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Nucleoporin gene expression in *Xenopus tropicalis* embryonic development

NOOREEN REZA, MUSTAFA K. KHOKHA and FLORENCIA DEL VISO*

Program in Vertebrate Developmental Biology. Department of Pediatrics and Genetics, Yale University School of Medicine, New Haven, CT, USA

ABSTRACT Nucleoporins (nups) compose the structure of the nuclear pore complex (NPC) of all cells, but several studies have illuminated nucleoporins' additional roles in development and the cell cycle. However, a comprehensive study of nup expression in embryonic development has not yet been reported. We synthesized antisense probes for all nup genes and used whole-mount *in situ* hybridization techniques to determine the expression pattern of all members of the nup family of genes at three different developmental stages in *Xenopus tropicalis.* We found that the expression of nups was not ubiquitous in embryos, but was localized to specific and distinguishable anatomical structures at all three stages tested. We also found that the expression patterns for nups within the same subcomplexes were not necessarily identical. Thus, nup expression is subject to a significant level of regulation during development. These results provide new information for functional studies of nups to unravel their roles in embryonic development.

KEY WORDS: *nups, NPC, nuclear pore complex, in situ hybridization*

The nuclear membrane forms an essential boundary for the nucleus and is a major site of macromolecular traffic in the cell, necessitating a mechanism for selective transport (Lusk *et al.*, 2007). The nuclear pore complex (NPC) is a cylindrical array of proteins, embedded in the nuclear envelope, which creates a portal for macromolecular transport. The structure of the NPC has been analyzed and categorized into a cytoplasmic ring, a central spoke ring, and a nuclear ring (Grossman *et al.*, 2012). Each of these subunits is composed of proteins called nucleoporins (nups), which form different subcomplexes: transmembrane, scaffold (outer and inner ring subcomplexes), cytoplasmic (ring/ linker), nuclear basket, and channel nups. Transmembrane nups anchor the NPC to the nuclear envelope, while scaffold and central channel nups make up the central structure of the transport complex (Fig. 1A). Several cytoplasmic, channel and nuclear basket nups have phenylalanine-glycine (FG) repeats that assist in trafficking molecules. For instance, central FG-repeat nups arrayed in the central pore create the permeability barrier of the NPC (Onischenko and Weis, 2011). A new study has recently elucidated the underlying protein-protein interaction network within the NPC. Analyzing the X-rays crystallographics of scaffold nucleoporins enabled the construction of full-length atomic structures, ultimately leading to a massive composite structure of the NPC (Lin *et al.*, 2016).

Aside from their role at the NPC, many nucleoporins have been shown to have functional roles in several cell processes influencing development (Raices and D'Angelo, 2012). For example, the Nup107-160 subcomplex is known to promote microtubule spindle assembly in mitosis by recruiting the gamma-tubulin ring complex to kinetochores (Mishra *et al.*, 2010). Other studies have associated nups like Nup98 and Nup153 with functions as diverse as HIV-1 import or integration (Di Nunzio *et al.*, 2013). In addition, *nup188* was identified as a candidate gene for Congenital Heart Disease, and its knockdown showed disrupted left-right patterning during *Xenopus* embryonic development (Fakhro *et al.*, 2011). While multiple nucleoporins have been the sole subjects of functional studies, a broad analysis of nucleoporin expression has not been undertaken. In addition, given that nup proteins are necessary in all nuclei, *a priori*, we anticipated that nups would be ubiquitously expressed during embryonic development where frequent cell divisions are regularly increasing overall nuclear envelope length. However, we were surprised to see that *nup188* had localized expression (Fakhro *et al.*, 2011). Moreover, we used ultra-high-frequency sampling of *Xenopus* embryos and absolute normalization of sequence reads

Abbreviations used in this paper: NPC, nuclear pore complex, nup, nucleoporin.

***Address correspondence to:** Florencia del Viso. Department of Pediatrics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT, 06520, USA. Tel: 1-203-737-4241. E-mail: fdv@stowers.org

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to show gene expression profiles in absolute transcript numbers (Owens *et al.*, 2016). Analyzing the nup expression profiles from this study we found dynamic temporal expression profiles during early development. Given these results, we sought to investigate the spatiotemporal expression of nups at different stages during embryonic development.

Using whole mount *in situ* hybridization, we analyzed the expression of all nups throughout *Xenopus tropicalis* development. In this study we show that nups are expressed in non-ubiquitous patterns across stages, revealing spatial regulation of nup expression during embryonic development.

Results

We began by analyzing an ultra-high resolution temporal expression profile. Using RNAseq and absolute normalization of mRNA levels, we calculated the number of transcripts per embryo during the first 66 hours post fertilization (stages 0 to 45) in *X. tropicalis* (Owens *et al.*, 2016). We generated temporal expression profiles for each of the nup subcomplexes revealing dynamic expression (Fig. 2). Based on this data, we sought to investigate the spatial expression profiles in order to identify any spatiotemporal regulation.

To study nup spatial expression during embryonic development, we focused on three stage-ranges: stages 16-18, 26-28, and 33-36. These are benchmark times of embryonic development after initial germ layer specification and encompass much organogenesis. We examined expression in multiple important anatomical structures such as the neural tube, eye, heart and visceral (branchial) arches, as well as other structures (Fig. 1 B,C,D). We present our results organized by NPC subcomplex.

Scaffold nucleoporins

We detected strong signal of scaffold nups in the following regions: neural tube, head, eyes, visceral arches, dorso-lateral plate mesoderm, and ventral mesoderm. Variations in these expression patterns are discussed in further detail below.

Outer ring: nups 37, 43, 85, 96, 107, 133, 160, sec13, seh1

At stages 16-18 all outer ring nups displayed expression in the anterior regions of the neural tube, and also near the eye anlages (Figs.1B and 3).

At stages 26-28, all nups in the subcomplex had signal in the eyes, midbrain and hindbrain. The eye signal was strong in *nup37, 107, sec13* and *seh1.* In addition, nups *37, 107* and *sec13* displayed strong signal in the forebrain. We also detected signal in the otic vesicle for all nups but *nup43* and *nup85. Nup133, sec 13* and *seh1* also had signal in the tailbud. All nups in this subcomplex were detected in the dorso-lateral plate mesoderm region, where pronephros will develop, with the exception of *nup37* and *nup85* (Fig. 3, lm). At these stages, only *seh1* was detected in the ventral mesoderm (Fig. 3, vm). *Sec13* was also detected strongly in the cement gland (Fig. 3, c).

