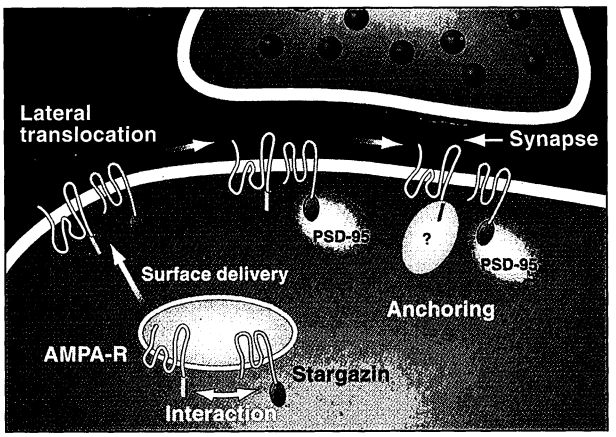
(which binds to the PDZ domains of PSD-95, a postsynaptic scaffold protein) still allowed rescue of the surface expression, but not the synaptic localization, of AMPA receptors.

The dissociation of synaptic and nonsynaptic delivery of AMPA receptors is an important conclusion of the Chen *et al.* work. It suggests a two-step model in which stargazin first conveys AMPA receptors to the neuronal surface and then sweeps them laterally into postsynaptic sites, the second step requiring an interaction of the carboxyl-terminus of stargazin with PSD-95 (or a similar synaptic anchoring protein). However, it is also possible that stargazin delivers AMPA receptors directly into the synapse, and that they disperse extrasynaptically if stargazin is unable to dock with PSD-95. Either way, stargazin presumably must bind to AMPA receptors to perform its task. Indeed, stargazin can interact with AMPA receptors when it is coexpressed in non-neuronal cultured cells (*1*). But, Chen *et al.* were unable to demonstrate a direct association in vivo, perhaps because the interaction between stargazin and AMPA receptors is weak or transient.



The benefits of stargazin. Synaptic targeting of AMPA receptors. The transmembrane protein stargazin interacts with AMPA receptors in an intracellular compartment of the neuron and promotes their delivery to the neuronal surface. The carboxyl terminus of stargazin binds specifically to an anchor protein called PSD-95 and mediates recruitment of the stargazin–AMPA receptor complex to postsynaptic sites. Additional interactions between the carboxyl terminus of AMPA receptor subunits and other anchoring proteins stabilize AMPA receptors in the postsynaptic membrane.

Why is the AMPA receptor defect observed in cerebellar granule cells but not in other regions of the *stargazer* mouse brain? The authors propose that members of the stargazin/γ-subunit family expressed in other types of neurons compensate for the loss of stargazin in these cells. Consistent with this notion, overexpression of a stargazin protein lacking a carboxyl terminus inhibited synaptic targeting of AMPA receptors in hippocampal neurons (*1*); presumably mutant stargazin had a "dominant interfering" effect on other stargazin-related proteins in the cell. This result suggests that proteins of the stargazin family are universally important for AMPA receptor trafficking.

The stargazin study raises several questions. How does stargazin bind to the AMPA receptor? Where does this interaction take place in the neuron, and how is it regulated? Synaptic accumulation of AMPA receptors is already known to depend on interactions of the cytoplasmic tails of their GluR1 and GluR2 subunits with the PDZ domains of other proteins (*6–9*). How do the direct interactions of AMPA receptor subunits fit into the *stargazer* model? Perhaps stargazin merely ushers AMPA receptors to the synapse. Once there, AMPA receptors might be released from stargazin to bind to a different set of PDZ-domain proteins that then anchor them in the postsynaptic membrane (see the figure).

Although it appears unimportant for synaptic delivery of another class of glutamate receptor (the NMDA receptor), stargazin is unlikely to be involved solely in the trafficking of AMPA receptors. Which other membrane proteins does stargazin convey to the cell surface and to synapses? And what is the connection between stargazin and neuronal calcium channels? Identifying other components of the AMPA receptor–stargazin protein complex should help to answer these questions and should shed further light on how membrane trafficking contributes to the formation and plasticity of synapses.

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PERSPECTIVES: CELL CYCLE

License Withheld—Geminin Blocks DNA Replication

Zoi Lygerou and Paul Nurse

For cells to survive they must receive a complete copy of their genome every time they divide. Two events enable dividing cells to achieve this goal—S phase (during which the DNA of the chromosomes is replicated) and M phase or mitosis (during which the replicated chromosomes segregate into the two newly divided cells). To ensure genomic stability, S phase is tightly regulated so that replication of the chromosomes is initiated only once in each cell cycle. A process called licensing ensures that chromatin becomes competent for a further round of DNA replication only after passage through mitosis (*1*). Building on the secure foundations provided by studies of prokaryotes and viruses, work in budding and fission yeasts and with frog egg extracts has identified and characterized many of the components that tightly regulate S-phase onset. Reports by Wohlschlegel *et al.* on page 2309 of this issue (*2*) and Tada *et al.* (*3*) in *Nature Cell Biology* now connect the activities of two of these components—the positive regulator Cdt1 and the negative regulator Geminin—and provide further insight into the licensing of DNA replication in human and frog cells.

