Geeniregulatsioon ja signaaliülekanne närvisüsteemis ja selle patoloogiates

Tõnis Timmusk
Keemia ja biotehnoloogia instituut
Tallinna Tehnikaülikool

Eesti teaduste akadeemia 24.10.2023

BIOGRAPHICAL SKETCH

NAME Timmusk, Tõnis	POSITION TITLE Professor of Molecular Biology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Tartu	M.Sc.	06/1982	Biology: cytology and genetics
Karolinska Institute	Ph.D.	06/1994	Molecular neurobiology
Karolinska Institute	Postdoctoral	11/1996	Molecular neurobiology

Positions and Employment

1996-1999 Research Assistant Professor, Department of Neuroscience, Uppsala University, Sweden

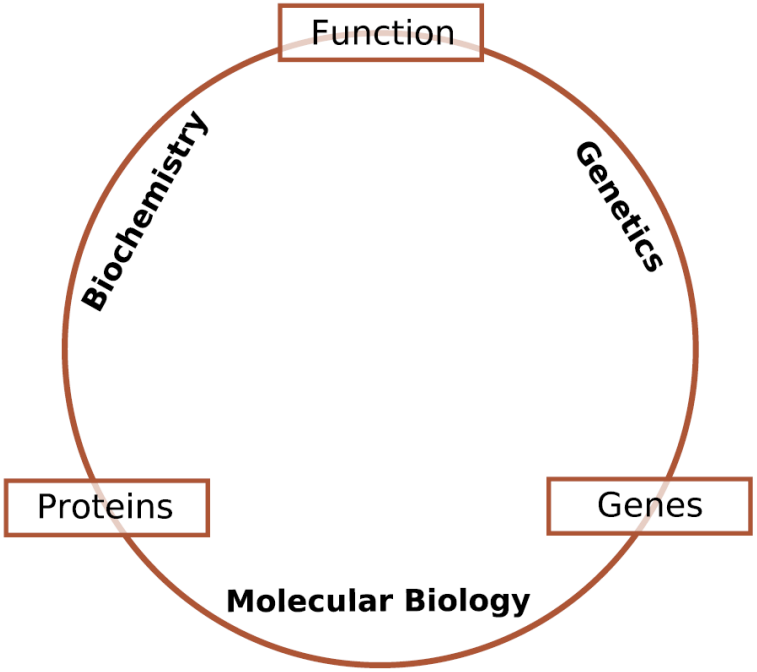
1999-2003 Senior Research Scientist, Institute of Biotechnology, University of Helsinki, Finland

2003-2008 Wellcome Trust International Senior Research Fellow, National Institute of Chemical Physics and Biophysics and Department of Gene Technology, Tallinn University of Technology, Estonia

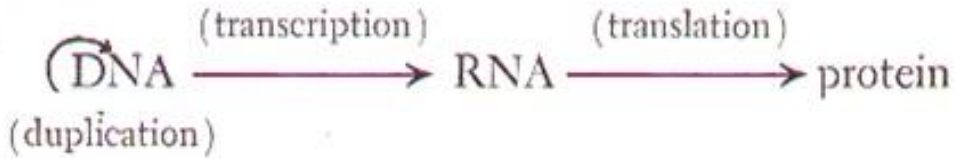
2003 - 2017 Professor, Chair of Molecular Biology, Department of Gene Technology, Tallinn University of Technology, Estonia

2017 - present Professor of Molecular Biology, Department of Chemistry and Biotechnology, Tallinn University of Technology, Estonia

Molekulaarbioloogia – mis see on?



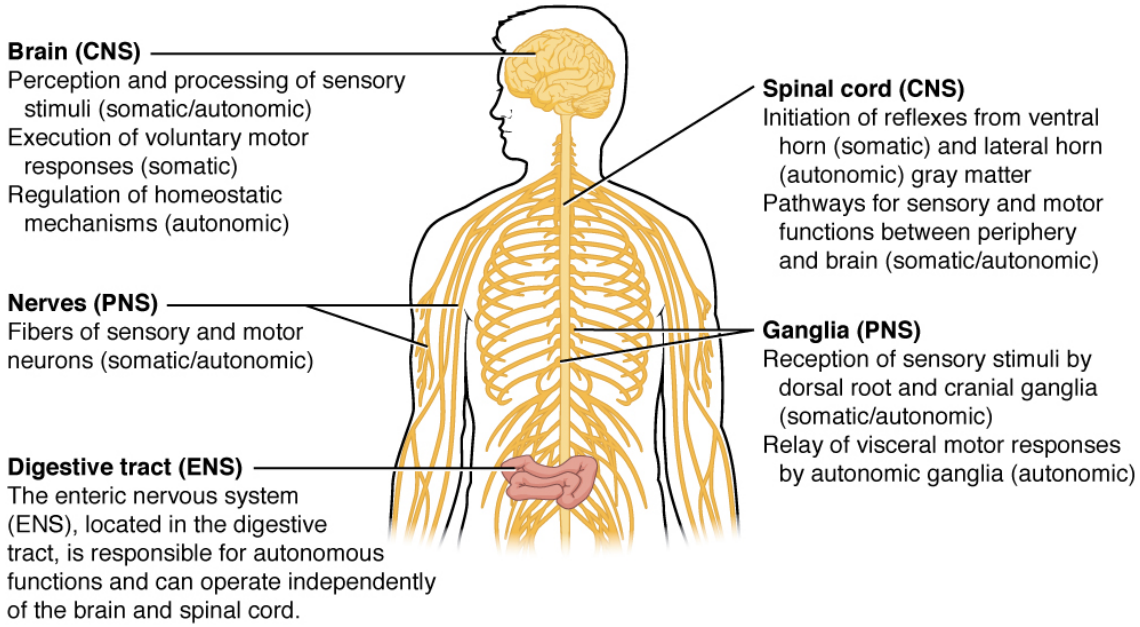
Molekulaarbioloogia dogma (lihtsustus)



Crick &
Watson, 1956

Närvisüsteem

- kesknärvisüsteem
  - peaaju
  - seljaaju
- perifeerne närvisüsteem
  - närvid
  - perifeersed ganglionid
- enteerne närvisüsteem
  - paikneb seedeelundkonnas
  - võib töötada sõltumatult kesknärvisüsteemist

