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Pore region of TRPV3 ion channel is specifically required for heat-activation

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Abstract

Ion-channels can be activated (gated) by a variety of stimuli including chemicals, voltage, mechanical force or temperature. Whereas molecular mechanisms of ion-channel gating by chemicals and voltage are in principal understood, the logic of temperature-activation has remained elusive. The transient receptor potential channel TRPV3 is a non-selective cation channel activated by warm temperatures and sensory chemicals such as camphor. Here, we screened ~14,000 random mutant clones and identified five single point-mutations that specifically abolish heat-activation, but do not perturb chemical-activation or voltage-modulation. Notably, all five mutations are located in the putative 6th transmembrane helix and the adjacent extracellular loop within the pore region of mouse TRPV3. Although distinct in sequence, we find that the corresponding loop of frog TRPV3 is also specifically required for heat-activation. The exchange of this domain from the heat-insensitive human TRPV2 into mouse TRPV3 specifically abolishes heat responses, emphasizing the requirement of this region for temperature activation. These findings demonstrate that temperature-sensitivity of TRPV3 is separable from all other known activation-mechanisms, and reveal a novel region implicated in sensing temperature.

Introduction

Sense of temperature plays a critical role for living organisms to interact with their environment. Sensory neurons in dorsal root and trigeminal ganglia detect changes of temperature and signal to the spinal cord and the brain[1]. Recently, several transient receptor potential (TRP) ion channels have been identified as receptors of physiological temperature, and gene ablation studies have revealed a requirement of these thermoTRPs in innocuous and noxious thermosensation[1,2]. At least six thermoTRPs have been identified that are activated by temperature, and are expressed in skin and/or sensory neurons. TRPV1 (approximate threshold 43°C), TRPV2 (52°C), TRPV3 (33°C) and TRPV4 (25–34°C) are activated by heating[3–9];TRPM8 (25°C) and TRPA1 (17°C), by cooling[10–13]. TRPV3 has been shown to be expressed in mouse skin keratinocytes and human sensory neurons of dorsal root ganglia, and TRPV3 knockout mice show impaired responses to innocuous and noxious heat[5,8,14]. Similar to other TRP channels, TRPV3 is a polymodal receptor also activated by chemical

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J.G. and H.H contributed equally to this work. J.G. and H.H designed the study, collected and analyzed data and wrote the manuscript. M.B. participated in designing the temperature device. B.B. conducted molecular modelling. M.S. performed biochemical experiments. M.P. participated in producing the random mutant library. A.P. designed the study and wrote the manuscript. All authors discussed results and commented on the manuscript.

ligands such as the natural compound camphor and a synthetic compound 2-aminoethoxydiphenyl borate (2APB)[15–17].

Most TRP channels are tetrameric calcium-permeable non-selective cation channels with six transmembrane (TM) domains and an ion-pore between TM5 and TM6[18,19]. Several domains/residues have been mapped within thermoTRPs required for chemical and voltage-dependent activation; however the mechanism for temperature-activation remains unsettled. Temperature activation of most thermoTRPs is retained in cell-free membranes, arguing for a mechanism independent of cytoplasmic processes[20]. Voltage-dependent gating has been proposed as a basic principle for temperature-sensing in heat-activated TRPV1 and cold-activated TRPM8 channels[21–23]. In this theory, temperature and chemical compounds shift the voltage-dependent activation curves of TRPV1 and TRPM8 towards physiological membrane potentials. Recently, voltage, temperature and chemicals have been proposed to act through independent mechanisms but interacting allosterically with each other[24–26]. Chimeras with swapped C-terminal domains between TRPM8 and TRPV1 have switched temperature phenotypes, suggesting that C-terminals of thermoTRPs might determine the directionality of the temperature response[27,28].

Little is known about the temperature activation underlying other thermoTRPs including TRPV3. Many ion channels appear to be modular proteins with specific structural domains required for sensing individual stimuli. For example, charged residues within specific domains render channels voltage-sensitive. We thus hypothesized that TRPV3 might posses residues or domains that specifically affect temperature activation without changing overall channel function and set out to identify these.

Results

Random mutagenesis screen

To find residues in TRPV3 that are required for heat-activation we constructed a mutant library of ~14,000 clones of mouse TRPV3, each containing on average 2.5 randomly introduced single point-mutations [29]. In a 384-well format we screened human embryo kidney (HEK) cells transiently transfected with DNA of these clones in a calcium-influx assay with a Fluorescent Imaging Plate Reader (FLIPR). A novel custom-designed device was used to rapidly and precisely change the temperature across a 384-well plate simultaneously to optical readout, hence enabling us to measure compound- and heat-activation of TRPV3 clones in a high-throughput manner (Fig. 1 and Supplementary Fig. 1).

Using temperature, 2APB and camphor as agonists, we identified, validated and sequenced 15 clones from this library that showed normal 2APB and camphor dose-response curves, but significantly reduced heat responses compared to wild-type TRPV3 (Supplementary Table 1). Since most of the clones (12 of 15) had a point mutation either in proximity of TM 1 or TM 6 we decided to engineer each of these mutations individually. Again, we measured full dose-response curves for 2APB and camphor, and activation by temperature. Of the twelve single-point mutations tested, only five (Asn643Ser, Ile644Ser, Asn647Tyr, Leu657Ile and Tyr661Cys) specifically affected heat-activation, but not activation by 2APB or camphor (Fig. 2). Importantly, all five are clustered in a small domain adjacent to the channel pore (see below).

