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Authors
Krauss, Sharon Wald
Spence, Jeffrey R.
Go, Minjoung M.

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PROTEIN 4.1R FUNCTIONS ARE CRITICAL FOR NORMAL NUCLEAR ASSEMBLY, NUCLEAR ARCHITECTURE AND CELL CYCLE PROGRESSION

Sharon Wald Krauss, Jeffrey R. Spence and Minjoung M. Go
Dept. Genome Biology, Univ. of California-LBNL, Berkeley, CA 94720

Protein 4.1R, a multifunctional structural protein, was initially identified in mature red cells as an adaptor protein stabilizing spectrin/actin interactions with plasma membrane-associated proteins. However, in nucleated cells, 4.1R localizes at multiple sites including nuclei where its interactions are not well characterized. To dissect its nuclear functions, we first established that 4.1 itself is critical for nuclear assembly by depletion/addback experiments using *Xenopus* egg extract nuclear reconstitution reactions. In additional dominant/negative peptide experiments in the *Xenopus* cell-free system, we showed that two distinct 4.1 domains (binding actin and binding NuMA) are crucial for proper lamin and nuclear pore organization, chromatin decondensation, and the capacity to synthesize DNA. Recently a 4.1 gene family was discovered necessitating refinement in identifying common as well as unique functions of its members. To specifically probe 4.1R (red cell) function *in vivo* in mammalian cells, we identified cells expressing only 4.1R and 4.1G (generally expressed). We then used RNA interference-mediated depletion of 4.1R in human cells and also analyzed 4.1R-deficient mouse embryonic fibroblasts. In both of these independent systems we found perturbed nuclear morphology, selective mislocalization of intranuclear as well as inner nuclear membrane proteins, and impaired cell cycle progression. After specific downregulation of 4.1R, increased numbers of nuclei become markedly blebbed and/or at least 2-fold larger or smaller with increased formation of binucleate and multinucleate cells. Notably NuMA, a 4.1 binding partner and a nuclear matrix protein, is also detected in cytoplasmic aggregates rather than being exclusively intranuclear, potentially reflecting a failure of proper anchorage of NuMA. Lamin A at the nuclear periphery and at intranuclear sites is perturbed. Emerin, LAP2alpha, MANI and nuclear pore proteins are partially mislocalized to the cytoplasm. However, LAP2beta remains at the nuclear membrane. Cell cycle analysis revealed that human or murine cells deficient in 4.1R expression have increased numbers of cells in S phase and decreased G2/M populations. Our data support the hypothesis that 4.1 interactions link structural components within nuclei, some potentially involving nuclear actin, and suggest that genetic defects affecting 4.1R function may lead to as yet unappreciated pathological consequences.