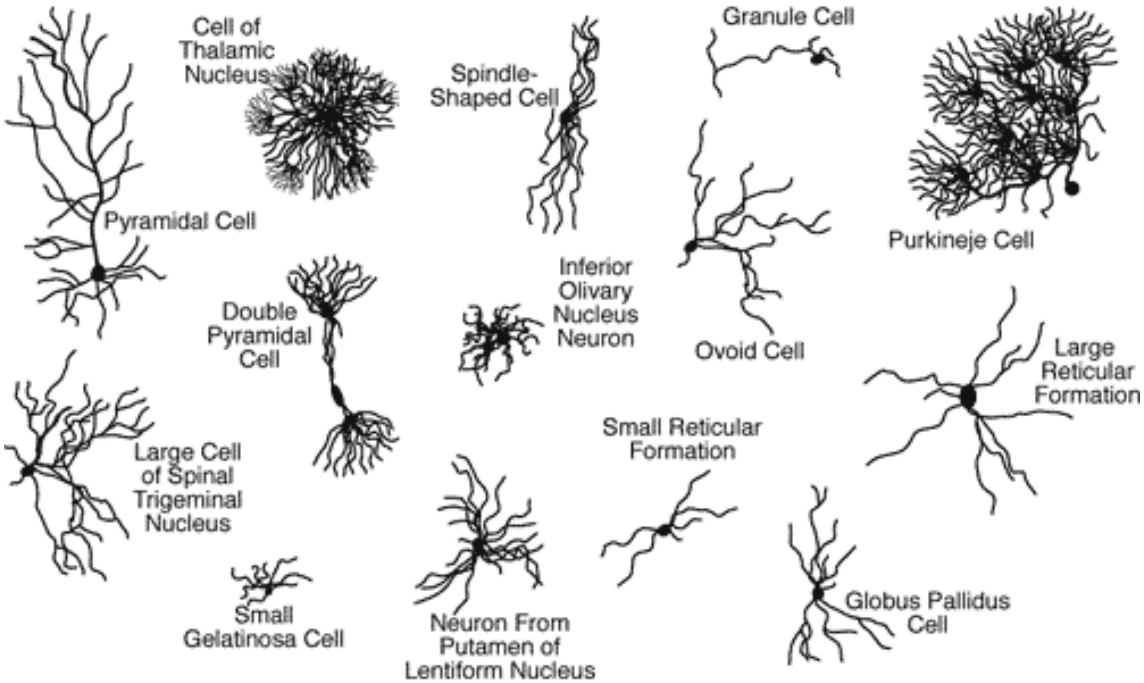


Närvikoe rakud

Neuronid ehk närvirakud - elektriliselt erutuvad rakud

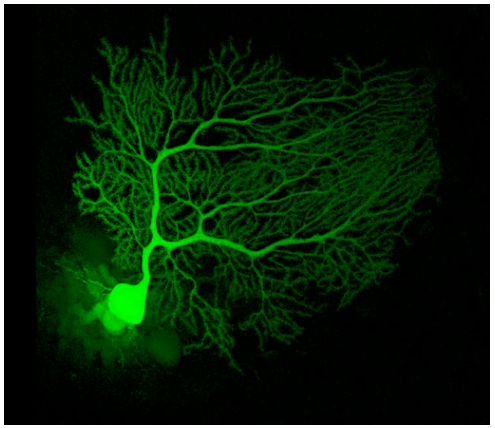
Gliia – mitte-erutuvad rakud

Neuronite arhitektuurne mitmekesisus



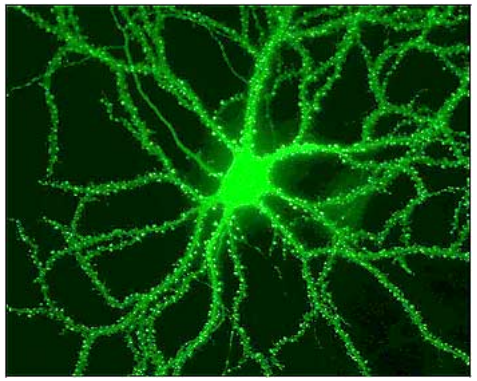
http://www.mind.ilstu.edu

väikeaju Purkinje neuron



wikipedia

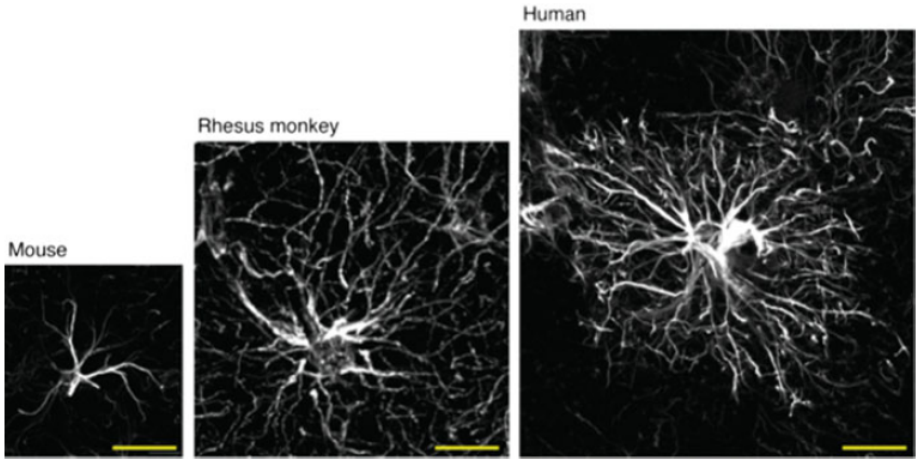
hipokampuse püramidaalneuron



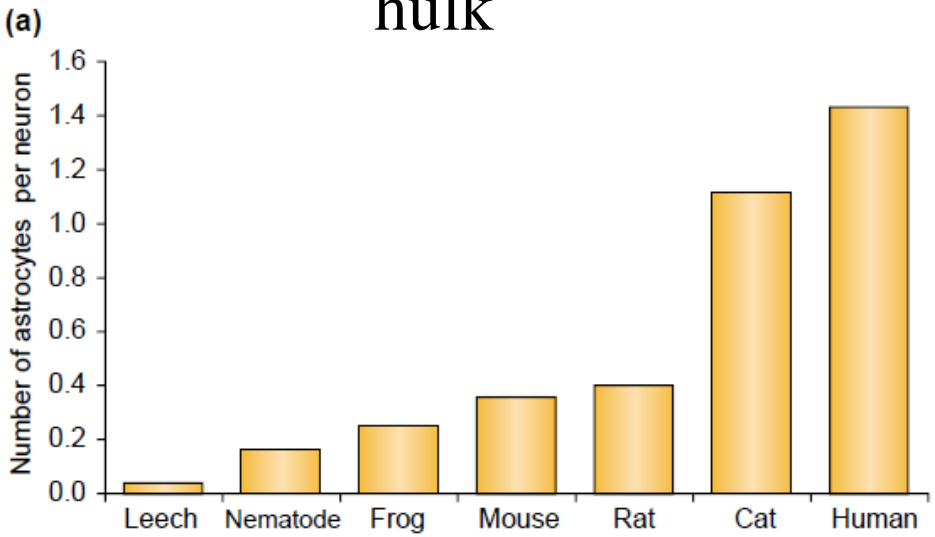
Rick Huganir; www.learner.org

Astrotsüüdid

Aju keerukuse suurenedes suurenevad astrotsüütide..ja suhteline hulk mõõtmed...



