and p53 in response to linear dsDNA to the same extent as the wild-type ATM[32]. Although we observed NOTCH1 inhibiting ATM-mediated p53 phosphorylation *in vitro*[24], in the light of the new results demonstrating disruption of KAT5 and FOXO3a from the AAC[1] it would be interesting to see if addition of: KAT5, FOXO3a, H3K9m3 and cAbl would give similar results in an *in vitro* ATM kinase assay in the presence of NOTCH1 and mutated ATM (S1981A).

It has been suggested that ATM autophosphorylation is necessary for ATM retention at the DSBs[33] because the ATM S1981A mutant, although recruited normally to DSBs, was not stabilized properly at the site of the damage[33]. In contrast we have reported that although NOTCH1 inhibited ATM autophosphorylation it did not affect ATM's retention at the DSBs[1]. To understand these apparently contradictory observations one should look not just at the ATM autophosphorylation. We have previously reported that NOTCH1 inhibits kinase activity of ATM[24], therefore it would be better to compare our results with the reports describing recruitment of the ATM kinase dead mutant (ATM KD). Indeed it has been shown that ATM KD mutant (that cannot undergo autophosphorylation) is recruited to the DSBs without any impairment of retention[34,35]. This is similar to our observation of the NOTCH1-mediated impact on ATM recruitment and autophosphorylation. Additionally, it was reported that cells carrying mutations in the KU70 and MRE11 nuclease actively recruit ATM to the DSBs (without retention impairment); although ATM does not undergo autophosphorylation in those conditions[36]. Overall, our results[1,24], together with above mentioned reports, show our still incomplete understanding of ATM autophosporylation and its role in the ATM activity and DDR, that needs further elucidation.

Meeting γH2AX at the FATC end

We have repeatedly observed that NOTCH1 inhibits ATM activation by blocking its autophosphorylation (Ser1981) and phosphorylation of downstream substrates such as KAP1, SMC1, p53, DNA-PKcs or CHK2[1,24](Adamowicz et al – in press). Additionally, we have observed in our initial study using *Xenopus laevis* egg extract that NOTCH1 blocked a substantial amount of ATM-mediated phosphorylation[24]. However, of all observed NOTCH1-mediated ATM phosphorylation defects, phosphorylation of ATM's main substrate, H2AX, remained unaffected.

It has been suggested that there is redundancy between PI3K-like kinases in terms of H2AX phosphorylation. DNA-PKcs or ATR kinases have been shown to phosphorylate H2AX[37,38]. Additionally, it has been reported that H2AX is phosphorylated to the same extent in the ATM WT and KO cells[37,39]. Indeed, experiments carried out in our laboratory showed that NOTCH1 neither blocks ATR nor DNA-PKcs kinase activity (Adamowicz et al – in press). However unexpectedly, when we performed analysis of the γH2AX foci formation in NOTCH1-expressing cells in the presence of either DNA-PKcs or ATR inhibitors we did not observe any difference in the H2AX phosphorylation (data unpublished – data available upon request).

These results suggest that H2AX can be phosphorylated by protein kinases other than ATM, ATR and DNA-PKcs. Indeed, it has been reported that JNK and p38 can phosphorylate H2AX in response of UV light irradiation or starvation respectively[40,41]. Additionally, it has been reported that VRK1 kinase can phosphorylate H2AX in response to IR in parallel to ATM kinase[42]. Moreover, VRK1 was shown to be necessary for the accumulation of DDR factors around DSBs, which implies more complex role of VRK1 in the DDR that needs further elucidation[43].

Because NOTCH1, unlike small molecule ATM inhibitors, cannot directly inhibit ATM kinase activity we can speculate that NOTCH1 binding to ATM could strongly impair ATM substrate recognition, resulting in an inhibition of phosphorylation of some substrates such as p53 or CHK2, but not H2AX. It has been already reported that NOTCH1 can bind and hence modulate the substrate recognition of LSD1 demethylase[44]. Therefore, it can be possible that by binding to the FATC domain of ATM, NOTCH1 would strongly impair substrate recognition of ATM. Interestingly, it has been shown in yeast that deletion of last 10 amino acids (aa) of the FATC domain can impair Tel1 phosphorylation of Rad53, which was connected with the loss in its ability to interact with MRX complex[45]. Moreover, MRN complex was shown to help ATM in the substrate recognition by stimulating ATM binding to its substrates like p53 or CHK2[46]. On the other hand it has been published that in human cells deletion of last 10aa of the FATC domain of ATM does not lead to the impairment of ATM MRN-mediated response, but rather its ability to activate upon oxidative stress[47]. We have shown that although NOTCH1 binds to the FATC domain it does not affect interaction between ATM and MRN complex[1]. This suggests that if by binding to the ATM FATC domain NOTCH1 is perturbing ATM substrate recognition this effect is rather mediated by inhibition of KAT5-mediated acetylation. Impairment of ATM acetylation will then block structural changes in ATM that would lead to its monomerization and activation, inhibiting this way release of the ATM kinase domain otherwise hindered inside of its dimer structure[17].

