The stress hormone cortisol induces virulence in the oral microbiome

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ABSTRACT

The human microbiome maintains under normal conditions a state of homeostasis. Disruption of homeostasis also referred to as dysbiosis, has been shown to be causally linked to the development of a variety of host diseases. These dysbiotic microbial communities exhibit synergistic interactions for enhanced protection from host defenses, nutrient acquisition, and persistence in an inflammatory environment. One factor that has been shown to lead to dysbiosis of the microbiome is exposure to psychological stressors. Throughout evolution microorganisms of the human microbiome have developed systems for sensing host-associated signals such as hormones associated with those stressors, enabling them to recognize essential changes in its environment thus changing its expression gene profile to fit the needs of the new environment. Cortisol or hydrocortisone is the primary hormone responsible for the stress response, and its levels increase in saliva and serum with the severity of periodontal disease. Most studies have focused on the effect of hormones on growth or virulence of individual organisms. Here we present the impact that cortisol had on the community-wide transcriptome of the oral community. We used a metatranscriptomic approach to obtain first insights into the metabolic changes induced by this stress hormone as well as which members of the oral microbiome respond to the presence of cortisol in the environment.

IMPORTANCE

Imbalances of the microbiome also referred to as microbial dysbiosis, lead to a series of different oral diseases. One fundamental question to be answered regarding the pathogenesis of polymicrobial diseases is what are the molecular mechanisms that lead to dysbiosis. The most widely accepted theory to explain the ability of hormones to influence the course of infection involves the suppression of the immune system. Commensal microbiota is involved in stressor-induced immunomodulation, but other biological effects are not yet known. These results bring new insights into the importance of stress

38 hormones such as cortisol as a signal used by the oral microbiome in periodontal disease. Our findings
39 suggest that cortisol can induce virulence of the oral microbiome directly and that Fusobacteria respond
40 rapidly to changes in concentrations of this stress hormone.

In recent years a considerable effort has been placed on characterizing the different microbial communities colonizing the human body (1). However, the nature of host-microbial interactions in the microbiome that allow for the maintenance of a stable microbiota is still poorly understood. Among the environmental factors that may alter the equilibrium in host-microbiome homeostasis, host-stress is a known risk factor for a variety of diseases. In case of acute stress, stress response may prepare the immune system for challenges such as infection, but when it becomes chronic, it may influence inflammatory processes leading to the development of systemic or local diseases such as rheumatoid arthritis (2), diabetes (3), or periodontitis (4). Furthermore, physiological stress can also alter the composition of the commensal microbiota in the human microbiome (5).

The most widely accepted theory to explain the ability of hormones to influence the course of infection involves the suppression of the immune system. According to this model, stress can activate the central nervous system and the hypothalamus releases corticotropin-releasing hormone and arginine vasopressin that stimulates the release of adrenocorticotropin from the pituitary, which in turn results in the production of cortisol by the adrenal cortex. Glucocorticoids, including cortisol, depress immunity by inhibiting the production of secretory immunoglobulins, and neutrophil functions, all of which may impair defense against infection by periodontal microorganisms (6). However, almost immediately following its first use, cases of adrenaline-associated sepsis were reported (7). It was demonstrated that the dose of *Clostridium* needed to cause infection was significantly smaller when was injected in the presence of a therapeutic level of adrenaline (8). Since then, there have been reports associating the levels of neuroendocrine hormones, such as adrenaline, with infectious diseases, suggesting organisms themselves directly respond to the presence of stress hormones. The study of these interactions has been termed ‘microbial endocrinology' (9, 10). Microorganisms that have evolved systems for sensing host-associated signals such as hormones would have an evolutionary advantage over those that have not. Detecting such signals enables the microbiome to recognize essential changes in its environment

thus changing its expression gene profile to fit the needs of the new environment.

Although most investigations of stress hormones induction of growth and virulence have been carried out with gut-associated bacteria, a few studies have shown that stress hormones have a significant effect on the growth of periodontal pathogens (11, 12). Cortisol or hydrocortisone is the primary hormone responsible for the stress response, and its levels increase in saliva and serum with the severity of periodontal disease (13, 14) Here we present the effect that cortisol had on the community-wide transcriptome of the oral community. We used a metatranscriptomic approach to obtain first insights into the metabolic changes induced by this stress hormone as well as which members of the oral microbiome respond to the presence of cortisol in the environment.

