This article is an attempt to formalize Chapter 17 of Keynes’s General Theory using a continuous dynamic optimization model with perfect foresight. I present two subjective interest rates: the time preference rate and the liquidity premium that, respectively, govern the consumption-saving and portfolio decisions. Under optimal household behavior, they are equalized to the market rate of interest. In the monetary economy described by Keynes, however, the equality can be inconsistent with the condition of market equilibrium, in which case persistent stagnation occurs. A new analytic method based on dynamic optimization is proposed as an alternative to LM analysis.

1. INTRODUCTION

Keynes extensively discussed various factors that prevent prices from reaching full equilibrium levels in The General Theory of Employment, Interest and Money. In the neoclassical literature, it is generally believed that, in the absence of permanent price and wage rigidities, market equilibrium eventually obtains. As a result, Keynesian stagnation traditionally has been analyzed either by assuming a priori some price rigidities or by imposing noncompetitive assumptions that might cause prices, wages, and/or the rate of interest to diverge from market-clearing levels. There is


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For example, monopolistic competition, kinked demand curves, efficiency wages, and asymmetric information have been posited and analyzed as possible causes. See Ball, Mankiw, and Romer (1988) for a survey of new Keynesian approaches and Stiglitz and Weiss (1981) and Mankiw (1986) for analyses of asymmetric information in credit markets.
A New Theory of Stagnation

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1. Theoretical background of research on stagnation

Since 1990 Japan has been in the worst stagnation after World War II. People do not consume enough to attain full employment. Surprisingly, economics has not yet fully solved why an overall shortage of demand occurs. In the history of economic thought there are lots of attempts to clarify the mechanism of stagnation. A typical one is Keynes (1936). However, later economists have found lots of contradictions to the rationality of consumers’ behavior in his theory. They criticize the economists that still follow Keynes’s theory and cynically call them old Keynesians. Furthermore, they have almost given up providing a model that accommodates stagnation caused by an overall demand shortage.

Consequently, some theorists (called neoclassical economists) say that involuntary unemployment never occurs and that the unemployed voluntarily choose the present situation. They concentrate on analyzing the dynamics of an economy with full employment and full market equilibrium. Others (called new Keynesians) believe that frictional price/wage adjustment causes a demand shortage in each individual market. Therefore, they analyze the mechanism of price/wage rigidities, e.g., monopolistic behavior of firms or workers, and imperfect information of the labor market.

Following these theories most economists, including Japanese and the U.S. ones, insist the following:

1) The present Japanese stagnation is caused not by a demand shortage but by production inefficiency. Therefore, inefficient firms should be removed and fiscal spending should be lowered.

2) The unemployed should be left as they are since they prefer being unemployed to employed.

3) Prices should be lowered more rapidly so that market equilibrium is smoothly reached.

In reality, however, lots of people are left unemployed although they are seriously searching for jobs. Prices continue to decline for more than 10 years and yet employment does not improve at all. A decrease in fiscal spending lowers national product. Therefore, policy makers cannot rely fully on the conventional theories and still use the old Keynesian analysis despite lots of shortcomings.

In this paper and Ono (1994) I establish a new stagnation theory that satisfies neoclassical criticisms against Keynes’s theory. In a competitive monetary economy with dynamically optimizing consumers and firms persistent stagnation is shown to occur although prices and wages continue to adjust. Preference for money/wealth holding plays a crucial role in generating stagnation. This stagnation is in sharp contrast to those of the old and new Keynesian theories since they either assume a priori some price rigidities or impose noncompetitive assumptions that might cause prices and/or wages to diverge from market-clearing levels.

This theory also requires a drastic change of neoclassical policy implications which most of Japanese policy makers believe in. For example, fiscal spending or monetary expansion is effective in increasing consumption and national product. Faster price/wage adjustment, leading to greater deflation, reduces employment.

2. Mechanism of stagnation

If people raise consumption, firms increase employment and investment to produce more, and a boom occurs. If they reduce consumption, firms decrease employment and investment, leading to stagnation. Thus, by considering how people determine consumption we can analyze the mechanism of business fluctuation.

Whether people consume or save depends on their asset holding and the attractiveness of commodities. If they save, they enjoy the utility of being rich at the cost of postponing consumption. If they consume, they immediately enjoy consumption at the cost of giving up wealth accumulation. Therefore, as they accumulate wealth and become richer, they increase consumption. If there are many attractive commodities, they consume them even though they are poor.

More precisely, when considering money allocation, people compare the utility of money holding, return from an interest-bearing asset and the utility of present consumption. If they save now, they have to give up interest earnings \( \pi \) or the utility of money holding measured by liquidity premium \( \delta \). If they save, they have to postpone consumption for a while. The loss of postponing consumption is given by the rate of time patience with respect to consumption measured in terms of money. It implies how much extra money is required to realize the same utility when consumption of unit money is postponed for a unit interval. Mathematically, it is given by the sum of subjective discount rate \( \rho \) and inflation (or deflation) rate \( \pi \).

\[
\text{Fig 1. Allocation of money and various interest rates.}
\]

Thus, the interest rate has three aspects, the premium of liquidity generated by money holding \( \delta \), the rate of asset return \( R \), and the rate of time patience with respect to consumption \( \rho + \pi \), as shown in figure 1. By comparing the three rates people determine consumption, saving and asset portfolio to realize the equality among the three:

\[
\rho + \pi = R = \delta.
\]

If there are lots of attractive commodities and people's desire for consumption dominates that for holding assets, they consume enough to attain full employment. If the desire is too much, demand exceeds supply and inflation occurs. Mathematically, it is the situation in which \( \rho + \pi \) exceeds the other two rates. As inflation goes on, the real value of money (viz. the nominal value divided by the general price level) declines, and hence people's preference for

1 See Blanchard and Fischer (1989) and Romer (1996) for these theories.
holding money compared to consumption gradually increases. Consequently, people decrease consumption and market equilibrium eventually obtains.

Conversely, if an economy’s overall productivity is so high that people’s desire for holding money dominates that for consuming commodities up to the level that attains full employment, a shortage of demand and unemployment occur, causing prices and wages to decline. Since deflation (viz. a continuous price/wage decline) raises the real value of money over time, it makes money holding more attractive and hence people reduce consumption. This is why consumption remains to be low under deflation.

Deflation also has a long-run positive effect on consumption. As prices decline, the real value of money increases. Since it makes people richer, they may raise consumption. If so, demand would gradually increase and eventually market equilibrium obtains.

However, such a long-run mechanism of recovering market equilibrium may not sufficiently work, as is now observed in Japan. This is because people’s desire for money holding is insatiable while that for consumption is satiable. Intuitively, no matter how much you like cakes or no matter how cheap they are, you would easily be satiated with them. This property is valid for any commodity.

In contrast, you would never be fed up with money holding. Even if you were a billionaire, you would be happier when you have more money - i.e., preference for money is insatiable, as Keynes (1936), Marx (1907) and Simmel (1978) state. Therefore, even if deflation continues and the real value of money expands, people’s desire for money does not decline enough. Consequently, the desire for money may exceed that for consumption especially in an economy with high production. In this case, only the negative effect of deflation works on consumption, and a demand shortage persists.

In this state we obtain the following results:
1) An increase in productivity expands the demand-supply discrepancy and raises deflation, causing consumption to decrease.
2) A rise in the price/wage adjustment speed increases deflation, which decreases consumption.

They are opposite to those obtained in the neoclassical theory.

3. Dynamics with persistent unemployment

The stagnation mechanism mentioned above is mathematically derived from the dynamic optimization behavior of consumers and firms. A representative consumer is considered to choose consumption, saving, and asset portfolio so as to maximize the integral of his (or her) lifetime utility stream subject to the intertemporal budget equation. The first-order optimal conditions of this problem are summarized as the equality among the three interest rates mentioned in figure 1. A representative firm is considered to determine its production schedule so as to maximize the integral of its future revenue stream.

From such rational consumer and firm behavior we derive differential equations with respect to consumption \( c \) and real money balance \( m \). Figure 2 illustrates the phase diagram of the dynamics in the case of high productivity and strong liquidity preference.\(^2\)

The arrow represents the dynamic path of consumption \( c \) and real money balance \( m \). Along this path consumption \( c \) cannot reach full-employment level \( y \) although real money balance \( m \) continues to expand as deflation goes on. Thus, stagnation persistently occurs.

\[ m = 0 \]

\[ y \]

\[ c \]

\[ \dot{c} = 0 \]

\[ \dot{m} \]

**Figure 2: Dynamics with persistent stagnation**

4. Policy implications

The present theory drastically revises neoclassical implications of fiscal and monetary policies.

Since a shortage of effective demand is not considered to exist in a neoclassical model, it is insisted that an increase in fiscal spending crowds out private spending. Intuitively, if the government hires labor that can be used in the private sector, private production decreases, forcing people to reduce consumption of private products. Therefore, unless the government supplies more valuable commodities or services than the private sector, people are worse off.

However, in the presence of involuntary unemployment, as is shown by the present theory, an increase in employment due to fiscal spending raises consumption by reducing the deflationary gap in the labor market. A decrease in deflation reduces the benefit of holding money and stimulates consumption. Thus, fiscal spending is effective only when it is large enough to reduce unemployment and the deflation rate significantly. Only a huge increase in fiscal spending, such as that in wartime, has a visible effect on national product.

However, even if fiscal spending has only a negligible effect, the government should increase fiscal spending under stagnation since it can produce social capital or public services without losing any private production in the presence of unemployment. This result significantly differs from the neoclassical one. In the neoclassical theory unemployment is ignored, and hence fiscal spending is considered to absorb workers from the private sector. Therefore, it is insisted that fiscal spending should be cut as much as possible.

The above result may look similar to the old Keynesian result. They think that fiscal spending raises national product through a multiplier effect - i.e., fiscal spending increases some people’s

\(^2\) See Ono (1994) for the other cases.
income and thus their consumption, which again increases others' income, and so on.

However, the argument of the multiplier effect has a significant contradiction. Fiscal spending is financed through either taxes or bonds. In the case of taxes people have to pay, in the case of bonds they will be taxed in the future to repay the bonds. Anyway, they know that their lifetime income will be unchanged. Therefore, they have an incentive to increase consumption. This contradiction does not exist in my theory. Fiscal spending stimulates consumption by reducing deflation.

The multiplier effect is derived from the ad hoc consumption function proposed by Keynes. Consumption is assumed to depend only on current income, regardless of asset holdings or future income. Neoclassical economists criticize this assumption and negate the multiplier effect. Note that the present theory also negates it and yet obtains the efficacy of fiscal spending in a dynamic optimization framework.

The implication of monetary expansion obtained from the present theory also differs from that of the neoclassical theory or that of the Keynesian theory. In the neoclassical and the Keynesian economics monetary policy has no effect on national product.

In the present theory, in contrast, monetary expansion reduces deflation, making it less advantageous for people to hold money, and thus people consume more. Consequently, employment and national product increase. This may provide a rationale for the inflation targeting policy that is nowadays broadly discussed as a possible policy instrument. However, as shown by the present theory, it works only if it can actually raise the inflation rate. We have to care about the danger that careless monetary expansion may seriously harm the credibility of money and eventually destroy the monetary system.

5. A New Method of Effective Demand Analysis

Using this theory we can propose a simple analytical method of effective demand as an alternative to the conventional Keynesian analysis that are seriously criticized. As mentioned in figure 1, the interest rate has three aspects, the rate of time patience, the rate of return from an interest-bearing asset and the liquidity premium of money. Consumption under stagnation is determined at the level where the rate of time patience (representing the desire of consumption) equals the liquidity premium (representing the desire of holding money).

Figure 3 illustrates these rates as functions of consumption. An increase in consumption raises inflation (or equivalently reduces deflation), and hence the desire of present consumption increases. It also turns people’s interest from consumption to money holding, causing the desire of money holding to increase. Thus, both rates are represented as increasing functions with respect to consumption. For the stability of the dynamics given in figure 2 the slope of the desire of consumption is shown to be less than that of the desire of holding money, as illustrated in figure 3. The equilibrium consumption level is given at the intersection point of the two curves. Note that $c_0$ and $y$ are respectively the same as those in figure 2.

Using this figure we can analyze the effect on consumption of fiscal spending or monetary expansion. These policies autonomously raise inflation and shift upward the curve that represents the desire for consumption without moving the curve that represents the desire of holding money. Consequently, the intersection point moves rightward i.e., these policies stimulate consumption.

6. Conclusion

Neoclassical economists think light of the utility of money holding. They consider that people eventually want to consume rather than hold money. In this case it is readily derived that people eventually spend money on consumption and hence a persistent demand shortage cannot occur. Thus, long-run stagnation cannot properly be treated in this framework.

This paper emphasizes the role of money. If people want to hold money so much that they consume little, a demand shortage and hence stagnation would occur. This paper models such liquidity preference in a dynamic optimization framework and provides a new method of effective demand analysis. It gives various policy implications that can be applied to a stagnant economy, such as the current Japanese economy.

References
Daisy Chain Necklace: Supramolecular Architecture Toward Molecular Composites and Devices

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Abstract
Cyclodextrins (CDs: cyclic glucose polymers) have been used as a cyclic building block for the construction of supramolecular architectures. Poly(rotaxanes), in which many CDs are imprisoned in a polymer chain, have been prepared. When a guest group, a molecule which can be included in a CD cavity, was attached on the cyclic host, the molecule formed intermolecular complexes to give supramolecular polymers, daisy chain. When the supramolecular polymers were treated with bulky stopper groups, they formed cyclic tri[2]rotaxane, daisy chain necklace. A cyclodextrin ring can move in some of their structures. Such compounds have potentials for molecular shuttles, motors, and machines. Design and synthesis of various supramolecular architectures using cyclodextrins as a cyclic component are described.

Introduction
Recently, much attention has been focused on nano-science and nano-technology, construction of nanometer-scale architectures from bulk materials by top-down engineering. Advances in VLSI (very large-scale integrated systems) production techniques (lithography) have moved processing down from the micrometer to the sub-micrometer level. Even smaller architectures have been realized for the construction of new materials and devices. However, there is a limit to top-down approaches for many reasons including the interference of light waves for lithography. Researchers exploit new ways to fabricate smaller architectures. Hence, the bottom-up procedure from a molecule to create new architecture has become an important approach. This approach has already been utilized by nature.

Synthetic polymers are now ubiquitous in the world, the rise of supramolecular chemistry has spurred investigations into alternative approaches for polymer synthesis. Whereas molecular polymerization relies upon the formation of covalent bonds between monomeric building blocks, the propagation step in a supramolecular polymerization proceeds via the formation of non-covalent bonds. When a guest part is attached on a cyclic host component, the conjugate may form intramolecular complexes or intermolecular complexes to give supramolecular polymers.

Experimental Procedure
We chose cyclodextrin (CD) as a cyclic component for the construction of supramolecular structures and various polymers as guest molecules. We found that CDs form complexes with some polymers with high selectivity to give supramolecular polymers. When we use CD as a host and a benzene as a guest moiety which is suitable for fitting in a cyclodextrin cavity, they formed intramolecular complexes or intermolecular complexes to give supramolecular polymers. When benzene is directly attached on CD, the compound did not form supramolecular polymers. This result suggests that some spacer groups are required for efficient formation of intermolecular complexes. We tested a hydrocinamomoyl group as a guest moiety. However, 6-hydrocinamomoyl [β-CD (6-HClOβ-CD)] formed an intramolecular complex and 6-HClO-α-cyclodextrin gave only weak intermolecular complexes. So we have decided to use cinamomoyl derivatives as a guest because cinamomoyl derivatives have a rigid carbon-carbon double bond. The synthetic route is shown in Scheme 1.
Supramolecules

Supramolecules are defined as the organized entities that result from the association of two or more chemical species held together by intermolecular forces. There are many types of supramolecular systems, put them together by hydrogen bonding, electrostatic interactions, hydrophobic interactions, coordination bonds, and host-guest interactions. Cyclodextrins are cyclic compounds consisting of 6 to 8 glucose units. They are called α-, β-, γ-cyclodextrin, respectively (Fig.1). They are known to form inclusion complexes with small molecules which can fit their internal cavity to give supramolecules. Since CDs were discovered, there have been a lot of papers on complex formation of CDs with various low molecular weight compounds. However, there were no reports on complex formation of CDs with polymers, when we started the project.

Complex Formation of CDs with Polymers

Previously, we reported that cyclodextrins form inclusion complexes with various polymers with high selectivity. This is the first observation that a ring molecule includes a polymer chain into cyclic cavity. Cyclodextrins were found to form inclusion complexes with various polymers with high selectivity. For example, α-cyclodextrin forms complexes with polyethylene and poly(ethylene glycol) to give crystalline compounds in high yields (Fig.2), β-cyclodextrin forms complexes with poly(propylene) and poly(propylene glycol), γ-Cyclodextrin forms complexes with poly(butadiene) and poly(isobutylene), (synthetic rubber) and poly(dimethyl silicone) (silicone oil). There is a good correlation between sizes of cyclodextrin cavities and the cross-sectional area of the polymers.

Rotaxane and catenanes

Cyclic compounds have been used for the construction of some interlocked molecules, such as rotaxane and catenanes (Fig.3). Rotaxanes are molecules which have a rotor and an axle in the molecule. Catenanes are chain molecules. CDs have been used as a cyclic component for the construction of rotaxane and catenanes. These compounds are important for the construction of molecular machines and devices, because a cyclic component of the molecules can move along an axle or other ring.

Polyrotaxanes

Polyrotaxanes in which many cyclodextrins are threaded onto a polymer chain have been prepared starting from poly(ethylene glycol) and α-cyclodextrin by capping the chain end by bulky stoppers (dinitrophenyl groups) (Fig. 4a). Cyclodextrin rings can be translocated along a polymer chain by chemical (acid/base) stimulation.

Recently, a cyclodextrin ring or two in a polyrotaxane have been found to be able to be manipulated by using the tip of STM (scanning tunneling micrography). One of the cyclodextrins in the polyrotaxane was mechanically pushed by the STM tip along the main chain of PEG. Upon moving the tip in the reverse direction, the α-cyclodextrin retracted its path and returned to its original position. Thus, the shuttling of one α-cyclodextrin could be repeated (Fig. 4b).
More recently, we found that cyclodextrins form complexes not only with non-ionic polymers but also with ionic polymers such as linear polymers consisting of biuridinium (viologen) bridged by polymethylene chains. Although these polymers do not give crystalline complexes with any cyclodextrins at all, cyclodextrins form complexes with these polymers in aqueous phase, because polymethylene units are favorable for complex formation with cyclodextrins. The $^1$H NMR spectra of this polymer in the presence of cyclodextrins proved their structures. On the bases of these observations, we have decided to make a molecular shuttle in which a cyclodextrin moves back and forth along a chain.

**Molecular Shuttles**

We have designed and prepared a molecular shuttle as follows: $\alpha$-cyclodextrin as a ring, two polymethylene chains as stations, 4,4'-biuridinium units as linkers, and dinitrophenyl groups as stoppers (Fig. 5). Shuttling behavior of the molecular shuttle is solvent-and temperature-sensitive and could be controlled by double interactions: hydrogenic interaction between a cyclodextrin ring and a station and a repulsive interaction between a cyclodextrin ring and a linker. This is a new method to control the mobility of a bead in a molecular shuttle.

While we were preparing a molecular shuttle, we found that a cyclodextrin ring did not escape from a linear axle with three cationic groups at the chain ends. Multi-cationic groups stabilize a rotaxane structure by inhibiting a cyclodextrin ring to come off (Fig. 6).

