# Molecular physiology of nonselective cation channels in metal permeation and cellular proliferation, survival, and death

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## Summary

There is ample evidence that continuous $Ca^{2+}$ influx critically controls the cell division cycle, and its abnormalities may be associated with aberrant cell growth such as tumors, hypertrophy and hyperplasia. However, few clues have been obtained for the molecular entity of the $Ca^{2+}$ influx pathway. Here we show the evidence that a homologue of the melastatin subfamily of transient receptor potential protein TRPM7, which was previously shown to be an intracellular $Mg^{2+}$/ATP-regulated, rare metal-permeable cation channel and implicated in cell viability, likely contributes to the growth control of human retinoblastoma (RB) cell. In RB cells, under voltage-clamp with the nystatin-perforated recording, a spontaneous cation current ($I_{spont}$) having outward-rectifying and $Ca^{2+}$/$Mg^{2+}$-inhibited but -permeating properties was recorded. The magnitude of $I_{spont}$ appeared well correlated with that of spontaneous $Ca^{2+}$ entry measured by $Ca^{2+}$ fluorescent imaging technique, and the rate of proliferation of RB cell, all of which were depressed by serum deprivation and various cation channel blockers such as $Gd^{3+}$, $La^{3+}$, LOE908 and 2-APB with similar efficacies. Excision of RB cell membrane (inside-out) into MgATP-free solution induced a 70pS single channel activity, which was effectively inhibited by $Mg^{2+}$ or MgATP complex in their millimolar range. RT-PCR and immunocytochemical experiments revealed abundant expression of TRPM7 mRNA and protein in RB cells and heterologous expression of TRPM7 in HEK293 cells reproduced the key features of $I_{spont}$. In contrast, elimination of this protein from RB cells by antisense oligonuceotides, RNA silencing, or co-transfection of loss-of function TRPM7 mutants markedly reduced the rate of spontaneous $Ca^{2+}$ entry and the density of $I_{spont}$, which was paralleled by a decrease in TRPM7-immunoreactivity and a retardation of $G_1$/S cell cycle progression. These results strongly suggest the essential importance of TRPM7 in RB cell proliferation as a component or regulator of spontaneously activated $Ca^{2+}$ influx pathway which turns active upon serum stimulation.