Our first experiments consisted of treating samples of oral biofilm with cortisol at a concentration found in the saliva of patients with periodontitis (13, 15). After only 2 hours of incubation in the presence of cortisol, we proceed to perform the analysis, avoiding possible changes related to the growth of individual members of the microbiome and not to the presence of the hormone itself (see Materials and Methods). We assigned the phylogenetic origin of those sequences using Kraken, and phylogenetic profiles were used to identify significant differences between active communities under the different conditions studied by performing linear discriminant analysis (LDA) effect size (LEfSe). Among all the organisms in the oral community, members of the phylum Fusobacteria (class Fusobacteriia and order Fusobacteriales) were significantly more active after the addition of cortisol (Fig. 1). One species, *Leptotrichia goodfellowii*, was substantially more active (Fig. 1b). Species belonging to the *Fusobacteriales*, such as *Fusobacterium nucleatum* have been associated with a wide variety of human diseases, other than periodontitis, including adverse pregnancy outcome, GI disorders (e.g., colorectal cancer, inflammatory bowel disease), cardiovascular disease, rheumatoid arthritis and respiratory tract infections (16). *Leptotrichia* species are typically part of the commensal flora in the oral cavity and genitourinary tract and are seldom found in clinically significant specimens. However,

Leptotrichia has been found to be in higher proportion in gingivitis (17, 18)

We then looked at how cortisol influenced the profiles of expression of the oral microbiome. To this end, we performed enrichment of Gene Ontology (GO) terms analysis, and we observed that, after only 2 hours of exposure to the hormone, the profiles of activities of the whole community were similar to what we previously found in periodontitis progression (19, 20). Interestingly, GO terms linked to host immune response were also over-represented when cortisol was present (Fig. 2a). These results agree with the observation that people with periodontal disease present higher levels of cortisol in the gingival crevicular fluid (13), a serum exudate in direct contact with the oral microbiome. As previously indicated potassium ion transport was significantly under-represented when cortisol was added (Fig. 2b), mimicking ours *in vivo* observations of periodontitis progression (21). In a follow-up manuscript, we demonstrated that ion potassium is a signal in functional dysbiosis of periodontal disease (22). Interestingly, when we look at the activities associated with putative virulence factors, we found a similar pattern of over-represented GO terms related to the addition of cortisol (Fig. S1). Moreover, the more significant fraction of up-regulated putative virulence factors seems to be synthesized by members of the genus Streptococcus (Fig. S2). We observed similar results in our two previous studies on periodontitis progression (21) and effect of ion potassium in functional dysbiosis of the oral microbiome (22). As a whole, these results seem to indicate that the presence of cortisol leads to a community-wide response very similar to the one observed *in vivo* during periodontitis.

We then performed transcriptome analysis of the effect of cortisol on organisms that we saw were more active in the presence of cortisol (Fig. 1): *Leptotrichia goodfellowii* and *Fusobacterium nucleatum*, which is a representative of the order Fusobacteriales and an essential member of the oral microbiome. In just 2 hours of exposure, there was a shift in their transcriptome profiles. *F. nucleatum* showed an increase in biological processes GO terms associated with proteolysis, cobalamin biosynthesis and iron transport (Fig. S3a), which we have previously found associated with the

progression of periodontal disease (19, 20). In the case of *L. goodfellowii*, we also found activities related to iron ion transport (Fig. S3b). The intersection of metabolic events that were commonly altered in *F. nucleatum* and *L. goodfellowii* was associated with the growth of the organisms such as lipid A biosynthesis, DNA replication or translation (Fig. S3), which indicates activation of their metabolism. These results are in agreement with the effects observed for the whole oral microbiome where members of the Fusobacteriales order are more active when cortisol was added to the medium, (Fig. 1). Likewise, the intersection of common molecular functions enriched with the addition of cortisol in *F. nucleatum* and *L. goodfellowii* include iron acquisition (iron-ion binding and iron-ion transmembrane transporter activity) and peptidase activities (serine-type endopeptidase activity) (Fig. S4).

