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Ion channels formed by transcription factors recognize consensus DNA sequences

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Abstract

Transcription factors (TFs) are proteins which bind to specific DNA sequences and thus participate in the regulation of the initiation of transcription. We report in this communication our observations that several of these proteins interact with lipid membranes and form ion-permeable channels. For each of the TFs that we studied, the single channel conductance was distinctively different, i.e. each TF had its own electrical signature. More importantly, we show for the first time that addition of cognate double-stranded DNA sequences leads to a specific response: an increase in the conductance of the TF-containing membrane. Strikingly, the effect of cognate DNA was observed when it was added to the *trans*-side of the membrane (opposite to where the TF was added), strongly suggesting that the TFs span the membrane and that the DNA-binding domain is *trans*-accessible. Alterations in the primary structure of the TF factors in their basic and DNA-binding regions change the characteristics of the conductance of the protein-containing membranes as well as the response to DNA addition, reinforcing the notion that the changes we measure are due to specific interactions.

Keywords: Transcription factor; Protein channel; DNA binding; Lipid bilayer

1. Introduction

Transcription factors (TFs) are proteins which recognize and interact with specific DNA sequences and thereby promote the transcription of genes under their regulatory control. Work in the past 20 years on the basic structural motifs which allow TFs to bind DNA in a sequence-specific manner has led to the classification of TFs into several families ([1] and references therein). These structural motifs (α-helical and β sheets) have also been found in several other classes of proteins which interact with membranes and form channels, as has been amply documented ([2–5] and references therein).

Based on these data, we decided to test the possibility that TFs might interact with membranes and form ion channels. The results that we present in this communication show that TF's do form ion permeable pathways in lipid membranes. The electrical characteristics of the channels are a unique property of each particular protein rather than a characteristic

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of the TF family. Moreover, this capacity to bind to lipid membranes and incorporate to form ion-permeable channels was found to be dependent on the presence of the appropriate dimerization partner. Thus, proteins which do not homodimerize, do not form channels and neither do proteins which require a heteropartner to dimerize, in the absence of their partners.

Strikingly, we have also found that the addition of the cognate DNA sequence(s) to the *trans*-side of TF-containing bilayers alters the channel's electrical characteristics, suggesting that DNA binding leads to a rearrangement of the architecture of the channel.

2. Materials and methods

2.1. Lipid bilayers

The lipid membranes were formed by apposition of two monolayers, across an aperture (100 μm diameter) on a teflon partition separating two aqueous solutions (1.5 ml), as described [6]. The lipid monolayers (1:1 phosphatidyl-ethanolamine:phosphatidylserine, Avanti Polar Lipids, Birmingham, AL, USA) were spread at the air–water interface from a solution of 12.5 mg/ml lipid in pentane (Fisher Chemicals, div Fisher Scientific Pittsburgh, PA, USA). The salt solutions contained 140 mM KCl, 140 mM NaCl, 1 mM EGTA, 10 mM Tris–MOPS pH 7.0 (room temperature). The addition of proteins and peptides was done on the *cis*-side under continuous stirring to final concentrations as indicated in the figure legends. Whenever used, DNA was added to the *trans*-side. The proteins were used within 1 week of synthesis and discarded after 10 days. Peptides were dissolved at 0.1 mg/ml in 20 mM NH_4acetate, 5 mM dithiothreitol and stored at −45°C. The connection from the solutions to the Ag–AgCl electrodes was done via agar bridges. Voltages are referenced to the *trans*-side (virtual ground).

2.2. Data acquisition and analysis

The data acquisition and analysis were performed using the equipment and methodology previously described [7]. Briefly, the value of the single channel conductance was calculated from the slope of the current–voltage curves (when linear) or from the chord conductance at low voltages (5–30 mV). The time constants (τ_0) were determined from the exponential fit to the log-bin distributions of open dwell-times [8] and the open probability (p(o)) was either calculated from the normalized amplitude histograms, or from the sum of open times divided by the total time, since both methods gave the same results. Each peptide (protein) was added to at least five different membranes. The electrical parameters shown in Tables 1 and 2 were calculated from data obtained in membranes in which 4–5 different voltages were applied at least twice at each of the different experimental conditions indicated.

