The Burst of Mitochondrial Diseases: Neurons and Calcium

Anna Fendyur MSc, Igor Kaiserman MD MSc MPA, Lior Kasinetz BSc and Rami Rahamimoff MD

Department of Physiology and Minerva Center for Cell Biophysics, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Key words: calcium, mitochondria, neurotoxicity, oxidative stress, genetic disease

IMAJ 2004;6:356–359

In the last decade there has been a tremendous burst of publications on mitochondrial diseases. In the past 5 years alone more than 5,000 articles have appeared on this subject [Figure 1]. We would like to restrict this review to two aspects: mitochondrial diseases of the nervous system, and their connection to intracellular calcium. Most of the diseases are due to neuronal cell death and are related to mitochondria and calcium dysregulation [1]. In some cases faulty intracellular regulation of calcium is the primary cause of cell death, but in others is secondary to genetic defects. We will focus on five diseases; two of them are fairly common: Parkinson's disease and Alzheimer's disease, while the other three are quite rare: Huntington's disease, Leber's hereditary optic neuropathy, and amyotrophic lateral sclerosis.

Ca^{2+} ions are of great importance in the normal function of the nervous system. They are involved in a large number of important cellular processes such as transmitter release [2,3], action potential conduction [4], and gene expression [3]. Large increases in the free intracellular calcium concentration ($[Ca^{2+}]_{in}$) cause cell death (apoptosis) [1]. There is a large number of cellular organelles that control $[Ca^{2+}]_{in}$ [for review see ref. 5]. These include, among others, the surface membrane [6,7], endoplasmic reticulum [8], nucleus [8], and secretory vesicles [9]. Of special importance in the context of this review is the regulation of intracellular calcium by the mitochondria. This was suggested more than 30 years ago [10], including their involvement in the function of the nervous system, by one of us [2,11]. However, this notion was not immediately accepted and only in the past decade has the basic importance of the mitochondrion in regulation of the intracellular calcium and neuronal function been shown [12].

Mitochondria are also involved in the generation of ATP [13], in intermediary metabolism, in building and recycling molecular building blocks [6], in protecting the cells from oxidative stress [14], and in production of reactive oxygen species [14]. The ATP molecules, generated by mitochondria, play two roles: ATP is a high energy molecule that participates in a large number of cellular processes. In addition, ATP stored in synaptic vesicles is released upon nerve stimulation and is considered to be one of the important elements of purinergic transmission [15].

The mitochondrion is an extremely versatile organelle with vital roles for cell function and survival. Any damage can result in cell dysfunction or a degenerative disorder. Identifying the cause of mitochondrial dysfunction may get us closer to understanding the pathogenesis and eventually to a rational treatment.

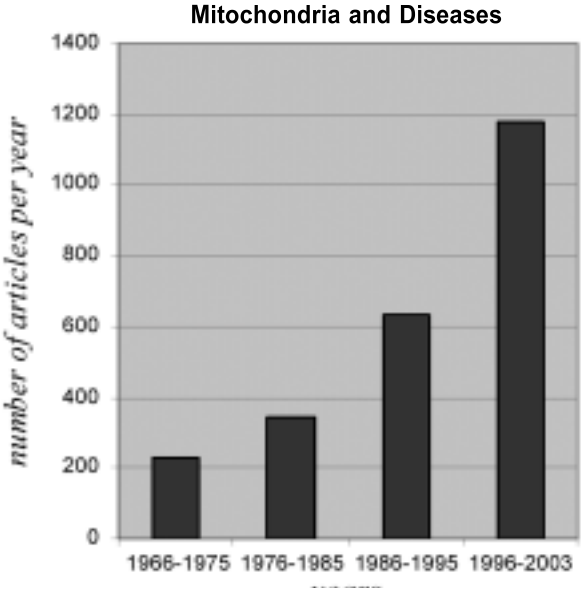

Figure 1. Graph showing the tremendous increase in recent years of the number of articles published on mitochondria and their involvement in various diseases.

