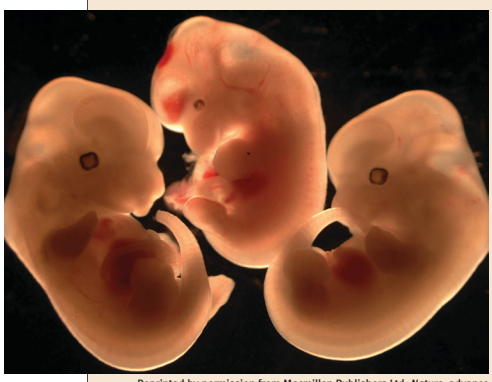
Spotlight

Cellular Reprogramming

Stem cell research is an area of tremendous promise in medicine, yet it engenders enormous controversy in society. Embryonic stem (ES) cells are pluripotent; that is, they are capable of differentiating into virtually every type of fetal or adult cell. It is this talent that has propelled them to celebrity status in the field of regenerative medicine, where they could help cure many devastating human conditions and diseases, such as paralysis, neurodegenerative disorders, and cancer. However, this tantalizing field is also engulfed in ethical debate, because the use of ES cells requires the destruction of human embryos. This has stimulated the search for alternative methods to create pluripotent cells. Three related papers by Wernig *et al.* (*Nature*, published online June 6, 2007; DOI: 10:1038/nature05944), Okita *et al.* (*Nature*, published online June 6, 2007; DOI: 10:1038/nature05934), and Maherali *et al.* (*Cell Stem Cell*, published online June 6 2007; DOI: 10:1016/j.stem.2007.05.014) now demonstrate that somatic cells, such as fibroblasts, can be reprogrammed into pluripotent cells that possess remarkable similarities to ES cells.



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The work in all three papers is based on the recent groundbreaking finding that mouse fibroblasts can be reprogrammed into pluripotent stem cells by retrovirus-mediated expression of four transcription factors, Oct4, Sox2, c-Myc, and Klf4. Subsequent selection of cells expressing the gene *Fbx15*, which is one of the targets of Oct4 and Sox2, resulted in the generation of cells capable of differentiating into diverse cell types. However, these cells exhibited significant differences from ES cells in their gene expression and epigenetic profiles. By selecting instead for cells expressing the transcription factor *Nanog*, which is also a target of Oct4 and Sox2 but is more tightly associated with pluripotency, all three groups demonstrated that the resulting cells, termed induced pluripotent stem (iPS) cells, were similar to ES cells in a number of important ways.

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Hydroxy Boron

Ribosomes, the molecular machines dedicated to protein synthesis, are often the target for antimicrobial agents. Some antibiotics bind where the transfer RNA (tRNA) substrates cycle through the ribosome, whereas others bind in the peptide exit tunnel to effectively clog up the machine's assembly line. Now, a new study by Rock *et al.* (*Science* 2007, *22*, 1759–1761) on a compound with antifungal properties demonstrates that yet another critical step in this pathway can be inhibited by a small-molecule drug. The authors began by mutating yeast cultures and screening for strains resistant to AN2690, a member of a broad-spectrum antifungal family known as benzoxaboroles. Mapping the isolated mutants to their corresponding gene loci yielded a striking commonality among the strains. All of the mutants were amino acid changes in the editing site of a leucine aminoacyl-tRNA synthetase. The editing site is essential for translational fidelity. It acts as the proofreader to detect and correct any mischarging events that occur on the tRNA. To test whether AN2690 did inhibit the editing activity, the authors mischarged leucine tRNA with isoleucine and then exposed it to the leucine synthetase. With AN2690 present, the rate of isoleucine hydrolysis was significantly lower, and the kinetics indicated that the drug binds slowly but tightly to a site other than the ATP or amino acid binding clefts. The drug also inhibited catalytic charging of the tRNA with leucine. To hone in on the molecular mechanism of AN2690's action on the editing site, the authors determined the X-ray crystal structure of a leucine aminoacyl-tRNA synthetase complexed with the antifungal molecule and a cognate tRNA. As implied by their name, the benzoxaborole drugs all contain a boron atom, and the structure of the bound form explained why this atom is essential for the drug's activity. The 3′ terminal adenosine of a tRNA, which accepts the amino acid during charging, was bound to the drug in a telling fashion. The boron atom coordinated both the 2′ and 3′ positions of the terminal ribose. This mode of action is consistent with AN2690's ability to inhibit both charging and editing. This study elegantly demonstrates how working back from a drug to its target can be a fruitful effort. Just as in bacteria, the translation machinery appears to be a good drug target in fungi. Jason G. Underwood, Ph.D.

