sequence leading to the enantioselective formation of the four-membered ring under the influence of the chiral ligand. Optimizing all the parameters of this photoredox-catalyzed reaction allowed products to be obtained typically in 50 to 80% synthetic yields and with consistently high enantiomeric excess of 85 to >95%. The utility of photoredox-catalyzed reactions has been demonstrated already for C–C bond–forming processes by MacMillan and co-worker (*14*). The attractiveness of the Du *et al*. procedure is that the process achieved via a redox sequence is a genuinely photochemical transformation, the [2 + 2] photocycloaddition.

The process reported mimics in its strategy the process of photosynthesis, which decouples the primary photochemical event from the utilization of the harnessed energy for synthetic transformations. The initial photochemical event creates a redox potential. The synthetic part harnesses the photochemical energy in creating energy-rich chemical structures. The results reported are notable because of the synthetic importance of the synthesized structures, but also because they allow studying the coupling of the energy collected from photons to the energy stored in interesting chemical structures.

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BIOPHYSICS

Silencing Neurons with Light

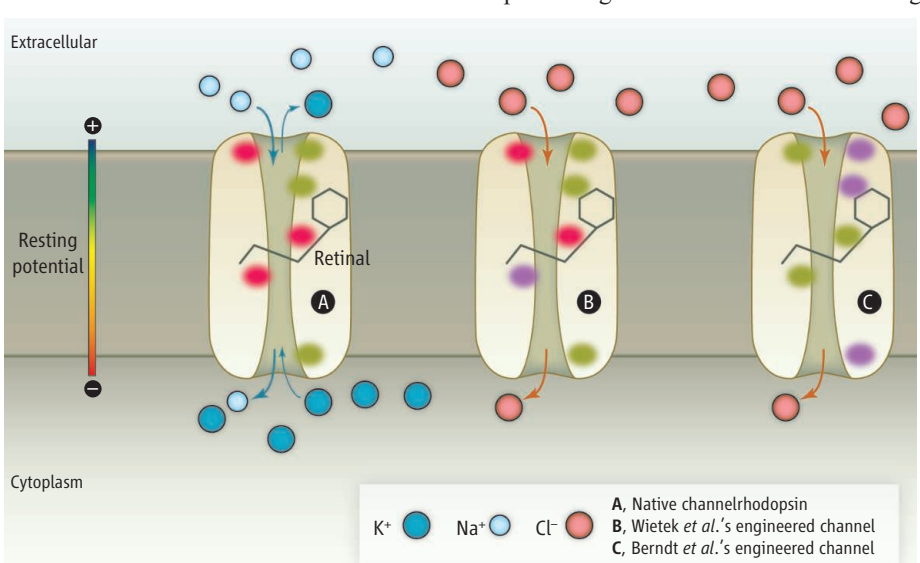
Engineered channelrhodopsins conduct anions rather than cations, changing the action potential of neurons.

Shigehiko Hayashi

Neural networks control the activity of living individuals as central processing units control the functions of modern computers. In a neuronal circuit, information is transmitted through neurons in the form of an action potential, which is the electric potential difference between the inside and the outside of a neuron. Ion channel proteins in the neuronal membrane act as molecular devices that create and regulate action potentials. A technology called optogenetics (*1*) allows neuronal circuits to be manipulated by a combination of optics and genetically targeted incorporation of microbial retinal binding proteins, called opsins (*2*), into neurons. On pages 409 and 420 of this issue, Wietek *et al*. and Berndt *et al*. (*3*, *4*) use structure-based molecular engineering to invert the charge selectivity of different opsins, channelrhodopsins from algae, resulting in much improved neuron silencers for use in optogenetics.

Through heterologous expression of light-sensitive opsins, researchers can control the electric signals of neurons underlying the activities of living animals with light. Optogenetics thus provides precise neuronal control that can resolve highly complex neuronal activity in the brain, contributing, for example, to understanding of psychiatric disease states. However, microbial opsins have limited functionality for optogenetics; for example, their ion conductance and ion selectivity are sufficient for microbial activity but are too low for the efficient control of animal neuronal activity. The recent determination of the three-dimensional atomic structure of a chimeric protein formed from parts of two different channelrhodopsins (*5*) from algae (*6*, *7*) has opened the way to rational molecular engineering of opsins with novel functionalities.

Ion selectivity, a key functional component of ion channels, enables the regulation of action potentials (see the figure). In the resting state of a neuron, the membrane is polarized, with a membrane potential of about –70 mV, through differences in ion concentrations maintained by energy-driven ion transporters. Upon light illumination, channelrhodopsins conduct cations but not anions (see the figure, panel A), thereby depolarizing the membrane and inducing



Better neuron silencers for optogenetics. Native channelrhodopsins conduct cations (**A**). In contrast, the channelrhodopsins engineered by Wietek *et al*. (**B**) and by Berndt *et al*. (**C**) conduct chlorine anions. Different residues were mutated to create the two Cl^- channels, with mutated residues shown in red (acidic), blue (basic), and green (neutral). Mutated residues that do not change their polarity are omitted.

