Yasuhara and Yoneda reply:

In mammals, *importin-α* genes comprise a multi-gene family that can be divided into three distinct subtypes: *importin-α1, -α3* and *-α5*. These subtypes are differentially expressed in various tissues, suggesting that these proteins may be involved in various tissue-specific regulation mechanisms[1,2]. Recently, we showed that the expression of importin-α subtypes is modulated during the neural differentiation of mouse embryonic stem (ES) cells. We also demonstrated that reproducing the switching of importin-α subtype expression in undifferentiated ES cells by *importin-α1*-specific RNAi in combination with the over-expression of importin-α5 induces neural differentiation. This result correlates with the regulated expression of transcription factors such as Oct3/4, Brn2, and SOX2[3]. These findings suggest that the subtype-switching in importin-α expression has a strong influence in the determination of cell fate through the regulated nuclear import of specific transcription factors. In addition, we found that in developing and adult mouse brains, importin-α5 was highly expressed, whereas the expression of importin-α1 was scarce, indicating the *in vitro* findings obtained by cultured ES cells may apply *in vivo*[3,4].

Given the data of Shmidt *et al.*[5], we examined in more detail the nuclear import of Brn2, a transcription factor for neural differentiation, to test the possibility of compensating for the transport of cargoes related to neural differentiation. We recently reported that the import of Brn2 was mainly dependent on importin-α5 when a digitonin-permeabilized *in vitro* transport assay was conducted under ordinary assay conditions and the cargo and importin-α ratio was 1:1 (ref. 3). In response to the study of Shmidt *et al.*, we performed the the *in vitro* transport assay in the presence of importin-α1, -α4, -α5 or -α7 against Brn2 at molar ratios of 2:1 and 1:2, as well as 1:1. When the concentration of Brn2 was raised by up to two fold, importin-α4 and -α7 were also able to import Brn2, although the import efficiencies were very low. In contrast, when the concentration of Brn2 was decreased to one half of that of importin-α, nuclear accumulation of Brn2 was not detected, indicating that the nuclear import of Brn2 by importin-α4 and -α7 could only be achieved under certain conditions. In contrast, consistent with previous results, we found that importin-α1 never imported Brn2, whereas importin-α5 efficiently imported Brn2 under any assay conditions. These results raise the possibility that importin-α4 can substitute for depleted importin-α5 and compensate for importin-α5 function during neural development. This is consistent with the ideas of Shmidt and colleagues. In order to elucidate this possibility, it will be necessary to analyse cargo specificities of importin-αs more extensively. Such a study will lead to the functional grouping of importin-α family members based on individual biological phenomena. It also implies that importin-α4 and -α5 may be classified into the same subgroup based on cell differentiation, despite the current classification based on the sequence homology categorizing importin-α4 and -α5 as distinct subtypes.

We have shown that during the neural differentiation, downregulation of importin-α1 expression occurs and is followed by gradual upregulation of importin-α5, although it is still not known how the upregulation of importin-α5 expression is induced after the downregulation of importin-α1. From these findings, we speculate that during neural differentiation in importin-α5-deficient mice, importin-α1 is first downregulated and concomitantly, importin-α4 expression is upregulated, although it is not known why only importin-α4 is selected as a substitute for importin-α5. To resolve this, it will be necessary to study the switching mechanism of importin-α subtype expression. Further studies will be required to examine whether, in importin-α5-deficient mice, the expression of transcription factors involved in neural differentiation such as Brn2 is upregulated to compensate for importin-α5-deficiency. This interest is based on our finding *in vitro* that a higher concentration of Brn2 was required for nuclear import mediated by importin-α4 compared with that induced by importin-α5.

As the study by Shmidt *et al.* suggests, a mechanism in mammals could exist by which the importin-α-mediated nucleo-cytoplasmic transport system is constantly maintained *in vivo*, perhaps a consequence of mammals being more adaptable to importin-α-deficiency than other organisms. This is demonstrated by the fact that certain importin-α subtypes compensate for each other in development, as indicated by the result that only importin-α4 was upregulated in the brain of all importin-α5-deficient mice. Although the over-expression of importin-α5 in combination with the downregulation of importin-α1 induced neural differentiation of ES cells, the results of Shmidt *et al.* imply that upregulation of importin-α5 is not a critical event for neural development. It will be important to test whether subtype-switching similar to that occurring in ES cells can trigger neural differentiation during development *in vivo*.

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