By stages 33-36, we detected strong signal for all nups in the head, eyes and visceral arches, with some variations in specific expression pattern. *Nup133* had a unique expression pattern in the brain at these stages. There was strong signal detected in the mid to hindbrain boundary (the isthmus, Fig. 3, i) whereas the immediate midbrain region showed comparatively weak signal; signal

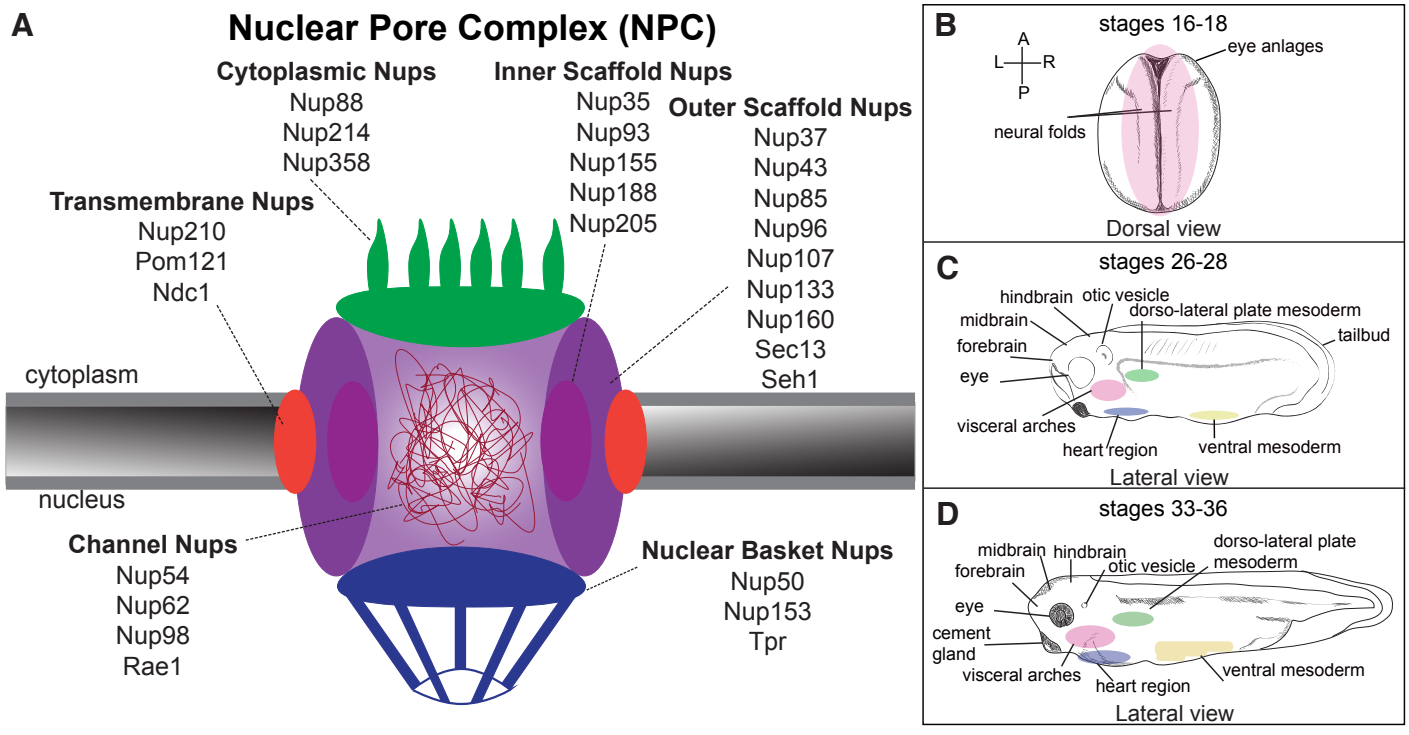


Fig. 1. (A) *Schematic of Nuclear Pore Complex (NPC). The different subcomplexes that form the NPC are shown.* **(B,C,D)** *Schematic of* X. tropicalis *embryos at stages 16-18* **(B),** *26-28* **(C),** *and 33-36* **(D).** *The embryo schematics have relevant anatomical structures highlighted and labeled. A, anterior; P, posterior; L, left; R, right.*

again increased towards the forebrain. Otherwise, all members of the subcomplex displayed signal in the brain with the exception of *nup43.* All nups were detected in the dorso-lateral plate mesoderm, with the exception of *nup37* and *nup85.* Signal in the heart progenitor field was detected for *sec13* and *seh1*. *Seh1* and *sec13* also showed signal in the ventral mesoderm.

Inner ring: nups 35, 93, 155, 188, 205

At stages 16-18, similarly to the outer ring nups, all the inner ring genes displayed significant expression in the anterior and posterior region of the neural tube (Fig. 4).

At stages 26-28, all nups displayed signal in the eyes that was particularly strong in nups *93, 188* and *205.* These three nups also share broad expression in the brain, visceral arches and otic vesicle. It is notable that nups *93, 188* and *205* display a common expression pattern at these stages, since these three nups were shown to interact with each other (Theerthagiri *et al.*, 2010). Dorso-lateral plate mesoderm signal was detected for all the nups in this subcomplex, with the exception of *nup35.* Heart progenitor field signal was observed in nups *93* and *155.* Ventral mesoderm signal was detected for *nup155* only. All nups showed signal at the tailbud.

At stages 33-36, the presence of signal in the head, brain, eyes, and visceral arches was maintained. Nups *93* and *155* in particular showed an expression pattern in the brain similar to *nup133* at these stages, with an area of strong signal in the isthmus (Fig. 4, i). Nups *93* and *188* had strong signal in the eye. Dorso-lateral plate mesoderm signal was detected in all the nups besides *nup35* and *nup205.* Heart progenitor field signal was maintained in nups *93* and *155* (Fig. 4, h). At this stage we detected ventral mesoderm signal only in *nup205.*

Channel nucleoporins: nups 54, 62, 98, rae1

In general, we detected signal in the head, eyes, somites, otic vesicle and visceral arches of the channel nups. At stages 16-18, all genes showed signal in the anterior regions around the eye anlages (Fig. 5). *Rae1* signal was detected strongly along the whole neural tube.

