Myotonic dystrophy associated expanded CUG repeat muscleblind positive ribonuclear foci are not toxic to Drosophila

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Myotonic dystrophy type 1 is an autosomal dominant disorder associated with the expansion of a CTG repeat in the 3′ untranslated region (UTR) of the DMPK gene. Recent data suggest that pathogenesis is predominantly mediated by a gain of function of the mutant transcript. In patients, these expanded CUG repeat-containing transcripts are sequestered into ribonuclear foci that also contain the muscleblind-like proteins. To provide further insights into muscleblind function and the pathogenesis of myotonic dystrophy, we generated Drosophila incorporating CTG repeats in the 3′-UTR of a reporter gene. As in patients, expanded CUG repeats form discrete ribonuclear foci in Drosophila

being rela ed onl b he na re of he ran cribed, b n ran la ed, CTG/CCTG repea . Secondl , mice e pre ing e panded CUG repea in he 3′-UTR of an nrela ed ran - gene de elop m o onia and a DM-like m opa h (5).

Do n ream pa holog in DM i linked i h plicing defec in a n mber of gene . Mo con incingl , mi - plicing of he *chloride channel subunit 1 (CLC1)* and *insulin receptor* ran crip almo cer ainl nderlie he ob er ed m o onia (6) and in lin in olerance (7). The pa h a ha link he e plicing defec o he primar CTG e pan ion are no e com- ple el nder ood b appear o in ol e o cla e of pro ein ha can bind CUG repea : he CUG-BP1 and ETR-3-like fac or (CELF) and he m cleblind-like (MBNL) pro ein (8). Bo h cla e of pro ein are reg la or of al erna i e plicing i h an agoni ic effec on a b e of al erna i el pliced gene . Con i en i h a direc role for CUG-BP1 in DM pa hogene i , DM plicing defec are mirrored in normal cell o er-e pre ing CUG-BP1 and n clear le el of CUG-BP1 are increa ed in DM pa ien cell (9). Similarl , con i en i h a direc role for he MBNL in DM pa hogene i , mice lacking *Mbnl1* de elop m opa h and m o onia and plicing defec in he *Clc1* ran crip (10).

Preci el ho he CELF and MBNL pro ein f nc ion are per rbed in pa ien cell i no comple el nder ood, b i a med o be rela ed o he ob er a ion ha e panded CUG repea RNA i rapped in di cre e foci in he n clei of pa ien cell (11). Al ho gh he e ribon clear foci do no con ain CUG-BP1 (12), he MBNL pro ein bind d CUG RNA and co-locali e i h he ribon clear foci, rongl p- por ing a role for MBNL i ra ion in he pa hogenic proce (13,14). Ne er hele , mice homo go n ll for *Mbnl1* are born heal h and do no pre en i h he congeni al form of DM. Th , i remain nclear a o ha e en MBNL i ra ion con rib e o ard pa hogene i and, impor an , he her e panded CUG repea RNA or ribon clear foci are inheren no io and ha e a direc o ic effec o er and abo e d reg la ion of al erna i e plicing. A more general o ic effec of e panded CUG repea RNA migh be media ed b he eq e ra ion of ran crip ion fac or , a ha been recen propo ed (15), and i ob er ed for high o ic pol gl amine e pan ion (16).

The ra e of progre in nder anding f ndamen al mech- ani m in DM i re ric ed b he comple i of anal ing pa ien ample , he inheren limi a ion of cell c l re model and he rela i e dif c l of genera ing addi ional mo e model . Signi can l , a n mber of riple repea di order ha e been cce f ll modelled in *Drosophila*, pro iding cri ical ne in igh in o he molec lar pa hogene i of he di ea e proce (17 20). We ha e, herefore, crea ed a *Drosophila* model of DM and pro ided in igh in o m cle- blind f nc ion b e pre ing CTGgl

ـigh m cle cell . In ere ingـ, $(CUG)_{162}$- peci c ran gene
RNA a al o de ec ed in he n clei of all lar al and ad l e

m cle, de pi e he pre ence of m cleblind pro ein and GFP in he e i e .