2.3. Proteins, peptides and oligonucleotides

The proteins used in this study: ADD1 (adipocyte determination- and differentiation-dependent factor-1), GCN4, GCN4-2AA, GCN4-N235S (yeast transcriptional activator protein and mutants described in Section 3), NF-AT4 (nuclear factor of activated T cells), Fos, Jun and Fos–Jun were expressed by in vitro transcription and translation reactions in the presence of trace amounts of ^{35}S methionine, using the TNT SP6 coupled reticulocyte lysate system (Promega, Madison, WI, USA) and used without further purification (unless otherwise stated). The expressed proteins, were then analyzed on a 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis from which the amount of protein expressed in the lysate system was estimated from the radioactivity present in the protein band. The final concentration of protein used is indicated in the figure legends. A peptide derived from ADD1(p278–403), which comprises the basic region and the DNA-binding domain was also cloned and expressed as above. Peptides containing the dimerization and DNA-binding domains from GCN4, (pGCNK58), Myo-D and E-47 were expressed and purified as described [9,10]. The oligonucleotides used were a gift from Dr. T. Ellenberger and contained the E-box motif (ABS: GATCCTGATCACGTGATCGAGGAG); the non E-box motif (SRE-1: GATCCTGATCACCCCAC TGAGGAG) and the AP-1 motif (TTCCTATGACTCATCAGTT) (only one strand is shown, we only used the double-stranded DNA sequences).

Table 1
Characteristics of lipid bilayers exposed to TFs

Protein	Conductance (pS ± S.E.M.)	Mean open time (ms ± S.E.M.)	p(o) ± S.E.M.
ADD1	243 ± 10 (8)	127 ± 10	
		5620 ± 50	0.97 ± 0.03
	114 ± 37 (4)*	60 ± 2*	0.04 ± 0.01*
(p278–403)	96 ± 8 (6)	3.6 ± 0.1	
		3100 ± 60	0.50 ± 0.10
GCN4-WT	15 ± 6 (3)	4 ± 2	
		31 ± 10	0.30 ± 0.18
GCN4-WT-P	18 ± 4 (3)	3 ± 0.5	
		26 ± 11	0.40 ± 0.08
pGCNK58	56 ± 10 (3)	14 ± 6	
		48 ± 8	0.33 ± 0.10
NF-AT4	145 ± 50 (2)	15 ± 8	
		330 ± 20	0.4 ± 0.06
Fos–Jun	120 ± 30 (4)	–	0.96 ± 0.1

Values of the conductance, of the mean open time and of the probability that the conductance state is open (p(o)) are given together with the standard error of the mean (S.E.M.). The values of the single channel conductance are for the most probable open state only, except for ADD1 in which a second state is shown and the value of the parameters indicated with (*). The number of different membranes in which the values were obtained is also shown (in parentheses).

2.4. Protein purification

The strategy followed in the purification of GCN4-WT was determined by the need to avoid denaturation of the protein and to avoid the presence of detergents in high concentrations ($>0.08\%$). Briefly, GCN4 was translated using the in vitro transcription/translation kit (Promega, Madison, WI, USA) in the presence of a trace amount of ^{35}S methionine. A portion of this mixture (10 μl) was separated to test its capacity to incorporate into lipid bilayers and form DNA-sensitive channels. An agarose conjugate of double-stranded DNA was equilibrated with DNA-binding buffer (see below) overnight and 250 μl 50% slurry of agarose-DNA was added to the diluted GCN4 in vitro translation product (200 μl to 10 ml DNA-binding buffer) and incubated for 4 h on a rocker. The mixture was then spun and washed 3× in DNA-binding buffer. The DNA-binding proteins were eluted in DNA-binding buffer after adjusting the NaCl concentration to 400 mM. The eluate was dialyzed twice against 500 ml of DNA-binding buffer without NP40 and benzamidine and was subsequently used for the bilayer studies (cf. Tables 1 and 2). All purification procedures were carried out at 4°C. Ten μl of the original in vitro translation product and 50 μl of the purified protein were run on 10% SDS gel, stained with Coomassie blue and the gel was exposed to a phosphoroimager cassette for 2 days. The bands were visualized using a BioRad phosphoroimager (Fig. 1). DNA-binding buffer: 20 mM Tris–HCl, pH 8, 1 mM EDTA, 10% glycerol, 0.05% NP-40, 1 mM benzamidine, 14 mM 2-mercaptoethanol and 100 mM NaCl).