**Supramolecular Polymers**

When a guest group is covalently attached to a cyclic host, the molecule may form an intramolecular complex or intermolecular complexes to give supramolecular polymers. When supramolecular polymers are treated with bulky stopper groups, they may form poly[2]rotaxane, daisy chains (Fig. 7). We have found that a cyclodextrin derivative which has a cinnamonol group as a guest part on the 6-position of cyclodextrin forms an oligomeric supramolecular structure in aqueous solutions and that the supramolecular structure could be stabilized by attaching bulky stoppers to give daisy chains. Poly[2]rotaxane are unique polymers, because the polymers can expand and shrink by external conditions. The behavior of the polymers reminds us of those of muscle fibrils (actins and myosins).

**Cyclic Daisy Chains**

Cyclic tri[2]rotaxanes (daisy chain necklace) containing cyclodextrins have been prepared by closing a tri[2]rotaxane containing $\alpha$-cyclodextrin and 6-(4-aminothiophenyl)benzoic acid sodium salt (Fig. 8) if the molecule changes its conformation (or co-conformation), the ring may expand or shrink by external conditions (temperature, solvents, photochemically, electrochemically). These compounds are important because the cycle can be used not only as a chemical valve as can be seen in ion channels in biological membranes.

![Cyclic Daisy Chain](image)

**Conclusion**

Recently, much attention has been paid on the construction of supramolecular structures by bottom-up procedures as an alternative for VLSI produced by classical lithography. Many attempts have been successfully made on the construction of mechanically interlocked molecular assemblies. We have used cyclodextrin as a building block for the construction of various supramolecular structures, such as rotaxanes, polyrotaxanes, molecular shuttles, and daisy chains. These supramolecular compounds have potentials for molecular motors, machines, and devices. Cyclodextrin derivatives can be used in our body such as drug delivery systems, because CDs are produced from amylose and made of glucose, so they are water-soluble and non-toxic. Another cyclodextrin derivatives, such as tubular polymers and double strand inclusion complexes, will be used as a component of molecular size devices.

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**References**

Optical patterning and photochemical fixation of polymer nanoparticles on glass substrates

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A method for fixing patterned nanoparticles onto a substrate was developed by combining photopolymerization with a laser manipulation technique. Nanoparticles were dispersed in ethylene glycol containing monomer, crosslinker, and photoinitiator, and gathered at the focal point of a trapping laser beam (1064 nm) just on a glass substrate. Local photopolymerization within and around the nanoparticles assembly was induced by additional irradiation of a pulsed-laser beam (355 nm), resulting in generation of polyacrylamide containing nanoparticles. The polymerized assembly was evaluated by atomic force microscopy observation. By scanning both trapping and excitation laser beams, patterned nanoparticles could also be fixed on a glass substrate. © 2001 American Institute of Physics. [DOI: 10.1063/1.1366646]

Since a single beam laser trapping of microparticles was demonstrated by Ashkin in 1986,1,2 it has received much attention in various fields of science and technology. In the past decade, laser trapping has been further developed to a three-dimensional (3D) laser micro-manipulation technique,3,4 which has been applied to chemistry, physics, biology, and so on.5 A single microparticle in solution can be trapped and manipulated, and a number of nanoparticles in solution can be gathered and localized at the small area with the size of beam spot of a trapping laser beam, and also patterned with the laser manipulation technique. Of course, it is necessary to irradiate continuously the nanoparticles for maintaining the trapping and localization. Indeed, once the laser beam is switched off, each particle returns to Brownian motion and the pattern disappears. To overcome this problem and to fix the particles onto substrates, a photothermal fixation method was already demonstrated for individual micrometer-sized polymer particles in water.6 The microparticle was trapped and pressed onto polymer film in water containing dye molecules, and then a 532 nm pulsed beam was focused to the interface between the microparticle and the film. It is important to understand that the pulse intensity is enhanced at the interface due to the Mie scattering effect. The dye molecules absorbed the pulsed light and converted its energy to heat, which led to local temperature elevation and local melting around the contact point. Thus, mutual adhesion was realized. The Mie scattering effect is not expected of the present nanoparticles, because nanoparticles are much smaller than the beam waist. Hence, it is necessary to develop alternative fixation methods.

The method we report here deals with photopolymerization around localized nanoparticles on substrate. Nanoparticles are gathered at the focal point by photon pressure of a trapping laser beam, and patterned onto a glass substrate. An excitation laser beam is additionally introduced and local photopolymerization is induced in the same area, where the nanoparticles are trapped, and it’s surroundings.

Consequently nanoparticles are fixed in polymerized gel on a substrate.

A 1064 nm Gaussian beam from a cw Ne:YAG (Yttrium aluminum-garnet; YAG) laser (Spectron Laser Systems, SL-902T 10W) was used as a trapping beam and focused onto a sample solution just on a glass substrate through an objective lens (×100, numerical aperture = 1.3), of an optical microscope (Nikon, Optiphot 2). Photopolymerization in a sample solution was induced by irradiation of a 355 nm pulsed-laser beam from a Ne:YAG laser (Spectron Laser Systems, SL-282G, pulse duration ~6 ns, repetition rate ~5 Hz). Trapping beam and excitation beam powers, Ptrap and Pexc, respectively, were determined by the reported method.7 For patterning and fixation of nanoparticles, we employed two sets of Galvano mirrors (GSI, G325DT), that were controlled by a personal computer and controllers (Marubun, TW25). A sample solution was placed between a glass substrate and a cover glass, and was set on a 3D stage of the microscope.

As a sample solution, polystyrene (PSI) latex particles with fluorescent dye (PolyScience, Fluoresbrite™ Yellow Green Carboxylate Microspheres, diameter ~220 nm) were dispersed in ethylene glycol containing acrylate acid amide (AA) (Fluka), N,N'-methylene bis(acrylamide) (MBA) (Fluka), and Irgacure2959 (Ciba Specialty Chemicals), which are polymerizable vinyl monomer, crosslinker, and radical photoinitiator, respectively. All the behaviors proceeding in a sample solution were monitored by a charge coupled device video camera. After the fixation procedure was applied, the sample solution was replaced with distilled water and the remaining nanoparticles assembly was observed with use of optical and fluorescence microscopes. Transmission and fluorescence images were acquired by using halogen and superhigh-pressure mercury (USH-102DH) lamps, respectively.

First we tried local photopolymerization just on a glass substrate. The polymerization without trapping nanoparticles was induced by irradiating only a 355 nm pulsed laser beam (P355 = 2.5×10−3 µJ/pulse) for 35 s. It was confirmed as shown in Fig. 1(a) that polyacrylamide gel remained on the sub-
Laser Manipulation and Fixation of Single Nanoparticles in Solution at Room Temperature

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Introduction

Recently, nanoparticles have received much attention and been investigated as one of representative materials in nanoscience and nanotechnology. They show interesting properties originating from intermediate size between single atoms and bulk. In many cases, studies on nanoparticles have been conducted for an ensemble of many particles, so that distributions in size, shape, defect, and so on cannot be analyzed directly. It is important and indispensable to manipulate, investigate spectroscopically, and fabricate nanoparticles one by one, and thus manipulation and fixation of individual nanoparticles is considered to be one of key technologies for various fields of nanoscience and nanotechnology. It is quite ordinary to apply scanning tunneling microscopy and atomic force microscopy (AFM) under vacuum at low temperature at the present stage of investigation, while manipulation, characterization, and fixation technique of individual nanoparticles in solution at room temperature is expected to be more fruitful.

Laser manipulation is a very useful method to handle fine objects in small domains, and has been developed to trap, characterize, modify, and fabricate small particles in solution under an optical microscope at room temperature [1-3]. Most studies have been concentrated on μm-sized objects such as polymer latex, crystals, bio-cells, and so forth, and application for nm-sized ones has been rarely explored because of the difficulty in identifying them by eye. However, we have already demonstrated that 10 ~ 20 nm sized entangled polymer chains could be trapped and assembled in solution at the focal point of a trapping beam [4]. Also, Svoboda and Block have shown to trap a single 36-nm-diameter gold particle in water [5].

In this report, we describe our recent results on laser manipulation and fixation of nanoparticles in solution at room temperature; (1) patterning of optically gathered fluorescent polymer nanoparticles, (2) fixation of a trapped single polymer nanoparticle by local photopolymerization, and (3) trapping of a single gold nanoparticle and its fixation by photothermal effect.

Experimental setup

An experimental setup for optical manipulation and fixation system is shown in Figure 1. A 1064 nm laser beam from a CW Nd³⁺:YAG laser was used as a trapping beam and focused into a sample solution just on a glass substrate through an objective lens (×100, NA = 1.30) of an optical microscope. Fixation of trapped nanoparticles in a sample solution was conducted by irradiation of a 355-nm pulsed laser beam from a Q-switched Nd³⁺:YAG laser (pulse duration ~ 6 ns, repetition rate ~ 5 Hz). Trapping and fixation beam powers, P_trapping and P_fixation, respectively, were determined by the reported method [6].

Laser patterning and fixation of plural polymer nanoparticles onto substrates

First, we tried to gather and fix nanoparticles on a glass substrate by means of local photopolymerization. As a sample solution, polystyrene latex particles with fluorescent dye (diameter ~ 220 nm, concentration ~ 2.5x10⁻⁷ mol/L) were dispersed in ethylene glycol containing acrylic acid amide (Fluka, ~ 31 wt%), N,N'-methylene bis(acrylamide) (Fluka, ~ 2.2 wt%), and Irgacure2959 (Ciba Specialty Chemicals, ~ 1.1 wt%), which are polymerizable vinyl monomer, crosslinker, and radical photoinitiator, respectively. The trapping laser beam (P_trapping ~ 45 mW) was irradiated for ~ 300 s in a sample solution, and consequently an assembly of nanoparticles was formed on a glass substrate. When the assembly was illuminated by blue light from a high-pressure mercury lamp, green fluorescence from dye molecules included in each nanoparticle was observed. Then, a fixation laser beam (PFixation ~ 0.657 mW) was additionally irradiated for ~ 13 s. As a result ~ 1.5 μm-sized acrylamide gel was prepared within and around the assembly. The gel containing nanoparticles remained on the substrate even after washing off the solution, which was confirmed by optical transmission and fluorescence images as in Figure 2a and 2b, respectively.

By scanning trapping and fixation laser beams with use of two pairs of Galvano mirrors nanoparticles could also be patterned and fixed on a substrate. The optical transmission and fluorescence images of the “H” patterned nanoparticles on a glass substrate are shown in Figure 2c and 2d, respectively. The letter “H” consisted of three straight lines of patterned and fixed nanoparticles. The trapping laser beam was scanned along each line with a length ~ 10 μm (P_trapping ~ 180 mW, repetition rate of the scanning ~ 30 Hz) on a glass.

![Fig 1. A block diagram of laser nanomanipulation-fixation system.](image-url)
substrate for ~ 300 s. Nanoparticles were gathered and patterned along the line locus of the focal point on the substrate. Then the excitation laser beam ($P_{1064} \sim 0.097 \mu J/pulse$, repetition rate 0.1 Hz) was additionally scanned for ~ 35 s. As a result, the straight lines of patterned nanoparticles were fixed on the substrate. Combining several simple fixed patterns, a more complex arrangement can be realized with use of the present manipulation and fixation techniques.

**Fixation of individual polymer nanoparticles**

As the next step, we tried to fix single polymer nanoparticles onto a substrate one by one by applying local photopolymerization. The same sample as described above was used. Brownian motion of individual nanoparticles in the solution at room temperature was observed when the sample was irradiated by Hg-lamp of fluorescence microscope. Then the trapping laser beam was introduced into the solution. A nanoparticle that accidentally entered the region irradiated by the laser beam was trapped at the focal point. The nanoparticle was moved onto the surface of the glass substrate by handling 3D stage of the microscope. The fixation laser beam ($P_{1064} \sim 0.03 \mu J$) was focused to the same point for ~ 10 s, which led to generate acrylamide gel just around the trapped nanoparticle. The trapped single polymer nanoparticle could be fixed onto the glass substrate. Repeating the procedure, single nanoparticles were successfully patterned on a glass substrate as in Figure 3 which is a fluorescent image of single nanoparticles patterned as a letter "H" in distilled water. A magnified AFM image of one of fixed nanoparticles is also shown, from which it was undoubtedly confirmed that only one polymer nanoparticle of 220 nm diameter was contained in polymerized acrylamide gel.

**Fixation of individual gold nanoparticles**

Quite recently, we have also succeeded in manipulation and fixation of single metallic nanoparticles in solution. As a sample, gold colloid (British BioCell, EM.GC80, diameter ~ 80 nm) was diluted with ethylene glycol. A single 80-nm-diameter gold particle could be observed like a black spot in transmission image of optical microscope due to their huge refractive index. The optically trapped ($P_{1064} \sim 36 mW$) single gold particle was transferred to a certain position on the surface of glass substrate in ethylene glycol. The focused 355-nm pulse was additionally irradiated on the pressed nanopar-
particle, which led to its fixation. The substrate was washed with distilled water to remove the sample solution, then dried and observed with the optical microscope and AFM. It was confirmed that at suitable laser fluence (32 - 60 mJ/cm²) a gold nanoparticle was fixed on the glass substrate without fragmentation.

The present results may be explained by the temperature rise of the individual gold nanoparticle. Interactions between gold nanoparticle and laser pulse have been well studied by El-Sayed et al. [7-9], Koda et al. [10, 11], and many other groups. Since electron-phonon relaxation time of gold nanoparticles is much shorter (few ps order) than phonon-phonon (lattice-environment) relaxation time (100 ps order), absorbed excitation energy is converted to heat efficiently in a gold nanoparticle, resulting in temperature elevation of the particle during the irradiation time (few ns). In fact, it was mentioned in their reports that gold nanoparticles were melt (with lower pulse energy) and broken into several smaller nanoparticles with size of a few nm to 20 nm (with higher pulse energy) when the laser pulse was irradiated to the particle. Although the results can not be simply compared with their results because of our different experimental conditions (pulse wavelength, particle size, particle shape, and so forth), the laser-induced adhesion is considered due to transient melting.

Repeating the same manipulation and fixation procedure, single nanoparticles could also be patterned on a glass substrate. Figure 4 shows an AFM image of successive spatial patterning of single 80 nm gold particles. With fixation laser fluence of ~ 45 mJ/cm² eight gold nanoparticles were patterned as a letter "I" whose size is ~ 2.5 µm on the glass substrate.

**Conclusion**

We have succeeded for the first time in developing laser manipulation and adhesion methods for nanoparticles in solution. Polymer nanoparticles are optically gathered and patterned, single polymer nanoparticles are locally polymerized, and single gold nanoparticles are adhered on substrates. The significant importance of the laser manipulation-fixation technique is that we can trap, manipulate, and fix single and/or many nanoparticles in solution at room temperature. We have already demonstrated that elongated polymer chains of 10 ~ 20 nm mean radius can be trapped and chromophores with high polarizability are preferentially attracted to the focal point. These various kinds of mesoscopic materials can be well manipulated. Thus, we foresee that the present nanomanipulation-fixation technique will be useful for studies and applications on further nanoscience and nanotechnology.

**Fig 4.** An AFM image of fixed and spatially patterned gold nanoparticles of ~ 80 nm on a glass substrate, as a letter "I".

**References**

A Combinatorial Strongly Polynomial Algorithm for Minimizing Submodular Functions

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A Combinatorial Strongly Polynomial Algorithm for Minimizing Submodular Functions

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1. Introduction

Submodular functions play fundamental roles in combinatorial optimization (see [7]) and submodular functions are discrete analogues of convex functions. There exist a lot of practical (efficient) minimization algorithms for ordinary convex functions, while for submodular functions there had been only one 'strongly polynomial' algorithm, due to Grötschel, Lovász, and Schrijver [9, 10], that utilizes the so-called ellipsoid method for linear programming, where 'strongly polynomial' and 'polynomial' are synonyms of 'efficient' in Computer Science. The ellipsoid method is far from efficient and is not a combinatorial one, so that the Grötschel-Lovász-Schrijver algorithm is a quite unsatisfactory one both theoretically and practically. Since 1981 it had been a long-standing open problem to devise a combinatorial efficient (polynomial-time) algorithm for minimizing submodular functions. Iwata, Fleischer, Fujishige [14] and Schrijver [16] independently and simultaneously succeeded in solving the open problem in July, 1999 by presenting combinatorial strongly polynomial algorithms for minimizing submodular functions.

We describe how submodular functions are related to convexity and sketch our algorithm [14] in the sequel.

2. Submodular functions and convexity

Let $V$ be a nonempty finite set and $2^V$ be the power set of $V$, i.e., $2^V = \{X | X \subseteq V\}$. Also let $\mathbb{R}$ be the set of reals and $\mathbb{R}_+$ the set of non-negative reals. We call a function $f : 2^V \rightarrow \mathbb{R}$ a submodular function if it satisfies

$$f(X) + f(Y) \geq f(X \cup Y) + f(X \cap Y) \quad (X, Y \subseteq V) \quad (1)$$

(see Fig.1). Without loss of generality we assume that $f(\emptyset) = 0$, where if necessary, we may consider the function $f'$ defined by $f'(X) = f(X) - f(\emptyset) (X \subseteq V)$. Then, we have

$$f(X) + f(Y) \geq f(X \cup Y) + f(X \cap Y) \quad \text{if} \quad X \cap Y = \emptyset. \quad (2)$$

A function $f$ satisfying (2) is called a subadditive function. Hence the class of submodular functions is a special class of subadditive functions. However, subadditive functions do not have as nice combinatorial structure as submodular functions.

Inequalities (1) can be rewritten as

$$f(X) - f(X \cap Y) \leq f(X \cup Y) - f(Y) \quad (X, Y \subseteq V). \quad (3)$$

This would remind us of concavity. In fact, if we are given a concave function $\varphi : \mathbb{R} \rightarrow \mathbb{R}$, then we get a submodular function $f : 2^V \rightarrow \mathbb{R}$ defined by

$$f(X) = \varphi(|X|) \quad \text{for any} \quad X \subseteq V, \text{where} \quad |X| \text{denotes the cardinality of} \ X. \text{Any submodular function of symmetric type can be represented in such a way. However, submodular functions are more closely related to convexity through the so-called Lovász extension of submodular functions. For any set function} \ f : 2^V \rightarrow \mathbb{R} \text{with} \ f(\emptyset) = 0 \text{define a function} \ f^\vee : \mathbb{R}_+^V \rightarrow \mathbb{R} \text{as follows. For a non zero vector} \ x \in \mathbb{R}_+^V \text{there uniquely exist a sequence}

$$C : S_1 \subset S_2 \subset \cdots \subset S_k \quad (5)$$

of nonempty subsets of $V$ and positive scalars $\lambda_i (i = 1, 2, \ldots, k)$ such that

$$x = \sum_{i=1}^{k} \lambda_i \chi_{S_i} \quad (6)$$

where $\chi_S$ is the characteristic vector of set $S$. By means of the unique representation (6) of $x$ define

$$f(x) = \sum_{i=1}^{k} \lambda_i f(S_i) \quad (7)$$

We also define $f(0) = 0$. The function $f^\vee$ is called the Lovász extension of $f$.