At stages 26-28, we detected signal in the brain, eyes, otic vesicle and visceral arches for all nups. The signal detected for *nup62* and *rae1* was broader around the head region and not clearly restricted to the visceral arches. They also showed strong signal along the entire length of the neural tube and in the somites. *Nup54* and *nup98* were expressed in the tailbud. It is interesting to note that the Nup96 and Nup98 proteins derive from a single protein precursor that is cleaved yielding two individual proteins. Regarding mRNAs, the long *nup98-nup96* mRNA precursor and another *nup98* mRNA, derived from an alternatively spliced pre-mRNA coexist (Fontoura *et al.*, 1999). Our probes were designed to hybridize to *nup96* and *nup98* separately; however due to the nature of these particular genes it is not possible to discern their individual expression pattern by *in situ* hybridization. As expected, these genes showed similar expression patterns (Figs. 3 and 5).

At stages 33-36, the trend of general expression in the brain and eyes was sustained. *Nup54* expression pattern in the brain is similar to

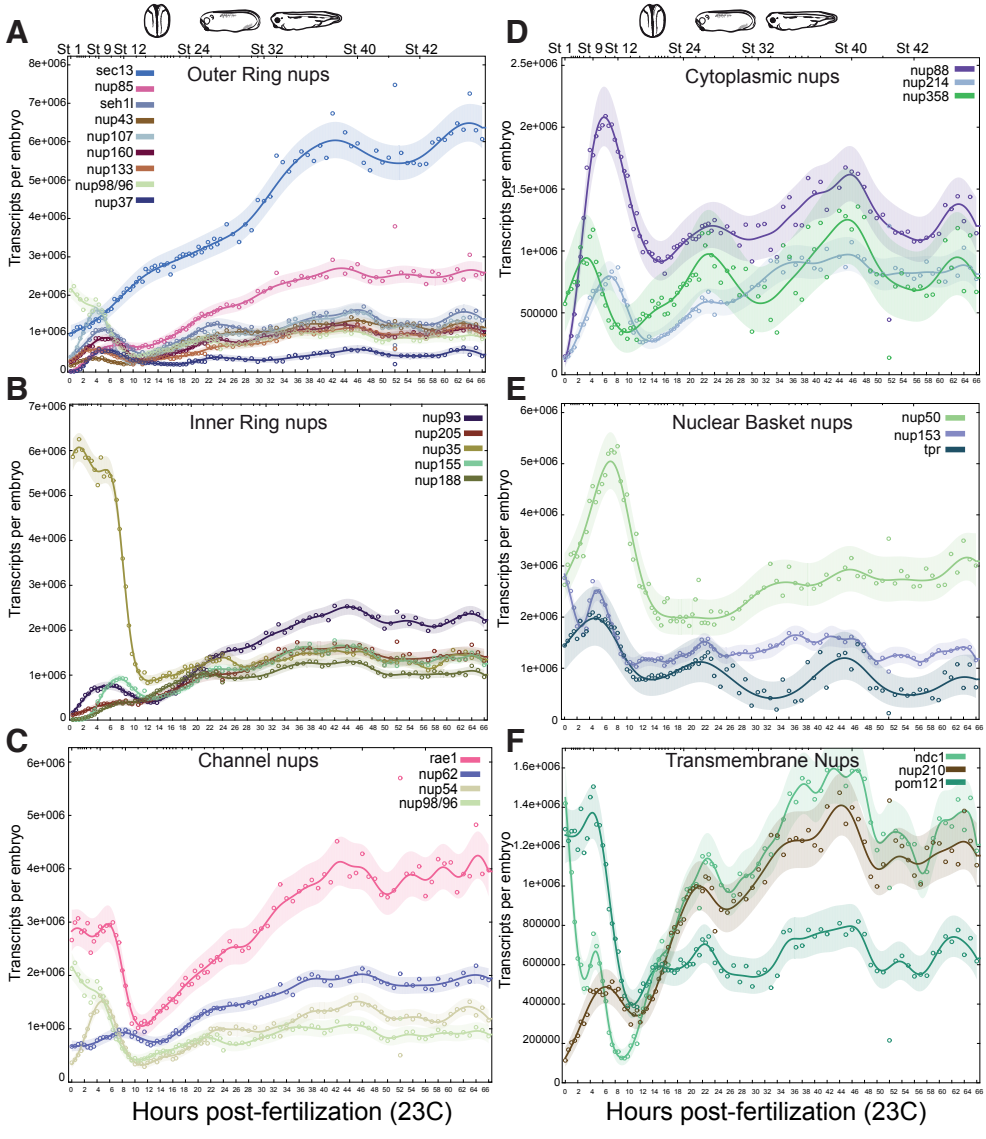


Fig. 2. RNA-seq profiles of nups during *X. tropicalis* development. *Outer ring* **(A)**, *inner ring* **(B)**, *channel* **(C)**, *cytoplasmic* **(D)**, *nuclear basket* **(E)** *and transmembrane nucleoporins* **(F)**. *Graphs show number of transcripts per embryo from 0 to 66 hours post fertilization (horizontal axis). Nieuwkoop and Faber (NF) developmental stage is shown on the top. Shaded regions mark Gaussian process 95% CIs of the data.*

nup133 (Fig. 3) with strong signal in the isthmus (Fig. 5, i). All genes in this subcomplex showed signal in the visceral arches and otic vesicle. We detected signal in the tail for *nup54.* We also detected strong signal in the neural tube and somites for *nup62* and *rae1* as in previous stages.

Cytoplasmic ring and nuclear basket nucleoporins: nups 50, 88, 153, 214, 358, tpr

These subcomplexes presented signal in the brain, eyes, visceral arches, dorsolateral plate mesoderm, heart, and otic vesicle. Variations in these observations are discussed below (Fig. 6 A,B).

