Supplemental Data (8 Supplemental Figures, 4 Supplemental Tables)

Mapping regions in Ste5 that support Msn5-dependent and -independent nuclear export

Zhenhua Hu^{1,§}, Yunmei Wang^{1,§§}, Lu Yu^{1,§§§}, Sanjoy K. Mahanty^{1,§§§§}, Natalia Mendoza^{§§§§§},
and Elaine A. Elion^{1,*}

¹Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical
School, Boston, MA 02115

Current addresses:

§ MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, The University of
Edinburgh, Edinburgh EH4 2XU, United Kingdom

§§ Department of Medicine, Cardiovascular Division, Case Western Reserve University, 2103
Wolstein Research Building, 4th floor, Room 4529, Cleveland, OH, U.S.A. 44106.

§§§ Paraxel, International, 101-105 Oxford Road, Uxbridge, Middlesex, UB8 1LZ, United
Kingdom

§§§§ MirCan Therapeutics, LLC, Chapel Hill, NC, U.S.A. 27517

§§§§§ Zuni Comprehensive Community Health Center, POB 467, Zuni, NM 87327

Running title: Msn5-dependent and independent cis elements regulate Ste5 export

#Corresponding author:

#Elaine A. Elion, Ph.D.

Supplemental References

Argueso, J.L., Carazzolle, M.F., Mieczkowski, P.A., Duarte, F.M., Netto, O.V.C. *et al.* 2009. Genome structure of a *Saccharomyces cerevisiae* strain widely used in bioethanol production. *Genome Res.* **19**(12): 2258-2270

Bakhrat, A., K. Baranes-Bachar, D. Reshef, O. Voloshin, O. Krichevsky et al. 2008. Nuclear export of Ho endonuclease of yeast via Msn5. *Curr Genet* **54**: 271-281.

Boratyn, G.M., Schaffer, A.A., Agarwala, R., Altschul, S.F., Lipman, D.J., Maddent, T.L. 2012. Domain enhanced lookup time accelerated BLAST. *Biol Direct.* **7**:12.

Borneman, A.R., Desany, B.A., Riches, D., Affourtit, J.P., Forgan, A.H., Pregorius, I.S., Egholm, M., Chambers, P.J. 2012. The genome sequence of the wine yeast VIN7 reveals an allotriploid hybrid genome with *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii* origins. *FEMS Yeast Research* **12** (1): 88-96. doi: 10.1111/j.1567-1364.2011.00773.x. Epub 2011 Dec 23.

Buchan, D.W.A., Minneci, F., Nugent T.C.O., Bryson, K., Jones, D.T. 2013. Scalable web services for the PSIPRED Protein Analysis Workbench. *Nucleic Acids Research* **41** (W1): W340-W348.

Cole, C., Barber, J.D. and Barton, G.J. 2008. The Jpred 3 secondary structure prediction server. *Nucleic Acids Research* **36**: W197-W201.

Elion, E.A., Satterberg, B. and Kranz, J.E. 1993. FUS3 phosphorylates multiple components of the mating signal transduction cascade: evidence for STE12 and FAR1. *Mol. Biol Cell* **4**: 495-510.

Feng, Y., Song, L.Y., Kincaid, E., Mahanty, S.K. and Elion, E.A. 1998. Functional binding between Gbeta and the LIM domain of Ste5 is required to activate the MEKK Ste11. *Curr Biol* **8**: 267-278.

Ferguson, K.A., Lemmon, M.A., Schlessinger, J. and Sigler, P.B. 1995. Structure of the high affinity complex of inositol trisphosphate with a phospholipase C pleckstrin homology domain. *Cell* **83**: 1037-1046.

Garrenton, L. S., Braunwarth, A., Irniger, S., Hurt, E., Kunzler, M. *et al.* 2009. Nucleus-specific and cell cycle-regulated degradation of mitogen-activated protein kinase scaffold protein Ste5 contributes to the control of signaling competence. *Mol Cell Biol* **29**: 582-601.

Garrenton, L.S., Young, S.L. and Thorner, J. 2006. Function of the MAPK scaffold protein, Ste5, requires a cryptic PH domain. *Genes and Dev*. **20**:1946-1958.

Good, M., G. Tang, J. Singleton, A. Remenyi and W. A. Lim, 2009. The Ste5 scaffold directs mating signaling by catalytically unlocking the Fus3 MAP kinase for activation. *Cell* **136**: 1085-1097.

Gordon, J.L., Armisen, D., Proux-Wera, E., OhElgeartaigh, S.S., Byrne, K.P., Wolfe, K.H. 2011. Evolutionary erosion of yeast sex chromosomes by mating-type switching accidents, *Proc Natl Acad Sci U. S. A.* Nov 28; **108**(50):20024-20029.

Kaffman, A., N. M. Rank, E. M. O'Neill, L. S. Huang and E. K. O'Shea 1998. The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus. *Nature* **396**: 482-486.

