Mapping the Interaction Site between $Na_V1.5$ and TRPM4

Comparison of TRPM4 Wild-Type and the Y790C TRPM4 Variant

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1. Abstract

This thesis examines the interaction between the human wild-type voltage-gated sodium channel ($Na_V1.5$) and the human wild-type Transient Receptor Potential Melastatin 4 (TRPM4) ion channels, which are crucial for regulating cardiac electrical signals. Mutations or changes in their interactions can lead to heart disease. The research group of Professor Dr. Abriel has demonstrated the interaction between both full-length $Na_V1.5$ and TRPM4 proteins using co-immunoprecipitation (Co-IP), which led to this experiment. TRPM4 is also suggested to modulate $Na_V1.5$ activity [1]. This basic research aims to understand these physiological interactions in greater detail to better comprehend heart diseases caused by mutations in these ion channels, with potential future therapeutic applications. The aim was to identify specific $Na_V1.5$ domains interacting with TRPM4 using techniques like Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), Western blot (WB), Co-IP, and cell culture with Human Embryonic Kidney 293 (HEK293) cells. Results showed inconsistent interactions, and it is unclear whether full-length $Na_V1.5$ interacts with TRPM4 or if $Na_V1.5$ parts 1, 6, and 7 do not. Further research is needed to fully understand these interactions.

2. Introduction

This thesis examines the transmembrane ion channels $Na_V1.5$ and TRPM4 in cardiac cells, essential for generating electrical signals that enable heart contractions and blood circulation [2]. Mutations in these channels can cause similar conduction disorders [3]. $Na_V1.5$ is found in ventricular and atrial muscle cells [4], part of the NaV1–NaV9 family, and is encoded by the SCN5A gene. $Na_V1.5$ plays a crucial role for initiating sodium currents in the heart's electrical activity and consists of four domains (DI-DIV) connected by interdomain linkers (N-terminal, ID 1-2, ID 2-3, and ID3-4, C-terminal), forming a circular structure [5]. It has a molecular weight of 220 kilodalton (kDa) [6]. TRPM4, part of the Transient Receptor Potential gene family, depolarizes the membrane and facilitates muscle contraction but its full function remains unclear. TRPM4 has a circular configuration [7] and a molecular weight of 130 kDa [8]. TRPM4 is activated by intracellular calcium but is impermeable to it.
TRPM4 knockout mice showed a 25-30% reduction in $Na_V1.5$-mediated sodium currents, suggesting TRPM4 modulates $Na_V1.5$. This interaction was confirmed by Co-IP[9].

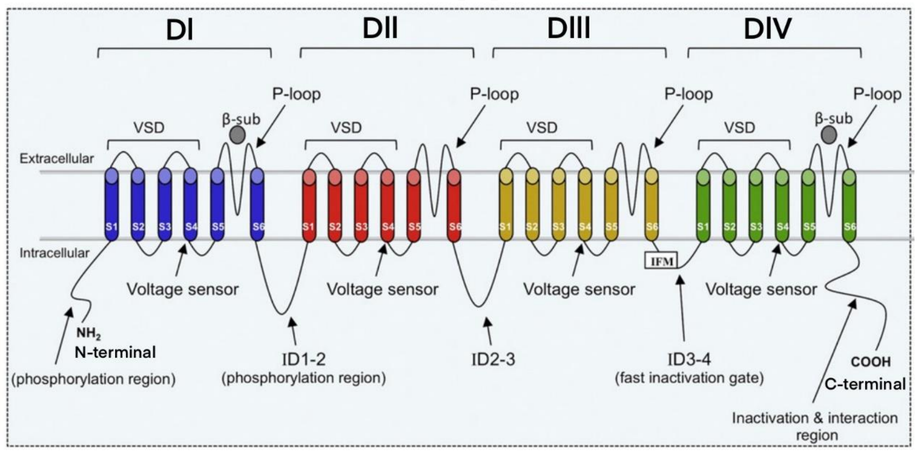

Figure 1: Representation of the full-length $Na_v1.5$ ion channel, detailing all domain and interdomain connections – adapted

3. Aim and leading question

The aim is to investigate the specific interaction domain between full-length TRPM4 channel and precisely defined domains of $Na_V1.5$ channel.

<u>Leading question:</u>

Which specific domain or domains of $Na_V1.5$ channel does full-length TRPM4 channel interact with?

4. Methods and Material

$Na_V1.5$ was divided into nine parts, each labelled with a Hemagglutinin (HA) tag. HEK293 cells were transfected with TRPM4 and one $Na_V1.5$ part. Co-IP was performed in two setups. Setup 1 used Pierce™ anti-HA magnetic Beads to isolate $Na_V1.5$ parts, and setup 2 used TRPM4 antibodies with Dynabeads™ Protein A to isolate TRPM4.Positive controls were used in both setups, while a negative control was used only in setup 1. An immunoglobulin G control verified the specificity of the TRPM4 antibody in setup 2. Actin served as a loading control in both setups, and syntrophin was used as a Co-IP control in setup 1. SDS-PAGE analysis was conducted, followed by WB. The nitrocellulose membrane was blocked, incubated with primary antibodies (TRPM4, HA, syntrophin, actin), and then treated with fluorescent secondary antibodies (anti-rabbit, anti-mouse).
The interactions were analysed using a Western blot imager, with results noted as "Yes" or "No".