In h man , MBNL1 i di rib ed hro gho he c- opla m and n cle i hin ild-- pe m cle cell b i recr i ed o he ribon clear foci in DM pa ien cell (13). Thi i a ion a replica ed in *Drosophila* hird in ar lar ae (Fig. 3A), b in ild-- pe and $(CTG)_{11}$ ad l , m cleblind a clear- loca ed in n clear foci in he ab ence of e panded repea RNA (Fig. 3B). Thi gge ha ei her m cleblind in racell lar locali a ion i de elopmen al- con rolled b- o her pro ein or ha de elopmen al- reg la ed m cleblind i oform differ in heir locali a ion. Fo r differen *muscleblind* mRNA i oform ha e been iden i ed in *Drosophila*: *mbl-A*

i h DAPI, b he ere no ob er ed in he n cleol . The herefore hared he in er-chroma in pace i h he pli-cing and mRNA e por machiner . The rela i e loca ion of ribon clear foci and molec lar marker of pliceo ome and e o ome ere anal ed. No co-locali a ion a ob er ed (Fig. 7A). The rela i e loca ion of he pro ea ome a al o in e iga ed o de ermine he her he pro ein pre en in he ribon clear foci con ain mi -folded pro ein and are arge ed for degrada ion, and again no co-locali a ion a ob er ed (Fig. 7A).

ne e ol ionari con er ed cell lar ra eg for dealing i h n clear d RNA i adeno ine o ino ine RNA edi ing (25). In er ebra e cell , i i kno n ha edi ing i follo ed b a achmen o he n clear ma ri b PSF and p54^{nrb} (26). Al ho gh d CUG repea RNA ho ld be imm ne o ch edi ing, he hairpin r c re ma none hele in erac i h pro ein in hi pa h a , o he rela i e loca ion of ribon c-lear foci and non-A, he *Drosophila* PSF/p54^{nrb} or holog e, ere anal ed (Fig. 7B). Co-locali a ion of he e elemen a er good, indica ing ha non-A and e panded repea RNA occ p he ame n clear region and probab in erac , direc or indirec , *in vivo.* To de ermine he her e panded CUG con aining ran crip ere edi ed b he non-A pa h a , RT. PCR ampli ed ran crip ere cloned and eq enced from $(CTG)_{162}$ e pre ing ie . RNA edi ing and re er e ran-crip ion con er edi ed adeno ine o g ano ine in he cDNA

To gain f r her in igh in o he organi a ion of ribon clear foci, heir pa ial po i ion in m cle n clei a anal ed. Ribon clear foci occ pied n clear region ligh ained

arra- in DM1 pa ien . Indeed, i ha been ho n ha addi ional eq ence elemen i hin he *DMPK* 3′-UTR modif- he effec of he e panded CUG repea on m- obla differen ia ion (31). None hele , he DM2 m a ion (3) and he CUG repea -e pre ing m- o onic mice (5) demon ra e ha an e panded repea rac in he ab ence of addi ional *DMPK* eq ence i f cien o media e DM pa holog- in mammalian cell .

In $(CTG)_{162}$ *Drosophila* e pre ing he ran gene biq i- o l-, ribon clear foci ere ob er ed onl- in ali ar- gland , lar al m cle cell and a b e of ad l m cle . Th , ribon clear foci forma ion i no an obliga e manife - a ion of e pre ing large e panded CUG repea arra- gge ing ha he hairpin r c re ch RNA adop (12) are no erical- blocked from e i ing he n cle , a ha been pre io l- propo ed (32). The e da a herefore indica e

be een e panded CUG repea RNA and n clear ma ri . We propo e ha in normal cell , ome MBNL i a ocia ed i h

Af er blocking for 30 min i h 5% normal er m (from he

pol- gl amine pro ein form n clear incl ion and ca e ne ral degenera ion in *Drosophila*. *Cell*, **93**