Table 2
Effect of addition of DNA on the conductance of bilayers in which TFs have been incorporated

Protein	DNA (concentration)	Conductance (pS ± S.E.M.)
Fos–Jun	AP-1 (10 nM)	2100 ± 110 (2)
ADD1	ABS (5 nM)	2200 ± 10 (2)
	SRE-1 (6 nM)	930 ± 11 (2)
p278–403	ABS (5 nM)	1500 ± 15 (4)
	SRE-1 (30 nM)	1200 ± 40 (3)
GCN4-WT	AP-1 (10 nM)	118 ± 30 (3)
GCN4-WT-P	AP-1 (10 nM)	124 ± 32 (2)
pGCNK58	AP-1 (10 nM)	1450 ± 45 (3)
Fos–JunGCN4-2AA		25 ± 10 (4)
	AP-1 (10 nM)	11 ± 5 (4)
GCN4-N235S		20 ± 9 (4)
	AP-1 (10 nM)	2100 ± 11025 ± 9 (24)

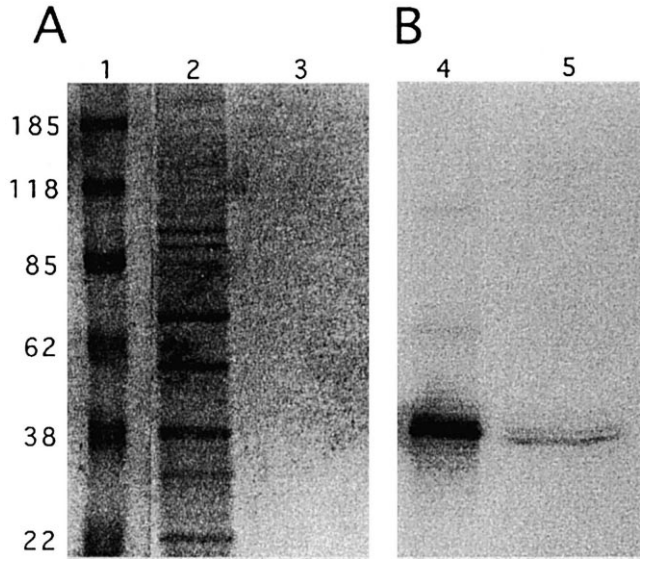

Fig. 1. Analytical gel of purified GCN4-WT. GCN4 was translated using the in vitro transcription/translation kit in the presence of a trace amount of ^{35}S methionine, as detailed in Section 2. (A) Coomassie blue stain of 10 μl of original in vitro translation product (lane 2) and 50 μl of purified protein (lane 3) separated on a 10% SDS gel. Lane 1: molecular weight marker. (B) Same gel exposed to phosphoroimager cassette for 2 days and bands visualized by phosphoroimager (BioRad Laboratories, Hercules, CA, USA); lane 4: 10 μl of original in vitro translation product; lane 5: 50 μl of purified protein. The purified protein runs as a wider lane due to high salt concentration.

2.5. Control experiments

Since the coupled transcription–translation system used to express the proteins under study contain many unrelated proteins, it was necessary to test the unprimed TNT SP6 reticulocyte system as to its capacity to form channels in lipid membranes. To this end, 20 μl of TNT SP6 (volume which is 20 times that used with primed TNT SP6) were added to a preformed lipid bilayer without alteration of the bilayer's electrical properties for up to 1 h after addition. This result implies that under our experimental conditions the channels are not produced by proteins derived from the reticulocyte system. We also found that Fos, as well as a mutated ADD1 (Y320→A320, mutation in the basic region which leads to loss of DNA binding) [11], failed to induce channels when added to a lipid bilayer at concentrations 10 times those used in the study, indicating that not all proteins (or primed TNT SP6) expressed using the coupled reticulocyte lysate system will bind to lipid bilayers and make channels (see below).

