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DECIPHERING THE SPATIO-TEMPORAL EXPRESSION AND STRESS REGULATION OF FAM107B, THE PARALOG OF THE RESILIENCE-PROMOTING PROTEIN DRR1 IN THE MOUSE BRAIN

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Abstract—Understanding the molecular mechanisms that promote stress resilience might open up new therapeutic avenues to prevent stress-related disorders. We recently characterized a stress and glucocorticoid-regulated gene, down-regulated in renal cell carcinoma – DRR1 (Fam107A). DRR1 is expressed in the mouse brain; it is up-regulated by stress and glucocorticoids and modulates neuronal actin dynamics. In the adult mouse, DRR1 was shown to facilitate specific behaviors which might be protective against some of the deleterious consequences of stress exposure: in the hippocampal CA3 region, DRR1 improved cognitive performance whereas in the septum, it specifically increased social behavior. Therefore DRR1 was suggested as a candidate protein promoting stress-resilience. Fam107B (family with sequence similarity 107, member B) is the unique paralog of DRR1, and both share high sequence similarities, predicted glucocorticoid response elements, heat-shock induction and tumor suppressor properties. So far, the role of Fam107B in the central nervous system was not studied. The aim of the present investigation, therefore, was to analyze whether Fam107B and DRR1 display comparable mRNA expression patterns in the brain and whether both are modulated by stress and glucocorticoids. Spatio-temporal mapping of Fam107B mRNA expression in the embryonic and adult mouse brain, by means of *in situ* hybridization, showed that Fam107B was expressed during embryogenesis and in the adulthood, with particularly high and specific expression in the forming telencephalon suggestive of an involvement in corticogenesis. In the adult mouse, expression was restricted to neurogenic niches, like the dentate gyrus. In contrast to DRR1, Fam107B mRNA expression failed to be modulated by glucocorticoids and social stress in the adult mouse. In summary, Fam107B and DRR1 show different spatio-temporal expression patterns in the central nervous system, suggesting at least partially different functional roles in the brain, and where the glucocorticoid receptor (GR)-induced regulation appears to be a unique property of DRR1.

Key words: DRR1 – Fam107A – Tu3A, Fam107B – HITS, stress, corticogenesis, glucocorticoid receptor.

INTRODUCTION

Stress activates a plethora of physiological, molecular and cellular mechanisms directed to adapt to changing environmental demands. However, severe and prolonged stressful episodes can also trigger deleterious consequences on brain function, which, in genetically predisposed individuals, increase the likelihood for the development of stress-related psychiatric disorders (de Kloet et al., 2005). Still, it remains poorly understood why some individuals are more vulnerable to stress whereas others remain resilient. Therefore, deciphering the specific mechanisms that activate adaptive (i.e. beneficial and protective) rather than maladaptive (i.e. aversive) consequences of stress might promise new insights into how stress-related mental dysfunctions can be prevented.

We recently identified a novel stress- and glucocorticoid-regulated gene, down-regulated in renal cell carcinoma 1 (DRR1, also named Fam107A and Tu3A) (Liebl et al., 2009; Schmidt et al., 2011).

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Abbreviations: 3V, third ventricle; aci, anterior commissure intrabulbar part; C, cortex; CA1, cornu ammonis area 1; CA3, cornu ammonis area 3; ChP, choroid plexus; CSDS, chronic social defeat stress; DG, dentate gyrus; DRR1, down-regulated in renal cell carcinoma 1 (also named Fam107A and Tu3A); E, embryonic day; Fam107B, family with sequence similarity 107, member B (also named HITS – heat-shock-inducible tumor small protein); GE, ganglionic eminences; GR, glucocorticoid receptor; GREs, glucocorticoid response elements; H, hippocampus; Hypoth, hypothalamic nuclei; IC, inferior colliculi; LV, lateral ventricle; MHb, medial habenular nuclei; Mo, molecular layer of the cerebellum; Nu, nuclear layer of the cerebellum; P, Purkinje cell layer; Pn, pontine nuclei; RtTg, reticulotegmental nucleus; S, septal region; T, tectum.

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Glucocorticoid receptor (GR) activation induces DRR1 expression in stress-relevant regions such as the CA3 region (Schmidt et al., 2011) and the lateral septum (Masana et al., 2014). Notably, virus-induced increase of DRR1 specifically in these brain regions promotes coping behaviors which might be protective against some of the aversive consequences of stress exposure: in the CA3 region, DRR1 improves cognitive performance (Schmidt et al., 2011), whereas in the septal region it specifically increases social behavior (Masana et al., 2014). As DRR1 is an actin-interacting protein, we proposed this conceptually novel link between stress and neuronal actin dynamics as a critical component of resilience mechanisms (Masana et al., 2014).