Electrophysiology on selected mutants

In order to validate our novel temperature screening method we performed patch-clamp experiments on HEK cells transiently transfected with DNA for the three single-point mutants (Ile644Ser, Asn647Tyr and Tyr661Cys) that showed complete and specific loss of temperature-activation. Three sequential heat-ramps followed by a pulse of 30 μM 2APB

confirmed that the heat response in these mutants was indistinguishable from pcDNA-transfected cells, whereas wild-type TRPV3 responses are large and sensitized by consecutives stimulations, as reported previously[30] (Fig. 3).

Since the identified mutations are located in the pore-region we performed single-channel and whole-cell recordings that demonstrate that the 2APB-dependent unitary conductance and the ion-selectivity of TRPV3 were unaltered by these mutations (Fig. 4a and b). Additionally, we observed that these mutants exhibit two distinct current-voltage phases during prolonged activation, a distinct feature that has been described previously for wild-type TRPV3 [31] (Fig. 4c and d). Together, these data demonstrate that these three point mutations do not alter the basic properties of TRPV3.

Voltage is a known modulator of TRPV3 activation, and we investigated the voltage-dependence for each mutant by a voltage-step protocol. From steady-state currents we calculated the voltage causing half-maximal activation (V_{half}) and found it identical to wild-type TRPV3 (Fig. 4e and f). Hence, these three mutations uncouple heat- from chemical- and voltage-sensitivity.

Detailed mutagenesis of a heat-specific domain

Our high-throughput random mutagenesis screen is not expected to be at saturation, as the library will not come close to carrying mutations in each amino acid with substitutions representing all other 19 residues. To explore the temperature-sensitive domain of TRPV3 in more detail, we generated a mutant library containing 45 random point-mutations for each position, between the selectivity filter and the last position identified in our initial screen (Leu642-Thr660) and tested it with temperature, 2APB and camphor. We found temperature-specific mutations in three additional positions (Supplementary Table 2), further highlighting the importance of this domain for temperature-sensitivity (Figure 5a). Interestingly, mutations within TM6 are located in a periodic pattern, potentially aligned on one side of an alpha-helical structure. Modeling the TRPV3 pore based on the crystal structure of Kv1.2[32] suggests that the three mutations are exposed to the lipid-facing side of TM6 (Figure 5b–e).

Xenopus TRPV3 and TRPV chimeras

TRPV3 sequence orthologs can be found in tetrapods but not in bony fishes or invertebrates. The most distant ortholog of mammalian TRPV3 with sequence information is *Xenopus tropicalis* (53% identity with mouse TRPV3). The extracellular loop region in question is highly divergent from mouse sequences, with all six amino-acids following the predicted selectivity-filter (E638-P643) being different in mouse and frog (Fig. 6a). This led us to speculate that xTRPV3 would be heat insensitive. Surprisingly, we found that frog TRPV3 responded to heat and 2APB. Although these data suggest that xTRPV3 is heat-gated, it is important to note that we added small N- and C-terminal mouse sequences to get a functional channel (see Supplemental Materials and Methods). To test directly whether extracellular loop residues of frog TRPV3 are involved in temperature-activation, we generated randomized single-point mutants for the six divergent positions and measured their sensitivity to heat and 2APB. Strikingly, we discovered heat-specific hits at every residue (Fig. 6b–c and Supplementary Table 3), demonstrating that this region, although highly different in primary sequence, is required for temperature-activation.

In addition to TRPV3, several other TRPV channels are activated by heat (TRPV1, ratTRPV2, TRPV4), whereas others are not (humanTRPV2, TRPV6)[1,9]. When aligning the amino-acid sequences of these channels we noticed that functional regions like pore, TMs and selectivity filter are well conserved, but the loop-region that contains many of the discovered heat-specific mutations varies largely (Supplementary Fig. 2a). We therefore engineered chimeric channels

where we placed different domains into a TRPV3 background and vice versa and tested their heat-sensitivity and response to their respective ligands (Supplementary Fig. 2b). As expected, our stringent requirement that chimeras have wild-type like responses to chemical stimulation in order to draw conclusions about the nature of heat-sensitivity was not fulfilled by most constructs (Supplementary Table 4). However, the exchange of a short domain of the heat-insensitive channel human TRPV2 into the background of TRPV3 was sufficient to completely abolish heat-activation, while not affecting 2APB- and camphor-sensitivity (Fig. 6d–e), underlining again the necessity of this region for heat-sensitivity in TRPV3.

Discussion

Categorized according to their mechanism of gating, ion channels can be activated by signals such as specific ligands, voltage, or mechanical force. To date, the mechanism underlying thermal activation of TRP channels represents a fundamental unknown in the field. Identification of structural elements in the channel involved in temperature sensing would be a first step towards a molecular and biophysical understanding of temperature gating. Our central finding here is the discovery of several single point-mutations clustered in a small domain in the pore region and TM6 that uncouple heat-activation of TRPV3 from all other known activation mechanisms.

Isolating temperature mutations with normal camphor and 2-APB responses ensures that the selected mutant channels are viable and basic properties such as expression level and overall functionality of TRPV3 is not perturbed. Furthermore, this approach directly tests the hypothesis whether activation of this channel by chemicals and heat can be separable. The fact that we have identified point mutants that are normal in all aspects of TRPV3 modulation (Fig. 3 and Fig. 4) except for heat responses strongly suggests that at least part of the heat-activation process is unique. This does not imply that chemical-activation and heat-activation are completely separable. Indeed, there is ample evidence that thermoTRPs are allosterically gated polymodal receptors [25].