Oberheim et al 2012



Nedergaard et al 2012

Neuron

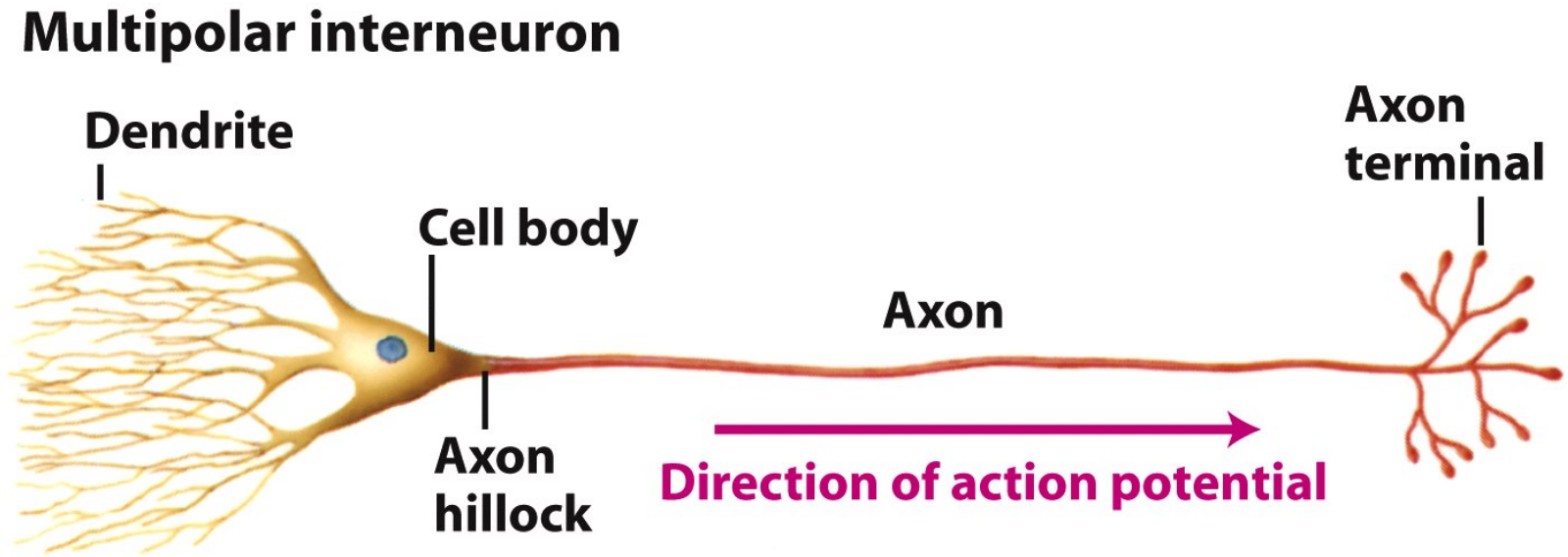


Figure 23-2a
Molecular Cell Biology, Sixth Edition

Aksoni otsas (terminalis) transformeeritakse elektriline signaal keemiliseks

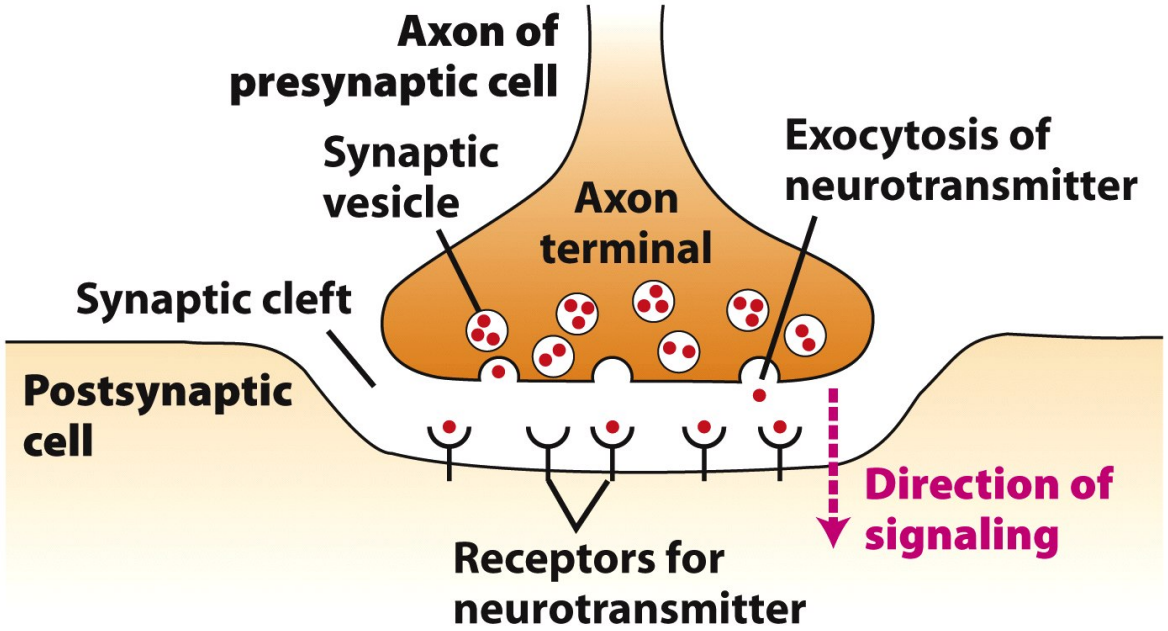
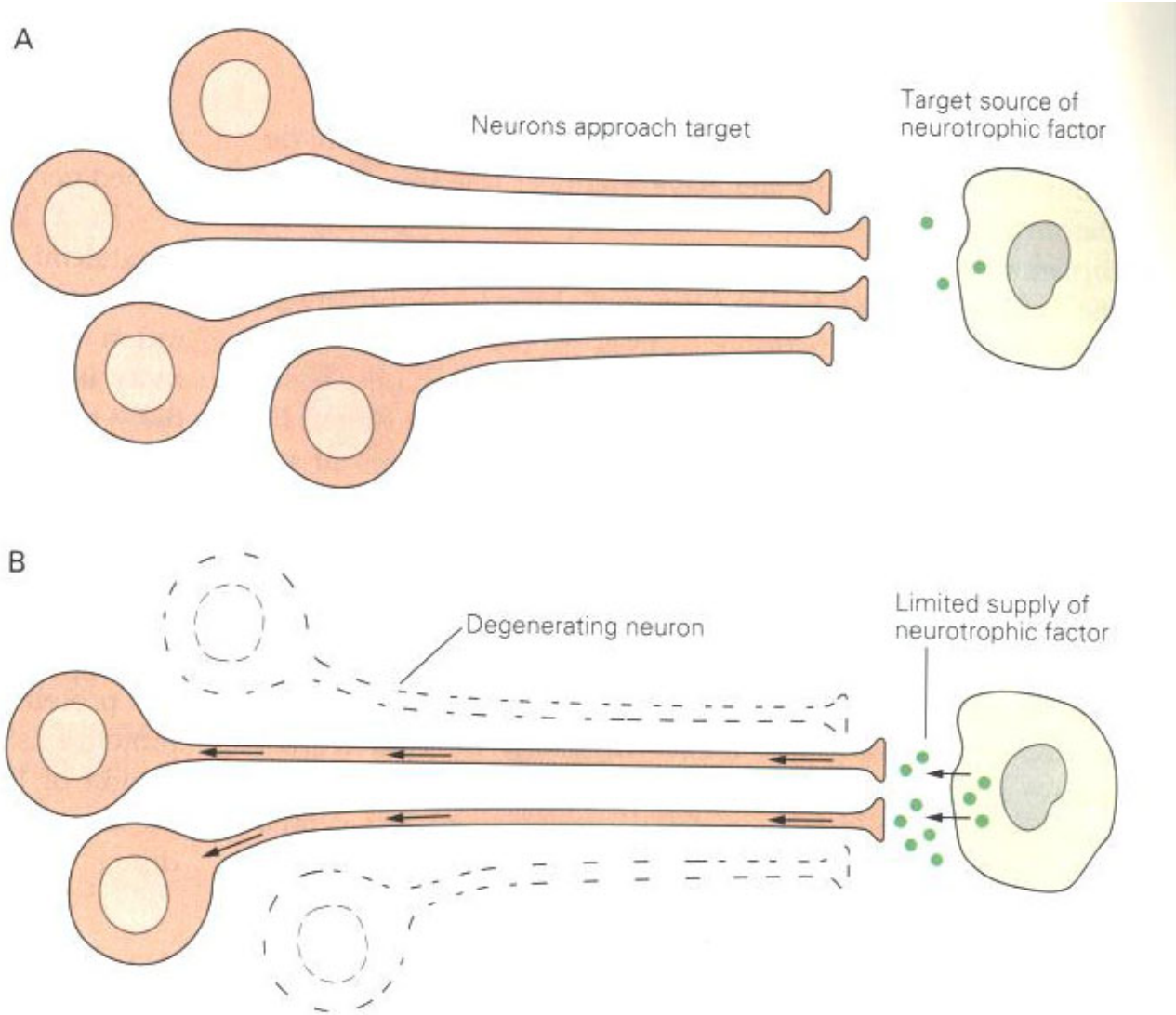
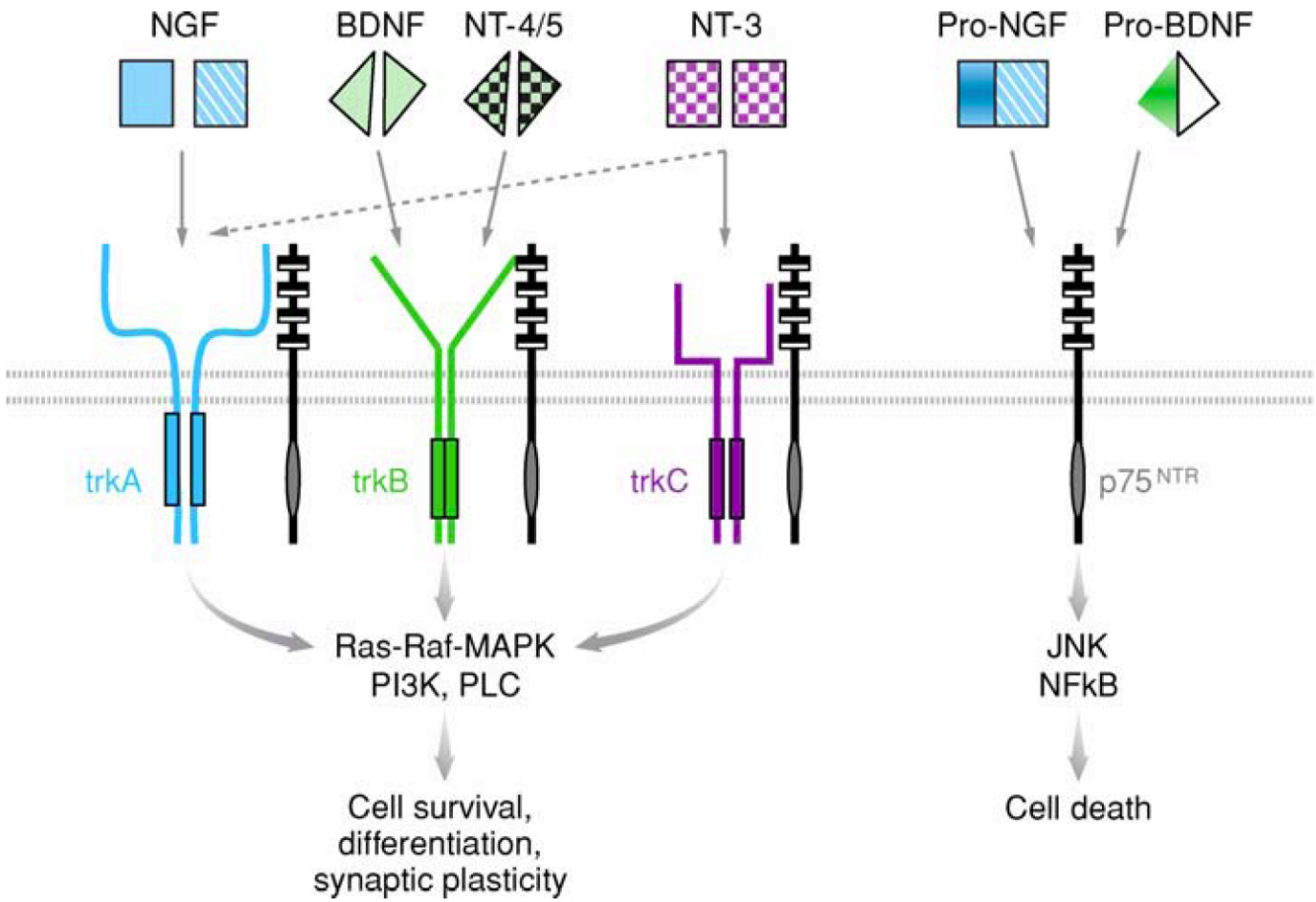