Theorem 1 (Lovász[15]): A set function $f : 2^V \rightarrow \mathbb{R}$ with $f(0) = 0$ is a submodular function if and only if the Lovász extension $f^\vee$ of $f$ is a convex function. \hspace{1cm} \Box

Associated with a submodular function $f : 2^V \rightarrow \mathbb{R}$, we define convex polyhedra $P(f)$ and $B(f)$, respectively, called the submodular polyhedron and the base polyhedron as

$$P(f) = \{x | x \in \mathbb{R}^V, \forall X \subseteq V : x(X) \leq f(X)\}, \quad (8)$$

$$B(f) = \{x, x(\emptyset) = f(\emptyset), x(V) = f(V)\}, \quad (9)$$

Associated with a submodular function $f : 2^V \rightarrow \mathbb{R}$, we define convex polyhedra $P(f)$ and $B(f)$, respectively, called the submodular polyhedron and the base polyhedron as

$$x(x) = \sum_{i \in X} x(i) \quad \text{(see [7])}. \text{A vector in} \ B(f) \text{is called a base. Both} \ P(f) \text{and} \ B(f) \text{uniquely determine} \ f \text{as follows (see Fig.2).} \text{The submodular polyhedron} \ P(f) \text{and the base polyhedron} \ B(f) \text{are also related to the Lovász extension} \ f^\vee \text{as follows. For a convex function} \ h : \mathbb{R}_+^V \rightarrow \mathbb{R} \cup \{+\infty\} \text{and a vector} \ x \text{with} \ h(x) = +\infty \text{the subdifferential} \partial h(x) \text{of} \ h \text{at} \ x \text{is defined by} \ \partial h(x) = \{z | z \in \mathbb{R}^V, \forall y \in \mathbb{R}^V : h(y + \langle z, y-x \rangle) \leq h(y)\}, \text{where} \ \langle \cdot, \cdot \rangle \text{is the canonical inner product.}
Theorem 2 (Fujishige [6]): We have
\[ P(f) = \partial \bar{f}(0), \quad D(f) = \partial \bar{f}(1), \]
where \( \partial f \) and \( \bar{f} \) in \( R^d \) and we assume \( \bar{f}(x) = +\infty \) for any \( x \in R^d \).\

Examples of a submodular function are the following.
(a) Cut functions for capacitated networks: Consider a capacitated network \( G = (V, A, c) \), where \( G' = (V, A) \) is the underlying graph with vertex set \( V \) and arc set \( A \) and \( c : A \to R \) is a non-negative capacity function. For each vertex subset \( U \subseteq V \) let \( \kappa_c(U) \) be the sum of the capacities of arcs leaving \( U \). Then \( \kappa_c : 2^V \to R \) is a submodular function (see Fig.3).

\[ U \]
\[ \kappa_c(U) \]

(b) Matroid rank functions, matrix rank functions, graph rank functions: Consider a (real) matrix \( M \) with a set \( E \) of columns. For any subset \( X \subseteq E \) define \( \rho_M(X) \) to be the rank of the submatrix \( M_X \) formed by columns in \( X \). Then \( \rho_M : 2^E \to R \) is a submodular function. For a graph \( G = (V, A) \) let \( M \) be the incidence matrix of \( G \). Then \( \rho_M \) is the graph rank function.

(c) Multi terminal flow-value functions: Consider a source \( s \)-sink \( t \) flow network \( G = (V, A, c) \), where \( G' = (V, A) \) is the underlying graph, \( c : A \to R \) is a capacity function, \( s \) is a source, and \( t \) is the set of sinks. For any \( X \subseteq S \) let \( f(X) \) be the maximum flow value from \( s \) to \( X \). Then \( f : 2^S \to R \) is a submodular function.

(d) Entropy functions: Let \( X_1, X_2, \ldots, X_n \) be random variables taking on values from a finite set. Put \( E = \{ X_1, X_2, \ldots, X_n \} \). For any \( X \subseteq E \) define \( h(X) \) to be the entropy of \( X \) in Shannon's sense. Then \( h : 2^E \to R \) is submodular.

One of the most general models of combinatorial optimization problems that can efficiently be solvable is the so-called submodular flow problem proposed by Edmonds-Giles [4] in 1977. The submodular flow problem includes a lot of graph and network optimization problems and the matroid-intersection problem. However, algorithms to solve the general submodular flow problem require an oracle (an efficient procedure) for submodular function minimization (see [8]). Combining our algorithm for submodular function minimization and the existing algorithms for submodular flows yields the first combinatorial strongly polynomial algorithms for the submodular flow problem.

It should also be noted that some combinatorial optimization problems such as a dynamic flow problem [11] and a location problem on trees [18] can be solved in strongly polynomial time only by using the general submodular function minimization algorithm.

3. Submodular function minimization

Our (strongly) polynomial algorithm [14] relies on the following min-max theorem. For any \( x \in R^d \) define \( \bar{x} \in R^d \) as \( \bar{x}(v) = \min\{x(v), 0\} \in V \).

Theorem 3 ([3], [7, Cor. 3.5]): For any submodular function \( f : 2^V \to R \) with \( f(\emptyset) = 0 \),
\[ \max\{x(V) \mid x \in B(f)\} = \min\{f(X) \mid X \subseteq V\}. \]
Moreover, if \( f \) is integer-valued, an integral \( x \) attains the maximum in the left-hand side of (11).

Our algorithm tries to find an approximate maximizer of the left-hand side of (11). When \( f \) is integer-valued, if we get a (not necessarily integral) base \( x \in B(f) \) and a set \( X \subseteq V \) such that \( x(V) > f(X) - 1 \), then we see that \( X \) is a minimizer of \( f \).

First, we suppose that \( f \) is integer-valued. We express a base \( x \) as a convex combination of extreme bases (extreme points of \( B(f) \)) \( y_i (i \in I) \) as \( x = \sum_{i \in I} \lambda_i y_i \) where \( \lambda_i \geq 0 \) (\( i \in I \)) and \( \sum_{i \in I} \lambda_i = 1 \). This is an approach taken by Cunningham ([1], [2]). By the greedy algorithm of Edmonds-Shapley ([3], [17]), each extreme base \( y_i \) is given in terms of a linear ordering \( L_i = \{v_1, v_2, \ldots, v_n\} \) of \( V \) as
\[ y_i(v_j) = f(L_i(v_j)) - f(L_i(v_j-1)) \quad (j = 1, 2, \ldots, n), \]
where \( L_i(v_j) = \{v_1, v_2, \ldots, v_j\} \) and \( n = |V| \). We increase \( x(V) \) by transforming \( y_i(v_j) \) to their adjacent extreme bases. This would provide us with a pseudo-polynomial algorithm of Cunningham [2].

We then employ the capacity scaling algorithm for submodular flows, due to Iwata [12]. We perturb the given submodular function \( f \) by means of the cut function \( \kappa_2 : 2^V \to R \)
\[ f(x) + \kappa_2(x) \quad (X \subseteq V), \]
where \( \kappa_2(X) = \delta |X| |V - X| \) and \( \kappa_2 \) is the cut function of the complete directed network \( \delta_2 \) with a parameter \( \delta > 0 \) and uniform capacities \( c(u, v) = \delta \) for arcs \( (u, v) \). A flow \( \varphi \) in \( \delta_2 \) is called \( \delta \)-feasible if \( 0 \leq \varphi(v, u) \leq \delta \) \( \forall (u, v) \in V \) and \( \sum_{v \in V} \varphi(u, v) = 0 \). The bound \( \delta \) is \( \varphi : V \to R \) is defined by \( \delta \varphi(v) = \sum_{u \in V} \varphi(u, v) - \sum_{u \in V} \varphi(v, u) \leq \delta \). Denote by \( \partial \varphi \) the set of all the boundaries of feasible flows in \( \delta_2 \). The polyhedron \( \partial \varphi \) is a base polyhedron and so is the Minkowski sum \( B(f) \oplus \partial \varphi \). Instead of the original min-max relation (11) we then consider
\[ \max\{|x(z) + z| \mid x \in B(f), z \in \partial \varphi \}, \]
and try to approximately maximize the left-hand side.

We call the procedure, given below, for a fixed \( \delta > 0 \) the \( \delta \)-scaling phase. The basic idea for the \( \delta \)-scaling phase is as follows. For a current base \( x = \sum_{i \in I} \lambda_i y_i \) and a current \( \delta \)-feasible flow \( \varphi \) we increase \( \delta \varphi \) for some \( s \in S = \{v \mid x(v) + \delta \varphi(v) \leq \delta \} \) by \( \delta \) and simultaneously decrease \( \delta \varphi \) for some \( t \in T = \{v \mid x(v) + \delta \varphi(v) \geq \delta \} \) by \( \delta \) while keeping other \( \delta \varphi(v) \) invariant. We call this operation \( \delta \)-augmentation. The \( \delta \)-augmentation can be carried out by finding a directed path in the so-called residual network associated with the current \( \delta \)-feasible flow \( \varphi \). We repeat the \( \delta \)-augmentation as far as possible.

If the \( \delta \)-augmentation becomes impossible, then let \( W \) be the set of vertices that can be reached from \( S \) in the residual network. If we have \( y_i(v) = f(W) \) for all \( i \in I \), then \( \partial \varphi \) and \( \delta \varphi \) and we finish the \( \delta \)-scaling phase (if necessary, put \( \delta = \delta - \delta \) and \( \delta \leftarrow \delta \) and perform the \( \delta \)-scaling phase for the new \( \delta \)). It should be noted that if \( W \) is the
set of elements in an initial segment of $L$, then we have $y_i(W) = f(W)$. While $W \cap T = \emptyset$ and $y_i(W) = f(W)$ for some $i \in I$, we modify $x = \sum_{i \in I} \lambda_i y_i$ and $\phi$, keeping $x + \partial \phi$ invariant. Though we omit the details of our algorithm (this part is the most complicated to describe and an idea from [5] is employed), in $O(n^2)$ time we obtain a set $W \subseteq V$ reachable from $S$ and a base $x = \sum_{i \in I} \lambda_i y_i$ with a new set of extreme bases $y_i (i \in I)$ such that $W \cap T = \emptyset$ or $y_i(W) = f(W)$ for each $i \in I$, where $|I| \leq 2n-1$, assuming $|1| \leq n$ at the beginning of the $\delta$-scaling phase. When $W \cap T = \emptyset$, we can carry out a $\delta$-augmentation.

After the $\delta$-augmentation we compute a new expression $x = \sum_{i \in I} \lambda_i y_i$ with $|I| \leq nh$ by using affinely independent $y_i$'s, which requires $O(n^2)$ time. We can show the following

**Lemma 4:** At the end of the $\delta$-scaling phase, $(x + \partial \phi)^* (V) \geq f(W) - n^2 \delta$ and hence $x^*(V) \geq f(W) - n^2 \delta$. □

The latter inequality shows that the difference between $f(W)$ and the minimum of $f$ is at most $n^2 \delta$, so that if $\delta \leq 1/n^2$, then $W$ is a minimizer of $f$, due to Theorem 3.

Since $x + \partial \phi \in B(f) + O(n^2)$, we have $(x + \partial \phi)^* (V) \leq f(W) + n^2 \delta|V|/4$. Therefore, it follows from Lemma 4 that after putting $\delta \leftarrow \delta/2$ and $\phi \leftarrow \phi/2$, we have

$$f(W) - 2n \delta - n^2 \delta/4 \leq (x + \partial \phi)^* (V) \leq f(W) + n^2 \delta|V|/4$$

(15)

at the beginning of the next $\delta$-scaling phase. Hence there are $O(n^2)$ $\delta$-augmentations in the next $\delta$-scaling phase. If we choose any extreme base as the initial $x$ and put $\phi \leftarrow 0$ and $\delta \leftarrow \min \{|x^*(V)|, x^*(V) / n^2\}$ where $x^*(V) = \max \{|x^*(V)|, x^*(V) / n^2\}$, then there are $O(n^2)$ $\delta$-augmentations in the initial $\delta$-scaling phase as well.

For an initial base $x$ and a set $X \subseteq \{v : \lambda(v) > 0\}$ we have $\min \{|x^*(V)|, x^*(V) / n^2\}$ is $x^*(X) = f(X)$. It follows that defining $M = \max \{|f(X)|, |X| \leq V\}$, we perform $O(\log M)$ scaling phases from the initial $\delta$ till $\delta < 1/n^2$.

Consequently,

1. there are $O(\log M)$ scaling phases,
2. there are $O(n^2)$ $\delta$-augmentations in each $\delta$-scaling phase,
3. each $\delta$-augmentation requires $O(n^2)$ time.

Hence the algorithm described above finds a minimizer of the integer-valued submodular function $f$ in $O(n^2 \log M)$ time. This is a combinatorial, weakly polynomial algorithm. We utilize the weakly polynomial algorithm to devise a strongly polynomial algorithm.

Using the weakly polynomial algorithm, we can achieve one of the following four:

1. We find that a base exists and $V$ is a minimizer. Here, $V$ may be modified by operations (ii) - (iv) given below.
2. After performing $O(\log n)$ scaling phases we find an element that does not belong to any minimizer of $f$. We delete such an element from the underlying set $V$.
3. After performing $O(\log n)$ scaling phases we find an element that belongs to a minimizer of $f$. We contract such an element.
4. After performing $O(\log n)$ scaling phases we find a pair of elements $(u, w)$ such that any minimizer of $f$ containing $u$ contains $w$. If we have a directed cycle formed by arcs represented by such pairs $(u, w)$, then we shrink elements lying on the cycle to a single element.

(i) and (iii) are repeated $O(n)$ times and (iv) is $O(n^2)$ times.

Each scaling phase requires $O(n^2)$ time and there are $O(\log n)$ scaling phases. Hence the total running time is $O(n^2 \log n)$. We thus get a strongly polynomial algorithm.

4. Concluding remarks

We have solved the long-standing open problem but as Schrijver [16] pointed out, both Schrijver's and our algorithms employ multiplications and/or divisions. It is desirable to construct a fully combinatorial polynomial algorithm for submodular function minimization that requires only additions and subtractions. Very recently Iwata [13] solved this problem.

Further research is extensively being made to improve the time complexity of the proposed algorithms and to simplify them.

References

A Novel Demetalation Process for Vanadyl- and Nickelporphyrins from Petroleum Residue by Photochemical Reaction and Liquid–Liquid Extraction

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A novel demetalation process for residue oils, based on a combination of photochemical reaction and liquid–liquid extraction, has been investigated. A simultaneous photoconversion and extraction process, employing an oil/water two-phase system, was first studied. The results for the demetalation, obtained for vanadyl(IV) and nickel(II)tetrathiaporphyrin dissolved in deuterium, were compared with those obtained for actual atmospheric residue oil. It was found that this first process was able to demetalize "free"-type metalloporphyrins, but had difficulty in demetalization of "bound"-type metalloporphyrins, which are associated strongly with the asphaltene molecules in residue oil. To weaken this association and thus convert the bound-type metalloporphyrins to the free-type ones, a hydrogen-donating polar solvent, 2-propanol, was added to the residue oil and photoreacted. The 2-propanol was then removed by evaporation, and the resulting residue oil was contacted with aqueous HCl solution, in which the resulting vanadium and nickel were successfully removed. According to the latter reaction, vanadium and nickel were recovered from atmospheric residue oil at 93% vanadium and 98% nickel were recovered from vacuum residue, respectively. The overall demetalation process, involving the recovery of the 2-propanol, has been formulated as an energy-saving and safe demetalation process, which is satisfactory for application in the upgrading of heavy residual feedstocks.

Introduction

Owing to the continuous depletion of petroleum resources and the consequent increase in oil prices, the use of heavy residual feedstocks is becoming increasingly more attractive as a precursor for catalytic-cracked gasoline and light gas oil. Petroleum residue, produced during the atmospheric and vacuum distillation of crude oil, contains a high proportion of sulfur, nitrogen, and the heavy metals. Vanadium and nickel are the most concentrated in residue oils and are known to occur naturally as vanadyl(IV) and nickel(II)porphyrins. These metalloporphyrins are difficult to be demetalated during the hydrotreatment (HDM) of heavy residue over sulfided CoMo or NiMo/Al2O3 catalyst and leave a metal sulfide deposit on the catalyst, thus causing a corresponding loss in catalyst activity.1,2 Catalyst regeneration must therefore be carried out. The demand for light fractions as motor fuels is expected to increase steadily, and the upgrading of heavy residual feedstocks will certainly be increased very significantly soon. An alternative demetalation process, able to be operated under moderate conditions and without the requirements for hydrogen and catalyst, is therefore strongly required.

The photochemical and photophysical properties of metalloporphyrins have been studied extensively, owing to the importance of the porphyrin ring system in photosynthetic processes and in biological systems.

Magnesium(II) protoporphyrin, when dissolved in benzene, is reported to be oxidized by photolysis in the presence of molecular oxygen, to form a demetalized protoporphyrinogen, via hydrogenation followed by oxidation of the porphyrin ring.6 Zinc(II)tetrathiaporphyrin, dissolved in benzene, is reported to be photodecomposed by sunlight irradiation in the presence of benzoin as a reducing agent, to form a free-base tetrathiaporphyrin.7 Also, Jones et al. reported recently that vanadyl(IV)tetrathiaporphyrin is photodecomposed in the gas phase by the irradiation of a 266-nm ionizing laser to cause the loss of the central vanadyl ion. If such photochemical processes can be applied to the demetalation of residue oils, a possible safe and energy-saving demetalation process may therefore be developed.

New deasphaltization processes for light gas oils and catalytically-cracked gasoline, based on photochemical reaction and liquid–liquid extraction, have been investigated in previous papers.8–10 In the present work, new photochemical demetalation processes for vanadyl- and nickelporphyrins from residue oils are studied as an extension of the above works, based on the concept of simultaneous photoconversion and extraction using an oil/water two-phase system. To clarify the photoactivity and photodecomposition pathway for the metalloporphyrins, tetradecin solutions, containing the pure metalloporphyrins, are used as model solutions representing the residue oils. The proposed process is also applied to the demetalation of real residue oils, and the feasibility of the process is studied further in detail. The nature of the process is clarified by ESR studies that show that the metalloporphyrins in the residue oils are associated strongly with the asphaltene molecules to form "bound-type metalloporphyrins. To weaken this association and...
A New Methodology Towards Demetalation of Petroleum Residue Based on a Combination of UV Irradiation and Liquid-Liquid Extraction

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Abstract
A new demetalation process for residue oil has been investigated, based on a combination of UV irradiation and liquid-liquid extraction. A simultaneous photoreaction and extraction process, using an oil/water system, was first studied. The demetalation results, for vanadyl(IV)tetraphenylporphyrin (VOTPP) and nickel(II)tetraphenylporphyrin (NITPP) dissolved in tetratin, were compared with those obtained for actual atmospheric residue (AR). ESR analysis showed that this first process was able to demetalize "free"-type metalloporphyrins, but had difficulty in the demetalation of "bound"-type ones, which are associated strongly with the asphaltene molecules in AR. To weaken this association and thus convert the bound-type metalloporphyrins to the free-type ones, polar 2-propanol solvent was added to the AR and photoirradiated. The 2-propanol was then removed by evaporation, and the resulting AR was contacted with aqueous HCl solution, into which the resulting vanadium and nickel were successfully removed. According to this latter development of the process, 93% vanadium and 98% nickel were recovered from AR.

Introduction
Petroleum residue, produced during atmospheric and vacuum distillation of crude oil, contains a high proportion of sulfur, nitrogen, and the heavy metals. Vanadium and nickel are most concentrated in residue oils and are known to occur naturally as vanadyl(IV)- and nickel(II)porphyrins [1]. These metalloporphyrins are difficult to demetalize during current hydrosolubilization processes and leave a metal sulfide deposit on the catalyst, thus causing a corresponding loss in the catalyst activity [2]. An alternative demetalation process, able to be operated under moderate conditions and without the requirements for hydrogen and catalyst, is therefore strongly required. In the present work, a new demetalation process for residue oil, based on photochemical decomposition of vanadyl- and nickelloporphyrins using UV light and extraction of the resulting materials, is studied, as one of our series works for desulfurization and denitrogenation of light oil and gasoline feedstocks [3, 4].

