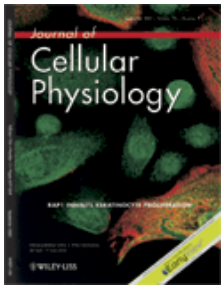

Journal of Cellular Physiology

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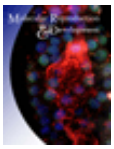

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Additional supporting information may be found in the online version of this article.

Supplementary Figure 1. Intracellular localization of CRF1a-EGFP isoforms in HaCaT keratinocytes. Keratinocytes were transiently transfected with CRF1a-GFP (green) and stained with organelle markers (red or violet) as described in Methods. Co-localization is shown in yellow.

Supplementary Figure 2. Intracellular localization of CRF1c-GFP isoforms in HaCaT keratinocytes. Keratinocytes were transiently transfected with CRF1c-GFP (green) and stained with organelle markers (red or violet). Co-localization is shown in yellow.

Supplementary Figure 3. Intracellular localization of CRF1d-GFP isoforms in HaCaT keratinocytes. Keratinocytes were transiently transfected with CRF1d-GFP (green) and stained with organelle markers (red or violet). Co-localization is shown in yellow.

Supplementary Figure 4. Intracellular localization of CRF1e-GFP isoforms in HaCaT keratinocytes. Keratinocytes were transiently transfected with CRF1e-EGFP (green) and stained with organelle markers (red or violet). Co-localization is shown in yellow.

Supplementary Figure 5. Intracellular localization of CRF1f-GFP isoforms in HaCaT keratinocytes. Keratinocytes were transiently transfected with CRF1f-EGFP (green) and stained with organelle markers (red or violet) . Co-localization is shown in yellow.

Supplementary Figure 6. Intracellular localization of CRF1g-GFP isoforms in HaCaT keratinocytes. Keratinocytes were transiently transfected with CRF1g-EGFP (green) and stained with organelle markers (red or violet) . Co-localization is shown in yellow.

Supplementary Figure 7. Intracellular localization of CRF1h-GFP isoforms in HaCaT keratinocytes. Keratinocytes were transiently transfected with CRF1h-GFP (green) and stained with organelle markers (red or violet . Co-localization is shown in yellow.

Supplementary Figure 8. Intracellular localization of GFP in HaCaT keratinocytes (control). Keratinocytes were transiently transfected with EGFP (green) and stained with organelle markers (red or violet).

Supplementary Figure 9. Detection of CRF1 immonoreactivity in HaCaT keratinocytes. (A) Non-transfected keratinocytes were stained with anti-CRF1 antibody (see Methods section for details). The experimental keratinocytes were transfected with DNA of plasmids carying CRF1 isoforms a (B), f (C) or h (D) with V5 tag on the C terminus. Immunoreactivity of V5 tag was detected using anti-V5 tag antibody and isoform a was found predominantly in the cell membrane, while isoform f and h showed intracellular localization.

Supplementary Figure 10. Binding of CRF to CRF1a-GFP fusion receptor. Keratinocytes were transfected with DNA of plasmid carying CRF1a-GFP construct and, after 24 hours, treated with 100 nM CRF. A. Transfected cell before treatment (control for panel B). B. Co-localization (yellow) of CRF1 (green) with CRF peptide fused with Rodamine B (Phoenix Pharmaceuticals, INC, Belmont, CA) 20 minutes after treatment. C. Transfected cell before treatment (control for panel D). D. Internalization of CRF1-GFP fusion receptor 20 minutes after treatment with CRF (Sigma, St. Louise, MO).

Table S1. Primers used in preparation of GFP, dsRED and V5 tagged receptors.

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- sm002.tif (515k) (TIFF image)
- sm003.tif (394k) (TIFF image)
- sm004.tif (524k) (TIFF image)
- sm005.tif (525k) (TIFF image)
- sm006.tif (384k) (TIFF image)
- sm007.tif (572k) (TIFF image)
- sm008.tif (506k) (TIFF image)
- sm009.tif (818k) (TIFF image)
- sm010.tif (349k) (TIFF image)
- sm011.doc (143k) (Microsoft Word)