

Neurobiology of Fragile X Syndrome: From Molecular Genetics to Neurobehavioral Phenotype

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Fragile X syndrome (FraX) is one of the most common genetic disorders associated with mental retardation (Moser, 1995). Recent developments in FraX research have led to an increased interest in the neurobiology of this condition; several factors contribute to this focus on FraX: its relatively high frequency, its association with a single gene defect (the *FMR1* gene), the molecular characterization of the *FMR1* gene product (Fragile X Mental Retardation Protein or FMRP), an increasing knowledge about the (specific) FraX neurobehavioral phenotype, and the development of transgenic animal models of *FMR1* and related genes. For all these reasons, FraX is considered as one of the best models for understanding how a genetic defect can lead to a severe behavioral and cognitive phenotype. This special issue, which includes a combination of topical reviews and original publications, intends to provide a broad perspective about how the discovery of *FMR1* in 1991 (Verkerk et al., 1991) has resulted in an explosion of research on FraX that has evolved from molecular genetics to neurobiology.

Although the genetics of FraX is not the subject of this issue, several important features should be noted. The mutation of the *FMR1* gene occurs "in stages"; the expansion of a CGG polymorphism within the 5' untranslated region of the gene is linked to an obvious phenotype, due to the lack of FMRP, only when it reaches a certain magnitude (full mutation allele). This process of CGG expansion appears to be gradual, evolving from normal to premutation to full mutation over several generations (Kaufmann and Reiss, 1999). There is still controversy about the existence of a phenotype associated with intermediate level mutations (i.e., premutation) (Sherman, 2000; Tassone et al., 2000; Kenneson et al., 2001). Nonetheless, if all patterns of *FMR1* mutation are taken into account, the condition affects as many as 1 in 25 subjects in the general population (Keneson et al., 2001). *FMR1* full mutation alleles are associated, in males, with severe reduction in FMRP levels (Kaufmann et al., 1999). This FMRP deficiency appears to be the most important factor determining the FraX phenotype. In this issue, Kaufmann and colleagues review the current knowledge about FMRP expression in human subjects, its possible molecular consequences, and provide novel data on the expression of other members of the family of RNA-binding proteins that includes FMRP. The next paper, by Feng, focuses on FMRP's structure and function with emphasis on its potential role in neuronal development and function. As Kaufmann and collaborators point out, the existence of a phenotype secondary to FMRP deficit implies that other proteins, with similar structure and function (to FMRP), are not capable of compensating for the lack of FMRP. This issue,

of critical importance for developing genetic therapeutic approaches for FraX, has led to a great interest in characterizing FMRP homologues: the Fragile X Related Proteins or FXRPs. Hoogeveen and colleagues provide a comprehensive review of this group of proteins, including their molecular and functional analogies with FMRP, pattern of expression, and potential for gene therapy. In addition, the paper presents an overview of knockout and transgenic mice of relevance to FraX. The first section of this issue is completed by the paper by Churchill and collaborators, in which the role of FMRP in neuronal function is revisited with a focus on the contribution of the *FMR1* knockout mouse to our understanding of the FraX neurobehavioral phenotype.

The second section of this special issue includes four papers dealing with the neurologic phenotype of FraX. These papers cover major neuroanatomic, neurophysiologic, and behavioral aspects of the FraX phenotype, which in recent years have begun to be examined in light of the molecular and cell biological advances illustrated in the previous section. While our current knowledge on the neurobiology of other developmental disorders associated with mental retardation (i.e., Down syndrome) has been significantly influenced by postmortem brain studies, in the case of FraX limited data are available on the neuropathology and neurochemistry of the condition (Kaufmann and Moser, 2000). For this reason, quantitative neuroimaging in vivo studies of FraX subjects offer a unique window into the basic substrate of the FraX phenotype. Kates and colleagues summarize today's knowledge about the neuroanatomy of FraX. In addition, this contribution includes original data on the early postnatal cerebral development of males with FraX. The results demonstrate that, despite common features with other developmental disorders, FraX is associated with unique cortical anomalies that, surprisingly, not only affect the gray matter but also the white matter. The next paper, by Hagerman and collaborators, presents novel data on the effect of stimulants on electrodermal responses in FraX subjects. This contribution represents an example of a relatively underdeveloped field of research in FraX, which includes neurophysiologic, neurochemical, and functional neuroimaging studies. The paper also enhances our understanding of pharmaco-

