Acta Biologica Hungarica 56 (1–2), pp. 109–117 (2005)

A PRELIMINARY STUDY ON ULTRA HIGH FREQUENCY ELECTROMAGNETIC FIELDS EFFECT ON BLACK LOCUST CHLOROPHYLLS

D. D. SANDU,[1] C. GOICEANU,[2] A. ISPAS,[1] I. CREANGA,[3]
S. MICLAUS[4] and DORINA E. CREANGA[1]*

[1] Faculty of Physics, "Al. I. Cuza" University, 11A Carol I Blvd., 6600 Iasi,
[2] Public Health Institute, Iasi 3 Blvd. Carol I, 6600 Iasi,
[3] Forestry Territorial Office Iasi, 28 Moara de Vint, 6600 Iasi,
[4] Terrestrial Troupe Academy, Sibiu, Romania

(Received: February 28, 2003; accepted: March 10, 2004)

Chlorophylls were quantitatively studied in the leaves of black locust (*Robinia pseudoacacia* L.) seedlings exposed to electromagnetic fields of high frequency. Exposure system was designed and built up to make possible simultaneous exposure of seedling lots (3 months old) to low power density electromagnetic fields corresponding to a frequency of 400 MHz. After three weeks of daily exposures (1, 2, 3 and 8 hours), chlorophyll levels were measured using adequate spectral device. Statistical analysis of experimental results was performed by means of *t*-test to identify significant modifications induced by electromagnetic treatment in exposed samples in comparison to the control. Chlorophyll-a as well as chlorophyll-b level was found to decrease except the exposure time of two hours, where a considerable enhancement was noticed. It was revealed that the ratio of the two main types of chlorophyll was decreasing logarithmically to the increase of daily exposure time.

Keywords: Black locust seedlings – chlorophyll – electromagnetic fields

INTRODUCTION

Black locust has become a basic agro-forestry species in the last about 350 year since it is cultivated [10, 16]. Being drought-tolerant and having the capacity of nitrogen fixing (as legumes) this tree species is grown in temperate and subtropical regions in U.S., Europe, New Zealand, India, China and Korea. In Europe considerable interest in studying and developing black locust tree plantations is shown by Hungary [21], Bulgaria [14] and Romania [7]. Among atmospheric factors threatening the health of superior biosphere level (the arbors), electromagnetic fields are considered more and more a remarkable pollutant. So, in the last decades, many multidisciplinary study groups have been interested in the biological effects of exposure to low-level electromagnetic fields upon seeds and plants, in both cases, favorable and adverse effects being searched [1, 2, 18]. There is a significant amount of literature concerning the effects of static and low frequency magnetic fields on grassy plant species, especially regarding seed germination or initial growth stages [6, 20, 24]. Few studies were

* Corresponding author; e-mail: emiliacreanga@bumerang.ro
Abbreviations: UHF – ultra high frequency, TEM – transvers electromagnetic cell

focused upon the germination of arbor species seeds after magnetic treatment [3, 4, 22, 23] or upon the photosynthetic activity changes induced in young plantlets exposed to electromagnetic waves [17, 19]. However it seems to be a significant lack of information regarding ultra high frequency (UHF) wave influence on arbor saplings. Organisms in early ontogenetic developmental stages being more sensitive to external stress than adult individuals, young seedlings of black locust were taken for experimental investigation.

Chlorophyll-a is the single pigment molecule directly involved in the transformation of solar visible radiation energy into chemical energy as catalyst. Secondary assimilatory pigments, indirectly implied in photosynthesis, are, mainly, chlorophyll-b and carotenoid pigments, located in the chloroplast membrane in the vicinity of chlorophyll-a molecules (forming molecular complexes with proteins of thylakoid membrane). They absorb solar electromagnetic energy and transfer it to chlorophyll-a. Recent studies demonstrated that microwaves (1–10 GHz frequency) are able to destroy the chemical bonds in nucleic acid structure [15]. The investigation presented in this article was designed to reveal, by quantitative spectral method, putative damages of chlorophyll molecules after young seedlings exposure to high frequency electromagnetic field.