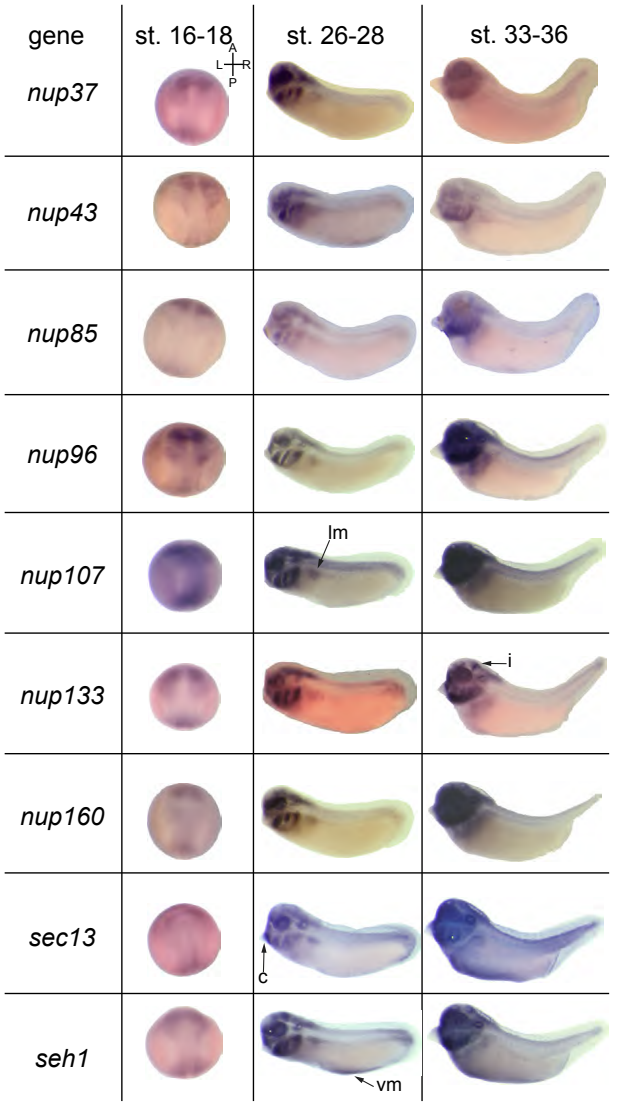
At stages 16-18, most of the genes showed signal in the anterior regions around the eye anlages coupled with visible expression in the posterior regions (Fig. 6B).

At stages 26-28, all nups were detected in the brain, eyes and visceral arches. We detected signal in the neural tube for *nup50* and *tpr.* There was also signal detected in the otic vesicle for *nup50.* Some signal was observed near the tail for *nup153.* All the nups in these subcomplexes displayed dorso-lateral plate mesoderm signal with the exception of *tpr. Nup88* was also strongly detected in the cement gland (Fig. 6A).

At stages 33-36 we detected brain expression for most genes. For *nup88*, in particular, we detected strong signal in the isthmus, similar to *nup133* (Fig. 6A). There was signal detected in the otic vesicle for nups *50, 88* and *153.* We also observed dorso-lateral plate mesoderm expression in all genes of these subcomplexes. Heart progenitor field signal was observed for *nup50, 214* and *tpr* (Fig. 6B). *Nup153* and *nup88* were the only genes detected in the ventral mesoderm in these subcomplexes, and only at these stages.

Transmembrane nucleoporins: nup210, pom121, ndc1

At stages 16-18, these three nups exhibited signal in the anterior regions surrounding the eye anlages and posterior region of the neural tube (Fig. 7).



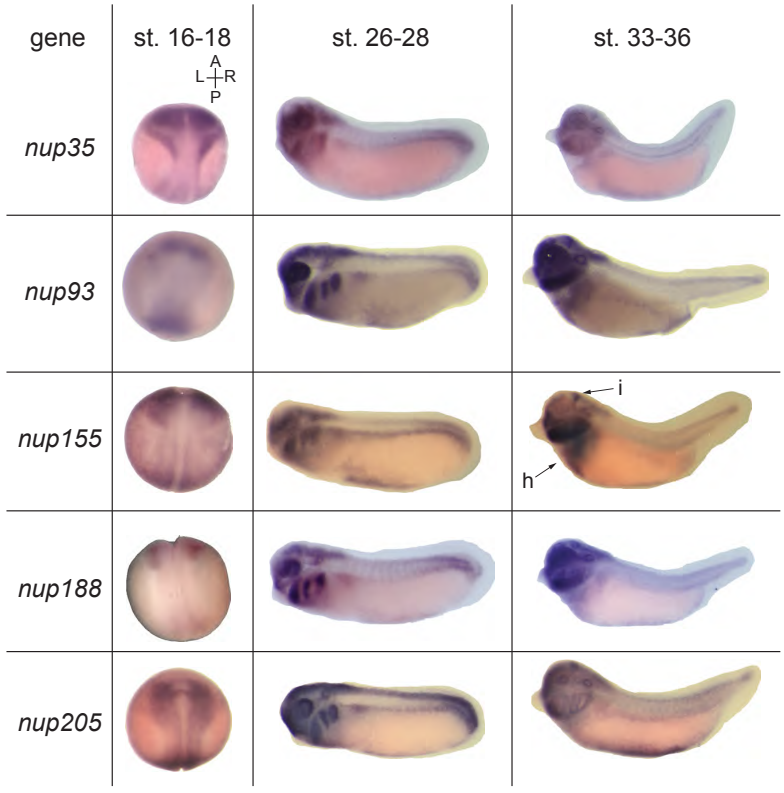


Fig. 3 (Left). Expression of outer ring scaffold nups in developing *X. tropicalis* embryos. *Expression was analyzed by whole mount* in situ *hybridization at 3 different stages of* X. tropicalis *development (16-18, 26-28 and 33-36, columns). Signal was detected for all the nups in the anterior regions of the neural tube and near the eye anlages at stages 16-18. At stages 26-28 and 33-36, signal was detected for all nups in the brain, eyes, and visceral arches. c, cement gland; i, isthmus or mid to hindbrain boundary; lm, dorsolateral plate mesoderm; vm, ventral mesoderm. A: anterior, P: posterior, L: left, R: right.*

Fig. 4 (Right). Expression of inner ring scaffold nups in developing *X. tropicalis* embryos. *Expression was analyzed by whole mount* in situ *hybridization at 3 different stages of* X. tropicalis *development (16-18, 26-28 and 33-36, columns). All nups were detected in the neural plate region at stages 16-18 and in the head, visceral arches and eyes at stages 26-28 and 33-36. h, heart progenitor field; i, isthmus or mid to hindbrain boundary. A: anterior, P: posterior, L: left, R: right.*

At stages 26-28 there was signal detected for all three genes in the brain, eyes and visceral arches (Fig. 7). For *pom121* we detected signal in the somites. *Nup210* and *ndc1* showed tailbud signal. There was also signal at the otic vesicle for *ndc1 and nup210.* In contrast to the other subcomplexes, we did not detect strong signal at the dorso-lateral plate mesoderm for these genes. However, strong ventral mesoderm signal was detected for *nup210* and *ndc1* at these stages.