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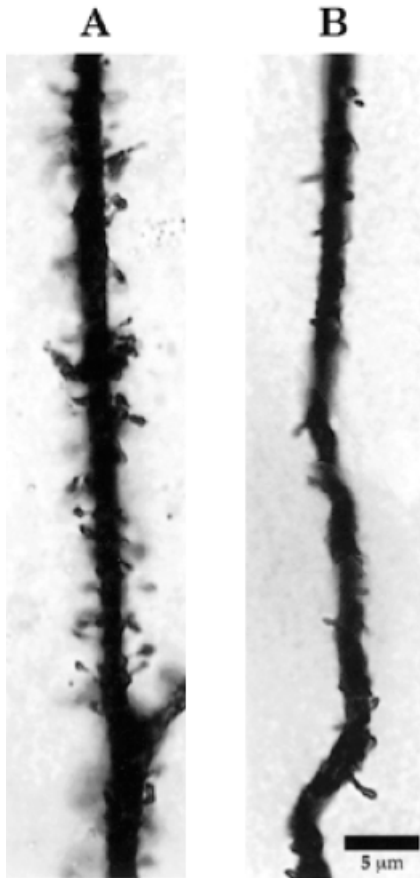


Fig. 1. Examples of typical spine morphologies on Golgi-impregnated dendrites from (A) a human afflicted by fragile-X syndrome and (B) an unaffected control. Dendrites are at extremes of density differences and not intended to depict the norm. Reproduced from Irwin et al. (2000) with permission of the publisher.

therapeutic approaches used to treat important manifestations of the FraX neurobehavioral phenotype, such as Attention Deficit Hyperactivity Disorder (ADHD). The last two papers of this special issue describe in detail salient aspects of the behavioral and cognitive phenotype of FraX. Kau and Kaufmann review the early development of boys with FraX. The data are organized in three major areas: motor, speech and language, and social behavior. Finally, Keysor and Mazzocco revise the psychological phenotype of females with FraX using a developmental perspective. These authors cover cognitive, neuropsychological, social, emotional, and behavioral aspects of the FraX phenotype. In contrast to the body of knowledge about the more severely affected males, research on females with FraX, particularly during early development, has been more limited. The relationship between more subtle behavioral features, such as shyness and social anxiety that affect females, and molecular and neuronal abnormalities in FraX probably represent one of the most challenging areas for the future of FraX research.

As the title of this introduction states, our understanding of the bases of the FraX phenotype is based on a continuum of research from molecular genetics (e.g.,

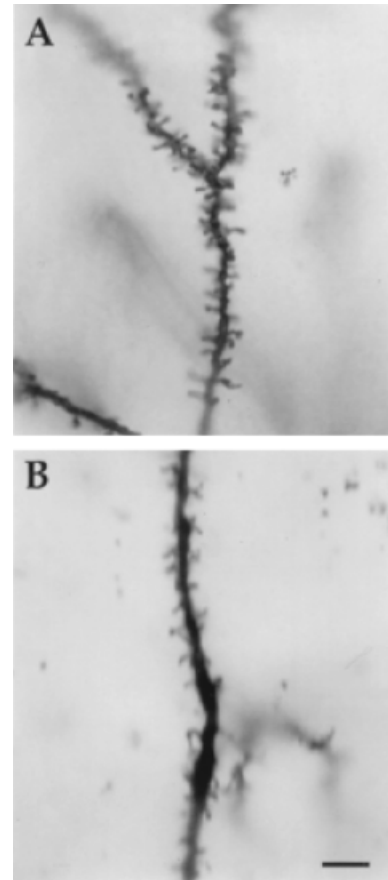
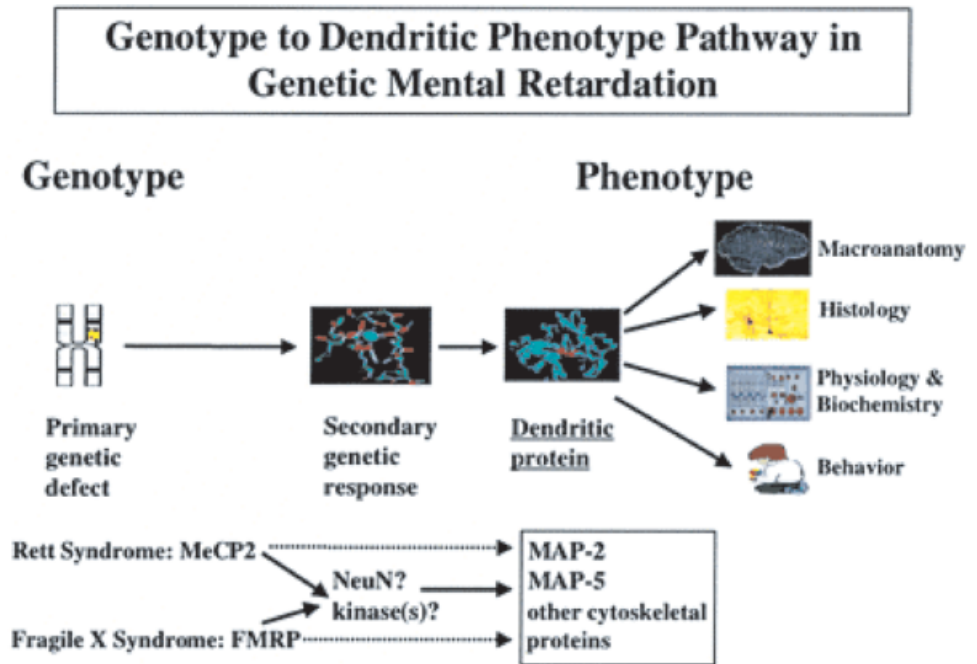


Fig. 2. Golgi-Cox impregnated apical dendrites in transgenic and wild-type mice. **A:** Segment of apical dendrite from layer V pyramidal neuron in *Fmr1* knockout mouse demonstrating both the increased incidence of long, thin dendritic spines and the increased spine density. **B:** Apical dendrite of layer V pyramidal neuron from wild-type mouse. (Bar = 10 μ m.). Reproduced from Comery et al. (1997) with permission of the publisher.