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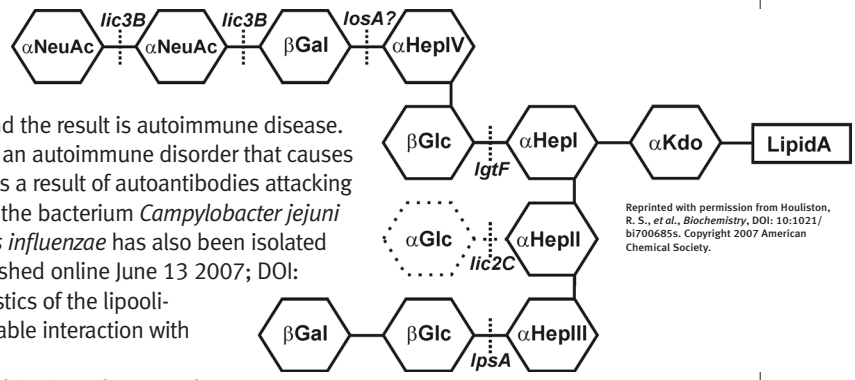
Ganging Up on Gangliosides



The immune system is incredible in its ability to attack and eliminate foreign substances. Unfortunately, sometimes the system goes awry and starts to gang up on its host's cells, and the result is autoimmune disease. Often triggered by bacterial infection, Fisher syndrome (FS) is an autoimmune disorder that causes paralysis of the eyes, among other muscular abnormalities, as a result of autoantibodies attacking cells presenting the ganglioside GQ1b. Although infection by the bacterium *Campylobacter jejuni* is most often associated with FS, the bacterium *Haemophilus influenzae* has also been isolated from individuals with FS. Houliston *et al.* (*Biochemistry*, published online June 13 2007; DOI: 10:1021/bi700685s) now delineate the structural characteristics of the lipooligosaccharide (LOS) from the *H. influenzae* strain DH1 that enable interaction with antibodies against GQ1b.

Purified core oligosaccharide from cultured DH1 and a combination of NMR and mass spectrometry analysis were used to determine the structure of the LOS from DH1. It is important to note that when DH1 cells were grown in the presence of sialic acid, the LOS contained both mono- and disialylated species. Inactivation of genes encoding key enzymes helped establish the specific sialylation pattern that was present and the glycosyltransferase responsible for it. The bifunctional sialyltransferase Lic3B was determined to furnish DH1 with a novel sialylation pattern, wherein two sialic acid residues are linked to galactose bound to the tetraheptosyl backbone of the LOS. It is notable that this sialylation pattern mimics the terminal trisaccharide unit of GQ1b. Serum antibodies from FS patients reacted to LOS extracts from DH1 cells, and the reactivity was dependent on the presence of sialylated species. This work provides critical insight into why infection by DH1 leads to production of autoantibodies against GQ1b. **Eva J. Gordon, Ph.D.**

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Several criteria were used to assess similarity to ES cells. Maherali and coworkers established that, like ES cells, iPS cells are capable of imposing an ES-like phenotype on somatic cells after cell fusion. Specific gene expression experiments and global DNA microarray analysis by all three groups revealed that iPS cells expressed ES cell marker genes and that global gene expression patterns of iPS cells clustered with ES cells. Another important indicator of cellular reprogramming is the epigenetic state of the cell, in which methylation of DNA and histones impact gene transcription. Examination of the histone methylation patterns in iPS cells revealed that the DNA methylation state of the promoter regions of both the *Oct4* and *Nanog* genes, as well as that of the entire genome, was reprogrammed to a state similar to that of ES cells. Okita *et al.* and Maherali *et al.* also evaluated the response of iPS cells to leukemia inhibitory factor (LIF) and retinoic acid. Like ES cells, iPS cells remained in an undifferentiated state in the presence of LIF when feeder cells were not present, while retinoic acid induced iPS cell differentiation. In addition, Maherali and coworkers investigated another characteristic of ES cells: the state of activation of the X chromosome. Analysis of specific X-linked gene expression, chromatin modifications, and the ability of the cells to undergo X inactivation upon differentiation enabled the researchers to conclude that female iPS cells display X chromosome inactivation dynamics similar to that of female ES cells. Finally, perhaps the most compelling evidence for the similarity of iPS cells to ES cells was established upon demonstrating their developmental potential. All three groups showed that iPS cells could differentiate into cell types representing all three embryonic germ layers. They all also showed that injection of iPS cells into blastocysts resulted in the generation of viable adult mice with contribution to diverse tissues, including oocytes and sperm.

