

Phytoremediation, or when the Sun assists in soil decontamination

Thanks to photosynthesis, made possible by sunlight, plants were able to colonize numerous territories. This tremendous biodiversity enables plants to grow on highly diverse soils, including those strongly contaminated by heavy metals, toxic to human health. Hence the concept of using plants for the depollution of contaminated soils. This technique is known as *phytoremediation*.



Selection of a transgenic plant expressing the YCF1 gene, through use of its properties of resistance to a selection marker. Investigations are aimed at providing certain plants with an enhanced heavy-metal accumulation capacity.

The impact of industrial and agricultural activities on the environment has kept growing ever since the industrial revolution. Whereas many organic molecules can be degraded, **heavy metal** contaminating soils and waters raise a human health issue, particularly owing to their entering into the trophic chain. Normally, these heavy metals undergo natural fixation in rocks, however human activities have an effect on their distribution and physical-chemical form. Thus, while a fraction of such heavy metals ends up directly in soils and waters, the largest part is initially released into the atmosphere, then becoming dispersed over very long distances from the emission area.

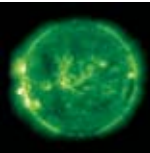
A report recently commissioned by the French Parliament⁽¹⁾ notes that heavy-metal discharges, in France, are of two types: physical, and atmospheric. *Physical discharges* concern mainly lead and, to a lesser extent, cadmium. They are due to metallurgical and mining activities, and to the end-of-life fate of products such as lead-charged car batteries (75,000 tonnes per year). *Atmospheric discharges* involve all metals, and amount to considerable masses, up to tens (mercury, arsenic, cadmium), hundreds (chromium) or thousands of tonnes (lead).

Plants as nutrient and pollutant pumps

The sheer concept of *bioremediation*, i.e. the utilization of living organisms such as **bacteria**, fungi or plants to assist in “cleaning up” the environment is no present-day novelty. The case of plants is of particular interest. Thanks to **photosynthesis** (see Box E, *The photosynthesis process*), plants only need to take up mineral substances from the ground to live, since their carbon source is taken from the atmosphere, in the form of **carbon dioxide**. Being autonomous, these organisms have the ability to colonize fairly quickly many biotopes,⁽²⁾ and, in a given soil, to explore large, and in depth polluted areas. This *phytoremediation* strategy is both ecological and economical, by contrast with conventional excavation and chemical soil-treatment strategies. Phytoremediation benefits from the fact that the plant, as a veritable photochemical fac-

(1) Report No 2979 of the French Assemblée nationale, *Les effets des métaux lourds sur l'environnement et la santé* (“Effects of heavy metals on the environment and health”), by Gérard Miquel (April 2001).

(2) Biotope: the ensemble of elements characterizing a given, uniform physical-chemical environment that is host to a specific flora and fauna.



tory where the solar panels are the leaves, acts as a nutritive mineral-element (nutrient) and heavy-metal pump. Using sunlight, photosynthesis takes place in the leaves, breaking up water molecules drawn from the ground and releasing oxygen into the atmosphere, while the hydrogen binds to the carbon dioxide absorbed by the leaves, yielding sugars. The energy supplied by these sugars and the evapotranspiration⁽³⁾ flux from the plant allow it to extract from the ground the mineral nutrients it requires, and thus indirectly to concentrate some heavy metals, such as cadmium, lead, chromium, arsenic... This singular property exhibited by plants is related to the mechanism of root uptake from the ground of the nutrients indispensable to the plant's growth. Uptake of these nutrients occurs by means of *membrane transporters*, specialized **proteins** embedded in the membranes, whose selectivity is imperfect. Thus, two homologous **cations** (i.e., exhibiting close physical-chemical properties), such as the calcium Ca^{2+} ion and cadmium Cd^{2+} ion, may be "mistaken" one for the other by the plant, which then indiscriminately takes up Ca^{2+} , a metal essential to life, or Cd^{2+} , an environmental toxin.