FMRP structure) to standard neurobiology (e.g., neuroanatomy of the *FMR1* knockout mouse). No other single feature of the FraX phenotype better symbolizes this integration than the study of dendritic anomalies in patients with FraX (Kaufmann and Moser, 2000). These postsynaptic abnormalities, which could be responsible for a wide variety of cognitive and behavioral deficits in FraX, are depicted in Figure 1. The illustration, reproduced from the recent review by Irwin and collaborators (2000) on the subject, demonstrates the long, tortuous, immature-looking dendritic spines that characterize the FraX cerebral cortex (Hinton et al., 1991; Irwin et al., 2001).

Similar dendritic spine abnormalities (Fig. 2) have been found in the cerebral cortex of *FMR1* knockout mice (Comery et al., 1997; Nimchinsky et al., 2001), establishing in such a way a strong association between FMRP deficit and dendritic pathology. Emphasizing the importance of this feature, several papers in this issue discuss the possible mechanisms by which FMRP deficit can lead to abnormal dendritic spine morphology. The fact that FMRP is both an RNA-bind-

Fig. 3. Hypothetical genotype-phenotype pathway. We postulate that a primary genetic defect, in the case of FraX a deficit in FMRP, will lead to changes in the expression of proteins that play a critical role in dendritic protein expression. Since several developmental disorders show similar dendritic anomalies, it is possible that several genetic abnormalities can converge at nodal points of signaling pathways that regulate the levels and conformation of key dendritic constituents. These changes in dendritic proteins will, in turn, have a spectrum of phenotypic features ranging from anatomical to behavioral. Identification of critical points in dendritic signaling pathways may help in designing specific therapies for these MR-associated disorders. The diagram exemplifies Rett syndrome and FraX. NT, neurotransmitter; GF, growth factor. Reproduced from Kaufmann and Moser (2000) with permission of the publisher. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]



ing protein and it localizes to dendrites and dendritic spines, where it possibly modulates protein synthesis in response to synaptic activity, creates an intriguing link between FMRP function and neuronal development and physiology.

Thus, the characterization of the molecular pathway between FMRP and dendritic spine formation (Fig. 3) represents a prime example of the multidisciplinary research needed for understanding and modifying the FraX phenotype. This collection of papers intends to foster this integrative approach to FraX research.

NOTE ADDED IN PROOF

Following the completion of this special issue, Brown and colleagues (Cell 107:477–487, 2001) have published a landmark study on the molecular bases of Fragile X syndrome. Given the importance of these data for the neurobiology of the disorder, the Guest Editor and one of the co-authors of the publication (Y. Feng) have summarized the results and their implications here. Using microarrays, Brown et al. identified mRNAs bound to the FMRP-ribonucleoprotein complex in mouse brains. A similar strategy was used to identify 251 transcripts with abnormal association to polyribosomes in lymphoblasts from FraX patients. Interestingly, of the 432 “FMRP targets” in brain, a substantial number was also found that displayed abnormal polyribosome profile in FraX lymphoblasts. Among those transcripts identified in both mouse brain and FraX lymphoblasts, most likely also abnormally regulated in brains from FraX patients, there are 14 coding for proteins that are critical for synaptic development and function. These include cytoskeletal dendritic and axonal proteins (e.g., MAP-1B, NAP-22), constituents of the postsynaptic dense zone (e.g., SAPAP4, ArgBP2), and a reg-

ulator of glutamate release (Munc13). Interestingly, more than half of these potential FMRP ligand mRNAs harbor a G quartet structure, which was identified for in vitro FMRP-binding (Darnell et al. Cell 107: 489–499). Furthermore, another recent study has confirmed in *Drosophila* the role of *FMR1* (dFXR) in negatively regulating the translation of the MAP1B (also known as MAP5) homolog Futsch (Zhang et al. Cell 107: 591–603, 2001), further emphasizing the importance of FMRP in dendritic and axonal development. These data altogether suggest that the predominantly neurologic phenotype of FraX is not only the consequence of indirect effects of FMRP deficit upon neural proteins, but also of direct translational misregulation within synaptic compartments. As postulated in this Editorial, the molecular pathways between FMRP and dendritic spine formation seem to be central to the pathogenesis of the FraX neurobehavioral phenotype.

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