Two key aspects of ion channel functionality are the ability of the proteins to sense stimuli and to gate (open) the channel pore. For example, TRP-related six transmembrane-containing voltage-activated ion channels sense voltage through positively-charged amino acids in the fourth transmembrane domain. The movement of this domain is thought to transduce a conformational change on nearby pore-spanning transmembrane domain six and directly gate the ion channel. Is there a similar temperature sensor domain in thermoTRPs? Is this domain localized or scattered throughout the protein? It is very striking that all the heat-specific mutations we identified are clustered within the pore region. We further show that a TRPV3 chimera carrying 28 amino acids of TRPV2 (TM6 and loop region) has normal chemical-activation but no heat-activation. This result further reinforces the conclusion that the TM6 and adjoining extracellular loop domain is specifically required for heat-sensitivity of TRPV3. Our data do not reveal specifically how these mutations affect temperature-activation mechanistically. Mutations could shift the activation-threshold to temperatures that are higher than the ones tested, or completely disrupt the temperature-sensing mechanism. In either case, the results suggest that TM6 and the adjacent extracellular loop are structures essential for temperature-sensing or for the gating-transition from an upstream temperature-sensing domain. Close proximity of this temperature module to the pore makes it tempting to speculate that temperature-induced conformational changes in this extracellular loop and adjoining TM6 helix may directly gate the channel.

Our studies have focused on TRPV3, and it may be that the mechanism of temperature sensitivity in other thermoTRPs is different [20,25]. However, it is also possible that the TM6 region identified here for TRPV3, and the C-terminal domain identified in TRPV1 and TRPM8

(which is mainly involved in determining the directionality of temperature gating), might work in concert[28]. The temperature domain isolated for mouse TRPV3 appears to be conserved in xenopus TRPV3, although the primary sequence of the loop region is not conserved between the two species. Perhaps biophysical characteristics of the loop region such as conformation or its flexibility determine the requirement for heat-sensitivity. Modelling of the TRPV3 pore suggests that the TM6 mutations are facing the membrane, and puts forward an alternative hypothesis that protein-lipid interactions might be involved in temperature-sensitivity. Future experiments can test these possibilities. For example, environmentally-sensitive labels could probe stimulus-dependent structural rearrangements of this region. Recordings in artificial membranes could test whether heat-activation of TRPV3 is dependent on the bilayer composition or structure. Finally, since temperature is equivalent to molecular motion, NMR experiments could be a promising approach to elucidate the mechanism of temperature activation.

Methods

See Supplementary Information for detailed Methods and Materials.

Screen

Wells in 384-well plates were challenged with a temperature step from 25°C to 42°C for 120s (Fig. 1). A final concentration of 25 μM 2APB was added on the same plates to determine the sensitivity to 2APB. Separate plates were prepared for stimulation by a final concentration of 1.75 mM camphor. Concentration-response curves were obtained from three wells for a given compound concentration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

1. Dhaka A, Viswanath V, Patapoutian A. TRP Ion Channels and Temperature Sensation. Annu Rev Neurosci. 2006
2. Caterina MJ. Transient receptor potential ion channels as participants in thermosensation and thermoregulation. Am J Physiol Regul Integr Comp Physiol 2007;292(1):R64–R76. [PubMed: 16973931]
3. Caterina MJ, et al. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997;389(6653):816–824. [PubMed: 9349813]
4. Guler AD, et al. Heat-evoked activation of the ion channel, TRPV4. J Neurosci 2002;22(15):6408–6414. [PubMed: 12151520]
5. Peier AM, et al. A heat-sensitive TRP channel expressed in keratinocytes. Science (New York, N.Y 2002;296(5575):2046–2049.
6. Xu H, et al. TRPV3 is a calcium-permeable temperature-sensitive cation channel. Nature 2002;418(6894):181–186. [PubMed: 12077604]
7. Watanabe H, et al. Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives. The Journal of biological chemistry 2002;277(16):13569–13577. [PubMed: 11827975]

8. Smith GD, et al. TRPV3 is a temperature-sensitive vanilloid receptor-like protein. Nature 2002;418 (6894):186–190. [PubMed: 12077606]
9. Neeper MP, et al. Activation properties of heterologously expressed mammalian TRPV2: evidence for species dependence. The Journal of biological chemistry 2007;282(21):15894–15902. [PubMed: 17395593]
10. McKemy DD, Neuhausser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 2002;416(6876):52–58. [PubMed: 11882888]
11. Peier AM, et al. A TRP channel that senses cold stimuli and menthol. Cell 2002;108(5):705–715. [PubMed: 11893340]
12. Story GM, et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 2003;112(6):819–829. [PubMed: 12654248]
13. Kobayashi K, et al. Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. The Journal of comparative neurology 2005;493(4):596–606. [PubMed: 16304633]
14. Moqrich A, et al. Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science (New York, N.Y 2005;307(5714):1468–1472.
15. Hu HZ, et al. 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3. The Journal of biological chemistry 2004;279(34):35741–35748. [PubMed: 15194687]
16. Vogt-Eisele AK, et al. Monoterpenoid agonists of TRPV3. British journal of pharmacology 2007;151 (4):530–540. [PubMed: 17420775]
17. Xu H, Blair NT, Clapham DE. Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. J Neurosci 2005;25(39): 8924–8937. [PubMed: 16192383]
18. Owsianik G, D'Hoedt D, Voets T, Nilius B. Structure-function relationship of the TRP channel superfamily. Reviews of physiology, biochemistry and pharmacology 2006;156:61–90.
19. Owsianik G, Talavera K, Voets T, Nilius B. Permeation and selectivity of TRP channels. Annual review of physiology 2006;68:685–717.
20. Bandell M, Macpherson LJ, Patapoutian A. From chills to chilis: mechanisms for thermosensation and chemesthesis via thermoTRPs. Current opinion in neurobiology 2007;17(4):490–497. [PubMed: 17706410]
21. Nilius B, et al. Gating of TRP channels: a voltage connection? The Journal of physiology 2005;567 (Pt 1):35–44. [PubMed: 15878939]
22. Voets T, et al. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 2004;430(7001):748–754. [PubMed: 15306801]
23. Voets T, et al. TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nature chemical biology 2007;3(3):174–182.
24. Brauchi S, Orio P, Latorre R. Clues to understanding cold sensation: thermodynamics and electrophysiological analysis of the cold receptor TRPM8. Proceedings of the National Academy of Sciences of the United States of America 2004;101(43):15494–15499. [PubMed: 15492228]
25. Latorre R, et al. ThermoTRP channels as modular proteins with allosteric gating. Cell calcium 2007;42 (4–5):427–438. [PubMed: 17499848]
26. Matta JA, Ahern GP. Voltage is a partial activator of thermo-sensitive TRP channels. The Journal of physiology. 2007
27. Brauchi S, et al. Dissection of the components for PIP2 activation and thermosensation in TRP channels. Proceedings of the National Academy of Sciences of the United States of America 2007;104 (24):10246–10251. [PubMed: 17548815]
28. Brauchi S, et al. A hot-sensing cold receptor: C-terminal domain determines thermosensation in transient receptor potential channels. J Neurosci 2006;26(18):4835–4840. [PubMed: 16672657]
29. Bandell M, et al. High-throughput random mutagenesis screen reveals TRPM8 residues specifically required for activation by menthol. Nature Neurosci 2006;9(4):493–500. [PubMed: 16520735]
30. Chung MK, et al. 2-aminoethoxydiphenyl borate activates and sensitizes the heat-gated ion channel TRPV3. J Neurosci 2004;24(22):5177–5182. [PubMed: 15175387]