These remarkable studies demonstrate the potential of obtaining pluripotent cells without destroying embryos. However, the retrovirus-mediated system used to accomplish this transformation is not without some inherent risks, most notably the potential for tumor development upon reactivation of the *c-myc* retrovirus established by Okita *et al.* Of significance is that the Wernig and Okita groups both suggest that small molecules may be able to replace retrovirus-mediated cellular reprogramming, underscoring the potential contributions that chemical biologists can make to the field. **Eva J. Gordon, Ph.D.**

Spotlight

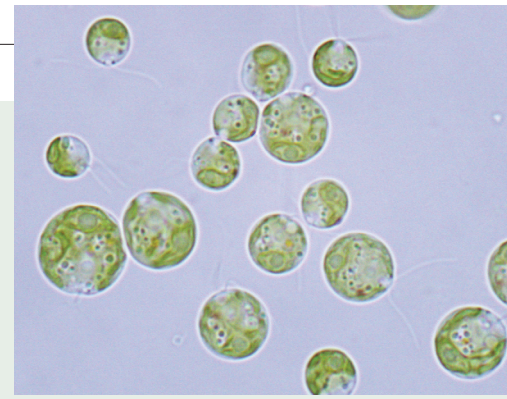

Image courtesy of Yijun Qi.

MicroRNAs Chlam Up

The tiny unicellular green alga *Chlamydomonas reinhardtii* has long been a workhorse organism for cell and molecular biologists interested in flagellar motors or the energy-producing organelle, the chloroplast. As an additional perk to scientists, these cells display a natural ability to synchronize their cell cycle in accordance with light and dark cycles. With a recent draft of the *C. reinhardtii* genome near completion, the study of this organism is poised to enter a new phase. The genome enables investigators to ask a new set of questions, and among the first queries were two studies aimed at the hot, emergent field of small RNAs.

Previous scans of other genomes indicated that hallmark RNA interference enzymes are present in some single-cell eukaryotes. The evidence was bolstered when an RNA cloning project in the protist *Tetrahymena* uncovered stable small RNAs. In *Tetrahymena*, the RNAs appeared to arise from bidirectional transcription of DNA to yield double-stranded RNAs that were cleaved into small products. No evidence was found for the single-strand transcription and fold-back RNA structures displayed by plant and animal microRNAs (miRNAs). Some scientists postulated that perhaps the fold-back miRNA trick was an innovation of multicellular organisms. Surprisingly, the new sequencing and genome alignments in *Chlamydomonas* indicated that some single-cell critters learned the miRNA gene regulation trick as well.

A study by Zhao *et al.* (*Genes Dev.* 2007, *15*, 1190–1203) began by purifying the 18–28 nucleotide small RNAs from *Chlamydomonas* and subjecting the pool to high-throughput sequencing. Most of the unique RNAs were 20–22 nucleotides in size, a characteristic of miRNA and small-interfering RNA (siRNA) products. Of the thousands of unique genome hits, 200 of the small RNAs seemed to be derived from fold-back miRNA-like structures that appear much like those found in multicellular eukaryotes. One unique finding was a longer stem structure that is cleaved to generate several miRNAs. The authors went on to investigate the activity of the miRNA products in *Chlamydomonas* by fractionating a cell extract by size exclusion chromatography. The 21 nucleotide RNAs were found in a peak at ~150 kDa, indicative of a large RNA–protein complex. Because miRNAs have been implicated in both cleavage of messenger RNAs (mRNAs) and translational repression, the partially purified fractions were tested for activity. Incubation of the fraction with a putative mRNA target displaying complementarity with one candidate miRNA resulted in cleavage of the mRNA. This indicates that the single-cell organism possesses the cutting machinery similar to that used by the siRNA pathway found in multicellular organisms.