How plants trap pollutants and make them less toxic

Once it is introduced into the plant cell, the heavy metal develops toxicity related to its low solubility, its **oxidizing** capacity and/or its ability to substitute to biological metals. The defense developed by plants in the course of evolution consists in making these metals

(3) Evapotranspiration: water evaporates through the leaves' stomata, the tiny microscopic pores through which carbon dioxide penetrates. This loss of water generates a suction force, causing more sap (consisting essentially of water and mineral elements) to rise up from the roots.

more soluble through alteration of their chemical form, by associating them to **peptides** (glutathione, phyto-chelatin...), and then displacing, by means of other transporters, the **complexes** thus generated to a compartment where they are trapped and their toxicity is reduced: the *vacuole*. The vacuole, a very large vesicle covered by a single membrane, occupying up to 90% of the cell's volume, is used more or less as the cell's "garbage can." The heavy-metal transporters sited on the vacuole membrane use energy supplied by **hydrolysis** of **ATP**, allowing more toxins to be accumulated than mere diffusion would achieve. This process is known as *hyperaccumulation*. After harvesting of the contaminated plants and incineration in controlled conditions, the metals recovered are recycled or removed.

Enhancing the plant's accumulation capacities

Some plants naturally exhibit heavy-metal accumulation properties, however their biomass often remains restricted, owing to the toxicity of the heavy metals accumulated. To obviate this problem, one strategy seeks to confer, through **genetic engineering**, hyperaccumulation properties to high-biomass plants (tobacco, mustard, poplar). As a prerequisite for such a step, it is necessary to identify **genes** having the capacity to bring to a plant hyperaccumulation properties. One recent instance of the pinpointing of such a gene was recently described, in the context of a collaboration between Zurich University (Switzerland), Pohang University (Republic of Korea), and CEA's Cadarache Center.⁽⁴⁾ For that purpose, the gene encoding for the cadmium transporter best characterized in Eukaryotae,⁽⁵⁾ the YCF1 in *yeast*, was introduced and over-expressed in the plant serving as a model in genetics, *Arabidopsis thaliana*. The **transgenic** plants thus obtained express the YCF1 transporter in large quantities, particularly within the vacuole membrane (see *Figure*). These plants developed an enhanced resistance capacity when exposed to lead and cadmium, and accumulate more heavy metals than control plants. These promising findings highlight the role played by transporters in compartmentalization of pollutants, one factor that had so far been a limiting one as regards improvement of phytoremediation properties in plants. Transposition of this technology to high-biomass plants such as poplar is currently being evaluated.

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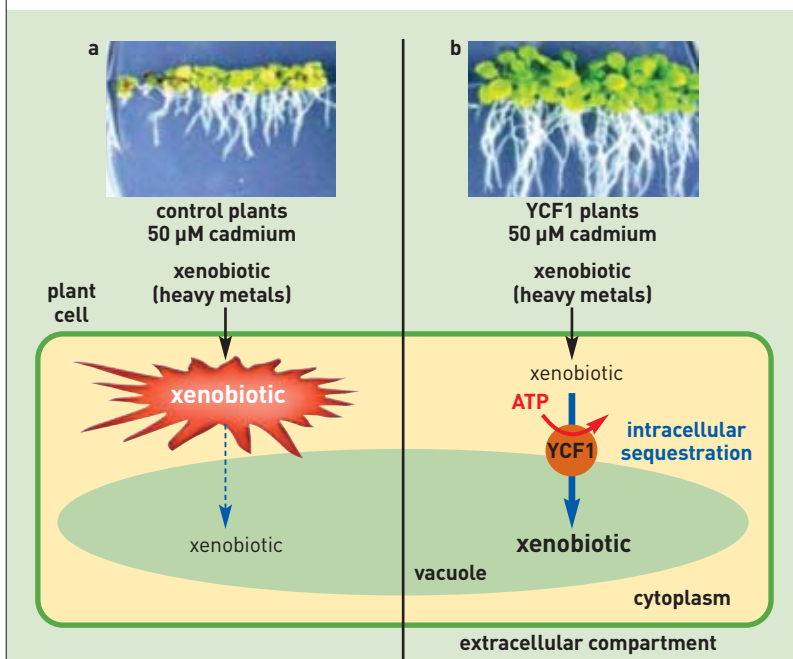


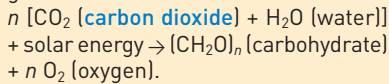
Figure. On a soil contaminated by 50 μM (micromole per liter) of cadmium, control plants grow poorly. Heavy metals are mainly located in the cell's **cytoplasm**, where they exert strong chemical toxicity (a). At (b), transgenic plants over-expressing the transporter YCF1 grow better, and accumulate more heavy metals, stored in the cell's vacuole.