At stages 33-36, we continued to observe general expression in the eyes, brain, otic vesicle and visceral arches for all three genes (Fig. 7). We observed *ndc1* signal along the length of the neural tube. Ventral mesoderm expression was observed for *nup210* and *ndc1.*

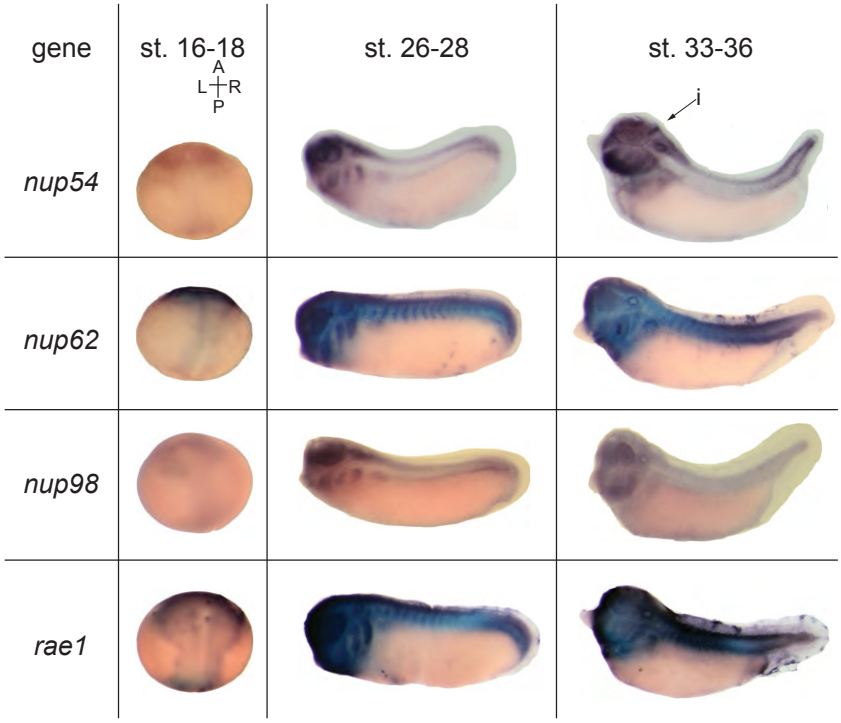


Fig. 5. Expression of Channel nups in developing *X. tropicalis* embryos. *Expression was analyzed by whole mount* in situ *hybridization at 3 different stages of* X. tropicalis *development (16-18, 26-28 and 33-36, columns). All nups were detected in neural plate region at stages 16-18; brain, eyes and visceral arches at stages 26-28. i, isthmus or mid to hindbrain boundary. A: anterior, P: posterior, L: left, R: right.*

RT-PCR validation

To assess if the specific expression detected by *in situ* hybridization correlates with mRNA transcript levels, especially in regions where penetration of probes could pose problems, we performed RT-PCR in two distinct regions of the embryo. We chose the head as a region where all the nups showed strong expression, and the ventral endomesoderm as a region where expression was very low or not detected for some nups. Additionally these two regions were easy to dissect from each other in a practical way given the small size of the embryos (Fig. 8A). We chose 2 nucleoporins from each subcomplex: one with strong expression in the ventral mesoderm and another where we could not detect expression by *in situ* hybridization. For example, for the outer ring nups, *seh1* shows strong expression in the ventral mesoderm, whereas for *nup133* we could not detect expression in the same region. RT-PCR showed strong expression of both nups in the head region, but we detected expression of *seh1* in ventral mesoderm, where *nup133* is reduced (Fig. 8B). Although RT-PCR is not quantitative, we were able to qualitatively correlate the expression with the *in situ* hybridization results, showing nups expression is highly regulated during development.

Discussion

In general, there are certain regions where nups are expressed across all subcomplexes; these include: the neural folds of early stage embryos, the eyes, visceral (branchial) arches and brain of later stage embryos. Other common areas of expression are the dorso-lateral plate mesoderm and otic vesicle. The question arises as to why signal was consistently and strongly seen in the eyes, brain and visceral arches across all nups. It may be possible that these regions of the embryo have a higher rate of cell proliferation, and therefore a higher rate of nup expression. However, the most striking feature of these expression patterns is the fact that we did not detect ubiquitous expression, despite the fact that nucleoporins are required in every cell. Previous *in situ* hybridization studies have shown a criteria to classified genes as ubiquitously expressed (Pollet *et al.*, 2005), which is clearly different from the *in situ* expression patterns we obtained for the nups. Within each subcomplex, one would expect a consistent pattern of expression based on the assumption that all genes within the subcomplex localize to the same structure. Therefore, we would expect a similar expression pattern for nups of the same subcomplex. The structural localization relationship should hold, but the intensity of signal detected for each nup may differ if the stoichiometric relationship between each nup protein in the complex is not 1:1. However, interestingly there is variation in localization of expression both between subcomplexes and within each subcomplex. This suggests a greater dynamism and mobility of nucleoporins than previously assumed. It also suggests that each nucleoporin may have several independent functions beyond their roles in the NPC. Whether or not the nup pool involved in function outside the NPC can account for the differential pattern of expression remains unclear.