Experimental Procedure
AR was dissolved in tetralin/decalin (1/1 v/v) mixture to ensure fluidity for the photoreaction studies. The solution was mixed vigorously with either water or alcoholic solvent by magnetic stirring, and were photoirradiated by the immersion of a high-pressure mercury lamp (100W) and combined with air bubbling at atmospheric pressure, as shown schematically in Figure 1. The temperature of the solutions, during photoirradiation, was about 303 K. To clarify the photoactivity for the metalloporphyrins, tetralin solutions, containing VOTPP and NITPP, are used as model solutions representing the residue oil.

Simultaneous Photoreaction and Extraction
The tetralin solution containing VOTPP or NITPP was mixed with distilled water and photoirradiated. With air bubbling, the VOTPP and NITPP concentrations and the total vanadium and nickel contents in tetralin were decreased successfully by irradiation time, and essentially most of the vanadium and nickel were removed into the water phase. Ion chromatography analyzes for the resulting aqueous solutions showed that the most of the vanadium and nickel are removed as VO₂⁻ ion and Ni(OH)₂.
The present process was then applied for the demetalization of AR. Figure 2a shows the time-course variation in the vanadium and nickel percentage remaining in the AR. Although the concentrations for each metal decrease with irradiation time, 36 h of photoirradiation achieved only 30% of the vanadium removal and 10% of the nickel removal. AR is known to be fractionated by the solvent densphalting procedure into two fractions, a "hexane-soluble" asphaltenic fraction and the other "hexane-insoluble" asphaltenic fraction" [5]. The former fraction contains relatively low molecular weight aromatics, while the latter contains heavy aromatics [6]. The 10 wt% concentration for each fraction, dissolved in a tetralin/decanol mixture, was photoirradiated as in the case for the total AR. As shown in Figure 2a, the demetalation of the asphaltenic fraction proceeded quite faster than that for the total AR. The demetalation of the asphaltenic fraction was, however, significantly more difficult, in that only 1% vanadium and 2% nickel were removed.

The macrostructure of asphaltenes in petroleum residue has been studied in detail by several researchers [5, 6]. It is established that the unit large aromatic sheets, having high molecular weights, are piled up on each other to form an unit cell and larger associated asphaltenic molecules. The metalloporphyrins are associated with the asphaltenic molecules via a π-electronic interaction to form "bound"-type metalloporphyrins, as schematically shown in Figure 3. The behavior of the metalloporphyrins during the present process was therefore studied by ESR measurement. As shown in Figure 4b, the AR dissolved in the tetralin/decanol mixture showed a 16-feature anisotropic hyperfine structure, thus indicating that most of the metalloporphyrins in AR occur as the bound-type [6]. The spectrum for the asphaltenic fraction was seen to be identical to that of AR. When the asphaltenic fraction was analyzed, new isotropic signals, No.2 at 3270 and No.5 at 3560 G, appeared, as shown in Figure 4c. These are attributable to "free"-type vanadylporphyrins, found in the spectrum for VOTPP dissolved in tetralin, as shown in Figure 4a, thus suggesting that the asphaltenic fraction contains free-type vanadylporphyrins, which do not associate with asphaltenic molecules, in addition to bound-type vanadylporphyrins. When the asphaltenic fraction was photoirradiated for 36 h together with distilled water, these isotropic signals disappeared, and only the anisotropic signals remained in the spectrum, as shown in Figure 4d. These results indicate that photoirradiation of the asphaltenic fraction tends to photodecompose the free-type vanadylporphyrins, but it is hardly able to demetalize the bound-type vanadylporphyrins.

**Photoreaction in the Presence of Alcohol Followed by Extraction**

To enhance the demetalation of "bound"-type metalloporphyrins, the interaction between the metalloporphyrins and asphaltenic molecules must therefore be weakened. The behavior of the metalloporphyrins in AR, when several polar solvents were added, was examined by ESR. As shown in Figure 4e, the 16-feature anisotropic signals, originally observed in the spectrum for AR in a tetralin/decanol mixture (Figure 4b), disappeared especially by the addition of 2-propanol, and the new isotropic signals, No.2 and No.5, apparently appeared. These results suggest that the bound-type metalloporphyrins that are associated with the asphaltenic molecules are dissociated by the addition of polar 2-propanol and thus converted to free-type metal-
lopophyrins. In addition, the photodecomposition of VOTPP and NiTTPP was found to be enhanced in the presence of 2-propanol.

On the basis of the above findings, a new demetalation process, consisting of the photodecomposition of metalloporphyrins in the presence of 2-propanol followed by extraction of the resulting products, was proposed, as shown in Figure 5. 2-Propanol (50 mL) was added to the AR (10 wt%) dissolved in a tetralin/decalin mixture (100 mL) and photoirradiated. The 2-propanol was then removed completely by evaporation, and the resulting solution was contacted with an equal volume of 1 mol/L HCl aqueous solution. Figure 2b shows the resulting time-course variation in the percentage vanadium and nickel remaining in the AR solution. The remaining percentage for both vanadium and nickel decreases drastically, in that 93% vanadium and 98% nickel were removed following 36 h of photoirradiation. Demetalation for the asphaltenic fraction of AR was also improved with the present process, and the remaining percentage for both metals was decreased to less than 25% following 36 h of photoirradiation. The results indicated that freetype metalloporphyrins, formed by the addition of 2-propanol, are then photodecomposed successively, thus resulting in the high demetalation yields for AR and for the asphaltenic fraction.

Conclusion

A new demetalation process for petroleum residue, based on UV irradiation followed by liquid-liquid extraction, has been developed. The relatively long photoirradiation time required in the present study may be expected to be reduced considerably for industrial application, via the development of a more efficient photoreactor of appropriate advanced design. Vanadium and nickel compounds in aqueous HCl solution, recovered from residue oil, may be used as rare metal resources. The proposed process comprises very simple stages, and is carried out under moderate conditions, with only air bubbling. Therefore, the present process will be applicable as a safe and energy-efficient upgrading process for petroleum residue oils.

References

Radiation Tolerance of Complex Oxides

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The radiation performance of a variety of complex oxides is predicted on the basis of a material's propensity to accommodate lattice point defects. The calculations indicate that a particular class of oxides possessing the fluorite crystal structure should accept radiation-induced defects into their lattices far more readily than a structurally similar class of oxides based on the pyrochlore crystal structure. Preliminary radiation damage experiments substantiate the prediction that fluorites are inherently more radiation resistant than pyrochlores. These results may permit the chemical durability and radiation tolerance of potential hosts for actinides and radioactive wastes to be tailored.

One of the principal factors complicating the selection of materials for nuclear waste storage is the susceptibility of waste forms to detrimental radiation damage effects. Several crystalline ceramics, such as zircon (ZrSiO₄) and the orthophosphate monazite (LaPO₄), exhibit both high chemical durability and volatility for actinides and other radionuclides, and are therefore attractive candidates for nuclear-waste host materials (1, 2). However, among the chemically stable host phases proposed for waste storage, there is a paucity of materials for which long-term stability can be anticipated. This is because radioactive constituents in high-level waste (HLW) can decay to produce numerous atomic defects. Most materials are destabilized by such defects, and if defect accumulation is allowed to proceed unchecked, crystallite oxides ultimately succumb to an amorphization transformation, often accompanied by significant volume changes (4) and concomitant microcracking (4).

The technical challenge for HLW storage has therefore been to identify materials for which deleterious radiation effects are averted even at very high self-radiation exposures. The principal consequence of a displaceable radiation environment is an elevated population in the lattice of Frenkel pairs (each pair consisting of an atomic interstitial and a lattice vacancy). Subsequent damage evolution hinges on two important factors. First is the degree to which lattice stability is affected by the accumulation of point defects. This factor influences a material's propensity to amorphize under irradiation. The second factor concerns the ultimate fate of radiation-induced point defects. Interstitials and vacancies can migrate and annihilate harmlessly by interstitial-vacancy (i-v) recombination (the reverse of a Frenkel reaction), or they can cluster with other interstitials and vacancies to precipitate interstitial clusters (3), which can voids. A material in which clustering occurs with ease will likely be susceptible to void swelling.

Many simple oxides such as magnesium (MgO) and alumina (Al₂O₃) are susceptible to void swelling (5). On the other hand, and perhaps surprisingly, a compound made from an equimolar mixture of MgO and Al₂O₃ (best known as the mineral spinel, MgAl₂O₄) is highly resistant to void swelling under neutron irradiation (5). The radiation-resistant behavior of spinel, which is exceptional for a ceramic, is most likely due to the following factors: (i) Complex chemistry causes the critical size of a dislocation loop nucleus to be unusually large (6). This necessarily suppresses loop nucleation. (ii) Complex structure generates constraints that prohibit dislocation loops from easily unknotted (7). Faulted interstitial loops remain poor sinks (compared to unknotted loops) for interstitial absorption. (iii) Some materials like spinel readily accommodate disordered defects within their structures. In fact, the cation sublattices in spinel can be completely disordered by high-fluence neutron irradiation (8). For all of these reasons, harmless i-v recombination (including cation antisite formation, i.e., swapping the position of one Mg cation with one Al cation) in spinel is a highly efficient point defect annihilation mechanism, and void swelling is negligible.

To test the generality of these attributes for radiation tolerance, we recently initiated an investigation into the radiation damage behavior of an extensive class of complex oxides known as pyrochlores. Oxide pyrochlores are typically ternary compounds of the general formula A₂B₂O₇ (where A and B are metallic cations). The simplest pyrochlore
Radiation Tolerant Material for Nuclear Waste Storage

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Safe container for nuclear waste

There exists a formidable challenge to the development of durable waste forms for the immobilization and long-term storage of surplus actinides and high-level wastes such as spent fuel from nuclear reactors. Currently, the waste, after spending years in cooling tanks, is stored in containers made from glass-like chemicals and sealed in metal drums, which are effective for only about 100 years. These containers are put into geologically stable places such as disposed salt mines, or buried very deep in the earth. The problem with containers that only last a hundred years is that some radioactive wastes have a half-life of millions and sometimes billions of years. For example, the half-life of the uranium-238 atom is 4.5 billion years.

The principal obstacle is the identification of a nuclear-waste host material that is not only chemically stable, but also able to withstand high doses of self-radiation. Typically, radioactive emissions jostle the atoms of a storage material out of their carefully ordered arrangement so that the material eventually becomes unstable and thus prone to cracking, swelling or structural change. If the structure of the waste container becomes severely damaged, highly toxic substances could leak into the ground and air. Stowing nuclear waste for the long run requires containment materials that can resist leaching and radiation damage for thousands of years.

The technical challenge for high-level waste storage has therefore been to identify materials for which deleterious radiation effects are averted even at very high self-radiation exposures.

New approach to develop radiation tolerant material

At the moment researchers have many different candidate materials for nuclear waste storage. Recent research has centered on a class of materials that are part of a larger group of ceramics called complex oxides. However, scientists have to try out all these materials one by one, to see how they react to radiation exposure. A new systematic way to assess an extensive group of complex oxide compounds is therefore strongly required. In this report, we demonstrate that we are able to predict the radiation performance of a variety of complex oxides, based on atomistic computer simulations.

We deal with oxide pyrochlores, typically ternary compounds of the general formula $AX_2BY_2O_7$ (where $A$ and $B$ are metallic cations). The amount of energy induced by irradiation is predicted to cause deleterious structural changes in the compounds. This energy, known as the defect energy, is then calculated for many chemical versions of different pyrochlores. Figure 1 shows a contour plot of the isolated cation antisite defect energy, as a function of $A$ and $B$ cation radii [1]. For a wide range of $A$ and $B$-ions, we considered cations ranging from lanthanum to lutetium on the $A$-site and titanium to cerium on the $B$-site. In pyrochlores, the cation antisite is the lowest energy intrinsic disorder mechanism. This mechanism involves the substitution of a $3^+$ ion onto a $B$ site and a $4^+$ ion onto an $A$ site. The contour plot in Fig. 1 provides insight into the stability range of the pyrochlore structure with respect to...
cation disorder. The plot indicates that antiste defect formation is accompanied by a high energy cost in compounds containing large A cations and comparatively small B cations. The lowest defect energies are associated with compounds in which A and B radii are similar. As cation antiste defects are an inevitable consequence of a displace radiation environment, Fig. 1 can also be interpreted as a predictor of radiation damage behavior: compounds with very dissimilar cationic radii should exhibit the greatest susceptibility to lattice destabilization (and possible amorphization), whereas compounds with similar radii should behave more robustly in a radiation environment.

Radiation resistant crystalline structure

We have included symbols in Fig. 1 to indicate whether a particular A:B:O compound is observed experimentally as a pyrochlore. When the pyrochlore structure is not observed experimentally, the fluoride (CaF$_2$) crystal structure is inevitably found in its place [2-4]. Figure 2 illustrates the crystallographic relationship between the cubic pyrochlore and cubic fluoride structures. The cation sublattices in both cases consist of atoms located at face-centered lattice positions. Pyrochlores and fluorites differ with regard to (1) the ordered arrangement of cations on the pyrochlore cation sublattice; and (2) the ordered arrangement of vacancies on the pyrochlore anion sublattice.

The symbols in Fig. 1 indicate that compounds with more similar cation radii are more likely to form as disordered fluorites than as ordered pyrochlores. The contours in the plot reveal why this is so: because the energy expended to form the kinds of defects that cause an ordered pyrochlore to resemble a disordered fluoride (cation antiste sites and anion Frenkel defects) is far lower for compounds of similar cation radii, compared with compounds containing A and B cations with highly disparate sizes. We anticipate that ordered pyrochlores in the left-hand portion of the plot in Fig. 1 behave poorly under irradiation, due to the high formation energies associated with disordering point defects. On the other hand, disordered fluorites located farther to the right-hand side in Fig. 1 may not be affected by irradiation.

A test of theoretical prediction

As a test of the predictive capabilities of our atomistic simulations, we performed ion irradiation experiments to determine the radiation performance of Er$_2$Ti$_2$O$_7$ and Er$_2$Zn$_2$O$_7$. We synthesized single crystals of Er$_2$Ti$_2$O$_7$ and Er$_2$Zn$_2$O$_7$ for this study and found that they crystallize as an ordered pyrochlore and a disordered fluoride, respectively, as anticipated by Fig. 1. Figure 3 shows the results of ion irradiation experiments on these two compounds. The results verify our prediction. Er$_2$Ti$_2$O$_7$ is amorphized by irradiation with heavy ions at a fairly low ion dose, while Er$_2$Zn$_2$O$_7$ remains crystalline to a high dose of ion irradiation, with no apparent change in crystal structure. These
results imply that the zirconate, which commenced existence as a disordered fluorite, is less perturbed by the introduction of defects due to irradiation than is the titanate, which began as a highly-ordered pyrochlore. In some sense, this comes as little surprise because even before exposure to ions, the zirconate resembled an irradiated compound.

\[ \text{Universal supposition} \]

Computational procedures verified by experiments for the first time allow us to predict the radiation performance of a variety of complex oxides, based on a material’s propensity to accommodate lattice point defects. Our model shows that a particular class of oxides possessing the fluorite crystal structure accepts radiation-induced defects into their lattices far more readily than a structurally similar class of oxides based on the pyrochlore crystal structure. Preliminary radiation damage experiments substantiate the prediction that fluorites are inherently more radiation resistant than pyrochlores. We think this might be a basic rule that applies to other materials those in this study: namely, other crystalline materials with relatively disordered structures may be resistant to radiation damage. This work will enable the exploration and development of new, chemically durable and radiation tolerant hosts for safe and reliable storage of radioactive wastes and surplus actinides.

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A Toll-like Receptor Recognizes Bacterial DNA

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DNA from bacteria has stimulatory effects on mammalian immune cells1,2, which depend on the presence of unmethylated Cpg dinucleotides in the bacterial DNA3. In contrast, mammalian DNA has a low frequency of Cpg dinucleotides, and these are mostly methylated; therefore, mammalian DNA does not have immune-stimulatory activity. Cpg DNA induces a strong T-helper-1-like inflammatory response4. Accumulating evidence has revealed the therapeutic potential of Cpg DNA as adjuvants for vaccination strategies for cancers, allergy and infectious diseases5-8. Despite its promising clinical use, the molecular mechanism by which Cpg DNA activates immune cells remains unclear. Here, we show that cellular response to Cpg DNA is mediated by a Toll-like receptor, TLR9. TLR9-deficient (TLR9−/−) mice did not show any response to Cpg DNA, including proliferation of splenocytes, inflammatory cytokine production from macrophages and maturation of dendritic cells. TLR9−/− mice showed resistance to the lethal effect of Cpg DNA without any elevation of serum pro-inflammatory cytokine levels. The in vivo Cpg DNA-mediated T-helper type 1 response was also abolished in TLR9−/− mice. Thus, vertebrate immune systems appear to have evolved a specific Toll-like receptor that distinguishes bacterial DNA from self-DNA.

The Toll-like receptor (TLR) family is a phylogenetically conserved mediator of innate immunity that is essential for microbial recognition11. Mammalian TLRs comprise a large family with extracellular leucine-rich repeats (LRRs) and a cytoplasmic Toll/interleukin-1 receptor (TIR) homology domain. To far, 11 members (TLR1-6) have been identified11, and (two additional) members have been deposited in GenBank as TLR7 and TLR8 (accession numbers AF240467 and AF248971, respectively). TLR2 and TLR4 are associated with immune responses to peptidoglycan (PGN) and lipopolysaccharide (LPS), respectively12-15.

By using a BLAST search, we identified an expressed sequence tag (EST) clone (AA275751; mouse) that showed high similarity with the previously identified TLRs. Using this fragment as a probe, we isolated a full-length complementary DNA from the mouse macrophage cDNA library. We also isolated the human counterpart. Sequence analysis revealed the presence of regions conserved in the TLR family, such as LRR and TIR domains (Fig. 1a,b). Therefore, we designated this gene TLR9. Northern blot analysis of various tissues indicated that mouse TLR9 transcripts were most abundantly expressed in the spleen (Fig. 1c).

To assess the biological function of TLR9, we generated TLR9−/− mice by homologous recombination in embryonic stem (ES) cells. The targeting vector was constructed to replace a 10.4-kb fragment of the mouse Tlr9 gene encoding a part of LRR with a neomycin resistance cassette (neo) (Fig. 2a). Correctly targeted ES cell clones were micro-injected into C57Bl/6 blastocysts, which contributed to transmission of the mutated allele through the germ line. We intercrossed heterozygotes to produce offspring that were homozygous for the disrupted Tlr9 allele (Fig. 2b). The mutant mice were...
A Toll-like Receptor Recognizes Bacterial DNA

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Toll-like receptor family plays crucial roles in innate immunity

The recognition of invading pathogen by host immune cells is a critical step for host defense in the multicellular organisms. Mammals have developed two types of immune system, innate and acquired immune system. The acquired immunity is based on lymphocytes with a huge repertoire of receptors that are generated through gene rearrangements such as B cell receptors and T cell receptors. On the other hand, a limited number of germline-encoded receptors are involved in the recognition of microorganisms in innate immunity (1). In order to detect many kinds of infectious agents, the innate immune system specifically recognizes structural patterns of microbial components, that are conserved among a wide variety of microorganisms. These structural patterns are referred as pathogen associated molecular patters (PAMPs), and include lipopolysaccharide (LPS), peptidoglycan (PGN), bacterial lipoproteins (BLP), mannans, bacterial DNA containing the CpG motif and so on (2). These PAMPs are essential for survival of microbes and are not expressed on the host. Once macrophages (Mφ) and dendritic cells (DC) recognize the existence of invading microbes, they activate subsequent acquired immunity by antigen presentation to antigen-specific lymphocytes as well as produce inflammatory cytokines such as tumor necrosis factor (TNF) -α, Interleukin (IL)-1β and nitric oxide (NO).