31. Chung MK, Guler AD, Caterina M. Biphasic currents evoked by chemical or thermal activation of the heat-gated ion channel, TRPV3. J. Biol. Chem 2005;260(16):15928–15941. [PubMed: 15722340]

32. Long SB, Campbell EB, MacKinnon R. Crystal Structure of a mammalian voltage-dependent Shaker family K+ channel. Science 2005;309(5736):897–903. [PubMed: 16002581]

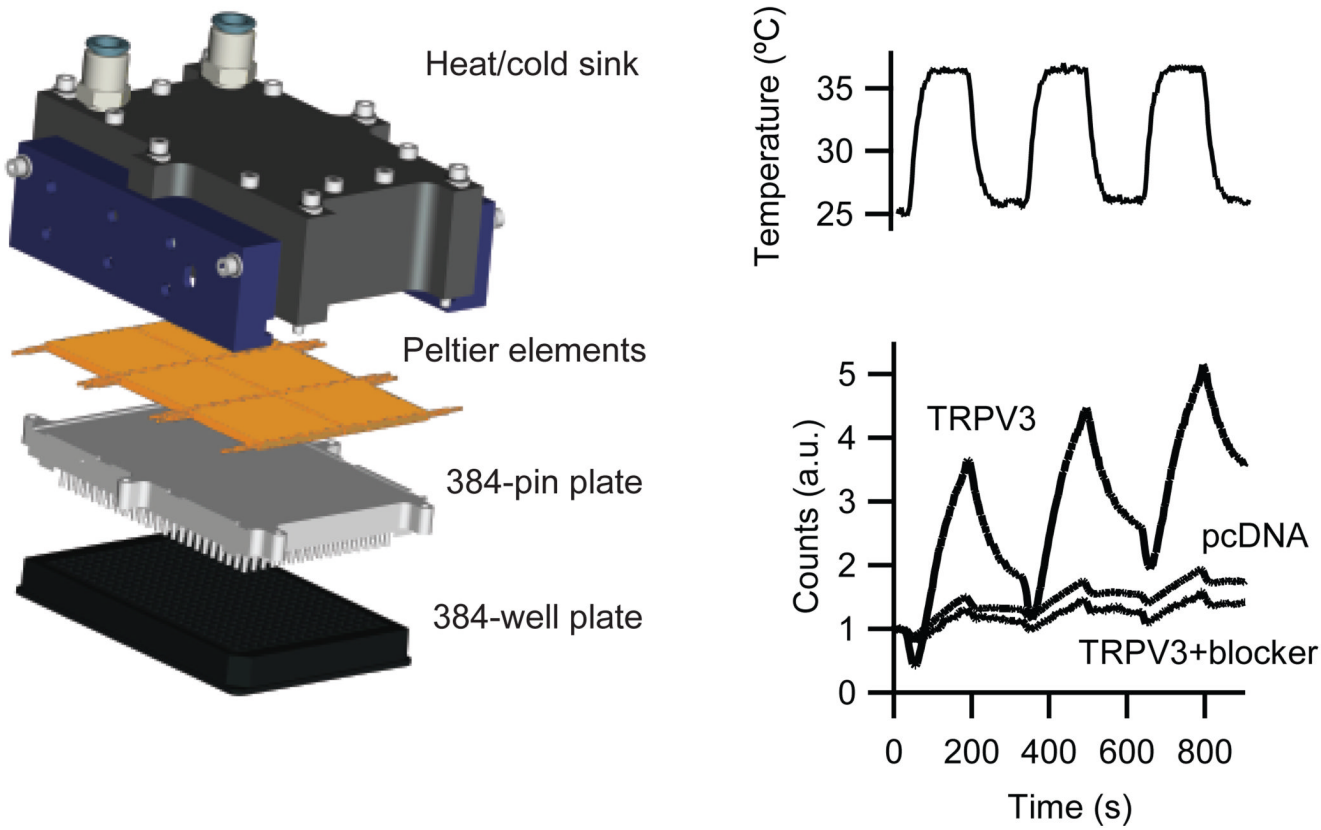

Fig. 1. TRPV3 mutant library screen

Image of custom-designed device that was used to control temperature across 384-well plates during optical readout in a FLIPR plate-reader. Peltier elements (orange) chilled by a water chamber on top (dark grey and blue) heat or cool a metal plate (light grey) with single pins penetrating into a 384-well plate (black). On the right, the activation profile of TRPV3 (red), its block by 10 μM ruthenium red (grey) and pcDNA transfected cells (blue) upon temperature-stimulation (above) is shown.