MATERIALS AND METHODS

Plant material

The experimental research was carried out on *Robinia pseudoacacia* L. (black locust) seedlings grown in laboratory. Selected seeds, with uniform genophond (from the same natural arbor population, with remarkable biological parameters, situated in the hill region of Iasi city), were adequately stored and then let to germinate in suitable vessels containing natural soil from that very forest, according to Muller and Bonnet-Massimbert's indication [29]. After germination, vessels were exposed to light (12 hours light/12 hours darkness) and maintained at constant temperature (25 °C). Seedlings have grown this way during three months in quasi-natural conditions of soil, water and temperature. Then assimilatory pigments were analyzed in the exposed seedling leaves, analyzing five replies for both control and exposed samples.

Exposure system

The exposure system used for the electromagnetic treatment of the seedling samples consists in a transverse electromagnetic (TEM) cell. The TEM cell is supplied by a UHF power generator via a bidirectional coupler. The other end of the TEM cell was terminated on a matched load [9]. The transversal dimensions of the TEM cell are $a = 715$ mm, $b = 340$ mm, $w = 450$ mm, where a is the width, b is the height of the rectangular zone and w is the width of the septum. These dimensions are calculated

to obtain a characteristic impedance $Z_{C0} = 50\ \Omega$. In fact the exposure cell represents a rectangular coaxial wave guide [5, 25, 28]; the inner conductor of the cell is connected directly to the inner conductor of the coaxial cable, therefore the dominant mode excited is transverse electromagnetic (TEM) [25, 27]. Inside the TEM cell false acacia seedlings were exposed to continuous traveling waves. At the frequency of 400 MHz, considering that almost the entire UHF power is propagating through the TEM cell by means of TEM mode, the electric field strength may be determined from the following expression [5], [28]:

$$E = \frac{(PZ_0)^{1/2}}{d}$$

where P is the transmitted power, Z_0 is the characteristic impedance of TEM cell and d is the distance between the septum and outer conductor of the cell. Forward, reflected and transmitted powers were measured using a bi-directional coupler and a RF power meter. According to the above formula, the required field level of about 60 V/m was obtained for approximately 2W transmitted power. TEM sizes are large enough to allow simultaneous exposure of a sufficient number of seedlings. It is made by aluminum and provided with small holes regularly distributed on all the walls, so that air and light can enter. *Artificial light sources were used to supplement plant illumination.* Environmental conditions of constant temperature (24 °C) and humidity were kept for exposed samples and controls.

Spectrophotometric assay

The content of chlorophyll-a and -b in the seedlings was measured, at about 24 hours after the last exposure was accomplished. Quantities of 0.02–0.03 g picked up from seedling leaves were crushed and acetone solution (90% in distilled water) was used for extraction according to standard procedure (Meyer-Bertenrath, modified by Stirban and Farcas [11]). Small aliquots of $CaCO_3$ and $MgCO_3$ were added during extraction in order to avoid chlorophyll transformation to pheophytine. Acetone extract was filtered through paper filter and quantitatively transferred to coated bottles of 25 ml. A C. Zeiss spectrophotometer type was used to record the spectra in acetone extracts. A Metrohm Herissau, spectrophotometer, type E-1009, with quartz cells of 1 cm width was used to measure light extinction to specific wavelengths. Computation of chlorophyll-a (Chl **a**) and chlorophyll-b (Chl **b**) contents (mg pigment/100 g green tissue) were performed by means of usual formulae [11]:

$$\text{Chl } \mathbf{a} = (9.784\ E_{662} - 0.99\ E_{644})\ 100\ \text{v/w}$$

$$\text{Chl } \mathbf{b} = (21.426\ E_{644} - 4.65\ E_{662})\ 100\ \text{v/w}$$

where: E_λ – light extinction at the wavelength λ, w – green tissue mass (g) and v – acetone extract (ml).

RESULTS AND DISCUSSION

The electronic absorption spectra of acetone extract for all samples and control have been recorded and comparatively studied. No significant shift of main spectra maxima were noticed (Fig. 1) except the blue range band, where carotenoid pigment absorption may be responsible for small intensity variations. So, one may conclude that no modification of electronic levels energy occur following UHF treatment, i.e. putative qualitative changes in chlorophyll molecules (chemical structure or spatial configuration), do not affect visibly electronic energetic states. Quantitative measurement of assimilatory pigment contents led to the results presented bellow. The average values obtained for five repetitions (five seedlings) representing the same exposed sample are represented in Figure 2. In this figure the sum of chlorophylls contents is also represented; except the net enhance corresponding to 2 h exposure time, the other exposed samples present diminished pigment content. Statistical analysis was accomplished by *t*-test, two tailed, pair type, for comparison of control data series and every exposed sample series (Table 1). Significant increase of chlorophyll-b can be seen for the exposure time of 2 hours ($p < 0.05$); the level of chloro-