Host organisms have developed a set of receptors that can recognize specifically PAMPs. As one of these receptors, Toll-like receptor (TLR) family has been well studied (3). TLR family is originally identified as homologues of Drosophila Toll protein (4). Drosophila Toll is involved in not only the establishment of dorsoventral axis in the early embryogenesis but also immune response to fungal infection in the adult. Toll and TLR family are characterized as a type I transmembrane protein with leucine-rich repeats (LRR) in the extracellular domain and a cytoplasmic Toll/interleukin (IL)-1 receptor homology (TIR) domain. So far, ten members of human TLR are identified. Biological functions of some TLR family members have been revealed (Fig. 1); TLR4 is responsible for the recognition of LPS and TLR2 is indispensable for that of PGN and LBP (3). Recently, it has been reported that TLR5 mediates the recognition of flagellin, one of principal components of bacterial flagella (5). Activation of TLRs induces recruitment of the adaptor protein MyD88 (myeloid differentiation factor 88) and sequential activation of signaling molecules such as IRAK (IL-1 receptor associated kinase) and TRAF6 (TNF receptor associated factor) (Fig. 1). Finally, transcription factors AP-1 (activating protein-1) and NF-κB (nuclear factor-κB) are activated and translocated to the nucleus, where they induce gene expression of cytokines such as IL-1β, IL-6, IL-12 and co-stimulatory molecules such as CD80 and CD86 (6).

Fig 1. Toll-like receptors recognize various bacterial components. See text for details.
**Table. The effect of CpG DNA on various immune cells**

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**Immunostimulatory DNA containing CpG motifs**

Bacterial DNA, one of PAMPs, has immunostimulatory activity on mammalian immune cells (Table). Various experimental evidences have shown that its immunostimulatory activity of bacterial DNA is responsible for the presence of the unmethylated CpG motif (7). These unmethylated CpG motifs are abundant in bacterial DNA; however, the frequency of the CpG motif is suppressed and highly methylated in mammalian DNA, so that mammalian DNA does not have any immunostimulatory activity. Synthetic oligodeoxynucleotides containing unmethylated CpG motifs can mimic immunostimulatory activity of bacterial DNA and can induce strong T helper (Th) 1-polarizing immune responses, which promote cellular immunity (8). Now, these immunostimulatory DNA containing the CpG motif (hereafter as CpG DNA) are becoming promising as vaccine adjuvants and as immunotherapies for treatment of human diseases such as cancer, allergy and infectious diseases. It is also known that a lot of oligodeoxynucleotides (ODN) containing different CpG motifs can activate mammalian immune cells, particularly human and mouse immune cells are optimally stimulated by slightly different CpG motifs, 5'GTCGTT-3' and 5'GACGTT-3' for human and mouse, respectively (7).

It has been shown that CpG DNA can induce activation of transcription factors such as NF-κB and AP-1, whereas protein(s) that is involved in the recognition of CpG DNA and activation of immune cells is remain unclear. Recently, it has been revealed that MyD88-deficient cells do not show any cellular responses to CpG DNA such as proliferation of splenocytes and production of inflammatory cytokines from Mφ(9, 10). Moreover, TLR2- or TLR4-deficient cells showed normal response to CpG DNA. These results suggest that TLR family member(s), other than TLR2 or TLR4, might mediate immune response to CpG DNA.

**TLR9 recognizes bacterial DNA**

We identified a novel TLR family member by a BLAST search. This novel gene showed high similarities with previously reported TLRs, which was characterized by the presence of conserved domains in TLRs, such as LRR in the extracellular regions and TIR domain in the cytoplasmic regions. To investigate physiological role of TLR9, we generated TLR9-deficient mice by gene targeting and evaluated immunological response to various microbial products. TLR9-deficient mice were born at the expected Mendelian ratio and did not show any abnormal morphology (11).

As mentioned above, TLR family members mediate immune cell activation via various bacterial components. So, cellular responses...
es to various bacterial components were measured in TLR9-deficient cell. Mφ from TLR9-deficient produced the same amount of TNF-α as that of wild type Mφ in response to LPS or PGN. In contrast, TLR9-deficient cells did not show any cytokine production in response to CpG DNA, even when cells were co-stimulated with interferon (IFN) -γ (Fig 2A). Spleenocytes and dendritic cells also did not show proliferative response and maturation in response to CpG DNA, respectively, but they did similar responses to that of wild-type cells in response to LPS. In addition, activation of intracellular signaling molecules such as IRAK, JNK and NF-κB induced by CpG DNA was also impaired in TLR9-deficient cells, whereas activation of these signaling molecules were induced by LPS stimulation. Thus, TLR9 are indispensable for the recognition of CpG DNA, but not for that of the other microbial components such as LPS and PGN.

Furthermore, TLR9-deficient mice did not respond to CpG DNA in vivo (Fig 2B). CpG DNA can induce the lethal shock in D-galactosamine-sensitized mice. In fact, wild-type mice died within 12 hours after CpG DNA plus D-galactosamine administration with marked elevation of serum cytokine levels. On the other hand, TLR9-deficient mice survived over 120 hours without any increase of serum concentrations of TNF-α, IL-6 and IL-12. It was also revealed that adjuvant effect of CpG DNA was also impaired in TLR9-deficient mice. These results indicate that TLR9 are essential for immunological effects of CpG DNA in vivo.

Conclusion

Activation of immune cells via CpG DNA has been shown to require cellular uptake by DNA sequence-independent endocytosis, and endosomal acidification/maturation (12). CpG DNA-induced activation of IRAK, as well as JNK activation, was slightly delayed as compared with LPS-induced activation (11). But TLR9 is predicted to express on the cell surface but not in the cytoplasm as is the case of other TLR family members, because TLR9 has a transmembrane domain and signal peptides. TLR9 may be internalized with CpG DNA or bacteria into endosomes via non-specific endocytosis and activated after endosomal maturation (Fig. 3). Although further investigations are required, the identification of signaling receptor for CpG DNA will add a new insight into our understandings on the mechanisms of CpG DNA recognition as well as into therapeutic applications of CpG DNA for cancer, infectious diseases and allergy.

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A ubiquitin-like system mediates protein lipidation


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Autophagy is a dynamic membrane phenomenon for bulk protein degradation in the lysosome/vacuole. Atg8/Aut7 is an essential factor for autophagy in yeast. We previously found that the carboxy-terminal arginine of nascent Atg8 is removed by Atg4/Aut2 protease, leaving a glycine residue at the C terminus. Atg8 is then converted to a form (Atg8-X) that is tightly bound to the membrane. Here we report a new mode of protein lipidation. Atg8 is covalently conjugated to phosphatidylethanolamine through an amide bond between the C-terminal glycine and the amino group of phosphatidylethanolamine. This lipidation is mediated by a ubiquitination-like system. Atg8 is a ubiquitin-like protein that is activated by an E1 protein, Atg7 (refs 7, 8), and is transferred subsequently to the E2 enzymes Atg3/Atg11 (ref. 9). Atg7 activates two different ubiquitin-like proteins, Atg12 (ref. 10) and Atg8, and assigns them to specific E2 enzymes, Atg10 (ref. 11) and Atg5, respectively. These reactions are necessary for the formation of Atg5-phosphatidylethanolamine. This lipidation is an essential role in membrane dynamics during autophagy.

Atg8 was the first molecule found to localize to the intermediate structures of the autophagosome, and is necessary for autophagosome formation. Atg8-X behaves like an integral membrane protein, even though Atg8 does not possess a membrane spanning region. Here we used phase partitioning with Triton X-114 to separate Atg8-X. In wild-type cells, a small amount of Atg8 was detected in the detergent phase—most of it was in the aqueous phase (Fig. 1a). In contrast, Atg7 cells resulted in the exclusive partitioning of Atg8 to the aqueous phase (Fig. 1a). Atg8-X accumulated in Δatg7Δatg8 cells expressing Atg8Fl (Atg8 lacking the C-terminal arginine), resulting in marked enhancement of detergent phase partitioning (Fig. 1a). These results indicate that Atg8 acquires sufficient hydrophobicity for membrane insertion after the attachment of molecule X.

To assess directly the structure of Atg8-X, we purified it from Δatg7Δatg8 cells expressing His6-Atg8FG. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) gave two signals at m/z 14,368 and 15,003 (Fig. 1b). The former value was assigned to the molecule His6-Atg8FG (14,297) modified with an acrylamide monomer (relative molecular mass (M) 71) derived from SDS/polyacrylamide gel electrophoresis (PAGE). The latter was assigned to the His6-Atg8-X. The difference in molecular mass (635) and hydrophobic nature of Atg8-X suggested that X is a glycerophospholipid. In fact, the His6-Atg8-
Analysis of Post-Translational Modifications by Mass Spectrometry

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(Institute for Protein Research)

Mass spectrometry (MS) represents a well-accepted and reliable method for the characterization of proteins. The method has great advantages in terms of throughput, accuracy, and sensitivity in measurements, which is well suited for the identification of a wide variety of proteins, such as those separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), and for the analysis of post-translational modifications of a protein, which play important roles in various biological events. Taking advantages of an accumulating protein sequence database, the former has been a routine task for overall profiling of proteins expressed in an organism or cell. The latter, especially, the analysis of unknown or multiple modifications is challenging and can be exhaustively achieved by tandem mass spectrometry (MS/MS) [1,2] (Fig. 1).
In this study, state-of-the-art MS techniques have been applied to the structural analysis of a new mode of protein lipidation found in a protein Apg8, an essential factor for autophagy [3-5], which is a dynamic membrane phenomenon for bulk protein degradation in the lysosome/vacuole [6,7]. The carboxyl-terminal Arg of nascent Apg8 is cut off by Apg4, leaving a Gly at the C terminus [8]. Apg8G is then converted by a ubiquitination-like system to a form (Apg8G-X) that is tightly bound to the membrane [8] (see Fig. 4), which was efficiently recovered from the detergent (Triton X-114) phase. The protein band corresponding to Apg8G-X was excised from a SDS polyacrylamide gel (Fig. 2a) and used for the following MS analysis. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) gave two signals at m/z 14,368 and 15,003 (Fig. 2b). The former value was assigned to the molecule His<sub>6</sub>-Apg8G (14,297) modified with an acrylamide monomer (Mr 71) derived from a gel. The latter was assigned to the His<sub>6</sub>-Apg8G-X. The difference in molecular mass (635) and hydrophobic nature of Apg8G-X suggested that X is a glycerophospholipid. In fact, the His<sub>6</sub>-Apg8G-X signal shifted to a lower molecular size on treatment with mild alkali, a process that removes fatty acids from glycerophospholipids. For elucidating the attachment site and exact chemical structure of X, Apg8G-X was treated in gel with mild alkali and digested by lysylendopeptidase (LEP). The resulting C-terminal peptide in the digest was subjected to electrospray ionization/tandem mass spectrometry (ESI-MS/MS) (Fig. 3). Every resulting fragment ion containing the C-terminal Gly was heavier by Mr 197 than the corresponding fragment ions from Apg8G (compare two series of y<sup>+</sup> ions in Fig. 3). This value (197) is consistent with the mass of a glycerophosphoethanolamine moiety. In addition, some y<sup>+</sup> ions from Apg8G-X accompanied signals reduced by Mr 172, which can be explained by loss of a glycerophosphate moiety (Fig. 3). It was also evidenced by gas chromatography/MS that saturated (C16:0 and C18:0) and unsaturated (C16:1 and C18:1) fatty acids were liberated from Apg8G-X by mild alkaline treatment. From the above evidence, it was concluded that phosphatidylethanolamine (PE) is covalently conjugated to the C-terminal Gly of Apg8 (Fig. 4). This is the first protein lipidation in which a protein conjugates to a phospholipid ubiquitously distributed in biological membranes [10].
Fig 3. MS/MS spectra of the C-terminal peptides (Apg8 Gly-Phe-Leu-Tyr-Val-Thr-Tyr-Asp-Gly-Asp-Thr-Phe-Gly) of Apg8G-X (upper) and Apg8G (lower). C-terminal fragments were generated from Apg8G-X and Apg8G by in gel digestion with LCP under the presence of 14C-labelled water. Each C-terminal fragment that was observed only as non-14C-labelled signals was subjected to MS/MS. The MS/MS spectra were interpreted by “SeqMID” [8], a software aid for de novo sequencing (http://protein.osaka-u.ac.jp/organic). The arrows show the sequences from the N and C terminus based on y1 and b ions, respectively, where w and f designate positions counted from the C and N terminus, which were produced by cleavage of peptide bonds during MS/MS. Amino acids shown in three-letter code denote immonium ions.

0.06 M NaOH in 30% CH3OH at 40 °C for 4h

Fig 4. A ubiquitin-like system mediates protein lipidation. PE: phosphatidylserine/olamine

References
Identification of CRE1 as a Cytokinin Receptor from Arabidopsis

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Cytokinins are a class of plant hormones that are central to the regulation of cell division and differentiation in plants. It has been proposed that they are detected by a two-component system, because overexpression of the histidine kinase gene CRE1 induces typical cytokinin responses and genes for a set of response regulators of the two-component systems can be induced by cytokinins. Two-component systems use a histidine kinase as an environmental sensor and rely on a phosphorelay for signal transduction. They are common in microorganisms and are also emerging as important signal detection routes in plants.

Here we report the identification of a cytokinin receptor. We identified Arabidopsis cre1 (cytokinin response 1) mutants, which exhibited reduced responses to cytokinins. The mutated gene CRE1 encodes a histidine kinase. CRE1 expression conferred a cytokinin-dependent growth phenotype on a yeast mutant that lacked the endogenous histidine kinase SLN1 (ref. 10), providing direct evidence that CRE1 is a cytokinin receptor. We also provide evidence that cytokinins can activate CRE1 to initiate phosphorelay signalling.

Generally, cytokinins induce cell division, chloroplast development and formation of shoots. We screened mutagenized Arabidopsis for mutants that were impaired in cytokinin responses, including rapid cell proliferation and shoot formation in tissue culture. We isolated a mutant designated cytokinin response 1-1 (cre1-1). We tested the responses of cre1-1 to auskin and cytokinin in tissue culture, using naphthalene acetic acid (NAA) as an auxin and kinetin as a cytokinin (Fig. 1). Wild-type explants responded to increasing levels of kinetin with rapid proliferation, greening and formation of shoots (Fig. 1a). By contrast, such cytokinin responses were not evident in cre1-1 (Fig. 1b). The mutant was also less responsive to other cytokinins, including trans-resorcylic acid adenine, benzyl adenine and the phosphorylated cytokinin thidiazuron (see Supplementary Information).

Next we tested the responses of cre1-1 to various plant hormones in a root elongation assay. External application of ethylene, auxin or abscisic acid inhibits root elongation. The root of the cre1-1 mutant was less sensitive to benzyl adenine than that of wild-type plants, but it responded normally to the ethylene precursor 1-amino-cyclopropane-1-carboxylic acid (ACC) and to the auxin indole-3-acetic acid (IAA) (Fig. 2a-c). The responses of cre1-1 to low levels of abscisic acid (ABA) were slightly higher than normal (Fig. 2d). The cytokinin responses of cre1-1 heterozygotes were intermediate between those of cre1-1 homoyzogotes and the wild type (see Supplementary Information).

We mapped the CRE1 locus to the top of chromosome 2 between the rga and nga1145 markers (see Supplementary Information). We searched the genome sequence of Arabidopsis between these markers for genes that could code for proteins involved in signal transduction. Among them was the putative gene AT2G01830, possibly coding for a histidine kinase. The nucleotide sequence of AT2G01830 revealed that this gene was mutated in the cre1-1 mutant. Hereafter we refer to this gene as CRE1. CRE1 is identical to WOL (see below) and AHR4 (ref. 16). A genomic fragment containing CRE1 was introduced into cre1-1 mutant calli. Wild-type calli that had been transformed with the control vector regenerated shoots when cultured in the presence of the...
Identification of a Receptor for the Plant Hormone Cytokinins.

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Introduction

Cytokinins are central to the regulation of numerous physiological processes including cell division and differentiation in plants. Cytokinins are necessary for cell division, induce formation of leaves and buds on a callus (an undifferentiated mass of cells), break lateral bud dormancy, regulate nutrient allocation and delay senescence. Despite the central importance of cytokinins in plant development, no cytokinin receptor has been identified yet.

Identification of an Arabidopsis cytokinin-resistant mutant

To identify important genes for cytokinin signal transduction, we have screened for mutants that are altered in cytokinin responses. Usually tissue segments excised from Arabidopsis grow rapidly with a green color reflecting chloroplast development, in the presence of a cytokinin and an auxin. Plants were grown from seeds of Arabidopsis that had been mutagenized with ethyl methane sulfonate, self-pollinated and then allowed to set seeds (M2 seeds). Hypocotyl segments were excised from M2 seedlings and cultured on plates that contain a cytokinin at a level that normally induce rapid growth and chloroplast development. From about 19,000 hypocotyl segments, we found a mutant that did not exhibit cytokinin responses. This mutant was designated crel-1 for cytokinin response I-1.

Characterization of the crel-1 mutant

The crel-1 mutant was recessive. We tested the responses of homozygous crel-1 to auxin and cytokinin in tissue culture, using naphthalene acetic acid as an auxin, and kinetin as a cytokinin. Wild-type explants responded to increasing levels of kinetin with rapid proliferation, greening and formation of shoots (Fig. 1a). By contrast, such cytokinin responses were not evident in crel-1 (Fig. 1b). This mutant was also less responsive to other cytokinins, including trans-zeatin, benzyl adenine, and a phenylurea type synthetic cytokinin, thidiazuron. Cytokinin responsiveness was also tested in another assay system. Cytokinins, auxins, ethylene and abscisic acid, which are all plant hormones, inhibit elongation of root cells. The crel-1 mutant was less responsive specifically to cytokinins for inhibition of root elongation.

Fig. 1. Responses of the cytokinin-resistant crel-1 mutant to auxin and cytokinin. Hypocotyl segments were excised and cultured on media containing different levels of kinetin and NAA. After 21 days in culture, the induced callus were arranged for photographing on the plates shown here. Wild-type explants (a) proliferated rapidly, turned green, and produced shoots in the presence of high concentrations of cytokinins. The crel-1 explants (b) did not.
**CRE1 gene codes for a histidine kinase**

We mapped the CRE1 locus to the top of chromosome 2 within several hundred kilobases, and found a nucleotide change in a gene in this region. This gene, named CRE1, codes for a protein with signature motifs of histidine kinases of the two component systems. Wild-type CRE1 gene complemented the crel phenotype confirming that CRE1 is the causal gene for the crel mutant. The two-component systems are prevalent in bacteria, and also present in fungi and plants. In the two-component system, a histidine kinase functions as an environmental sensor and autophosphorylates at an invariant histidine residue when it perceives a signal. The phosphoryl group is then transferred to a response regulator, which modulate the activity of the output domain of the response regulator. In many cases, the output domains of response regulators are DNA-binding domains (Fig. 2).