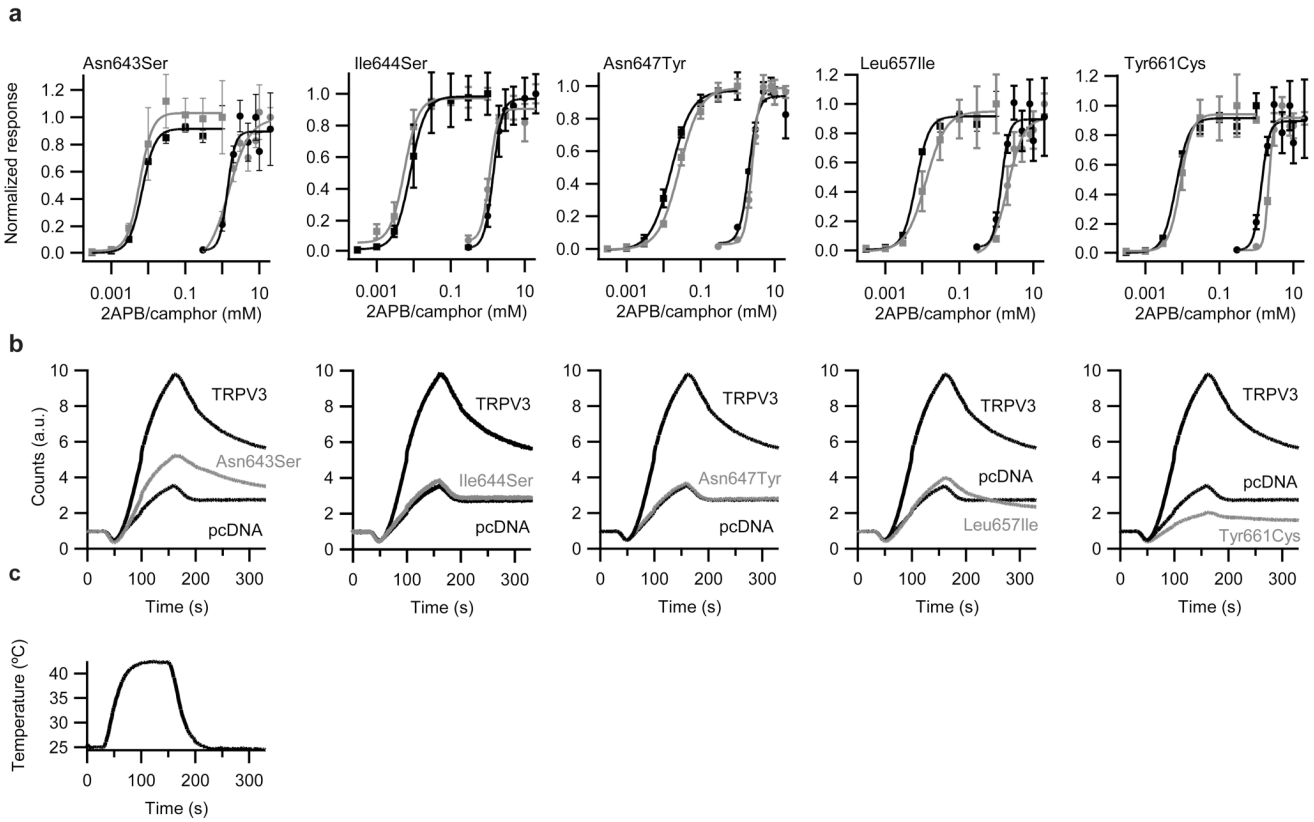

Fig. 2. Concentration-responses and heat-activation curves for single-point mutants

a, Normalized dose-responses of 2APB (squares) and camphor (circles) for single-point mutants Asn643Ser, Ile644Ser, Asn647Tyr, Leu657Ile andTyr661Cys (all grey) and TRPV3 wild-type (red). Curves are fits of Hill-equations to data that were obtained from multiple plates. Error bars indicate s.d., n=3.

b, Average responses of respective mutants (grey), TRPV3 wild-type (red) and pcDNA (blue) upon heat-stimulation (c), n=16.