Figure 23-4a
Molecular Cell Biology, Sixth Edition

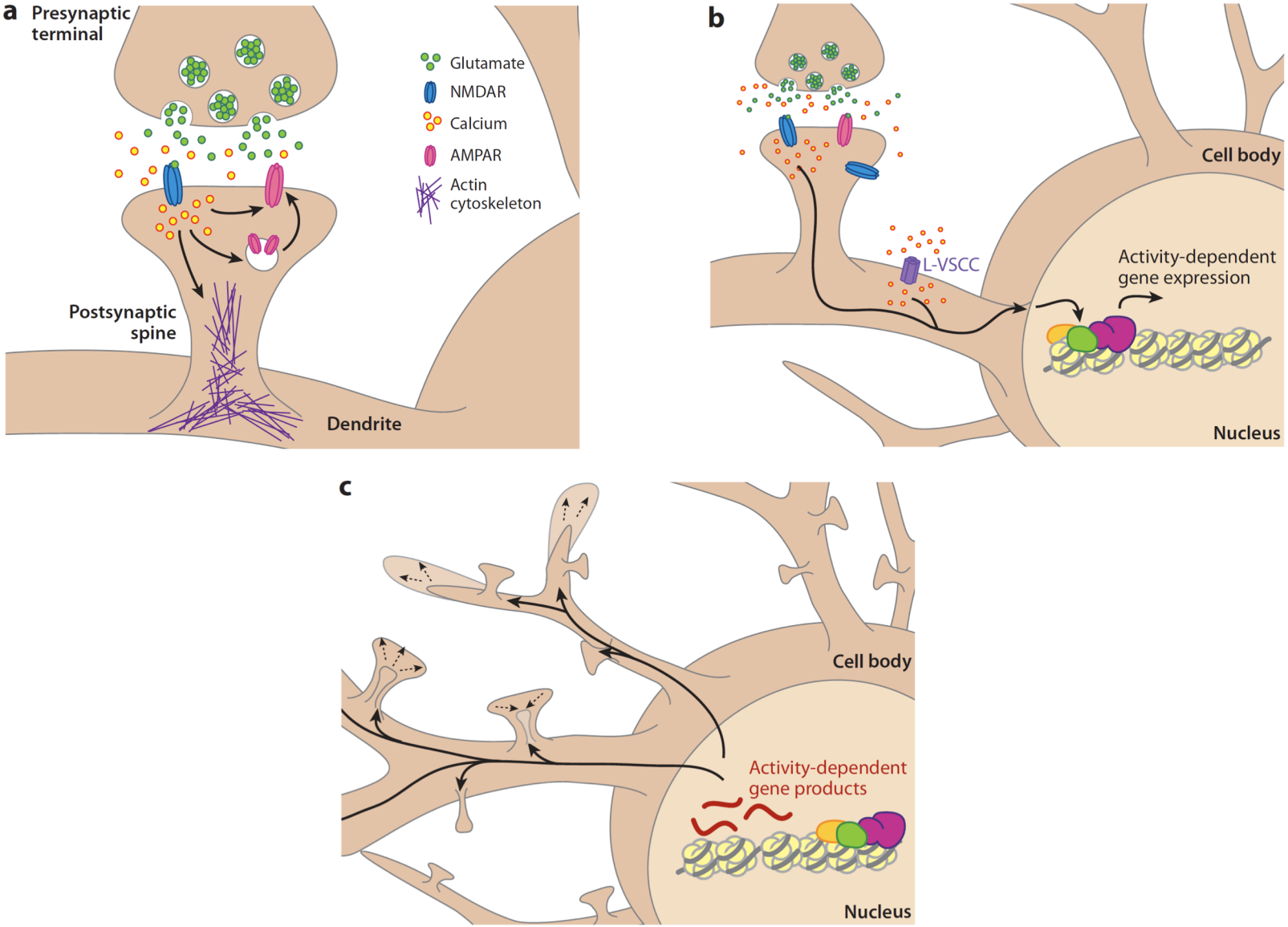
Neurotroofne hüpotees



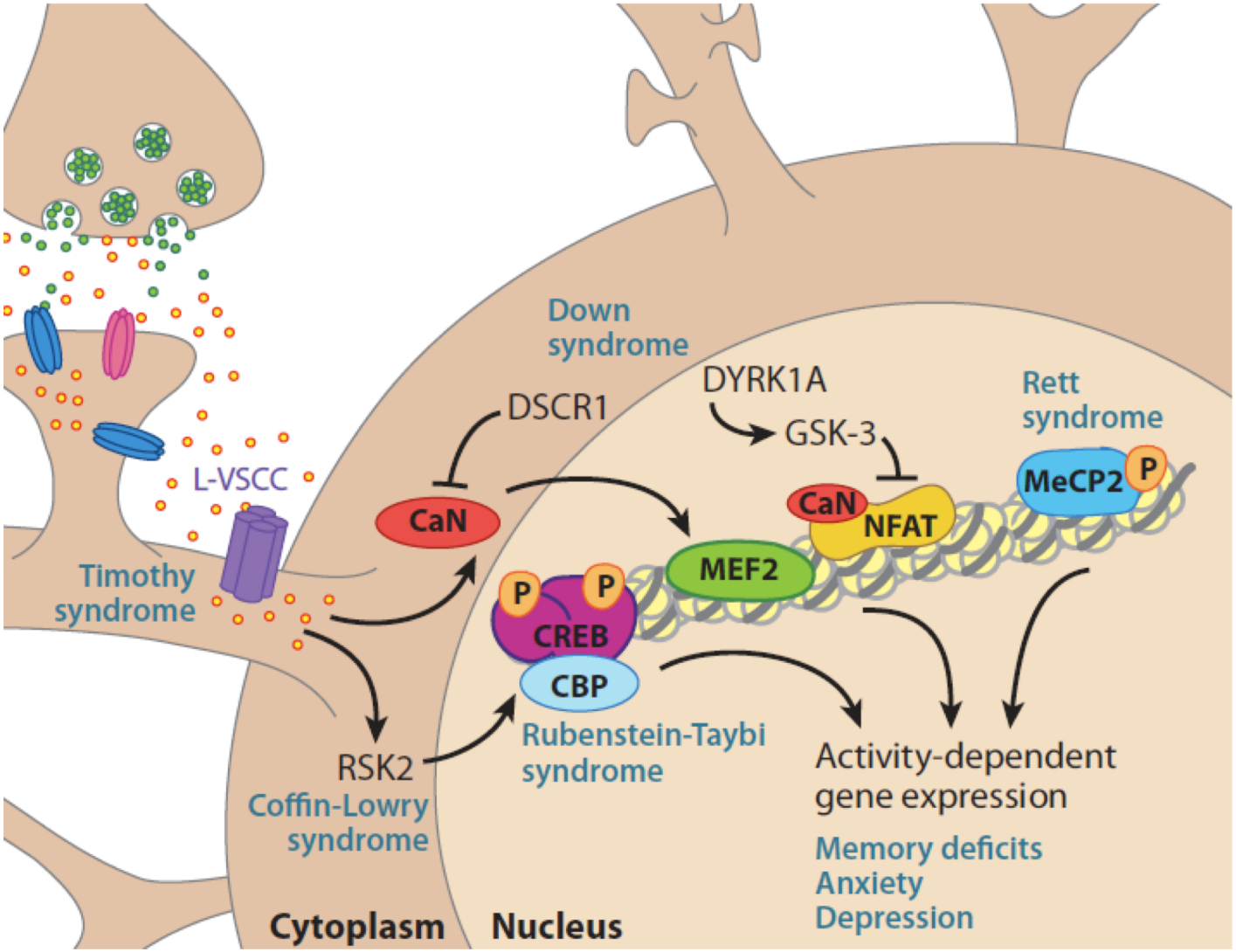
Neurotrofiinid ja nende retseptorid



Sünapsite ja rakutuuma kahesuunaline kommunikatsioon vahendab neuraalset arengut ja plastilisust



Mutatsioonid sünapsite funktsioneerimises osalevates geenides põhjustavad kognitiivsete häiretega närvisüsteemi haigusi



Neurotroofsed tegurid ja närvisüsteemi haigused

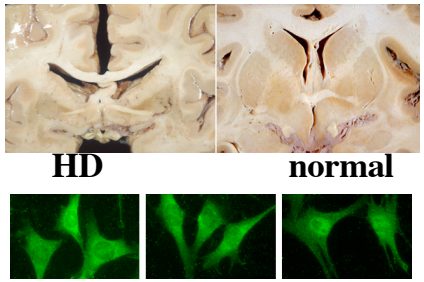
Häired neurotroofsete faktorite funktsioonis arvatakse olevat paljude närvisüsteemi haiguste aluseks

- Neurodegeneratiivsed haigused
- Meeleolu- ja ärevushäired
- Perifeersed neuropaatiad
- Seljaaju traumad
- Rasvumine

Neurodegeneratiivsed häred ja neurotroofsed tegurid

BDNF

Nucleus caudatus and putamen

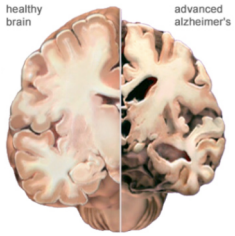


HD **normal**

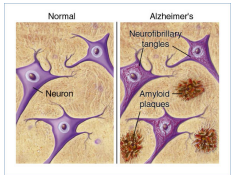
- Zuccato et al *Science* 2001
- Zuccato et al *Nature Genetics* 2003
- Kannike et al *J. Biol. Chem*. 2014
- Nurm et al *eNeuro* 2021