Mitochondria may be involved in calcium metabolism in two different ways. First, mitochondria can take up calcium by an energy-dependent process and thus reduce $[Ca^{2+}]_{in}$. Second, mitochondria can release their calcium content upon appropriate stimulation. These calcium-regulatory processes occur via a specific transporter, the sodium-calcium exchange [Figure 2], and via channels that permit the flow of calcium ions through the mitochondrial membranes. We show in this review that both these functions are involved in a number of mitochondrial diseases of the nervous system. Impaired mitochondrial function causes an increase in $[Ca^{2+}]_{in}$ and quite frequently cell death (for detailed review of the role of mitochondria in apoptosis see ref. 16]. Several toxins [17–19] exert their role by affecting the calcium metabolism of the mitochondria. For instance, manganese (Mn^{2+}) shares the uniport-mechanism of mitochondrial Ca^{2+} influx [Figure 2] and, when in excess, was found to be toxic to neurons of the globus pallidus, leading to a Parkinson-like syndrome [19].

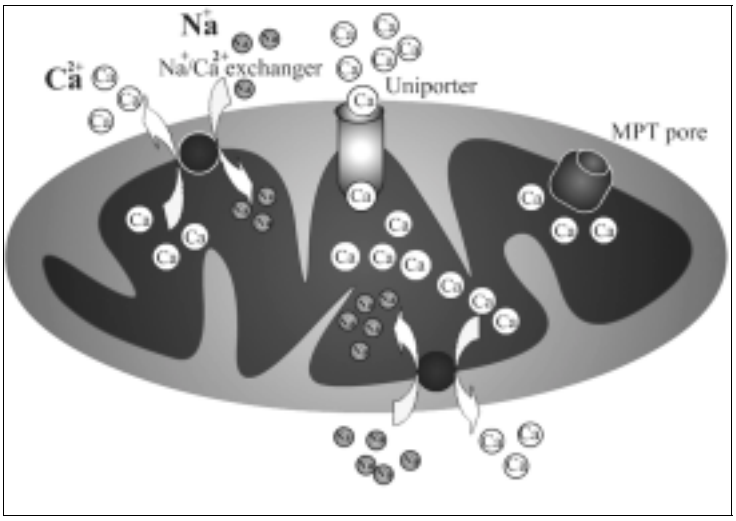

Figure 2. Schematic view of the different stages of mitochondrial calcium handling. Calcium ions enter the mitochondria through the uniporter and can exit the mitochondria through a Na^+/Ca^{2+} exchanger and the mitochondrial permeability transition (MPT) pore. Various factors, including high mitochondrial Ca^{2+} and mitochondrial depolarization, trigger MPT pore opening, whereas the opposite conditions stabilize it in the closed state. Prolonged opening of this pore has been associated with the release of apoptotic factors and cell death.

Huntington's disease

One of the diseases where a clear connection has been established between mitochondria, calcium and neurodegeneration is Huntington. This is a dominant autosomal neurodegenerative disorder showing an expansion of a CAG-trinucleotide repeat in the first exon of the HD gene [20]. This expansion induces formation of a mutant HD protein ($huntingtin_m$). It has been shown [21] that mitochondria from patients with HD have lower membrane potentials and depolarize at a lower calcium load than mitochondria from controls. These changes were found in liver and in brain mitochondria and were suggested to associate with direct interaction of $huntingtin_m$ with the mitochondrial membrane [21]. Moreover, incubating normal mitochondria with the fusion protein containing abnormally long polyglutamine repeats, as it appears in $huntingtin_m$, induces the mitochondrial calcium defect observed in human patients and in transgenic animals with HD [20]. It was shown that $huntingtin_m$ causes an increased sensitivity of the IP_3-receptor to IP_3-mediated Ca^{2+} signaling in neurons by binding to the neuronal isoform of IP_3-receptor [22]. Hence, it appears that neuronal cell loss found in HD is the result of $huntingtin_m$, which causes an increased calcium release from IP_3-receptor that might be responsible for apoptosis of specific neurons containing relevant IP_3-receptor isoform.