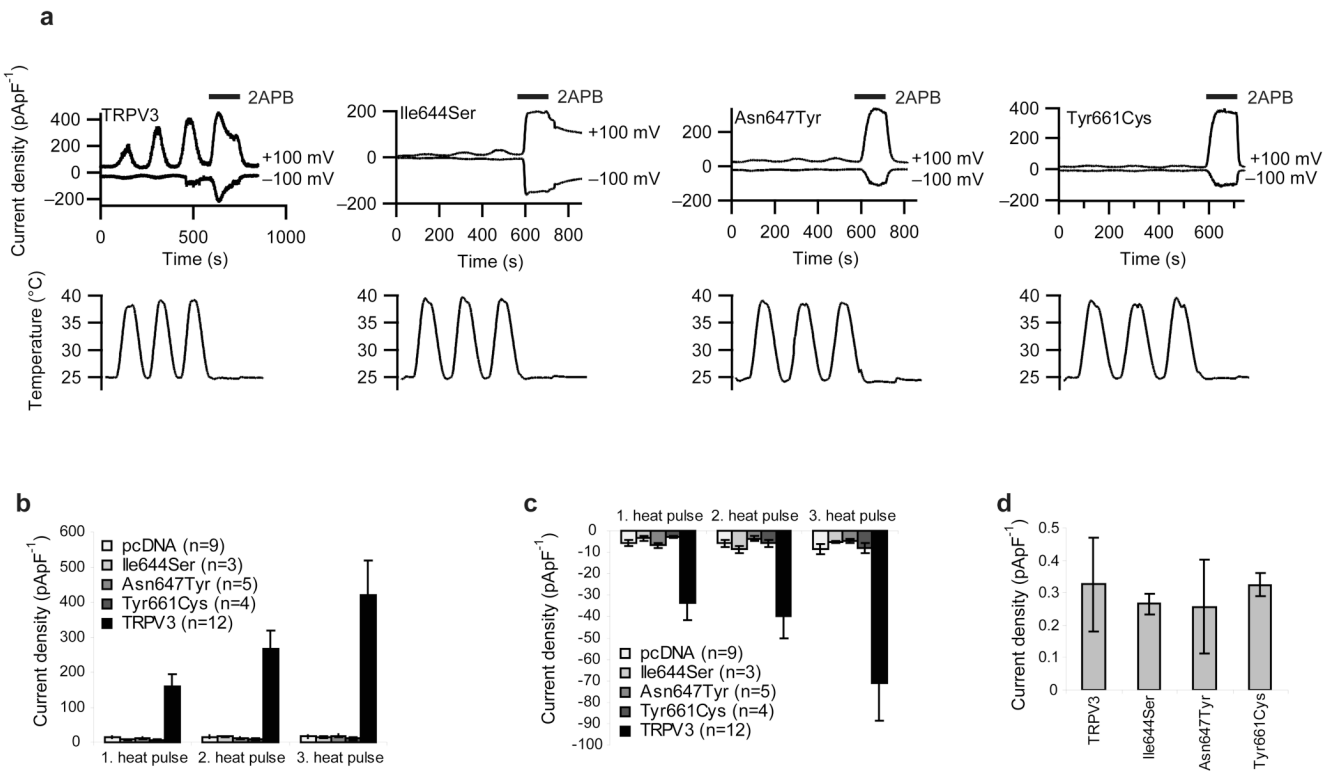

Fig. 3. Electrophysiology of heat-sensitivity of single-point mutants Ile644Ser, Asn647Tyr, Tyr661Cys and wild-type TRPV3

a, An example of current-density traces of whole-cell recordings from transiently transfected HEK293 cells at +100 mV and −100 mV. Cells were stimulated with three consecutive heat ramps (25 to 39°C) and subsequently challenged with 30 μM 2APB to determine channel expression.

Averaged maximal current densities for each heat pulse at +100 mV (b) and −100 mV (c). Error bars are s.e.m. Numbers (n) of individually tested cells are indicated in Fig.s (b) and (c). d, Average current-densities evoked by 30 μM 2APB without prior heat-stimulation. Errors are s.d. (n=3)

