Messenger RNA (mRNA) localization plays an important role in various cellular functions. To date, two general mechanisms have been identified for intracellular mRNA localization. The first one was identified by Blobel and colleagues more than three decades ago, by which mRNAs encoding for membrane and secreted proteins are targeted to the endoplasmic reticulum (ER) in a signal peptide dependent manner. The second mechanism is for the intracellular targeting of mRNAs encoding cytosolic proteins, which is dependent on specific sequence on the mRNA called zipcode.

Recently, we have identified a new mechanism which targets Dia1 mRNA to the perinuclear ER in a zipcode-independent manner, even though the mRNA encodes a cytosolic protein. Here, we provide an updated discussion on how the Dia1 mRNA is targeted and what might be its physiological significance.

How and why does Dia1 mRNA localize?

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Key words: formin, endoplasmic reticulum, translation, Rho, cytoskeleton, IQGAP

Submitted: 04/11/11
Accepted: 04/11/11
DOI: 10.4161/cib.4.5.15794

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Does IQGAP1 Play a Role in Dia1 mRNA Localization?

We have demonstrated that localization of Dia1 mRNA to the perinuclear ER requires a Dia1 nascent peptide which must contain both the Rho GTPase binding domain (GBD) and diaphanous inhibitory domain (DID). While it is clear that GBD is for binding to Rho-GTP, the role of DID in Dia1 mRNA localization is less clear. In our previous working model we speculated that DID might be required to enhance or stabilize the interactions between the GBD of Dia1 nascent peptide and Rho-GTP given that DID can physically contact with Rho-GTP in a crystal structure. However, it is equally possible that the DID is required for binding to another protein on the ER. It is therefore interesting to notice that Brandt and Grosse reported that IQGAP1 interacts with the DID of Dia1. IQGAP1 is a scaffold protein with multiple functions, which localizes to the perinuclear region and leading edge in fibroblasts. Its binding to DID of Dia1 requires Rho binding to the GBD of Dia1. Given that the GBD of Dia1 nascent peptide binds to Rho-GTP, this will permit the interactions between IQGAP1 and the DID. Hence, interactions between IQGAP1 and the DID of Dia1 nascent peptide is expected to enhance the anchorage of the ribosome-mRNA-nascent peptide complex on the ER.

Prompt Initiation of Translation and Pausing of Translational Elongation

When exogenous Dia1 was expressed in transfected fibroblasts, it was often
observed that the ectopically expressed Dia1 mRNA was enriched in a narrow zone surrounding the nucleus.\(^7\) We predict that this is due to a prompt translation of the Dia1 mRNA once it exits the nuclear pore as translation is necessary for the Dia1 mRNA localization. It is unlikely that such pattern of mRNA localization could be resulted from a pool of Dia1 mRNA which is first diffused and translated in the cytoplasm then is transported to the perinuclear ER.\(^8\) This argument is based on that if Dia1 mRNA is first translated in the cytoplasm, the relatively enriched Rho-GTP in the cytoplasm and plasma membrane may keep the mRNA there because interactions between Dia1 nascent peptide and Rho-GTP dictates Dia1 mRNA localization. A test for this question would be to determine whether delocalized Dia1 mRNA can localize to the perinuclear ER by first delocalizing the Dia1 mRNA using puromycin and then washing-off the drug (in the presence of transcription inhibitor to prevent the accumulation of newly transcribed Dia1 mRNA). In addition to prompt translation, pausing during translational elongation may play a role in Dia1 mRNA localization. It is known that translation of mRNAs encoding membrane and secreted proteins pauses after the signal peptide is synthesized.\(^9\) This pausing is important for the subsequent co-translational localization of these mRNAs on the ER. In another case, a nascent peptide motif pauses the translation of XBPlu mRNA for the splicing of an intron on the ER.\(^10\) It is generally believed that translation pausing helps co-translational folding of nascent peptides and even interactions of the folded domain with binding partners for regulatory purposes.\(^11\)\(^12\) Our model predicts that a Dia1-mRNA-ribosome complex is tethered on the ER through Dia1 nascent peptides.\(^3\) Therefore multiple nascent-peptide-ribosome complexes on each Dia1 mRNA are required to ensure that at any given moment there is at least one nascent peptide which is bridging the ribosome-mRNA and the ER (also see Fig. 1). It is feasible that translation pausing of Dia1 mRNA will increase the number of the ribosome on each mRNA. In addition, the pausing may provide more time for the folding of Dia1 nascent peptide hence facilitating its interactions with Rho-GTP. In this regard, it is interesting to notice that there is some similarity between a short sequence in the GBD of Dia1 and the translation pausing motif of XBPlu\(^10\) (Fig. 2). Given that the GBD-containing N-terminal fragment of Dia1, which is predicted to have much fewer number of ribosome on each mRNA due to its much shorter length, is sufficient for its mRNA localization,\(^3\) this further raises the possibility that translation pausing plays a role in Dia1 mRNA localization.

### The Physiological Significance of Dia1 mRNA Localization

The perinuclear ER localization of Dia1 mRNA was a surprise to us given that no role has been proposed for Dia1 protein on the ER. Rather, Dia1 has been proposed to work in other compartments such as the leading edge.\(^6\) Then why do the cells localize the Dia1 mRNA and synthetize Dia1 protein on the ER instead of localizing the Dia1 mRNA to its site of function at the leading edge as in the case for the Arp2/3 mRNAs?\(^13\)\(^14\) Although there is currently no clear answer to this question, our preliminary data do suggest that the perinuclear ER localization of Dia1 mRNA may be important for cellular function as de-localization of Dia1 mRNA in fibroblasts could impair cell migration (Liao G and Liu G, unpublished). Because one of the functions of mRNA localization is to avoid inappropriate protein interactions, it is possible that local synthesis of Dia1 on the ER is to prevent its premature interactions with other proteins until it is functionally ready (e.g., folded and formation of dimer). Another possible reason for the local synthesis of Dia1 on the ER is its potential involvement in ER expansion. It is known that Dia1 regulates microtubules (MT) stability and MT regulate ER extension.\(^15\)\(^16\) Therefore, the first job of the newly synthesized Dia1 might be involved in the interactions with MT in the perinuclear region. Finally, because IQGAP1 is required for the recruitment of Dia1 to the leading edge of fibroblasts,\(^6\) the above mentioned local interactions between the DID of Dia1 nascent peptide and IQGAP1 on the ER may precondition IQGAP1 for this purpose as the nascent Dia1 peptides are in a Rho-GTP bound state which is a prerequisite for the DID-IQGAP1 interactions.

### Acknowledgments

This work is supported by NIH grant R01GM070560. We thank Dr. Qingfen Li for proof reading.

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Figure 2. Predicted potential translation pausing motif (CTR) in chicken and human Dial protein sequences. The CTR sequences are from reference 10. The CTR in both chicken and human Dial are overlapped with a portion of the GBD.

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