Nucleoporin proteins of the Nup107-160 complex have been shown to play active roles in mitosis and kinetochore formation (Mishra *et al.*, 2010). In addition, *nup107* depleted cells were defective in NPC formation, as in its absence several other nup proteins failed to assemble into the NPC complex (Boehmer *et al.*, 2003). This specific role for *nup107* may explain its apparently stronger expression in our *in situs,* since it plays more than a structural role in a single subcomplex. In another study, *seh1* was shown to be necessary for correct chromosome alignment in mitosis and for completion of cytokinesis (Platani *et al.*, 2009). This suggests that in addition to the role that the 107-160 subcomplex has as a unit, individual members of the subcomplex also have unique functions. In a study of neuronal development in mice, *nup133* null mutants showed significant defects in neural tube formation that resulted in embryonic lethality (Lupu *et al.*, 2008). In our results, *nup133*

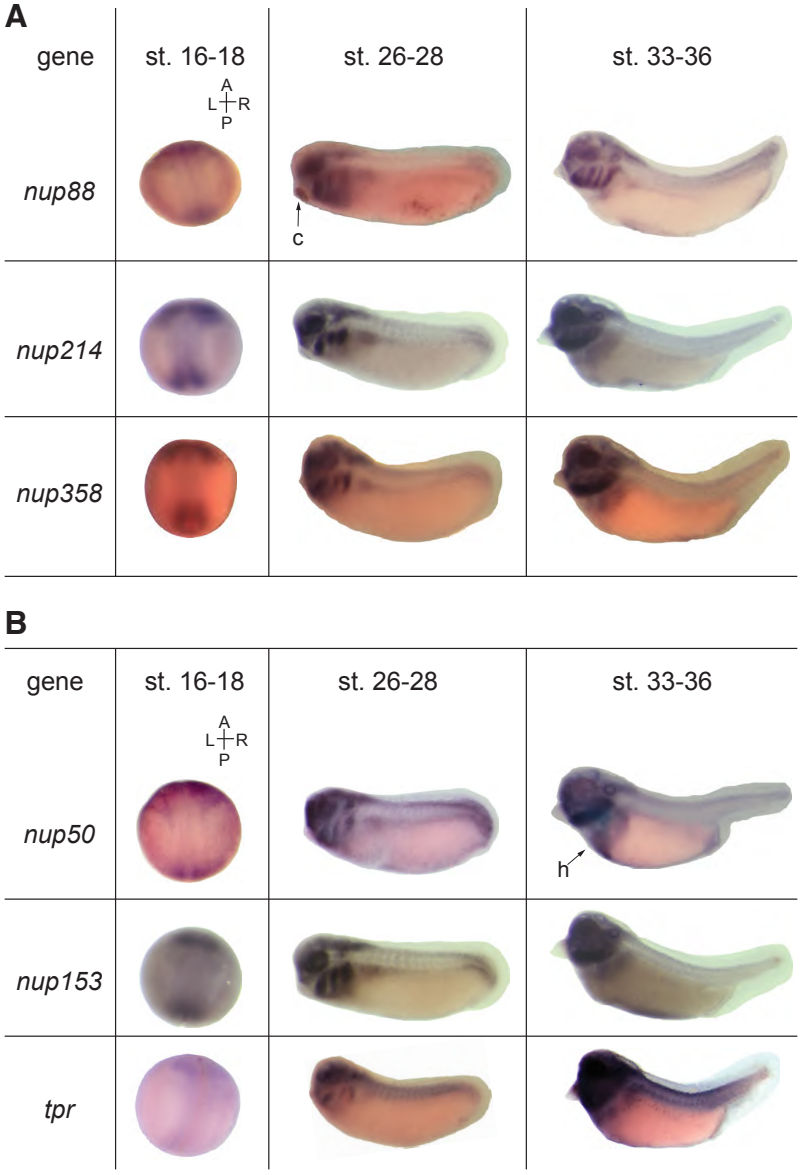


Fig. 6. Expression of cytoplasmic and nuclear basket nups in developing *X. tropicalis* embryos. *Expression was analyzed by whole mount* in situ *hybridization at 3 different stages of* X. tropicalis *development (16-18, 26-28 and 33-36, columns).* **(A)** *Cytoplasmic nups and* **(B)** *Nuclear basket nups expression patterns. Neural tube signal was detected all nups at stages 16-18. At stages 26-28 and 33-36 transcript expression was detected in the head, eyes, and visceral arches in all* nups. *h, heart progenitor field; c, cement gland. A: anterior, P: posterior, L: left, R: right.*

showed a unique expression pattern (Fig. 3), with strong expression in the mid to hind brain boundary at stages 33-36, indicating that it may be a candidate for further study of neuronal development in *Xenopus* as well. Given their similar expression patterns within the brain to *nup133,* nups *54, 88, 93* and *155* may also be candidates for further study in this regard. Recently, Niu *et al.,* showed that *sec13* plays independent functional roles in the NPC (affecting retinal development) and in the COPII complex of the ER, which is responsible for protein trafficking to the Golgi apparatus (Niu *et al.*, 2014). These results may point to a similar role for *sec13* in *Xenopus,* given its strong expression in the eye regions in our *in situs*. These studies and many others showed that nucleoporins play an important role in embryonic development (Raices and D'Angelo, 2012).

Interestingly, functional studies on nucleoporins seemed to have focused largely on the scaffold nucleoporins, leaving a large portion of the nup family genes still open for further experimentation. For instance, *tpr,* a member of the nuclear ring subcomplex, has been shown to play a role in the formation of heterochromatin exclusion zones proximal to NPCs in somatic cells (Krull *et al.*, 2010). In addition, *rae1* null mutants in mice were found to be embryonic lethal. Moreover, haploinsufficient *rae1* mutants were shown to have mitotic checkpoint and chromosomal segregation abnormalities, similar to the mutant phenotypes found in functional studies of the scaffold subcomplex nups (Babu *et al.*, 2003). Furthermore, *nup62* depletion induced cell cycle arrest and affected centriole and centrosome function (Hashizume *et al.*, 2013).

Given the diverse roles of nucleoporins determined thus far, it can be expected that other nups may possess additional functions beyond a structural role in the NPC. Our study suggests that from a developmental perspective, there may be certain embryonic regions that are differentially affected by nucleoporin function. However, further studies are needed to illuminate what these potential roles are.