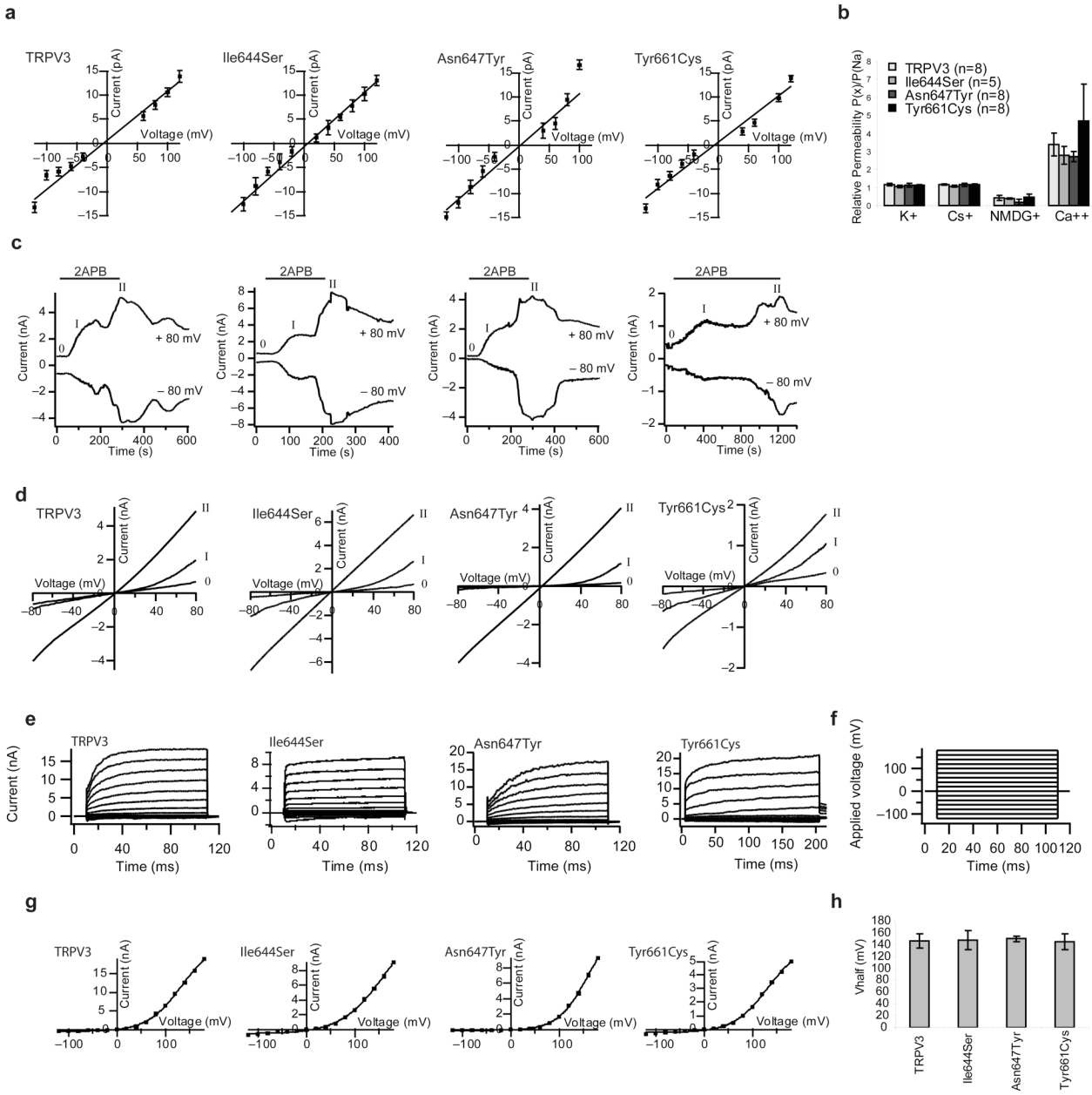

Figure 4. Basic channel properties of Ile644Ser, Asn647Tyr, Tyr661Cys and wild-type TRPV3

a, Single-channel current-amplitudes obtained from Gaussian fits to current-histograms as a function of voltage. Error bars are s.d., straight lines linear fits to the data. Average values±s.d. of unitary conductance are 112±4 pS (Ile644Ser), 109±6 pS (Asn647Tyr), 96±6 pS (Tyr661Cys) and 101±5 pS (wild-type TRPV3).

b, Calculated relative permeabilities of TRPV3 and mutant channels for selected ions. Numbers (n) are indicated for each channel, error bars are s.e.d.

c, Examples of whole-cell currents (n=3) from cells transfected with TRPV3 or mutant channels, continuously stimulated by 25 μM 2APB, at −80mV and +80mV. Respective current-voltage measurements before (0), and during (phase I and phase II) stimulation are shown in (d). Note that mutant Tyr661Cys enters phase II only after prolonged application of 2APB as compared to wild-type TRPV3.

e, current-voltage plots from steady-state currents of whole-cell patches, stimulated by 30 μM 2APB during a voltage-step protocol. Lines show sigmoid fits to the data points.
f, Average values±s.d. (n=3) of voltage that causes half-maximal steady-state currents (V_{half}).

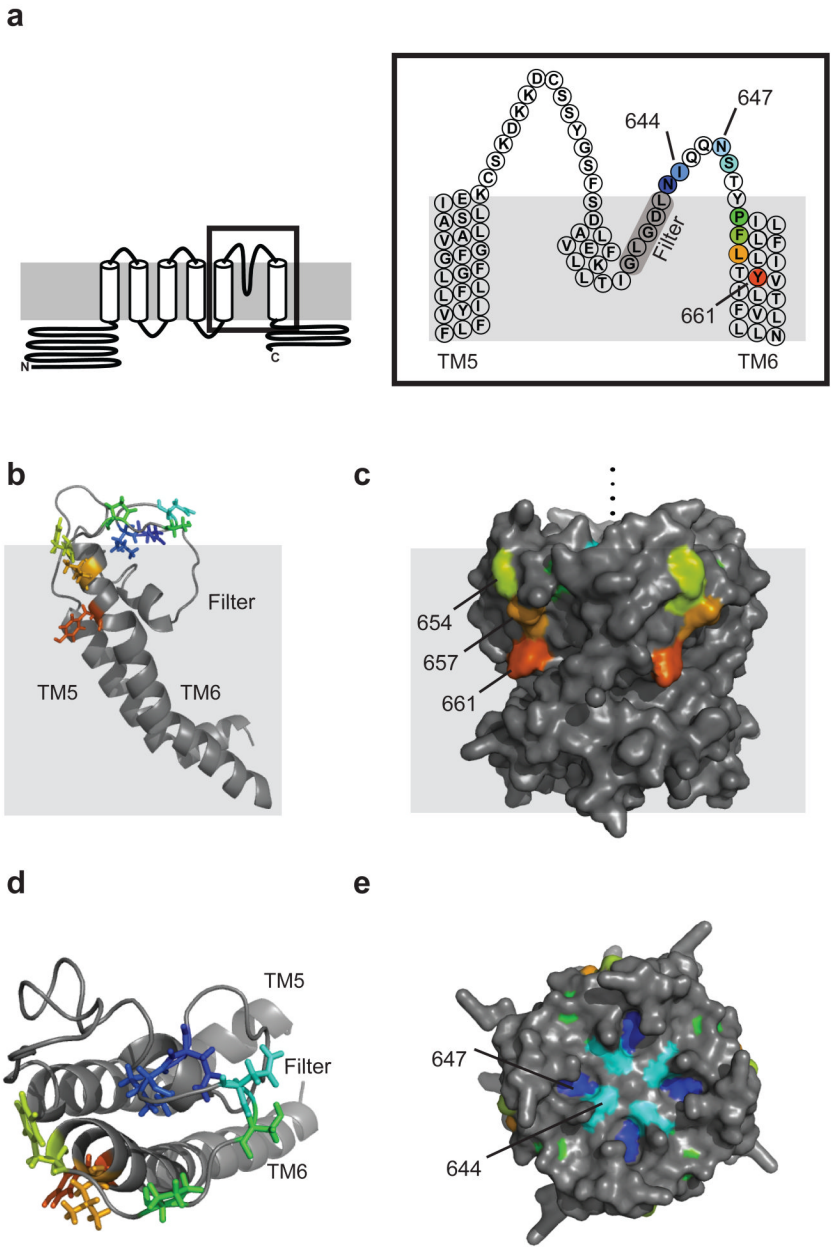

Fig. 5. Location of the heat-sensitive domain

a, Schematic of TRPV3 channel topology with positions of heat-specific mutations indicated in colours. Residues contributing to the selectivity filter are highlighted grey.