Altogether, these results show that exposure to cortisol in the oral microbiome increases its virulence, mimicking previous observations on periodontitis *in vivo*. Our results also highlight the importance of *Fusobacteria* and *Leptotrichia* as the first members of the community to respond to the increase of cortisol in the environment.

Data Availability. The sequence datasets used in these analyses were deposited at the Human Oral Microbiome Database (HOMD) under the submission number 20180522 (ftp://homd.org/publication_data/20180522/).

ACKNOWLEDGMENTS

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Figure Legends

Figure 1. Statistical differences in the phylogenetic composition of active communities. Metatranscriptome hit counts were obtained using Kraken against an oral microbiome database. Counts were then analyzed using LEfSe to identify significant differences at the species level between the microbial communities compared. a) Taxonomic representation of statistically and biologically consistent differences between controls without cortisol [green] vs. samples where cortisol was added [red]. b) Histogram of the LDA scores computed for features deferentially abundant between controls without cortisol [green] vs. samples where cortisol was added [red].

Figure 2. GO enrichment analysis comparing oral microbiome response to the presence and absence of added cortisol to the medium. Enriched terms obtained using GOseq were summarized and visualized as a scatter plot using REVIGO. Only GO terms with FDR adjusted p-value < 0.05 in the 'GOseq' analysis were used. A) Summarized GO terms related to biological processes after addition of cortisol. B) Summarized GO terms related to biological processes with no cortisol added. Circle size is proportional to the frequency of the GO terms; color indicates the log10 p-value (red higher, blue lower). The distance between circles represents GO terms' semantic similarities. Each of the circles represents a GO term, which depending on the similarity in the terms included in them they will be closer or more distant in the graph.

Supporting Information Legends

Figure S1. Community-wide GO enrichment analysis of deferentially expressed virulence factors in response to the presence and absence of added cortisol to the medium. Putative virulence factors were identified by alignment of the protein sequences from the different genomes against the Virulence Factors Database (VFDB) as described in the methods section. Enriched terms obtained using GOseq were summarized and visualized as a scatter plot using REVIGO. Only GO terms with FDR adjusted p-value < 0.05 in the 'GOseq' analysis were used. A) Summarized GO terms related to biological processes after addition of cortisol. B) Summarized GO terms related to biological processes with no cortisol added. Circle size is proportional to the frequency of the GO terms; color indicates the log10 p-value (red higher, blue lower). The distance between circles represents GO terms' semantic similarities. Each of the circles represents a GO term, which depending on the similarity in the terms included in them they will be closer or more distant in the graph. In red are activities we have previously seen associated with periodontitis (19, 21).

Figure S2. Ranked species by the number of up-regulated putative virulence factors in the metatranscriptome. Putative virulence factors were identified by alignment of the protein sequences from the different genomes against the Virulence Factors Database (VFDB) as described in the methods section. Numbers in the graph refer to the percentage of hits for the different species for the putative virulence factors identified. We selected only species whose percentage of putative virulence factors from the total of the community was higher than 1%. We included also results from 2 previous studies one on periodontal disease progression (21) and another where we showed that potassium was a crucial signal in dysbiosis (22). In red, species that were ranked at the top of the three studies.

Figure S3. Biological processes GO terms associated with changes in gene expression profiles in *Fusobacterium nucleatum* and *Leptotrichia goodfellowii*. GO terms were assigned to deferentially expressed genes due to the addition of cortisol and summarized using REVIGO. a) GO terms associated with up-regulated genes in *F. nucleatum* b) GO terms associated with down-regulated genes *L. goodfellowii*. In green are metabolic activities that were associated with up-regulated genes in both *F. nucleatum* and *L. goodfellowii*. In red are activities we have previously seen associated with periodontitis (19, 21).

Figure S4. Common molecular function GO terms associated with changes in gene expression profiles in *Fusobacterium nucleatum* and *Leptotrichia goodfellowii*. GO terms were assigned to deferentially expressed genes due to the addition of cortisol and summarized using REVIGO. Networks of over-represented molecular functions from *F. nucleatum* and *L. goodfellowii* were then uploaded to Cytoscape, and the intersection of the two networks was extracted and plotted as shown in the figure. In red are activities we have previously seen associated with periodontitis (1, 22).