To address the issue of purified proteins versus proteins in the TNT SP6 system, we purified and tested GCN4-WT protein (GCN4-WT-P) for its ability to induce DNA-sensitive channels in lipid bilayers. As illustrated in Table 1, the channels induced by the purified protein are indistinguishable from those formed by GCN4-WT, protein obtained with the in vitro transcription and translation reaction, and used without further purification. Moreover, as illustrated in Table 2, the channels formed by the purified protein respond to the addition of the cognate DNA (as do the channels induced by GCN4-WT). We feel that these control experiments validate the use of proteins expressed using the TNT SP6 coupled reticulocyte lysate system without further purification. Finally, addition of DNA (up to 100 nM) to a bilayer without incorporated TFs, did not produce changes in the conductance of the lipid bilayer.

3. Results and discussion

3.1. TFs form channels in lipid bilayers

To determine if TFs bind to lipid bilayers and insert so as to promote changes in the electrical properties of the membranes, we chose to work with proteins belonging to different families: the yeast transcriptional activator protein (GCN4) and the adipocyte determination- and differentiation-dependent factor-1 (ADD1). GCN4 is a member of the (bLZ) family, which homodimerizes via the leucine zipper (LZ) adjacent to the basic region directly in contact with the AP-1 motif [12]. ADD1 is a member of the (bHLH-LZ: basic helix loop helix-LZ) family, the murine homolog of SREBP-1c from the SREBP (sterol regulatory element binding proteins) family of proteins, [11,13–15] and, as a member of this family, capable of forming homodimers. Unlike other bHLH-LZ proteins, however, the ADD1 homodimer has unique dual DNA-binding specificities, with the E-box motif, which is the typical binding site of all the bHLH proteins, and the non-box sequence, SRE-1 [13].

We have routinely detected the presence of chan-

nels such as those depicted in Fig. 2, upon unilateral exposure of lipid bilayers to GCN4 or to ADD1. Fig. 2A,B,D show representative traces of the time course of the current across a bilayer in response to an applied voltage pulse (−30 mV). Fig. 2A shows the current response versus time obtained from an unmodified membrane and the traces in Fig. 2B show the current response of the same membrane (and same applied potential) after unilateral exposure to GCN4. The traces in Fig. 2D show the current response to the same applied potential from another membrane after unilateral exposure to ADD1. Clearly, addition of these TFs produces channels which have different conductance states as well as

