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## Preface

Two decades have passed since trinucleotide repeat expansion was first discovered in 1991 in genes responsible for certain neurological diseases. The disease-responsible genes include a CGG repeat expansion in fragile X syndrome, a GAA repeat expansion in Friedreich's ataxia, a CTG repeat expansion in myotonic dystrophy, and CAG repeats in X-linked spinal and bulbar muscular atrophy, Huntington's disease (HD), spinocerebellar ataxia, and Dentatorubral-pallidoluysian atrophy (DRPLA). The neurological diseases associated with CAG repeat expansion are also called polyglutamine repeat diseases, because the CAG repeats are located within the coding regions of the responsible genes, translated as polyglutamine in their protein products. The first edition of *Trinucleotide Repeat Protocols* in 2004 (Kohwi, Y., Editor) focused on the analyses of trinucleotide repeat expansion in vitro and in vivo. By 2011, new technologies have developed and innovative concepts have emerged, which may prove useful in devising therapeutic approaches to neurological diseases. Therefore, in this new edition of *Trinucleotide Repeat Protocols*, I wish to address, as our new theme, "from mechanisms to cure." Under this theme, we not only need to deepen our understanding of the mechanisms of trinucleotide repeat diseases, but we must also explore possible therapeutic approaches and/or seek targets. Such efforts are still in their infancy in the trinucleotide disease fields.

This new edition of *Trinucleotide Repeat Protocols* is divided into six parts. Each chapter follows a concise but well-described format that can stand alone as a tool for a specific area of research on trinucleotide repeat diseases, including the history behind trinucleotide repeat expansion, the technical advances made, as well as current examples and discussions relevant to each chapter topic. In the first part of the introductory Part I, Dr. McMurray's group provides an overview of trinucleotide repeat diseases. This is followed by Dr. Watt's group, giving us a summary of the past and present and the future prospects in the HD field. Because analysis of synaptic plasticity is important for understanding mechanisms for brain function and in monitoring neuronal responses to damage or to stimulation-dependent improvement, we invited Dr. Gan's group and Dr. Zuo's group each to contribute a chapter in Part II, based on their excellent work on dynamic monitoring of synaptic plasticity in vivo. In Part III, Dr. Chan's group and Dr. Nolte's group describe embryonic stem (ES) cell-related protocols, focusing on HD. These are followed in Part IV by RNA-related protocols, from Dr. Deglon's group (RNAi), Dr. Siomi's group (RNAi), and Dr. Tanese's group (FISH). Neurological diseases may be influenced not only by genetic mutation but also by DNA/protein modification. Recent evidence strongly suggests that epigenetic modification is an important factor in neurological disorders. In Part V, Dr. Neri's group and Dr. Oostra's group describe analysis of epigenetic modification in fragile X syndrome, including DNA methylation, histone modification, and chromatin immunoprecipitation. Dr. Segovia's group addresses the oxidation damage pathway of protein in the HD animal model system and seeks for therapeutic strategies, and Dr. Orr's group focuses on the importance of phosphorylation of the causal protein ataxin-1 in SCA1 disease. Finally, in

Part VI Dr. McMurray's group, Dr. Gourdon's group, Dr. Koob's group, Dr. Patterson's group, and Dr. Tsuji's group have taken on the challenge of providing a better way for the analysis of trinucleotide repeat expansion in vitro and in vivo in different trinucleotide repeat diseases.

As briefly summarized above, this new Edition of these Protocols covers not only direct analysis of trinucleotide repeat diseases but also alternative approaches for the analysis of trinucleotide repeat diseases, with the hope that this will result in better understanding of the mechanisms and future therapeutic prospects for treatment of these diseases.

I wish to take this opportunity to express my great appreciation for the efforts of all authors for establishing the new edition and thank Dr. Asmita Patel and Mr. Kevin Peet for their editorial help.

Dr. Neri's group, Dr. Goudon's group and Dr. Patterson's group each provided us their opinions about our main theme, "From mechanisms to Cure," in the Preface section below.