In another recent study, Molnar *et al.* (*Nature* 2007, *28*, 1126–1129) start with a similar sequencing effort and uncover even more unique small RNAs. In this study, most of the predicted miRNAs were derived from longer stem loops that were cleaved at multiple positions to yield several small RNAs, so this appears to be the norm rather than the exception for these cells. This study went on to compare the *Chlamydomonas* results with those found in the well-characterized plant system

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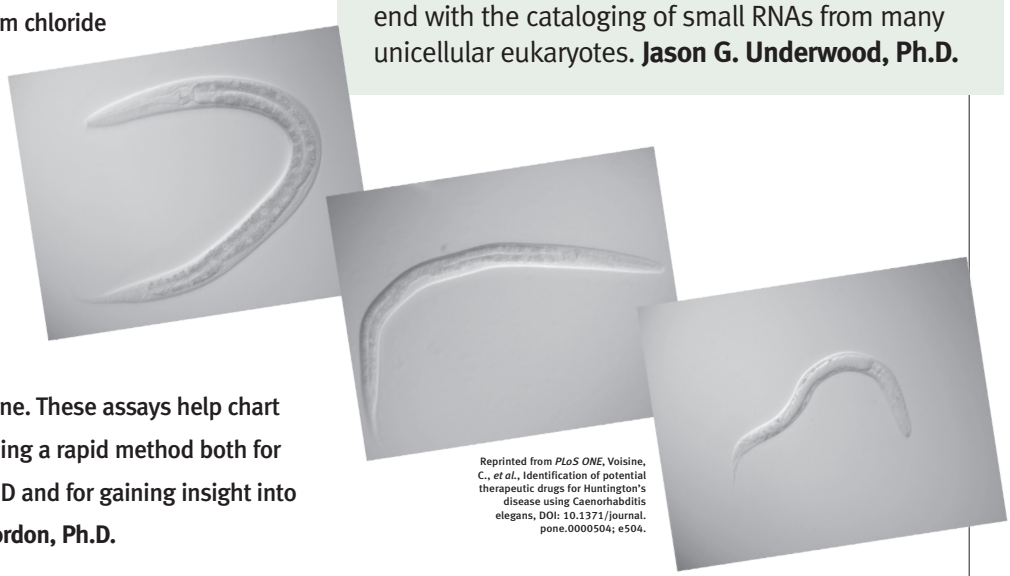
Hunting for Huntington's Drugs

The hunt for drugs to treat Huntington's disease (HD) is especially cryptic because of the multiple pathways, including aging and neurodegeneration, that are involved in the pathogenesis of the disease. HD progression depends on the length of a polyglutamine (polyQ) tract in the huntingtin protein and on age. Thus, HD model systems with short lifecycles, such as in the worm *Caenorhabditis elegans*, are attractive for drug screening because the effects of aging can be evaluated much more rapidly. Voisine *et al.* (*PloS ONE* 2007, *2*; e504) describe several new assays developed in *C. elegans* to aid in the pursuit of new drugs for HD.

The authors used a collection of compounds with demonstrated activity in established assays targeted at HD to help develop additional screens for compounds effective against HD. One assay, the food clearance assay, enables rapid assessment of the optimal compound concentration for screening. A second assay employs a genetic mutant background that accelerates polyQ-mediated neurodegeneration and cell death, cutting the time required to test drug efficacy from 1 week to 2–3 d. In three additional assays, mutant strains were also utilized, and assay conditions were developed to confirm the efficacy of the hits and decouple the effects of growth, development, and aging from neurodegeneration. With these assays, lithium chloride and mithramycin emerged as two compounds that exert a protective effect on neurons in a manner that is independent of growth, development, and aging. It is notable that when lithium chloride and mithramycin were tested in combination, neuronal survival was increased more than with either compound alone. These assays help chart the hunt for HD treatments by providing a rapid method both for identifying potential new drugs for HD and for gaining insight into their mechanisms of action. **Eva J. Gordon, Ph.D.**



Reprinted from *PLoS ONE*, Voisine, C., *et al.*, Identification of potential therapeutic drugs for Huntington's disease using Caenorhabditis elegans, DOI: 10.1371/journal.pone.0000504; e504.