NGF, BDNF

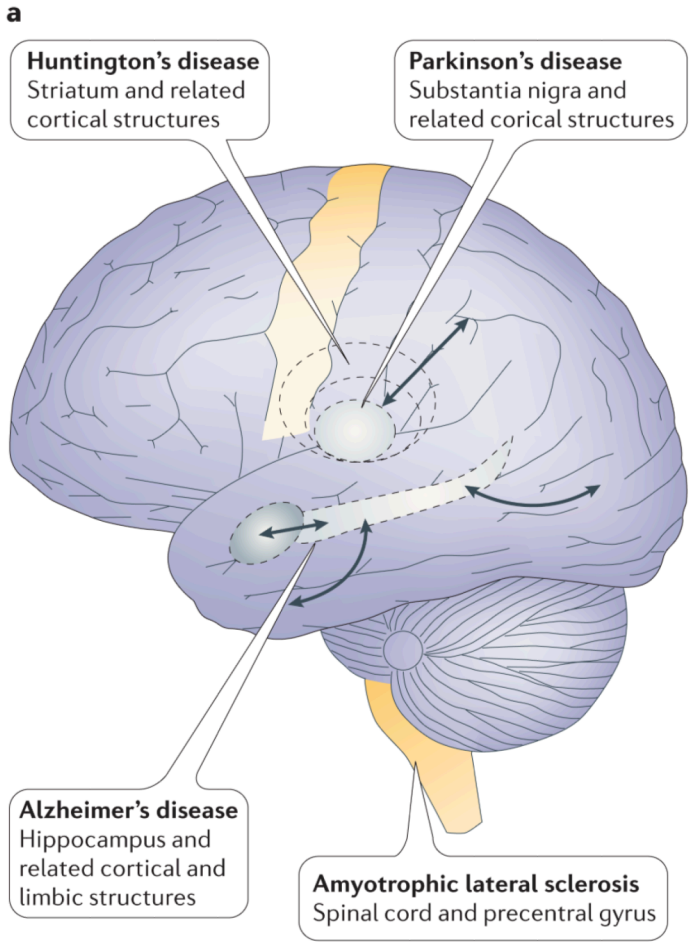
hippocampus and cerebral cortex

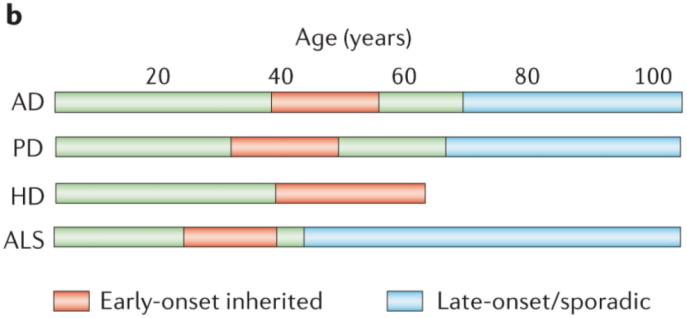


normal **AD**



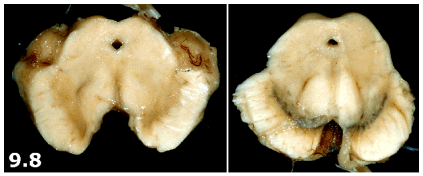
- Kumar et al., *Neuron* 2023



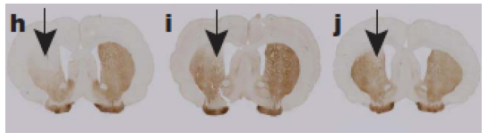


GDNF, NRTN, BDNF, CDNF

substantia nigra



PD **normal**

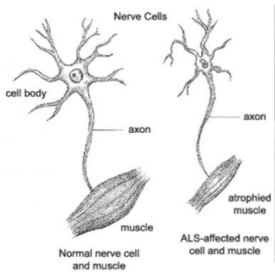


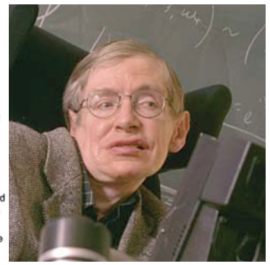
6-OHDA **GDNF** **CDNF**

- Lindholm et al., *Nature*, 2007

GDNF, BDNF

spinal cord

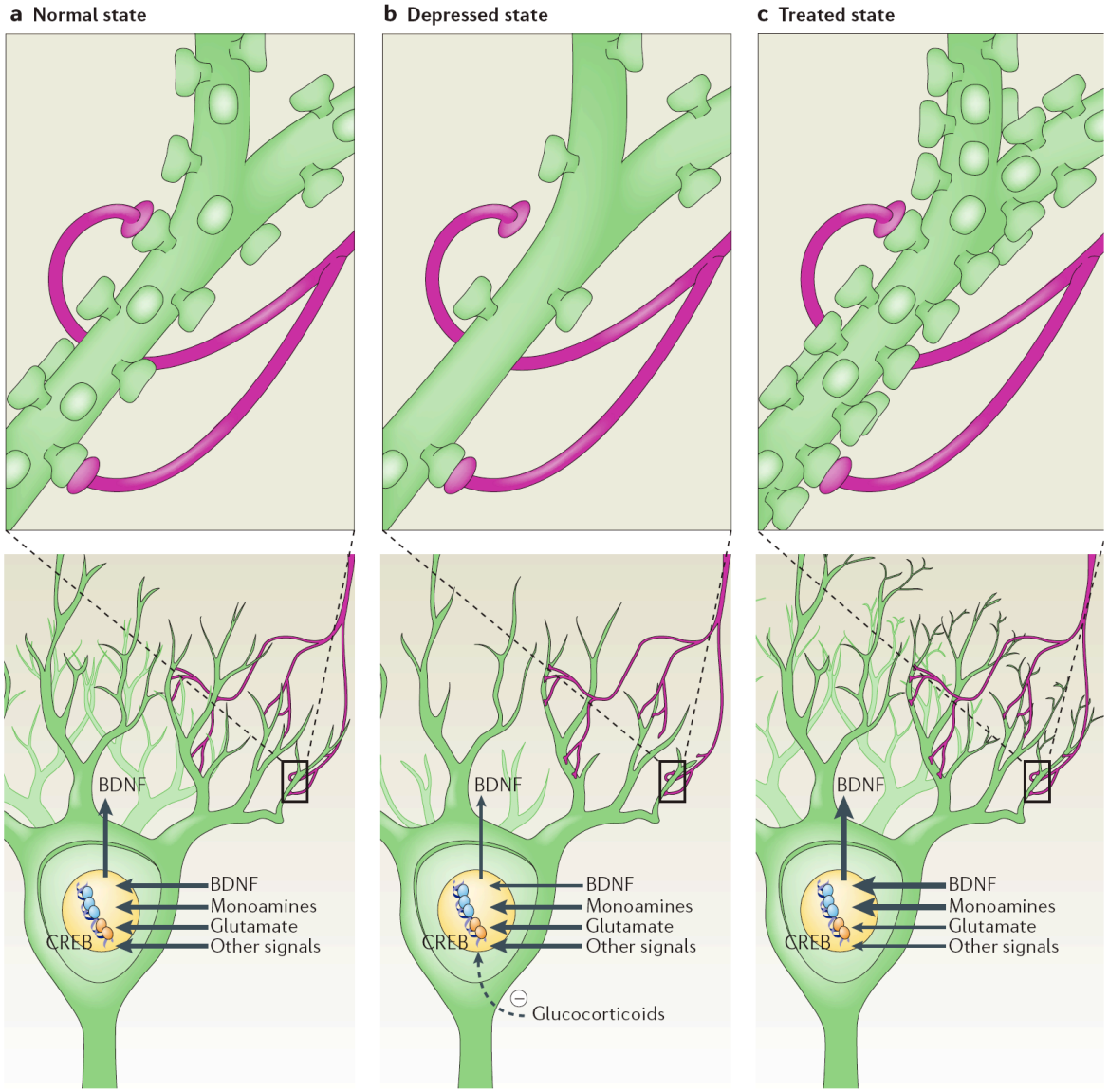




normal **ALS** **Stephen Hawking**

- Trupp et al., *J Cell Biol* 1995

BDNF ja depressioon



Olulisemad uuringud/projektid I

1. **Neurotrofiin BDNF geeni regulatsiooni** mehhanismide väljaselgitamine

(Timmusk et al ***Neuron*** 1994, ***J Cell Biol*** 1995, ***J Biol Chem*** 1999, Palm et ***al J Neurosci*** 1998; Shieh et al ***Neuron*** 1998; Pruunsild et al ***J Neurosci*** 2011; Tuvikene et al ***J Neurosci*** 2016, Esvald et al ***J Neurosci*** 2020, 2022; Tuvikene et al ***eLife*** 2020; Koppel et al ***Glia*** 2018; Avarlaid et al ***Glia*** 2023; ***Lekk*** et al J Biol Chem 2023)

2. **Närvisüsteemi traumad** ja närvirakkude regeneratsiooni molekulaarsed alused

(Funakoshi et al., ***J Cell Biol*** 1993, ***PNAS*** 1998; Lauren et al., ***Genomics*** 2003, ***Mol Cell Neurosci*** 2003)

3. **Huntingtoni tõve** molekulaarsete aluste uuringud

(Zuccato et al ***Science*** 2001; ***Nature Genetics*** 2003; Kannike et al ***J. Biol. Chem***. 2014; Nurm et al ***eNeuro*** 2021)

4. Neurotrofiin BDNF ja **Alzheimeri tõbi**

(Kumar et al., ***Neuron*** 2023)

Olulisemad uuringud/projektid II

5. Uued neurotroofsed tegurid ja nende kasutamine **Parkinsoni tõves** surevate neuronite elushoidmisel

(Trupp et al., ***J Cell Biol*** 1995; Lindholm et al., ***Nature*** 2007; Lindholm et al., ***Mol Cell Neurosci*** *2008*)

6. Neurotrofiinide retseptorite antagonistid kui potentsiaalsed **valuvaigistid**

(Tammiku-–Taul et al ***Eur J Med Chem*** 2016)

7. **Skisofreenia ja intellektuaalse puudega** seotud geenid ja nende regulatsioon

(Francks et al ***Mol Psych*** 2007; Sepp et al., ***Plos One*** 2011, ***Hum Mol Genet*** 2012, **J Neurosci** 2017; Karis et al., ***Front Mol Neurosci*** 2018; Sirp et al ***J Biol Chem*** 2021; Tamberg et al Biol Open 2015, ***Dis Mod Mech*** 2020; Sirp et al ***Front Mol Neurosci*** 2022)

8. **Fuchs'i sarvkesta düstroofiat** põhjustava TCF4 geeni mutasioonide uuringud

(Sirp et al ***Sci Rep*** 2020)

Neurotrofiin BDNF: neuronite ellujäämise/surma, sünapsite plastilisuse, õppimise ja mälu molekulaarne alus