Overall, our results have shown that nup expression is subject to a high degree of spatial regulation and transcripts

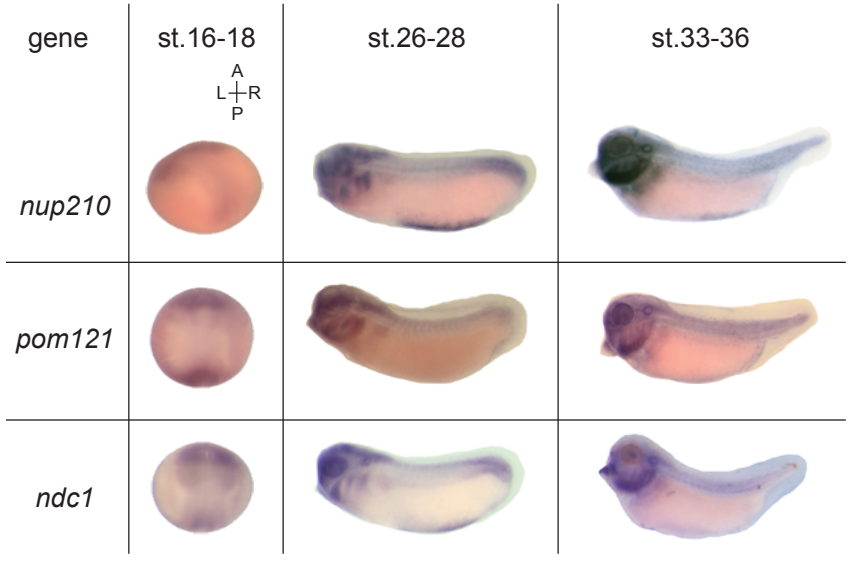


Fig. 7. Expression of Transmembrane nups in developing *X. tropicalis* embryos. *Expression was analyzed by whole mount* in situ *hybridization at 3 different stages of* X. tropicalis *development (16-18, 26-28 and 33-36, columns). At stages 16-18 expression was detected for all genes in the neural tube. All nups showed expression in the head, eyes, and visceral arches at stages 26-28 and 33-36. A: anterior, P: posterior, L: left, R: right.*

are produced in a much higher amount in specific regions of the embryo, even if the ubiquitous presence of nucleoporins is assumed in cells. Furthermore, absolute quantification of nup transcripts per embryo also shows dynamic transcriptional regulation during early development (Fig. 2, (Owens *et al*., 2016). Knowledge of these expression patterns provides great insight into potential directions of experimentation and study of nucleoporin localization and function.

Materials and Methods

Animal husbandry

X. tropicalis were housed and cared for in our aquatics facility according to established protocols that were approved by Yale IACUC.

RNA-seq data analysis

RNA-seq profiles of nucleoporins during *X. tropicalis* development were obtained from http://genomics.crick.ac.uk/cgi-bin/profile-search.exe, (Owens *et al*., 2016). Graphs in Fig. 2 show the expression profiles grouped by subcomplex. This RNA-Seq profile represent an absolute quantification of transcripts per embryo from 0 to 66 hours post fertilization (horizontal axis) with 95% confidence interval depicted in the shaded region.

Obtaining and fixing embryos

WT Embryos were obtained from *Xenopus* natural matings (del Viso and Khokha, 2012). Embryos were allowed to grow in 1/9X MR supplemented with 50ug/ml of gentamycin to the desired stages (16-18, 26-28 and 33-36), that were scored using the Normal Table of *Xenopus laevis* (Daudin) (Nieuwkoop and Faber, 1994). Embryos were fixed in MEMFA (0.1M MOPS (pH7.4), 2mM EGTA, 1mM MgSO4, 3.7% v/v formaldehyde) for 2 hours at room temperature, and subsequently dehydrated in 100% ethanol overnight. Vitelline membrane was removed manually using forceps. Stocks were stored at -20°C. Around 1000 embryos of each range of stages were fixed and used for *in situ* hybridizations.

Whole-mount in situ *hybridization*

We obtained clones from our own cDNA library stocks (Sanger Clone Library), Open Biosystems (Thermo Scientific) and the Harland Lab at the University of California-Berkeley (Table 1). A portion of the *nup188* clone was amplified by PCR using the primers Nup188F1: CCAGCAGGATCTT-GTAGACACAGC and Nup188R1: CAGGGGATAAGTTGAGGATTGCAT to obtain a 1kb segment for *in situ* probe synthesis. RNA probe synthesis was done via *in vitro* transcription using T7 High Yield RNA Synthesis Kit (E2040S) from New England Biolabs, using digoxigenin labeled dUTP. To synthesize RNA sense probes we used the same clones and the Maxiscript SP6 (AM1308) from Ambion, using digoxigenin labeled dUTP. The whole-mount *in situ* hybridization procedure was done according to the standard protocol (Khokha *et al*., 2002). Embryos were stained with BM Purple at room temperature or 4°C depending on ideal conditions for each probe. Embryos were then bleached and suspended in glycerol for long-term storage. Images were taken with a Canon DS126201 camera and Zeiss Discovery.V8 SteREO scope. Exposure settings were kept constant for all images. *In situ* hybridizations were done between 2 and 3 times for each nucleoporin. After we detected strong signal for each gene, we fixed half of the embryos and continued developing the other half to asses if the signal would become ubiquitous. Developing embryos further did not change the original pattern of expression and in some cases lead to background due to inappropriate precipitation of the substrate. As negative controls we used sense RNA probes for at least one representative of each subcomplex (Supplementary Fig. S1). Sense probes were developed for the same amount of time as the respective antisense probe.

RT-PCR assays

Embryos were raised to the desired stages (26-28 or 33-36) and two regions were isolated: the head and the ventral mesoderm (Fig. 8A). Tissue was immediately immersed in Trizol Reagent (Life Technologies) and RNA was extracted following the manufacturer's instructions. The Superscript™ IV Reverse Transcriptase (Thermofisher) was used to synthesize cDNA using 1 μg of total RNA from each tissue. PCR was performed using Phusion High