**Functional analysis of the CRE1 gene**

That CRE1 belongs to the histidine kinase family and the cytokinin resistant phenotype of the crel-1 mutant raised the possibility that CRE1 is a cytokinin receptor. To explore the function of the CRE1 gene, we introduced CRE1 into an yeast strain deficient in the SLN1 gene (Fig.3), which codes for a histidine kinase with the osmosensing function. At normal osmolarity, SLN1 autophosphorylates the conserved histidine residue. The phosphoryl group is then transferred to the conserved aspartate residue in the receiver domain of the same protein, then to the phosphotransfer mediator, YPD1, and finally to the SSK1 response regulator. This in turn inhibits the ability of SSK1 to activate the downstream mitogen-activated protein (MAP) kinase pathway. The sln1Δ mutant is lethal because the downstream SSK1 response regulator is always dephosphorylated, which over activates the downstream MAPK pathway. The sln1Δ mutant carrying the CRE1 gene, which would express the CRE1 gene, was still lethal without cytokinins. However, surprisingly, it grew at a normal rate, if trans-zeatin, a native cytokinin, was included in the medium. It is noteworthy that the active cytokinin trans-zeatin was effective in this yeast system, but the much less active cytokinin cis-zeatin was ineffective in this system (Fig. 3a). Other classes of plant hormones, auxin, gibberelllic acid, and abscisic acid had no effect.

To know the signalling pathway initiated by CRE1 in yeast, CRE1 was also introduced into the ypd1Δ mutant (Fig. 3a). CRE1 did not suppress the lethality of ypd1Δ, indicating that signal transduction from CRE1 was mediated by YPD1 in yeast. We also tested several mutant CRE1 genes in the sln1Δ mutant (Fig. 3b). The crel-1 mutant, which was found in the Arabidopsis crel-1 mutant, did not suppress the sln1Δ mutant, suggesting that crel-1 product does not have histidine kinase activity. Also, mutations that change the conserved His459 and Asp973 phosphorylation sites destroyed the ability of the CRE1 gene to suppress the sln1Δ lethality. These results indicate that cytokinins activate the histidine kinase activity of CRE1, and the signal is transmitted through YPD1 to SSK1. Arabidopsis also has phosphotransfer mediators, which resemble YPD1, and has response regulators. Therefore, cytokinins probably activate CRE1, which in turn initiate the phosphorelay-mediated signalling that governs cytokinin responses.

**CRE1 is required for cell division and differentiation in the root vascular tissue**

The allele crel-1 and wol mutants are impaired in specific cell divisions in provascular tissues. Also, these mutants do not form the phloem, but misspecification of the phloem could be a secondary effect of the deficiency in the cell division (unpublished results). These results suggest that cytokinins, through CRE1, regulate cell divisions in the provascular tissue and possibly cell specification. The lack of more diverse effect of the lack of a cytokinin receptor, despite cytokinin regulating many physiological processes, could be attributed to the presence of redundant genes. Indeed, two genes of Arabidopsis that resemble CRE1 rendered the sln1Δ yeast cytokinin responsive (unpublished).
Conclusion

We have identified cytokinin-insensitive Arabidopsis mutant, crel-1. The causal gene CRE1 coded for a histidine kinase of the two-component systems. CRE1 suppressed the lethality of the sln1Δ mutant of S. cerevisiae, which lacks the only one histidine kinase gene of yeast, in a cytokinin-dependent manner. These results indicate that CRE1 is a cytokinin receptor, and histidine kinase activity of CRE1 is activated by cytokinins.

References
Cigarette Smoking and Risk for Impaired Fasting Glucose and Type 2 Diabetes in Middle-Aged Japanese Men

Background: The contribution of cigarette smoking to development of impaired fasting glucose and type 2 diabetes remains unclear.

Objective: To investigate the association of cigarette smoking with development of impaired fasting glucose and type 2 diabetes.

Design: Prospective cohort study.

Setting: Work site in Osaka, Japan.

Participants: 1266 Japanese male office workers 35 to 59 years of age who did not have impaired fasting glucose or type 2 diabetes and were not taking medication for hypertension at study entry.

Measurements: Fasting plasma glucose levels were measured at annual health examinations from May 1994 through May 1999. Impaired fasting glucose was defined as a fasting glucose level of at least 6.1 mmol/L (110 mg/dl) but less than 7.0 mmol/L (126 mg/dl). Type 2 diabetes was defined as a fasting glucose level of 7.0 mmol/L or more or current receipt of hypoglycemic medication.

Results: 87 and 54 men developed impaired fasting glucose and type 2 diabetes during 5817 and 5937 person-years follow-up, respectively. After controlling for potential predictors of diabetes, the relative risk for impaired fasting glucose compared with never-smokers was 1.62 (95% CI, 0.85 to 3.10) for ever-smokers, 1.14 (CI, 0.58 to 2.25) for persons who smoked 1 to 20 cigarettes/d, 1.33 (CI, 0.63 to 2.80) for those who smoked 21 to 30 cigarettes/d, 1.84 (CI, 1.32 to 2.56) for those who smoked 31 to 50 cigarettes/d, and 2.44 (CI, 1.32 to 4.60) for those who smoked 51 or more cigarettes/d. The respective multivariable-adjusted relative risks for type 2 diabetes compared with never-smokers were 1.08 (CI, 0.34 to 3.42), 1.88 (CI, 0.71 to 5.00), 3.02 (CI, 1.15 to 7.94), and 4.09 (CI, 1.82 to 10.29) for those who smoked 1 to 20 cigarettes/d, respectively. The relative risk for impaired fasting glucose and type 2 diabetes in current smokers versus never-smokers was stronger among men with a body mass index less than 24.2 kg/m² than among men with a body mass index of 22.4 kg/m² or more, although the absolute risk was greater in middle-aged Japanese men.

Conclusion: The number of cigarettes smoked daily and the number of pack-years of exposure seem to be associated with development of impaired fasting glucose and type 2 diabetes in middle-aged Japanese men.

The prevalence of type 2 diabetes in Japan has increased in the past decade, in tandem with the rapid westernization of lifestyle. This disorder of impaired insulin secretion and insulin resistance is associated with increased risk for cardiovascular disease, renal disease, and retinopathy. Although age, family history of diabetes, obesity, alcohol consumption, and reduced physical activity are well-known risk factors for type 2 diabetes, the association of smoking with development of type 2 diabetes is not well understood.

Longitudinal studies from the Netherlands, the United States, and Japan have reported that cigarette smoking may be an independent risk factor for type 2 diabetes. However, one study found a monotonic association between cigarette smoking and type 2 diabetes, but two found a nonmonotonic association. Furthermore, a cohort study in the United Kingdom failed to show an independent association between cigarette smoking and type 2 diabetes. These inconclusive results may have resulted in part from ethnic or lifestyle differences in the study samples but also may have been strongly influenced by different methods used to diagnose type 2 diabetes. In the Western studies, the diagnosis of type 2 diabetes was ascertained by a mailed questionnaire. In one Japanese study, type 2 diabetes was diagnosed by measuring the 75-g oral glucose tolerance test in persons with both glucosuria and a fasting plasma glucose level of 6.1 mmol/L (110 mg/dl) or more. In another Japanese study, type 2 diabetes was defined according to newer criteria (a fasting plasma glucose level ≥ 7.0 mmol/L [126 mg/dL]) in 1997 and the World Health Organization (WHO) in 1985. This page is made by NAKANISHI Noriyuki as the reprinting of the original paper was not permitted.
Cigarette Smoking and Risk for Diabetes in Japanese Men

NAKANISHI Noriyuki
(Graduate School of Medicine)

Introduction

Type 2 diabetes is a common disease in industrialized countries and there are approximately seven million patients in Japan. This common disease characterized by impaired insulin secretion and insulin resistance is associated with increased risk for cardiovascular disease, renal disease, and retinopathy. Although age, family history of diabetes, obesity, alcohol consumption, and reduced physical activity are well-known risk factors for type 2 diabetes, the contribution of cigarette smoking to development of type 2 diabetes remains to be elucidated.

Previous longitudinal studies from the Netherlands, the United States, and Japan have reported that cigarette smoking may be an independent risk factor for type 2 diabetes (1-4). However, the effects of smoking on type 2 diabetes have been variously found to be monotonic (4) or not (2, 3). Furthermore, a large cohort study in the United Kingdom failed to show an independent association between cigarette smoking and type 2 diabetes (5). These discrepancies may reflect differences in investigational design, methods, and populations; in some, the diagnosis of type 2 diabetes was ascertained by a mailed questionnaire; and in others, there has been insufficient control for putative risk factors for diabetes.

The American Diabetes Association (ADA) (6) and the World Health Organization (WHO) (7) have recently recommended that estimates of diabetes incidence in epidemiologic studies should be based on the fasting plasma glucose level. Using serial annual health examinations at the workplace and the new ADA and WHO criteria (6, 7), we performed a longitudinal population study followed from 1994 through 1999 to prospectively examine the association of cigarette smoking with development of impaired fasting glucose and type 2 diabetes in middle-aged Japanese men.

Methods of this study

1266 Japanese male office workers aged 35 to 59 years and free of impaired fasting glucose, type 2 diabetes, and medication for hypertension partook in a survey. Fasting glucose levels were measured at annual health examinations from May 1994 through May 2000. Normal fasting glucose, impaired fasting glucose, and type 2 diabetes were defined by using the ADA and WHO criteria (6, 7). Normal fasting glucose was defined as a fasting plasma glucose level less than 6.1 mmol/L (110 mg/dL). Impaired fasting glucose was defined as a fasting plasma glucose level of 6.1 but less than 7.0 mmol/L (126 mg/dL). Type 2 diabetes was defined as a fasting plasma glucose level 7.0 mmol/L or greater or receipt of hypoglycemic medications (because not every participant underwent an oral glucose tolerance test). Men in whom impaired fasting glucose and type 2 diabetes were found during repeated surveys through May 1999 were defined as incident cases of impaired fasting glucose and type 2 diabetes. To determine the incidence of type 2 diabetes, incident cases of impaired fasting glucose were followed and were considered type 2 diabetes if this condition developed.

For each participant, person-years of follow-up were calculated from the date of enrollment to the date of diagnosis of impaired fasting glucose or type 2 diabetes or the date of follow-up, whichever came first. The follow-up rate was 95.6% of the potential person-years of follow-up. Cox proportional hazards models were used to evaluate the association between smoking status and the development of impaired fasting glucose or type 2 diabetes, controlling for the following potential confounders: age; body mass index; alcohol consumption; physical activity; family history of diabetes; systolic and diastolic blood pressure; levels of fasting plasma glucose, total cholesterol, high-density lipoprotein cholesterol, triglyceride, and uric acid; and hematocrit at study entry.
Table 1. Smoking Status and Risk for Impaired Fasting Glucose and Type 2 Diabetes in the Study Sample

<table>
<thead>
<tr>
<th>Condition</th>
<th>Never-Smokers</th>
<th>Ex-Smokers</th>
<th>Current Smokers</th>
<th>P Value for Trend *</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-20 Cigarettes/d</td>
<td>21-30 Cigarettes/d</td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
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<td></td>
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<td>Cases, n</td>
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<td>1877</td>
<td>1906</td>
<td>1906</td>
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<tr>
<td>Person-years</td>
<td>20</td>
<td>18</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>10.7</td>
<td>18.4</td>
<td>12.06</td>
<td>892</td>
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<td>Age-adjusted relative risk (95% CI)</td>
<td>1.0 (referent)</td>
<td>1.6 (0.9-3.0)</td>
<td>1.2 (0.6-2.3)</td>
<td>2.2 (1.2-4.1)</td>
</tr>
<tr>
<td>Multivariate-adjusted relative risk (95% CI)</td>
<td>1.0 (referent)</td>
<td>1.6 (0.9-3.1)</td>
<td>1.1 (0.6-2.3)</td>
<td>2.6 (1.3-5.0)</td>
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<td>Type 2 diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cases, n</td>
<td>7</td>
<td>5</td>
<td>11</td>
<td>12</td>
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<tr>
<td>Person-years</td>
<td>1906</td>
<td>1014</td>
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<tr>
<td>Rate per 1000 person-years</td>
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<tr>
<td>Multivariate-adjusted relative risk (95% CI)</td>
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<td>1.1 (0.3-3.4)</td>
<td>1.9 (0.6-7.0)</td>
<td>4.1 (1.6-10.3)</td>
</tr>
</tbody>
</table>

* Calculated across increasing categories of smoking for current smokers only.
† Controls for age, body mass index, alcohol consumption, regular physical exercise, family history of diabetes, systolic and diastolic blood pressure, fasting plasma glucose level, total cholesterol level, high-density lipoprotein cholesterol level, triglyceride level, uric acid level, and hemoglobin at study entry.

Results of this study

During 3 years of follow-up representing 5817 and 5937 person-years, 87 and 54 men developed impaired fasting glucose and type 2 diabetes (Table 1). The multivariate-adjusted relative risk for impaired fasting glucose compared with never smokers was 1.6 (95% CI, 0.9 to 3.1) for ever-smokers, 1.1 (CI, 0.6 to 2.3) for those who smoked 1 to 20 cigarettes/d, 1.3 (CI, 0.6 to 2.8) for those who smoked 21 to 30 cigarettes/d, and 2.6 (CI, 1.3 to 5.0) for those who smoked 30 or more cigarettes/d (P for trend for current smokers = 0.013). The respective multivariate-adjusted relative risks for type 2 diabetes compared with never smokers were 1.1 (CI, 0.3 to 3.4), 1.9 (CI, 0.7 to 5.0), 3.0 (CI, 1.2 to 7.9), and 4.1 (CI, 1.6 to 10.3) (P for trend for current smokers < 0.001).

To evaluate the long-term effect of cigarette smoking on development of impaired fasting glucose and type 2 diabetes, we assessed the relation between pack-years of exposure and risk for impaired fasting glucose and type 2 diabetes (Table 2). The multivariate-adjusted relative risk for impaired fasting glucose compared with never smokers was 1.9 (CI, 0.4 to 2.5) for those whose cumulative lifetime exposure was 0.1 to 20.0 pack-years, 1.1 (CI, 0.5 to 2.5) for those whose cumulative lifetime exposure was 20.1 to 30.0 pack-years, 1.8 (CI, 0.5 to 2.5) for those whose cumulative lifetime exposure was 30.1 to 40.0 pack-years, and 2.0 (CI, 1.1 to 3.8) for those whose cumulative lifetime exposure was 40.1 or more pack-years (P for trend = 0.039).

We assessed the effect of obesity on the association between cigarette smoking and risk for development of impaired fasting glucose or type 2 diabetes (Figure 1). Among men with a body mass index less than 24.2 kg/m², the multivariate-adjusted relative risk for impaired fasting glucose or type 2 diabetes compared with never smokers was 0.9 (CI, 0.4 to 2.2) for ever-smokers, 1.5 (CI, 0.7 to 3.1) for those who smoked 1 to 20 cigarettes/d, 2.2 (CI, 1.1 to 4.6) for those who smoked 21 to 30 cigarettes/d, and 3.8 (CI, 1.9 to 7.6) for those who smoked 31 or more cigarettes/d (P for trend for current smokers < 0.001).

Among men with a body mass index 24.2 kg/m² or more, the respective multivariate-adjusted relative risks for impaired fasting glucose or type 2 diabetes compared with never smokers were 2.0 (CI, 0.9 to 4.5), 1.3 (CI, 0.5 to 3.1), 1.7 (CI, 0.7 to 4.4), and 2.4 (CI, 1.0 to 5.6) (P for trend for current smokers = 0.031). As the effect of obesi-
Table 2. Pack-Years of Cigarette Smoking and Risk for Impaired Fasting Glucose and Type 2 Diabetes in the Study Sample

<table>
<thead>
<tr>
<th>Exposure to Cigarette Smoking</th>
<th>0 Pack-Years</th>
<th>0.1-20.0 Pack-Years</th>
<th>20.1-30.0 Pack-Years</th>
<th>30.1-40.0 Pack-Years</th>
<th>≥ 40.1 Pack-Years</th>
<th>for Trend *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impaired fasting glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases, n</td>
<td>20</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Person-years</td>
<td>1877</td>
<td>644</td>
<td>670</td>
<td>611</td>
<td>1037</td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>10.7</td>
<td>10.9</td>
<td>13.4</td>
<td>14.7</td>
<td>23.2</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted relative risk (95% CI)</td>
<td>1.0 (referent)</td>
<td>1.1 (0.5-2.6)</td>
<td>1.2 (0.6-2.7)</td>
<td>1.3 (0.6-2.9)</td>
<td>2.0 (1.1-3.6)</td>
<td>0.032</td>
</tr>
<tr>
<td>Multivariate-adjusted relative risk (95% CI)</td>
<td>1.0 (referent)</td>
<td>1.0 (0.4-2.5)</td>
<td>1.1 (0.5-2.5)</td>
<td>1.8 (0.5-2.5)</td>
<td>2.0 (1.1-3.8)</td>
<td>0.039</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases, n</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Person-years</td>
<td>1906</td>
<td>654</td>
<td>678</td>
<td>612</td>
<td>1074</td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>3.7</td>
<td>9.2</td>
<td>10.3</td>
<td>11.4</td>
<td>20.5</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted relative risk (95% CI)</td>
<td>1.0 (referent)</td>
<td>2.5 (0.7-7.5)</td>
<td>2.8 (1.0-8.0)</td>
<td>3.1 (1.1-8.8)</td>
<td>5.4 (2.3-12.8)</td>
<td>~ 0.001</td>
</tr>
<tr>
<td>Multivariate-adjusted relative risk (95% CI)</td>
<td>1.0 (referent)</td>
<td>2.3 (0.7-6.9)</td>
<td>2.4 (0.8-11.1)</td>
<td>2.4 (0.8-11.1)</td>
<td>4.1 (1.1-10.2)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Calculated across increasing categories of smoking for current smokers only.
† Controls for age, body mass index, alcohol consumption, regular physical exercise, family history of diabetes, systolic and diastolic blood pressure, fasting plasma glucose level, total cholesterol level, high-density lipoprotein cholesterol level, triglyceride level,_title: smoking cessation and obesity

Commonly, Most (1-4) but not all (5) previous epidemiologic studies found smoking to be positively associated with risk for type 2 diabetes. However, the studies conflicted; one found that the relation between the number of cigarettes smoked per day and the risk for type 2 diabetes was monotonous (4) and two found that it was not monotonous (2, 3). Our results are consistent with the findings of another Japanese study (4) that used the new ADA and WHO criteria (6, 7) for an epidemiologic study. Our data also agree with those from a U.S. study (2) in which the relative risk for diabetes associated with cigarette smoking was stronger in leaner men. Thus, obesity may modify the association between smoking and the risk for impaired fasting glucose and type 2 diabetes, and the impact of smoking on risk for impaired fasting glucose and type 2 diabetes may be stronger in leaner men. The U.S. study (2) also reported that the risk for type 2 diabetes was higher among past smokers than among never-smokers but decreased with years since quitting smoking. Because smoking cessation is more common and may lead to weight gain, which increases the risk for diabetes. Inclusion of persons who were smokers at baseline but who quit and became more obese during follow-up might have falsely elevated the risk associated with smoking at baseline; however, this would not explain fully the increased risk seen in ex-smokers. These results suggest that we need not only to encourage smoking cessation but also to concomitantly help patients avoid obesity.