MicroRNAs Chlam Up,

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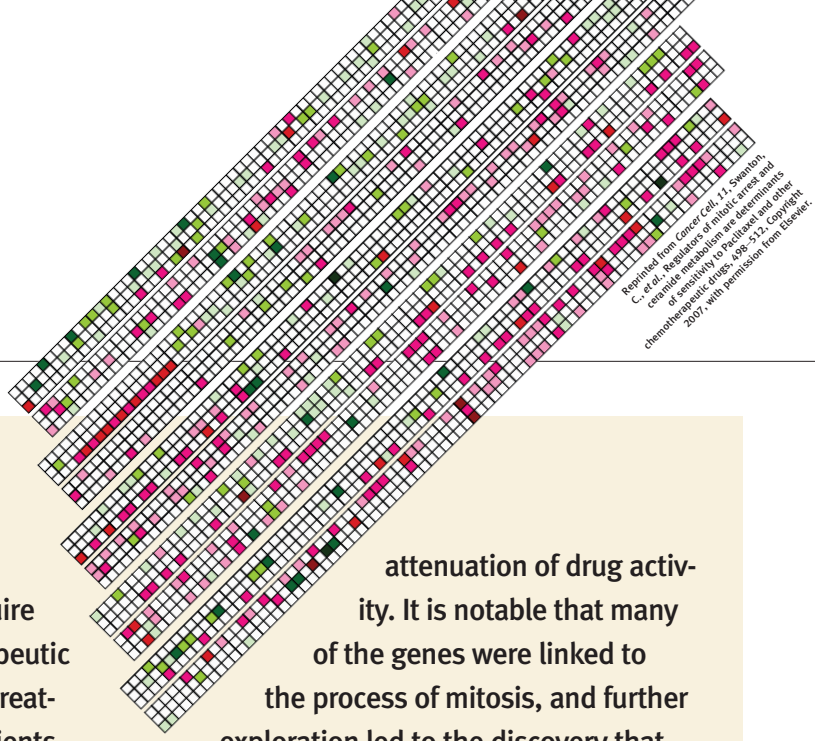
Arabidopsis. No miRNAs were found to be shared between the species, but an interesting modification was conserved between the two. Both display a unique 2′ methyl group on the 3′ end of the final small-RNA product. In plants, this protects the RNA from degradation, so a similar regulation mechanism is probably at work in the little light-harvester, *Chlamydomonas*, as well.

This pair of studies opens up a new field of inquiry into the evolutionary history of small-RNA pathways. Since the algae appear to have components of both siRNA and miRNA pathways, these innovations were not coincident with multicellularity. Though the Zhao *et al.* study displays cleavage of an mRNA, this does not exclude the possibility that translational regulation may play into small-RNA-mediated regulation in this unicellular alga as well. At some point in history, there was probably a transition from small-RNA pathways serving as a simple immune system to a mechanism for endogenous mRNA regulation. This will be a central question as more unicellular genome sequences are deposited into databases. The next step may start with a pond-water fishing expedition and end with the cataloging of small RNAs from many unicellular eukaryotes. **Jason G. Underwood, Ph.D.**



siRNAs . . . Systematically

Cancer cells have the maddening ability to acquire resistance to chemotherapeutic agents, a major cause of treatment failure in cancer patients. Although this problem is being attacked from many angles, the variety of mechanisms used by cancer cells to escape death and the difficulties in examining sufficient numbers of relevant tumor samples make it a significant scientific challenge. Use of short interfering RNAs (siRNAs) provides an extraordinarily powerful method for silencing mammalian genes and, when combined with high-throughput screening technology, enables implementation of systematic loss-of-function screens. Using a collection of siRNAs that target all human kinases, kinase-associated proteins, and proteins involved in ceramide lipid metabolism, Swanton *et al.* (*Cancer Cell* 2007, *11*, 498–512) apply this technology to methodically search for genes in cancer cells that contribute to acquired resistance to chemotherapy drugs.