Leber's hereditary optic neuropathy

While the mutation leading to HD is found in nuclear DNA, there are also neuropathies associated with mutations in mitochondrial DNA. Mitochondria are the only cellular organelles known to have their own DNA. It has been shown that mtDNA undergoes mutations at a rate five to ten times faster than nuclear DNA [23] and mitochondrial ability to repair mutations is very low. One of the mitochondrial mutation diseases is LHON. This condition, described in 1871 by the German ophthalmologist Theodore Leber, is characterized by a bilateral degeneration of the optic nerves with an incidence of 1 in 25,000 [24]. This maternally inherited mitochondrial disease is due to one of three mitochondrial DNA point mutations (G3460A, G11778A, T14484C) that affect different subunits of complex I [25] [Figure 3], which is of key importance for normal mitochondrial function. LHON accounts for about 3% of cases of blindness in young adult males. Pathologic studies have shown degeneration of both the ganglion cell layer and the optic nerve without any signs of inflammation [26]. In addition, there is marked degeneration of the lateral geniculate nucleus. Electron microscopy shows swollen ganglion cells containing both swollen mitochondria and Ca^{2+}-filled double-membrane-bound structures, most probably damaged mitochondria [26]. The molecular mechanism responsible for the increased accumulation of calcium in mitochondria is not yet known. Cells from LHON patients are especially sensitive to oxidative stress. Depletion of Ca^{2+} from the medium protects those cells from oxidative stress *in vitro*. Indirect evidence suggested that the defect is in the mitochondrial permeability transition pore [Figure 2]. This pore is known to be inhibited by cyclosporin A, and treatment of LHON cells with cyclosporin A significantly rescued them from oxidative damage [27].

Parkinson's disease

PD is a chronic progressive disease and its main symptoms are tremor, rigidity, bradykinesia, and a characteristic disturbance of gait and posture due to selective degeneration of nigro-striatal dopamine neurons [28]. There is strong evidence suggesting the involvement of mitochondria and impaired calcium metabolism in PD. It has been observed that in PD there are dysfunctional mitochondria with reduced activities of complex I and of NADH cytochrome c reductase in neurons from substantia nigra [28]. Complex I is part of the mitochondrial energy-producing apparatus – the electron transport chain. It was found that toxic substances such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine – a by-product of a synthetic heroin) cause Parkinson-like symptoms [17] [Figure 3]. MPTP undergoes oxidation, leading to 1-methyl-4-phenylpyridinium-cation (MPP^+). MPP^+ causes inhibition of mitochondrial complex I [29] that, in turn, causes excessive generation of ROS, which are known to be toxic to many cells [30]. Calcium ions play an important role in ROS generation [30]. Dysfunctional mitochondria in PD cells fail to regulate $[Ca^{2+}]_{in}$. Increased calcium levels can stimulate ROS production [30] [Figure 3]. MPTP is not the only toxic substance that affects the mitochondria and calcium metabolism. Rotenone [18] and manganese [19] also affect

HD = Huntington's disease
mtDNA = mitochondrial DNA
LHON = Leber's hereditary optic neuropathy

