PERSPECTIVES: BIOMEDICINE

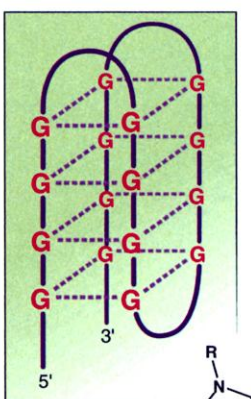
Do G Quartets Orchestrate Fragile X Pathology?

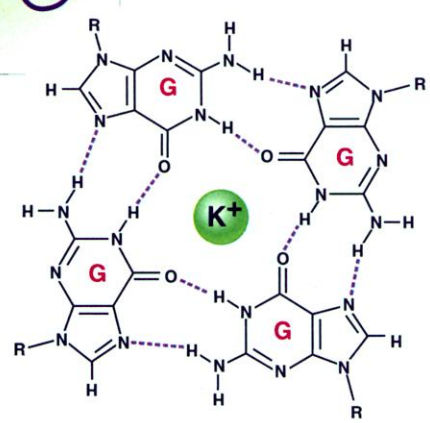
Hervé Moine and Jean-Louis Mandel

It is exactly 10 years since a series of *Science* and *Cell* papers showed that the fragile X syndrome is caused by expansion of a CGG repeat in the 5′-untranslated region of the *FMR1* gene. This triplet repeat expansion leads to abnormal local DNA methylation and to shutdown of *FMR1* gene transcription (*1, 2*). Absence of the FMRP protein encoded by this gene is responsible for the disabling cognitive deficits that characterize this disease, considered to be the most common cause of inherited mental retardation (*3*).

Fast forward a decade, and now we know numerous facts about FMRP, a protein produced by many cell types that is particularly abundant in neurons. FMRP contains domains—two KH domains and an RGG box—characteristic of an RNA binding protein, and shuttles between the nucleus and the cytoplasm (*1, 2*). When bound to mRNAs within ribonucleoprotein particles, FMRP associates with polysomes, the strings of ribosomes that translate mRNAs into proteins. FMRP can pair with itself to form a homodimer, or with the related proteins FXR1P and FXR2P (fragile X related proteins 1 and 2) to form heterodimers. Together these findings suggest that FMRP may be involved in the transport of mRNAs or in the regulation of their translation into proteins. Three recent papers in *Cell* (*4–6*) report encouraging progress in determining the specific mRNAs targeted by FMRP.

There is strong evidence to support the notion that FMRP is important in regulating mRNA translation. Preincubation of mRNAs with purified FMRP inhibits their translation in cell-free systems and in frog oocytes. This in vitro inhibitory effect has little (*7*) or no (*8*) specificity regarding the nature of the mRNAs. However, this inhibition is specific for FMRP, because FXR1P and FXR2P do not block mRNA translation, nor does FMRP that carries a missense mutation preventing this protein from pairing with itself (*8*). In neurons, FMRP is found at postsynaptic sites enriched in ribosomes where some mRNAs (including *FMR1* mRNA) are translated in response to synaptic activation. This response appears impaired in mice that lack FMRP (*9*). In the brains of FMRP-deficient mice and of the small number of fragile X patients examined, there are subtle anomalies in the number and morphology of dendritic spines, the small protuberances on neuronal cell body processes (dendrites) that form synapses with other neurons (*9, 10*). These observations suggest that by modulating mRNA translation and consequently protein synthesis, FMRP is important for the formation and pruning of synapses during development and for synaptic activity in the adult. These processes provide the plasticity necessary for learning and long-term memory.





Structure of a G quartet. In the G quartet, four guanines form hydrogen bonds with each other in a symmetrical square planar arrangement. The overall structure of a G quartet is stabilized by coordination of cations, such as K^+, within the central cavity of the quadruplex, and by stacking several G quartet layers on top of each other.

