Down syndrome DSCR1 causes spine pathology via the Fragile X-related protein FMRP

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A new study by Wang *et al* (2012) published in *The EMBO Journal* reports that the Down Syndrome Critical Region 1 (DSCR1) protein interacts with the Fragile X-related protein (FMRP) to regulate spine morphology and local protein synthesis. These findings highlight a convergence of Down syndrome (DS) and Fragile X pathogenic pathways, and provide insights on potential therapeutic approaches.

DS is among the most common causes of intellectual disability of genetic origin. It is caused by the triplication of chromosome 21 (HSA21q), and is clinically characterized by phenotypes affecting multiple organs and systems, with prominent cognitive impairments. Although the chromosomal region 21q contains >350 genes, only a few of them have been linked to specific features of DS. With respect to intellectual disability, the analysis of partial triplications has pinpointed a number of DS critical regions, whose genes are involved in brain function (Patterson, 2009). Studies in transgenic mice and in human samples have uncovered a number of synaptic features disrupted in DS: spine densities are reduced, spines display abnormally large bulbous heads

(Wang *et al*, 2012) and synaptic plasticity is altered. HSAq21 genes involved in synaptic physiology are therefore attractive candidates to account for intellectual disability in DS. Among them, *DSCR1* (also known as *RCAN1*) is strongly expressed in the brain and its overexpression or knockout causes memory impairment and LTP derangement in mice. *DSCR1* is a negative regulator of calcineurin phosphatase and, as such, has been implicated in a large number of functions both in and outside the CNS; however, the mechanisms linking *DSCR1* to intellectual disability have not been identified unambiguously.

Wang *et al* (2012) now show that *DSCR1* interacts with, regulates and exerts its effects via the Fragile X mental retardation protein (FMRP). This remarkable finding reveals that two major causes of intellectual disability converge onto the regulation of local protein synthesis at synapses. *Fmr1* gene is targeted by a triplet-expansion mutation that, by effectively preventing its transcription, causes the Fragile X syndrome (FXS, the most common cause of autism). FMRP is an RNA binding protein enriched at synapses. It regulates the transport of a subgroup of mRNAs coding for synaptic

Figure 1 DSCR1 regulates mRNA translation at synapses through FMRP. (A) At rest, DSCR1 inhibits calcineurin that, in turn, does not dephosphorylate FMRP. Both mGluR5 and mTOR signalling cascades contribute to regulate FMRP phosphorylation; in its phosphorylated form, FMRP depresses mRNA translation. (B) Upon BDNF application, DSCR1 is phosphorylated and shifts to activate calcineurin, which efficiently dephosphorylates FMRP, relieving its inhibitory effect on mRNA translation.

proteins, and, upon phosphorylation, reversibly represses their translation by recruiting the RISC and microRNAs (Muddashetty *et al*, 2011). In the absence of FMRP, protein translation goes on unchecked in synapses causing amplified LTD after mGluR activation and abnormally thin spine morphologies (Osterweil *et al*, 2010).

In their study, Wang *et al* (2012) exploit genetic models, RNA interference and biochemical tools to demonstrate the genetic and functional interaction of *DSCR1* and *fmr1* in spine morphogenesis. The study first investigated the role of the two proteins on spine density and morphology. Overexpression of *DSCR1* increased spine size and density, and that a 50% decrease in FMRP effectively rescued this phenotype. On the other hand, knockdown of either *DSCR1* or *fmr1* decreased spine size and density; and loss of both *DSCR1* and FMRP did not aggravate this phenotype. Thus, while *DSCR1* overexpression is compensated by *fmr1* knockout, *DSCR1* knockout occluded any further effect of *fmr1* knockout. Since the actin cytoskeleton plays a prominent role in spine morphology, the actin-binding protein cofilin was a natural target of investigation to link *DSCR1* to deranged spine structure. Dephosphorylated cofilin severs actin filaments and promote actin turnover, driving the formation of thin spines; phosphorylation blocks cofilin function and high level of phospho-cofilin results in the build-up of actin filaments, leading to increased spine size (Hotulainen *et al*, 2009). Consistent with morphological findings, phospho-cofilin level was upregulated by *DSCR1* overexpression and, reciprocally, was downregulated by *DSCR1* knockout. Cofilin phosphorylation is reversed by calcineurin, thus *DSCR1* may modulate phospho-cofilin level by directly affecting calcineurin activity. However, FMRP decrease rescues *DSCR1*-induced phospho-cofilin upregulation, suggesting that local protein synthesis plays a major role in *DSCR1*-induced actin cytoskeleton remodelling.

