Nucleo-cytoplasmic shuttling of murine RBPJ by Hairless protein matches that of Su(H) protein in the model system Drosophila melanogaster

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Abstract

CSL transcription factors are central to signal transduction in the highly conserved Notch signaling pathway. CSL acts as a molecular switch: depending on the cofactors recruited, CSL induces either activation or repression of Notch target genes. Unexpectedly, CSL depends on its cofactors for nuclear entry, despite its role as gene regulator. In Drosophila, the CSL homologue Suppressor of Hairless (Su(H)), recruits Hairless (H) for repressor complex assembly, and eventually for nuclear import. We recently found that Su(H) is subjected to a dynamic nucleo-cytoplasmic shuttling, thereby strictly following H subcellular distribution. Hence, regulation of nuclear availability of Su(H) by H may represent a new layer of control of Notch signaling activity. Here we extended this work on the murine CSL homologue RBPJ. Using a 'murinized' fly model bearing RBPJ\textsubscript{wt} in place of Su(H) at the endogenous locus we demonstrate that RBPJ protein likewise follows H subcellular distribution. For example, overexpression of a H\textsubscript{NLS3} protein variant defective of nuclear import resulted in a cytosolic localization of RBPJ protein, whereas the overexpression of a H\textsubscript{NES} protein variant defective in the nuclear export signal caused the accumulation of RBPJ protein in the nucleus. Evidently, RBPJ is exported from the nucleus as well. Overall these data demonstrate that in our fly model, RBPJ is subjected to H-mediated nucleo-cytoplasmic shuttling as is Su(H). These data raise the possibility that nuclear availability of mammalian CSL proteins is likewise restricted by cofactors, and may hence present a more general mode of regulating Notch signaling activity.

Keywords: Notch signal transduction, Hairless, CSL, CBF1, RBPJ, Su(H), Protein availability, Nucleo-cytoplasmic transport, Transcription repression, Drosophila

Background

Development as well as tissue homeostasis of higher eumetazoa depends on inter-cellular communication mediated by the Notch signaling pathway. Accordingly, the Notch signaling pathway is highly conserved in the evolution of invertebrates and vertebrates alike [1–3]. Upon binding of one of its ligands, the Notch receptor undergoes cleavage releasing the Notch intracellular domain NICD. Together with several cofactors, NICD assembles a transcriptional activator complex switching gene expression, and eventually cell fate, in the signal-receiving cell [3–7]. Pivotal to Notch target gene regulation is the DNA binding protein CSL; CSL is an acronym for mammalian CBF1 / RBPJ, for Drosophila Su(H) and for Caenorhabditis Lag1. Crystal structure analyses of the trimeric activator complex revealed that NICD contacts CSL with its RAM-domain and ankyrin repeats [8]. In the absence of signal, CSL engages in Notch target gene inhibition by

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forming a repressor complex on Notch target gene promoters [9, 10]. Several corepressors have been identified in mammals, which compete with NICD for the RAMP binding site within the beta-trefoil domain of CSL [9–12]. The major antagonist of the Notch signaling pathway in Drosophila is named Hairless (H) [13]. Contrary to most of the mammalian CSL corepressors, H contacts the C-terminal domain of the fly CSL homologue named Suppressor of Hairless (Su(H)) [14–16]. By recruiting two general corepressors, Groucho and C-terminal binding protein, the Su(H)-H repressor complex eventually silences Notch target genes [13, 17–19]. With SHARP (also named MINT), a functional homologue of H has been identified in vertebrates [9, 11, 20, 21]. SHARP binds CSL in a bipartite manner, i.e. both within the beta-trefoil domain and the C-terminal domain, resembling the interaction of mammalian corepressors as well as of H with CSL [22].

Unexpected for a transcription factor, CSL apparently relies on its cofactors for nuclear entry. For example, mutations of CBF1/RBPJ in the beta-trefoil domain affecting both, the binding of NICD as well as of corepressors, prevented nuclear entry and Notch target gene activation [23]. Similarly, Su(H) nuclear entry depended on NICD in a Drosophila cell culture system; hence it may not enter the nuclear compartment on its own [24, 25]. Moreover, tissue-specific overexpression of H protein caused Su(H) nuclear accumulation, whereas Su(H) protein levels appeared reduced in the absence of H protein [26–28]. In fact, it was demonstrated that Su(H) protein stability depends on formation of transcription complexes together with H and NICD, respectively [28].