The mechanisms leading to the initiation of DNA replication depend on the sequential association of proteins with chromatin. A collection of proteins called the origin recognition complex (ORC) (*4*), which is thought to bind to origins of replication in the chromatin, is associated with chromatin throughout the cell cycle (see the figure). This association is necessary for the binding of other replication proteins but does not appear to regulate the timing of S-phase onset. The onset of S phase appears to be controlled by six proteins that form the MCM (minichromosome maintenance) complex. As the cells exit from mitosis, the MCM interacts with chromatin and licenses the DNA for replication. Although the molecular basis

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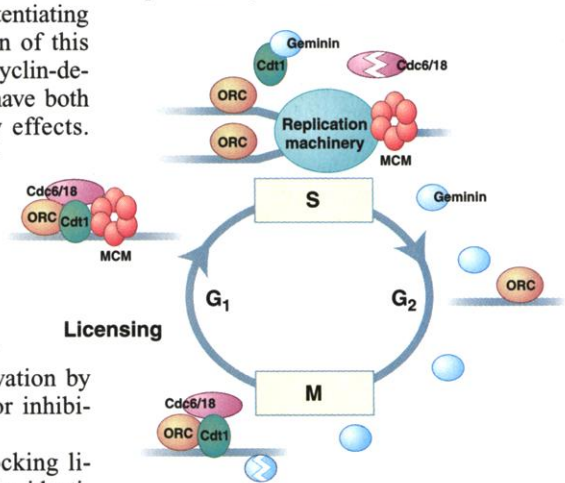
for MCM action is still not clear, the complex is likely to open up the chromatin, providing access for enzymes that replicate DNA. This activity is consistent with the finding that MCM family members are helicase enzymes that unwind DNA.

The loading of MCM proteins onto chromatin is, therefore, a key step in controlling the initiation of DNA replication. Loading requires the initiating factor Cdc6/18 (*5*, *6*), which accumulates in the nucleus as cells exit mitosis and enter G_1; an increase in the amount of Cdc6/18 prompts inappropriate entry into S phase (*7*). A second MCM loading factor, Cdt1, has been identified in fission yeast (*8*), the fruit fly *Drosophila* (where it is called DUP) (*9*), and the frog *Xenopus* (*10*). Cdt1 is expressed when cells exit mitosis, becomes associated with chromatin, and can form a complex with Cdc6/18, potentiating its activity. The overall regulation of this sequence of events involves the cyclin-dependent kinases (CDKs), which have both positive and negative regulatory effects. The G_1/S CDK promotes progression through G_1 into S phase, whereas the G_2/M CDK inhibits the licensing of DNA for replication during G_2. The G_2/M CDK does this by phosphorylating, among other proteins, Cdc6/18 and MCM family members, leading to their inactivation by degradation, nuclear exclusion, or inhibition of chromatin binding.

A candidate molecule for blocking licensing is a protein called Geminin, identified in *Xenopus* and human cells (*11*). Geminin is present in the cell nucleus from S phase until mitosis and is degraded as cells complete mitosis (see the figure). Addition of Geminin to an in vitro replication assay containing *Xenopus* egg extracts blocks the association of MCM proteins with G_1 chromatin, thereby inhibiting DNA replication. The addition of Geminin blocks initiation of DNA replication at the same stage as does depletion of Cdt1 from *Xenopus* extracts. Wohlschlegel *et al.* (*2*) and Tada *et al.* (*3*) now extend these findings by showing that Geminin stably interacts with Cdt1 in *Xenopus* and human cells.

Wohlschlegel *et al.* looked for proteins that would form a complex with Geminin. They found two proteins (with molecular sizes of 130 kD and 65 kD) that could be coprecipitated with Geminin from extracts of cultured human HeLa cells. The 65-kD protein was identified as the human homolog of Cdt1, but the identity of the 130-kD protein remains unknown. Human Cdt1 coprecipitated with a single major protein of 35 kD from human cell extracts, which was found to be Geminin. The authors cloned a full-length Cdt1 cDNA and showed that the protein it encodes interacts with Geminin in vitro. The amino-terminal 101 amino acids of human Cdt1 are sufficient to ensure binding to Geminin; this region is the least conserved among Cdt1 homologs from different species and was not found in a previously identified partial human cDNA.