Our findings, which were obtained from a cohort of middle-aged Japanese men, provide evidence that cigarette smoking is closely associated with the risk for impaired fasting glucose and type 2 diabetes according to the new ADA and WHO criteria for epidemiologic studies.

References
Notomi Kajiro: An Industrial Art Pioneer and the First Design Educator of Modern Japan

FUJITA Harukiko
(Graduate School of Letters)
Design Issues, 17, 1-11 (2001)

It was around 1900 when design started to be taught at a few higher educational institutions in Tokyo and Kyoto. However, Japan's history of design education goes all the way back to 1897, when Notomi Kajiro (1844-1915, Fig.1) established a municipal technical school in Karazawa (Fig.2), which was followed by similar schools in Takamatsu (Fig.3), Takamatsu (Fig.4), and Arita (Fig.5), all founded by Notomi.

In 1895, an American architect, Frank Lloyd Wright (1867-1959) first visited the Takamatsu school and was very much impressed with its "pure Japanese" education. Wright seems to have shared the same ideal of art education with Kururi Yasa (1895-1904), the successor to Notomi as director. Wright who had revolutionized the Arts and Crafts Theory by a 1901 lecture, seems to have changed his attitude toward the machine after his first visit to Japan. While Notomi's schools in local cities remained preferential for secondary education, the two government schools in Tokyo and Osaka evolved into institutions for higher education, and the third government school in Kyoto was established as the first "koto-gakko", higher school of technology and design. Notomi's schools were more or less against his own will, partly becoming preparatory schools for these higher schools and particularly for the Tokyo Byo-Bu Gakko which started to introduce Western art education and were called "these delayed schools" by Frank Lloyd Wright.

The West Uighur Kingdom and Turfan, Temples and Tombs in the 10th-11th Centuries

MORIYASU Takao
(Graduate School of Letters)

In the West Uighur Kingdom which existed in the modern Chinese Turkestan during the 8th-13th centuries, the Manichaean church had enjoyed national support at the beginning but it was little by little enrooted and finally lost its religious vitality. On the other hand, Turfanian and Chinese Buddhists who had entered the Uighur ruling class were eager to convert the Manichaeans in Uighurs. When did the West Uighurs begin being converted to Buddhism? To answer this question, the discovery of Buddhist Manichaean double-walled cisterns (Plate A 1 - 2) is the most remarkable event in Turfan. In this article, the main parts of the Tufan Buddhist wall-paintings should date to the 10-13th centuries. This view of mine has partly been proved by the radiocarbon test. Prof. M. Yassig, who is the director of the Museum für Ostasiatische Kunst in Berlin, has announced that the Manichaean form of the Uighur inscriptions on the older wall-paintings of this cave. There is no doubt that its contents were

Plate A 1 - 2. Buddhist Manichaean double-walled cisterns

Plate B. Manichaean wall-paintings in Grünewald's Cave No. 25 at Bezeklik

Plate C. Buddhist wall-painting at Bezeklik

Grünewald and Le Coq, kindly informed me the result of the radiocarbon test in the report on June 9th, 1978: I got the first C14 dates of the wall-paintings. You might remember that in our museum (MAK 281564) depicting the Buddhist grotto from Bezeklik, Tempel 4. Traditionally it had been dated to the 9th century. A.D. This radionuclide analysis gives us a date A.D. 1140 +/- 30, i.e. three hundred years later. Tempel 4 in Bezeklik is also dated much later, i.e. A.D. 1078 +/- 28.
Formation of Initial Perturbation of Rayleigh-Taylor Instability in Supernovae and Laser-Irradiated Targets — Is There Any Similarity?

AZECHI Hiroshi
(Institute of Laser Engineering)

In this paper, we suggest that the formation of initial hydrodynamic perturbation in the very early phase of supernova explosions is analogous to the initial "imprint" process of laser non-uniformity on a target for inertial confinement fusion. We have presented a reasonably simple imprint model, test it in the laser experiments, and apply on the supernova process. If this analogy is correct, the velocity perturbation in supernovae may be significantly amplified compared with a secular distortion of the pressure non-uniformity.

Reference data

uniform laser on modulated target

Imprint data

modulated laser on uniform target

Space (500 μm)

Time (2.3ns)

Morphology and Photonic Band Structure Modification of Polystyrene Particle Layers by Reactive Ion Etching

ITOH Tadashi
(Graduate School of Engineering Science)
*Applied Physics Letters, 78, 1478-1480 (2001)*

A self-assembled, hexagonally-close-packed monolayer of sub-μm latex particles on a glass substrate is expected to show a characteristic phonon dispersion along the layer called quasi-2D photonic band. In the present work, the particle size has been modified by reactive ion etching without changing the position of the particles, as shown in the SEM images from (a) to (f) on the left. Optical transmission spectra in oblique incidence have been analyzed to investigate the modification of photonic bands. As the particle size reduces, the main band shows high frequency shift and band narrowing as illustrated in the red zones of the dispersion curves from (a) to (c) on the upper right. The change is caused by the increase of the frequency of single-particle eigenmodes called Whispering Gallery Modes and by the decrease of mode transfer between the adjacent particles, that clearly demonstrates the applicability of the light binding model to photonic bands, just in the case of electronic energy bands. The lower-right pictures show (1) topographic and (2) fluorescence-excitation images taken by a scanning near-field optical microscope (SNOM), where the light propagation is directional along the array of the microparticles.
Solar Wind Record on the Moon: Deciphering Presolar from Planetary Nitrogen

HASHIZUME Ko
(Graduate School of Science)

Moon’s surface, exposed to the solar wind, offers us a unique opportunity to study the isotopic composition of the Sun. Through a new method, we identified the isotopic composition of the solar nitrogen implanted to the lunar soil grains. This N is depleted in $^{15}\text{N}$ by about 25% compared to terrestrial N. The systematic enrichment of $^{15}\text{N}$ relative to the solar composition observed on Earth, on Mars, and in meteorites probably shows survival in the protosolar nebula of presolar nitrogen-bearing dusts having inherited interstellar enrichment in $^{15}\text{N}$ from isotopic fractionation that took place in the interstellar medium. Electronic reprint is available from http://www.cbg.cnrs-nancy.fr/Science/Koisol-lunaire.html.

Solar N isotopic composition

Itinerant f-electron System of Cerium and Uranium Compounds

OUNKI Yoshichika
(Graduate School of Science)

The f-electron systems of the cerium and uranium compounds have attracted attention in the context of heavy fermion and anisotropic superconductivity. UPt$_3$, with the hexagonal structure is a prime candidate in which the unconventional pairing state (odd parity) in superconductivity is realized. We have succeeded in growing a high-quality single crystal with a residual resistivity ratio of 700, and measured the de Haas-van Alphen (dHvA) effect. Figure A shows the angular dependence of the dHvA frequency, shown by red circles, which are well explained by the $\delta$-invariant band model, shown by blue solid lines. The origin of the dHvA branches is identified, shown in Figures B - D. All branches are, however, surprisingly heavy, with cyclotron masses of 15 - 105 m$_e$ (m$_e$: rest mass of an electron), i.e. ten to twenty times larger than the corresponding band masses.

[Diagram of solar wind and depth from mineral surface]

[Graph showing isotopic composition of nitrogen over time]

[Diagram of itinerant f-electron system of cerium and uranium compounds]
Chiral Discrimination of Fructo-oligosaccharides toward Amino Acid Derivatives by Induced-fitting Chiral Recognition

SAWADA Masami and TAKAI Yoshio
(Institute of Scientific and Industrial Research)

Dynamic Enantioselective Complexation

(M)-Enantiomer

(R)-Enantiomer

MeFruNys Chiral Ammonium Ions

Induced-fitting Molecular Recognition Model

Host Guest

Dynamic Conformation Change

Induced-fitting Complex

Mass, NMR, and UV-visible spectrometries clarified that one of the linear fructo-oligosaccharide derivatives, permethylated fructosylose (MeFruNys), showed higher degrees of enantioselectivity toward binding some amino acid derivatives based on the induced-fitting chiral recognition mechanism. Here, the higher chiral selectivity of the fructo-oligosaccharide, to which much attention had not been paid so far, was discovered through mass spectrometric screening of combinatorial mixtures.

Construction of a New Multi-turn Time-of-flight Mass Spectrometer

TOYODA Michisato
(Graduate School of Science)


A new type of multi-turn time-of-flight (TOF) mass spectrometer was developed and reconstructed for cometary and planetary explorations. The size and weight of such instrument are limited when it is carried on the spacecraft. On the other hand, the mass resolution of a TOF mass spectrometer is proportional to the path length. A large instrument is generally required. In this small instrument, however, the high mass resolution can be achieved because ions pass through the same path many times. This instrument consists of four cylindrical electric sectors and 28 electric quadrupole lenses. The size of the vacuum chamber was 60 cm × 70 cm × 90 cm. It was demonstrated that the mass resolution can be increased according to the number of cycles of the ions through the ion optical system.
Anisotropic Mechanical Properties of Porous Copper Fabricated by Unidirectional Solidification

NAKAJIMA Hideo
(The Institute of Scientific and Industrial Research)
Materials Science and Engineering 4, 299, 741-748 (2001)

Lotus-structured porous copper whose long cylindrical pores are aligned in one direction has been fabricated by unidirectional solidification of the melt in a mixture gas of hydrogen and argon. The anisotropy in the uniaxial tensile behavior of the porous copper is examined. The ultimate tensile strength of the porous copper with the cylindrical pores orientated parallel to the tensile direction decreases linearly with increasing porosity; the specific tensile strength does not change even by the porosity increase. For the porous copper whose pore axes are perpendicular to the tensile direction, the ultimate tensile strength decreases significantly with increasing porosity at low porosity, which is explained in terms of the stress concentration induced in the vicinity of the pores during tensile deformation.

Merry-Go-Round Multiple Alkylation on Aromatic Rings via Rhodium Catalysis

MIURA Masahiro and NOMUKA Masakatsu
(Graduate School of Engineering)

Aromatic substitution is one of the most important and fundamental reactions in organic chemistry. We discovered a new, unusual catalytic multiple alkylation on aromatic rings. For example, phenylboronic acid selectively undergoes four times sequential substitution with 2-nitrobenzene in the presence of a rhodium catalyst. In this reaction, rhodium metal turns around the aromatic ring adding the alkyl groups, and thus, we call this "merry-go-round reaction." This sequential reaction provides a straightforward method for the synthesis of a unique class of sterically encumbered aromatic molecules.

\[
\text{B(OH)}_2 + \text{Ph} \rightarrow \text{PhB(OH)}_2 + \text{HX}
\]

Pathway / Transition
Tissue Classification Based on 3D Local Intensity Structures for Volume Rendering

SATO Yoshinobu
(Graduate School of Medicine)

A novel approach to tissue classification using three-dimensional (3D) derivatives features in the volume rendering pipeline has been developed. In conventional tissue classification for a certain volume, tissue of interest is characterized by an opacity transfer function defined as a one-dimensional (1D) function of the original volume intensity. To overcome the limitations inherent to conventional 1D opacity functions, we propose a tissue classification method that employs a multidimensional opacity function, which is a function of the 3D derivative features calculated from a scalar volume as well as the volume intensity. Tissues of interest are characterized by explicitly defined classification rules based on 3D filter responses highlighting local structures such as, edge, line, and blob, which typically correspond to tissue boundaries, cortices, vessels, and nodules respectively in medical volume data. The 3D local structure filters are formulated using the gradient vector and Hessian matrix of the volume intensity function combined with isotropic Gaussian blurring. These filter responses and the original intensity define a multidimensional feature space, in which multi-channel tissue classification strategies are designed. The figure shows the results of the classification of the lung from CT data aimed at computer-aided diagnosis of cancer detection.

Effects of Activating Flux on Arc Phenomena in Gas Tungsten Arc Welding

TANAKA Manabu
(Joining and Welding Research Institute)
Science and Technology of Welding and Joining, 5, 397-402 (2000)

Dramatic increases in the depth of weld bead penetration have been demonstrated by welding stainless steel using the gas tungsten arc (GTA) process with activating fluxes consisting of oxides and halides. We proposed a mechanism for the effect of flux on GTA welding on basis of experimental observations of interactive phenomena between the arc plasma which reached a high temperature exceeding 10000 K and the molten metal of which temperature was about 2000 K. Figure shows characteristic appearances of the arc in helium shielded GTA welding, with and without flux, for a 200 A welding current. The distributed region of blue luminous plasma is greatly enhanced by the flux. It was spectroscopically confirmed that this blue luminous plasma was mainly composed of metal vapor from the weld pool. The presence of the flux causes a change in the direction of the convection flow in the weld pool from being radially outward to the surface to being radially inward, and thus leads to different temperature distributions in the weld pool. Consequently, the different distributions of blue luminous plasma are formed, and the anode spot is changed on the weld pool surface. The combined effect of the change in the flow direction in the weld pool and the difference in the anode spot size changes the depth of weld penetration.
Maskless Fabrication of Field-Emitter Array by Focused Ion and Electron Beam
YAYAS Oguz, OCHIAI Chikako, TAKAI Mikio, HOSONO Akihiko and OKUDA Soichiro
(Research Center for Materials Science at Extreme Conditions and Graduate School of Engineering Science)

Field emitter fabricated by ion and electron beams

Pt pillars fabricated by electron beam

Ion Ga
Beam energy 30 keV
Beam current 1～11500 pA
Ion Column
Beam energy 1～30 keV
Beam current 200～400 pA
Beam spot size 3 nm
Gas Injection System
Stage
Sample
Beam scan raster scan

Nobelium-gated field-emitter arrays with Pt tips were fabricated using focused ion and electron beams. An approach, based on a two-step etch process in a Nb/SiO$_2$/Si structure, has been implemented for the regeneration of beam-induced damage and contamination in the processed area during the production of the gate openings. Only the top Nb layer was removed for gate openings by physical sputtering using the focused ion beam. The underlying SiO$_2$ was subsequently removed by wet etching. Deposition of Pt pillars into these gate openings using electron-beam-induced chemical reaction resulted in field emission at an applied gate bias of 60 V even without any thermal annealing process.

Atomic Resolution Imaging on Si(100)2×1 and Si(100)2×1:H Surfaces with Noncontact Atomic Force Microscopy
MORITA Seizo and SUGAWARA Yasuhiro
(Graduate School of Engineering)

Si(100)2×1

Si(100)2×1:H

Using the noncontact atomic force microscopy (NC-AFM), we succeeded clearly to discriminate dimer structures and precisely to determine the lengths 3.2±0.1Å and 3.5±0.1Å of imaged dimers on a clean active Si(100)2×1 surface with tilted dangling bonds and on even a hydrogen passivated inert Si(100)2×1:H monohydride surface without dangling bonds, respectively. By comparing each length of imaged dimer with the corresponding structure model of both Si(100)2×1 and Si(100)2×1:H surfaces, we confirmed that tilted dangling bond on Si(100)2×1 surface and individual hydrogen atom on Si(100)2×1:H surface were observed for the first time.

**A Truncated Isoform of the B56 Regulatory Subunit of Protein Phosphatase 2A Promotes Cell Motility Through Paxillin Phosphorylation**

ITO Akihiko and NOJIMA Hiroshi
(Graduate School of Medicine and Research Institute for Meningeal Diseases)
*The EMBO Journal, 19, 562-571 (2000)*

**Transcription Factor ERG Variants and Functional Diversification of Chondrocytes during Limb Long Bone Development**

IWAMOTO Masahiro
(Graduate School of Dentistry)

**B**

[Images of protein phosphorylation and cellular responses]

**B**

In human melanoma A431 and NIH-3T3 cells, metastatic behavior changes after intravenous injection, but only B6.5 cells are metastatic after subcutaneous injection. We found a retrotransposon insertion that produced an N-terminally truncated form of B56 isoform (2γ1) of the B56 regulatory subunit isoform of protein phosphatase (PP2A) in B6.5 cells, but not in NIH-3T3 cells. In addition, we found a biochemical interaction between PP2A and a cytoskeletal protein, paxillin (Fig. A) by immunoprecipitation, indicating direct dephosphorylation of paxillin by PP2A. We also showed their subcellular relocalizations, where γ1 behaved similarly to B56γ1 (Fig. B). The results indicate that B6.5 cells could contribute to a highly malignant phenotype in tumor cells.

**Transcription Factor ERG Variants and Functional Diversification of Chondrocytes during Limb Long Bone Development**

During limb development, chondrocytes located at the epiphysial layer of long bone models give rise to articular tissue, whereas the more numerous chondrocytes in the shaft undergo maturation, hypertrophy, and mineralization and are replaced by bone cells. It is not understood how chondrocytes follow these alternative pathways to distinguish and functions. In this study, we describe the cloning of C-1-1, a novel variant of the ets transcription factor c-ERG. C-1-1 expression characterizes developing articular chondrocytes, whereas c-ERG expression is particularly prominent in prehypertrophic chondrocytes in the growth plate. To analyze the function of C-1-1 and c-ERG, viral vectors were used to constitutively express each factor in developing chick leg buds and cultured chondrocytes. Overexpression of C-1-1 maintained chondrocytes in a stable and immature phenotype, blocked their maturation into hypertrophic cells, and prevented the replacement of cartilage with bone (see Figure). In contrast, viral driven expression of c-ERG significantly stimulated chondrocyte maturation in culture. The data suggest that C-1-1 and c-ERG have diverse biological properties and distinct expression patterns during skeletogenesis, and are part of molecular mechanisms by which limb chondrocytes follow alternative developmental pathways.

**Diaphyseal Portion of Tibiotarsus**

**Diaphyseal Portion of Tibiotarsus**

**Control**

**C-1-1 Virus-Infected**
Downregulation of an AIM-1 Kinases Couples with Megakaryocytic Polyploidyization of Human Hematopoietic Cells

KANAKURA Yutaka
(Graduate School of Medicine)
The Journal of Cell Biology, 152, 275-287 (2001)

During the late phase of megakaryopoiesis, megakaryocytes undergo polyploidyization, which is characterized by DNA duplication without concomitant cell division. AIM-1, that belongs to an Aurora/AK serum/threonine kinase family, was restrictedly observed at G2/M junction of cell cycle during proliferation, and was continuously repressed during megakaryocytic polyploidyization of human hematopoietic cell lines as well as normal hematopoietic progenitor cells. Supplementation of AIM-1 activity by the enforced expression of AIM-1 WT canceled TPA-induced polyploidyization in hematopoietic cell lines (panels A-D); the suppression of AIM-1 activities by dominant-negative AIM-1 (KRF) led to polyploidyization of the cells (panels E-H at 72h; I-L at 96h). These results suggested that downregulation of AIM-1 at G2/M phase may be involved in abortive mitotic and polyploid formation of megakaryocytes. Bar, 5µm.