Subcellular localization of Hairless and suppressor of Hairless protein

We recently addressed the subcellular localization of H and Su(H) proteins in Drosophila tissue and showed that Su(H) protein strictly follows the subcellular localization of H [29]. H protein contains three potential nuclear localization signals NLS1–3, with NLS3 being the most effectual. Accordingly, H NLS3 mutant protein defective in NLS3 accumulated within the cytosol. In addition, a nuclear export signal NES, juxtaposed to NLS3, proved relevant for the export of H protein from the nuclear compartment. Mutation of the NES resulted in nuclear retention of H NES protein in larval tissues. Endogenous Su(H) protein co-localized with H protein, i.e. it was cytosolic when the H NLS3 mutant was overexpressed and nuclear in cells expressing H NES [29]. A double mutant H NLS3 NES had an intermediate effect, and either protein distribution resembled the wild type situation, demonstrating the importance of the NES in H and Su(H) export. Overall our data implied, that H mediated shuttling of Su(H) between the nucleo-cytosolic compartments provided a means of regulating Notch activity by restricting nuclear availability of Su(H). Here we asked, whether mammalian CSL protein might be subjected to a similar mode of regulation. The fact that nuclear import of CBF1/RBPJ is dependent on its cofactors as well makes this hypothesis very likely. Moreover, in yeast two-hybrid assays murine CBF1/RBPJ was shown capable of binding H with its C-terminal domain similar to Su(H) [14, 15, 30].

Murine RBPJ protein follows the subcellular distribution of Hairless protein in the fly

To address the potential role for H on nucleo-cytoplasmic shuttling of mammalian CSL, we made use of a ‘murinized’ fly model which we recently established [30]. In these flies, the endogenous Su(H) locus has been replaced by the murine CSL orthologue RBPJ using genome engineering. Interestingly, RBPJ flies are viable with subtle phenotypes, demonstrating that the murine CSL orthologue can replace the majority of Su(H) activities during fly development [30]. The RBPJ flies allowed us to test, whether RBPJ protein is subjected to H-mediated nuclear localization like its fly homologue Su(H), i.e. nuclear import – as expected by a likewise nuclear import of CBF1/RBPJ by corepressors – as well as nuclear export, as uncovered for Su(H) in Drosophila.

We applied the Gal4-UAS system [31] for a tissue specific overexpression of H protein mutant in a nuclear translocation signal, as this setting allows following the distribution of endogenous CSL protein within larval tissue [29]. For the overexpression, we used sd-Gal4 [32] driving UAS-H NES transgene expression in the larval salivary glands, where subcellular protein localization can be easily visualized in the cytoplasm and nuclei of the giant cells [29]. To this end, we first combined the RBPJ flies bearing 2nd chromosome with the sd-Gal4 line and the UAS-H NES transgenes, respectively, to generate driver and effector lines in the RBPJ genetic background (Fig. 1). Four RBPJ bearing effector lines were established: UAS-H NES as control, UAS-H NES NES defective for nuclear import, UAS-H NES NES defective for nuclear export, and UAS-H NES NES affecting both, import- and export signal [29].

Each effector line was crossed with the sd-Gal4; RBPJ fliesdriver line to induce the overexpression of the respective H NES protein in the salivary glands of RBPJ larvae. Staining of the salivary glands then revealed the subcellular distribution of H NES and RBPJ NES protein, respectively (Fig. 2a). As shown earlier [29], H NES protein was mostly cytoplasmic, whereas all other H NES variants were detected in both, cytosolic and nuclear compartment. Notably, H NES appeared more strongly enriched in the nucleus than H NES and H NES NES protein (Fig. 2a). As predicted from the subcellular localization signals NLS3, with NLS3 being the most effectual. Accordingly, H NLS3 mutant protein defective in NLS3 accumulated within the cytosol. In addition, a nuclear export signal NES, juxtaposed to NLS3, proved relevant for the export of H protein from the nuclear compartment. Mutation of the NES resulted in nuclear retention of H NES protein in larval tissues. Endogenous Su(H) protein co-localized with H protein, i.e. it was cytosolic when the H NLS3 mutant was overexpressed and nuclear in cells expressing H NES [29]. A double mutant H NLS3 NES had an intermediate effect, and either protein distribution resembled the wild type situation, demonstrating the importance of the NES in H and Su(H) export. Overall our data implied, that H mediated shuttling of Su(H) between the nucleo-cytosolic compartments provided a means of regulating Notch activity by restricting nuclear availability of Su(H). Here we asked, whether mammalian CSL protein might be subjected to a similar mode of regulation. The fact that nuclear import of CBF1/RBPJ is dependent on its cofactors as well makes this hypothesis very likely. Moreover, in yeast two-hybrid assays murine CBF1/RBPJ was shown capable of binding H with its C-terminal domain similar to Su(H) [14, 15, 30].

