# Is *FURIN* gene expression in salivary glands related to SARS-CoV-2 infectivity through saliva?

Luisa Zupin ,[1] Lorella Pascolo,[2] Sergio Crovella[1,3]

[1]Genetic Immunology Laboratory, Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste, Italy
[2]Gynecology and Obstetrics Department, Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste, Italy
[3]Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy

**Correspondence to**
Dr Luisa Zupin, Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste 34137, Italy; luisa.zupin@burlo.trieste.it

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Unravelling the SARS-CoV-2 mechanism of entry into host cells is engaging the endeavours of researchers worldwide and, although angiotensin-converting enzyme 2 (ACE2) is recognised as the primary receptor, many issues remain to be investigated.[1]

Remarkably, the interaction between ACE2 and the spike (S) glycosylated protein of SARS-CoV-2 necessary for viral entry has been discovered by employing crystallography. S protein presents a receptor binding domain (RBD) and more specifically a receptor binding motif (RBM) which mediates the attachment to two virus-binding hotspots within ACE2 surface. The aminoacidic constitution of SARS-CoV-2 RBM is highly homologous to that of SARS-CoV but shows some differences, specifically a four-residue motif at 482–485 (Gly-Val-Glu-Gly) that confers more affinity for ACE2 resulting in a tight relation between the two molecules.[2]

However, S protein, a trimeric class I fusion protein, is maintained in a metastable prefusion conformation and the RBD is largely in a lying-down status, a receptor inaccessible condition, facilitating immune escape, that needs to pass through a massive reconfiguration to trigger the viral-cell fusion.[3]

Recent evidence demonstrated that other molecular strategies are exploited by SARS-CoV-2 to enter host cells, thus explaining its high infectivity. Interestingly, Shang *et al* reported the possible pre-activation of the virus by furin, acting in synergy with Transmembrane protease, serine 2 (TMPRSS2) and cathepsins.[4]

Furin is a member of the proprotein convertase family whose components mediate the regulation of different proteins. Furin is able to cleave different substrates, both endogenous and exogenous, as bacterial toxins and viral molecules; therefore, it is involved in many infectious diseases including those caused by coronavirus.[5]

*FURIN* expression is rather ubiquitary, but different isoform combinations can be found in human tissues.[6]

Notably, *FURIN* gene presents three promoters (P1, P1A, P1B) generating three different alternative transcription starting sites and 8 *FURIN* isoforms. Intriguingly, while P1A and P1B are similar to those of constitutively housekeeping genes, P1 can be stimulated by cytokines as IFN-gamma, TGF-beta and IL12. Furin is post-translational modified and activated by cleavage, then it is localised both intracellularly in endosome and *trans*-Golgi network but also at the level of the plasma membrane, and it can be secreted in the extracellular environment.[5]

Here, we investigate *FURIN* gene expression in different human tissues inquiring different online repositories, namely, the Human Protein Atlas (HPA),[6] the Genotype-Tissue Expression GTEx[7] and Functional Annotation of Mammalian Genomes 5 (FANTOM5).[8]

First, the consensus dataset, generating from the three repositories previously mentioned, has been interrogated: *FURIN* is mostly expressed at the level of the salivary glands (337 NX=normalised expression), correlated with a high protein production by glandular cells.[6]

Based on this finding, we focused on the three datasets singularly to further explore *FURIN* expression patterns in salivary glands.

HPA shows that *FURIN* is mainly produced by salivary gland (141.1 one million transcripts per kilobase million—pTPM); specifically, 45%–55% is found in glandular cells, 15%–25% in ductal cells, 5% in adipocytes and 5% in endothelial cells, being also 20% of the quantity transcribed by unidentified cell types[6] (https://www.proteinatlas.org/about/assays+annotation).

FANTOM5 describes the *FURIN* expression in different related districts, that is, parathyroid gland (251.5 scaled tags per million), salivary gland (128.5 scaled tags per million) and submandibular gland (85.6 scaled tags per million)[8] (https://fantom.gsc.riken.jp/5/datahub/description.html).

GTEx instead exhibits an average pTPM of 60.9 in minor salivary glands and most interestingly identifies the expression of the 8 *FURIN* isoforms. The ENST00000610579.4 isoform is rather ubiquitous; the others are restricted to few districts, including minor salivary glands. Indeed, in this region, five isoforms are expressed, namely ENST00000610579.4 (read count 43.3; transcribed exons 2, 4–16), ENST00000268171.7 (read count 2.87; transcribed exons 1, 4–16), ENST00000558794.1 (read count 0.455; transcribed exons 10–15), ENST00000618099.4 (read count 0.350; transcribed exons 3, 4–16) and ENST00000560018.1 (read count 0.150; transcribed exons 13 and 14)[7] (https://www.gtexportal.org/home/documentationPage).

When looking in detail at the different isoforms, the first three encode the same protein in terms of aminoacidic sequence (794 aa) which is predicted to have a transmembrane region, presumably localising at the level of the cellular plasma membrane prior to the secretion in the extracellular milieu. The other two isoforms are estimated to yield a mRNA subjected to nonsense