(4) W. Y. Song, E. J. Sohn, E. Martinoia, Y. J. Lee, Y. Y. Yang, M. Jasinski, C. Forestier, I. Hwang, Y. Lee, "Engineering tolerance and accumulation of lead and cadmium in transgenic plants", in *Nat. Biotechnol.* 21, 8, 2003, pp. 914-919.

(5) Eukaryotae: living organisms comprising one or any number of cells having a distinct nucleus and **cytoplasm**. The Eukaryotae lineage includes all life forms, with the exception of bacteria, some lower algae, and viruses.

E The photosynthesis process

Photosynthesis is the biological process by means of which solar energy is used by living cells for their own energy requirements. This highly complex process is carried out by plants, but equally by algae and numerous **cyanobacteria**. Among the latter, **cyanobacteria** (see Box 1, p. 88) make use of the same kind of photosynthesis as plants and algae, enabling them to **oxidize** water and release oxygen. These photosynthetic organisms are **autotrophic**: they synthesize their own organic material from the mineral substances they draw from the ground or the aquatic environment (water and mineral salts). In symbolic representation, formulation of this so-called oxygen photosynthesis goes as follows:



All the stages of photosynthesis take place in the **chloroplasts**, which are internal organelles in plant cells. The chloroplasts, just as the cyanobacteria, contain specialized membranes in which are found all the molecular structures required for the initial steps of photosynthesis. These membranes are organized to form closed structures (a form of flat vesicle, these are known as **thylakoids** in chloroplasts).

Visible light from the Sun is **absorbed** by colored molecules: mainly **chlorophylls**, the universal photosynthesis pigment, and a variety of molecules, which differ according to the organism (carotenoids, phycobilins, phycocyanins...). These photoreceptor pigments are bound to **proteins**, which are in

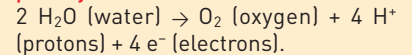
turn, in most cases, embedded in the photosynthetic membrane. These proteins, together with their pigments, are associated into vast arrays comprising around 300 chlorophylls, known as **photosystems**. Each photosystem is structured round a central region, the **reaction center**, the core of which is formed by a specialized pair of chlorophyll molecules. Only this pair is chemically reactive. It receives, in the form of electronic excitation, the energy from the solar **photons** captured by all of the pigments in the photosystem. The latter thus have a function as **harvesting antenna** elements, also carrying out an electron-energy transfer function. Thus excited, the "special" chlorophyll pair has the ability to transfer an **electron** to a succession of electron-acceptor and -donor sites present in the reaction center. It is consequently termed the "**primary electron donor**."

Electron transfers follow a precise organization within the photosynthetic membrane, to achieve, on the one hand, chemical **reduction** of one compound, nicotinamide adenine dinucleotide phosphate (**NADP⁺**), by an **enzyme**, NADP-reductase (also known as **FNR**, or ferredoxin-NADP-reductase), and, on the other, storage of **protons H⁺** in the thylakoids' internal space. The chemical energy potential resulting from this difference in proton concentration on either face of the photosynthetic membrane is used by a membrane protein, **ATP-synthase**, to synthesize adenosine triphosphate (**ATP**), subsequent **hydrolysis** of which will release, in controlled fashion, the chemical energy thus stored. Ulti-

mately, the energy from solar photons is converted into two forms of chemical energy, in reduced **NADP⁺** (**NADPH**) and in **ATP**.

Photosynthetic organisms having the ability to release oxygen have two quite distinct types of photosystems, designated as I and II. Associated within the same photosynthetic membrane, they work to couple the energy from their two photochemical reactions into the electron-transfer chain going from oxidation of water into oxygen through to **NADPH** and **ATP** synthesis. They thus work in series, in energy terms (see **Figure**).