Wang *et al* (2012) elegantly explore *DSCR1* effects on synaptic protein synthesis exploiting a Dendra-based reporter targeted to spines. By photoconverting Dendra before BDNF application, any newly (i.e., not photoconverted) synthesized fluorescent protein could be identified and quantified with high spatial accuracy. In contrast to conclusions from previous reports (Osterweil *et al*, 2010), basal translation rates in *DSCR1* or *fmr1* knockout mice were normal; this discrepancy may be due to the intrinsically lower sensitivity of the imaging approach compared to metabolic labelling methods, or to some peculiarities in reporter design. By contrast, *DSCR1* overexpression greatly enhanced BDNF-induced protein synthesis, whereas *DSCR1* knockout completely abolished the effects of BDNF. Interestingly, FMRP knockdown did not modulate *DSCR1*-enhanced protein synthesis, indicating that overexpression of *DSCR1* is sufficient to occlude any further stimulation of local synthesis. Nevertheless, FMRP knockdown did shorten the duration of protein synthesis, supporting a role of FMRP in regulating not only translation rates but also the local availability of mRNAs.

Mechanistically, *DSCR1* is shown to interact directly with phosphorylated FMRP, but not with the unphosphorylated protein. *DSCR1* inhibits calcineurin activity, thus increasing phospho-FMRP level and repressing translation (Figure 1A). *DSCR1* phosphorylation, on the other hand, promotes

activation of calcineurin. Activated calcineurin would then dephosphorylates both FMRP (causing the dissociation of the *DSCR1*–FMRP complex and the upregulation of translation; Figure 1B) and its own activator *DSCR1* (closing a negative feedback loop).

Intriguingly, FMRP knockout can rescue synaptic plasticity and behavioural impairment also in the *Tsc2* +/– mouse, a model of intellectual disability associated with tuberous sclerosis. Despite mTOR activity upregulation, synaptic protein synthesis is reduced in *Tsc2* +/– neurons (possibly because of FMRP hyperphosphorylation; Figure 1A) but it is restored to normal levels by *fmr1* knockout (Auerbach *et al*, 2011). Thus, *DSCR1* and *Tsc2* +/– transgenic mice display a similar FMRP-dependent downregulation of protein synthesis at synapses; in both cases, FMRP phosphorylation is critical in repressing translation. In contrast, if the FXS FMRP is strongly reduced and protein synthesis is constitutively upregulated. Therefore, altered local protein synthesis (either increase or decrease) emerges as a key player in the pathophysiology of intellectual disabilities.

The convergence of *DSCR1* and *Tsc2* on FMRP suggests potential therapeutic interventions. In *Tsc2* +/– mice, mGluR5-positive allosteric modulators restore synaptic protein synthesis and ameliorate memory impairment (Auerbach *et al*, 2011). Furthermore, inhibition of overactive mTOR by rapamycin also improves electrophysiological and behavioural readouts in *Tsc2* mice (Ehninger *et al*, 2008). On the other hand, in *fmr1* knockout mice, mGluR5 antagonists repress deregulated protein synthesis and reverse behavioural abnormalities (Michalon *et al*, 2012). Since deregulated protein synthesis is also a major consequence of *DSCR1* downregulation, compounds targeting mGluR5 and mTOR may prove equally beneficial. However, the overexpression of *DSCR1* may result in both strong suppression of protein synthesis at rest and in excessive synthesis upon stimulation; the contribution of these two processes to the derangement of synaptic physiology deserves further investigations.

Additional phenotypes resulting from *DSCR1* overexpression have been described, including reduced neuronal density, defective neurogenesis and altered neuronal Ca^{2+} dynamics (Martin *et al*, 2012); the contribution of these phenotypes to the pathogenesis of intellectual disability and the involvement of FMRP remain to be elucidated.

A growing number of autistic disorders have been linked to mutations in synaptic scaffold proteins (e.g., Shank and SAPAP proteins). Several of these proteins are regulated by local translation and may be critically affected by the *DSCR1*–FMRP pathway. Recently, *fmr1* knockout was found to result in the disruption of the interaction of mGluR5 with Shank-interacting scaffold protein Homer. It is therefore conceivable that further convergences of pathogenic pathways may be uncovered in the near future. For the time being, Wang *et al* (2012) provide a critical step towards the integration of different genetic causes of intellectual disability into a single, unified pathogenic model.

Conflict of interest

The author declares that he has no conflict of interest.

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