In a parallel set of experiments, Tada and co-workers set out to identify the targets of Geminin using a *Xenopus* in vitro DNA replication assay. The investigators showed that Geminin binds to RLF-B, a licensing activity present in *Xenopus* egg extracts, the components of which have not yet been identified. They then demonstrated that RLF-B is, in fact, Cdt1. Both groups used the *Xenopus* in vitro DNA replication system to show that addition of excess Cdt1 reverses the block on licensing imposed by Geminin, and restores DNA replication. Thus, Cdt1 can bind to and block Geminin activity, suggesting that Geminin inhibits the initiation of DNA replication by binding to and inactivating Cdt1. It is rather more difficult to establish unambiguously that Geminin acts through, and only through, Cdt1. The Geminin interaction domain is at the end of Cdt1's amino terminus. To establish whether Geminin acts only through Cdt1, one could engineer a truncated Cdt1 protein missing its amino-terminal domain and then see whether this protein is able to support DNA replication in vitro, even in the presence of excess Geminin.



Geminin regulates S-phase onset. Before a cell can duplicate its genome during S phase, DNA must be licensed for replication. This requires the assembly of protein complexes on chromatin: the origin recognition complex (ORC), the loading factors Cdc6/18 and Cdt1, and the six MCM proteins. ORC is present throughout the cell cycle, whereas the other proteins are loaded stepwise as cells exit mitosis. Activation of proteins at the G_1/S transition results in engagement of the replication machinery and the initiation of DNA replication. In metazoan cells, Geminin is expressed in S phase, and its association with Cdt1 may prevent replication origins from refiring. Origin refiring is also prevented by Cdk-dependent phosphorylation, nuclear exclusion, and degradation of Cdc6/18. In G_2 phase, only ORC is bound to chromatin, and the presence of Geminin may ensure that inappropriate expression of Cdc6/18 and Cdt1 does not lead to aberrant licensing. Geminin accumulates in mitosis (M) where it blocks premature licensing. It is degraded at the metaphase-to-anaphase transition of mitosis, and at this time the Cdc6/18 and Cdt1 proteins accumulate in the nucleus and associate with chromatin. This permits licensing and results in the initiation of a new round of DNA replication.

What can we say about Geminin's job in vivo? Geminin accumulates when *Xenopus* eggs are arrested in metaphase, just before the separation of chromosomes to the two daughter cells, and is degraded when cell division proceeds (*11*). Tada *et al.* show that depletion of Geminin from metaphase extracts prompted licensing of chromatin for DNA replication. Geminin could therefore be important for repressing DNA licensing until cell division is complete. Geminin also accumulates in the nucleus of G_2 cells, and could act redundantly to ensure inhibition of S phase if licensing factors were to become inappropriately expressed in G_2 cells. Geminin is also present in S-phase cells—here, it may bind to and inactivate Cdt1 to ensure that DNA replication is not reinitiated at origins that have just replicated (a process called origin refiring). Geminin may also be important after DNA damage, halting S phase to give cells time to repair their DNA. It will be interesting to see whether Geminin expression is induced in cells arrested in G_1, due to either DNA damage or withdrawal of growth factors. Genetic approaches will help to clarify the various tasks of Geminin in vivo.

Is Geminin's regulatory role conserved in evolution? Geminin homologs are present in mammals and *Xenopus*. We have identified a putative *Drosophila* Geminin homolog in DNA databases (unpublished observations), and *Drosophila* Cdt1 has an amino-terminal domain that could potentially interact with Geminin. This suggests that Geminin's job may be conserved among all metazoans (multicelled organisms). If a *Drosophila* homolog of Geminin exists, then flies carrying mutant forms of this protein could be engineered enabling the different functions of Geminin to be elucidated.

Cdt1 homologs from fission yeast and the plant *Arabidopsis* are truncated at their amino termini, in contrast to their metazoan orthologs, and no proteins with significant similarity to Geminin have

been identified in these organisms. They may yet turn out to have divergent Geminin homologs that operate through a different Cdt1 domain or a different protein, but it is possible that Geminin exists only in Metazoa. Geminin may have evolved to couple S-phase regulation to developmental and growth signals found only in metazoans. Given the fact that Geminin is a crucial negative regulator of the cell cycle, it will be important to establish whether it operates as a tumor-suppressor protein and whether it is mutated in cancer cells.

