Addendum: Heme is involved in microRNA processing

Michael Faller, Michio Matsunaga, Sheng Yin, Joseph A Loo & Feng Guo

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Here we report that the calibration peaks of size-exclusion chromatography (SEC) were misassigned in our paper. The estimated molecular weights based on the new calibration curve are larger than those reported (Table 1). However, our conclusions about oligomerization states of the heme-bound and heme-free NC1 and the NC9 proteins are supported by additional experimental evidence, and are unchanged. The heme-bound NC1 is a dimer, as was directly confirmed using ESI MS. NC9, and the heme-free NC1, is largely monomeric, as indicated by our unpublished ESI mass spectra. In addition, NC9 is similar to the monomeric DGCR8 core, as demonstrated by its crystal structure (*Nat. Struct. Mol. Biol.* **14**, 847–853, 2007). In our paper, we proposed a cooperative trimer model in which the heme-free NC1 dimer, as well as NC9, further trimerizes upon association with the pri-miR-30a RNA. This model is supported by the new SEC estimates. Therefore, SEC seems to systematically overestimate the molecular weights of DGCR8 proteins and protein–RNA complexes, probably due to their elongated shapes. The new estimated molecular weight of the heme-free NC1 in complex with pri-miR-30a is consistent with a model in which three heme-free NC1 molecules associate with one pri-miRNA, but this estimate is not conclusive. Further corroboration on the oligomerization state of the heme-free NC1 upon binding to pri-miRNAs is needed.

Table 1 Summary of SEC results with new estimates of molecular weights

Protein or protein–RNA complexes	Figures in paper	Elution volume (ml)	Previous estimates (kDa)	New estimates (kDa) ^a	Predicted molecular weight (kDa)
Heme-bound NC1	2a, 2b	10.9	122	181	108
Heme-free NC1	2a	12.1	78	120	54
NC1 C352A	3b	11.9	84	129	54
Pri-miR-30a	4c	11.6	70	121	48
Heme-bound NC1–pri-miR-30a	4c	8.5	310	379	372
Heme-free NC1–pri-miR-30a	4c	9.8	170	234	156 ^b or 210 ^c
NC9	6a	15.3	23	40	29
NC9–pri-miR-30a	6d	10.7	111	168	135

^aThe new calibration curves are $y = -0.1485x + 6.877$ for high-salt buffer with a 500- μ l injection loop, and $y = -0.1603x + 6.941$ for low-salt buffer with a 100- μ l injection loop, where x = elution volume and y = $\log(\text{molecular weight})$. ^bBased on a cooperative dimer model, in which two heme-free NC1 molecules bind to one pri-miR-30a RNA. ^cBased on a cooperative trimer model, in which three heme-free NC1 associate with a pri-miR-30a.