Photosystem II, **PSII**, has a primary electron donor designated as **P680** (as this pair of chlorophylls absorbs light at 680 **nm**). Photo-induced departure of one electron leaves P680 bearing a positive charge, i.e. in an oxidized state. In order to return the P680⁺ to its neutral state, and allow a new photochemical cycle to occur, a major step of photosynthesis now takes place within PSII, **photolysis of water**:



The electrons originating in water photolysis are transferred through to P680, and gaseous oxygen is released. The electrons stripped from P680 travel through to photosystem I, **PSI**, by way of a sequence of oxidation-reduction reactions involving electron transporters bound to the PSII core (see **Photosynthesis and oxygen production**). They exit PSII in the guise of doubly-reduced plastoquinones, **PQH₂**, that diffuse in the membrane, and transfer this reduction potential to a membrane cytochrome (**cyt b₆f**), and

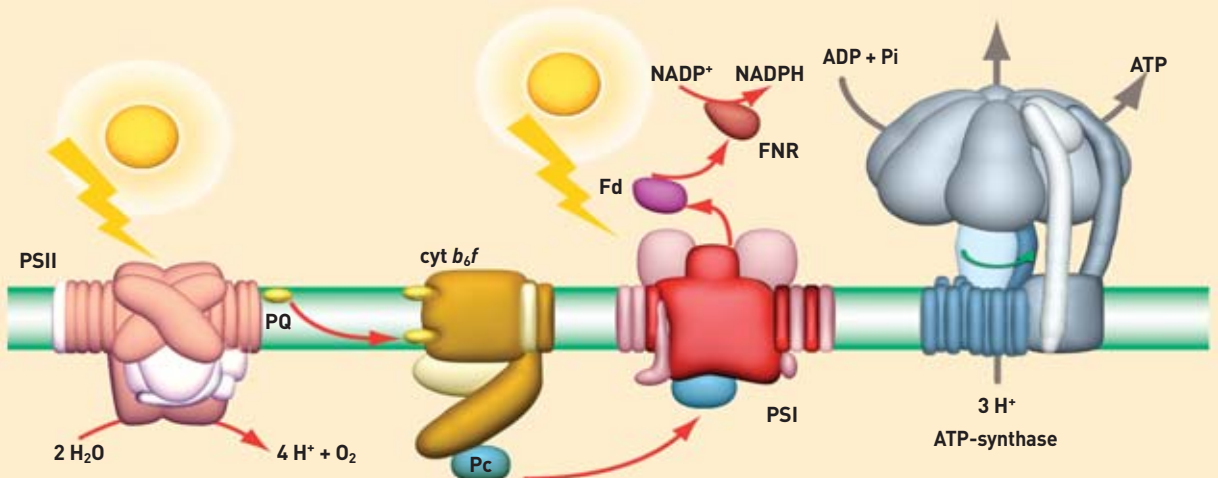


Figure.

Les étapes membranaires du processus de photosynthèse se déroulant chez les plantes vertes, les algues et les cyanobactéries.

E (next)

subsequently to a plastocyanin, **Pc**, a small, copper-containing soluble molecule.

When the electron transmitted by PSII by way of the plastocyanin reaches PSI, it replaces the electron lost by that photosystem's primary donor, **P700**, this also having been indirectly excited by a second photon. The electron yielded by PSI follows a chain of transporters within the reaction center, ultimately being transferred to a ferredoxin, **Fd**, a small iron-containing molecule. The electron is then transferred on to the NADP-reductase, this reducing NADP^+ into NADPH.

The chemical energy thus generated by the membrane-located photosynthesis processes just described allows reactions of organic molecule synthesis (including carbohydrate synthesis) to occur inside the cell. These reactions, which require input of energy, and take

place in the aqueous phase, constitute the *Calvin cycle*.

This final stage of photosynthesis involves the ATP and NADPH generated by the in-membrane reactions. The main carbon-fixation reaction, whereby an **atom** of inorganic carbon is converted into organic carbon, unfolds as follows: atmospheric CO_2 reacts with a sugar, ribulose 1,5-bisphosphate, or RuBP, and water, to yield two molecules of 3-phosphoglycerate. This carbon-fixation reaction is **catalyzed** by a large enzyme, ribulose biphosphate carboxylase, or **Rubisco**. The remaining steps in the cycle result in regeneration of RuBP. For every molecule of CO_2 converted into a carbohydrate, three molecules of ATP and two molecules of NADPH are consumed. The overall equation for the Calvin cycle may thus be set out as follows:

$3 \text{CO}_2 + 9 \text{ATP} + 6 \text{NADPH} + \text{water} \rightarrow \text{glyceraldehyde 3-phosphate} + 8 \text{Pi}$ (inorganic phosphate) + 9 ADP (adenosine diphosphate) + 6 NADP^+ .