Taking ATM down a NOTCH

NOTCH1 was very early connected to the tumorogenesis and marked as an oncogene due to its ability to induce tumour growth[48]. Activating mutations in NOTCH1 are present in many T-cell acute lymphoblastic leukemias (T-ALL)[49] or breast cancers[50]. Indeed, we have found that

NOTCH1 expression was negatively correlated with the ATM activation in the human breast cancer patients[24]. At the same time, other group reported that ectopic expression of NOTCH1 in cancer cells lead to their increased resistance to DNA damage in vivo[51]. Those data show that expression of high levels of NOTCH1 (due to activating mutations or ectopic expression) stimulate radioresistance and survival in cancer cells, resulting probably from the inhibition of p53-mediated apoptosis. Additionally, increased levels of NOTCH1 induce faster proliferation[52] leading to replication stress. Although we showed that NOTCH1 inhibits ATM activation, this is not true for ATR kinase (Adamowicz et al – in press) resulting in the protection of NOTCH1-driven cancers from replication stress.

Neural stem cells (NSC) are known to express moderate levels of activated NOTCH1, which is necessary for their proliferation[53]. Interestingly it has been reported that induction of DDR in NSC leads to their spontaneous differentiation to astrocytes, which is dependent on ATM activation[54]. It is therefore possible to speculate that NOTCH1-mediated downregulation of ATM activation could tip the balance allowing for DNA damage repair without inducing differentiation. It is important to remember that physiological levels of NOTCH1 are low as compared to those observed in T-ALL cells or those achieved by ectopic expression, therefore observed effects of NOTCH1 activation might be very mild. Additionally, observed results might be an outcome of many different factors impacting at the same time on DDR. Indeed, it has been shown that SALL4 transcription factor expressed in stem cells favours ATM activation by its binding to MRN complex[55].

In summary, I would like to propose that the physiological role of NOTCH1 is not to inhibit fully ATM activation, but rather to induce its mild impairment, to modulate a balance between the amount of DNA damage and DDR signalling. This would result in the suppression of DNA damage induced apoptosis or differentiation, giving time for necessary repair.

There is plenty more ATM in the sea

Formation of AAC is necessary for ATM activation at DSBs and DDR[1,24]. Apart from AAC, ATM relies also on MRN complex, which allows proper AAC localization and substrate recognition. Interestingly, in the nucleus, ATM has been described to exist in two different complexes. In has been shown that there is competition between MRN complex and ATMIN for binding to ATM (**Figure 3**)[56,57]. Studies have found that while MRN complex guides ATM in the response to DSBs, ATMIN is necessary during oxidative and hypotonic stresses[58,59]. It is possible that like MRN, ATMIN by binding to ATM regulates it substrate recognition and therefore its kinase activity in response to different stimuli. It would be interesting to see if the structure of AAC is preserved while complexed with ATMIN, and if so how it is involved in the ATM activation (**Figure 3**).

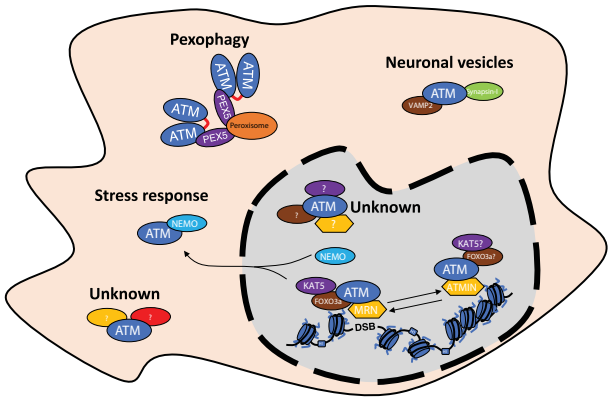

Figure 3. Existence of different ATM complexes in the cells. Schematic representation of the existence of different ATM complexes both in the nucleus as well as in the cytosol. For details please look into the text.

We tend to think about ATM through its role in DDR regulation, although ATM has been described to be involved in many more cellular processes like: stress response[60], neuronal signal transmission[61] or pexophagy[62,63](**Figure 3**), which in every case requires its presence outside the nucleus. This implies that ATM is not always in complex with KAT5 or MRN complex. The presence of ATM in peroxisomes is a result of its interaction with PEX5, which is responsible for ATM peroxisome localization. In peroxisomes, ATM is activated by reactive oxygen species and formation of an active dimer, allowing ATM to control peroxisome phagocytosis[63]. The involvement of ATM in the stress response is connected to its interaction with NEMO and with shuttling between nucleus and cytosol[60]. Additionally, ATM has been described to localize in the cytosol of neuronal cells[64] and has been implicated in the neuronal signal transmission by its interaction with VAMP2 and Synapsin-I[61]. It would be interesting to see if NOTCH1, which has very strong affinity to the FATC domain of ATM[1], could be used as a tool for identifying new regulatory components of ATM complexes in the cytosol.

It is thought-provoking to picture ATM in different complexes that differentially regulate its activity and substrate recognition. The identification of different active ATM complexes opens new and exciting areas of research and raises even more fascinating questions. For example, how is ATM activation in those complexes stimulated, and how is ATM substrate recognition and kinase activity regulated? Hopefully, in the near future we will know the answers.

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Conflict of Interest

I declare no conflict of interest.

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