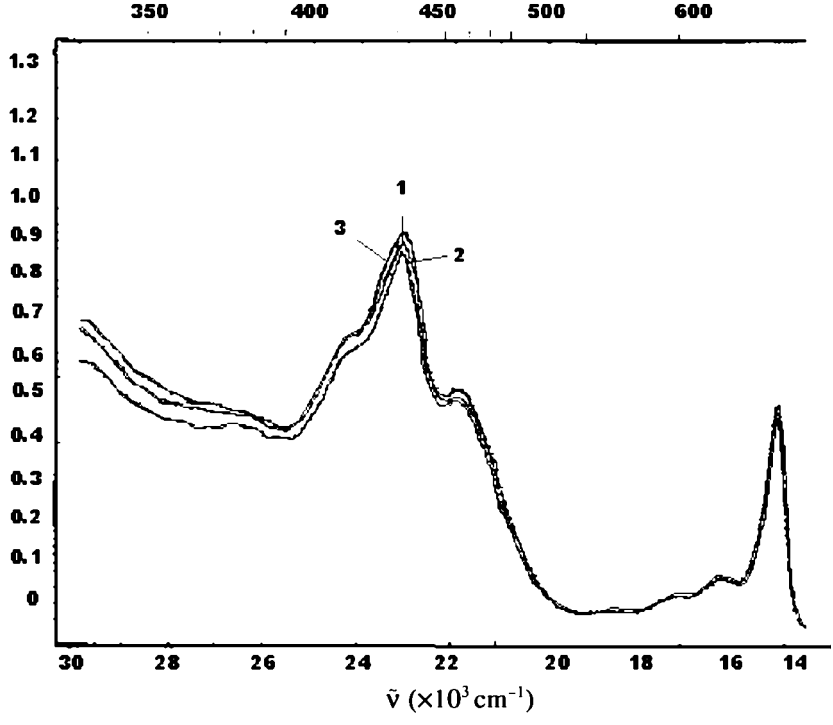

Fig. 1. Electronic absorption spectra obtained in acetone extract for control (1), the sample corresponding to 2 hours exposure time (2) and the sample corresponding to 8 hours exposure time (3) (ṽ-wave number, λ-wavelength). No spectral shift was noticed

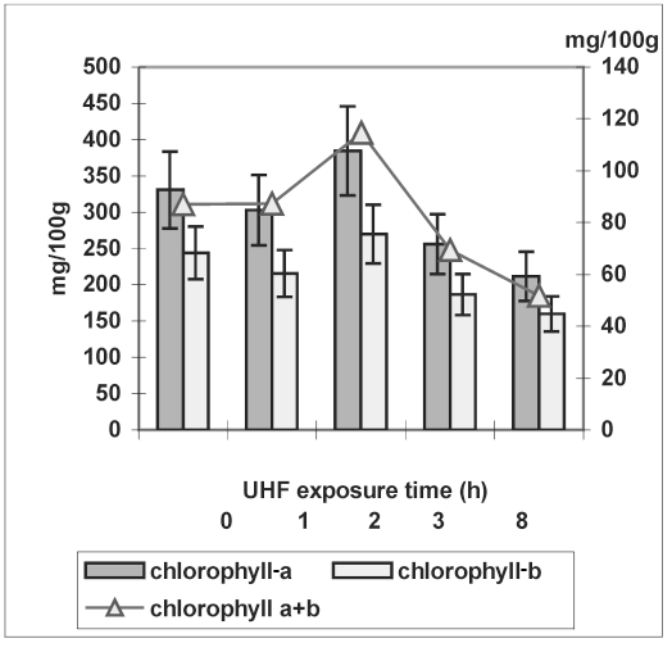

Fig. 2. Average values and average standard deviations of chlorophyll contents depending on the UHF exposure time