PD = Parkinson's disease
ROS = reactive oxygen species

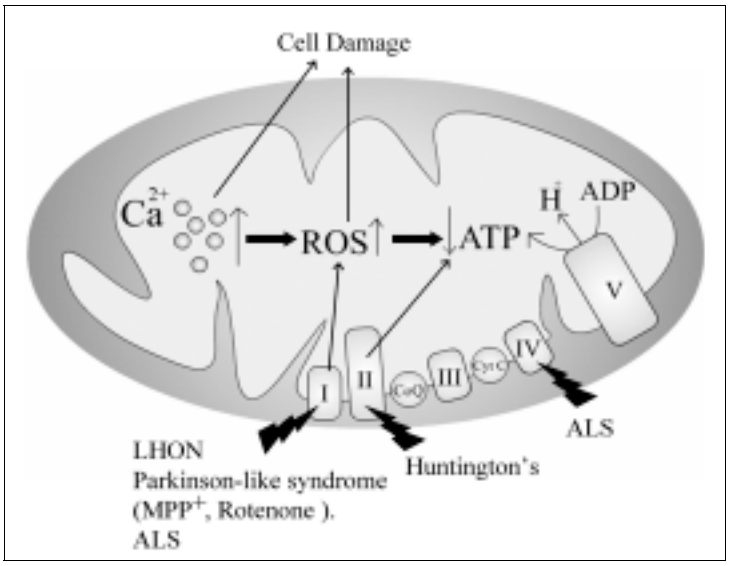

Figure 3. Summary of events associated with mitochondrial impairment, which eventually cause cell damage and neurodegeneration. Different neurologic disorders can be associated with the defect in the mitochondrial electron transport chain (Complexes I-V) from both genetic (mutations in the complex proteins) and toxic (Rotenone, MPP^+) origin. Dysfunction in electron transport chain causes an elevation in the damaging by-products production (ROS?) and inadequate supply of energy to the cell (ATP?). Calcium ions are important regulators of mitochondrial function, hence calcium dysregulation can also cause oxidative stress and decreased ATP supply. The consequences of calcium dysregulation and oxidative stress are usually cell damage and eventually cell death.

mitochondria and neuronal function and produce Parkinson-like symptoms. Important components of Lewy bodies, inclusions found in PD cells, are α-synuclein proteins [31]. These proteins were shown to have a Ca^{2+} binding domain, which ties the Lewy bodies with calcium metabolism in the affected neurons [32]. PD is one of the more common neurodegenerative diseases in which there is a clear link between mitochondria, calcium and pathophysiologic manifestations.

Alzheimer's disease

AD is a common, progressive neurodegenerative disease, characterized by selective neuronal loss in discrete regions of the brain. In addition, there is accumulation of intraneuronal and extracellular fibrils, neurofibrillary tangles and senile plaques [33]. Cell death in AD is presumed to be apoptotic in nature and most likely results from mitochondrial dysfunction [34]. Defects in calcium metabolism and in ROS generation were implicated in the pathophysiology of AD [34]. Fibroblast mitochondria isolated from AD patients take up less Ca^{2+} compared to normal controls and they sequester more Ca^{2+} following oxidative stress [35]. It was shown [36] that pyramidal hippocampal neurons, which are affected in AD, show increased levels of mtDNA and proteins in the cytoplasm and in lysosomes – suggested in previous studies to be the site of mitochondrial degradation by autophagy.

AD = Alzheimer's disease
ALS = amyotrophic lateral sclerosis
SOD = superoxide dismutase

Amyotrophic lateral sclerosis

ALS is an age-dependent neurodegenerative disorder. The neurons that suffer the most in this disorder are large neurons in the cerebral cortex, brain stem, and motor neurons in the spinal cord [37], leading to progressive paralysis and death within 3–5 years. Electron microscopy studies have shown bizarre giant mitochondria and intramitochondrial paracrystalline inclusions [38]. Studies have shown that brain tissue of patients with ALS suffer from a damaging effect of ROS [39], which might be due to mutations in the superoxide dismutase gene in familial ALS. The normal function of SOD1 is to eliminate superoxide anion radicals and hydrogen peroxide and to protect the cell from oxidative damage [40]. In sporadic ALS, decreased activity of complexes I and IV was observed [Figure 3]. In addition, motor nerve terminals from ALS specimens contain significantly increased intracellular calcium levels, which probably result from a defect in glutamate transport [40]. As previously described, $[Ca^{2+}]_{in}$ elevation also contributes to ROS production by mitochondria and to increased motor neuron toxicity. To summarize the cascade of damaging events: mutations in SOD1 (or in other defense processes against oxidative damage) cause intracellular oxidative stress that can damage mitochondrial and cellular membranes; thus causing disruption of calcium homeostasis and glutamate transport, hence increasing the sensitivity of motor neurons to exitotoxicity

Conclusions

In addition to being the cellular powerhouse, mitochondria are important Ca^{2+} regulators. Malfunction of the mitochondria can lead to various neurodegenerative diseases, all showing signs of impaired Ca^{2+} homeostasis. Better understanding of these processes might be the first step towards finding a possible cure for these diseases.

In summary, it seems that in many neurologic disorders – of both genetic and toxic origin – mitochondrial disruption leads to an altered calcium metabolism and thus to cell death. This raises the possibility that various protective agents, such as antioxidants, that improve mitochondrial function efficiency, may be of benefit in high risk individuals.