Neuron, Vol. 10, 475–489, March, 1993, Copyright © 1993 by Cell Press

Multiple Promoters Direct Tissue-Specific Expression of the Rat BDNF Gene

Tõnis Timmusk,*†‡ Kaia Palm,†‡ Madis Metsis,*‡ Tõnu Reintam,‡ Viiu Paalme,‡ Mart Saarma,†‡ and Håkan Persson*
*Department of Medical Chemistry (II)
Laboratory of Molecular Neurobiology
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Finland
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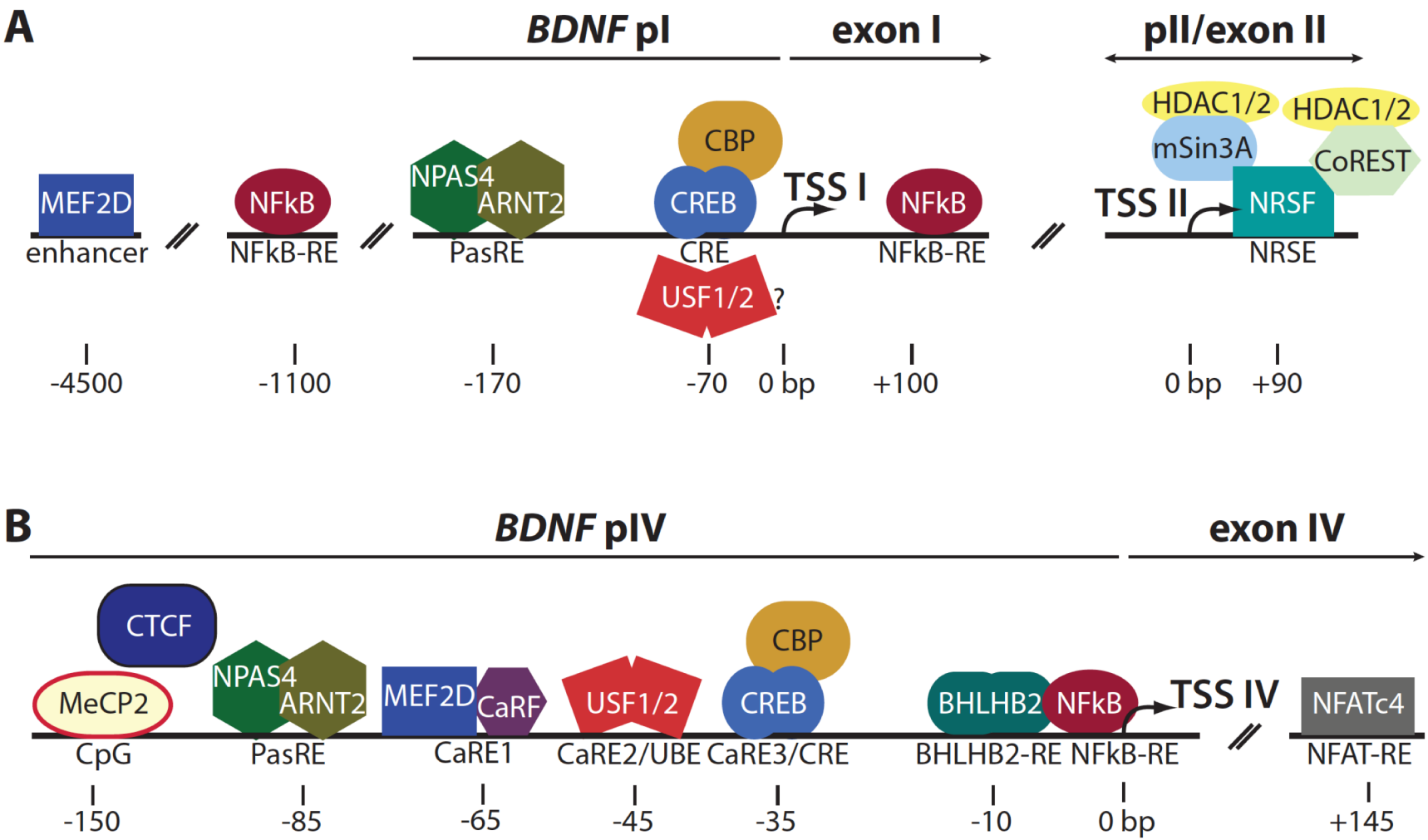
Tallinn EE0026
Estonia

rotrophic factor that supports peripheral sympathetic and neural crest–derived sensory neurons as well as central cholinergic neurons (Levi-Montalcini, 1987). Based on this sequence similarity, additional members of a family of structurally related genes coding for neurotrophin-3 (Ernfors et al., 1990a; Hohn et al., 1990; Jones and Reichardt, 1990; Kaisho et al., 1990; Maisonpierre et al., 1990b; Rosenthal et al., 1990) and neurotrophin-4 (Hallböök et al., 1991; Ip et al., 1992), also named neurotrophin-5 (Berkemeier et al., 1991), have been isolated. All four neurotrophins are synthesized as preproproteins of similar sizes that are proteolytically cleaved to release the mature neurotrophins, which comprise essentially the 120 carboxy-terminal amino acids of each precursor. In agreement with their important role in the vertebrate nervous

Transcription factors involved in neuronal-activity regulated expression of BDNF gene



West, Pruunsild and Timmusk, *Handb. Exp. Pharmacol.*, 2014

Received: 12 October 2022 | Revised: 10 August 2023 | Accepted: 14 August 2023
DOI: 10.1002/glia.24463

GLIA
WILEY

RESEARCH ARTICLE

An 840 kb distant upstream enhancer is a crucial regulator of catecholamine-dependent expression of the *Bdnf* gene in astrocytes

Annela Avarlaid[1] | Eli-Eelika Esvald[1,2] | Indrek Koppel[1] | Annabel Parkman[1] | Anna Zhuravskaya[3] | Eugene V. Makeyev[3] | Jürgen Tuvikene[1,2] | Tõnis Timmusk[1,2]

[1]Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia

[2]Protobios LLC, Tallinn, Estonia

[3]Centre for Developmental Neurobiology, King's College London, London, UK

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Email: annela.avarlaid@taltech.ee; tonis.timmusk@taltech.ee

Funding information
Biotechnology and Biological Sciences Research Council, Grant/Award Numbers: BB/R001049/1, BB/V006258/1; Estonian Research Council, Grant/Award Numbers: IUT19-18, PRG805; European Regional Development Fund, Grant/Award Numbers: 2014-2020.4.01.15-0012, ASTRA 2014-2020.4.01.16-0032; HORIZON EUROPE

Abstract

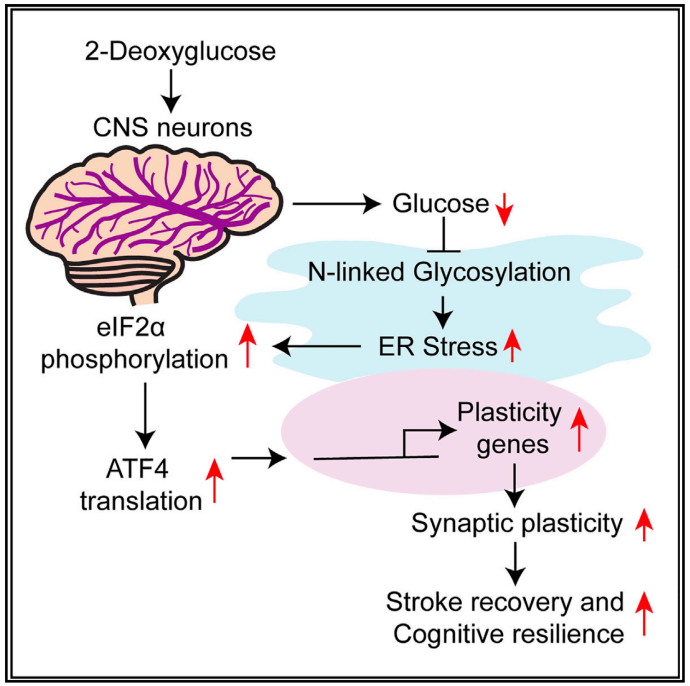
Brain-derived neurotrophic factor (BDNF) plays a fundamental role in the developing and adult nervous system, contributing to neuronal survival, differentiation, and synaptic plasticity. Dysregulation of BDNF synthesis, secretion or signaling has been associated with many neurodevelopmental, neuropsychiatric, and neurodegenerative disorders. Although the transcriptional regulation of the *Bdnf* gene has been extensively studied in neurons, less is known about the regulation and function of BDNF in non-neuronal cells. The most abundant type of non-neuronal cells in the brain, astrocytes, express BDNF in response to catecholamines. However, genetic elements responsible for this regulation have not been identified. Here, we investigated four potential *Bdnf* enhancer regions and based on reporter gene assays, CRISPR/Cas9 engineering and CAPTURE-3C-sequencing we conclude that a region 840 kb upstream of the *Bdnf* gene regulates catecholamine-dependent expression of *Bdnf* in rodent astrocytes. We also provide evidence that this regulation is mediated by CREB and AP1 family transcription factors. This is the first report of an enhancer coordinating the transcription of *Bdnf* gene in non-neuronal cells.

Neuron

Article

2-Deoxyglucose drives plasticity via an adaptive ER stress-ATF4 pathway and elicits stroke recovery and Alzheimer's resilience

Graphical abstract



Authors

Amit Kumar, Saravanan S. Karuppagounder, Yingxin Chen, ..., Tõnis Timmusk, Daniel H. Geschwind, Rajiv R. Ratan

Correspondence

rrr2001@med.cornell.edu

In brief

Intermittent fasting (IF) is a nutritional paradigm that forestalls cognitive aging, stroke disability, and Alzheimer's progression. How glucose restriction, an aspect of IF, contributes to IF-induced benefits is unclear. Here, we used 2-deoxyglucose to elucidate how low glucose engages an evolutionarily conserved ER stress response pathway to stimulate brain plasticity and treat stroke and AD.