DRR1 has only one known paralog, Fam107B (Family with sequence similarity 107, member B), also named HITS (heat-shock-inducible tumor small protein) (Nakajima et al., 2010). Interestingly, Fam107B, like its paralog DRR1, shows heat-shock induction and tumor suppressor properties (Yamato et al., 1999; Wang et al., 2000; Nakajima et al., 2010, 2012). DRR1 and Fam107B also show a high sequence similarity (Nakajima et al., 2010) and both have predicted glucocorticoid response elements (GREs) (Nakajima and Koizumi, 2014). Fam107B expression was described in different types of human tissue, including digestive, respiratory, genital and lymphoid organs, with especially high expression in the breast, thyroid, uterine cervix and testis, and moderate Fam107B expression in the brain (Nakajima et al., 2012). However, a thorough analysis of the neuroanatomical expression pattern of Fam107B has not been performed to date, and data on its putative role in central nervous system function or involvement in stress-associated processes are still lacking.

Considering the interesting role of DRR1 in stress resilience, it is therefore intriguing to study whether its paralog, Fam107B, shows a comparable pattern of expression in the central nervous system and similar stress and glucocorticoid-dependent regulation and function. In the present work, we describe the expression pattern of Fam107B mRNA during different stages of brain development and in the adult central nervous system of the mouse. To decipher the putative function of Fam107B in the adult brain, we continued to investigate whether Fam107B, in comparison with DRR1, is also regulated in a glucocorticoid- or stress-dependent manner.

EXPERIMENTAL PROCEDURES

Animals

Male C57Bl/6N mice (Charles River Laboratories, Germany; > 12 weeks old) were used for all adult mouse experiments. Animals were allowed to rest at least 1 week upon arrival before the beginning of the experiments. Male CD1 mice (16–18 weeks old) served as resident mice in the acute and chronic social defeat paradigm (see below). Animals were singly housed and kept on a 12-h light/dark cycle (lights on at 7:00 AM), at room temperature of 23 ± 2 °C, with food and water provided *ad libitum*. Resident mice were allowed to habituate to the social defeat cage for two weeks before the experiment. The experiments were carried out in accordance with European Communities Council Directive 2010/63/EU. All efforts were made to minimize animal suffering during the experiments. All procedures using adult mice were carried out in the animal facilities of the Max Planck Institute of Psychiatry in Munich, Germany. The protocols were approved by the committee for the Care and Use of Laboratory Animals of the Government of Upper Bavaria, Germany. For studies on mouse embryos, CD-1 mice (Harlan, Israel) were used for timed mating. Mice were kept in a temperature-controlled (21–22 °C) room under a 12-h light/dark cycle (lights were turned on at 6:00 a.m.), with free access to food and water. The procedures and experiments with these animals were conducted in accordance with Ethics Committee approval IL-13-03-2010, Ben-Gurion University.

Dexamethasone treatment

Dexamethasone-21-dihydrogen-phosphate disodium salt (Fortecortin®-Inject 100 mg (10 ml), Merck Pharma GmbH, Germany), a potent synthetic agonist of the GR, was diluted using 0.9% saline to a final concentration of 2 mg/ml and injected sub-cutaneously (s.c.) with a single dosage of 10 mg/kg body weight (~130 µl) between 8 and 9 am. Vehicle-treated animals (Control) were injected with the same volume (~130 µl) of 0.9% saline. Animals were sacrificed 8 h post injection, and brains processed for *in situ* hybridization analysis (n = 6 mice/group).

Acute social defeat

The acute social defeat paradigm was performed as previously described (Wagner et al., 2013). Briefly, animals were exposed for 5 min to an aggressive CD1 resident mouse with short attack latency, and then returned to their home cage until sacrifice. Control animals were allowed to explore a novel empty cage similar to the resident cage for 5 min. Experimental animals were sacrificed 1 h, 4 h, 8 h or 24 h after the acute social defeat, and brains processed for *in situ* hybridization (n = 4–10 mice/group).

Chronic social defeat stress

The chronic social defeat stress (CSDS) paradigm lasted for 21 days and was conducted as described previously (Wagner et al., 2012). Briefly, the experimental mice were introduced into the home cage (45 × 25 cm) of a dominant resident mouse and defeated shortly after. When the defeat was achieved, the animals were separated by a wire mesh, preventing physical but allowing sensory contact for 24 h. Each day, stressed animals were defeated by another unfamiliar, dominant resident mouse in order to exclude a repeated encounter throughout the experiment. The daily encounter was performed between 11 and 16 h. Experimental mice were always defeated by resident males during the entire stress period. Control mice were housed in their home cages during the course of experiment. Some of the control and CSDS mouse groups were sacrificed on day 22, ~24 h after the last