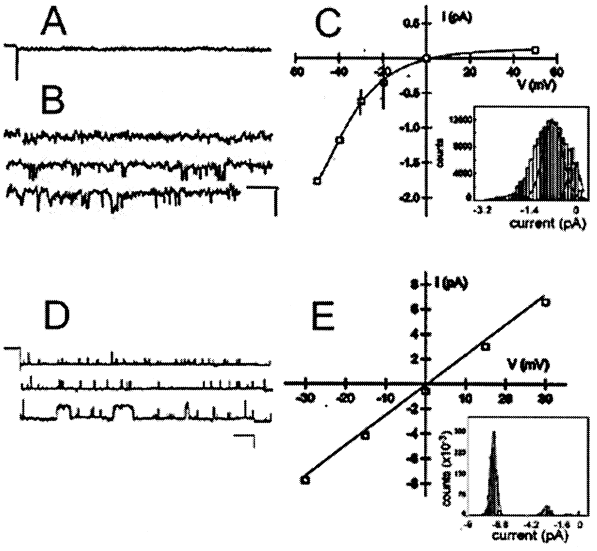

Fig. 2. TFs form channels in lipid bilayers. The current versus time traces in the figure begin 2–30 min after addition of protein to the lipid membrane and show the current response to a −30 mV pulse (duration 60 s), upon addition of: (A) control, no additions, (B) GCN4 (2 ng/ml) and (D) ADD1 (0.8 ng/ml). (C) and (E) Current–voltage plots of the channels formed by TFs from data such as the ones shown in (B) and (D), respectively, collected at various applied potentials. The points in the graph represent the mean value of the most probable current peak at each potential and determined as indicated in Section 2 from the fit to the current amplitude distributions (see insets, current amplitude distributions for the traces in (B) and (D)). The vertical bars show the standard error of the single channel current; the error bars in the case of (E) are as large as the data markers. Data were sampled at 2 kHz and are shown filtered at 180 Hz. Value of the conductance in the absence of protein: 2 pS. The capacitative surge indicates the beginning of the pulse. Time scale: 200 ms (A and B); 5000 ms (D). Current scale: 1.5 pA (A and B); 6 pA (D).

different kinetic characteristics (cf. Table 1). From traces such as those shown in Fig. 2 it is possible to obtain the current amplitude distribution curves of the channels at different voltages (e.g. insets in Fig. 2C,E obtained at −30 mV). Fig. 2C shows the mean values of the current of the most probable open state as a function of voltage of the channels induced in bilayers exposed to GCN4. The figure shows that channel openings are absent (or too small to be detected) when positive potentials are applied and that they open at negative potentials, suggesting that the channel current (conductance) is strongly rectifying. By contrast, Fig. 2E shows that the opening of channels induced by ADD1 in lipid bilayers is voltage-independent, thus yielding a linear (i versus V) curve for the most probable state of the channel. Thus, the conducting pathway promoted by the incorporation of GCN4 results in a strong asymmetry in the flow of current, whereas incorporation of ADD1 yields a constant value of conductance with voltage, given by the slope of the line of current versus voltage. The independence of the conductance on voltage extends to all of the conductance states of ADD1 (cf. Table 1).

In order to determine if the dimerization and binding domain(s) of the TFs is sufficient to produce channels in lipid bilayers, we tested the capacity of a peptide comprising the dimerization and binding domains of GCN4 (residues 226–281, pGCNK58) [9] for its ability to form channels. The data shown in Table 1 indicate that pGCNK58 incorporates and forms channels which have a somewhat larger conductance and longer lifetime than those formed by the incorporation of the full-length GCN4 protein. The reason for this difference in the conductance of the single channel is not clear at the moment.

To determine the minimum domain(s) in ADD1 sufficient to produce changes in the conductance of lipid membranes, we expressed several truncated forms of the protein, all containing the dimerization and binding domains. Removing the C-terminal end alone or the C-terminal end plus the transmembrane domain of ADD1 yields peptides which, upon interaction with lipid bilayers form channels with a conductance almost identical to that of ADD1 (not shown) thus indicating that the transmembrane domain is not necessary for channel formation. A 20 kDa fragment containing solely the dimerization and

binding domain of ADD1, ADD1(p278–403), incorporates into lipid membranes, and forms channels whose major conductance state coincides with one of the conductance states of ADD1 cf. Table 1). These results then indicate that for ADD1 as well, the binding and dimerization domain(s) is sufficient to produce channels of the same qualitative electrical properties as those of the full-length protein. The results further indicate that binding of the dimerization and binding domains of the TFs to lipid membranes leads to the formation of channels, which resemble those formed by the full-length proteins, thus suggesting that these domains might form an integral part of the ion-permeable pathways.

The fact that the conductance of the ADD1 fragment is lower than that of the full-length protein might be a consequence of the lack of the *N*-terminal domain in this peptide, as suggested by the fact that one of two other fragments derived from ADD1 containing the *N*-terminal domain through the binding and dimerization domains induces channels with the same conductance as that of ADD1-WT (not shown).