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an action potential. However, light-sensitive ion channels for silencing neurons must be selective for chloride (Cl^-) or potassium (K^+) to further decrease the membrane potential (hyperpolarization) and thereby turn off the action potential. Such ion channels have not yet been found in natural species. In optogenetics, light-driven chloride and proton pumps from archaeal halobacteria have been used instead; however, pumps conduct only a single ion per photon and are therefore less efficient than channels.

Wietek *et al*. and Berndt *et al*. now provide drastic solutions to this problem. They use molecular engineering to convert cation-conducting channelrhodopsins into light-gated ion channels that selectively conduct Cl^-. Further, they show that these engineered opsins can be used to efficiently hyperpolarize the membrane.

The conversions of ion selectivity obtained by the two groups occur in completely different ways (see the figure). Wietek *et al*. convert the ion selectivity of their channel through a single mutation, replacing an acidic residue with a positively charged arginine at a gating site located in the middle of the putative pore (see the figure, panel B). This observation suggests that the gating site may act as a selectivity filter. In contrast, Berndt *et al*. achieve Cl^- selectivity through multiple mutations that alter the electrostatic environment along an extended region of the pore, while replacing the acidic residue at the gating site by a neutral one (see the figure, panel C). The mechanisms underlying the Cl^- selectivity of the two engineered channels are thus remarkably different.

Ion channels are thought to achieve high ion selectivity through precisely defined pore architectures that efficiently conduct particular ions, as seen in tetrameric K^+ selective channels (*8*). However, the different ion selectivity mechanisms of the two engineered channelrhodopsins show that charge selectivity of ion channels can be controlled in more variable ways. A clue to the permissive feature of channelrhodopsins may come from experimental evidence that channelrhodopsin is a light-driven proton pump as well as an ion channel (*9*) and that a single mutation in the middle of the proton channel can convert another light-driven opsin proton pump into a Cl^- pump (*10*). In both systems, the proton pump function is fulfilled by concerted motion of protons between acidic residues, positively charged basic side chains, and water molecules in the pore (*11*); the flexibility of these residues may contribute to making channelrhodopsins amenable to molecular engineering.

The highly controllable charge selectivity of engineered channelrhodopsins demonstrated by Wietek *et al*. and Berndt *et al*. should encourage researchers to further augment and improve functionalities of channelrhodopsins for optogenetics applications. Understanding of the physicochemical mechanism by which channelrhodopsins operate will also help to guide the design of novel ion channels with controllable selectivity through de novo protein design (*12*), synthetic engineering (*13*), and biomimetic synthesis of artificial macromolecules.

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STRUCTURAL BIOLOGY

The 30-nm Fiber Redux

Do alternating stacking modes of nucleosomes underlie the compaction of chromatin?

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The DNA of eukaryotic cells is packaged onto nucleosomes—complexes composed of histone proteins—that together form chromatin, enabling tight packing of the genome within the cell nucleus. In the first folded structure of chromatin to be characterized, nucleosomes were coiled into a ~30-nm-diameter helix, with the "linker" histone located in the interior of the fiber (*1*). The precise molecular organization of this 30-nm fiber has long been extensively debated. Initially, structural studies on fibers assembled on natural DNA sequences were hampered by variation in the length of the linker DNA between nucleosomes. However, more recently, the construction of regularly spaced tandem DNA repeats for precise nucleosome positioning (*2*) has revolutionized analysis. On page 376 of this issue, Song *et al*. (*3*) determine by cryo–electron microscopy the 11 Å–resolution structure of 30-nm fibers assembled from arrays of 12 nucleosomes.

Song *et al*. unequivocally identify the path of linker DNA. They show that in the fiber, a linear array of nucleosomes is packed in two interwound left-handed helical stacks with a straight linker DNA between successive nucleosomes in the array crossing the interior of the fiber. In agreement with an emerging consensus, this finding resolves the fundamental issue as to whether the fiber is built from one nucleosome stack—a solenoid—or from two. However, importantly, the new structure differs in one largely unanticipated aspect from most previous models. Instead of the monotonous helix previously imposed by the limitations of the available information, the 30-nm fiber is formed by the tight helical packing of a tetranucleosome unit. Within this unit, first observed by the crystallization of a tetranucleosome lacking linker histone (*4*), the two opposing nucleosome dimers are fully stacked on each other with only a small angular separation. Between the units, the angular separation is larger and each unit is staggered relative to its neighbor. The structure agrees well with all other direct measurements but does not exclude the possibility that a nucleosome array may still have the potential to fold into other helical forms.

The first hint of a lack of structural uniformity of nucleosomes in the folded fiber came from the observation of alternating pattern of deoxyribonuclease I (DNase I) digestion in successive nucleosomes (*5*). Subsequent modeling of the fiber showed that retention of the tetranucleosome unit necessitated the

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