193 **References**

1. Human Microbiome Project Consortium. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486:207–214.

2. McCray CJ, Agarwal SK. 2011. Stress and autoimmunity. Immunol Allergy Clin North Am 31:1–18.

3. Marcovecchio ML, Chiarelli F. 2012. The effects of acute and chronic stress on diabetes control. Sci Signal 5:pt10.

4. Akcali A, Huck O, Tenenbaum H, Davideau JL, Buduneli N. 2013. Periodontal diseases and stress: a brief review. J Oral Rehabil 40:60–68.

5. Gur TL, Worly BL, Bailey MT. 2015. Stress and the Commensal Microbiota: Importance in Parturition and Infant Neurodevelopment. Front Psychiatry 6.

6. Genco RJ, Ho AW, Kopman J, Grossi SG, Dunford RG, Tedesco LA. 1998. Models to evaluate the role of stress in periodontal disease. Ann Periodontol 3:288–302.

7. Freestone P. 2013. Communication between Bacteria and Their Hosts. Scientifica (Cairo) 2013:361073.

8. Evans DG, Miles AA, Niven JSF. 1948. The enhancement of bacterial infections by adrenaline. Br J Exp Pathol 29:20–39.

9. Lyte M. 2013. Microbial Endocrinology in the Microbiome-Gut-Brain Axis: How Bacterial Production and Utilization of Neurochemicals Influence Behavior. PLoS Pathog 9.

10. Freestone PPE, Sandrini SM, Haigh RD, Lyte M. 2008. Microbial endocrinology: how stress influences susceptibility to infection. Trends Microbiol 16:55–64.

11. Jentsch HFR, März D, Krüger M. 2013. The effects of stress hormones on growth of selected periodontitis related bacteria. Anaerobe 24:49–54.

12. Roberts A, Matthews JB, Socransky SS, Freestone PPE, Williams PH, Chapple ILC. 2002. Stress and the periodontal diseases: effects of catecholamines on the growth of periodontal bacteria in vitro. Oral Microbiol Immunol 17:296–303.

13. Rai B, Kaur J, Anand SC, Jacobs R. 2011. Salivary stress markers, stress, and periodontitis: a pilot study. J Periodontol 82:287–292.

14. Ishisaka A, Ansai T, Soh I, Inenaga K, Awano S, Yoshida A, Hamasaki T, Sonoki K, Takata Y, Nishihara T, Takehara T. 2008. Association of cortisol and dehydroepiandrosterone sulphate levels in serum with periodontal status in older Japanese adults. J Clin Periodontol 35:853–861.

15. Cakmak O, Tasdemir Z, Aral CA, Dundar S, Koca HB. 2016. Gingival crevicular fluid and saliva stress hormone levels in patients with chronic and aggressive periodontitis. J Clin Periodontol 43:1024–1031.

16. Han YW. 2015. Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol 23C:141–147.

17. Park O-J, Yi H, Jeon JH, Kang S-S, Koo K-T, Kum K-Y, Chun J, Yun C-H, Han SH. 2015. Pyrosequencing Analysis of Subgingival Microbiota in Distinct Periodontal Conditions. J Dent Res 94:921–927.

18. Huang S, Yang F, Zeng X, Chen J, Li R, Wen T, Li C, Wei W, Liu J, Chen L, Davis C, Xu J. 2011. Preliminary characterization of the oral microbiota of Chinese adults with and without gingivitis. BMC Oral Health 11:33.

19. Duran-Pinedo AE, Chen T, Teles R, Starr JR, Wang X, Krishnan K, Frias-Lopez J. 2014. Community-wide transcriptome of the oral microbiome in subjects with and without periodontitis. ISME J 8:1659–1672.

20. Yost S, Duran-Pinedo AE, Teles R, Krishnan K, Frias-Lopez J. 2015. Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis. Genome Medicine In press.