Kim, J, Lee, CD, Rath, A, Davidson, AR 2008. Recognition of non-canonical peptides by the yeast Fus1p SH3 domain: elucidation of a common mechanism for diverse SH3 domain specificities. *J. Mol. Biol.* **377**: 889-901.

Kutay, U. and Guttinger, S. 2005. Leucine-rich nuclear-export signals: born to be weak. *Trends Cell Biol* **15**: 121-124.

la Cour, T., Kierner, L, Molgaard, A, Gupta, R, Skriver, K and Brunak, S 2004. Analysis and prediction of leucine-rich nuclear export signals. *Protein Eng. Des. Sel.* **17**(6): 527-536.

Leberer, E., Dignard, D., Marcus, D., Hougan, L., Whiteway, M., Thomas, D.Y. 1993. Cloning of *Saccharomyces STE5* as a suppressor of a Ste20 protein kinase mutant: Structural and functional similarity of Ste5 to Far1. *Mol. Gen. Genet.* **241**: 241-253.

Mahanty, S. K., Y. Wang, Farley, F.W. and Elion, E.A, 1999. Nuclear shuttling of yeast scaffold Ste5 is required for its recruitment to the plasma membrane and activation of the mating MAPK cascade. *Cell* **98**: 501-512.

Mukai, Y., Harashima, S. and Oshima, Y. 1993. Function of the ste signal transduction pathway for mating pheromones sustains MAT alpha 1 transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13**(4): 2050-2060.

Papadopoulos, J.S. and Agarwala, R. 2007. COBALT: constraint-based alignment tool for multiple protein sequences. Bioinformatics **23**: 1073-1079.

Perlman, R., Yablonski, D., Simchen, G. and Levitzki, A. 1993. Cloning of the STE5 gene of *Saccharomyces cerevisiae* as a suppressor of the mating defect of cdc25 temperature-sensitive mutants Proc. Natl. Acad. Sci. U.S.A. **90**(12): 5474-5478.

Printen, J.A. and Sprague, G.F. Jr. 1994. Protein-protein interactions in the yeast pheromone response pathway: Ste5p interacts with all members of the MAP kinase cascade. Genetics **138**: 609-619.

Slabinski, L., Jaroszewski, L., Rychlewski, L., Wilson, I.A, Lesley, S.A and Godzik, A. 2007. XtalPred: a web server for prediction of protein crystallization. Bioinformatics **23**: 3403-3405.

Strickfaden, S. C., M. J. Winters, G. Ben-Ari, R. E. Lamson, M. Tyers et al. 2007 A mechanism for cell-cycle regulation of MAP kinase signaling in a yeast differentiation pathway. Cell **128**: 519-531.

Walczak, MJ, Samatanga, B, van Drogen, F, Peter, M, Jelesarov, I and Wider, G (2013) The

RING domain of the scaffold protein Ste5 adopts a molten globular character with high thermal and chemical stability Angew. Chem. Int. Ed **52**: 1-5.

Wang, Y., and E. A. Elion 2003. Nuclear export and plasma membrane recruitment of the Ste5 scaffold are coordinated with oligomerization and association with signal transduction components. Mol Biol Cell **14**: 2543-2558.

Wen, W., Meinkoth, JL, Tsien, RY and Taylor SS (1995) Identification of a signal for rapid export of proteins from the nucleus. Cell **82**: 463-473.

Wei, W. *et al.* 2007. Genome sequencing and comparative analysis of *Saccharomyces cerevisiae* strain YJM789 Proc. Natl.Acad. Sci. U.S.A. **104**(31): 12825-12830.

Wen, W., J. L. Meinkoth, R. Y. Tsien and S. S. Taylor, 1995. Identification of a signal for rapid export of proteins from the nucleus. Cell **82**: 463-473.

Xu, D., Marquis, K., Pei, J., Fu, S-C., Cagatay, T., Grishin, N.V. and Chook, Y.M. 2014.
LocNES: A computational tool for locating classical NESs in CRM1 cargo proteins.
Bioinformatics December 15 ahead of print pii: btu826

Zalatan, J.G., Coyle, S.M., Rajan, S., Sidhu, S.S., Lim, W.A. 2012. Conformational control of the Ste5 scaffold protein insulates against MAP kinase misactivation. Science **337**: 1218-1222