Table 1
Statistic analysis of chlorophyll absorption measurements

	Exposure time (hours)				
Clorophyll-a (mg/100 g)	0	1	2	3	8
Average (mg/100 g)	243.8755	215.5147	269.6993	186.5548	159.9709
Standard deviation (mg/100 g)	32.4915	26.4706	27.8386	14.8092	69.8059
	t-test	0.03213	0.05987	0.014512	0.04121
Clorophyll-b (mg/100 g)	0	1	2	3	8
Average (mg/100 g)	86.9816	87.2953	114.6688	69.5061	51.72603
Standard deviation (mg/100 g)	7.8555	8.3871	18.5514	17.3756	23.0601
	t-test	0.2613	0.0000124	0.023141	0.03213

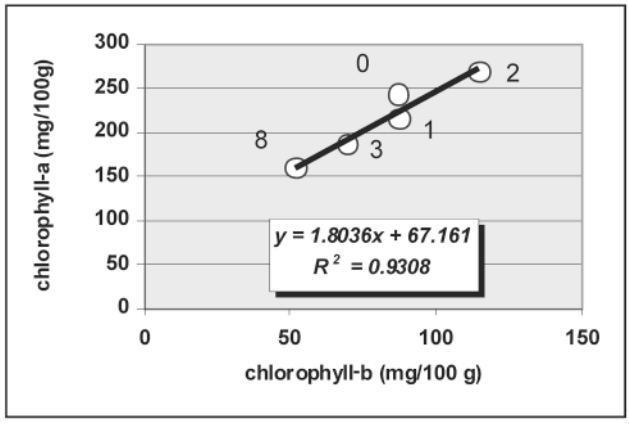

Fig. 3. Linear dependence of chlorophyll-a on chlorophyll-b (exposure times are written near the corresponding points)

phyll-a seems also to be enhanced though *t*-test gave non-significant p-value (0.059) – but not much different to the limit of 0.05. For longer exposure time an inhibitory effect is supposed to underline the decreasing of both types of chlorophyll being statistically significant ($p<0.05$). In Figure 3 the correlation between chlorophyll-a and chlorophyll-b is given. It is visible a very good linear dependence of chlorophyll-a on chlorophyll-b (correlation coefficient, R^2, higher than 0.9). Studying pigment ratio (Fig. 4) a slight diminution of chlorophyll-a content compared to chlorophyll-b content for all exposure times except the 8 hours where a slight enhance was noticed. So, we can conclude that UHF waves have a negative influence in the assimilatory pigment balance in the young seedlings of this arbor species when repeated exposures occur. Thermal effect, quite low in the case of power density of 1 mW/cm^2, is expected to cause a slight increase of temperature in the irradiated samples (due to the dielectric relaxation of water molecules), which is usually associated with a stimulation of biosynthesis, in this case the assimilatory pigment biosynthesis. The decrease of assimilatory pigment levels needs to be related to a non-thermal, specific, effect of microwaves in living tissues, coexisting with the thermal one, and dominating it especially for long exposure time. Inhibitory action resulted from the non-thermal UHF waves effect, may consist in the perturbation of ion channels functions at the level of membrane structures from the cell. Mainly ion transport through chloroplast membranes can be invoked in this case, though ion channels of plasma membrane and other cytoplasmatic structures are also supposed to experience microwave action [12]. Perturbation of ion transport may lead to perturbations of various biochemical reactions which are controlled by ion messengers (especially calcium ions are known as intra-cellular messengers); so, it is possible that biochemical reactions involved in the assimilatory pigment biosynthesis are delayed for the duration of UHF action. The energy level of a UHF photon is approximately a million times lower than the energy required to break a covalent bond. Based on this fact, it has been stated in the

literature that "microwaves are incapable of breaking the covalent bonds of DNA" [8, 13], but this has apparently occurred in the Kakita [15] experiment, even though this may be only an indirect, complex effect of the microwaves. Still, no theory currently exists to explain the phenomenon of DNA fragmentation by microwaves although research is ongoing which may elucidate the mechanism. Regarding chemical bonds assuring pigment molecule primary structure, it is not impossible that electromagnetic fields are able to destroy them, too, since recent studies demonstrated such destruction for nucleic acid chemical bonds when exposed to microwaves [15]. We need to consider also the role of light harvesting protein complex of photosynthetic system II (LHC II). Encoded in the nucleus it is known to exhibit remarkable structural flexibility upon changes in the environmental conditions (such as, in the present case, the electromagnetic environmental component); so, environmental factors are considered able to regulate LHC II like phytochromes, redox, diurnal cycle, etc. are doing, too. LHC II is slightly involved in plant capacity of light harvesting as well as in regulatory process such as via phosphorilation and non-photochemical quenching. Consequently the chlorophyll a/b ratio, much dependent upon the LHC II content in the thylakoid membranes, can be affected by microwave exposure, at least during very early ontogenetic stages of vegetal organisms. In this frame, we need to mention also the enzyme capacity to capture and transmit free energy from oscillating electric fields, as assumed by Westerhoff et al. [30]. For older seedlings (two or three years) of forestry species (spruce and beech) continuously exposed to microwaves, Schmutz et al. [26] recorded no significant changes in chlorophyll content (fluorescence measurements).

