between WT and 102 subunits. In previous experiments using these cRNAs (10), coinjected 102 subunits decreased the level of expression of channels from WT subunits, but did not alter the expression of similarly coinjected Na^+ channel RNA. Using injections in the linear range of channel expression, the coinjection of WT (0.25 ng) and 102 RNA (0.5 ng) produced ~30% of the current observed for injection of WT RNA (0.25 ng) alone. Thus, there appears to be a specific association between WT and 102 subunits. The observed degree of current suppression was, however, less dramatic than would be expected if 102 subunits combine with normal affinity to WT subunits.

It is thought that the H5 regions of the subunits of the tetrameric channel together form a portion of the channel pore, and thus, the H5 region may be an important determinant in the folding and assembly of subunits into the channel complex as well. Since 102 subunits lack the H5 and other downstream regions they might be incapable of channel assembly. It is not known whether 102 subunits or WT/102 hybrids are expressed in the plasma membrane; it is possible that the association of WT and 102 subunits results in multimeric hybrids that fail to assemble correctly and are not transported out of the endoplasmic reticulum (23, 24).

If 102 subunits compete with WT subunits at the level of monomer-monomer association, rather than in higher order multimers, a lower level of inhibition would result (25), more similar to that observed here. In nicotinic acetylcholine (ACh) receptors, truncated subunits appear to be able to associate with wild type subunits and decrease the expression of cell surface ACh receptors through the formation of complexes between truncated and wild type subunits which do not assemble properly (26). Moreover, the moderate amount of inhibition found for these truncated subunits (~50% for ~1–4 times as much of the truncated subunit α, γ or δ, than the same wild type subunit α, γ or δ, respectively) is comparable to that found for the inhibition of WT *Sh* expression by coinjection of 102 subunits.

In summary, we suggest that the tandem linkage of subunit cDNAs does not necessarily ensure a uniform subunit stoichiometry. The WT-102 tandem experiment casts doubt on the idea that linked subunits are con-

strained to be incorporated into the same oligomeric complex, while the WT-V2 and V2-WT experiments show that the ordering of subunits in a tandem construct can have a large effect on channel properties. These phenomena are best explained by distortions of the channel symmetry or other structural constraints imposed by the covalent linkage and/or the partial degradation of the injected RNA and subsequent translation of the incomplete transcripts. Both of these effects may contribute to the formation of oligomers with other than the expected stoichiometries. We therefore suggest that results obtained with linked channel subunits be interpreted with caution unless the stoichiometry of the expressed complexes can be established.