3.2. Cognate DNA sequences increase the conductance of TF-induced channels

Having shown that the DNA-binding element is an intrinsic part of the TF channels, we decided to test the possibility that specific DNA sequences could bind to the channel and change their permeability, through conformational changes arising from the interaction. To test this idea, we measured the electrical properties of TF-containing bilayers after asymmetrical addition of specific DNA sequences. Fig. 3 shows the current versus time traces which are obtained in this type of experiment, for two different membranes. As shown in Fig. 3B,C, the current increases abruptly to its final value in one or two steps and remains at this value for the duration of the experiment. Fig. 3A shows the *i* versus *t* trace of a membrane containing GCN4-WT-P (the purified GCN4-WT protein), just before addition of DNA. The traces shown in Fig. 3B were obtained in the same membrane 7 min after addition of AP-1 (10 nM). At this time, the conductance had already increased to an intermediate value of 73 pS with a second increase occurring about 1 min later (indicated by the arrow) to a final value of the conductance (133 pS). The same type of response occurs when bilayers containing either GCN4-WT or pGCNK58 are exposed to the AP-1 site (not shown). In all the membranes tested as described, the final conductance value remained constant for at least 25 min.

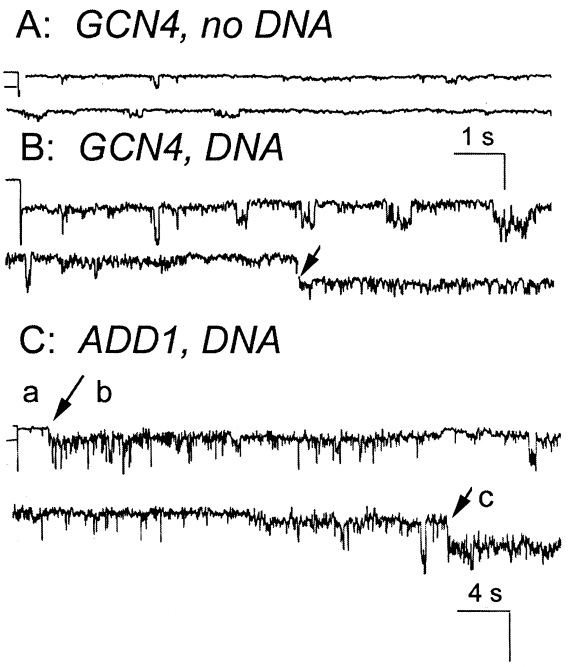

Fig. 3. DNA increases the conductance of bilayers containing TFs. The current versus time traces of membranes exposed to TFs and DNA in response to $V=-30$ mV. (A) Current response of a bilayer containing GCN4-WT-P. (B) The response of the same membrane 7 min after addition of the AP-1 motif (10 nM). The initial current corresponds to an increase from 0.5 pA to an intermediate value of 2.2 pA; at the arrow, another increase to the final value occurred (4 pA). This last one remained open for another 25 min (and the experiment terminated). In one separate experiment (out of four in total), the channel did not remain open all the time, but rather the single channel conductance increased to the value shown in this trace, and fluctuated between open and closed states. Current scale: 3 pA. (C) The temporal response of the current across an ADD1-containing bilayer to a pulse applied 5 min after addition of the double-stranded E-box motif (ABS, 50 nM). The current increased from its value without DNA ('a': 6.2 pA) to 12 pA ('b') and a final increase after approximately 1 min to 66 pA ('c'), which remained unaltered for at least another 30 min. Current scale: 60 pA. All other conditions as in Fig. 2.

The traces in Fig. 3C show that the same type of sudden increase in conductance occurs in response to the addition of ABS (50 nM) to a bilayer with incorporated ADD1-WT. The recording begins 6 min

after addition of the DNA sequence. The segment labeled 'a' corresponds to a channel conductance of 207 pS, obtained in the absence of DNA. At the arrow, there is a rather abrupt increase to about 400 pS ('b') which lasts just over 1 min, followed by the last increase ('c') corresponding to a conductance of about 2000 pS. The same type of response was observed when bilayers containing ADD1(p278–403) were exposed to either ABS or SRE (not shown).