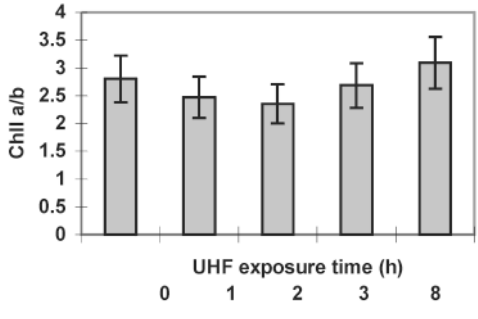

Fig. 4. Chlorophyll a/b ratio

CONCLUSIONS

Young plantlets of black locust (3 months old) are sensitive to the action of low power density UHF waves, when repeated exposures of several hours daily occur. Biochemical parameters such as the chlorophyll levels in the leaves are significantly modified, as *t*-test for statistical significance showed. Most sensitive appeared to be

the level of chlorophyll-b which first is enhanced (2 hours exposure) and then diminished (3 and 8 hours exposure). The ratio between chlorophyll-a and chlorophyll-b is slightly diminished following UHF waves exposure. Non-thermal effect of UHF waves in ion channels from cell system of membranes is supposed to be induced but the complexity of metabolism processes related to photosynthesis involve further experiments, based on diversified analysis scheme.

REFERENCES

1. Aksyonov, S. I., Bulychev, A. A., Grunina, T. Yu., Goryachev, S. N., Turovetsky, V. B. (2001) Effects of ELF-ELM treatment on wheat seeds at different stages of germination and possible mechanisms of their origin. *Electro-Magnetobiol. 20,* 231–253.
2. Carbonell, M. V., Martinez, E., Amaya, J. M. (2000) Stimulation of germination in rice (*Oriza sativa* L.) by a static magnetic field. *Electro-Magnetobiol. 19,* 121–128.
3. Celestino, C., Picazo, M. L., Toribio, M. (2000) Influence of chronic exposure to an electromagnetic field to germination and early growth of *Quercus suber* seeds: preliminary study. *Electro-Magnetobiol. 19*, 115–120.
4. Celestino, C., Picazo, M. L., Toribio, M., Alvarez-Ude, J. A. (1998) Influence of 50 Hz electromagnetic fields on reccurent embriogenesis and germination of cork oak somatic embryos. *Plant Cell, Tissue Organ Cult. 54*, 65–69.
5. Crawford, M. L. (1974) Generation of standard EM Fields Using TEM transmission cells. *IEEE Trans. Electromagnetic Compatibility 16*, 189–195.
6. Dayal, S., Shing, R. P. (1986) Effect of seed exposure to magnetic field on the height of tomato plants. *Ind. J. Agric. Sci. 56*, 483–486.
7. Enescu, V. (1989) Micropropagarea *in vitro* şi ameliorarea prin selecţie clonală a salcâmului (*Robinia pseudoacacia* L.). *Revista pădurilor 104*, 6–9.
8. Fujikawa, H., Ushioda, H., Kudo, Y. (1992) Kinetics of *Escherichia coli* destruction by microwave irradiation. *Appl. Environ. Microbiol. 58*, 920–924.
9. Goiceanu, C., Arteni, A., Avadanei, O. V., Artenie, V., Creanga, D. E. (2001) Some evidence of biological effects of ultrahigh frequency fields in *Triticum aestivum*. *Proc. 9th Mediterranean Conf. Medical and Biological Engineering and Computing*, pp. 781–784.
10. Hanover, J. W., Mebrahtu, T., Bloese, P. (1989) Genetic improvement of black locust: A prime agroforestry species. *Proc. 1st Conf. Agroforestry in Nord America*, Ontario, Canada.
11. Hager, A., Meyer-Bertenrath, T. (1966) Die Isolierung und quantitative Bestimmung der Carotenoide und Chlorophylle von Blättern, Algen und isolierten Chloroplasten mit Hilfe dünnschichtchromatographischer Methoden. *Planta 69*, 128–217.
12. Hinrikus, H. (1999) Low-level microwave field effects on living systems. *Med. Biol. Eng. Comput. 37,* 45–49.
13. Jeng, D. K. H., Kaczmarek, K. A., Woodworth, A. G., Balasky, G. (1987) Mechanism of microwave sterilization in the dry state. *Appl. Environ. Microbiol. 53*, 2133–2137.
14. Kalmukov, K. (2002) Selection and technological capacity of *Robinia pseudoacacia* L. for intensive culture production. *Proc. IUFRO Meeting "Management of fast growing plantations",* Izmit, Turkey, pp. 276–281.
15. Kakita, Y., Kashige, N., Murata, K., Kuroiwa, A., Funatsu, M., Watanabe, K. (1995) Inactivation of Lactobacillus bacteriophage PL-1 by microwave irradiation. *Microbiol. Immunol. 39,* 571–576.
16. Keresztesi, B. (ed.) (1988) *The black locust*. Akadémiai Kiadó, Budapest, Hungary.
17. Lebedev, I. S., Litvinenko, L. G., Shiyan, L. T. (1977) After-effect of a permanent magnetic field on photochemical activity of chloroplasts. *Sov. Plant Physiol. 24*, 394–395.
18. Namba, K., Sasao, A., Shibushava, S. (1995) Effect of magnetic field on germination and plant growth. *Acta. Hortic. 399*, 143–147.