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REFERENCES

1. Christie, M. J., R. A. North, P. B. Osborne, J. Douglass, and J. P. Adelman. 1990. Heteropolymeric potassium channels expressed in *Xenopus* oocytes from cloned subunits. *Neuron.* 4:405–411.
2. Isacoff, E. Y., Y. N. Jan, and L. Y. Jan. 1990. Evidence for the formation of heteromultimeric potassium channels in *Xenopus* oocytes. *Nature (Lond.).* 345:530–534.
3. McCormack, K., J. W. Lin, L. E. Iverson, and B. Rudy. 1990. *Shaker* K^+ channel subunits form heteromultimeric channels with novel functional properties. *Biochem. Biophys. Res. Comm.* 171:1361–1371.
4. Ruppersburg, J. P., K. H. Schroter, B. Sakmann, M. Stocker, S. Sewing, and O. Pongs. 1990. Heteromultimeric channels formed by rat brain potassium-channel proteins. *Nature (Lond.).* 345:535–537.
5. MacKinnon, R. 1991. Determination of the subunit stoichiometry of a voltage-activated potassium channel. *Nature (Lond.).* 350:232–235.
6. Catterall, W. A. 1988. Structure and function of voltage-sensitive ion channels. *Science (Wash. DC).* 242:50–61.
7. Heginbotham, L., and R. MacKinnon. 1992. The aromatic binding site for tetraethylammonium ion on potassium channels. *Neuron.* 8:483–491.
8. Kavanaugh, M. P., R. S. Hurst, J. Yakel, M. D. Varnum, J. P. Adelman, and R. A. North. 1992. Multiple subunits of a voltage-dependent potassium channel contribute to the binding site for tetraethylammonium. *Neuron.* 8:493–497.
9. Tytgat, J., and P. Hess. 1992. Dimeric and tetrameric channels made from wildtype and mutant RCK1 K channels. *Biophys. J.* 61:426*a*. (Abstr.)
10. McCormack, K., M. A. Tanouye, L. E. Iverson, J. W. Lin, M. Ramaswami, T. McCormack, J. T. Campanelli, M. K. Mathew, and B. Rudy. 1991. A role for hydrophobic residues in the voltage-dependent gating of *Shaker* K^+ channels. *Proc. Natl. Acad. Sci. USA.* 88:2931–2935.
11. Schoppa, N. E., K. McCormack, M. A. Tanouye, and F. J. Sigworth. 1992. Size of gating charge in wild type and mutant *Shaker* potassium channels. *Science (Wash. DC).* 255:1712–1715.
12. Iverson, L. E., and B. Rudy. 1990. The role of the divergent amino and carboxyl domains on the inactivation properties of potassium channels derived from the *Shaker* gene of *Drosophila. J. Neurosci.* 10:2903–2916.
13. Gisselman, G., S. Sewing, B. W. Madsen, A. Mallart, A. Anguat-Petit, F. Muller-Holtkamp, A. Ferrus, and O. Pongs. 1989. The interference of truncated with normal potassium channel subunits leads to abnormal behavior in transgenic *Drosophila melanogaster. EMBO (Eur. Mol. Biol. Org.) J.* 8:2359–2364.
14. Gautam, M. and M. A. Tanouye. 1990. Alterations of K^+ channel gating: molecular analysis of the *Drosophila* Sh^5 mutation. *Neuron.* 5:67–73.
15. Yool, A. J. and T. L. Schwarz. 1991. Alteration of ionic selectivity of a K^+ channel by mutation of the H5 region. *Nature (Lond.).* 349:700–704.
16. Hartmann, H. A., G. E. Kirsch, J. A. Drewe, M. Taglialatella, R. H. Joho, and A. M. Brown. 1991. Exchange of conduction pathways between two related K^+ channels. *Science (Wash. DC).* 251:942–944.
17. Yellen, G., M. E. Jurman, T. Abramson, and R. MacKinnon. 1991. Mutations affecting internal TEA blockade identify the probable pore-forming region of a K^+ channel. *Science (Wash. DC).* 251:939–942.
18. Hoshi, T., W. N. Zagotta, and R. W. Aldrich. 1990. Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. *Science (Wash. DC).* 250:533–538.
19. Timpe, L. C., Y. N. Jan, and L. Y. Jan. 1988. Four cDNA clones from the *Shaker* locus of *Drosophila* induce kinetically distinct A-type potassium currents in *Xenopus* oocytes. *Neuron.* 1:659–667.
20. Kamb, A., J. Tseng-Crank, and M. A. Tanouye. 1988. Multiple products of the *Drosophila Shaker* gene may contribute to potassium channel diversity. *Neuron.* 1:421–430.
21. Richter, J. and L. Smith. 1981. Differential capacity for translation and lack of competition between mRNAs that segregate to free and membrane-bound polysomes. *Cell.* 27:183–191.
22. Soreq, H. 1985. The biosynthesis of biologically active proteins in mRNA-microinjected *Xenopus* oocytes. *CRC Crit. Rev. Biochem.* 18:199–238.
23. Hurtley, S. M. and A. Helenius. 1989. Protein oligomerization in the endoplasmic reticulum. *Annu. Rev. Cell. Biol.* 5:277–307.
24. Klausner, R. D. and R. Sitia. 1990. Protein degradation in the endoplasmic reticulum. *Cell.* 62:611–614.
25. McCormack, K. 1991. Structure-function studies of *Drosophila Shaker* potassium channels. Ph.D. thesis, California Institute of Technology.
26. Verall, S., and Z. Hall. 1992. The N-terminal domains of acetylcholine receptor subunits contain recognition signals for the initial steps of receptor assembly. *Cell.* 68:23–31.