Addition of non-cognate DNA sequences to TF-containing membranes either do not modify the existing conductance or produce closure of the channels (not shown). These results then suggest that when TFs incorporate into lipid bilayers promoting the formation of channels, the DNA-binding region within the channel architecture is accessible to nucleotides added to the aqueous phase surrounding the membrane. It is also interesting to point out that addition of either SRE-1 (6 nM) or ABS (5 nM) to bilayers containing channels formed by the full-length protein ADD1 leads to an increase in the conductance of the membrane, as shown in Table 2. The channels induced by ADD1(p278–403), which respond to the addition of ABS (5 nM), require about six times as much SRE-1 to produce the conductance increase (cf. Table 2). This suggests that the affinity of the site for SRE-1 is lower in the incorporated peptide (p278–403) than in the incorporated full-length protein (ADD1). A similar type of change in the binding affinity for DNA has been previously reported for the segment containing the DNA-binding domain of NFTc [16].

3.3. The dimerization and binding domains are important for channel formation and DNA recognition

To explore further the specificity and importance of the dimerization and binding domains both to channel formation and to DNA binding, we took advantage of existent mutants of GCN4, which contain alterations in the LZ–basic region [17,18]. The mutant proteins with various insertions of amino acids between the basic and the LZ regions produce changes in the conductance of bilayers with varying phenotypes. Thus, (GCN4-2AA) a mutant with a two amino acid insert between the basic region and the LZ resulting in loss of function of GCN4, induces channels in bilayers. The value of the most probable conductance is somewhat larger than the most probably conductance of the channel formed by GCN4-WT (cf. Table 2) and with a longer lifetime (not shown). Addition of DNA, however, produces a partial blockage of the channel, since the conductance of the GCN4-2AA-containing bilayer changes from about 25 pS to a value around 10 pS (cf. Table 2, 'GCN4-2AA'). Addition of GCN4-N235S, a mutant with an N235 substitution for S235, which can dimerize but is devoid of DNA binding, results in channels which lack recognition of DNA, since the value of the most probable conductance state is not changed upon addition of AP-1, as shown in Table 2 'GCN4-N235S'. These results then show that changes in the regions important for DNA recognition also have consequences for the DNA recognition in the in vitro system.

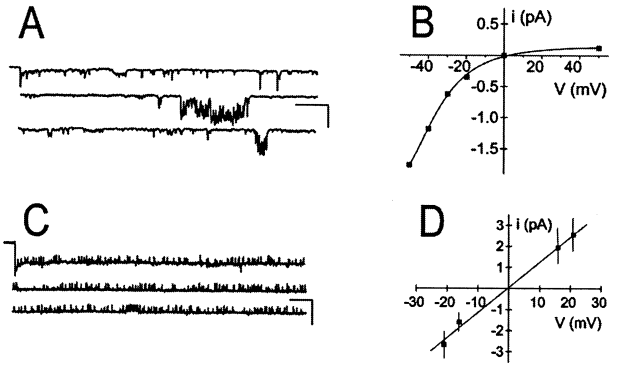

Fig. 4. Channels induced by other TFs. Same conditions as described in legend to Fig. 2. (A) Current response of a bilayer containing NF-AT4 (3 ng/ml) and (B) the current–voltage curve from several membranes. (C) The current response of a membrane exposed to Fos–Jun (2 ng/ml) and (D) the current–voltage curve composite of several membranes. Time scale: 1000 ms (A); 200 ms (C). Current scale: 6 pA.

3.4. Effects of other TFs

To generalize the results obtained thus far, we studied the effects on the conductance of lipid bilayers induced by the addition of other TFs (or the peptides containing the dimerization and binding domains), either from the same or from different families to those already tested. Fig. 4A shows current versus time traces from a bilayer containing NF-AT4

and the averaged results obtained at various applied voltages in several membranes are shown in the current–voltage curves in Fig. 4B. Fig. 4C shows the current versus time from another membrane containing Fos–Jun and the (average current) versus voltage curve for the heterodimer is shown in Fig. 4D. Similarly to the channels induced by GCN4, the *i–V* curve of the channels formed by NF-AT4 shows strong rectification, whereas the *i–V* plot of membranes containing Fos–Jun is linear, as is the case for the *i–V*) plot of membranes containing ADD1 (cf. Fig. 2).

We also attempted to incorporate peptides comprising the dimerization and binding regions of the E-box proteins Myo-D and E47 which contain the bHLH DNA-binding domain. These proteins, which do not homodimerize and do not form dimers with other bHLH proteins [10], do not form channels when added to lipid membranes and addition of specific DNA fails to produce a change in the conductance (not shown).