19. Phirke, P. S., Kubde, A. B., Umbakar, S. P. (1996) The influence of magnetic field on plant growth. *Seed Sci. Technol*. *24*, 375–392.
20. Pietruszewski, S. (1996) Effects of magnetic biostimulation of wheat seeds on germination, yield and proteins. *Int. Agrophys*. *10*, 51–55.
21. Redei, K. (2002) Improvement of black locust (*Robinia pseudoacacia* L.) in Hungary. *Proc. IUFRO Meeting on "Management of fast growing plantations*", Izmit, Turkey, pp. 166–174.
22. Ružič, R., Jerman, I., Jelig, A., Fefer, D. (1992) Electromagnetic stimulation of buds of *Castanea sativa* Mill. in tissue culture. *Electro-Magnetobiol. 11*, 145–153.
23. Ružič, R., Vodnik, D., Jerman, I. (1998) Effects of weak low magnetic fields on spruce seeds germination under acid conditions. *Can. J. For. Res*. *28*, 609–616.
24. Ružič, R., Jerman, I. (2002) Weak magnetic field decreases heat stress in cress seedlings. *Electromag. Biol. Med. 21,* 69–80.
25. Sandu, D. D. (2000) *Transmission lines*. Internal Research Report, "Al. I. Cuza" University, Iasi.
26. Schmutz, P., Siegenthaler, J., Stager, C., Tarjan, D., Bucher, J. B. (1996) Long-term exposure of young spruce and beech trees to 2450-MHz microwave radiation. *Sci. Tot. Environ. 180*, 43–48.
27. Stuchly, M. A., Stuchly, S. S. (1986) Experimental radio and microwave dosimetry. In: Polk, C., Postow, E. (ed.) *Handbook of Biological Effects of Electromagnetic Fields*. CRC Press, Boca Raton, pp. 229–272.
28. Stuchly, M. A., Stuchly, S. S. (1987) Measurements of electromagnetic fields in biomedical applications. *CRC Crit. Rev. Biomed. Eng. 14*, 241–88.
29. Suszka, B., Muller, C., Bonnet-Massimbert, M. (1994) *Les chaines des arbres forestieres*. INRA, Orleans.
30. Westerhoff, H. V., Tsong, T. Y., Chock, P. B., Chen, Y. D., Astumian, R. D. (1986) How enzymes can capture and transmit free-energy from an oscillating electric field. *Proc. Natl. Acad. Sci. USA 83*, 4734–4738.