Supplemental Figures and Legends for FigS1-FigS8

Supplemental Figure S1

Fig. S1. Localization of Ste5-Myc9. A. Tally of Ste5-Myc9 localization enriched in nuclei and cytoplasm. *MATa bar1 Δ ste5 Δ* (EY1775) cells harboring Ste5-Myc9 on a 2μ *URA3* plasmid (pSKM12 was used to better visualize the nuclear pool of Ste5) were grown in SC-uracil containing 2% dextrose to an A600 of 0.5 at 30°C then centrifuged and resuspended in fresh media at the same density with or without 50 nM α factor and incubated with shaking at 30°C. Aliquots (5 ml) were removed and fixed with 5% formaldehyde for 1 hour at room temperature then spheroplasted with lyticase and prepared for indirect immunofluorescence staining for the Myc epitope with anti-Myc monoclonal 9E10 antibody, tubulin with YOL1/34 monoclonal

antibody and DNA with DAPI. See Mahanty *et al.* 1999 for methods and secondary antibodies. Samples were tallied for nuclear versus cytoplasmic distribution of Ste5-Myc9 as a function of cell morphology, using tubulin, DAPI and bud morphology as guides. Numbers are percentages derived from averages of two transformants, several hundred cells counted. N is predominantly nuclear Ste5-Myc9 distribution and C is predominantly cytoplasmic distribution. Top row cartoons of cell division morphology abbreviations are UB for unbudded cell, SB for small budded cell, BB for large budded cell, 2C for two cells post cytokinesis, Shm for shmoo and Nuc for nucleus. The circles indicate nuclei; lines drawn from circles indicate microtubule morphology through the cell cycle. The length of α factor (α F) induction is none, 10 and 180 minutes. B. Representative cells from the experiment described in A. The Ste5-Myc9 – α factor panel shows three unbudded cell with predominantly nuclear staining and one small budded and one large budded cells with cytoplasmic staining. The Ste5-Myc9 + α factor 2.5 hours shows three shmoos with cytoplasmic staining and more intense nuclear staining.

Supplemental Figure S2

Fig. S2. Localization of Ste5(1-242)-GFP2, Ste5(1-176)-GFP2 and Ste5(1-176Δ49-66)-GFP2. *MATa bar1Δ ste5Δ* (EY1775) and *MATa bar1Δ STE5* (EY957) cells harboring *ADHIpromoter-STE5-supergloGFP URA3 CEN* fusion plasmids were grown in SC-uracil medium containing 2% dextrose to an A600 of 0.4-0.6 at 30°C with shaking then α factor was added (50 nM α factor dissolved in methanol) or methanol alone as the negative control. Samples were removed, centrifuged to concentrate the cells, resuspended in a smaller volume of medium and visualized live on a slide under an unsealed glass coverslip on the laboratory Axioskop as described in Materials and Methods.

Supplemental Figure S3

A. LY series in EY1775 *ste5Δ* -αF
TAgNLS-GFP2

TAgNLS-Ste5(627-644)-GFP2

TAgNLS-Ste5(264-361)-GFP2

TAgNLS-Ste5(602-639)-GFP2

TAgNLS-Ste5(466-645)-GFP2

TAgNLS-Ste5(602-618)-GFP2

TAgNLSSte5(475-533)-GFP2

TAgNLS-Ste5(602-618,L610/614A,Q615H)-GFP2

B. LYseries in EY1775 *ste5Δ* -/+αF
TAgNLS-Ste5(733-916)-GFP2
-αF

TAgNLS-Ste5(733-916)-GFP2
+αF 1 hr

C. LY series in EY1775 *ste5Δ* -αF

TAgNLS-Ste5(336-601)-GFP2

TAgNLS-Ste5(475-491)-GFP2

TAgNLS-Ste5(508-533)-GFP2

TAgNLS-Ste5(508-639)-GFP2

D. -/+αF MSN5 and *msn5Δ*

LY157 [EY957, WT +
TAgNLS-Ste5(627-644)-GFP2]

-αF

+αF

LY171 [EYL357, *msn5Δ* +
TAgNLS-(627-644)-GFP2]

-αF

+αF

E.

Nuclear accumulation of LNES(627-644)
in MSN5 and *msn5Δ* strains

msn5 localization

TAgNLS-GFP2
TAgNLS-Ste5(336-601)-GFP2
TAgNLS-Ste5(475-533)-GFP2
TAgNLS-Ste5(602-618)-GFP2
TAgNLS-Ste5(602-639)-GFP2
TAgNLS-Ste5(602-639)-GFP2
TAgNLS-Ste5(627-644)-GFP2

N>> C
C>>N
C>>N
C>>N
C>>N
C>>N
C>>N

Fig. S3. Localization of SV40TA_gNLS-Ste5-GFP-GFP fusions in *bar1Δ STE5*, *bar1Δ ste5Δ*, *msn5Δ* cells. The indicated superglo GFP2 fusion proteins were expressed in EY1775, EY957 and EYL357 *msn5Δ* cells grown to an A₆₀₀ of 0.4-0.8 in SC-uracil containing 2% dextrose medium at 30°C and treated with 50 nM α factor for *bar1Δ* and 5 mM α factor for *BAR1* cells or methanol carrier as negative control where indicated (- α F). Aliquots were removed, centrifuged and visualized on the Harvard Medical School/Dept. Cell Biology Nikon microscopes as described in Materials and Methods and Yu *et al.* 2008. See main text, Table 1, Table 2 for description of methods and strains and plasmids.