b, Side-view of homology model structure of pore-region of one subunit. Side-chains of heat-specific mutations are coloured individually.

c, Side view of the predicted surface of the TRPV3 pore region homology model. Temperature-specific mutations (Phe654Ser, Leu657Glu, Tyr661Cys) are lining the putative membrane exposed part of TM6 and are coloured individually.

d, Top view of homology model structure of pore-region of one subunit.

e, Top view of the predicted surface of the TRPV3 pore region homology model.

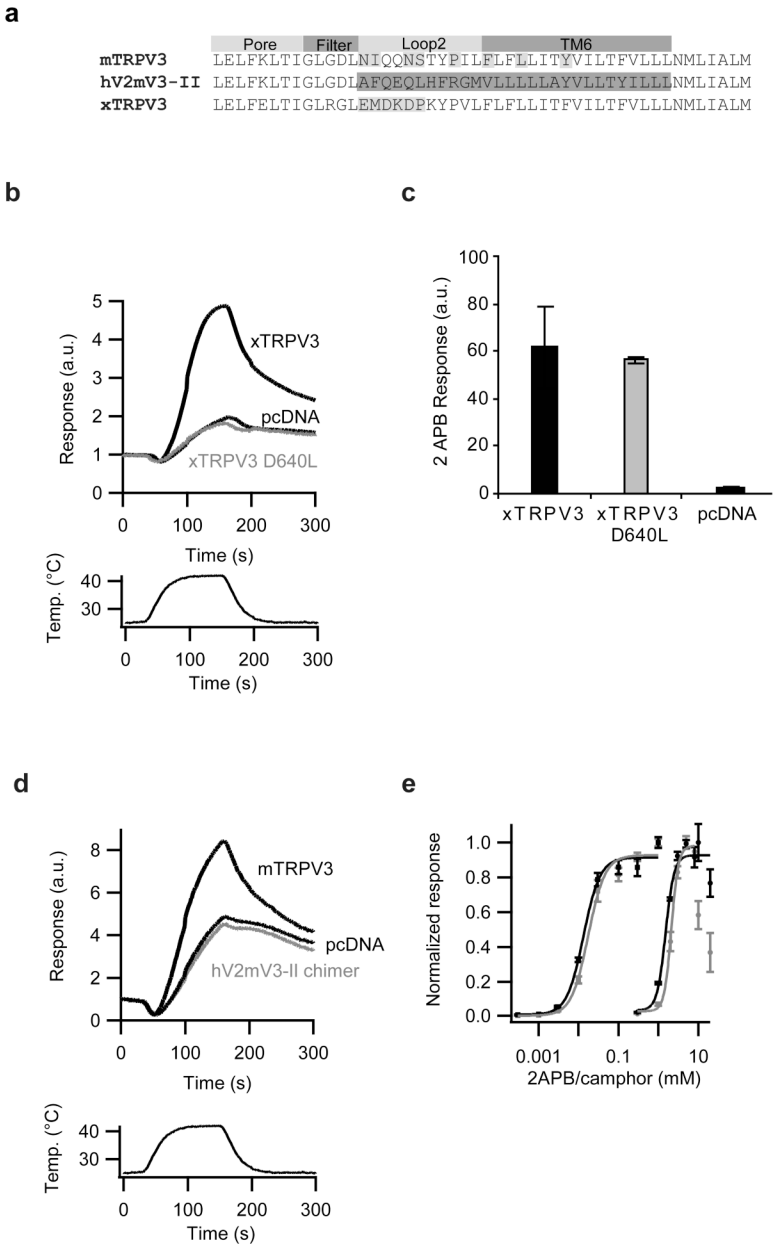

Fig. 6. Xenopus TRPV3 and TRPV chimeric channels

a, Alignment of amino-acid sequences of mouse TRPV3, the chimeric construct hV2mV3-II and xenopus TRPV3 from the pore-region to TM6. Locations of different functional domains are indicated by coloured boxes above alignment. Locations of heat-specific single-point mutations are labelled red. The yellow box marks the swapped domain of the chimeric construct.

b, Average FLIPR responses of xenopus TRPV3 (red), pcDNA (blue) (n=64 each) and one of the heat-specific mutants xTRPV3 D640L (grey) (n=4) upon heat-stimulation (below).

c, Average FLIPR response of xenopus TRPV3 (red) (n=64), pcDNA (blue) (n=12) and the mutant D640L (grey) (n=4) upon stimulation with 25 μM 2APB. Error bars indicate s.d.

d, Average FLIPR responses of the chimera hV2mV3-II (grey), TRPV3 wild-type (red) and pcDNA (blue) upon heat-stimulation (below), n=48.

e, Normalized FLIPR dose-response for chimera hV2mV3-II (grey) and TRPV3 wild-type (red) for 2APB (squares) and camphor (circles). Lines are fits of Hill-equations to the data. Error bars indicate s.d., n=3.