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localization of Su(H) [29], RBPJ<sub>wt</sub> protein was cytosolic when H<sup>*NLS3</sup> protein was overexpressed, and detected in the nuclear compartment as well in the presence of any other H<sup>*</sup> protein variant (Fig. 2a). To confirm the visual impression, we quantified the staining intensities of confocal micrographs on eight specimen each for every genotype, comprising a minimum of 160 nuclei. Composite Z-stacks crossing the entire gland were segmented into nuclei and cytoplasm, and mean grey values were recorded. The results confirm that murine RBPJ<sub>wt</sub> protein is shuttled by H protein the same way as is Su(H) protein (Fig. 2b). Overexpression of the wild type protein isoform H<sup>cwt</sup> caused strong accumulation of RBPJ<sub>wt</sub> protein in the nucleus, and even stronger, when H<sup>*NES</sup> was overexpressed. In contrast, overexpression of H<sup>*NLS3</sup> resulted in the retention of RBPJ<sub>wt</sub> in the cytoplasm, whereas that of H<sup>*NLS3*NES</sup> allowed RBPJ<sub>wt</sub> protein to re-enter the nucleus (Fig. 2b). Briefly, we observed a nucleo-cytosolic shuttling of RBPJ<sub>wt</sub> protein, which strictly followed H protein distribution in the salivary glands of <em>Drosophila</em> larvae.

RBPJ<sub>wt</sub> protein accumulated significantly stronger in nuclei upon the overexpression of H<sup>*NES</sup> (Fig. 2) which is defective in the nuclear export signal [29]. Evidently, RBPJ<sub>wt</sub> is subjected to nuclear export by wild type H protein similar to Su(H). The importance of nuclear export of CSL-H has not been elucidated yet. We know, however, that the H-NES is relevant for fly survival, as in its absence, only a fraction of the animals developed to adulthood [29]. In mouse cells, the tubulin-binding protein RITA induced nuclear export of RBPJ<sub>wt</sub>, thereby downregulating Notch-mediated transcription [33]. The more important roles of RITA, however, lie in the regulation of microtubule dynamics during mitosis and cell motility [34, 35]. Albeit its high conservation in the animal kingdom, RITA has no fly homologue. Accordingly, despite binding to Su(H) and tubulin, human RITA has no biological effect on the subcellular distribution or the stability of Su(H) protein in the fly [36]. In contrast to mammalian cells, sequestration of Su(H) by a tubulin-tether in the cytosolic compartment does not occur [36]. Nevertheless, regulation of nuclear availability of CSL proteins appears an important layer of regulation during the transduction of Notch signals in vertebrates and equally in invertebrates.

**Conclusion**

Nucleo-cyttoplasmic shuttling of Su(H) as a means of regulating Notch signaling activity in the fly has been already shown. Here we demonstrate that murine RBPJ<sub>wt</sub> is subjected to a likewise dynamic nucleo-cyttoplasmic shuttling by H protein in vivo in <em>Drosophila</em> tissue.
These data support the hypothesis that nuclear availability of mammalian CSL proteins is restricted by their cofactors, on which they depend for nuclear import. Moreover, murine RBPJ protein is also subjected to nuclear export by H protein. Overall, our data demonstrate the requirement of corepressors for CLS nuclear translocation, emphasizing the additional layer of regulation at the level of nuclear availability.