## **Opinions on Trinucleotide Repeat Diseases, from Mechanisms to Cure**

***By Drs. Ali Khoshnaw and Paul H. Patterson***

Polyglutamine (polyQ) expansion in huntingtin is a major determinant of HD pathology. However, the age of onset varies greatly among individuals with similar polyQ length. This supports the notion that other genetic and environmental factors influence the onset and progression of HD pathogenesis. Several important studies have identified the epitopes flanking the polyQ as modifiers of HD pathology. For example, the proline repeats and the proline-rich motif downstream of polyQ, as well as the N-terminal 17 amino acids of Htt, regulate oligomerization and toxicity of mutant Htt. Thus, binding of cellular proteins to these domains may significantly influence the neurotoxicity of mutant Htt. Intrabodies have been instrumental in characterizing and identifying these pathogenic epitopes. Moreover, the intrabodies that bind to these epitopes are neuroprotective and are emerging as novel therapeutic molecules. Environmental factors such as neuroinflammation and DNA damage may also impact the onset and the severity of HD, and represent examples of how gene-environment interactions regulate neurodegeneration. Inflammation and DNA damage can potentially trigger the cleavage of mutant Htt, which is one of the earliest events in HD pathology and a prerequisite for generating amyloidogenic N-terminal fragments. These findings offer therapeutic windows to ameliorate neurotoxicity in HD. The I $\kappa$ B kinase  $\beta$  (IKK $\beta$ ) holds great promise as a novel therapeutic target for HD, as a regulator of inflammation and DNA damage-induced Htt cleavage.

***By Drs. Elisabetta Tabolacci and Giovanni Neri***

Trinucleotide repeat diseases are a group of genetic disorders caused by trinucleotide repeat expansions, exceeding the threshold typical of normal alleles. These tandem repeats are interspersed throughout the genome. If the repeat is present in a gene, a dynamic mutation may increase the repeat number and result in a defective gene.

Since the early 1990s, a new class of molecular diseases has been characterized, based on the presence of unstable expansions of DNA-triplets (trinucleotides). The first triplet disease to be identified was the fragile X syndrome (FXS), an X-linked condition affecting mostly males, the main clinical manifestations of which are intellectual disability, a characteristically elongated face with large ears, and macro-orchidism. Subsequently, several other genetic diseases have been shown to be caused by unstable mutation of short repeat sequences, including myotonic dystrophy, Huntington's disease, Friedreich ataxia, spinocerebellar ataxias, Kennedy disease, etc. Trinucleotide repeat disorders generally show the phenomenon of genetic anticipation, likely explained by further expansion of the repeats in successive generations.

An interesting question is why three nucleotides are expanded, rather than two or four or some other number. If expansions fall near coding regions of the genome, repeats that are not multiples of three could cause frameshift mutations. If these mutations altered the expression of developmentally obligatory pathways, the non-trinucleotide repeats would be masked by prenatal lethality.

The prospect of treating single-gene disorders by gene replacement therapy has turned into a major disappointment, with very few exceptions. Wherever possible, alternative approaches, mostly drug-based, are being sought. FXS is one notable example, as testified by the interest taken in this condition by small orphan drug companies, as well as the big pharma industry. Two alternative approaches are currently being pursued. The first aims at correcting the effects from lacking the *FMRI* protein, for example on the activity of neuronal metabotropic glutamate receptors. The other approach aims at restoring the activity of the gene itself, given that the transcriptional block of *FMRI* is epigenetic and therefore reversible. We have already demonstrated that the activity of the gene can be restored in vitro by certain drugs. We also recently performed in vivo clinical trials, the results of which suggest that effective pharmacological treatment of FXS is a realistic goal.

***By Drs. Judith R. Brouwer, Laurent Foiry, and Geneviève Gourdon***

Understanding the mechanisms underlying diseases is essential for engineering therapeutic strategies. Trinucleotide repeat disorders line up in the category of complex diseases, and involve many fundamental cellular factors and processes, such as DNA, RNA, and protein metabolisms. After the discovery of the first pathology associated with a dynamic expansion and the characterization of many more in the 1990s, it took a while to acknowledge the major physiological consequences of triplet expansions. Although there is still more to learn about these diseases, we have reached a stage in our knowledge which allows the design of therapeutic strategies aiming at remedying at least some of the cascade effects of the mutation. Although the majority of ongoing strategies are still at the preclinical level, there is more hope for patients today than when researchers were facing the black hole between the mutation and symptoms.

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