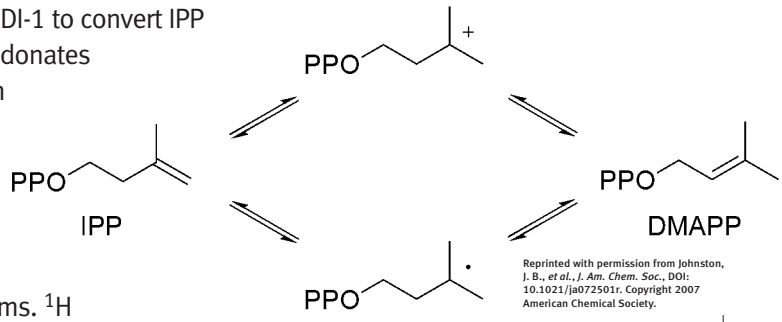
Treatment of three cancer cell lines with the siRNAs in the presence of the chemotherapeutic agent paclitaxel led to the identification of several genes whose knockdown was associated with attenuation of drug activity. It is notable that many of the genes were linked to the process of mitosis, and further exploration led to the discovery that, in the absence of drug, the siRNAs induced polyploidy (the presence of more than two sets of chromosomes) and other nuclear abnormalities. Taken together, the data suggest a correlation between chromosomal instability and resistance to paclitaxel, pointing to a situation that cancer cells may exploit to circumvent drug activity. In addition, when the screen was carried out with four different chemotherapeutics agents, knockdown of *COL4A3BP*, a gene involved in ceramide transport from the endoplasmic reticulum to the Golgi apparatus, resulted in sensitization of the cells to drugs. Consistent with these data, COL4A3BP protein expression is increased in drug-resistant cell lines, an indication that it could be a target for chemotherapy-resistant cancers. This systematic approach to deciphering the complex code of acquired resistance provides both mechanistic insight into drug resistance and potential new therapeutic strategies with which to attack it.

Eva J. Gordon, Ph.D.

Isolating the Activity of an Isomerase

Isoprenoids, small organic compounds found in all living things, exhibit considerable diversity in both structure and function. These assorted molecules have demonstrated medicinal potential in addition to their aromatic, flavorful, and colorful properties. Conversion of isopentenyl diphosphate (IPP) to dimethylallyl diphosphate (DMAPP) is an important step in isoprenoid biosynthesis, and two different enzymes, types 1 and 2 IPP isomerase (IDI-1 and IDI-2), are known to perform this transformation. IDI-1 isomerizes IPP to DMAPP through a proton addition–elimination mechanism, but two potential mechanisms have been proposed for IDI-2. Johnston *et al.* (*J. Am. Chem. Soc.*, published online June 5, 2007, DOI: 10.1021/ja072501r) create two mechanism-based inhibitors to help elucidate the mode of action of IDI-2.

It is interesting that IDI-1 and IDI-2 have no sequence or structural similarities, and unlike IDI-1, IDI-2 is a flavoprotein that requires NADPH (the reduced form of NADP) to reduce the flavin mononucleotide cofactor to the dihydro form needed by the enzyme for catalysis. Nevertheless, IDI-2 could use a mechanism similar to that of IDI-1 to convert IPP to DMAPP. However, it is also possible that the cofactor donates a hydrogen atom to the substrate, followed by hydrogen atom abstraction to generate the final product. The authors synthesized cyclopropyl and epoxy analogues of IPP, and the differential reactivities of these compounds were exploited to help distinguish between the proton addition–elimination and the hydrogen atom addition–abstraction mechanisms. ^{1}H NMR spectroscopy, reversed-phase HPLC, HPLC/MS, and deuterium-exchange experiments provided compelling evidence that IDI-2 uses a proton addition–elimination mechanism similar to that of IDI-1 in the conversion of IPP to DMAPP. The mechanistic detail gained through these experiments demonstrates the power of using small-molecule irreversible inhibitors for delineating enzyme mechanisms. **Eva J. Gordon, Ph.D.**



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Biochemical Society

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