**Methods**

The genome engineered fly stock \( RBPJ^{mut} / \text{CyO-GFP} \) contains murine RBPJ cDNA (isoform 1; the N-terminal 128 codons are derived from Su(H) fused at Val-codon 81 to RBPJ) in place of wild type Su(H) \([30]\). The stock was combined with \( \text{sd-Gal4} \) (BL8609) to generate a driver line, and with either \( \text{UAS-H}_{cwt}, \text{UAS-H}^{NLS3}, \text{UAS-H}^{NES} \) or \( \text{UAS-H}^{NLS3\_NES} \) [29] to generate an effector line, by standard genetic crosses as outlined in Fig. 1. To this end, we made use of the dominant markers \( \text{sha}^{Sec} \) (BL9325) and \( L^2 \) (BL319) [37], and a doubly balanced \( \text{cyc}^{HR7} \) allele [38], to be able to follow unambiguously every chromosome through all generations. Driver and effector lines were crossed, and offspring reared at 25 °C to eventually analyse the salivary glands at third instar larval stage. The homozygous \( RBPJ^{mut} \) animals were recognized by the lack of GFP, otherwise marking the heterozygous siblings due to the CyO-GFP (BL9325) marker. A Leica MZ FLIII UV stereo-microscope (Leica, Wetzlar, Germany) illuminated with CoolLED pE-300 white (AHF, Tübingen, Germany) was used for the purpose of selecting the larvae.

Respective UAS-constructs were expressed in the developing salivary glands using \( \text{sd-Gal4} \). To visualize H and RBPJ protein expression, immuno-cytochemistry on third instar larval salivary glands was performed as outlined before, with a 20 min fixation with 4%
paraformaldehyde [29]. As primary antibodies, we used guinea pig anti-Hairless A (1:500) [27] and rabbit anti-RBPSUH (1:200) (D10A4; Cell Signaling Technology, Cambridge, UK). Goat secondary antibodies (1:250), coupled to FITC or Cy3 were obtained from Jackson Immuno-Research (Dianova, Hamburg, Germany). Fluorescently labelled tissue was mounted in Vectashield (Vector labs, Eching, Germany). Pictures were taken with a Zeiss Axioskop (Carl Zeiss, Jena, Germany), coupled to a BioRad MRC1024 confocal microscope (Carl Zeiss, Jena, Germany; O.S.T.I. microscopy, Milano, Italy) using LaserSharp 2000™ software. The presented figures were created using ImageJ, PhotoPaint and CorelDraw software.

Quantification of H and Su(H) protein in salivary glands overexpressing the specific H* nuclear localization mutant was performed based on confocal micrographs using Image J software. Z-stacks crossing the entire glands with 1 μm increments were used for maximum projection. Composite images were segmented into nuclei and cytoplasm. Nuclei were defined as region of interest, and subtracted from the outline of the whole gland, defining the cytoplasm. Mean grey values of nuclei and corresponding cytoplasm of the entire gland were recorded [29]. Eight glands each with a total of at least 160 nuclei were analyzed. Statistical significance was determined by ANOVA two-tailed Dunnett’s approach for multiple comparisons.

**Abbreviations**

CBF1: C promoter binding factor 1 (mammalian); CSL: CBF1/RBPJ, Su(H), Lag-1 (acronym); Gal4: galactose responsive transcription factor (from S. cerevisiae); GFP: Green fluorescent protein (from A. Victoria); H: Hairless (from D. melanogaster); HDAC1: Histone deacetylase 1 (mammalian); Lag1: Abnormal cell lineage 12 (lin-12) and abnormal germ line proliferation phenotype-a (glp-1) (from C. elegans); Mam: Mastermind from D. melanogaster; corresponds to mammalian Maml (mastermind like transcriptional coactivator); MINT: Msh2-interacting nuclear target (mammalian); NCoR2: Nuclear receptor co-repressor 2 (mammalian); NES: Nuclear export signal; NIDC: Notch intracellular domain; NLS: Nuclear localization signal; RAM: RBP-JKappa-associated module (part of NIDC); RBPJ: Recombination signal binding protein for immunoglobulin kappa J region (mammalian); RITA: RBPJ interacting and tubulin associated, also named ZNF331 (mammalian); SHARP: SMRT/HDAC1-associated repressor protein (mammalian); SMRT (or NCoR2): Silencing mediator for retinoid and thyroid hormone receptors (mammalian); Suppressor of Hairless from D. melanogaster; UAS: Upstream activating sequences (Gal4 target sequence); ZNF331: Zinc finger protein 331 (mammalian)