21. Yost S, Duran-Pinedo AE, Teles R, Krishnan K, Frias-Lopez J. 2015. Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis. Genome Med 7:27.

22. Yost S, Duran-Pinedo AE, Krishnan K, Frias-Lopez J. 2017. Potassium is a key signal in host-microbiome dysbiosis in periodontitis. PLOS Pathogens 13:e1006457.

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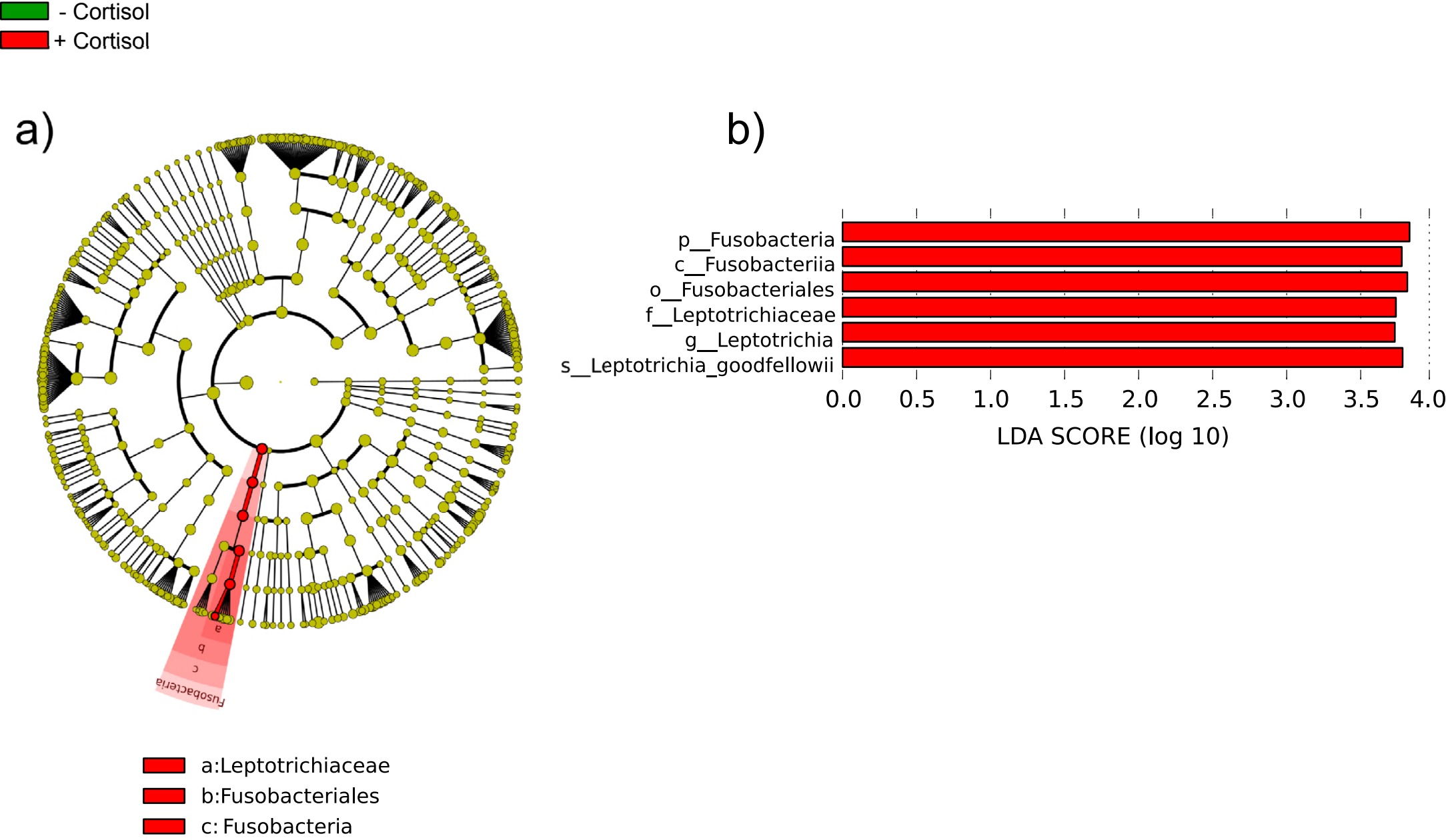