The key issues are to determine whether FMRP binds to specific sets of mRNAs, and whether its absence affects the distribution of proteins encoded by these mRNAs, in cells from fragile X patients and FMRP-deficient mice. Originally, FMRP was estimated to bind to only about 4% of brain mRNAs (*11*), but the identity of these mRNAs was not known. Enter Darnell *et al.* (*4*) and Schaeffer *et al.* (*12*), Brown and colleagues (*5*), and Zhang and co-workers (*6*) with their identification of mRNAs that are preferentially bound by FMRP. Using different approaches, the Darnell laboratory (*4*) and our group (*12*) showed that purified FMRP binds with high affinity to mRNAs through a very peculiar secondary structure called a G quartet. The structure of an RNA G quartet was recently solved by x-ray crystallography at the remarkable resolution of 0.61 Å (*13*) (see the figure). Darnell *et al.* used an iterative in vitro selection strategy (SELEX) to identify FMRP-bound RNA sequences, starting from a randomly synthesized RNA pool. In contrast, we detected and analyzed a high-affinity binding site for FMRP within its own mRNA. In both cases, the high G-base content of the RNAs bound to FMRP and the cationic specificity of binding implied that FMRP bound to RNA G quartets. Unexpectedly, Darnell *et al.* discovered that specific RNA binding required the RGG domain of FMRP, but not the KH domain.

Although direct proof of their existence in living cells is lacking, G quartets are thought to be of physiological importance. In DNA, G quartets may form at the ends of chromosomes to stabilize them. They may also participate in immunoglobulin gene recombination at switch regions. The G quartets in RNA have been implicated in the repression of mRNA translation and in mRNA turnover. The structures of RNA and DNA G quartets differ sufficiently (particularly regarding sugar conformation) such that a protein binding to the former would not necessarily bind to the latter.

Brown *et al.*'s systematic microarray-based search for mRNAs associated with FMRP complements the work on FMRP binding to G quartets (*5*). These investigators looked for mRNAs in the mouse brain that were selectively immunoprecipitated in FMRP ribonucleoprotein particles. They also sought mRNAs that appeared more or less frequently in the polysomes of lymphoblast cells from fragile X patients. With these two approaches, they identified 432 mouse mRNAs and 251 human mRNAs, respectively, as potential targets of FMRP. Of the 12 mRNAs identified in both data sets, 8 showed a potential G quartet according to computer predictions (whereas only 4% of random mRNAs would be predicted to contain such a structure), and sev-

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eral of them bound FMRP with high affinity in vitro. The microarray data and computer predictions garnered a list of mRNAs that are potential targets of FMRP. Many of these mRNAs encode important neuronal proteins such as semaphorin and the microtubule-associated protein MAP1B. Remarkably, Zhang *et al.* (*6*), working in a fly model of fragile X syndrome, show that the fly homolog of FMRP, dFXR, binds to and represses the translation of an mRNA encoding the fly homolog of MAP1B. They suggest that the regulation of MAP1B by dFXR is necessary for normal synaptic activity, because a MAP1B mutant is able to correct the synaptic anomalies elicited by dFXR deficiency.

The next step will be to analyze how the proposed mRNA targets are affected by FMRP deficiency or overexpression. The effects of binding to FMRP may vary for different mRNAs, because the G quartets can be found either in their coding sequences or in their 5′- or 3′-untranslated regions. Furthermore, of the 251 mRNAs whose distribution on polysomes was significantly altered in fragile X cells, half were found at an increased frequency and half at a decreased frequency, arguing against the possibility that FMRP is always a translational repressor. Indeed, the presence of a G quartet in an mRNA may provide a zip code indicating that the mRNA should be transported to a postsynaptic or other selected site in the cell.

Our understanding of the complexity and subtlety of the fragile X phenotype induced by FMRP's absence will certainly benefit from analysis of the mRNA targets of this protein. We also need to clarify whether FMRP shares some of its activities with its close relatives, FXR1P and FXR2P. The new work unveils exciting avenues for future research into fragile X syndrome.