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**Authors’ contributions**

DM and ACN conceived of the work. DW performed the histological examination of the specimen. DW, DM and ACN analyzed, interpreted and validated the data. DM and ACN supervised the work and provided resources and funding. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Kelleher FC, Fennelly D, Rafferty M. Common critical pathways in embryogenesis and cancer. Acta Oncol. 2006;45(4):375–88. https://doi.org/10.1080/0284186060062946.
2. Siebel C, Lendahl U. Notch signaling in development, tissue homeostasis, and disease. Physiol Rev. 2017;97(4):1235–94. https://doi.org/10.1152/physrev.00005.2017.
3. Kolan R, Ilagan MXG. The canonical Notch signaling pathway: unfolding the activation mechanism. Cell. 2009;137(2):216–33. https://doi.org/10.1016/j.cell.2009.03.048.
4. Bray SJ. Notch signalling in context. Nat Rev Mol Cell Biol. 2016;17(11):722–35. https://doi.org/10.1038/nrm.2016.94.
5. Bray SJ, Gomez-Lamarca M. Notch after cleavage. Curr Opin Cell Biol. 2018;51:103–9. https://doi.org/10.1016/j.celrep.2017.12.008.
6. Sjoqvist M, Andersson ER. Do as I say, Not (ch) as I do: lateral control of cell fate. Dev Biol. 2019;447(1):58–70. https://doi.org/10.1016/j.ydbio.2019.07.032.
7. Gordon WR, Arnett KL, Blacklow SC. The molecular logic of Notch signalling—a structural and biochemical perspective. J Cell Sci. 2008;121(19):3109–19. https://doi.org/10.1242/jcs.035683.
8. Koval RA, Blacklow SC. Mechanistic insights into Notch receptor signaling from structural and biochemical studies. Curr Top Dev Biol. 2010;92:31–71. https://doi.org/10.1016/S0070-2153(09)02002-4.
9. Borggreve T, Oswald F. The Notch signaling pathway: transcriptional regulation at Notch target genes. Cell Mol Life Sci. 2009;66(10):1631–46. https://doi.org/10.1007/s00018-009-8668-7.
10. Wang H, Zang C, Liu XS, Aster JC. The role of Notch receptors in transcriptional regulation. J Cell Physiol. 2015;230(5):982–8. https://doi.org/10.1002/jcp.24872.
11. Maier D. The evolution of transcriptional repressors in the Notch signalling pathway: a computational analysis. Hereditas. 2019;156(1).5. https://doi.org/10.11186/s41066-019-0081-0.
12. Hall DP, Koval RA. Structurally conserved binding motifs of transcriptional regulators to Notch nuclear effector CSL. Exp Biol Med (Maywood). 2019;244:1520–9.
13. Maier D. Hairless: the ignored antagonist of the Notch signalling pathway. Hereditas. 2006;143(2006):212–21. https://doi.org/10.1111/j.2007.0018-0661.01971.x.
14. Maier D, Kurth P, Schulz A, Russell A, Yuan Z, Gruber K, Koval RA, Preiss A. Structural and functional analysis of the repressor complex in the Notch signaling pathway of Drosophila melanogaster. Mol Biol Cell. 2011;22(17):3542–52. https://doi.org/10.1091/mbc.e11-05-0420.
15. Yuan Z, Praxenthaler H, Tabaja N, Torella R, Preiss A, Maier D, Koval RA. Structure and function of the Su(H)-Hairless repressor complex, the major antagonist of Notch signaling in Drosophila melanogaster. Mol Biol Cell. 2016;14(7):e1002509. https://doi.org/10.1017/jcb.2016.1002509.
16. Borggreve T, Oswald F. Setting the stage for Notch: the Hairless-Su(H)-Hairless repressor complex. PLoS Biol. 2016;14(7):e1002524. https://doi.org/10.1371/journal.pbio.1002524.
17. Morel V, Lecourtou M, Massioni O, Maier D, Preiss A, Schweisguth F. Transcriptional repression by Suppressor of Hairless involves the binding of a Hairless-dCtBP complex in Drosophila. Curr Biol. 2001;11(10):789–92. https://doi.org/10.1016/S0960-9822(01)00224-X.
18. Barolo S, Stone T, Bang AG, Posakony JW. Default repression and Notch signaling: Hairless acts as an adaptor to recruit the corepressors Groucho and dCBP to Suppressor of Hairless. Genes Dev. 2002;16(15):1964–76. https://doi.org/10.1101/gad.987402.