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a) Taxonomic representation of statistically and biologically consistent differences between controls without cortisol [green] vs. samples where cortisol was added [red].
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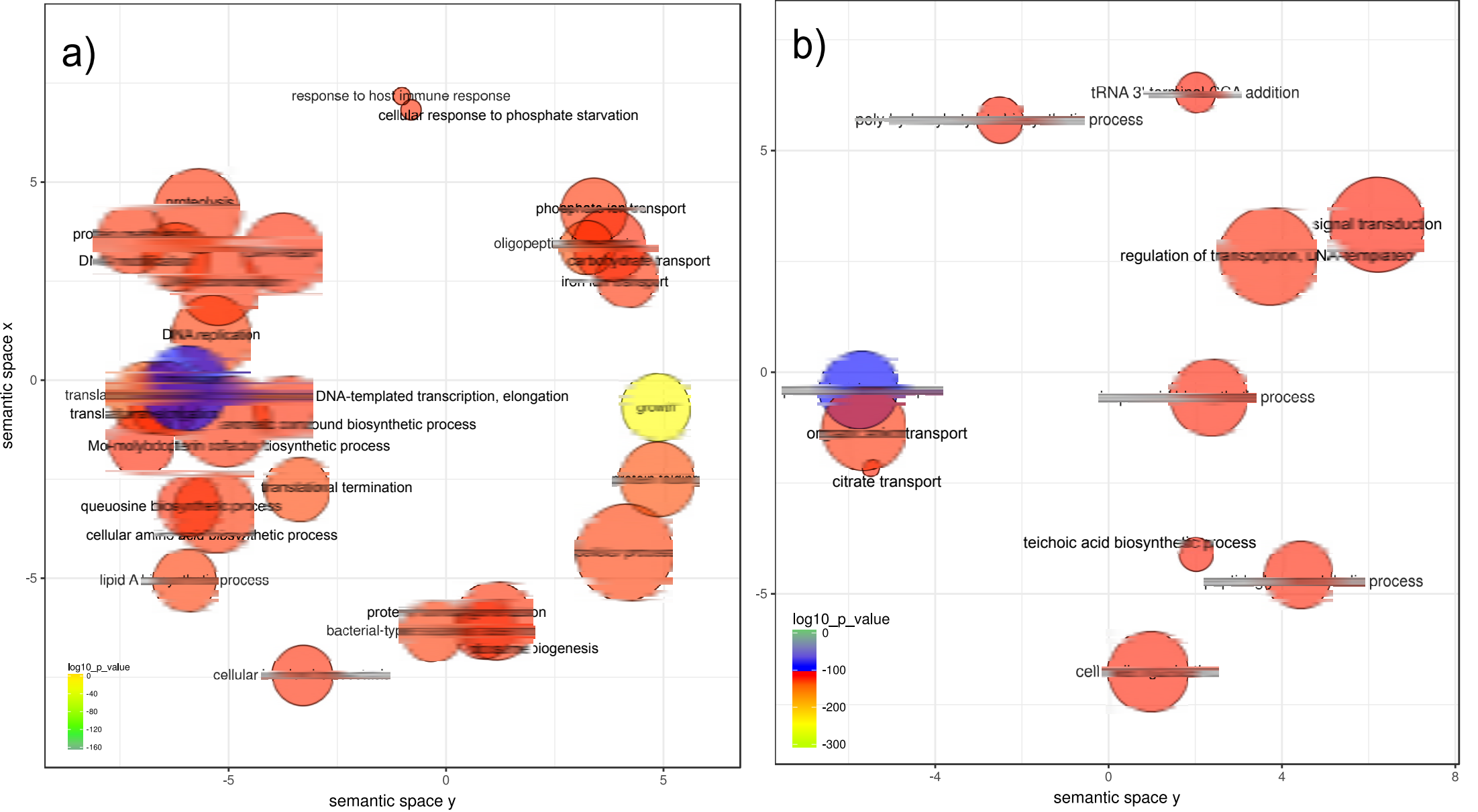

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