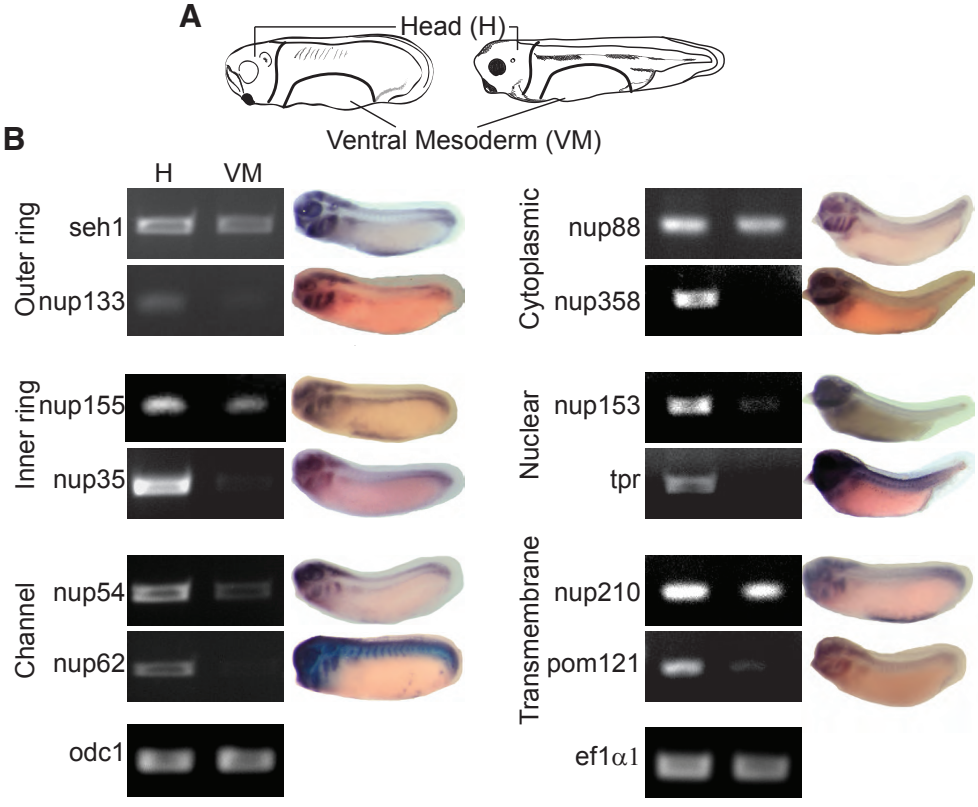


Fig. 8. Correlation of nup expression between *in situ* hybridization and RT-PCR. (A) *Schematics of stages 28 and 33 depicting Head (H) and ventral mesoderm (VM) regions that were dissected and used for RNA extractions and subsequent RT-PCR.* **(B)** *Expression by RT-PCR of 2 nups for each subcomplex, in each of the dissected tissues.* In situ *hybridization images from previous figures are shown for illustration. Odc1 (ornithine decarboxylase 1) and ef1α1 (eukaryotic translation elongation factor 1 alpha 1) were used as positive controls of RT-PCR. The primer sequences for each nup are shown in Table 1.*

TABLE 1

LIST OF ALL NUP GENES TESTED WITH CLONE ID INFORMATION AND PRIMERS USED FOR RT-PCR

Gene Name	Clone ID	RT-PCR primers
Nup35	TEgg013j23	XtNup35F1: 5' CATGGAGCCTATGGGTGCTG XtNup35R1: 5' CAACAGACTGTGTAGGAGTCCCTA
Nup37	TGas082o16	
Nup43	TEgg125d07	
Nup50	TNeu054d12	
Nup54	TGas114o17	XtNup54F1: 5' AACCAATCTGTTCAAAGCACGCC XtNup54R1: 5' TGTTGCTTTGTCATCTGATCTTGGA
Nup62	7605025	XtNup62F1: 5' AGGCGGTCTTACCTTTGGTG XtNup62R1: 5' GCGTTGACCTGAGTTGCTTG
Nup85	TNeu102j12	
Nup88	TGas106b12	XtNup88F1: 5' CTGGAGATTCCTAAACGCTGGGG XtNup88R1: 5' GCAATGGTATCGGGATGGGC
Nup93	5379233	
Nup96	8889890	
Nup98	7686621	
Nup107	8850047	
Nup133	7623820	XtNup133F2: 5' AAAGATTAATCAACGTATTCTGCCG XtNup133R2: 5' TATCTCCTTGTGCTTCAAATGGAAT
Nup153	8851980	XtNup153F1: 5' CAAACGCTCAGCAGTATGGGG XtNup153R1: 5' TCCTAAGGTGACCTTTACAGGGG
Nup155	TGas122g21	XtNup155F1: 5' GTGCTTGCTGTGCTCAGTTTGC XtNup155R1: 5' GGCAAGGAATATAGCGGGTCTG
Nup160	8851700	
Nup188	7675066	
Nup205	7684539	
Nup210	7554222	XtNup210F1: 5' TTAGAGCTAAAATGCTGGAGCCG XtNup210R1: 5' TGGGAACGTAAGAATTTTAGGGG
Nup214	8851888	
Nup358	7586397	XtNup358F1: 5' ACAGATTTAGAATGGTGCTCCCG XtNup358R1: 5' AAGTCTTGAAGTTTCCTGGGGC
Ndc1	TEgg045l09	
Rae1	7604803	
Sec13	TGas091g04	
Seh1	TGas096l04	XtSeh1F1: 5' GGGACAAAAGTGAAAATGGCG XtSeh1R1: 5' TGTGCCAGTGATGTTCCAACTTAC
Tpr	TNeu065h23	XtTprF1: 5' AATGGAATCCGTGAAAGCCCG XtTprR1: 5' TTGTGAAGCTCCTCCACTGCCC
Pom121	TGas001m22	XtPom121F2: 5' ATCTGGAACCAGCAGCCTTC XtPom121R2: 5' ACCAAACGTAGGCTGAGTGG
ODC1		ODC1-F: 5' TGTTCTGCGCATAGCAACTG ODC1-R: 5' ACATCGTGCATCTGAGACAGC
Ef1α1		Ef1α1-F: 5' CCCTGCTGGAAGCTCTTGAC Ef1α1-R: 5' GGACACCAGTCTCCACACGA

Fidelity DNA polymerase (New England Biolabs) and the following program: 98°C for 30s, 22 cycles of 98°C for 15s, 60-66°C for 30s, 72°C for 45 sec, and a final elongation at 72°C for 5 min. Annealing temperature varied according to each pair of primers. Odc1 (ornithine decarboxylase 1) and ef1α (eukaryotic translation elongation factor 1 alpha 1) were used as positive controls of RT-PCR. The primers used for each nup are shown in Table 1.

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