Acknowledgments. We thank Dr. Halina Meiri for her helpful remarks. We also thank Mrs. Laura Brendel for her unfailing administrative help. The work in our laboratory was supported by grants from the Israel Science Foundation, the U.S.-Israel Binational Science Foundation, and the Israel Ministry of Health.

References

1. Richter C. Pro-oxidants and mitochondrial Ca^{2+}: their relationship to apoptosis and oncogenesis. *FEBS Lett* 1993;325(1-2):104–7.
2. Rahamimoff R, Erulkar SD, Alnaes E, Meiri H, Rotshenker S, Rahamimoff H. Modulation of transmitter release by calcium ions and nerve impulses. *Cold Spring Harb Symp Quant Biol* 1976;40:107–16.
3. Hardingham GE, Bading H. Calcium as a versatile second messenger in the control of gene expression. *Microsc Res Tech* 1999;46(6):348–55.

4. Sugiura H, Joyner RW. Action potential conduction between guinea pig ventricular cells can be modulated by calcium current. *Am J Physiol* 1992;263(5 Pt 2):H1591–604.
5. Kraus D, Khoury S, Fendyur A, Kachalsky SG, Abu-Hatoum T, Rahamimoff R. Intracellular calcium dynamics-sparks of insight. *J Basic Clin Physiol Pharmacol* 2000;11(4):331–65.
6. Li SW, Westwick J, Poll CT. Receptor-operated Ca^{2+} influx channels in leukocytes: a therapeutic target? *Trends Pharmacol Sci* 2002;23(2):63–70.
7. Blaustein MP, Lederer WJ. Sodium/calcium exchange: its physiological implications. *Physiol Rev* 1999;79:763–854.
8. Malviya AN, Rogue PJ. "Tell me where is calcium bred": clarifying the roles of nuclear calcium. *Cell* 1998;92(1):17–23.
9. Pappas GD, Rose S. Localization of calcium deposits in the frog neuromuscular junction at rest and following stimulation. *Brain Res* 1976;103(2):362–5.
10. Lehninger AL. Ca^{2+} transport by mitochondria and its possible role in the cardiac contraction-relaxation cycle. *Circ Res* 1974;35(Suppl 3):83–90.
11. Rahamimoff R, Erulkar SD, Lev Tov A, Meiri H. Intracellular and extracellular calcium ions in transmitter release at the neuromuscular synapse. *Ann N Y Acad Sci* 1978;307:583–98.
12. David G, Barret JN, Barret EF. Evidence that mitochondria buffer physiological Ca^{2+} loads in lizard motor nerve terminals. *J Physiol (Lond)* 1998;509(1):59–65.
13. Gunter TE, Gunter KK, Sheu SS, Gavin CE. Mitochondrial calcium transport: physiological and pathological relevance. *Am J Physiol* 1994;267(2 Pt 1):C313–39.
14. Arai M, Imai H, Koumura T, et al. Mitochondrial phospholipid hydroperoxide glutathione peroxidase plays a major role in preventing oxidative injury to cells. *J Biol Chem* 1999;274(8):4924–33.
15. Sneddon P, Westfall TD, Todorov LD, et al. Modulation of purinergic neurotransmission. *Prog Brain Res* 1999;120:11–20.
16. Kroemer G, Dallaporta B, Resche-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis. *Annu Rev Physiol* 1998;60:619–42.
17. Bergman H, Deuschl G. Pathophysiology of Parkinson's disease: from clinical neurology to basic neuroscience and back. *Mov Disord* 2002;17(Suppl 3):S28–40.
18. Alam M, Schmidt WJ. Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. *Behav Brain Res* 2002;136(1):317–24.
19. Verity MA. Manganese neurotoxicity: a mechanistic hypothesis. *Neurotoxicology* 1999;20(2-3):489–97.
20. Panov AV, Burke JR, Strittmatter WJ, Greenamyre JT. In vitro effects of polyglutamine tracts on Ca^{2+}-dependent depolarization of rat and human mitochondria: relevance to Huntington's disease. *Arch Biochem Biophys* 2003;410(1):1–6.
21. Panov AV, Gutekunst CA, Leavitt BR, et al. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat Neurosci* 2002;5(8):731–6.