The glyceraldehyde 3-phosphate goes on to be transformed into saccharose (sucrose), and starch, these constituting the energy reserves of plant cells. Rubisco is a bifunctional enzyme, being equally able to effect fixation of oxygen O_2 . This reaction yields 2-phosphoglycolate, which is not involved in the Calvin cycle. It would thus seem to be of no use to the plant. It sets off a complex process, known as **photorespiration**, for the purposes of consuming the 2-phosphoglycolate. Some plants, such as corn (maize), have developed leaf structures and biochemical pathways that concentrate CO_2 in the vicinity of Rubisco, thus restricting its utilization of oxygen.

Cyanobacteria, privileged organisms for photosynthesis research

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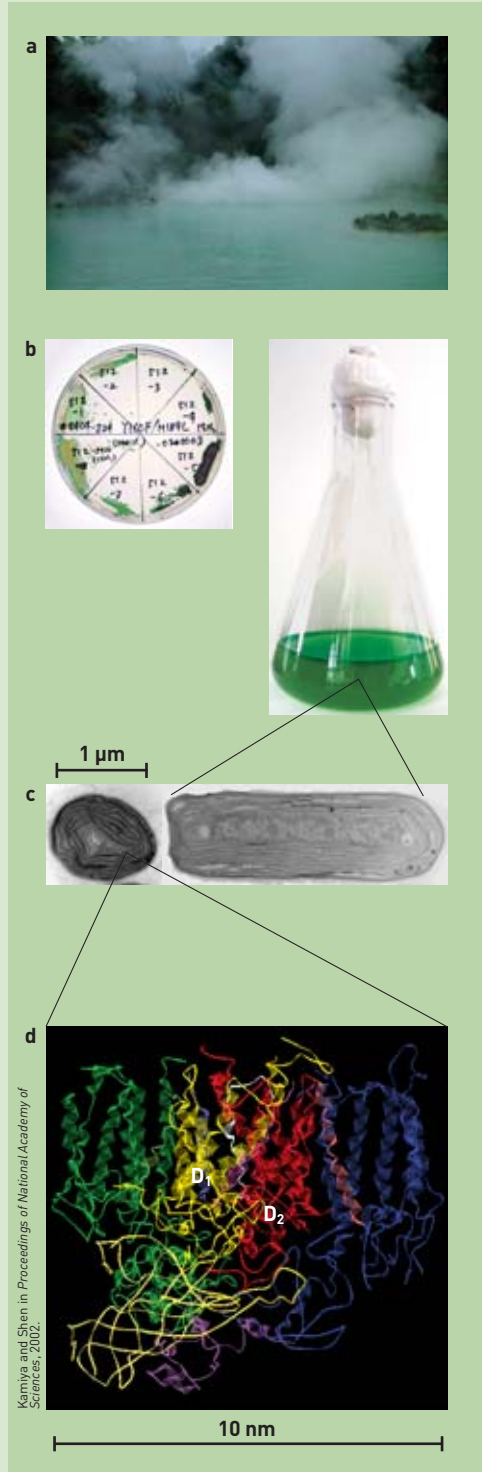
Cyanobacteria are the originators of the planet's oxygen. They are exemplars of the extreme adaptability shown by **photosynthetic** organisms. The thermophile bacterium *Thermosynechococcus elongatus* (*T. elongatus*) is of particular interest. It was discovered at Beppu, in Japan, in a hot spring. In this species, the oxygen-evolving **enzyme** (photosystem II, **PSII**) is far more stable than that found in plants, even though it is practically identical to it. Quite recently, its **genome** was fully **sequenced**. This cyanobacterium is deemed to be similar to the first organisms to have acquired the ability to **oxidize** water. The enzyme has changed little over the course of evolution.