5. Results

In setup 1, the positive control and Co-IP control did not always work, preventing some results from being interpreted. An interaction between all $Na_V1.5$ parts and TRPM4 was observed. In setup 2, all controls worked correctly, but inconsistent interactions were seen for $Na_V1.5$ parts 1 and 6, and no interaction was detected for part 7.

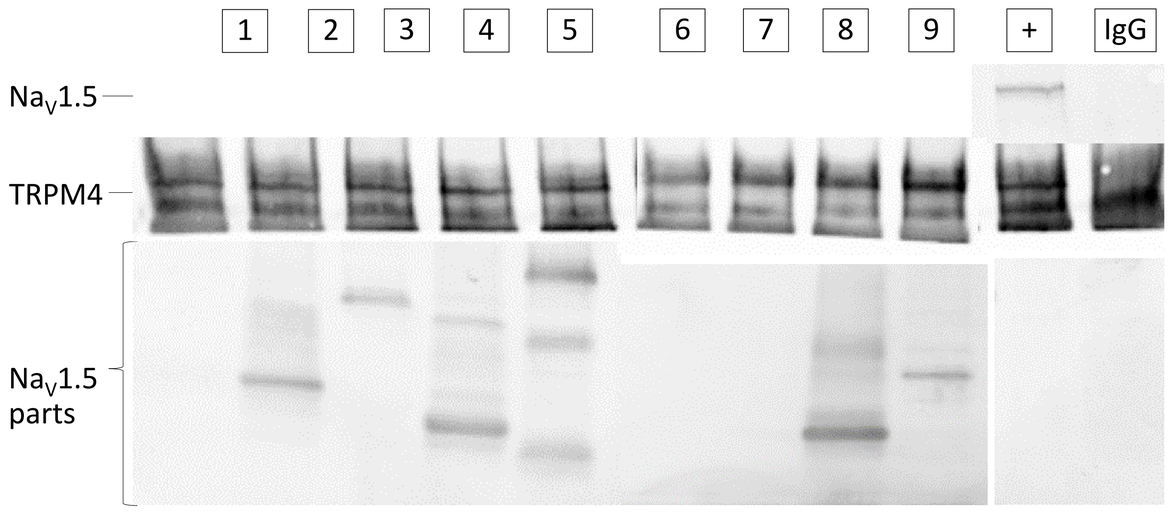

Figure 2: summarizes the results from Table 8 of the thesis. It shows Co-IP Setup 2, Series 1, with $Na_V1.5$ parts 1-9 interacting with full-length TRPM4. The numbers 1-9 correspond to $Na_V1.5$ parts 1-9, while "+" represents the positive control and "IgG" denotes the immunoglobulin G control. Actin and syntrophin are not displayed for clarity in this image

6. Discussion

In Setup 1 positive control had issues, likely due to $Na_V1.5$-HA expression problems or detection difficulties, while Co-IP control issues may have been caused by protein damage during cell lysis. Setup 2 showed inconsistent interactions in parts 1 and 6, possibly due to variable expression levels or structural differences compared to full-length $Na_V1.5$. No interaction was seen with part 7, possibly due to its small size, loss during Co-IP or structural changes. The results suggest either full-length $Na_V1.5$ interacts with TRPM4, or only parts 2, 3, 4, 5, 8, and 9 interact. Further research is needed to confirm these interactions. Professor Dr. Abriel's group is working on this interaction using cryo-electron microscopy.

List of references

[1] Ozhathil et al., 2021, pp. 10–13.
[2] Grant, 2009, p. 185
[3] Daimi et al., 2022, pp. 1–25.
[4] Boron et al., 2009, p. 506.
[5] Grant, 2009, pp. 186–188
[6] Abriel et al., 2015, p. 1972.
[7] Abriel et al., 2012, pp. 873–874
[8] Daumy et al., 2016, p. 355
[9] Ozhathil et al., 2021, pp. 10–13

List of figures

Figure 1: Detta et al., 2015, p. 1503
Figure 2: Järmann, J. (2024). *summarizes the results from Table 8 of the thesis. It shows Co-IP Setup 2, Series 1, with Na $_V$ 1.5 parts 1-9 interacting with full-length TRPM4. The numbers 1-9 correspond to Na $_V$ 1.5 parts 1-9, while "+" represents the positive control and "IgG" denotes the immunoglobulin G control. Actin and syntrophin are not displayed for clarity in this image*. medi.