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PERSPECTIVES: SURFACE SCIENCE

Hitting the Surface—Softly

Daniel J. Auerbach

In many chemical reactions, reactants first adsorb on a solid surface and then interact to form products. Such reactions play an important role in the industrial-scale production of chemicals, materials processing, atmospheric chemistry, biochemistry, and environmental science.

Adsorption—the process whereby an atom or molecule in the gas or liquid phase loses some of its initial energy and becomes bound on a surface—is a prerequisite to all these reactions. In many-body systems, energy can be lost through a variety of loss channels. Understanding the relative importance of these channels in the adsorption process is thus key to understanding these reactions. On page 2521 of this issue (*1*), Gergen *et al.* show that energy loss from electronic excitation of the solid may play a more pervasive role in adsorption than was previously believed.

As an incident particle approaches a solid surface (see the figure), it interacts with the electrons of the solid. By perturbing these electrons, it also generates interactions with the ion cores in the lattice. Two broad classes of energy-dissipation channels arise from these interactions: excitation of lattice vibrations (or phonons) and electronic excitations, which include particle emission, photon emission, and the excitation of electron-hole pairs.

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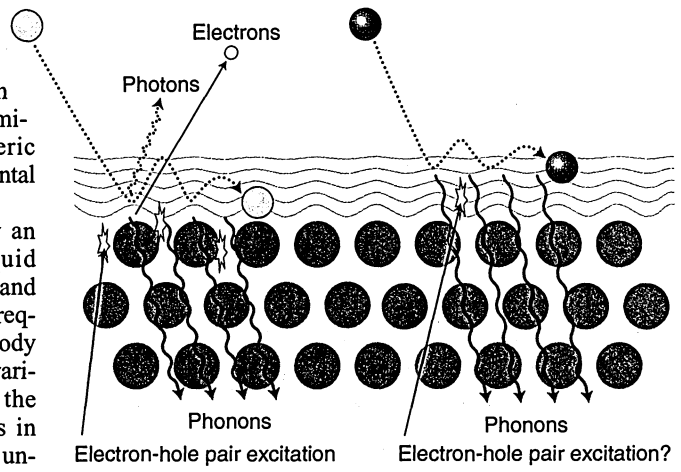

Energy-dissipation channels in adsorption. At high adsorption energies (**left**), many energy-dissipation channels are available, whereas at lower energies (**right**), only phonons and electron-hole pair generation are possible.

At high interaction energies (see the left part of the figure), there is abundant evidence for electronic excitation. For some channels, particles or photons can be observed directly and their participation in the adsorption process established unambiguously. As early as 1905, Thomson (*2*) observed the emission of negative particles following adsorption of various gases on an alkali metal surface. Many examples of emission of electrons and ions (exoemission) and of photons (chemiluminescence) in highly exothermic reactions at surfaces are now known (*3*).

At lower energies (see the right part of the figure), the importance of electronic excitations in adsorption tends to decrease for several reasons. Processes like exoemmision and chemiluminescence cannot occur below a certain energy threshold. Hence, only phonons and electron-hole pairs are available to dissipate energy. Furthermore, the electrons in the solid have more time to adjust smoothly to the perturbation of a slow incoming particle. The system therefore tends to evolve adiabatically, with energy dissipated to phonons rather than to nonadiabatic electronic excitations.

Direct observation of phonons or electron-hole pairs has been difficult. Most of our knowledge of their role in adsorption comes from comparison of theoretical calculations for these channels with experiments in which molecules are scattered from surfaces under well-defined incidence conditions (*4*, *5*). For example, measured adsorption probabilities and angular and velocity distributions of Ar and Xe scattered from a platinum surface (*6*, *7*) are in reasonable agreement with calculations that include phonon excitation but ignore electron-hole pair excitation (*8*). Similarly, adsorption probabilities of alkanes on the same platinum surface are in good agreement with calculations that omit electron-hole pairs (*5*).

These and related, more recent studies