Activity-Dependent Transfer of Brain-Derived Neurotrophic Factor to Postsynaptic Neurons

KOHARA Keigo and TSUMOTO Tadaharu
(Graduate School of Medicine)
Science, 291, 2419-2423 (2001)

As neurons grow, they extend nerve processes called axons towards other cells to which they will send neural signals. These postsynaptic neurons have been thought to release neurotrophic factors which promote extension of axons and differentiation or survival of presynaptic neurons. We found, however, that brain-derived neurotrophic factor (BDNF) transfers in the opposite direction, from pre- to postsynaptic neurons, in an activity-dependent manner. To see location and movement of BDNF in living cortical neurons, we simultaneously injected two different plasmids into the nucleus of a single neuron; one encoding BDNF tagged with green fluorescence protein (GFP) and the other encoding another fluorescence protein called DsRed (A). A horizontally running axon marked with DsRed (B) makes synaptic contacts with a postsynaptic neuron which is visualized with antibody against microtubule-associated protein 2 (MAP2) (C). BDNF-GFP signals are detected in the cell body of the postsynaptic neuron (D). This is confirmed in a superimposed figure (E). These results indicate that BDNF transfers from the presynaptic axon to the postsynaptic neuron because only the presynaptic neuron has received the injection of plasmid. Also we found that such a transneuronal transfer of BDNF is dependent on neuronal activity.
Structure of the Electron Transfer Complex Between Ferredoxin and Ferredoxin-NADP⁺ Reductase

KUSUNOKI Masami
(Institute for Protein Research)

Nature Structural Biology, 8, 117-121 (2001)

The reducing power of higher plants derived by oxygenic photosynthesis is utilized by combinations of a single multihistidine electron center protein, ferredoxin (Fd), and several Fd-dependent oxidoreductases. Here, we describe the first crystal structure of the complex between maize leaf Fd and Fd-NADP⁺ oxidoreductase (FNR). In the complex, the two redox centers—the FeS-85 cluster of Fd and flavin adenine dinucleotide (FAD) of FNR—are in close proximity; the shortest distance is 6.9 Å. Whereas the interactions between the two molecules are mainly electrostatic through salt bridges, the interface around the prosthetic groups is hydrophobic. The FNR recognition site on Fd identified in the crystal structure were confirmed by NMR experiments in solution. It is noted that the structures of Fd and FNR in the complex and in the free state are different in several respects. For example, the active site of FNR, complex formation involves the formation of a new hydrogen bond between side chains of Glu 312 and Ser 96 FNR. These results strongly indicate that this type of molecular communication not only determines the optimal orientations of the two proteins for electron transfer, but also contributes to the modulation of the enzymatic properties of FNR.

Structural and Kinetic Characterization of Early Folding Events in β-Lactoglobulin

GOTOYuji and HOSHIKOMasaru
(Institute for Protein Research)

Nature Structural Biology, 8, 151-155 (2001)

The folding of globular proteins is an intricate process that involves local and non-local interactions. The folding behavior of bovine β-lactoglobulin, a predominant protein in cow's milk, highlights the interplay between local and non-local interactions in protein folding. Within 2 ms of refolding, a stable core domain comprising β-strands F, G and H, and the main α-helix is formed. At the same time, local interactions stabilize marginally stable non-native α-helix near the N-terminus. The non-native α-helix is slowly transformed to the native β-strand, which is the structure including non-local interactions. The folding reaction of β-lactoglobulin is an important model to understand the α-helix → β-sheet transition as observed for various amyloidogenic proteins.
An Auxiliary Mode of Apoptotic DNA Fragmentation Provided by Phagocytes

NAGATA Shigekazu
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Apoptotic DNA fragmentation is mediated by CAD (caspase-activated DNAase). When the wild mice (left panel) were irradiated by x-ray, many thymocytes become TUNEL-positive (stained green) indicating that the thymocytes underwent DNA fragmentation. In addition to the cell-autonomous DNA fragmentation, many TUNEL-positive thymocytes could be found in macrophages stained with F4/80 antigen (red). In CAD-deficient mice (right panel), no cell-autonomous DNA fragmentation occurs, but DNA in apoptotic cells was degraded in macrophages.

Critical Roles of Glycosylphosphatidylinositol for Trypanosoma Brucel

NAGAMUNE Kishiburo and KINOSHITA Taroh
(Research Institute for Microbial Diseases)
Proceeding of the National Academy of Sciences of USA, 97, 10336-10341 (2000)

Trypanosoma brucei, is a protozoan parasite responsible for sleeping sickness in human and Nagana in domestic animals. Bloodstream form of the parasite evades the immune response of the mammalian host by means of the coat protein, VSG. Procyclic form, an insect stage parasite, escapes from the digestion in the gut of tsetse fly, by the coat protein procyclins. These coat proteins are tethered to the cell surface via glycosylphosphatidylinositol (GPI) anchors (upper left). To evaluate the importance of GPI for parasite survival, we cloned and disrupted a trypanosomal gene, TbGPI10, involved in biosynthesis of GPI (upper right). Bloodstream form of T. brucei could not lose TbGPI10 (lower left), therefore, GPI synthesis is essential for growth of the pathogenic stage parasites. Procyclic form parasites lacking the surface coat proteins were viable, but infectivity to tsetse flies was impaired (lower right). Therefore, parasite-specific inhibition of GPI biosynthesis should be an effective chemotherapy target against African trypanosomiasis.
Essential Role of Voltage-dependent Anion Channel in various forms of Apoptosis in Mammalian Cells
SHIMIZU Shigemori and TSUJIMOTO Yoshihide
(Graduate School of Medicine)

Apoptosis is a gene-regulated mechanism of cell death that is essential for elimination of unwanted cells in various biological systems in most metazoans. Mitochondria play a crucial role in apoptosis by releasing apoptogenic factors into the cytoplasm, and Bcl-2 family proteins, well-characterized regulators of apoptosis, directly control mitochondrial membrane permeability through interacting with the mitochondrial channel VDAC.

Microinjection of the specific anti-VDAC antibodies, that inhibit VDAC activity, inhibits apoptosis induced by a variety of stimuli, indicating that VDAC plays an essential role in apoptosis in mammalian cells.

Crystal Structure of a Repair Enzyme of an Oxidatively Damaged DNA, MutM (Fpg), from an Extreme Thermophile, Thermus thermophilus HB8
FUKUYAMA Keiichi and KURAMITSU Seiki
(Graduate School of Science)
The EMBO Journal, 19, 3857-3869 (2000)

The MutM (Fpg: formamidopyrimidine DNA glycosylase) protein is a bifunctional DNA base-excision-repair enzyme, which removes a wide range of oxidatively damaged bases (6-4 photoproducts) and cleaves both the S- and O- glycosylated bonds of the resulting apurinic/apyrimidinic site (AP-lyase activity). The crystal structure of MutM from an extreme thermophile, Thermus thermophilus HB8, was determined at 1.9A resolution with MAD phasing using the intrinsic Zn^{2+} ion of the zinc finger. The complex between MutM in the absence from and the flipped-out linked DNA is drawn as a CPK model with backbones colored in steel blue and with bases in steel gray. C and GO bases and their sugar residues before and after flipping out are shown by ball-and-stick models. All the four conserved regions (1) turn β5-β6 and (2) β8-β9 in the sky-blue circle, (2) the catalytic site in the pink circle, (3) the H2TH motif in the yellow circle, and (4) the zinc finger motif in the light-green circle are in the large cleft of the MutM molecule. The N-terminal domain can access the major groove of DNA and the zinc finger motif of the C-terminal domain can access the minor groove. The H2TH motif of the C-terminal domain is situated near the active site, and interacts with the DNA backbone with the damaged base.
Two Cell Adhesion Molecules, Nectin and Cadherin, Interact through Their Cytoplasmic Domain-associated Proteins

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The Journal of Cell Biology, 150, 1161-1175 (2000)

**E-Cadherin**

Nectin is an immunoglobulin-like cell-cell adhesion molecule localized at cadherin-based cell-cell adhesion junctions (A26). We examined whether nectin recruits cadherin to nectin-based cell-cell adhesion sites, using two cell lines from cadherin-deficient L cells: one was an L cell line stably expressing nectin-1α and the other was an L cell line stably expressing both nectin-1α and E-cadherin (nectin-1α-EL cells). These two cell lines were co-cultured, followed by immunostaining for nectin-1α and E-cadherin. Nectin-1α was concentrated at adhesion sites between the same type of cells and between the two different types of cells. E-cadherin was concentrated at adhesion sites between nectin-1α-EL cells. In addition, E-cadherin was concentrated at nectin-1α-EL and -1α-EL cells. These results indicate that nectin recruits E-cadherin to nectin-based cell-cell adhesion sites without the trans-interaction of E-cadherin, and suggest that nectin plays a key role in formation of cadherin-based A26. L, nectin-1α-EL cells. Arrows, cell-cell adhesion sites between two nectin-1α-EL cells. Double arrowheads, cell-cell adhesion sites between nectin-1α-EL and -1α-EL cells. Scale Bar, 10 μm.

Complete Cysteine-Scanning Mutagenesis and Site-Directed Chemical Modification of The Tn10-Encoded Metal-Tetracycline/H⁺ Antipporter

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(Institute of Scientific and Industrial Research)

The Journal of Biological Chemistry, 276, 27101-107133 (2001)

**Bacterial metal-tetracycline/H⁺ antipporter (TetAAB) was the first found drug exporter and has been studied as a paradigm of antipporter-type major facilitator superfamily transporters. Here the 400 amino acid residues of this protein were individually replaced by cysteine except for the initial methionine. As a result, we could obtain a complete map of the functionally or structurally important residues. In addition, we could determine the precise topology on the basis of the reactivity with N-ethylmaleimide (NEM). The NEM-binding results indicated the presence of a transmembrane water-filled channel in the transporter.**

**Model of the transmembrane helix arrangement of metal-tetracycline/H⁺ transporter TetA/AB**

**A** Cytosolic side

**B** Periplasmic side

Principle of topology determination by competitive site-directed chemical modification

**Membrane-impermeable SH reagent**

**Membrane-permeable SH reagent**
Ectodomain Shedding of Epidermal Growth Factor Receptor Ligands is Required for Keratinocyte Migration in Cutaneous Wound Healing

HIGASHIYAMA Shigeki
(School of Allied Health Sciences)

Wound stimuli induce keratinocyte shedding of EGFR ligands in vitro, particularly the ligand hepatocyte growth factor (HB-EGF). The resulting soluble ligands stimulated transient activation of EGFR and the signal transduction molecule Stat3 (a vs b, Green color: Stat3, Orange color: Nucleus). OSU8-1, a newly developed inhibitor of HB-EGF shedding, abrogated the wound-induced activation of EGFR and Stat3 (a vs c), and increased expression of keratinocyte migration in vitro (a vs f). The application of OSU8-1 to wound sites in vivo greatly retarded neophagocytotic migration as the result of a failure in keratinocyte migration, but this effect could be overcome by recombinant soluble HB-EGF. EGF was added along with OSU8-1 (f vs g). These findings indicate that the shedding of EGFR ligands represents a critical event in keratinocyte migration, and suggest their possible use as an effective clinical treatment in the early phases of wound healing.

Three Subunit α Isoforms of Mouse Vacuolar H⁺-ATPase: Preferential Expression of the α3 Isoform During Osteoclast Differentiation

FUTAI Masamitsu
(Institute of Scientific and Industrial Research)

V-ATPase is a highly-conserved multimeric enzyme that catalyzes the translocation of protons across the membranes of eukaryotic cells. Its α subunit of transmembraneous Vc sector occurs in tissue and organelle-specific isoforms and thus may be involved in targeting the enzyme or modulating its function. We have identified three isoforms of V-ATPase α subunit in mouse, which were named α1, α2 and α3.

The V-ATPase in osteoclast-mediated bone resorption is observed on the ruffled bone membrane and intracellular components. Immunohistochemical analysis is efficient means to detect the subcellular localization in vivo, and reveals that the α3 is required for V-ATPase in the plasma membrane of osteoclasts. As a result, we displayed that the α3 subunit is related to the localization of V-ATPase.
List of 100 Papers Selections

* Researchers in bold italic letters are faculty members of Osaka University, and their institutions are indicated in parentheses.
* Shaded papers are included in the “10 Papers Selections”.

- Humanities & Social Sciences .......................... 5
- Science .................................................. 23
- Engineering ........................................... 35
- Biology .................................................. 37
  Total 100

Humanities & Social Sciences

1. Fujita, H. (Graduate School of Letters)
   Notomi Kajiro: An Industrial Art Pioneer and the First Design Educator of Modern Japan
   Design Issues, 17, 17-31 (2001)

2. Ikeda, S. (Institute of Social and Economic Research)
   Weakly Non-Separable Preferences and the Herberger-Lauesen-Meitzer Effect

3. Kano, Y.; Harada, A. (Graduate School of Human Sciences)
   Stepwise Variable Selection in Factor Analysis
   Psychometrika, 65, 7-22 (2000)

4. Moriyasu, T. (Graduate School of Letters)
   The West Uighur Kingdom and Tun-huang around the 10th-11th Centuries

5. Ono, Y. (Institute of Social and Economic Research)
   A Reinterpretation of Chapter 17 of Keynes’s General Theory: Effective Demand Shortage under Dynamic Optimization

Science

   Observation of Spin-Orbit Splitting in A Single-Particle States

2. Ashino, R.; Heil, C.; Nagase, M.; Vaillancourt, R. (Graduate School of Science)
   Microlocal Filtering with Multwavelets
   Computers and Mathematics with Applications, 41, 111-133 (2001)

   Formation of Initial Perturbation of Rayleigh-Taylor Instability in Supernovae and Laser-Irradiated Targets: Is There Any Similarity?

4. Chen, B.; Itaya, H.; Matsuo, T.; Murakami, K. (Graduate School of Science)
   p-p System with B Field, Branes at Angles and Noncommutative Geometry

   Morphology and Photonic Band Structure Modification of Polystyrene Particle Layers by Reactive Ion Etching
6. Hashizume, K.; Chauvédon, M.; Martz, B.; Robert, F. (Graduate School of Science) 
Solar Wind Record on the Moon: Deciphering Presolar from Planetary Nitrogen

7. Hayashi, N.; Numakura, P. I. (Graduate School of Science) 
On the Quadratic Nonlinear Schrödinger Equation in Three Space Dimensions

Daisy Chain Necklaces: Tris(2,2’-bicoloxane) Containing Cyclodextrins 
Journal of the American Chemical Society, 122, 9876-9877 (2000)

9. Ishihara, H.; Amakata, T.; Cho, K. (Graduate School of Engineering Science) 
Size Dependence of Degenerate Four-wave Mixing Signal due to Enhancement of Internal Field in Mesostructured Systems

10. Kato, K.; Usui, S. (Graduate School of Science) 
Logarithmic Hodge Structures and Classifying Spaces 
Centre de Recherches Mathématiques CRM Proceedings and Lecture Notes, 24, 115-130 (2000)

11. Kohno, H.; Iwasaki, T.; Takeda, S. (Graduate School of Science) 
Metal-mediated Growth of Alternate Semiconductor-insulator Nanostructures

Anisotropic Coulomb Explosion of Cu-Irradiated with a High-intensity Femtosecond Laser Pulse

Electromagnetic Moments of β-Emitting Nucleus 16N
Physical Review Letters, 80, 3733-3735 (2000)

New Persistent Radicals: Synthesis and Electronic Spin Structure of 2,5-Di-tert-Butyl-6-Oxophenaloxonol Derivatives
Journal of the American Chemical Society, 122, 4825-4826 (2000)

15. Obika, S.; Hari, Y.; Sekiguchi, M.; Imamichi, T. (Graduate School of Pharmaceutical Sciences) 
A2, 4’-Bridged Nucleic Acid Containing 2-Pyridone as a Nucleobase: Efficient Recognition of a C·G Interruption by Triplex Formation with a Pyrimidine Motif

Itinerant f-4-4 electron System of Cerium and Uranium Compounds

Strong coupling between local moments and superconducting ‘heavy’ electrons in UPdAl

A Novel Class of Emitting Amorphous Molecular Materials as Bipolar Radical Formans:2-[4-(Bis[4-methylphenyl)aminophenyl]-5-(dimethylamino)boryl)] 2-thiophene and 2-[4-(Bis[9,9-dimethylfluorenyl)aminophenyl]-5-(dimethylamino)boryl] 2-thiophene 

Chiral Discrimination of Fructose-oligosaccharides toward Amino Acid Derivatives by Induced-Filling Chiral Resonating Junction of the Chemical Society, Perkin Transactions 2, 592-601 (2001)

20. Toki, H.; Suganuma, H. (Research Center for Nuclear Physics) 
Dual Ginzburg-Landau Theory for Confinement and Chiral Symmetry Breaking 
Progress in Particle and Nuclear Physics, 45, S397-S482 (2000)

Construction of a New Multi-turn Time-of-flight Mass Spectrometer 

First Total Synthesis of the Re-Type Lipopolyascharide 

Superconducting Fluctuations and the Pseudogap in the Slightly Overdoped High-Tc Superconductor TlSr2CaCu2O8+x High Magnetic Field NMR Studies 
Engineering

1. Abe, M.; Adan, W.; Ino, Y.; Nishina, M. (Graduate School of Engineering) Stereocchemical Deuteration Labeling as Mechanistic Probe for Differentiating the Singlet- and Triplet-Excited States in the Rearrangement of the 2-Spiropoxy-1,3-cycloptantanediyl to Oxetanes Journal of the American Chemical Society, 122, 6508-6509 (2000)


12. Kawakami, T.; *1; Ohtake, H.; *2; Arakawa, H.; *2; Okachi, T.; *2; Imada, Y.; *2; Murakami, S.; *2 (*1 Institute for Protein Research, and *2 Graduate School of Engineering) Asymmetric Synthesis of β-Amino Acids by Addition of Chiral Enolates to Nitrones via N-Acylhydrazinyl Ions Bulletin of the Chemical Society of Japan, 73, 2423-2444 (2000)


15. Kobayashi, H.; *1; Sakurai, T.; *1; Nishimura, M.; *2 (*1 Institute of Scientific and Industrial Research, and *2 Central Research Laboratory) Formation of SiOx/SiC Structure at 203 °C by use of Perchloric Acid Applied Physics Letters, 78, 2356-2358 (2000)


23. Shihata, I., Suma, T., Ryu, K., Baha, A. (Graduate School of Engineering) Selective α-Stannylated Addition of Di-n-butyloliodin Hydride At Complex to Simple Aliphatic Alkynes Journal of the American Chemical Society, 123, 4101-4102 (2001)


34. Yokozuma, K.; Ochi, T.; Yoshimoto, A.; Sugawara, Y.; Morita, S. (Graduate School of Engineering) Atom Probe Toward Imaging on Si(100)×1 and Si(100)×1-H Surfaces with Noncontact Atomic Force Microscopy Japanese Journal of Applied Physics, 39, L113-L115 (2000)

Biology


2. Emura, R.*1; Ashida, N.*1; Higashii, T.*1; Takach, T.*2. (1* Graduate School of Medicine, and 2* Low Temperature Center) Orientation of Bif Sperms in Static Magnetic Fields Bioelectromagnetics, 22, 60-65 (2001)


7. Ito, A.*1; Kataoka, T.*1; Watanabe, M.; Nishiyama, K.*2; Mazaki, Y.; Sabe, H.; Kitauma, Y.*1; Noimura, H.*1 (Graduate School of Medicine, and 2* Research Institute for Microbial Diseases) A Transected Isoform of the PP2A B56 Subunit Promotes Cell Motility Through Pixinin Phosphorylation The EMBO Journal, 19, 562-570 (2001)


34. Waga, S.; Masuda, T.; Take, T.; Sugino, A. *; (1 Research Institute for Microbial Diseases, and *2 Graduate School of Science) DNA polymerase ε is Required for Coordinated and Efficient Chromosomal DNA Replication in Xeropus Egg Extracts Proceedings of the National Academy of Sciences of the United States, 98, 4973-4983 (2001)

35. Yamamoto, M.; Yoshida, K.; Kishimoto, T.; Inoue, H. *; (1 Graduate School of Medicine, *2 School of Health and Sport Sciences, and *3 President) IL-6 Is Required for the Development of Th1 Cell-mediated Murine Collitis The Journal of Immunology, 164, 4876-4882 (2000)