22. Tang TS, Tu H, Chan EY, et al. Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. *Neuron* 2003;39(2):227–39.
23. Luft R. Historical overview of mitochondrial diseases. In: Beal MF, Howell N, Bodis-Wollner I, eds. Mitochondria and Free Radicals in Neurodegenerative Diseases. New York: Wiley-Liss, 1997:3–14.
24. Man PY, Turnbull DM, Chinnery PF. Leber hereditary optic neuropathy. *J Med Genet* 2002;39(3):162–9.
25. Mackey DA, Oostra RJ, Rosenberg T, et al. Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. *Am J Hum Genet* 1996;59(2):481–5.
26. Kerrison JB, Howell N, Miller R, Hirst L, Green WR. Leber hereditary optic neuropathy. Electron microscopy and molecular genetic analysis of a case. *Ophthalmology* 1995;102(10):1509–16.
27. Wong A, Cortopassi G. mtDNA mutations confer cellular sensitivity to oxidant stress that is partially rescued by calcium depletion and cyclosporin A. *Biochem Biophys Res Commun* 1997;239(1):139–45.
28. Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* 1990;54(3):823–7.
29. Smith TS, Bennett JP Jr. Mitochondrial toxins in models of neurodegenerative diseases. I: In vivo brain hydroxyl radical production during systemic MPTP treatment or following microdialysis infusion of methylpyridinium or azide ions. *Brain Res* 1997;765(2):183–8.
30. Sousa SC, Maciel EN, Vercesi AE, Castilho RF. Ca^{2+}-induced oxidative stress in brain mitochondria treated with the respiratory chain inhibitor rotenone. *FEBS Lett* 2003;543(1-3):179–83.
31. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. Alpha-synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc Natl Acad Sci USA* 1998;95(11):6469–73.
32. Nielsen MS, Vorum H, Lindersson E, Jensen PH. Ca^{2+} binding to alpha-synuclein regulates ligand binding and oligomerization. *J Biol Chem* 2001;276(25):22680–4.
33. Khachaturian ZS. Diagnosis of Alzheimer's disease. *Arch Neurol* 1985;42(11):1097–105.
34. Sheehan JP, Swerdlow RH, Miller SW, et al. Calcium homeostasis and reactive oxygen species production in cells transformed by mitochondria from individuals with sporadic Alzheimer's disease. *J Neurosci* 1997;17(12):4612–22.
35. Kumar U, Dunlop DM, Richardson JS. Mitochondria from Alzheimer's fibroblasts show decreased uptake of calcium and increased sensitivity to free radicals. *Life Sci* 1994;54(24):1855–60.
36. Hirai K, Aliev G, Nunomura A, et al. Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci* 2001;21(9):3017–23.
37. Tanaka J, Nakamura H, Tabuchi Y, Takahashi K. Familial amyotrophic lateral sclerosis: features of multisystem degeneration. *Acta Neuropathol (Berl)* 1984;64(1):22–9.
38. Nakano Y, Hirayama K, Terao K. Hepatic ultrastructural changes and liver dysfunction in amyotrophic lateral sclerosis. *Arch Neurol* 1987;44(1):103–6.
39. Bowling AC, Schulz JB, Brown RH Jr, Beal MF. Superoxide dismutase activity, oxidative damage, and mitochondrial energy metabolism in familial and sporadic amyotrophic lateral sclerosis. *J Neurochem* 1993;61(6):2322–5.
40. Kruman II, Pedersen WA, Springer JE, Mattson MP. ALS-linked Cu/Zn-SOD mutation increases vulnerability of motor neurons to excitotoxicity by a mechanism involving increased oxidative stress and perturbed calcium homeostasis. *Exp Neurol* 1999;160(1):28–39.

Correspondence: Dr. R. Rahamimoff, Dept. of Physiology and Minerva Center for Cell Biophysics, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel.
Phone: (972-2) 675-8527
Fax: (972-2) 643-9736
email: ramir@cc.huji.ac.il