This cyanobacterium can be dish-grown, and its **DNA** may be manipulated. Appropriate protein-engineering techniques have enabled a purification protocol to be arrived at for the PSII enzyme. This is a membrane **complex** of about 300 kDa (1 dalton = $1.66 \cdot 10^{-27}$ kg), comprising at least 17 subunits. It contains at least 13 cofactors ⁽¹⁾ active in **electron** transport, and over 35 **chlorophylls**. The stability of the purified PSII means it is wholly suited for the carrying out of enzymology experiments in which extreme biochemical constraints may be used, to trap intermediate states in the key water-oxidation reactions. This enzyme's three-dimensional structure has recently been elucidated.

Thus, because its genome is known, because its PSII enzyme is easily manipulated, and because the latter's three-dimensional structure is known, the *T. elongatus* cyanobacterium is a privileged organism for photosynthesis research.

(1) Cofactors: small organic molecules or inorganic ions bound to a protein, acting as part of its structure, and in most cases required for its activity. In photosystem II, the main cofactors involved, directly or indirectly, in electron transport are chlorophylls, pheophytins, plastoquinones, and manganese and calcium ions.

At (a), hot spring at Beppu, in Japan, where cyanobacterium *T. elongatus* was discovered. It cells may be grown on a Petri dish or in a liquid medium (b). At (c), cross-sectional and longitudinal views of a cell, in electron microscopy. Highly elongated in shape, it is replete with lipid membranes carrying chlorophyll-containing proteins together with the other proteins involved in the energy machinery (see Box E, **The photosynthesis process**). At (d), structure of the PSII core, obtained by X-ray crystallography. The oxygen-evolving enzyme contains over twenty different proteins. The two central subunits (D₁ and D₂) hold all the important cofactors, and are host to the photochemical and catalytic reactions.



C Elementary particles and fundamental interactions

Neutrinos are the stealthiest particles in the **standard model of particle physics**, the theoretical framework describing all known **elementary particles** and the **fundamental interactions** they mediate (see Table). The basic constituents of matter, **fermions**, are partitioned into two main categories:

leptons, which do not respond to **strong interaction**, and **quarks**, which are subject to all of the interactions. The six quarks form three pairs (up/down, charmed/strange, beauty/top). In the lepton category, the **charged leptons** (**electron** e^- , **muon** μ , **tau** τ) are involved in the **electromagnetic interaction** and the

weak interaction, while **neutral leptons** (**electron neutrino** ν_e , **muon neutrino** ν_μ , **tau neutrino** ν_τ) are only subject to weak interaction. In the standard model, neutrinos have zero mass, however experiments have shown they do have some mass, though very small, the exact value of which is as yet unknown. Involvement

	leptons able to move freely		quarks held captive in larger particles, they are not observed separately	
Fermions Common matter is made up of particles from this group. Most of these particles were around just after the Big Bang. Presently only to be found in cosmic rays, and around accelerators.	first family electron Responsible for electricity and chemical reactions. Charge: -1 . mass: $0.511 \text{ MeV}/c^2$	electron neutrino Has no electric charge, and interacts very seldom with the ambient medium.	down Electric charge: $-1/3$. The proton holds one, the neutron two. mass: $5 - 8,5 \text{ MeV}/c^2$	up Electric charge: $+2/3$. The proton holds two, the neutron one. mass: $1,5 - 4,5 \text{ MeV}/c^2$
	second family muon A more massive companion to the electron. mass: $105,658 \text{ MeV}/c^2$	muon neutrino Properties similar to those of the electron neutrino.	strange A heavier companion to "up." mass: $80 - 155 \text{ MeV}/c^2$	charmed A heavier companion to "down." mass: $1000 - 1400 \text{ MeV}/c^2$
	third family tau Heavier still. mass: $1777 \text{ MeV}/c^2$	tau neutrino Properties similar to those of the electron neutrino.	beauty Heavier still. mass: $4000 - 4500 \text{ MeV}/c^2$	top Heaviest in the family (observed in 1985): 180 times the proton mass. mass: $174300 \pm 5100 \text{ MeV}/c^2$
Vector Bosons Fundamental particles carrying out transmission of all natural forces.	photon Elementary grain of light, vector for the electromagnetic force.	gluon Bearer of the strong force between quarks.	W^\pm, Z^0 Bearers of the weak force, responsible for some forms of radioactive decay.	
Higgs Boson?	Responsible for "electroweak symmetry breaking."			