These data reinforce the notion that TFs capable of homodimerizing form channels in lipid membranes, since NF-AT4 (which forms homodimers) also forms channels in lipid bilayers (Fig. 4A), as do GCN4 and ADD-1 (cf. Fig. 2). The heterodimerizing proteins Fos or Jun failed to form channels when present alone (not shown). However, if both partners are present, the proteins form channels (Fig. 4C) capable of recognizing specific DNA sequences (cf. Table 2). Formation of channels in the presence of Jun alone, however, was detected if the concentration of the protein was approximately five times that required to produce the Fos–Jun heterodimers. This suggests that even in the presence of membranes, Jun has a lower affinity for homodimerization than for heterodimerization, as has already been described [19]. Thus, taken altogether, the results presented suggest that the channel formation induced by TFs only occurs if the appropriate dimerization partners are present.

A comparison of the traces shown in Fig. 2A (and C) with those shown in Fig. 4A (and C) reveals that the channels produced by the various TFs tested are different in their amplitude and kinetic characteristics (cf. Table 1). Comparison of the plots in Fig. 2C (and E) with those in Fig. 4B (and D) further show that the voltage dependence of the conductance induced by the different proteins vary as well. Thus, one of the TFs containing the LZ motif (GCN4) and the β-sheet representative (NF-AT4) induce a voltage-dependent conductance when incorporated into lipid membranes (cf. Figs. 2C and 4B). However, this does not seem to be a characteristic of the LZ motif, since other members of this family, Fos–Jun, interact with lipid bilayers and produce channels with a linear response of the current with voltage, as does the bHLH-LZ protein, ADD1 (cf. Figs. 2E and 4D).

To summarize, the data we have presented confirm the hypothesis that TFs form channels in lipid bilayers. The particular electrical characteristics of these channels were found to be different for each of the particular proteins. Apparently, the electrical properties of the channels are subtle manifestations of slight differences in the conformations of each particular protein (cf. Figs. 2 and 4), as has been found to be the case in studies of the structure–function relation of voltage-gated peptides [20,21].

Moreover, the data obtained with the mutants of GCN4, have underscored the importance of the distance between the basic region (DNA-binding) and the dimerization region for channel formation. Thus, modifications in the distance between these regions via addition of amino acids (spacers) result in altered channel characteristics as well as altered DNA binding (cf. Table 2).

The data in this communication further reveal an interesting property of the channels induced by the dimerization and binding domains of the transcriptions factors tested: *the recognition of specific DNA sequences*. Recent reports have shown that DNA can translocate across planar lipid bilayers containing large channels formed by porin (a protein from the outer wall of bacteria) or by α-hemolysin (from *Staphylococcus aureus*), producing transient closures of the channels in their transit [5,22]. The results of our experiments suggest that the channels formed by TFs are specific DNA-recognizing channels rather than DNA-translocating ones, based on the fact that addition of specific DNA sequences leads to an *increase* in the channel conductance (cf. Fig. 3 and Table 2), and that this increase in the conductance only happens upon addition of cognate DNA sequences, irrespective of the size of the channel conductance (cf. Tables 1 and 2). This contrasts with the studies on the translocation of DNA through porin

and α-hemolysin channels, since in the small-sized channels (10–40 pS) the DNA sequences (no specificity required) cannot penetrate into the channel and produce the characteristic transient changes in current [5]. In contrast, in our experiments the conductance of the TF-induced channels does not determine the events which follow the addition of DNA sequences. The increase in the conductance of the protein channels occurs *only* upon addition of cognate DNA sequences. Thus, we would suggest that the increase in the conductance which we observe upon addition of the specific DNA sequences to TF-induced channels is due to the binding of DNA and rearrangement of the channel's architecture to accommodate the bound DNA molecule(s). The fact that the signal(s) which this process produces are specific for cognate DNA-binding domains could be utilized to determine the existence and nature of as yet undetermined binding sequences in proteins.

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