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PERSPECTIVES: QUANTUM CRYPTOGRAPHY

Single Photons "on Demand"

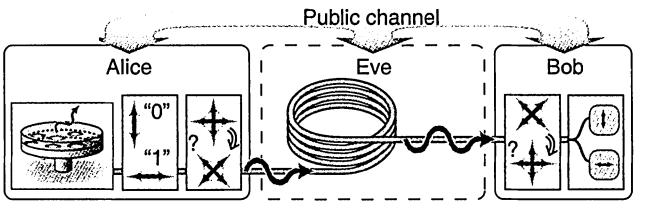
Simon Benjamin

No information without representation! This is the fundamental principle behind quantum information (*1*), a new, rapidly evolving field of physics (*2*). Information cannot exist without a physical system to represent it, be it chalk marks on a stone tablet or aligned spins in atomic nuclei. And because the laws of physics govern any such system, physics ultimately determines both the nature of information and how it can be manipulated. Quantum physics enables fundamentally new ways of information processing, such as procedures for "teleporting" states between remote locations, highly efficient algorithms for seeking solutions to equations and for factorization, and protocols for perfectly secure data transmission.

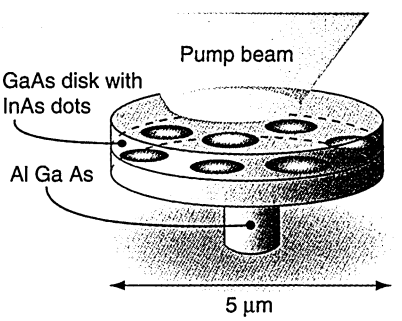
The last of these, quantum cryptography, has been fully demonstrated experimentally (*2–4*), but several obstacles have prevented its practical implementation. One of these has now been surmounted, as reported by Michler *et al.* on page 2282 of this issue (*5*) and by Lounis and Moerner in a recent issue of *Nature* (*6*). These authors have achieved, in two very different experimental setups, the generation of individual photons "on demand," thus making it essentially impossible to eavesdrop on quantum cryptographic information transfer without being noticed.

To understand why reliable generation of single photons increases the security of quantum cryptography, consider the following scenario, which is based on the protocol for quantum cryptography by Bennett *et al.* (*3*).

Alice wants to share a secret "key" with Bob. This key is simply a random sequence of bits; Bob will use it to encode a message, making it incomprehensible to anyone except Alice. But what if Eve tries to monitor the key's sequence without Alice and Bob's knowledge? If they exploit the physics of quantum information, Eve's eavesdropping will leave a mark. Alice distributes key bits by setting the polarization of photons horizontal or vertical (see the figure, above). She also applies a 45° "twist" to the polarization of half of the photons, chosen at random. At the receiving end, Bob also twists 50% of the photons before measuring their polarization. Then Alice and Bob openly tell each other which photons they twisted. They discard those bits that Alice has twisted and Bob has not, or vice versa. Their remaining keys will agree exactly—unless Eve has been snooping. For example, if she measured each photon and created a new one to match the result, then inevitably, 25% of Bob's final key bits will differ from Alice's. This is because Eve does not know which photons have been twisted, and so cannot untwist the right photons prior to measuring their polarization. No amount of additional cleverness or resources can avoid this effect (*7*). By publicly comparing (and then discarding) a subset of bits from their keys, Alice and Bob can thus be certain that Eve wasn't snooping, because any significant tampering will cause substantial discrepancies between those bits. Alice must not, however, send two (or more) photons at a time, because Eve could then use a simple "beam splitter attack" to measure one photon while leaving the other undisturbed (*8*).



Eavesdroppers beware! Alice, Eve, and Bob are the usual participants in quantum cryptography experiments. Eve tries to spy on the information transmitted between Alice and Bob. Controlled single-photon generation will increase the security of quantum cryptography substantially, thus moving the approach one step closer to practical implementation.



Controlled photon release from quantum dots (QDs). A QD is a region where charge carriers become so strictly trapped that their energy levels are fully quantized, much as they are in atoms. In Michler *et al.*'s system, QDs form naturally during the growth of the layered material in which they are embedded. The material is then etched to produce a 5-μm disk containing several QDs, connected by a 0.5-μm post to the bulk sample below.

Until very recently, single photons could not be produced with very high probability. One could adjust the average number of photons in a light pulse, but a good probability of producing one photon meant that there was a similar chance of producing zero or two photons (*2*). Experimental demonstrations of quantum cryptography schemes have used pulses with an average number of photons as low as 0.1, thus minimizing the chances of multiphotons at the cost that 9 in 10 pulses contain no photons at all. Even then, however, 5% of the populated pulses will contain more than one photon. Because the pulses containing multiple photons could potentially be read undetected and Alice and Bob do not know which pulses have multiple photons, they must correspondingly shorten their key to reduce the security risk (*2*).

Michler *et al.* obtain their single photons from a quantum dot (QD) embedded in a microdisk (see the figure, left) (*5*). A related structure has been proposed by one of the authors as a possible candidate for a fully fledged quantum computer (*9*). The disk is illuminated by a laser pulse, which excites electrons in the GaAs matrix surrounding the QDs. The electrons become trapped in the QDs, together

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