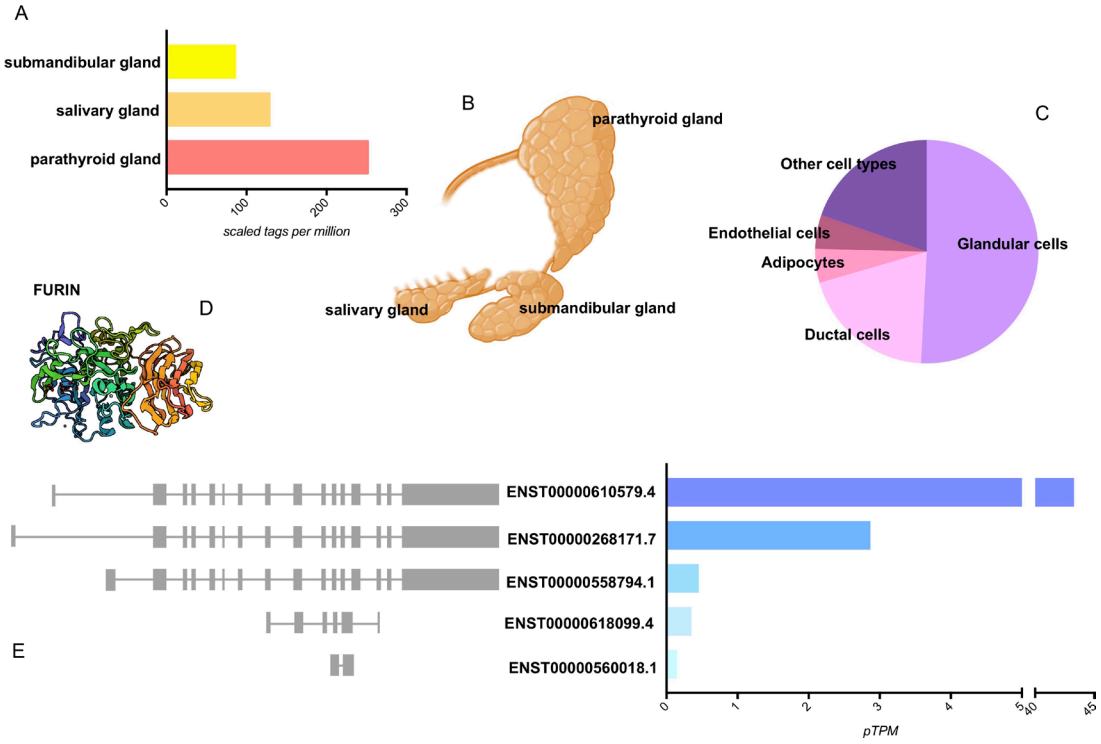


**Figure 1** *FURIN* gene expression. (A) FURIN gene expression in submandibular, salivary and parathyroid glands according with FANTOM5 repository. The data are reported a scaled tags per million (https://fantom.gsc.riken.jp/5/datahub/description.html) (B) anatomic illustration of submandibular, salivary and parathyroid glands (C) the distribution of FURIN gene expression in the different subpopulations of cells within salivary gland, namely, glandular cells, ductal cells, adipocytes and endothelial cells, unidentified cell types, accordingly with HPA repository. The data are reported as parts of a whole (percentage). (https://www.proteinatlas.org/about/assays+annotation) (D) 3D representation of Furin protein. Protein data bank accession number 4Z2A.[14] (E) The schematic representation of the 5 FURIN isoforms (on the left) and their expression (on the right) at the level of minor salivary gland accordingly with GTEx repository. The data are reported as read count (https://www.gtexportal.org/home/documentationPage). FANTOM5, Functional Annotation of Mammalian Genomes 5; GTEx, Genotype-Tissue Expression; HPA, Human Protein Atlas.

mediated decay and an intron retained form not transduced, respectively.[9]

Intriguingly, in the other three isoforms, ENST00000559353 and ENST00000560824 are supposed to be intracellular and the ENST00000640725 to be secreted,[9] although on GTEx their expression is not detected in any tissues.[7]

These observations point out that salivary glands are a highly probable and important target of SARS-CoV-2 since they express a high level of *FURIN*, besides *ACE2* and *TMPRSS2*,[10] and led us to hypothesise the significance of the possible spread of viral particles through the saliva.

Notably, the appearance of furin cleavage sites is linked to a dramatic increment of pathogenicity of other viruses, such as the highly pathogenic avian influenza A viruses (HPAIVs). HPAIV, with respect to low pathogenic influenza A viruses, lodges more furin sites in the influenza A virus hemagglutinin (HA) that should be cut to allow viral binding to the cellular membrane and the fusion. Moreover, considering another virus, the human papilloma virus (HPV), the furin action on HPV L2 protein in target cells is pivotal to allow the viral entry into the host. Furin intracellular activity is also critical for HIV-1 infection; indeed, furin motif is also present on the viral envelope protein, whose precursor gp160 is processed by furin to harbour gp120 and gp41 prior to virus assembly, but also for Flavivirus, which after the incorporation of prM protein in new virions needs the split of mature pr and M proteins by furin.[5]

Furin cleavage pattern was observed on S protein of other coronaviruses, such as avian infectious bronchitis virus, mouse hepatitis virus[5] and MERS,[11] but not on SARS-CoV.[12] These observations could suggest that the high transmissibility of SARS-CoV-2 could depend also on the exploitation of the strong expression of furin throughout the human body, especially in the respiratory tract, where it co-localises with ACE2 and TMPRSS2.[5]

SARS-CoV-2 RNA is found in saliva, resulting in a possible diagnostic biological fluid to assess coronavirus disease 2019 (COVID-19); moreover, remarkably, saliva withdrawn directly from saliva gland ducts is correlated with severe disease,[13] suggesting the invasiveness of the virus through the mucosal barrier, possibly expedited by furin, as our in silico findings also indicated. Moreover, the furin presence in saliva could avail a virus pre-activation, thus favouring the prompt spread through saliva droplets of more infectious virions than those through sneezing and coughing (figure 1).

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**ORCID iD**
Luisa Zupin http://orcid.org/0000-0001-5886-9129

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