Tableau.
Constituants élémentaires.

of the various elementary constituents in the fundamental interactions is governed by their quantum numbers, or interaction charges (electric charge, color charge ⁽¹⁾...). To every constituent of matter is associated its **antiparticle**, a particle having the same mass and opposite charges. The **gravitational force**, which is not included in the standard model, acts on all fermions in proportion to their mass. The table of elementary constituents of matter manifests another classification – independently from their involvement in fundamental interactions – into three generations, or families. From one family to the next, charged quarks and leptons having the same charges only differ by their mass. The electron, up quark and down quark, which all belong to the first family, are the lightest massive particles. They are stable particles, and the constituents of common matter. For instance, the **proton** is made up of two up quarks and one down quark; the **neutron**, of two down quarks and one up quark. Particles in the other two families are unstable, and rapidly decay into

(1) Color charge: a quantum number that determines whether a particle is involved in strong interaction. The color charge can take on three values: “red,” “green,” or “blue” – such colors bearing no relation to visible colors. Every quark bears one of the three color charges, every antiquark one of the three anticolor charges. Gluons bear double color–anticolor charges (eight possible combinations).

stable first-generation particles. This is why all the stable matter in the Universe is made up from constituents from the first family.

According to quantum mechanics, for an interaction to take place, at least one elementary particle, a **boson**, must be emitted, absorbed or exchanged. The **photon** is the vector for the electromagnetic interaction, the **W⁺**, **W⁻** and **Z⁰** mediate the weak interaction, and **gluons** act as messengers for the strong interaction. Quarks and charged leptons exchange photons, but conserve their electric charge after the exchange, the photon having no electric charge. Since the photon’s mass is zero, the electromagnetic interaction’s range is infinite. Having no electric charge, neutrinos are the only elementary fermions that are not subject to electromagnetic interaction.

In the electroweak theory (a unification of the weak and electromagnetic interactions), the weak interaction has two aspects: charged-current weak interaction, for which the interaction vectors are the **W⁺** and **W⁻**; and neutral-current weak interaction, for which the mediator is **Z⁰**. These two forms of weak interaction are active between all elementary fermions (quarks, charged leptons and neutrinos). The mass of these bosons being very large (80,000 MeV/c² for **W[±]**, 91,180 MeV/c² for **Z⁰**), the range of the weak interaction is tiny – of the order of

10⁻¹⁸ m. Since **W[±]** bosons have a non-zero electric charge, fermions exchanging such bosons undergo a change in electric charge, as of nature (flavor). Conversely, since the **Z⁰** boson has no electric charge, fermions exchanging one undergo no change in nature. In effect, neutral-current weak interaction is somewhat akin to exchanging a photon. As a general rule, if two fermions are able to exchange a photon, they can also exchange a **Z⁰**. On the other hand, a neutrino has the ability to exchange a **Z⁰** with another particle, though not a photon. Only those quarks that have a color charge exchange gluons, these in turn being bearers of a color charge. Thus, when a gluon exchange takes place between quarks, the latter exchange their respective colors. Gluons have zero mass, however, since they do bear a color charge, they are able to interact. The range of the strong interaction is consequently very restricted – of the order of 10⁻¹⁵ m.

The graviton, the vector for gravitational interaction, has not so far been observed.

Theory predicts that another fundamental interaction mechanism exists, responsible for the mass of elementary particles, for which the messenger is the Higgs boson, which remains as yet undiscovered. This boson makes it possible to assign a mass to elementary fermions of zero mass that interact with it.

fundamental interaction	messenger	actions
gravitational	graviton?	responsible for the mutual attraction of any two masses and for the law of falling bodies
electromagnetic	photon	responsible for the attraction between electrons and atomic nuclei, hence for the cohesion of atoms and molecules
weak	W ⁺ , W ⁻ , Z ⁰	the root cause of thermonuclear fusion inside the Sun, ensuring its longevity. β ⁻ and β ⁺ radioactivity, and reactions involving neutrinos are weak interactions
strong	gluons	ensures the cohesion of the atomic nucleus

Table. Fundamental interaction and elementary constituents.