Highlights

- 2-deoxyglucose (2-DG), a glucose-restriction mimetic, drives *Bdnf* transcription
- 2-DG reduces disability after ischemic stroke and improves cognition in AD
- 2-DG inhibits N-glycosylation to induce ER stress, ATF4, and *Bdnf* transcription
- N-glycosylation senses low glucose to drive adaptation to stroke and AD

Kumar et al., 2023, Neuron *111*, 2831–2846
September 20, 2023
https://doi.org/10.1016/j.neuron.2023.06.013

Geenide sisselülitamist kontrolliv valk TCF4/E2-2 ja selle seos vaimse alaarengu ja skisofreeniaga

```
E47:  RERRMANNARERVRVRDINEAFRELGRMCQMHLKSDKAQTKLLILQQAVQVILGLEQQ
TCF4: KERRMANNARERLRVRDINEAFKELGRMVQLHLKSDKPQTKLLILHQAVAVILSLEQQ
      :***********:*********:***** *:******.*******:*** ***.****
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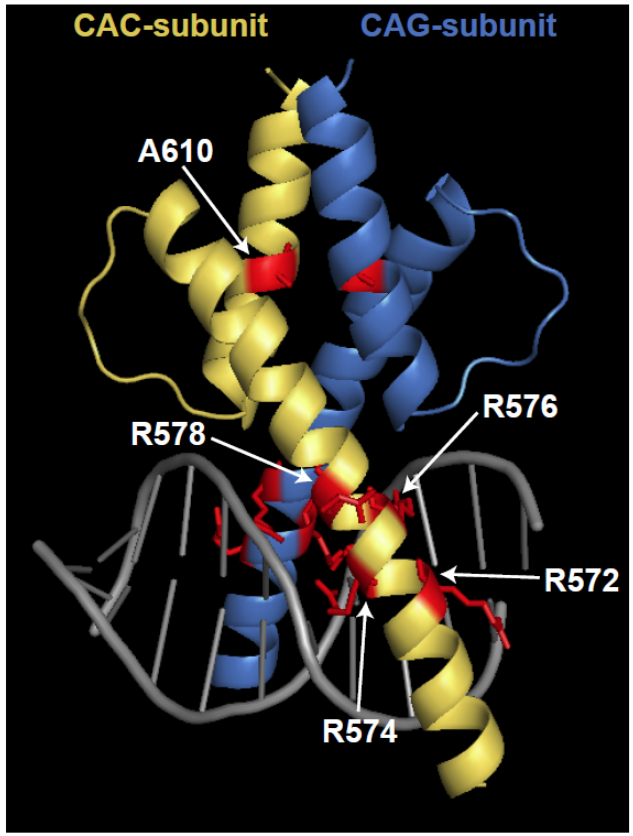

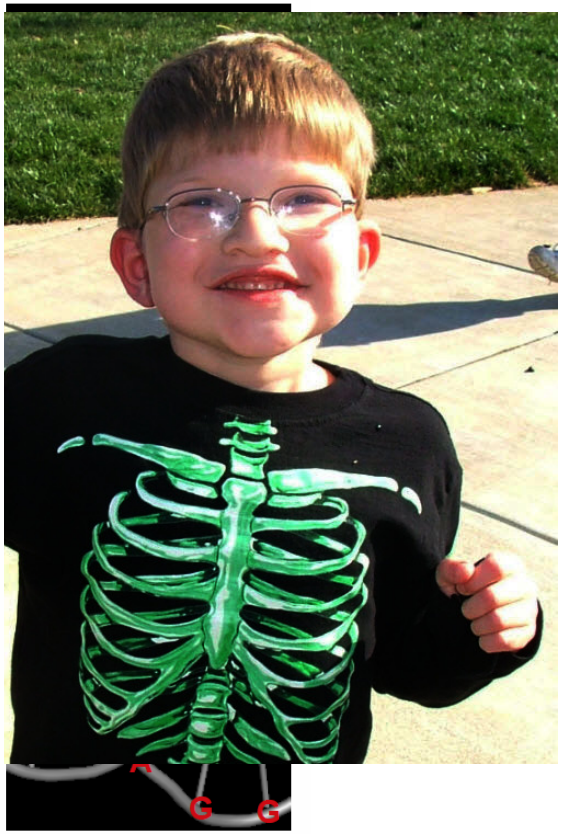

- Avaldunud paljudes kudedes, kõige rohkem närvisüsteemis
- Klass I bHLH ehk E-valk, seondub E-boksile (CANNTG)
- Heterosügootsed deletsioonid või mutatsioonid põhjustavad **Pitt-Hopkins'i Sündroomi**, (PTHS), mis on arenguline närvisüsteemi haigus, millega kaasneb vaimne alaareng, mikrotsefaalia, epilepsia.
- Ühenukleotiidne polümorfism TCF4 geenis on seotud suurenenud **skisofreenia ja depressiooni** riskiga. No 2 top skisofreenia geen (GWAS)! Leitud ka mutatsioonid skisofreenia patsientides.

Sepp M., Kannike K., Eesmaa A., Urb M., **Timmusk T.**. ***PLoS ONE***, 2011, 6, e22138.
Sepp M., Pruunsild P., **Timmusk T.**. ***Hum. Mol. Genet.,*** 2012, 21, 2873-2888.
Tamberg L, Sepp M, **Timmusk T**., Palgi M. ***Biol. Open***, 2015, 4, 1762-1771.
Tamberg L., Jaago M., Säälik K., Sirp A., Tuvikene J., Shubina A., Kiir C. S., Nurm K., Sepp M., **Timmusk T.,** Palgi M.. Dis Model Mech, 2020, 13: dmm042747.
Sirp A., Roots K., Nurm K., Tuvikene J., Sepp M. **Timmusk T**. J. Biol. Chem., 2021, 297, 101381.

Cellular/Molecular

The Intellectual Disability and Schizophrenia Associated Transcription Factor TCF4 Is Regulated by Neuronal Activity and Protein Kinase A

Mari Sepp,[1] Hanna Vihma,[1]* Kaja Nurm,[1]* Mari Urb,[1]* Stephanie Cerceo Page,[2] Kaisa Roots,[1] Anu Hark,[1] Brady J. Maher,[2,3,4] Priit Pruunsild,[1] and Tõnis Timmusk[1]

[1]Department of Chemistry and Biotechnology, Tallinn University of Technology, 12618 Tallinn, Estonia, [2]Lieber Institute for Brain Development, Johns Hopkins Medical Campus, Baltimore, Maryland 21205, and [3]Department of Psychiatry and Behavioral Sciences and [4]Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, Maryland 21205

Transcription factor 4 (TCF4 also known as ITF2 or E2-2) is a basic helix-loop-helix (bHLH) protein associated with Pitt–Hopkins syndrome, intellectual disability, and schizophrenia (SCZ). Here, we show that TCF4-dependent transcription in cortical neurons cultured from embryonic rats of both sexes is induced by neuronal activity via soluble adenylyl cyclase and protein kinase A (PKA) signaling. PKA phosphorylates TCF4 directly and a PKA phosphorylation site in TCF4 is necessary for its transcriptional activity in cultured neurons and in the developing brain *in vivo*. We also demonstrate that *Gadd45g* (growth arrest and DNA damage inducible gamma) is a direct target of neuronal-activity-induced, TCF4-dependent transcriptional regulation and that TCF4 missense variations identified in SCZ patients alter the transcriptional activity of TCF4 in neurons. This study identifies a new role for TCF4 as a neuronal-activity-regulated transcription factor, offering a novel perspective on the association of TCF4 with cognitive disorders.

Key words: bHLH; E2-2; ITF2; neuronal activity; Pitt–Hopkins syndrome; schizophrenia

Significance Statement

The importance of the basic helix-loop-helix transcription factor transcription factor 4 (TCF4) in the nervous system is underlined by its association with common and rare cognitive disorders. In the current study, we show that TCF4-controlled transcription in primary cortical neurons is induced by neuronal activity and protein kinase A. Our results support the hypotheses that dysregulation of neuronal-activity-dependent signaling plays a significant part in the etiology of neuropsychiatric and neurodevelopmental disorders.

Olulisemad publikatsioonid

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