19. Nagel AC, Kiecić A, Terin G, Bravo-Patiño A, Bray S, Maier D, Preiss A. Hairless-mediated repression of Notch target genes requires the combined activity of Groucho and CBP corepressors. Mol Cell Biol. 2005;25(23):10433–41. https://doi.org/10.1128/MCB.25.23.10433-10441.2005.

20. Oswald F, Kostedka U, Astrahantseff K, Bouettee S, Dillinge K, Zechner U, Ludwig L, Wilda M, Hameister H, Köchel W, Lipay S, Schmid RM. SHARP is a novel component of the Notch/RBP-Jkappa signaling pathway. EMBO J. 2002;21(20):5417–27. https://doi.org/10.1093/emboj/cdf549.

21. Kurosaki K, Han H, Tani S, Tanigaki K, Tsum T, Funakawa T, Taniguchi Y, Kurooka H, Harnada T, Toyokuni S, Honjo T. Regulation of marginal zone B cell development by MINT, a suppressor of Notch/RBP-J signaling pathway. Immunity. 2003;18(2):301–12. https://doi.org/10.1016/S1074-7613(03)00029-3.

22. Yuan Z, VanderWelen BD, Gaiomo BD, Pan L, Collins CE, Turkiewicz A, Hein K, Oswald F, Borggreve T, Novall RA. Structural and functional studies of the RBPI-SHARP complex reveal a conserved corepressor binding site. Cell Rep. 2019;26:845–854.e6.

23. Zhou S, Hayward SD. Nuclear localization of CBFI is regulated by interactions with the SMRT corepressor complex. Mol Cell Biol. 2001;21(18):6222–32. https://doi.org/10.1128/MCB.21.18.6222-6232.2001.

24. Fortini ME, Artavanis-Tsakonas S. The Suppressor of Hairless protein participates in Notch receptor signaling. Cell. 1994;79(2):273–82. https://doi.org/10.1016/0092-8674(94)90196-1.

25. Furriols M, Bray S. Dissecting the mechanisms of Suppressor of Hairless function. Dev Biol. 2000;227(2):520–32. https://doi.org/10.1006/dbio.2000.9923.

26. Maier D, Nagel AC, Johannes B, Preiss A. Subcellular localization of Hairless protein shows a major focus of activity within the nucleus. Mech Dev. 1999; 89(1-2):195–9. https://doi.org/10.1016/S0925-4773(99)00208-7.

27. Maier D, Praxenthaler H, Schulz A, Preiss A. Gain of function Notch phenotypes associated with ectopic expression of the Su(H) C-terminal domain illustrate separability of Notch and Hairless-mediated activities. PLoS One. 2013;8(11):e81578. https://doi.org/10.1371/journal.pone.0081578.

28. Praxenthaler H, Nagel AC, Schulz A, Zimmermann M, Meier M, Schmid H, Preiss A, Maier D. Hairless-binding deficient Suppressor of Hairless alleles reveal Su(H) protein levels are dependent on complex formation with Hairless. PLoS Genet. 2017;13(5):e1006774. https://doi.org/10.1371/journal.pgen.1006774.

29. Gahr BM, Brändle F, Zimmermann M, Nagel AC. An RBPI-Drosophila model reveals the dependence of RBPJ protein stability on the formation of transcription-regulator complexes. Cells. 2019;8(10):1252. https://doi.org/10.3390/cells8101252.

30. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 1993;118(2):401–15.

31. Roy S, Shashidhara LS, VijayRaghavan K. Muscles in the Drosophila second thoracic segment are patterned independently of autonomous homeotic gene function. Curr Biol. 1997;7(4):222–7. https://doi.org/10.1016/S0960-9822(06)0117-3.

32. Wacker SA, Alvarado C, von Wichert G, Knippschild U, Wiedenmann J, Clauss M. Hereditas. 2019;156(1):37. https://doi.org/10.1186/s41065-019-0113-9.

33. Brockmann B, Mastel H, Oswald F, Maier D. Analysis of the interaction between human RITA and Drosophila Suppressor of Hairless. Hereditas. 2014;151(6):209–19. https://doi.org/10.1111/hrd.200074.

34. Maier D, Nagel AC, Preiss A. Genetic interactions between Protein Kinase D and Lobe mutants during eye development of Drosophila melanogaster. Hereditas. 2016;159(1):37. https://doi.org/10.1111/hrd.12555–63. https://doi.org/10.1242/jcs.113902.

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